2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 1: 53-60; January 27, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.7

EFFECTS OF PROBIOTIC *Enterococcus faecium* AND RAW, SPROUTED AND FERMENTED PEARL MILLET BASED DIETS ON PERFORMANCES, CARCASS TRAITS, HEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILER CHICKENS

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Supporting Information

RESEARCH ARTICLE

PII: S2228770124000007-14

Received: August 14, 2023

Revised: November 30, 2023

Accepted: December 03, 2023

ABSTRACT: Probiotics, recognized as a safe substitute for antibiotics in the animal industry, have been acknowledged for their growth-enhancing properties. This study assessed the impact of *Enterococcus faecium* strain NCIMB 11181 and diets incorporating Raw, Sprouted, and Fermented pearl millet on the performance, carcass traits, organ weights, and blood parameters of broiler chickens. In a randomized design, 120 one-day-old Arbor Acre broiler chickens were assigned to five groups: 1) No supplement, negative control (N-con); 2) Control + antibiotics, positive control (P-con); 3) Raw pearl millet + probiotics in drinking water (RPM + PRO); 4) Sprouted pearl millet + probiotics in drinking water (SPM+PRO); 5) Fermented pearl millet + probiotics in drinking water (FPM + PRO). Probiotic supplementation did not significantly impact body weight gain (BWG) but influenced feed intake (FI) ($P<0.05$). FPM+PRO increased feed conversion ratio (FCR), thigh yield, and drumstick yield. Thymus weight is reduced in the RPM+PRO and SPM+PRO groups compared to the control groups. Serum high-density lipoprotein (HDL) levels decreased ($P<0.01$) in the P-con and FPM+PRO groups. No treatment effect ($P>0.05$) was observed on hematological indices. Overall, pearl millet diets supplemented with probiotics demonstrated no adverse effects on the health status of broiler chickens, suggesting their potential as viable alternatives to antibiotics.

Keywords: Pearl millet, Broiler chickens, Blood, Carcass traits, Growth performance, Probiotics.

INTRODUCTION

Antibiotics gained popularity in the poultry industry for promoting growth and maintaining poultry health. However, their extensive use led to concerns about residues in livestock products, antibiotic-resistant gene transfer, and negative effects on human health and safety (Ronquillo and Hernandez, 2017; Vieco-Siaz et al., 2019). Consequently, antibiotic use in food animals faced restrictions (Vieco-Siaz et al., 2019).

Probiotics, non-pathogenic microorganisms in the intestinal flora, offer an alternative by benefiting host physiology and health. They stabilize intestinal microbiota, enhance carcass traits, intestinal morphology, gut microbial population, modulate the immune response, and strengthen the mucosal barrier (Attia et al., 2017; Wu et al., 2019; Zhang et al., 2021). Probiotics may replace antibiotics as growth promoters in poultry (Suryadi and Prasetyo, 2018). Notably, different probiotic strains within the same genus and species can have varying clinical effects (Vieira et al., 2013). The European Food Safety Authority (EFSA) approved *Enterococcus faecium* strain NCIMB 11181 as an animal feed additive to improve growth performance (EFSA, 2012).

Enterococcus faecium, a lactic acid bacterium, is a natural intestinal inhabitant resistant to acidic conditions and bile salts. It produces enterocins, antimicrobial substances serving as poultry probiotics (Zommiti et al., 2018). The *E. faecium* strain 11181 has demonstrated efficacy in improving feed conversion ratio, daily weight gain, and gut health, inhibiting gut pathogen proliferation, and stimulating the immune system (Wu et al., 2019; Shao et al., 2022). Adding *E. faecium* to broiler feed or drinking water enhances intestinal morphology, modulates microflora, and inhibits pathogen proliferation, including Salmonella (Cao et al., 2013; Zheng et al., 2016; Wu et al., 2019; Shao et al., 2022).

This study focuses on Pearl Millet (PM), a nutrient-rich grain abundant in the Sahel region, especially Nigeria. PM boasts protein, amino acids, vitamins, minerals, fiber, fat, energy, ash, and antioxidants, with fewer anti-nutritional factors than other cereals (Uppal et al., 2015; Weckwerth et al., 2020). Furthermore, PM includes fewer anti-nutritional factors, compared to other cereals (Kaushik and Grewal, 2017; Boncompagni et al., 2018; Punia, 2020).

Different processing techniques, including sprouting and fermentation, enhance nutrient availability in PM (Gowda et al., 2022). Our prior study confirmed that processed PM did not negatively impact the physiology and welfare of broiler chickens (Olasehinde and Aderemi, 2023). Yet, the potential benefits of supplementing processed PM diets with probiotics on broiler chicken growth performance and blood metabolites remain unexplored. This study aimed to address

Citation: Olasehinde O and Aderemi F (2024). Effects of probiotic *Enterococcus faecium* and raw, fermented and sprouted pearl millet based diets on performances, carcass traits, hematological and biochemical indices of broiler chickens. *Online J. Anim. Feed Res.*, 14(1): 53-60. DOI: https://dx.doi.org/10.51227/ojafr.2024.7

this gap by investigating the effects of unprocessed and processed PM, supplemented with probiotic *E. faecium*, on various aspects health and performance of broiler chickens.

MATERIALS AND METHODS

All experimental procedures were approved by Bowen University's committee for research and ethics. The birds were managed and handled following standard guidelines of the University and ARRIVE 2.0 and National Research Council (NRC) Committee guidelines (du Sert et al., 2020), which reduced pain and discomfort on the birds.

Source of probiotics supplementation

The *E. faecium* NCIMB 11181 strain (Protexin) used in the study was a commercial product from Probiotics International Ltd (Lopen Head Somerset, UK) which contained a total bacteria count ≥ 2.0 x 10 11 CFU/kg. The probiotic product was added to drinking water on daily basis for 42 d according to manufacturer instruction. Broilers on antibiotics treated group receive colistin (as sulphate, 4,800,000 IU, Kepro, Holland) daily through drinking water for 42 d.

Animal, design, and diets

A total of 120 one-day-old Arbor Acres chicks were individually weighed, labelled, and randomly allocated, following a completely randomized design, to 5 dietary treatment groups each comprising 4 replicate cages with 6 birds per cage. The basal diet was isocaloric and met or exceeded NRC (1994) nutrient requirements for starter (day 1 to 21) and finisher (day 22 to 42) phases. Pear millet replaced 25% maize in the basal diet. The composition and nutrient levels of the basal diet of maize or PM + soybean meal-based is presented in Table 1. The treatments consisted of the following: 1) basal diet without supplementation, negative control (N-con); 2) basal diet with antibiotics supplementation, positive control (P-con); 3) Raw pearl millet + probiotics supplementation (RPM + Pro); 4) Sprouted pearl millet + probiotics supplementation (SPM+Pro); 5) Fermented pearl millet + probiotics supplementation (FPM+Pro). Antibiotics and probiotics were administered according to the manufacturer's recommendations through drinking water. The broiler chickens on N-con, P-con and probiotics were placed in separate rooms to prevent contamination. The rooms were identical in environmental configuration throughout the study. The lighting programs during the study were 1 hour of darkness (0 - 7 days after hatch) and 4 hours of darkness (8 - 42 days, experimental period). The ambient temperature ranged between 25^{o}C and 33^{o}C during the experimental period. Temperature within the pen was regulated and was carried out through use of natural and mechanical means within the pen.

Table 1 - Basal diet formulation and composition

Ingredient (%)	Starter (1 to 21 d)					Finisher (22 – 42 d)				
	N-con	P-con	RPM+PRO	SPM+PRO	FPM+PRO	N-con	P-con	RPM+PRO	SPM+PRO	FPM+PRO
Maize	52.10	52.10	39.78	39.60	39.28	59.78	59.78	46.97	45.49	45.77
Pearl millet	-	-	14.42	14.29	14.81	-	-	14.94	16.14	16.20
Soybean meal	40.89	40.89	39.24	39.66	39.52	34.00	34.00	32.33	32.61	32.50
Soybean oil	2.68	2.68	2.20	2.26	2.03	2.17	2.17	1.68	1.69	1.46
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	2.16	2.16	2.16	2.15	2.14	1.95	1.95	1.95	1.93	1.92
Limestone	1.49	1.49	1.50	1.51	1.52	1.39	1.39	1.40	1.41	1.42
Vit-Min Premix [1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine		-	0.03	0.03	0.03	0.05	0.05	0.08	0.08	0.08
Methionine	0.18	0.18	0.17	0. 17	0.17	0.16	0.16	0.15	0.15	0.15
Calculated composition (%)										
ME (kcal/kg)	2970	2970	2970	2970	2970	3004	3004	3004	3004	3004
Protein	22.5	22.5	22.5	22.5	22.5	20.00	20.00	20.00	20.00	20.00
Methionine	0.53	0.53	0.53	0.53	0.53	0.45	0.45	0.45	0.45	0.45
Lysine	1.21	1.21	1.21	1.21	1.21	1.10	1.10	1.10	1.10	1.10
Calcium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phosphorus	0.50	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.45

N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; ME = Metabolizable energy; 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.

Growth performance, carcass traits and organ measurements

Body weight (BW) was recorded daily to calculate body weight gain (BWG) for starter (1 to 21 days), finisher (22 to 42 days) and for the overall (1 to 42 days) growth period. Feed intake (FI) per cage was measured daily to calculate FI for each growth period. Feed conversion ratio (FCR) was determined for the starter, finisher, and overall growth period. At the end of starter and finisher growth period, carcass and organ variables were sampled and measured as we previously described (Olasehinde and Aderemi, 2023).

Blood analysis

At the end of finisher growth period, blood samples were drawn from wing 8 birds for each treatment and analyzed as described by Olasehinde and Aderemi (2023).

Statistical analysis

Data were analyzed as a one-way analysis of variance (ANOVA) using the general analysis of variance model of statistical software GenStat version 21 (VSN international). Where there are significant differences at the P<0.05 level, the treatment means were assessed using Tukey's test. Data are presented as means and pooled standard error of mean (SEM).

RESULTS

The BWG by broilers was not altered (P>0.05) by dietary treatments in the starter, finisher, and overall growth periods (Table 2). Broilers in FPM+PRO group consumed most amount of feed during the starter period, while addition of antibiotic decreased (P<0.001) feed intake. During the finisher phase, lowest FI was observed for SPM+PRO group while highest FI was recorded for broiler chickens in the FPM+PRO group (Table 2). There was no difference in the FI of broilers chickens in the N-con, P-con and RPM+PRO groups. When considering the entire experimental period, FI reduced significantly in the SPM+PRO and P-con group compared with the N-con and RPM+PRO group. The highest FI was obtained in broiler chickens in the FPM+PRO group (Table 2). Dietary treatments had no significant effect on FCR during the starter period (Table 2). However, during the finisher period, FCR was significantly higher in the FPM+PRO group (P=0.034) compared to the rest of the treatment groups. In the overall experiment period, FCR of broiler chickens in the FPM+PRO group was significantly higher than the N-con, P-con and SPM+PRO groups (Table 2).

Table 2 - Effect of dietary treatments on performance of broiler chickens

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
BWG (g/bird)							
Starter phase	649	652	633	659	667	15.300	0.839
Finisher phase	1080	1056	1056	1011	1002	11.400	0.124
Overall phase	1729	1708	1689	1670	1669	21.100	0.688
FI (g/bird)							
Starter phase	545^{b}	512^{a}	552^{b}	552^{b}	589^{c}	7.110	<.001
Finisher phase	1435^{b}	1434^{b}	1446^{b}	1366^{a}	1494^{c}	11.900	0.001
Overall phase	1980^{bc}	1946^{ab}	1999^{c}	1918^{a}	2083^{d}	15.200	<.001
FCR							
Starter phase	0.84	0.80	0.89	0.85	0.89	0.025	0.392
Finisher phase	1.33^{a}	1.36^{a}	1.37^{a}	1.35^{a}	1.49^{b}	0.018	0.034
Overall phase	1.15^{a}	1.14^{a}	1.19^{ab}	1.15^{a}	1.25^{b}	0.016	0.047

Means with different letters in the same row differ significantly (p≤0.05, 0.001); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; BWG = Body weight gain; FI = Feed intake; FCR = Feed conversion ratio; g = gram; SEM = Standard error of the mean.

There were no significant differences between treatments for all carcass traits, except abdominal fat, at the end of the starter period (Table 3). Abdominal fat in the RPM+PRO group increased significantly compared with the N-con and the SPM+PRO treatment groups. FPM+PRO increased drumstick and thigh weights of broiler chickens on finisher diets while the addition of antibiotic decreased (P<0.01) drumstick weight. However, there was no effect (P>0.05) of dietary treatments on carcass yield, breast, wing, and abdominal fat at the end of the finisher period (Table 3). There was no significant effect of dietary treatments on digestive and immune organ weights at the end of the starter period (Table 4).

Similarly, dietary treatments did not influence the relative weights of the pancreas, gizzard, proventriculus, liver, bursa, and spleen at the finisher period. However, relative weight of thymus of broiler chickens in the SPM + PRO group decreased ($P<0.05$) compared with the control groups (Table 4).

The hematology indicators presented in Table 5 were not affected by dietary treatments. Similarly, there were no significant treatment effects on albumin, AST, globulin, serum protein, LDL-cholesterol, triglycerides (Table 6). Glucose concentration in PM-based diets supplemented with probiotic showed a decreased trend ($P<0.05$) in contrast to the control groups. However, HDL-cholesterol concentration increased ($P<0.01$) in the SPM+PRO group compared with the control groups. HDL of broiler chickens in the RPM+PRO also increased compared to broilers chickens in the P-con group (Table 6).

Table 3 - Effect of dietary treatments on carcass characteristics of broiler chickens.

Parameters (%)	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Starter phase							
Carcass yield	53.70	54.40	56.20	54.90	50.70	1.380	0.685
Breast	19.24	14.97	19.10	18.82	18.58	0.560	0.069
Drumstick	9.44	8.73	10.11	8.96	8.76	0.240	0.393
Thigh	9.77	9.05	10.29	9.92	8.88	0.230	0.313
Wing	6.61	5.66	6.74	6.15	6.16	0.160	0.124
Abdominal fat	0.24^{a}	0.75^{ab}	1.40^{b}	0.60^{a}	0.76^{ab}	0.110	0.018
Finisher phase							
Carcass yield	60.52	60.11	61.79	60.16	60.24	0.480	0.790
Breast	25.20	25.50	25.50	24.10	23.10	0.490	0.401
Drumstick	10.38^{b}	9.65^{a}	10.03^{ab}	10.41^{b}	11.14^{c}	0.140	0.007
Thigh	10.39^{a}	10.73^{a}	10.92^{ab}	10.69^{a}	11.39^{b}	0.099	0.011
Wing	6.48	6.33	6.37	6.50	6.52	0.056	0.779
Abdominal fat	0.35	0.57	0.54	0.67	0.57	0.110	0.908

Means with different letters in the same row differ significantly ($p\leq0.05$, 0.01); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; BWG = Body weight gain; FI = Feed intake; FCR = Feed conversion ratio; g = gram; SEM = Standard error of the mean.

Table 4 - Effect of dietary treatments on digestive organ weights of broiler chickens.

Parameters (%)	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Starter phase							
Pancreas	0.40	0.30	0.31	0.32	0.33	0.013	0.076
Gizzard	2.07	1.81	1.83	1.97	1.90	0.055	0.647
Proventriculus	0.54	0.50	0.49	0.47	0.41	0.019	0.345
Liver	2.81	3.00	3.10	2.62	2.61	0.083	0.250
Bursa	0.26	0.24	0.20	0.23	0.30	0.016	0.062
Spleen	0.10	0.10	0.11	0.07	0.10	0.009	0.698
Thymus	0.41	0.44	0.41	0.42	0.44	0.020	0.992
Finisher phase							
Pancreas	0.18	0.17	0.20	0.16	0.15	0.007	0.170
Gizzard	1.27	1.19	1.19	1.28	1.21	0.030	0.716
Proventriculus	0.26	0.23	0.24	0.24	0.23	0.009	0.785
Liver	1.76	1.64	1.75	1.84	1.70	0.036	0.587
Bursa	0.15	0.12	0.07	0.06	0.09	0.011	0.068
Spleen	0.07	0.08	0.07	0.07	0.05	0.006	0.636
Thymus	0.29^{c}	0.23^{bc}	0.14^{ab}	0.11^{a}	0.19^{abc}	0.020	0.030

Means with different letters in the same row differ significantly ($p\leq0.05$); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean.

Table 5 - Effect of dietary treatments on hematological profile of broiler chickens.

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Hemoglobin (g/dl)	9.47	9.18	10.10	9.85	9.25	0.270	0.848
Heterophil (%)	31.80	34.20	29.50	32.20	33.00	1.300	0.894
Lymphocytes (%)	61.00	58.80	62.00	61.00	59.80	1.310	0.969
Monocytes (%)	3.50	3.25	2.75	3.25	3.00	0.200	0.846
PCV (%)	29.75	28.00	30.50	30.00	28.00	0.850	0.877
RBC (x 10^6/µL)	3.00	2.84	2.82	2.95	2.93	0.082	0.969
WBC (x 10^3/µL)	15675	14888	16100	14975	15738	331	0.780

Means with different letters in the same row differ significantly (p≤0.05); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean; PCV = Packed cell volume; RBC = Red blood cell; WBC = White blood cell; µL= microliter; g = gram; mg = milligram; dl = deciliter.

Table 6 - Effect of treatments on serum biochemical indices of broiler chickens.

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Albumin (g/dl)	1.57	1.57	1.64	1.50	1.59	0.052	0.949
AST (µ/L)	81.90	91.60	103.10	92.40	82.80	3.250	0.228
Globulin (g/dl)	1.25	0.90	1.06	1.38	1.24	0.088	0.538
Glucose (mg/dl)	235.20	253.80	228.10	203.60	217.00	6.220	0.087
HDL (mg/dl)	32.40^{ab}	29.63^{a}	33.26^{b}	35.04^{b}	29.96^{a}	0.690	0.008
LDL (mg/dl)	16.32	15.38	16.66	16.77	16.29	0.450	0.892
Triglycerides (mg/dl)	39.60	61.80	74.50	54.10	69.20	6.220	0.377
Total Protein (g/dl)	2.81	2.46	2.75	2.88	2.82	0.096	0.755

Means with different letters in the same row differ significantly (p≤0.05, 0.01); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean; AST = Aspartate Transaminase; HDL = High density lipoprotein; LDL = Low density lipoprotein; µL= microliter; g = gram; mg = milligram; dl = deciliter.

DISCUSSION

Growth performance

Probiotics, known for maintaining gut health and enhancing productivity, were studied in broiler chicken diets. Previous research showed no performance impact on chickens fed processed PM (Olasehinde and Aderemi, 2023). In our study, PM with probiotics did not affect BWG, aligning with findings by Shao et al. (2022) who reported no significant change in BWG with probiotic *E. faecium* NCIMB 11181. During the starter period, PM diets with probiotics had no effect on FCR. However, raw and sprouted PM diets with probiotics influenced FCR in the finisher and overall growth phases, consistent with previous reports (Marcato et al., 2023; Awad et al., 2015).

Probiotics influenced FI in our study, in line with existing literature (Rehman et al., 2020; Zhang et al., 2021). However, disparities in FI effects may stem from feed type, probiotic characteristics, and environmental factors. Fermented PM increased FI, but when supplemented with probiotics, it adversely impacted overall performance. In contrast, sprouted PM with probiotics reduced feed consumption without negative effects on FCR and BWG, suggesting potential economic advantages. The impact of probiotic supplementation on broiler growth performance varies across studies (Rehman et al., 2020; Zou et al., 2022). Factors like probiotic type, dosage, diet composition, and animal health status contribute to these discrepancies. Further exploration is needed to clarify these influences and enhance our understanding of probiotics' role in broiler diets.

Carcass traits

Assessing carcass traits is crucial for evaluating broiler chicken quality. In this study, the starter diet showed no significant impact on carcass traits. Olasehinde and Aderemi (2023) indicated that sprouted PM had no effect on broiler

chicken carcass traits. Similarly, fermented PM did not increase carcass yield, though a dose effect was noted in broilers on finisher diets (Olasehinde and Aderemi, 2023). However, adding probiotics to the finisher's diet with fermented PM increased drumstick and thigh weights, suggesting a positive effect on carcass traits of broiler chickens. This aligns with studies by Ghasemi-Sadabadi et al. (2019) and Salehizadeh et al. (2019), demonstrating that probiotics improved carcass and thigh yield. On the contrary, Pelicano et al. (2003) reported that probiotics did not enhance weights of carcass, thigh, breast, and liver in broiler chickens.

Abdominal fat is a key indicator of lipid accumulation in broiler chickens. Our previous work (Olasehinde and Aderemi, 2023) demonstrated that sprouted or fermented PM in diets did not affect abdominal fat of broiler chicken. In this study, broiler chickens on processed PM with probiotics had similar abdominal fat levels to those on control diets, while raw PM with probiotics increased abdominal fat. In contrast, studies by Agboola et al. (2015) reported that probiotic supplementation could reduce abdominal fat in broiler chickens, similar to findings with *E. faecium* as a probiotic supplement (Demeterova, 2009; Weis, 2011). Variations in these results may stem from differences in basal diet, bacterial type and strain, chicken breed, and environmental conditions.

Organ weights

The thymus, spleen, and bursa of Fabricius play crucial roles in coordinating immune functions. Chen et al. (2020) suggested that increased weight of these organs in broilers may indicate the development and proliferation of immune cells. However, our results showed no impact of supplementation on the weight of the spleen and bursa of Fabricius. While various studies have reported positive effects of probiotic supplementation on poultry immune organ weight (Hidayat et al., 2020; Zhang et al., 2021), our study observed a reduction in thymus weight in broilers fed sprouted PM diets with probiotics compared to the control groups.

The thymus, a key lymphoid organ, is essential for the development and maturation of T-lymphocytes that regulate immune protection (Farley et al., 2013). A decrease in thymus weight may indicate a suppression of adaptive immunity (Sharma and Moroni, 2021). Notably, the reduction in thymus weight in broilers fed sprouted PM with probiotics was accompanied by an increase in HDL concentration without adversely affecting performance. HDL's role in removing excess cholesterol from peripheral cells has implications for immune cell activation (Pradhan et al., 2021). The interaction between HDL and immune cells may influence immune cell development and response. Therefore, our results suggest that probiotic supplementation in sprouted PM diets may impact

Hematological and biochemical content

No significant differences were observed among dietary treatments for the studied hematological indices in broiler chickens. This aligns with findings from Alkhalf et al. (2010) and Abdel-Hafeez et al. (2017), who reported positive effects of probiotics on packed cell volume and hemoglobin concentration. However, Beski and Al-Sardary (2015) noted a significant increase in hemoglobin concentration and a reduction in the heterophil to lymphocyte ratio, differing from our results. The variation might be attributed to differences in probiotic bacteria, basal diet, and the birds' physiological and nutritional status (Etim et al., 2014).

In broiler chickens, serum HDL-cholesterol concentration was higher in those on the negative control diet, raw PM with probiotics, and sprouted PM with probiotics compared to the control diet with antibiotics. HDL plays a crucial role in transporting free cholesterol for disposal in the liver (Zannis et al., 2015). Additionally, HDL is involved in glucose homeostasis through insulin secretion, direct glucose uptake in muscles, and potentially increased insulin sensitivity (Han et al., 2007; Drew et al., 2012; Haase et al., 2015). Despite probiotic supplementation, a decreasing trend in glucose concentration in PM diets may be related to pearl millet's intrinsic property of lowering blood glucose due to its low glycemic index (Dias-Martins et al., 2018). These findings suggest that probiotic supplementation may not have influenced energy metabolism in broiler chickens.

CONCLUSION

In our study, diets containing raw PM and sprouted PM, both supplemented with probiotics *Enterococcus faecium*, showed no significant impact on the performance, HDL-cholesterol concentration, or blood indices of broiler chickens. However, fermented PM with probiotics improved carcass traits, thigh and drumstick yields but decreased HDL concentration. Despite these variations, PM diets with probiotics did not adversely affect the overall health of broiler chickens, possibly due to the non-pathological conditions of the birds in our study. Our findings suggest the importance of exploring these treatments under pathological conditions for a comprehensive understanding.

DECLARATION

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Author's contribution

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

Consent to publish

Not applicable.

Competing interest

The authors declare that there is no conflict of interests.

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