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Original Research Article

PLEUROTUS OSTREATUS-FERMENTED RICE MILLING WASTE AS PARTIAL FEED REPLACEMENT IN GROWER PIGS: HAEMATOLOGICAL AND SERUM BIOCHEMICAL RESPONSES

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ABSTRACT

A feeding trial was conducted to evaluate the effects of partial replacement of basal grower pigs diet with solid-state fermented rice milling waste (FRMW) with or without enzymes (Enerzyme®) supplementation on haematological and serum biochemical parameters of grower pigs. Fresh rice milling waste from a milling centre was collected, pasteurized, and fermented for three weeks using *Pleurotus ostreatus*. The FRMW was subsequently used to replace varied percentages (15%, 25% and 50%) of basal diet formulated with maize, soybean cake, and other standard ingredients, with or without enzymes (Enerzyme®) supplementation. Twenty-one Large White weaners (8 weeks old) were divided into 7 treatment groups (3 weaners per group) after 2 weeks acclimatization, and fed for 8 weeks. Haematological parameters (Hb, PCV, RBC, WBC), erythrocytic indices (MCV, MCH, MCHC), serum biochemistry (total protein, albumin, globulin, AST, ALT, ALP, bilirubin, urea, creatinine, glucose), and lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C) were measured at the end of the trial. Results showed statistically significant dietary effects on haemoglobin ($P = 0.05$), but not on other haematological parameters, no significant changes in most serum biochemical indices except for glucose ($P=0.02$), and significant reductions in total cholesterol, triglycerides, LDL-C and VLDL-C in groups receiving higher FRMW, particularly the 50% replacement ($P < 0.00$). The findings suggest that *P. ostreatus* FRMW can be used up to 50% for replacement of conventional grower diet without adverse effects on key blood health indicators in pigs and may beneficially modulate lipid metabolism.

Keywords: Fermentation; Haematology; *Pleurotus ostreatus*; Rice milling waste; Serum biochemistry

INTRODUCTION

Livestock production contributes significantly to global agricultural output and underpins the livelihoods and food security of nearly one billion people (FAO, 2009). The increasing demand for animal protein in developing countries is spurred by rising incomes, urbanization, and shifts toward more diversified and high-value diets (FAO, 2009; FAO, 2011). However, average daily protein consumption in Nigeria has been estimated at about 45-54g per capita, with only 6-9g derived from animal sources, which is below recommended levels (FAO, 2019; NBS, 2020). The global human population is projected to reach 11 billion by 2100, with much of that growth occurring in developing regions (Dorling, 2021). This demographic trend has created pressure for a "livestock revolution" in developing countries, particularly in the monogastric sectors such as swine and poultry (Delgado, 2003; FAO, 2020).

In Nigeria, as in many tropical countries, pig production holds promise for narrowing the animal protein supply gap,

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because of the pig's high feed efficiency, prolificacy, and shorter generation interval (Adesehinwa *et al*., 2024). However, feed constitutes up to 75% of production cost in swine production, with energy and protein feedstuffs being the major cost drivers (Uddin & Osasogie, 2016). In many developing countries, the cost and scarcity of conventional feedstuffs such as maize, soybean meal, and cassava drive the search for alternative, locally available feed resources (Dumitru *et al*., 2024).

One abundant, yet underutilized agro-industrial by-product in Nigeria is rice milling waste (RMW), which consists of rice husks, bran, and broken grains. Despite its availability, this resource is often discarded or periodically burned, thereby contributing to environmental pollution (Ifeanyichukwu *et al*., 2019). The high lignin content of rice milling waste (RMW) significantly reduces its digestibility, limits protein availability, and diminishes palatability for monogastric animals (Andlar *et al*., 2018; Sadh *et al*., 2018). Chemical treatments of such lignocelluloses (e.g. with an alkali) may improve digestibility, but such methods are costly; can introduce toxic residues, and often fail to remove lignin entirely (Belewu & Babalola, 2009). In contrast, biological treatment using white-rot fungi such as *Pleurotus* spp. offers a promising approach to degrade lignin, liberate cellulose and hemicellulose, and upgrade the nutritive value of fibrous residues (Yilkal, 2015; Wang *et al*., 2023). Indeed, solid-state fermentation (SSF) using *P. ostreatus* has been shown to increase protein content, reduce lignin, and improve digestibility of various substrates (Heidari *et al*., 2022; Sánchez-García *et al*., 2022; Iqbal *et al*., 2024).

Egwu *et al*. (2026) reported that partially substituting basal pig diet with rice milling waste (RMW) fermented with *Pleurotus ostreatus* at inclusion levels of 15–25%, with or without enzymes supplementation, produced growth performance comparable to that of pigs on control diet. Comparable outcomes were also documented by Wang *et al*. (2011) and Sun *et al*. (2024), who found that solid-state fermented feeds enhanced both nutrient digestibility and growth in swine. However, higher inclusion rates (50%) resulted in reduced weight gain and feed efficiency, likely because of residual lignin and fiber, which restricted nutrient utilization (Egwu *et al*., 2026). From an economic perspective, Egwu *et al*. (2026) further demonstrated that diets containing 15–25% fermented RMW, regardless of enzyme addition, achieved similar or superior benefit-cost ratios compared to control diet. These findings are consistent with those of Sime & Duguma (2025), who emphasized the role of alternative feed resources in lowering production expenses.

While many studies have focused on growth performance and digestibility of fermented feeds, fewer have examined their effects on the health status of animals via haematological and serum biochemical parameters. Haematological and serum biochemical parameters are important diagnostic tools for assessing the wellbeing, nutritional status, and disease state of animals (Perri *et al*., 2017; Olajide *et al*., 2021). These parameters are sensitive to diet and stress (Abonyi *et al*., 2016; Lindholm-Perry *et al*., 2021). For example, changes in RBC, Hb, PCV, WBC, and liver enzymes may signal physiological stress, compromised health, or metabolic disturbances (Thorn, 2010; Norbury & Moyer, 2015; Golinar *et al*., 2022).

Therefore, this study was designed to evaluate the effects of partially replacing conventional feed with *P. ostreatus* solid-state fermented RMW (FRMW) at 15%, 25%, and 50% levels, with and without enzyme supplementation, on haematological, and serum biochemical parameters of grower pigs.

MATERIALS AND METHODS

ANIMALS AND OTHER MATERIALS

Twenty-one 8-week old Yorkshire weaners (7 males and 14 females), were sourced from a reputable pig farm, and used for the study. Freshly produced RMW was obtained from a rice milling centre in Abia State, and conveyed to the study centre for fermentation, and subsequently for partial replacement of the basal diet. Grain spawns of *Pleurotus ostreatus* were sourced from commercial dealer; multiplied, and used as RMW fermentation agent, while Enerzyme® (a cocktail of cellulase 10,000UI; Beta-glucanase 200UI; Xylanase 10,000UI, and Phytase 2,500UI), was purchased from a commercial dealer for the enrichment of some groups' diets.

FERMENTATION PROCEDURE AND DIET FORMULATION PREPARATION AND SOLID-STATE FERMENTATION OF RICE MILLING WASTE

Fermentation of rice milling waste (RMW) was carried out using two plastic containers, each with a capacity of 200kg. Prior to use, the containers were decontaminated with 70% ethanol applied at a rate of 3 ml per 10 cm² in accordance with AOAC (2000) guidelines.

The fermentation procedure followed the method described by Olagunju *et al.* (2023). Fresh RMW was pasteurized by thoroughly mixing 30kg of the substrate with 21 liters of water in a metal vat earlier heated to 70°C. The mixture was maintained at this temperature for 15 minutes to reduce microbial contamination.

After pasteurization, 30kg of the treated substrate was aseptically transferred into each disinfected fermentation container. The substrate was then inoculated with *Pleurotus ostreatus* grain spawn at an inclusion rate of 2.5% (w/w, wet basis). The inoculum was uniformly mixed into the substrate to ensure effective fungal colonization.

Each container was covered to establish suitable solid-state fermentation conditions. Following an initial incubation period of 48 hours, the containers were opened at 24-hour intervals, and the substrate was manually stirred to improve aeration, prevent compaction, and promote uniform mycelial growth. The fermentation process lasted for 21 days. During this period, environmental parameters were carefully controlled: substrate moisture content was maintained between 50–70%, and temperature was regulated within the range of 28-30°C to optimize fungal metabolic activity and enzymatic degradation

HARVESTING AND PROCESSING OF FERMENTED RICE MILLING WASTE (FRMW)

At the end of the 21-day fermentation period, the process was terminated. The fermented rice milling waste (FRMW) was spread thinly under direct sunlight and dried to constant weight to reduce moisture content and prevent post-fermentation spoilage. The dried FRMW was subsequently packaged in clean polypropylene bags and stored on wooden pallets in a cool, dry, well-ventilated, and rodent-free storage facility until incorporation into the experimental diets.

FORMULATION OF BASAL RATION

The proximate composition of the principal feed ingredients: maize, soybean cake, and palm kernel cake (PKC) was determined prior to diet formulation using standard analytical procedures. The analyzed nutrient values served as the basis for accurate ration balancing to ensure that the targeted nutrient specifications for the experimental pigs were met. Based on the analytical results, the basal ration was formulated in accordance with the nutrient requirements recommended by the Pig Improvement Company (PIC®) Nutrition and Feeding Guidelines (2021) for growing pigs. Specifically, the basal diet comprised maize, soybean cake, palm kernel cake (PKC), synthetic lysine, synthetic methionine, common salt (NaCl), and a vitamin-mineral premix. The formulated diet was designed to contain 20.1% crude protein and 2,985kcal/kg metabolizable energy. Subsequently, fermented rice milling waste (FRMW) was incorporated into the basal diet as a test ingredient, replacing portions of the conventional feed at graded inclusion levels of 15%, 25%, and 50%. In addition, dietary treatments were prepared either with or without exogenous enzyme supplementation. The enzyme preparation (Enerzyme®) was included at a rate of 1g per 10kg of formulated feed (0.01%) (Unigwe *et al*., 2017).

TABLE I: ANALYZED COMPOSITION OF THE EXPERIMENTAL DIETS

Composition	Basal Diet	Partial Replacement of Basal Diet		
		15 % FRMW	25% FRMW	50% FRMW
Moisture (%)	08.30	07.90	07.40	08.51
Dry Matter (%)	91.70	92.10	92.60	91.49
Crude Protein (%)	20.10	18.70	18.60	18.52
Ether Extract (%)	03.20	02.70	02.70	00.59
Ash (%)	10.80	12.28	10.52	08.39
Crude Fibre (%)	07.30	06.90	07.40	17.23
Nitrogen-Free Extract (%)	49.70	48.48	46.62	46.76
Metabolizable Energy (Kcal/kg)	2,985.96	2,915.96	2,906.36	2,670.80

STUDY DESIGN

The experiment was conducted using the completely randomized design (CRD). There were seven dietary treatments (A-G) as presented in Table II below.

TABLE II: EXPERIMENTAL GROUPS

Group	Conventional Feed	FRMW inclusion	*Enzymes (Enerzyme®)
A (Control)	100 %	0 %	–
B	85 %	15 %	–
C	75 %	25 %	–
D	50 %	50 %	–
E	85 %	15 %	+
F	75 %	25 %	+
G	50 %	50 %	+

Enerzyme® was supplemented at 1g/10kg (0.01%) of formulated feed (Unigwe *et al*., 2017).

MANAGEMENT OF EXPERIMENTAL ANIMALS

The 21 weaners were acclimatized for two weeks on arrival at the experimental farm. During this period, Albendazole was administered orally at a dosage of 10mg/kg body weight as a single dose. At the end of the acclimatization period, the weaners were weighed, and the initial body weights recorded. They were then assigned to the experimental treatments using the completely randomized design. Each treatment group consisted of three weaners (one male and two females), with each weaner serving as a replicate. They were provided feed and clean drinking water *ad libitum* throughout the experiment, and the feeding trial lasted for eight weeks.

HAEMATOLOGY AND SERUM BIOCHEMICAL ANALYSES

Blood samples were collected from the pigs belonging to each experimental group via the external jugular vein into dry sterilized sample bottles with and without anticoagulant. Haematological parameters, including haemoglobin concentration (Hb), red blood cell count (RBC), packed cell volume (PCV), and total and differential white blood cell counts (WBC), were determined immediately after blood collection using standard procedures as described by Weiss and Wardrop (2010) and Bain *et al*., (2017). Erythrocytic indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1993) and Weiss and Wardrop (2010).

Mean Corpuscular Volume (MCV (fL) = $\frac{PCV(\%)\times 10}{RBC(10^6\text{mm}^3)}$

Mean Corpuscular Haemoglobin (MCH (ρg) = $\frac{Hb(\text{g/dL})\times 10}{RBC(10^6\text{mm}^3)}$

Mean Corpuscular Haemoglobin Concentration MCHC (g/dL) = $\frac{\text{MCH x 10}}{\text{MCV}}$

Blood samples collected into plain tubes without anticoagulant were allowed to clot and subsequently centrifuged at 3,000 × *g* for 5 minutes to obtain serum. Serum was harvested and used for the determination of serum biochemical parameters. Blood glucose (GLUC), total protein (TP), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), albumin (ALB), globulin (GBL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CRE) were measured using a fully automated clinical chemistry analyser (TBA-120FR, Toshiba Medical Systems Corporation, Tochigi, Japan) and test kits specific to each biochemical parameter following the manufacturers' instructions.

STATISTICAL ANALYSIS

Data generated were subjected to one-way analysis of variance (ANOVA) using "R" statistical software, version 4.4.2. Means were separated using the least significant difference (LSD) method, and significance accepted at P ≤ 0.05.

RESULTS

HEMATOLOGICAL RESPONSES OF PIGS TO DIETARY TREATMENTS

The results of haematological evaluation in Table III revealed no significant differences (P > 0.05) in most blood parameters among pigs fed diets containing graded levels of fermented rice milling waste (FRMW) up to 50%, with or without enzyme supplementation. Packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values remained statistically comparable across all dietary treatments (P > 0.05).

Although haemoglobin (Hb) concentration showed a slight, but overlapping statistical variation among groups (P = 0.05), the values ranged from 14.47 to 15.47g/dL, but remained within normal physiological reference ranges for pigs as shown in Table IV above. The highest Hb was observed in group B (15.47 ± 0.31g/dl), while the lowest was in group F (14.47 ± 0.23g/dL). Overall, there were no observable pathological deviations or indicators of haematological disorder attributable to FRMW inclusion at any of the tested levels.

The results of serum biochemical responses of pigs to the dietary treatments in Table V showed no significant (P > 0.05) differences among dietary treatments in most evaluated parameters. Hepatic enzymes: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) as well as protein fractions (total protein, albumin, and globulin), renal function indices (urea and creatinine), and bilirubin concentrations were statistically comparable across all groups.

Although mean ALP and globulin values in the treatment groups were slightly lower than standard reference ranges, they remained within physiologically acceptable limits and were not associated with any observable clinical abnormalities. A statistically significant difference (P < 0.05) was observed in serum glucose concentration among the groups, with pigs in Group F recording the highest mean value. However, all glucose values remained within normal physiological ranges for pigs.

The results of serum lipid profile of pigs in response to dietary treatments in Table VII showed that dietary inclusion of fermented rice milling waste (FRMW) significantly (P < 0.05) influenced all measured serum lipid parameters in the pigs. Serum total cholesterol concentrations decreased progressively with increasing levels of FRMW in the diets. The lowest total cholesterol value (81.88 ± 9.60 mg/dL) was observed in pigs in Group D, which received 50% FRMW without enzyme supplementation. Similarly, serum total triglyceride concentrations declined significantly as the proportion of FRMW replacing the basal diet increased.

TABLE III: HEMATOLOGICAL RESPONSES OF PIGS TO THE DIETARY TREATMENTS

Group	Hb (g/dL)	PCV (%)	RBC ($x10^6 mm^3$)	WBC ($x10^3 mm^3$)	MCV (fL)	MCH (pg)	MCHC (g/dL)
A	15.27±0.31^a	40.67±1.15	06.64±0.14	12.18±1.52	61.27±0.49	23.01±0.25	37.55± 0.43
B	15.47±0.31^a	41.67±0.58	06.74±0.08	13.80±2.52	61.79±0.22	22.94±0.37	37.12± 0.48
C	14.87±0.23^a	38.67±1.53	06.28±0.25	10.87±0.99	61.61±0.16	23.70±0.59	38.47± 0.98
D	15.13±0.42^a	40.33±1.53	06.55±0.18	11.95±0.61	61.57±0.64	23.11±0.03	37.53 ±0.41
E	15.13±0.61^a	40.00±2.00	06.51± 0.30	12.43±2.46	61.47±0.26	23.27±0.27	37.85±0.50
F	14.47±0.23^{ab}	37.67±2.52	06.16±0.42	11.52±0.10	61.12±0.06	23.53±1.27	38.50 ±2.04
G	14.53±0.42^{ab}	37.67±3.51	06.16±0.55	11.83±1.20	61.13±0.39	23.68±1.52	38.75 ±2.69
P_value	0.05	0.18	0.20	0.50	0.27	0.82	0.73

Means within the same column with different superscripts are significantly different ($P \leq 0.05$)

TABLE IV: REFERENCE VALUES FOR COMMON HAEMATOLOGICAL PARAMETERS IN PIGS

Parameter	Reference Range
Hemoglobin (Hb)	10 – 16 g/dL
Packed Cell Volume (PCV/Hct)	32 – 50%
Red Blood Cells (RBC)	5 – $8\times10^6/\mu L$
White Blood Cells (WBC)	11– $22\times10^3/\mu L$
Mean Corpuscular Volume (MCV)	50 – 68 fL
Mean Corpuscular Hemoglobin (MCH)	17 – 21 pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	30 – 34 g/dL

Fielder, S. E. (2024)

TABLE V: SERUM BIOCHEMICAL RESPONSES OF PIGS TO THE DIETARYTREATMENTS

Groups	Parameters T.Prot. (g/dl)	Alb (g/dl)	Glob (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilr. (mg/dl)	Urea (mg/dl)	Crt (mg/dl)	Glu. (mg/dl)
A	6.33 ± 0.99	3.26 ± 0.50	3.07 ± 0.50	58.03 ± 2.39	36.75 ± 2.17	95.45 ± 1.53	0.44 ± 0.06	18.95 ± 1.77	1.56 ± 0.52	92.35 ± 3.79^c
B	7.19 ± 0.32	3.68 ± 0.15	3.51 ± 0.17	58.15 ± 4.13	36.15 ± 1.66	93.05 ± 2.30	0.38 ± 0.03	19.42 ± 0.58	1.67 ± 0.04	107.03 ± 5.87^{ab}
C	6.78 ± 0.47	3.48 ± 0.21	3.3 ± 0.26	57.46 ± 1.97	38.34 ± 0.98	95.04 ± 1.45	0.31 ± 0.03	19.32 ± 1.57	1.16 ± 0.34	105.19 ± 6.07^{ab}
D	6.42 ± 0.65	3.3 ± 0.33	3.12 ± 0.32	56.49 ± 1.63	37.55 ± 2.54	94.6 ± 1.70	0.44 ± 0.10	18.61 ± 1.01	1.82 ± 0.16	102.55 ± 3.55^{bc}
E	7.22 ± 0.28	3.57 ± 0.37	3.38 ± 0.38	58.08 ± 2.56	37.7 ± 2.02	94.89 ± 1.11	0.41 ± 0.01	19.99 ± 0.51	1.53 ± 0.59	98.72 ± 0.08^{bc}
F	6.75 ± 0.60	3.45 ± 0.32	3.29 ± 0.27	55.61 ± 4.95	35.75 ± 3.16	94.65 ± 2.24	0.38 ± 0.08	17.38 ± 2.04	1.53 ± 0.48	114.22 ± 11.21^a
G	5.96 ± 0.41	3.09 ± 0.22	2.88 ± 0.20	55.59 ± 0.70	35.12 ± 0.54	94.26 ± 2.55	0.44 ± 0.02	18.2 ± 1.98	1.5 ± 0.47	97.69 ± 8.00^{bc}
P_value	0.15	0.35	0.30	0.83	0.48	0.81	0.11	0.45	0.65	0.02

Means within the same column with different superscripts are significantly different ($P \leq 0.05$)

In contrast, high-density lipoprotein cholesterol (HDL-C) exhibited an increasing trend with higher dietary inclusion levels of FRMW. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were the most responsive lipid fractions to dietary treatment. Pigs in Group D recorded markedly lower LDL-C (19.05 ± 9.40mg/dL) and VLDL-C (11.64 ± 0.52mg/dL) compared with the control group, which had corresponding values of 49.63 ± 7.11mg/dL and 13.99 ± 0.59mg/dL respectively.

Other FRMW-fed groups (E, F, and G) also demonstrated reductions in LDL-C and VLDL-C relative to the control group, although the magnitude of reduction was less pronounced than that observed in Group D. Overall, the 50% FRMW inclusion level without enzyme supplementation produced the most pronounced hypolipidemic effect among all treatment groups.

TABLE VI: REFERENCE VALUES FOR SERUM BIOCHEMICAL PARAMETERS IN PIGS

Parameter	Reference Range
Total Protein	7.9 – 8.9 g/dL
Albumin	1.9 – 3.9 g/dL
Globulin	5.3 – 6.4 g/dL
ALT	31 – 58 U/L
AST	32 – 84 U/L
ALP	118 – 395 U/L
Creatinine	1.6 – 2.7 mg/dL
Total Bilirubin	0 – 1.0 mg/dL
Urea (BUN)	10 – 30 mg/dL
Blood Glucose	85 – 150 mg/dL

Fielder, S. E. (2024)

across dietary treatment groups. From a nutritional perspective, PCV serves as an integrative indicator of erythropoietic adequacy, hydration status, and nutrient sufficiency, particularly protein, iron, and essential micronutrients required for effective red blood cell production (Balarajan *et al*., 2011). The findings are consistent with previous reports by Sorhue *et al.* (2022) and Lian *et al*. (2024) who observed no significant haematological disturbances in pigs fed non-conventional or fermented feed resources.

Furthermore, Lindholm-Perry *et al.* (2021) highlighted that erythrocyte status, particularly haematocrit, may serve as supplementary phenotypic indicators of feed efficiency. In the present study, the absence of adverse deviations in PCV or related erythrocytic indices (MCV, MCH, and MCHC) suggests that graded FRMW inclusion did not impair nutrient utilization efficiency or physiological resilience.

TABLE VII: SERUM LIPID PROFILE OF PIGS IN RESPONSE TO DIETARY TREATMENTS

	Parameters				
Group	T_Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
A	106.38 ± 9.74^{a}	69.97 ± 2.99^{a}	42.76 ± 2.63^{c}	49.63 ± 7.11^{a}	13.99 ± 0.59^{a}
B	105.03 ± 4.00^{ab}	69.73 ± 1.49^{a}	47.28 ± 0.52^{b}	43.13 ± 3.01^{ab}	13.95 ± 0.30^{a}
C	103.38 ± 1.83^{ab}	69.76 ± 1.10^{a}	48.88 ± 1.48^{ab}	40.55 ± 0.42^{abc}	13.95 ± 0.22^{a}
D	81.88 ± 9.60^{d}	58.21 ± 2.58^{c}	51.18 ± 2.60^{a}	19.05 ± 9.40^{e}	11.64 ± 0.52^{c}
E	96.75 ± 6.57^{abc}	65.6 ± 4.64^{a}	47.43 ± 1.76^{b}	36.22 ± 4.40^{bc}	13.12 ± 0.93^{a}
F	93.50 ± 6.00^{bcd}	64.59 ± 4.51^{ab}	48.76 ± 2.03^{ab}	31.83 ± 3.54^{cd}	12.92 ± 0.90^{ab}
G	86.39 ± 5.40^{cd}	59.32 ± 2.31^{bc}	49.57 ± 1.78^{ab}	24.95 ± 6.30^{de}	11.87 ± 0.47^{bc}
P_value	0.00	0.00	0.00	0.00	0.00

Means within the same column with different superscripts are significantly different ($P \leq 0.05$)

DISCUSSION

The absence of significant alterations in most haematological parameters indicates that dietary inclusion of FRMW up to 50%, irrespective of enzyme supplementation neither adversely affected hematopoietic function nor induced overt physiological stress on the pigs. Haematological indices are reliable indicators of the physiological, nutritional, and health status of animals (Thrall *et al*., 2012). Therefore, their stability in this study suggests that FRMW did not compromise systemic health.

The observed variation in haemoglobin concentration, though statistically significant, remained within the established physiological range for pigs and is therefore considered biologically normal (MSD Veterinary Manual, 2023). This suggests that the variation was not clinically relevant, and did not indicate anaemia or impaired oxygen transport capacity.

The maintenance of stable RBC and WBC counts, together with within-range PCV values, further confirms that oxygen-carrying capacity and immune competence were preserved

Generally, these findings confirm that FRMW can be incorporated into pig diets up to 50% inclusion without compromising haematological integrity, thereby supporting its nutritional safety and physiological compatibility at the evaluated levels. The significant variation in serum glucose concentration suggests possible metabolic adjustments among dietary treatments. The relatively higher glucose concentration in Group F may be associated with improved carbohydrate digestibility or enhanced gluconeogenic activity, potentially influenced by the synergistic effect of fermentation and enzyme supplementation. However, since all glucose values remained within normal physiological ranges, the biological relevance of this difference appears minimal and unlikely to reflect pathological alterations. These findings are consistent with previous observations by Lian *et al*. (2024) who reported that fermented feed ingredients did not significantly alter major serum biochemical parameters in pigs.

Generally, the results support the metabolic safety and physiological compatibility of FRMW-based diets under the conditions of the present study, confirming that graded inclusion levels did not adversely affect systemic biochemical integrity. The significant reduction in total cholesterol, triglycerides, LDL-C, and VLDL-C in pigs fed 50% FRMW suggests that dietary fermentation of rice milling waste positively modulated lipid metabolism. The progressive decline in serum cholesterol and triglycerides with increasing FRMW inclusion indicates a dose-dependent effect of the fermented substrate.

The observed hypocholesterolemic effect may be attributed to bioactive compounds generated during fungal solid-state fermentation with *Pleurotus ostreatus*. Fermentation has been reported to enhance the production of β-glucans, phenolic compounds, and to modify fibre fractions, all of which are associated with cholesterol-lowering properties (Heidari *et al*., 2022; Sánchez-García *et al*., 2022; He *et al*., 2024; Iqbal *et al*., 2024). β-glucans in particular, are known to bind bile acids in the intestine, thereby increasing cholesterol excretion and reducing circulating LDL concentrations (Gunness *et al*., 2016). Additionally, improved fibre degradability and reduced lignin and anti-nutritional factors following fermentation with *P. ostreatus* may enhance nutrient utilization and metabolic efficiency (Sufyan *et al*., 2022).

The increase in HDL-C concentration further supports a favourable shift in lipid metabolism. The HDL-C plays a role in reverse cholesterol transport, facilitating the removal of excess cholesterol from peripheral tissues. Although lipid profile modulation is not typically the primary objective in livestock nutrition where growth performance and feed efficiency are prioritized, the improved lipid profile may suggest reduced fat deposition and potentially improved cardiovascular and metabolic health of the animals over time.

The ability of grower pigs to tolerate up to 50% replacement of the basal diet with FRMW without adverse effects on health indices demonstrates the nutritional viability of this fermented by-product. This finding aligns with previous reports in which solid-state fermented agro-industrial by-products, such as cassava peel meal, were incorporated into pig diets without detrimental effects on haematological parameters while reducing feed costs (Irekhore *et al*., 2015; Williams *et al*., 2023).

Overall, the present findings extend existing knowledge on the nutritional and metabolic benefits of *P. ostreatus*-fermented substrates and support the potential of FRMW as a functional feed ingredient capable of improving lipid metabolism while contributing to sustainable and cost-effective pig production systems.

CONCLUSION

This experiment demonstrates that up to 50% of conventional growers feed can be replaced with *P. ostreatus* solid-state fermented rice milling waste (FRMW), with or without enzymes supplementation without causing deleterious effects on key haematological and serum biochemical indicators. Moreover, high inclusion of FRMW induced favourable changes in lipid profile (lower total cholesterol, triglycerides, LDL-C, and VLDL-C; increased HDL-C). These findings support the potential use of fermented rice milling waste as a viable, health-compatible feed ingredient in pig production, with the dual benefit of improved lipid metabolism and waste valorization.

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