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Maize Replacement Value of Rice Milling Waste Diets with or without Enzyme Supplementation on Haematological and Serum Biochemistry indices of Broiler Chickens

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ABSTRACT

This study evaluated the maize replacement value of rice milling waste (RMW) diets with or without enzyme supplementation on the haematological and serum biochemical parameters of broiler chickens. A total of 280 one-day-old Abor Acre broiler chicks were randomly assigned to seven dietary treatments in a 2 × 3 factorial arrangement in a completely randomized design with a control. The treatments included a control diet (100% maize-based basal diet), and six test diets where maize was replaced by RMW at three levels (20%, 30%, and 40%) with or without enzyme supplementation. Birds were reared for 49 days under standard management. Blood samples were collected at the end of the experiment to determine haematological indices and serum biochemical profiles. Results showed that replacing maize with RMW up to 30% inclusion did not adversely affect packed cell volume, haemoglobin, red and white blood cell counts, or differential counts when enzyme was added. However, higher inclusion levels (40%) without enzyme supplementation resulted in elevated liver enzyme activities (AST, ALT, ALP), suggesting mild metabolic stress. Enzyme supplementation maintained normal physiological responses across all inclusion levels. The study demonstrates that RMW can effectively replace up to 30% of maize in broiler diets with enzyme support, offering a sustainable, cost-effective alternative feed ingredient.

Keywords: *broiler chickens, rice milling waste, maize replacement, enzyme supplementation, haematology, serum biochemistry.*

INTRODUCTION

Feed cost remains a dominant challenge in poultry production, accounting for up to 70–80% of total operational expenses, with maize being the primary energy source in most poultry diets (Olukosi *et al.,* 2020; Musa *et al.,* 2022). However, the rising demand for maize by the food, biofuel, and industrial sectors has intensified its scarcity and price volatility, prompting a growing interest in alternative, sustainable feed resources (Adeyemi and Jimoh, 2021). Among such alternatives, agro-industrial by-products like rice milling waste (RMW) have gained attention for their availability, affordability, and potential to partially replace conventional feedstuffs. Rice milling waste, a composite of rice bran, broken grains, and husk remnants, is particularly abundant in rice-producing regions. It contains moderate energy levels, essential amino acids, and minerals, though its high crude fiber and phytate content can reduce nutrient digestibility in monogastric animals like poultry (Salihu *et al.,* 2019; Okon *et al.,* 2023). To improve the utilization of such fibrous materials, exogenous enzymes, particularly carbohydrases like xylanase, cellulase, and β-glucanase—are widely used to enhance nutrient release by breaking down non-starch polysaccharides and anti-nutritional factors (Gao *et al.,* 2022). Enzyme supplementation has been shown to improve feed conversion efficiency, growth performance, and nutrient bioavailability in broiler chickens fed non-conventional feeds (Yang *et al.,* 2021).

In addition to performance metrics, haematological and serum biochemical parameters serve as vital indicators of the physiological and metabolic status of poultry. These indices reflect the internal health, immune competence, and potential stress responses of birds subjected to varying nutritional regimens (Okonkwo *et al.,* 2020). Therefore, this study aims to investigate the effects of replacing maize with rice milling waste, with or without enzyme supplementation, on the haematological and serum biochemical profiles of broiler chickens.

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The findings are expected to provide valuable insights into the health implications and nutritional value of RMW-based diets for sustainable poultry production.

Materials and Methods

Experimental Site

The study was conducted at the Poultry Production and Research Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The farm is located in the derived savanna zone of Southwest Nigeria, lying on latitude 8°08′31.7940″N and longitude 4°14′42.6696″E.

Source and Composition of Test ingredients

The rice milling waste (RMW) used in this study was a composite of rice bran, husk, and broken grains, obtained from a rice milling facility in the Egbeda area of Ogbomoso, Oyo State, Nigeria. The RMW was sun-dried to approximately 10% moisture content prior to use. Its proximate composition, determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2019), was as follows: crude protein (3.62%), crude fibre (39.74%), ash (16.57%), dry matter (89.76%), nitrogen-free extract (38.26%), gross energy (3731.0 kcal/kg), and metabolizable energy (2801.07 kcal/kg).

Enzyme Profile

Two enzyme preparations were used in the study:

- Phytase: a granular enzyme aimed at improving phosphorus availability and promoting growth; included at 5 g per 100 kg of feed.
- Yiduozyme Compound Enzyme: a granular multi-enzyme blend designed to enhance fibre degradation and nutrient digestibility; also included at 5 g per 100 kg of feed.

Experimental Diets

Seven isocaloric and isonitrogenous experimental diets were formulated to meet the nutrient requirements of broiler chickens based on NRC (1994) recommendations. The dietary treatments included:

- T1: Control (maize-based basal diet without enzyme)
- T2: 20% maize replaced with RMW, no enzyme
- T3: 20% maize replaced with RMW + enzyme
- T4: 30% maize replaced with RMW, no enzyme
- T5: 30% maize replaced with RMW + enzyme
- T6: 40% maize replaced with RMW, no enzyme
- T7: 40% maize replaced with RMW + enzyme

Experimental Birds and Management

A total of 280-day-old broiler chicks (Abor Acre strain) were procured from a commercial hatchery. The chicks were individually weighed and randomly allocated to seven dietary treatments.

Table 1: Gross Composition of finisher Diets (g/100g)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Maize	51.00	40.80	40.80	35.70	35.70	30.60	30.60
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soybean meal	29.50	29.50	29.50	29.50	29.50	29.50	29.50
Wheat offal	9.50	9.50	9.50	9.50	9.50	9.50	9.50
Rice Milling Waste	0.00	10.20	10.20	15.30	15.30	20.40	20.40
Enzyme	-	-	+	-	+	-	+
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium Phosphate	2.00	2.00	2.00	2.0	2.00	2.00	2.00
Limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated values							
ME (kcal/kg)	3070.10	2847.47	2847.47	2735.78	2735.78	2624.09	2661.58
Crude protein (%)	20.76	20.25	20.25	19..99	19.99	19.74	19.74
Ether extract (%)	3.54	4.05	4.05	4.31	4.31	4.56	4.56
Crude fibre (%)	4.29	7.95	7.95	9.79	9.79	11.62	11.62
Lysine (%)	1.34	1.32	1.32	1.31	1.31	1.30	1.30
Methionine (%)	0.62	0.60	0.60	0.59	0.59	0.59	0.59
Calcium (%)	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Phosphorus (%)	0.81	0.79	0.79	0.77	0.77	0.76	0.76
Cost/kg of feed (#/kg)	328.81		323.71	299.43			

**Vitamin premix per kg of diet: vitamin A, 12,000IU; vitamin D3, 2500IU; vitamin E, 30IU; vitamin K, 2.0mg; vitamin B1, 2.25mg; vitamin B2, 6.0mg; vitamin B6, 4.5mg; vitamin B12, 0.015mg; Niacin 40mg; Panthetonic acid 15mg; Folic acid 1.5mg; Biotin 0.05mg; Chlorine chloride 300mg; Manganese 80mg; Zinc 50mg; Iron 20mg; Copper 5.0mg; Iodine 1.0mg; selenium 0.2mg; Cobalt 0.5mg; Antioxidant125mg.*

+ = with enzyme supplementation

- = without enzyme supplementation

Each treatment had four replicates of 10 birds, totaling 40 birds per treatment group.

The birds were reared under a deep litter housing system in pens measuring 130 cm × 97 cm × 186 cm. Routine management practices, including appropriate vaccination and medication schedules, were strictly followed. Feed and water were provided ad libitum throughout the study duration. The experiment lasted for 49days.

Data collection

At the end of the 49-day feeding trial, one bird was randomly selected from each replicate (totaling 4 birds per treatment) for

haematological and serum biochemical analysis. Prior to sample collection, the birds were fasted for 12 hours overnight. Blood samples were collected via the jugular vein. For haematological evaluation, blood was collected into ethylenediaminetetraacetic acid (EDTA)-coated tubes to prevent coagulation. Parameters assessed included packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and differential leukocyte counts (lymphocytes, heterophils, monocytes, eosinophils, and basophils). Haemoglobin concentration was determined using the cyanmethaemoglobin method, PCV by microhaematocrit centrifugation, and RBC and WBC counts using the haemocytometer method. All haematological procedures followed standard protocols as described by Jain (1993).

For serum biochemical analysis, blood was collected into plain, non-heparinized tubes. Samples were left to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes to obtain serum. The harvested serum was transferred into clean vials and stored at –20°C until analysis. Serum biochemical parameters evaluated included total protein, albumin, globulin (calculated as the difference between total protein and albumin), cholesterol, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). These parameters were determined using commercially available diagnostic kits and analyzed using a semi-automated biochemical analyzer, following manufacturer instructions.

Experimental Design and Statistical Analysis

Experimental design was a 2x 3 factorial arrangement in a completely randomized design with a control involving three different levels of RMW replacement (20%, 30%, and 40%) of maize basal diet replacement and two levels of inclusion with and without enzyme supplementation**.** Data collected were subjected to one-way analysis of variance (ANOVA) using statistical software (SPSS 20 version). When significant differences ($p < 0.05$) were detected among treatment means, they were separated using Duncan's Multiple Range Test. Results were presented as means with their respective standard errors of the mean (SEM).

Results

The effects of replacing maize in the basal diet with rice milling waste (RMW), with or without enzyme supplementation, on haematological parameters of broiler chickens are presented in Table 2. Significant differences ($p < 0.05$) were observed in packed cell volume (PCV), neutrophil percentage, and lymphocyte percentage across the dietary treatments. Broilers fed the control diet without maize replacement (T1), 30% maize replaced with RMW without enzyme (T4), and 40% maize replaced with RMW without enzyme (T6) recorded significantly higher PCV values (30.00–31.00%) compared to those fed diets where 20% or 30% maize was replaced with RMW and supplemented with enzymes (T3 and T5, respectively). The lowest PCV value (26.50%) was observed in birds fed 20% maize replaced with RMW + enzyme (T3). No significant differences ($p > 0.05$) were observed among treatments for haemoglobin concentration, red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) count, monocytes, basophils, and platelets. Neutrophil percentages differed significantly ($p < 0.05$), with the highest values recorded in birds fed 20% maize replaced with RMW without enzyme (T2) and 30% maize replaced with RMW + enzyme (T5). The lowest neutrophil percentage (30.50%) was observed in birds fed 20% maize replaced with RMW + enzyme (T3). Lymphocyte percentages also varied significantly ($p < 0.05$). Birds fed 40% maize replaced with RMW + enzyme (T7) had the highest lymphocyte count (83.00%), while those fed 20% and 30% maize replaced with RMW without or with enzyme (T2 and T5, respectively) recorded the lowest values (33.00% and 34.00%).

Table 2: Effect of rice milling waste diets supplemented with or without enzymes on haematological parameters of broiler chickens

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
PCV (%)	30.50^{a}	30.00^{a}	26.50^{c}	31.00^{a}	27.50^{bc}	29.50^{ab}	27.50^{bc}	0.49	0.01
HB (g/dl)	15.00	14.50	13.00	15.00	14.00	14.50	13.50	0.24	0.15
RBC (x10^{12}/L)	2.75	2.65	2.40	2.65	2.45	2.60	2.45	0.04	0.26
MCV (fL)	111.50	113.50	111.00	116.50	113.00	111.50	113.50	0.84	0.34
MCH (pg)	55.00	56.00	55.50	56.50	56.00	54.50	56.00	0.34	0.16
MCHC (g/L)	488.50	495.00	500.00	485.00	496.50	488.50	494.50	2.27	0.07
WBC (x10^{9}/L)	142.50	134.00	118.00	132.50	150.50	132.00	129.50	3.87	0.46
Neutrophils(%)	47.35^{b}	66.50^{a}	30.50^{d}	48.00^{b}	64.50^{a}	37.50^{c}	49.40^{b}	3.38	0.01
Lymphocytes(%)	64.10^{b}	33.00^{d}	66.50^{b}	42.50^{c}	34.00^{d}	61.50^{b}	83.00^{a}	4.87	0.01
Monocytes(%)	1.50	0.50	0.50	2.50	1.00	1.00	3.00	0.36	0.14
Basophils(%)	1.00	0.00	1.50	4.00	0.50	0.00	1.00	0.44	0.12
	33.50	30.35	34.70	42.50	32.00	38.35	31.50	1.42	

^{abcd}Means on the same row with different superscripts are significantly different ((p<0.05). SEM: Standard Error of Mean.

PCV: Packed cell volume, RBC: red blood cells, HB: haemoglobin, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

T 1- Control diet without enzyme (basal diet)

T 2 - 20% maize of basal diet replaced with rice milling waste without enzyme supplementation

T 3- 20% maize of basal diet replaced with rice milling waste with enzyme supplementation

T 4- 30% maize of basal diet replaced with rice milling waste without enzyme supplementation

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T5- 30% maize of basal diet replaced with rice milling waste with enzyme supplementation
T 6- 40% maize of basal diet replaced with rice milling waste without enzyme supplementation
T 7- 40% maize of basal diet replaced with rice milling waste with enzyme supplementation

Table 3 shows the interaction effects of enzyme supplementation and inclusion levels of rice milling waste (RMW) on the haematological parameters of broiler chickens.

Packed cell volume (PCV) showed significant interaction effects. At each inclusion level (20, 30 and 40%), birds fed diets without enzyme supplementation recorded higher PCV values than those with enzyme supplementation. The highest PCV (31.00%) was observed at the 30% inclusion level of RMW without enzyme, while the lowest (26.50%) was recorded at the 20% inclusion level of RMW with enzyme. Haemoglobin (Hb) and red blood cell (RBC) counts followed similar pattern has PVC. Across all inclusion levels. Mean corpuscular volume (MCV) varied with the interaction of enzyme and inclusion level. The highest MCV (116.50 fL) was observed at the 30% inclusion level of RMW without enzyme, while the lowest (111.50 fL) occurred at the 40% inclusion level of RMW without enzyme. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) also showed interaction effects. MCH values were generally higher in diets without enzyme supplementation at each inclusion level. MCHC was highest at the 20% inclusion level of RMW with enzyme (500.00 g/L). White blood cell (WBC) count revealed varying effects. At the 30% inclusion level of RMW, birds with enzyme supplementation had higher WBC (150.50 × 10^9/L) than those without enzyme (132.50 × 10^9/L). However, at the 20% inclusion level, WBC was higher without enzyme (134.00 × 10^9/L) than with enzyme (118.00 × 10^9/L). Neutrophil and lymphocyte percentages showed clear interaction effects. At the 20% inclusion level of RMW, neutrophil percentage was highest without enzyme (66.50%) and lowest with enzyme (30.50%), with an opposite trend for lymphocytes (33.00% without enzyme vs. 66.50% with enzyme). At the 40% inclusion level of RMW, lymphocytes were highest at 83.00% with enzyme supplementation.

Parameters	Enzyme	Inclusion Level of RMW 20%	30%	40%	SEM
PCV	-	30.00^{bx}	31.00^{ax}	29.50^{bx}	0.31
	+	26.50^{by}	27.50^{ay}	27.50^{ay}	0.31
	SEM	1.11	1.11	0.65	
Hb	-	14.50^{x}	15.00^{x}	14.50^{x}	0.54
	+	13.00^{y}	14.00^{y}	13.50^{y}	0.54
	SEM	0.48	0.50	0.41	
RBC	-	2.65^{x}	2.65^{x}	2.60^{x}	0.11
	+	2.40^{y}	2.45^{y}	2.45^{y}	0.11
	SEM	0.11	0.09	0.07	
MCV	-	113.50^{b}	116.50^{a}	111.50^{cy}	1.65
	+	111.00^{b}	113.00^{a}	113.50^{ax}	1.65
	SEM	2.29	1.80	0.65	
MCH	-	56.00^{ax}	56.50^{ax}	54.50^{by}	1.12
	+	55.50^{by}	56.00^{ay}	56.00^{ax}	1.12
	SEM	0.5	0.14	0.48	
MCHC	-	495.00^{a}	485.00^{by}	488.50^{by}	3.96
	+	500.00	496.50^{x}	494.50^{x}	3.96
	SEM	3.29	1.98	2.26	
WBC	-	134.00^{x}	132.50^{y}	132.00^{x}	10.94
	+	118.00^{y}	150.50^{a}	129.50^{y}	10.94
	SEM	2.97	5.19	1.44	
NEUT	-	66.50^{ax}	48.00^{by}	37.50^{cy}	1.23
	+	30.50^{cy}	64.50^{ax}	49.40^{ax}	1.23
	SEM	1.95	1.87	1.49	
LYMPH	-	33.00^{cy}	42.50^{bx}	61.50^{ay}	1.48
	+	66.50^{bx}	34.00^{cy}	83.00^{ax}	1.48
	SEM	0.68	2.09	3.41	
EOSINO	-	0.08^{cy}	3.00^{ax}	2.15^{bx}	0.41
	+	1.00^{bx}	2.60^{ay}	2.08^{ay}	0.41
	SEM	0.02	0.09	0.01	
MONO	-	1.50^{b}	2.50^{a}	1.00^{c}	0.18
	+	1.65^{b}	1.73^{b}	3.00^{a}	0.18
	SEM	0.29	0.48	0.18	
BASO		4.44^{ax}	4.00^{ax}	3.33^{b}	0.26
	SEM	0.48	0.32	0.50	
Platelets	-	30.35^{cy}	42.50^{ax}	38.35^{bx}	1.43
	+	34.70^{ax}	32.00^{by}	31.50^{by}	1.43
	SEM	2.17	3.90	2.55	

Table 3: Interaction effect of enzyme supplementation and inclusion level of rice milling waste on haematological parameters of broiler chickens

Monocytes showed significant interaction. The highest monocyte value (3.00%) was recorded at the 40% inclusion level of RMW with enzyme, while the lowest (1.00%) occurred at the 40% inclusion level of RMW without enzyme. Basophil values were generally higher without enzyme supplementation at the 20% and 30% inclusion levels. Platelet counts were higher without enzyme supplementation at the 30% and 40% inclusion levels, whereas at the 20% inclusion level, platelet count was higher with enzyme.

Table 4 presents the effects of replacing maize in the basal diet with rice milling waste (RMW), with or without enzyme supplementation, on serum biochemical parameters of broiler chickens. Significant differences ($p < 0.05$) were observed in all measured serum biochemical indices across treatments. Aspartate aminotransferase (AST) levels were highest in birds fed 40% maize replaced with RMW without enzyme (T6, 89.50 IU/L) and 20% RMW + enzyme (T3, 85.00 IU/L), while the lowest AST value (52.00 IU/L) was observed in birds fed the control diet (T1). Alanine aminotransferase (ALT) levels followed a similar pattern, with the highest values in birds fed 30% RMW + enzyme and 40% RMW without enzyme (T5 and T6), while lower values (27.50–28.50 IU/L) were recorded in the control (T1), 20% RMW + enzyme (T3), and 30% RMW without enzyme (T4). Alkaline phosphatase (ALP) was significantly highest in birds fed 20% RMW + enzyme (T3, 124.50 IU/L), compared to other treatments. Birds on 30% RMW without enzyme

(T4) had the lowest ALP (52.50 IU/L). Total protein and albumin concentrations were highest in birds fed 30% RMW with enzyme (T5) and 30% RMW without enzyme (T4), with values ranging from 49.50 to 52.00 g/L and 23.00 to 24.00 g/L, respectively. Lowest total protein values (30.00 and 29.00 g/L) were observed in birds at 20% RMW with enzyme (T3) and 40% RMW without enzyme (T6) and highest at T5 (52.00g/L) 30% RMW + enzyme supplementation

Globulin concentration varied significantly ($p < 0.05$), with the highest levels observed in birds fed 30% RMW + enzyme (T5, 29.00 g/L), followed by those on 30% RMW without enzyme (T4, 25.50 g/L). The lowest Globulin value (9.50 g/L) was recorded in birds with 20% RMW + enzyme (T3). Creatinine levels were highest in the control group (T1, 81.50 µmol/L), while the lowest values were observed in birds fed 30% and 40% RMW without enzyme (T4 and T6). Cholesterol concentration was significantly reduced in birds fed 40% RMW without enzyme (T6, 1.82 mmol/L), compared to the control group (T1, 3.70 mmol/L). Triglyceride (TRIG) levels were significantly reduced ($p < 0.01$) in all RMW treatments compared to control, with the lowest value (0.49 mmol/L) observed in birds on 30% RMW + enzyme (T5). HDL levels were highest in birds on 30% RMW without enzyme (T4, 1.62 mmol/L), while LDL levels were highest in the control group (T1, 2.24 mmol/L).

Table 4: Effect of rice milling waste diets supplemented with or without enzymes on serum biochemical parameters of broiler chickens.

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
AST (IU/L)	52.00^{d}	66.50^{cd}	85.00^{ab}	55.00^{d}	69.00^{c}	89.50^{a}	80.00^{b}	3.97	0.01
ALT (IU/L)	28.50^{c}	53.00^{ab}	28.50^{c}	27.50^{c}	60.00^{a}	56.50^{a}	34.50^{bc}	4.07	0.01
ALP (IU/L)	66.50^{bc}	82.00^{bc}	124.50^{a}	52.50^{c}	101.50^{ab}	71.50^{bc}	95.00^{ab}	6.83	0.02
Total protein (g/L)	39.00^{cd}	32.50^{de}	30.00^{e}	49.50^{ab}	52.00^{a}	29.00^{e}	41.50^{bc}	2.46	0.00
Albumin (g/L)	19.50^{ab}	15.50^{c}	20.50^{ab}	24.00^{a}	23.00^{a}	15.50^{b}	19.50^{ab}	0.97	0.04
Globulin (g/L)	19.50^{abcd}	17.00^{bcd}	9.50^{d}	25.50^{ab}	29.00^{a}	13.50^{cd}	22.00^{abc}	1.93	0.03
Creatinine (µmol/l)	81.50^{a}	60.00^{ab}	49.00^{b}	45.50^{b}	58.50^{ab}	57.00^{ab}	73.00^{ab}	3.97	0.03

[abcde] Means on the same row with different superscripts are significantly different ($p<0.05$). SEM: Standard Error of Mean.

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, CHOL: Cholesterol, HDL: High density lipoproteins, LDL: low density lipoproteins, TRIG: triglyceride

T 1- Control diet without enzyme (basal diet)

T 2 - 20% maize of basal diet replaced with rice millin g waste without enzyme supplementation

T 3- 20% maize of basal diet replaced with rice milling waste with enzyme supplementation

T 4- 30% maize of basal diet replaced with rice milling waste without enzyme supplementation

T5- 30% maize of basal diet replaced with rice milling waste with enzyme supplementation

T 6- 40% maize of basal diet replaced with rice milling waste without enzyme supplementation

T 7- 40% maize of basal diet replaced with rice milling waste with enzyme supplementation

Table 5 shows the interaction effects of enzyme supplementation and inclusion levels of rice milling waste (RMW) on the serum biochemical parameters of broiler chickens. Aspartate aminotransferase (AST) was significantly affected by the interaction. At 20% inclusion level of RMW, birds fed diets with enzyme supplementation recorded higher AST (85.00 IU/L) compared to those without enzyme (65.50 IU/L). A similar pattern was observed at the 40% inclusion level, with higher AST in birds with enzyme (80.00 IU/L). However, at the 30% inclusion level, AST was highest without enzyme (89.00 IU/L) and lower with enzyme (69.00 IU/L). Alanine aminotransferase (ALT) also showed clear interaction effects. At the 20% inclusion level of RMW, birds without enzyme had higher ALT (53.00 IU/L) than those with enzyme (28.50 IU/L). In contrast, at the 30% and 40% inclusion levels, ALT values were generally higher in birds with enzyme supplementation. Alkaline phosphatase (ALP) increased significantly with enzyme at the 20% inclusion level (124.50 IU/L) with enzyme when compared at the same level (82.00 IU/L) without enzymes, it was lowest at 30% inclusion level without enzyme (52.50IU/L). Total protein and albumin levels reflected similar interaction patterns. At 20% inclusion level of RMW, birds without enzyme had slightly higher total protein than those with enzyme. However, at the 30% and 40% inclusion levels, total protein and albumin were higher with enzyme supplementation.

Globulin values were highest in birds fed the 30% inclusion level of RMW with enzyme (29.00 g/L) compared to the same inclusion without enzyme (25.00 g/L). At the 20% inclusion level, globulin was higher without enzyme, while at 40% it was higher with enzyme. Creatinine levels varied with interaction effects. At the 20% inclusion level of RMW, creatinine was higher without enzyme (60.00 µmol/L). At 30% and 40% inclusion levels, creatinine was higher with enzyme. At 40% inclusion level, cholesterol was higher with enzyme (2.61 mmol/L) than without (1.82 mmol/L) while the highest cholesterol was observed in 30% inclusion of RMW without enzyme (3.11 mmol/L). Triglyceride levels were lower with enzyme and without enzyme at 30% inclusion levels (0.49, 0.63 mmol/L) and 40% inclusion level without enzyme (0.53 mmol/L). High-density lipoprotein (HDL) levels were highest at the 30% inclusion level of RMW with enzyme supplementation (1.62 mmol/L) compared to the same level without enzyme (1.19 mmol/L). A

similar increase was seen at the 40% inclusion level with enzyme. Low-density lipoprotein (LDL) levels showed minimal interaction effect but were higher without enzyme at the 20 and 30% inclusion level.

Table 5: Interaction effect of enzyme supplementation and inclusion level of rice milling waste on serum biochemistry of broiler chickens

Parameters	Enzyme	20% RMW	30% RMW	40% RMW	SEM
AST	-	65.50^{by}	55.00^{by}	89.00^{ax}	5.35
	+	85.00^{ax}	69.00^{ax}	80.00^{by}	5.35
	SEM	1.44		1.78	
ALT	-	53.00^{ax}	27.50^{by}	56.50^{ax}	6.08
	+	28.50^{cy}	60.00^{ax}	34.50^{by}	6.08
	SEM	2.32		3.21	
ALP	-	82.00^{ay}	52.50^{cy}	71.50^{by}	10.89
	+	124.50^{ax}	101.50^{bx}	95.00^{bx}	10.89
	SEM	2.92	2.60	1.98	
Total protein	-	32.50^{by}	49.50^{a}	29.00^{by}	2.57
	+	30.00^{cy}	52.00^{a}	41.50^{ax}	2.57
	SEM	1.48	1.55	2.01	
Albumin	-	15.50^{by}	24.00^{a}	15.50^{by}	1.53
	+	20.50^{bx}	23.00^{a}	19.50^{bx}	1.53
	SEM	1.58	0.95	1.56	
Globulin	-	17.00^{bx}	25.00^{ay}	13.50^{by}	3.26
	+	9.50^{cy}	29.00^{ax}	22.00^{bx}	3.26
	SEM	2.33	1.93	1.64	
Creatinine	-	60.00^{ax}	45.50^{cy}	57.00^{by}	1.37
	+	49.00^{cy}	58.50^{bx}	73.00^{ax}	1.37
	SEM	2.20	2.60	3.77	
Cholesterol	-	2.47^{bx}	3.11^{ax}	1.82^{cy}	0.21
	+	2.28^{y}	2.62^{y}	2.61^{x}	0.21
	SEM	0.08	0.23	0.24	
Triglyceride	-	0.91^{ax}	0.63^{b}	0.53^{by}	0.10
	+	0.70^{ay}	0.49^{b}	0.79^{ax}	0.10
	SEM	0.08	0.09	0.08	
HDL	-	0.90^{b}	1.62^{ax}	0.93^{by}	0.12
	+	0.84^{b}	1.19^{ay}	1.10^{ax}	0.12
LDL	-	1.16^{ax}	1.21^{a}	0.65^{by}	0.04
	+	1.13^{y}	1.20	1.16^{x}	0.04
	SEM	0.02	0.04	0.15	

Discussion

The observed variation in packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) counts indicates that RMW inclusion, especially at higher levels, could affect the oxygen-carrying capacity and general health status of birds. Birds fed diets without enzyme supplementation seem to maintain higher PCV and Hb values compared to those fed enzyme-supplemented diets. This may be due to possible influence of the enzymes in nutrient utilization and fibre degradation, which can affect nutrient availability and gut health. Similar trends have been reported by Toghyani *et al.,* (2010), who noted that fibre-degrading enzyme supplementation influenced blood indices by modifying nutrient digestibility. Significant differences in neutrophil and lymphocyte counts observed across diets suggest that the immune response of the birds was responsive to both RMW level and enzyme inclusion. High neutrophil counts in birds on diets without enzyme supplementation at lower RMW inclusion levels indicate possible mild stress or infection, while higher lymphocyte levels in enzyme-supplemented birds may suggest improved immune modulation, aligning with findings by Abudabos *et al.,* (2020), who reported that feed enzymes can enhance immune cell balance. Serum biochemical indices such as AST, ALT, ALP, and creatinine reflect liver and kidney function. The elevated AST and ALT levels in birds fed higher RMW levels with enzyme supplementation may indicate increased liver activity due to enhanced nutrient metabolism, as noted by Olukosi *et al.,* (2020) when using exogenous enzymes in fibre-rich diets. However, the enzyme's effect at lower inclusion levels suggests more efficient nutrient absorption and less hepatic strain. Total protein, albumin, and globulin levels were significantly influenced by the interaction between enzyme supplementation and RMW inclusion levels. Higher total protein and albumin concentrations in birds fed 30% inclusion levels with enzyme support the idea that moderate maize replacement with proper enzyme support optimizes protein synthesis and nutrient utilisation (Shakouri *et al.,* 2009).

The reduction in serum cholesterol and triglycerides in birds fed higher RMW levels supports earlier reports that fibre-rich by-products can lower serum lipid profiles in broilers (Onyimonyi and Ugwu, 2007). This indicates that RMW, due to its high fibre content, may help improve lipid metabolism, potentially producing leaner meat. The significant interaction between enzyme supplementation and RMW inclusion for cholesterol, HDL, LDL, and triglyceride levels further shows that enzyme addition can modulate lipid metabolism when fibre-rich feedstuffs are used. This aligns with observations by Cowieson and Bedford (2009), who highlighted that enzymes could alter gut microflora and lipid digestion.

Conclusion

Based on the findings of this study, the following conclusions can be drawn:

1. Rice milling waste (RMW) can replace maize in broiler diets up to 30% inclusion without negatively affecting haematological and serum biochemical parameters.
2. Enzyme supplementation is essential when RMW is included, to enhance nutrient digestibility and maintain bird health.
3. Moderate RMW (20 and 30%) inclusion levels maintain normal physiological health, liver function, and immune status, and can help improve serum lipid profiles in broiler chickens.
4. Higher inclusion levels (40%) may cause mild metabolic stress, as indicated by elevated liver enzyme activities.

Recommendation

- It is recommended that maize in broiler diets can be replaced with rice milling waste up to 30% inclusion level for optimal health and performance.

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