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Haematological, Serum biochemical indices and lipid profile of finisher broiler chickens fed red and white sorghum based diets, supplemented with tannase enzyme

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Abstract: A 28 days study was carried out to investigate the effects of red sorghum (RS) and white sorghum (WS) based diets, with or without tannase supplementation on the haematological, serum biochemical indices and lipid profile of finisher broiler chickens. Two hundred and forty (240) 4 weeks old Arbor Acre broiler chicks were allotted to eight (8) dietary treatments, which consisted of 30 birds, having 3 replicates of 10 birds each. Diets were formulated to replace maize with red and white sorghum at 4 levels (0%, 100%RS, 100%WS and 50%RS:50%WS), with or without tannase enzyme to have a 4x2 factorial arrangement in a completely randomized design. On 28th day, blood was collected for determination of haematological, serum biochemical and lipid profile. Birds fed 100% RS diet and those fed 50RS: 50WS had the highest ($P<0.05$) white blood cell count (WBC), while the least WBC value was recorded for birds fed maize-based diet. Haematological indices were not significantly ($P>0.05$) influenced by the interaction between the sorghum type and tannase supplementation. Birds fed the maize-based diet without tannase had the highest ($P<0.05$) level of albumin (4.87g/dl), while birds fed 100%WS diet without tannase had the lowest value (3.33g/dl). The lipid profile of boiler chickens was not significantly ($P>0.05$) influenced by sorghum type, tannase supplementation and their interaction. It was concluded from the study that RS and WS, with or without tannase supplementation, can totally replace maize in the diet of finisher broilers without detrimental effect on the haematological, serum-biochemical indices and lipid profile.

Keywords: Broiler finisher; sorghum; tannase enzyme; haematology; serum

INTRODUCTION

The poultry industry is presently being faced with the challenge of high feed cost due to high cost of maize, which constitutes about 60-70% component of poultry feed (Thirumalaisamy *et al.,* 2016). There is need to source for alternative energy feedstuffs that may replace maize to reduce over dependence on maize to supply the energy requirement of poultry, especially broiler chickens (Fasuyi, 2010). Such alternative energy feedstuff is sorghum. The utilization of sorghum for food, feed, forage, silage and fuel mostly in the semi-arid tropics of Asia, Africa, America and Australia (Reddy *et al*., 2010) is partly due to its ability to grow in areas where maize may not grow and has helped in reducing food and livestock feed shortage (Legodimo and Madibela,

2013). The presence of anti-nutritional factors, such as high tannin and phytates in sorghum, is a major constraint in the utilization of sorghum by poultry (Manyelo *et al*., 2019). This can be reduced or eliminated by fermentation, grinding, use of activated charcoal, alkali, microbial phytase, which may hydrolyse phytate and improve phosphorus absorption and use of tannase (Okot and Mujabi, 2001). Tannase, as an inducible enzyme, can be used in the preparation of animal feed to catalyse the hydrolysis of hydrolysable tannins, such as tannic acid releasing glucose and gallic acid (Govindarajan *et al*., 2016). This study was conducted to determine the haematological, serum biochemical indices and lipid profile of finisher broiler chickens fed red and white sorghum-based diet supplemented, with or without tannase.

MATERIALS AND METHODS

Preparation of test ingredients

The red and white sorghum were sourced from the open market, while maize was sourced from a reputable feed mill. The red sorghum and white sorghum were crushed using the mechanical crusher and enriched with tannase enzyme during the formulation of the diets. Tannase enzymes were prepared at the Animal Nutrition Laboratory, Federal University of Agriculture Abeokuta, by obtaining cultured fungal spores (*Aspergillus spp*.) from microbiology laboratory. The fungal spores were screened and the best clear zone producing isolates were selected for tannase production, using methods described by Abou-Bakr *et al*. (2013). Isolates were separated, then optimization study was done for temperature, pH, incubation period for maximum enzyme production and activity (Yao *et al*., 2014).

Experimental animals, diets and design

A total of 240 four weeks old unsexed Arbor Acre broiler chicks were randomly allotted into 8 dietary treatments of 30 birds, and each treatment replicated 3 times with 10 birds each in a 4x2 factorial arrangement of four replacement levels of maize (0, 100RS, 100%WS and 50%RS:50%WS), and two levels of tannase enzyme supplementation (with or without), using completely randomized design. Eight experimental diets were formulated for the finisher broilers (28-56 days) as is shown in Tables 1, where maize was replaced with red sorghum (RS), white sorghum (WS) at 0% (diet 1), 100% RS (Diet 2), 100% WS (diet 3), and 50RS: 50WS% (diet 4). Diets 1, 2, 3 and 4 were without tannase, while diets 5, 6, 7 and 8 had the same composition as diets l, 2, 3 and 4, but supplemented with tannase enzyme at 0.5g/kg feed. The birds were offered the formulated diets and clean water *ad libitum* for 28 days.

Data collection

Blood samples were obtained via the wing vein puncture (Kelly and Alworth, 2013) from 2 birds per replicate, at the end of the 56th day into well labeled bottles containing ethylene diamine tetra acetate (EDTA) as anti-coagulant for determining the haematological indices (red blood cell (RBC), Haemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC)). Another set of blood samples were collected into plain empty bottles for serum bio-chemical analysis of glucose, albumin, globulin, total protein (TP), alanine amino-transferase (ALT), aspartate amino transferase (AST) and lipid profile (cholesterol, triglyceride, high density lipoprotein (HDL), and very low-density lipoprotein (VLDL).

RBC, PCV and WBC count of the blood samples were determined in wintrobe haematocrit tube according to the method of Schalm *et al*. (1975). Haemoglobin concentration was estimated using the cyanmethaemoglobin method (Cannon, 1958).

Statistical analysis

Data obtained were subjected to two-way analysis of variance in a 4 x 2 factorial arrangement, using Minitab statistical package (Minitab, 2017), to determine the main effect of maize replacement levels with sorghum, tannase enzyme supplementation and interactive effect of maize replacement levels with sorghum and tannase enzyme supplementation. The significant ($p<0.05$)

Table 1. Composition of experimental broiler finisher diets (5-8weeks)

	Without Tannase enzyme				*With Tannase enzyme*			
Ingredients	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Maize	57.00	-	-	-	57.00	-	-	-
Red sorghum	-	57.00	-	28.50	-	57.00	-	28.50
White sorghum	-	-	57.00	28.50	-	-	57.00	28.50
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soyabean meal	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat offal	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bonemeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Tannase	-	-	-	-	+	+	+	+
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrient values								
Metabolisable Energy (kcal/kg)	2936.50	2873.81	2816.80	2845.31	2936.50	2873.81	2816.80	2845.31
Crude Protein (%)	21.64	22.21	21.81	22.01	21.64	22.21	21.81	22.01
Crude Fat (%)	3.65	2.97	3.08	3.03	3.65	2.97	3.08	3.03
Crude Fibre (%)	3.24	3.13	3.21	3.19	3.24	3.13	3.21	3.19
Calcium (%)	1.16	1.15	1.18	1.17	1.16	1.15	1.18	1.17

1kg of broiler premix contains: Vitamin A 10,000,000 IU; Vitamin E 20,000 IU; Vitamin K 2,250mg; Thiamine 1750mg; Riboflavin 5000mg; Pyridoxine 2,750mg; Niacin 27,500mg; Vitamin B12 15mg; Pantothenic acid 7500mg; Biotin 50mg; Choline chloride 400g; Antioxidant 125g; Magnesium 80g; Zinc 50mg; Iron 20g; Copper 5g; Iodine 1.2g; Selenium 200mg; Cobalt 200mg.

difference among means were separated by Tukey's test procedure of the same package.

The experimental model is:

$Y_{ijk} = \mu + S_i + T_j + (ST)_{ij} + \sum_{ijk}$

Y_{ijk} = observed value of dependent variable

μ= population mean (overall mean)

Si= Effect of main energy sources (Maize, RS, WS and RS+WS)

T_J = Effect of tannase supplementation (without or with)

$(ST)_{ij}$ = Interaction effects of sorghum and tannase supplementation

$\sum_{ijk}$ = Random residual error

RESULTS

Table 2 shows the effect of sorghum type with or without tannase supplementation on haema-

tological parameters of finisher broiler chickens. The WBC were significantly (P<0.05) influenced by sorghum type and tannase supplementation. However, the haematological parameters measured were not significantly (P>0.05) affected by the interaction of the sorghum type and tannase supplementation. Birds fed 100% RS diet and those fed 50RS:50WS had the highest (P<0.05) WBC count and the least value ($11.83x10^{9/L}$) was obtained for broilers fed diet without 0% sorghum, while it was intermediate for broilers fed 100% WS diet. Broilers fed diets without tannase supplementation had higher value ($13.23x10^{9/L}$), compared to a lower value of $12.49x10^{9/L}$ recorded for birds fed tannase supplemented diets.

Serum biochemical indices of broiler chickens is shown in Table 3. Only albumin level was significantly (P<0.05) influenced by the main effect of sorghum type, tannase supplementation and interaction of sorghum type and tannase supplementation, while other parameters were not significantly (P>0.05) affected. The interactive effect shows that higher (P<0.05) level of albumin (4.87g/dl) was obtained in birds fed the control diet (100% maize) without tannase, compared to other treatments. The main effect of sorghum type reveals that broilers fed diet with 0% sorghum had the highest (P<0.05) albumin (4.25g/dl) and birds fed 100% WS diet had the lowest albumin (3.62 g/dl), while those fed 100% RS and

Table 2. Effect of sorghum type with or without tannase supplementation on haematological parameters of finisher broiler chickens

Sorghum Level (%) **Interaction effect**	Tannase	PCV (%)	Hb (g/dl)	RBC ($x10^{12/L}$)	WBC ($x10^{9/L}$)
0	+ Tannase	37.67	12.40	3.17	11.40
100RS	+ Tannase	36.67	12.10	3.03	13.40
100WS	+ Tannase	37.67	12.53	3.13	11.97
50RS: 50WS	+ Tannase	35.67	11.83	2.97	13.20
0	No Tannase	38.33	12.80	3.20	12.27
100RS	No Tannase	35.33	11.77	3.00	13.93
100WS	No tannase	39.00	13.00	3.30	13.27
50RS: 50WS	No Tannase	34.33	11.47	2.80	13.47
Pooled SEM		0.57	0.19	0.06	0.22
Main effect					
0	-	38.00	12.60	3.18	11.83^{b}
100RS	-	36.00	11.93	3.02	13.67^{a}
100WS	-	38.33	12.77	3.72	12.62^{ab}
50RS: 50WS	-	35.00	11.65	2.88	13.33^{a}
SEM level		1.15	0.37	0.32	0.32
-	+ Tannase	36.92	12.22	3.08	12.49^{b}
-	No Tannase	36.75	12.26	3.08	13.23^{a}
SEM Tannase		0.81	0.26	0.08	0.22
Normal Range		23-55	7-18.6	1.3-4.5	11.40-13.93

^{ab}mean values in the same column having different superscripts are significantly (P<0.05) different.

SEM: Standard error of mean, PCV: Packed Cell Volume, Hb: Haemoglobin, RBC: Red Blood Cell, WBC: White Blood Cell.

those fed 50%RS: 50%WS had intermediate albumin content. There was no significant (P>0.05) effect tannase supplementation on albumin content.

The main and interaction effects of sorghum type and tannase supplementation as is shown in Table 4 were not significant (P<0.05) for all the lipid profile indices (Cholesterol, Triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein) measured in the broiler chickens.

DISCUSSION

The haematological parameters show that the main effect of sorghum type influenced WBC concentration, which increased for broilers fed 100% RS diet and those fed 50%RS:50%WS diet. However, the values obtained were within the normal range of WBC ($11.40x10^{9/L}$ - $13.93x10^{9/L}$) by Pollack et al. (2005), which indicated sorghum can sufficiently replace maize in the diet of finisher broilers without causing infection since the values were within the normal range. The increased WBC values, obtained in birds fed the sorghum based diet, could be a reaction of the birds to foreign body, which could probably be tannin, there by having higher tendency of producing more antigens. White blood cells are a part of immune system that helps fight infection and defend the body against other foreign materials (Eldridge, 2022).

Table 3. Effects of sorghum type with or without tannase supplementation on serum biochemical indices of finisher broiler chickens

Sorghum Level (%) **Interaction effect**	Tannase	TP (g/dl)	ALB (g/dl)	GLOB (g/dl)	GLUC (mmol/l)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
0	+ Tannase	6.40	3.63^{b}	2.77	65.50	133.67	25.00	46.67
100RS	+ Tannase	6.53	3.73^{b}	2.83	73.20	143.00	24.67	48.33
100WS	+ Tannase	6.67	3.90^{b}	2.77	75.27	137.33	25.33	47.00
50RS: 50WS	+ Tannase	6.30	4.00^{b}	2.27	74.20	143.00	25.67	38.67
0	No Tannase	6.50	4.87^{a}	1.63	70.50	133.00	28.33	28.00
100RS	No Tannase	6.43	3.93^{b}	2.50	85.47	129.33	25.67	42.33
100WS	No Tannase	6.50	3.33^{b}	3.00	58.07	143.00	25.33	51.00
50RS: 50WS	No Tannase	6.02	3.80^{b}	1.90	81.20	138.00	25.67	32.33
Pooled SEM		0.10	0.10	0.13	3.00	2.75	0.51	2.26
Main effect								
0	-	6.45	4.25^{a}	2.20	68.00	138.50	26.67	37.33
100RS	-	6.50	3.83^{ab}	2.67	79.33	136.17	25.17	45.33
100WS	-	6.50	3.62^{b}	2.88	66.67	140.17	25.33	49.00
50RS: 50WS	-	5.98	3.90^{ab}	2.08	77.70	135.50	25.17	35.50
SEM level		0.19	0.11	0.23	5.97	6.12	1.09	3.83
-	+ Tannase	6.48	3.82	2.66	72.04	136.76	25.17	45.17
-	No Tannase	6.25	3.98	2.26	73.81	138.42	22.25	38.42
SEM Tannase		0.13	0.08	0.16	4.22	4.33	0.77	2.71

^{ab} mean values in the same column having different superscripts are significantly (p<0.05) different.
SEM: Standard error of mean, TP: Total Protein, ALB: Albumin, GLOB: Globulin, GLUC: Glucose, AST: Aspartate amino transferase, ALT:Alanine amino-transferase, ALP: Alkaline phosphatase

Table 4. Effects of sorghum type with or without tannase supplementation on lipid profile of finisher broiler chickens

Sorghum Level (%) **Interaction effect**	Tannase	CHOL (mg/dl)	TRIG (mg/dl)	HDL (Mg/dl)	LDL) Mg/dl)	VLDL (mg/dl)
0	+ Tannase	98.57	111.93	49.28	26.90	22.39
100RS	+ Tannase	102.10	108.27	51.05	29.40	21.65
100WS	+ Tannase	110.00	113.63	55.00	32.27	22.73
50RS: 50WS	+ Tannase	101.33	106.27	50.67	29.41	21.25
0	No Tannase	107.57	103.70	53.78	33.04	20.74
100RS	No Tannase	95.07	118.47	47.53	23.84	23.69
100WS	No Tannase	99.90	107.33	49.95	28.48	21.47
50RS: 50WS	No Tannase	107.33	103.43	53.67	32.98	20.69
Pooled SEM		1.83	2.19	0.92	0.99	0.44
Main effect						
0	-	103.07	107.82	51.33	29.97	21.56
100RS	-	98.58	113.37	49.29	26.62	22.67
100WS	-	104.95	110.48	52.47	30.38	22.10
50RS: 50WS	-	104.33	104.85	52.17	31.20	20.97
SEM level		3.69	4.65	1.84	1.85	0.93
	+ Tannase	103.00	110.02	51.50	29.50	22.01
	No Tannase	102.47	108.23	51.50	29.59	21.65
SEM Tannase		2.61	3.29	1.30	1.31	0.66

SEM: Standard error of mean, CHOL: Cholesterol, TRIG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

The serum biochemical indices of broilers in this study show that only serum albumin content was significantly influenced by dietary treatments, which indicates that for most of the parameters feeding of maize based diet, or sorghum based diet, with or without tannase enzyme supplementation, did not elicit any significant changes, which suggests suitability of diets for the finisher broilers. This is in agreement with the findings of Lakurbe et al. (2018) and Esiegwu (2017) that stated there were no significant differences in serum biochemical indices (albumin, globulin, total protein, glucose and uric acid) of broiler chickens fed sorghum grain as a replacement for maize in their diet, and the serum-biochemical indices were not adversely affected by the diet. The highest serum albumin content obtained in birds fed the control diet, might be an indication of availability of more protein to the birds than those fed sorghum based diet. Sorghum has been reported to contain Kafirin, phytates and other phenolic compounds, which interferes with protein metabolism (Farahat *et al*., 2020). However, the observed improved and normal range of the serum albumin in this study (3.33-4.87g/dL), which is higher than those reported by Wikivet (2012) at a range of 3.28 to 3.48/dl, is an indication that normal protein level was sustained in the blood of the birds across all the dietary treatments.

Similarly, there was no significant changes in the activities of the serum enzymes (AST, ALT and ALP), this indicated that the sorghum based diets did not cause hepatic toxicity in the chick-

ens. An AST value greater than 800 IU/L is an indication of severe hepatic disorder (Melillo, 2013). The non-significant effect of dietary treatment on these liver enzymes suggests there is no alteration in normal metabolism, which may adversely affect the health status of the birds. The non-significant effect of tannase supplementation on the serum bio-chemical indices of broiler chickens indicated the adequacy of nutrients in the diets of birds fed sorghum based diets, with or without tannase. The non-significant main and interaction effects on the lipid profile of broiler chickens fed sorghum based diets supplemented, with or without tannase suggests that replacement of maize with sorghum in broiler diet had no negative effect on lipid profile. This study agrees with the report of Gidado *et al.* (2020), who reported that feeding of sorghum based diets supplemented with enzyme had no adverse effect on lipid profile of broiler chickens.

CONCLUSION

From the result of the study, it can be concluded that maize can be replaced with 100% red or white sorghum, with or without tannase supplementation in the diet of finisher broiler chickens, without adverse effects on the haematological, serum biochemical indices and lipid profile.

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