



## Research Article

**Crude Fibre Versus Roxazyme® G<sup>2</sup>G Inclusion Levels in Sub Optimum Energy Diets for Broiler Chickens: Effect on Blood Profile**<sup>1</sup>Salami RI and <sup>2</sup>Odunsi AA

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**ABSTRACT**

The improved nutritional value of 2600ME (Kcal/kg) diet at the instance of 8% optimum dietary Crude Fibre (CF) level sequel to enzyme supplementation for improved broiler performance may also provoke changes in the blood profile, especially serum cholesterol and glucose. Hence, this study was conducted to monitor the blood profile of unsexed Obamarshal strain of broiler chickens fed multi-fibre source-based 2600ME (Kcal/Kg) diets at varying CF (4, 8, and 12%) and Roxazyme®G<sup>2</sup>G inclusion levels (0, 200 and 400mg/kg diet) per CF level in a 3X3 factorial arrangement to produce 9 treatment diets labeled A to I in ascending order of CF and enzyme levels. Birds were provided test diets and water *ad libitum* in the starter and finisher phases. Blood samples were drawn from the sampled experimental birds from the neck vein at 50 days of age and were subsequently used for evaluation of haematological and serum biochemical indices in triplicate using standard procedures. The values of haematological indices for the treatment groups were generally not significantly affected by the individual variable factors and their interaction. The range of values of haematological indices was within the range for normal chickens. Similarly, the varying CF and enzyme levels and their interaction except cholesterol and glucose did not affect the serum metabolites. The values of serum cholesterol in the birds ranged from 75mg/dl for diet H (12% CF) to 97mg/dl for diet C (4% CF), which were within the range of 92 to 100mg/dl for normal chickens. However, serum glucose concentration in the birds ranged from 133mg/dl for diet A (4% CF) to 195mg/dl for diet H (12% CF) (which is outside the normal range of 167mg/dl) for normal chickens. Thus, 12% dietary CF level might have implications for lower carcass fat and higher carcass glycogen irrespective of enzyme inclusion level, which, are worthy of consideration in broiler production.

**Key words:** Broiler chicken, Crude fibre, Metabolisable energy, Roxazyme®G<sup>2</sup>G, Blood profile

**INTRODUCTION**

The essentiality of Crude Fibre (CF) as a “forgotten” nutrient in poultry nutrition has been demonstrated and it has also been exploited to reduce feed cost (Salami, 2016; Isikwenu, 2011). Similarly, the adequacy of 2800 and 3000ME (Kcal/Kg) diet for broiler chicken has been revalidated against the recommendation of Low Energy-Low Protein (LELP) diets for raising broilers (Dairo *et al.*, 2010). It has also been shown that increasing dietary CF levels lowered carcass fat and blood cholesterol while increasing dietary ME levels increased both of them in the broiler chickens (Salami and Odunsi, 2017a&b). Though it was cheaper, 2600 ME (Kcal/Kg) diet at all CF levels was

inadequate as compared with 2800 and 3000 ME (Kcal/Kg) diets for broiler chickens at the starter and finisher phases for optimum performance (Salami 2016).

However, Salami, (2016) in another study, indicated that the nutritional value of 2600ME (Kcal/Kg) diet was improved when supplemented with 200mg Roxazyme® G<sup>2</sup>G per kg diet at 8% dietary CF for improved performance and carcass yield in broiler starters and finishers. This improved nutritional value is expected to reflect in the blood profile, especially serum cholesterol and glucose. Thus, it becomes pertinent to verify this assumption. Therefore, this study, aimed at investigating the changes provoked in the blood profile of broiler chickens by the variable factors of CF and enzyme at various inclusion levels at 50 days of age.

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## MATERIALS AND METHODS

### Experimental Site, Diets and Management

The study was conducted at the Poultry unit of the Teaching and Research farm, Emmanuel Alayande College of Education, Oyo, Nigeria. Oyo is located approximately along latitude 7° 51' North of the equator and longitude 3° 57' East of the Greenwich meridian and 850m above sea level. The annual average rainfall is 1163mm while annual mean temperature is 27°C and annual mean relative humidity is 82% (Iwena, 2012).

Nine test diets labeled A to I (Table 1) each for the starter and finisher phases of the same formulation and chemical composition (Salami, 2016) were applied to the treatment groups of Obamarshal Strain of broiler chickens for the evaluation of blood profile. There were 3 replicates per test diet arranged in a completely randomised design. Experimental stock was housed and managed as described by Salami and Odunsi (2017c).

### Blood collection and evaluation

Blood was collected aseptically from the neck veins of the sampled birds at 50 days of age for the measurement of haematological indices and serum metabolites. Heparinized bottles were used for collection of 2mls blood samples per treatment group for haematological indices while bottles without anti-coagulant were used for collecting 3mls blood samples from 3 birds per test diet for serum metabolites which were subsequently determined in triplicate.

The Packed Cell Volume (PCV) and Haemoglobin (Hb) were measured using the micro haematocrit and cyanomethemoglobin methods respectively as described by Mitruka and Rawnsley (1977). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer

haemocytometer after the appropriate dilution (Schalm *et al.*, 1975). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalm *et al.*, 1975).

Glucose and cholesterol were measured by spectrophotometric method according to Barham and Trinder (1972) and Flegg (1973) respectively. Urea and creatinine were by urease and Folin-Wu filterate methods respectively as described by Toro and Ackermann (1975). Total serum protein was determined using the biuret method as described by Reinhold (1953) while albumin was evaluated using the Bromocresol Green (BCG) method as described by Peters *et al.* (1982).

### Statistical analysis

Data collected were subjected to analysis of variance in accordance with 3X3 factorial design comprising 3 levels of CF (4, 8 and 12%) and 3 levels of Roxazyme®G<sup>2</sup>G (0, 200 and 400mg/kg) per CF level using SAS (2000) statistical package. Means were also separated using Duncan's Multiple Range Test (1955) of the same package at 5% probability level.

## RESULTS

Dietary crude fibre levels did not generally affect haematological indices (Table 2) except for inconsistent effect on the eosinophils and heterophils. The value of heterophils (33%) was higher ( $P < 0.05$ ) at 8% CF than at 4 and 12% CF levels which was 27%. The eosinophils could not be detected in the birds fed 12% CF diet while the values for 4 and 8% CF diets were similar ( $P > 0.05$ ). Likewise, enzyme supplementation of the diets did not affect ( $P > 0.05$ ) the haematological indices of the birds except for inconsistent effect on eosinophils, which was lower ( $P < 0.05$ ) at the highest enzyme inclusion level.

**Table 1:** Percentage Composition of Basal Starter and Finisher Diets

Feed Ingredients	Broiler Starter Diets			Broiler Finisher Diets		
	Dietary crude fibre level (%)			Dietary crude fibre level (%)		
Maize (2%CF)	4	8	12	4	8	12
Rice Offal (38%CF)	40	33	14	46	39	20
Wheat Offal (8.5%CF)	2	12	23	2	12	23
Palm kernel Cake (17.5%CF)	18	18	18	18	18	17
Groundnut Cake (5%CF)	2	2	2	2	2	3
Palm Oil	16	17	20	14	15	18
Sterilised Sand	2	3	8	1	2	7
<sup>a</sup> Fixed Ingredients	5	1	1	5	1	1
Total	14	14	14	11	11	11
Calculated Fractions:	100	100	100	100	100	100
Metabolisable energy (Kcal/kg)	2622.3	2628.3	2609.0	2611.9	2621.9	2604.9
Lysine (%)	1.10	1.10	1.10	0.89	0.90	0.91
Methionine (%)	0.31	0.30	0.28	0.32	0.31	0.30
<sup>c</sup> Determined chemical fractions (%):						
Dry matter	90.01	89.92	89.76	90.21	89.96	89.42
Crude protein	20.89	21.11	21.19	18.31	18.53	18.65
Crude fat	5.31	7.09	10.31	4.47	6.42	11.26
Nitrogen – free extract	50.62	47.65	38.82	54.03	47.21	42.57
Crude fibre	4.03	7.98	11.95	3.78	7.88	12.04
Ash	12.93	10.68	11.23	12.38	11.42	11.27
Calcium	2.48	2.58	2.39	2.44	2.56	2.37
Phosphorus	0.69	0.68	0.71	0.77	0.83	0.73

<sup>a</sup>Made up of 2.5% bone meal, 2% oyster shell, 0.25% salt and 0.25% broiler premix in the starter and finisher diets while the starter and finisher diets contained 6 and 4% Blood Meal and 3 and 2% Fish Meal respectively. <sup>c</sup>Means of triplicate determinations.

Haemoglobin concentration, packed cell volume, red blood cells and white blood cells of the experimental birds were not affected by the interaction of fibre and enzyme levels in the treatment diets (Table 3), indicating that their health was not challenged by the treatments applied. However, eosinophils decreased ( $P < 0.05$ ) as CF level increased irrespective of enzyme inclusion level. Furthermore, birds fed 8% CF diet without enzyme had higher value ( $P < 0.05$ ) of heterophils while birds fed 4 and 12% CF diets with or without enzyme had similar ( $P > 0.05$ ) but lower ( $P < 0.05$ ) values of heterophils.

Except for serum cholesterol and glucose, there was no significant effect of CF on other serum metabolites (Table 4). Serum cholesterol was reduced ( $P < 0.05$ ) while serum glucose was increased ( $P < 0.05$ ) with increasing dietary CF levels. Similarly, enzyme inclusion levels had no significant effect on the serum metabolites except cholesterol and glucose which were reduced ( $P < 0.05$ ) in the birds fed diets containing 400mg Roxazyme®G<sup>2</sup>G per Kg diet.

There was no interaction effect ( $P > 0.05$ ) on serum total protein (albumin and creatinine) and urea (Table 5), thereby suggesting no adverse effect on protein

metabolism in the birds. However, irrespective of enzyme inclusion levels, serum cholesterol was decreased ( $P < 0.05$ ) while serum glucose was increased ( $P < 0.05$ ) with increasing dietary CF levels in favour of 12% CF diet for producing leaner broiler carcass since fat is distasteful to consumers of animal products.

### DISCUSSION

There was no depression in the values of haematological indices within the range of crude fibre levels fed (4 to 12%), which is at variance with the observation of Bamgbose *et al.*, (2004) who fed cockerels with diets whose CF content ranged from 13 to 19%. The values of haematological indices recorded for the treatment groups agreed with the values obtained by Salami and Odunsi (2017a) which fell within the standard values reported by Mitruka and Rawnsley (1977) and those values obtained for Isa Brown grower pullets fed 4 to 12% CF diets by Alade (2014). The reported values and those obtained in this study suggested that the birds were nutritionally and physiologically comfortable.

**Table 2:** Effects of varying dietary crude fibre and enzyme inclusion levels on haematological parameters of broiler finishers at 50 days of age

Parameters	Dietary crude fibre level (%)			± SEM
	4	8	12	
Packed cell volume (%)	30.11	29.56	28.11	1.80
Haemoglobin concentration (mg/dl)	10.04	10.07	9.37	1.04
Red blood cell (x10 <sup>6</sup> /ml)	3.45	2.99	3.19	0.59
White blood cell (x10 <sup>3</sup> /ml)	25.98	23.31	23.07	1.64
Heterophils (%)	26.56 <sup>b</sup>	33.34 <sup>a</sup>	27.45 <sup>b</sup>	1.79
Lymphocytes (%)	67.66	61.78	66.67	2.69
Monocytes (%)	2.56	2.33	3.56	0.56
Eosinophils (%)	3.11 <sup>a</sup>	2.33 <sup>a</sup>	0.00 <sup>b</sup>	0.45
Basophils (%)	0.11	0.22	0.00	0.11
Parameters	Dietary enzyme level (mg/Kg diet)			± SEM
	0	200	400	
Packed cell volume (%)	30.00	29.00	28.78	1.80
Haemoglobin concentration (mg/dl)	9.99	9.66	9.81	1.04
Red blood cell (x10 <sup>6</sup> /ml)	3.24	3.43	3.08	0.59
White blood cell (x10 <sup>3</sup> /ml)	24.90	24.24	23.22	1.64
Heterophils (%)	29.34	28.56	29.45	1.79
Lymphocytes (%)	64.78	66.55	64.78	2.69
Monocytes (%)	2.67	2.11	3.67	0.56
Eosinophils (%)	2.00 <sup>ab</sup>	3.00 <sup>a</sup>	1.40 <sup>b</sup>	0.45
Basophils (%)	0.11	0.11	0.11	0.11

<sup>a,b</sup>Means within the same row bearing identical or no superscript are similar ( $P > 0.05$ ) while those with unidentical superscripts differ significantly ( $P < 0.05$ ).

**Table 3:** Interaction effects of varying dietary enzyme and crude fibre levels on haematological parameters of broiler finishers at 50 days of age

Parameters	0			200			400mg/Kg			± SEM
	@ 4%CF			@ 8%CF			@ 12%CF			
	A	B	C	D	E	F	G	H	I	
Packed cell volume (%)	29.67	31.00	29.67	31.67	29.00	28.00	28.67	27.00	28.67	3.13
Haemoglobin concentration (mg/dl)	9.89	10.33	9.89	10.55	9.66	10.00	9.55	9.00	9.55	1.86
Red blood cell (x10 <sup>6</sup> /ml)	3.62	3.82	2.90	3.04	3.19	2.73	3.05	3.27	3.24	1.03
White blood cell (x10 <sup>3</sup> /ml)	26.54	25.27	26.12	24.83	24.32	28.78	23.33	23.12	22.75	2.83
Heterophils (%)	26.67 <sup>b</sup>	26.00 <sup>b</sup>	27.00 <sup>b</sup>	35.67 <sup>a</sup>	31.67 <sup>ab</sup>	32.67 <sup>ab</sup>	25.67 <sup>b</sup>	28.00 <sup>b</sup>	28.67 <sup>b</sup>	3.11
Lymphocytes (%)	67.33	68.33	67.33	60.00	63.00	62.33	67.00	68.33	64.67	4.67
Monocytes (%)	2.33 <sup>b</sup>	2.67 <sup>b</sup>	2.67 <sup>b</sup>	1.67 <sup>b</sup>	2.00 <sup>b</sup>	3.33 <sup>ab</sup>	4.00 <sup>a</sup>	1.67 <sup>b</sup>	5.00 <sup>a</sup>	0.97
Eosinophils (%)	3.33 <sup>a</sup>	3.00 <sup>a</sup>	3.00 <sup>a</sup>	2.67 <sup>b</sup>	3.00 <sup>a</sup>	1.33 <sup>bc</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.78
Basophils (%)	0.33	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.18

<sup>a,b,c</sup>Means within the same row bearing identical or no superscript are similar ( $P > 0.05$ ) while those with unidentical superscripts differ significantly ( $P < 0.05$ ). \*Enzyme inclusion rate.

**Table 4:** Effects of varying of crude fibre and enzyme inclusion levels on serum metabolites of broiler finishers

Metabolites (mg/dl)	Dietary crude fibre level (%)			± SEM
	4	8	12	
Total protein	4.06	4.23	4.28	0.68
Albumin	1.18	1.20	1.13	0.37
Creatinine	0.68	0.77	0.67	0.28
Urea	2.29	2.24	2.42	0.51
Glucose	133.40 <sup>c</sup>	165.37 <sup>b</sup>	188.68 <sup>a</sup>	4.25
Cholesterol	95.46 <sup>a</sup>	84.72 <sup>b</sup>	75.56 <sup>c</sup>	3.08
	Dietary enzyme level (mg/Kg diet)			
	0	200	400	
Total protein	4.05	4.24	4.29	0.68
Albumin	1.16	1.14	1.22	0.37
Creatinine	0.58	0.79	0.75	0.28
Urea	2.47	2.34	2.14	0.51
Glucose	165.59 <sup>a</sup>	171.37 <sup>a</sup>	150.49 <sup>b</sup>	4.25
Cholesterol	84.94 <sup>a</sup>	84.81 <sup>a</sup>	60.62 <sup>b</sup>	3.08

<sup>a,b,c</sup>Means within the same row bearing identical or no superscript are similar ( $P>0.05$ ) while those with unidentical superscripts differ significantly ( $P<0.05$ ).

Neither CF nor enzyme inclusion levels affected the serum metabolites of the treatment groups except serum cholesterol and glucose which is also evident in their interaction effects. The values of total protein, albumin, creatinine and urea obtained for the treatment groups were within the normal range of values reported by Mitruka and Rawnsley (1977) and Salami and Odunsi (2017a). This shows that CF and enzyme levels did not adversely interfere with the protein metabolic status of the birds.

There was tendency for blood cholesterol reduction as CF and enzyme levels increased in the diets as evident in the abdominal fat and gizzard fat (Salami, 2016). The cholesterol-lowering effect of increasing CF level is caused by the binding action of soluble fibres and lignin with the bile acids for excretion (Shang *et al.*, 2010; Alade, 2014; Salami and Odunsi, 2017a). The values of serum cholesterol ranged from 75mg/dl for diet H (12%CF) to 97mg/dl for diet C (4%CF), which were within the range (92 to 100mg/dl) for normal chickens (Mitruka and Rawnsley, 1977).

Serum glucose increased with increasing CF level and reduced with increasing enzyme level. The values ranged from 133mg/dl for diet A (4%CF) to 195mg/dl for diet H (12% CF) which was outside the range of 167mg/dl for normal chickens (Mitruka and Rawnsley, 1977). This result is at variance with the findings of Alade (2014),

who observed stabilized serum glucose in Isa Brown grower pullets fed 4 to 12 CF diets during 11 to 20 weeks of age and the observation of Salami and Odunsi (2017a) in Obamarshal broiler chickens fed 4 to 12% CF diets at 2600, 2800 and 3000ME (Kcal/Kg) from day-old to 8 weeks of age. However, given the low-energy content of the test diets (2600ME Kcal/Kg) with or without enzyme in this study, the birds might have been challenged to derive glucose from CF via depolymerisation and fermentation of fibre NSP as well as gluconeogenic pathways to produce glucose to generate calorie resulting in increased blood glucose. The exogenous enzyme (Roxazyme<sup>®</sup>G<sup>2</sup>G) might have also stimulated insulin activity in the pancreas towards the removal of blood glucose with resultant reduction in blood glucose (John 1979; Ikegami *et al.*, 1999; Dietary Fibre and Resistant Starch, 2001).

It is noteworthy that 12% CF diet with supplemental enzyme, which minimized serum cholesterol in this study, could not sustain maximum dressed carcass weight according to Salami (2016).

### Conclusion and recommendation

Findings from this study indicated that:

- Neither CF nor enzyme inclusion levels and their interaction adversely affected haematological indices.
- Serum metabolites except cholesterol and glucose were not affected significantly by varying inclusion levels of CF and enzyme and likewise their interaction.
- Serum cholesterol was significantly reduced with increasing CF and enzyme levels while serum glucose was increased with increasing CF level and reduced with increasing enzyme level.
- 12% CF diet minimized serum cholesterol while it maximized serum glucose with attendant implications for producing broiler carcass of lower fat and higher carcass glycogen.

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**Table 5:** Interaction effects of varying dietary exogenous enzyme and crude fibre levels on serum metabolites of broiler finishers at 50 days of age

Metabolites (mg/dl)	0@A	200	400mg/Kg	0@D	200	400mg/Kg	0@G	200	400mg/Kg	± SEM
		4% CF	diet*		8% CF	diet*		12% CF	diet*	
	B	C		E	F		H	I		
Total protein	3.55	4.69	3.94	4.47	4.08	4.15	4.12	3.96	4.77	1.18
Albumin	1.18 <sup>ab</sup>	0.99 <sup>b</sup>	1.38 <sup>a</sup>	1.23 <sup>ab</sup>	1.30 <sup>ab</sup>	1.08 <sup>ab</sup>	1.07 <sup>ab</sup>	1.12 <sup>ab</sup>	1.20 <sup>ab</sup>	0.63
Creatinine	0.69 <sup>abc</sup>	0.78 <sup>ab</sup>	0.56 <sup>bc</sup>	0.36 <sup>c</sup>	1.02 <sup>a</sup>	0.94 <sup>ab</sup>	0.69 <sup>ab</sup>	0.59 <sup>bc</sup>	0.74 <sup>abc</sup>	0.48
Urea	2.89	2.20	1.80	2.31	2.46	1.95	2.22	2.36	2.67	0.88
Glucose	132.70 <sup>c</sup>	141.97 <sup>bc</sup>	125.53 <sup>c</sup>	180.20 <sup>a</sup>	180.20 <sup>a</sup>	138.47 <sup>bc</sup>	183.87 <sup>a</sup>	194.70 <sup>a</sup>	187.47 <sup>a</sup>	7.36
Cholesterol	94.61 <sup>a</sup>	95.23 <sup>a</sup>	96.55 <sup>a</sup>	84.61 <sup>b</sup>	84.61 <sup>b</sup>	85.30 <sup>b</sup>	75.60 <sup>c</sup>	75.01 <sup>c</sup>	76.06 <sup>c</sup>	5.33

<sup>a,b,c</sup>Means within the same row bearing identical or no superscript are similar ( $P>0.05$ ) while those with unidentical superscripts differ significantly ( $P<0.05$ ). \*Enzyme inclusion rate.

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