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Full length Research

Blood profile variations in Ross 308 broiler chickens fed dietary cocoa (Theobroma cacao) bean shell fermented with bovine rumen filtrate

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ABSTRACT: The nutritional impact of fermented cocoa bean shell (bovine rumen filtrate) on the blood biochemistry of Ross 308 broiler chickens was assessed over a 56-day trial. Rumen filtrate, obtained at a ratio of 5:5, was combined with fresh cocoa bean shells, fermented, and allowed to air dry. Five different experimental diets were used with varying inclusion levels of dried fermented cocoa bean shell (FCBS) (0, 5, 10, 15, and 20%, respectively). With three replicates of 24 birds each, a total of 360 broiler chickens were randomly assigned into five treatment groups, consisting of seventy-two (72) birds per treatment in a completely randomised design. Following the feeding trial, two (2) birds per replicate were randomly chosen and bled (6 mL) via the jugular veins using a new sterile hypodermic needle and syringe for the blood profile analysis following standard procedure. Data on haematological and serum biochemical indices were subjected to ANOVA at α=0.05 and treatment means were separated using the Duncan's Multiple Range Test. Result revealed that dietary FCBS supplementation had no significant effect (p>0.05) on the haematological indices of broiler chicken. The packed cell volume, haemoglobin, RBC and WBC of the broiler chicken obtained in this study ranged from 23.67 – 26.00%, 7.50 – 8.27 g/dL, 1.92 – 2.42 × 10^6 µL and 12.75 – 13.45 × 10^9 µL, respectively. Dietary FCBS had no significant influence (p>0.05) on the serum biochemical indices of broiler chicken except the alanine amino transferase (ALT), alkaline phosphatase (ALP), and cholesterol. The cholesterol values increased as the inclusion levels of FCBS increased across the dietary treatments. The ALT (25.33 – 37.00 iu/L) and ALP (200.67 – 361.00 IU/L) values obtained in this study ranged significantly (p<0.05) across the treatment groups. These findings suggest that FCBS can be incorporated up to 10% in broiler chickens' diet for the improved health status of the broiler chickens.

Keywords: Ross 308 genotype, physiological response, rumen filtrate, theobromine, unconventional feedstuffs.

INTRODUCTION

The cost of producing animal feed is increasing, which in turn raises the price of animal protein (Adeyeye *et al*., 2017). Nworgu *et al.* (1999) reported that 60–70% of the total production costs of monogastric animals are related to feeding expenses. As a result, local communities can no longer afford to consume the 8–15 g of animal protein per day that many African countries consume (Ogunsipe *et al*., 2017; Oloruntola *et al*., 2016).

Cocoa bean shell (CBS), a by-product of breaking and winnowing cocoa beans, has substantial nutritional value and can be used as animal feed source. Farmers' improper disposal of waste raises environmental issues, which are addressed by its widespread availability. Conventional feed components are becoming more and more expensive

in intensive animal production systems, and there is a growing preference for using unconventional feedstuffs in animal nutrition (Fakhlaei *et al*., 2020).

The direct use of the 14.5% crude protein, 18.3% crude fibre, and 1.3–2.0% theobromine content of the CBS in animal feed is restricted due to theobromine's inherent toxicity and antinutritional properties (Abiola and Tewe, 1991). Even at low inclusion levels like 5%, its theobromine content discourages its use in poultry feed (Yoeng *et al*., 1989). Solid-state fermentation has been studied as a way to enhance the nutritional profile of CBS meals, according to Olugosi *et al*. (2019). These modifications are meant to enhance its potential for improving blood profiles in chicken stocks, according to Ladokun *et al*. (2018). Bonadiman *et al.* (2009) state that these blood profile features not only aid in the diagnosis of poultry diseases but also offer essential information for research on comparative pathology and avian.

Generally speaking, haematological and serum biochemical indices are significant markers of an animal's physiological well-being that reveal how the animal responds to various environmental and dietary stimuli (Khan and Zafar, 2005). Animals' dietary or environmental influences are often indicated by changes in these parameters. Therefore, it is essential to examine the nutritional effects of cocoa bean shells fermented by bovine rumen filtrate on grill chickens' serum biochemical and haematological parameters.

MATERIALS AND METHODS

Experimental site

This study was conducted at the poultry unit of the Teaching and Research Farm of the Animal Science Department, Faculty of Agriculture, University of Abuja, and the experiment lasted 56 days.

Collection and processing of test ingredients and diet preparation

The bovine rumen content was extracted from four randomly chosen slaughtered cattle at Gwagwalada's abattoir in the Federal Capital Territory, Abuja, while fresh cocoa bean shells (CBS) were purchased from cocoa bean processors in Ondo State. Rumen filtrate (RF) was created by mixing the rumen and CBS at a 1:1 ratio with potable water, stirring, and sieving (Ewuola *et al.,* 2024).

The resulting RF was then mixed with CBS at a 5:5 ratio (Ewuola *et al.,* 2024). The manual mixing was followed by packing the mixture into polythene bags, securing the open end with ties, and allowing it to be fermented for 24 hours under the shade of a tree according to the procedure of Odunlade *et al.* (2020). Subsequently, the fermented mixture was sun-dried within 48 hours to achieve a moisture content of less than 10%, following the procedure outlined by previous researchers which was originally described for fermenting sweet orange (*Citrus sinensis*) peels with bovine rumen filtrate for use in broilers, pullets, and rabbits. The resulting dried fermented cocoa bean shell (FCBS) was then incorporated into the experimental diets at varying levels: 0, 5, 10, 15, and 20%, as outlined in Table 1. Additional ingredients included in the diets are presented in Tables 1 and 2.

Experimental animals and management

A total of 360 birds (day-old chicks) were procured and used for this study. Two weeks prior to the arrival of the experimental birds, the pen and facilities (equipment and metabolic cages) were prepared cleaned, thoroughly washed, and disinfected. The chicks were brooded for 2 weeks, and all necessary vaccinations and medications were administered following the manufacturer's protocols. Feed and water were supplied *ad-libitum.* Other routine management operations were carried out as outlined according to Olumide and Hamzat (2021). The birds were weighed on a group basis and the mean weight was determined to obtain individual weight and randomly allotted to five treatment groups with seventy-two (72) birds per treatment and three replicates containing 24 birds each in a completely randomized design.

Data collection

At exactly 56 days, blood samples were collected from two (2) birds randomly selected per replicate via the jugular vein using a sterile hypodermic needle and syringe. About 6 mL of blood samples were collected from each bird for the evaluation of haematological and serum biochemical parameters. Approximately 3 mL of blood sample collected was released into a labeled sample bottle containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and the bottle was gently rocked to ensure proper mixing of the blood with EDTA to prevent coagulation. Blood samples were then analyzed for full haematological parameters such as packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cells (RBC), and white blood cells (WBC). The remaining 3 mL was then released into a labeled sample bottles without anticoagulants for serum chemistry analysis. The serum biochemical indices examined were cholesterol, creatinine, aspartate transaminase, alanine transaminase, and total protein. The serum samples were kept in sterile tubes and stored at -20°C prior to analysis to determine lipid profiles as outlined by Roschlau *et al.* (1974), creatinine and bilirubin were assessed by the colometric method described by Newman and Price (1999). Aspartate transaminase and alanine transaminase levels were determined as described by Huang *et al*. (2006). The total protein was determined using the method described by Peters (1968).

Table 1. Gross and chemical composition of experimental broiler starter diets.

Ingredients	Fermented Cocoa Bean Shell				
	T_1(Control)	T_2 (5%)	T_3 (10%)	T_4 (15%)	T_5 (20%)
Maize	57.00	54.15	51.30	48.45	45.60
FCBS	0.00	2.85	5.70	8.55	11.40
SBM	28.00	28.00	28.00	28.00	28.00
Fish meal	3.00	3.00	3.00	3.00	3.00
BDG	5.00	5.00	5.00	5.00	5.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Methionine	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Determined Analysis (%)					
Dry matter	87.55	87.94	88.60	91.40	92.40
Crude Protein	21.97	21.04	20.94	20.87	20.87
Crude fibre	4.20	4.20	4.71	4.20	4.16
Ether extract	3.93	3.95	3.80	3.95	2.60
Ash	3.40	3.43	4.60	5.16	5.90
NFE	50.23	55.98	54.45	55.98	54.31

FCBS: Fermented Cocoa Bean Shell, GNC: Groundnut cake, NFE: Nitrogen Free Extract.

Table 2. Gross composition of experimental broiler finisher diets.

Ingredient	Fermented Cocoa Bean Shell				
	T_1(Control)	T_2 (5%)	T_3 (10%)	T_4 (15%)	T_5 (20%)
Maize	55.00	52.25	49.50	46.75	44.00
FCBS	0.00	2.75	5.50	8.25	11.00
Blood meal	3.00	3.00	3.00	3.00	3.00
Soya bean meal	19.00	19.00	19.00	19.00	19.00
Fish meal	3.00	3.00	3.00	3.00	3.00
W/offal	5.00	5.00	5.00	5.00	5.00
GNC	10.00	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Determined Analysis					
Dry matter	88.55	87.94	89.60	90.40	90.40
Crude Protein	23.04	22.97	22.94	22.87	22.87
Crude fibre	4.29	4.20	4.71	4.20	4.16
Ether extract	3.93	3.95	3.80	3.95	2.60
Ash	5.40	5.40	6.60	7.16	7.90
NFE	50.23	53.98	51.45	53.98	50.31

FCBS: Fermented Cocoa Bean Shell, BDG: Brewer Dry Grain, NFE: Nitrogen Free Extract.

Statistical analysis

Data obtained was subjected to analysis of variance using the statistical package of SAS (2005). The means among the variables were separated using the Duncan multiple range test of the same statistical package

RESULTS

Dietary effect of bovine rumen filtrate fermented cocoa bean shell on the haematological indices of broiler chickens

Indicated in Table 3 is the effect of dietary bovine rumen filtrate fermented cocoa bean shells on the haematological indices of broiler chickens. Dietary treatment had no significant ($p>0.05$) influence on the haematological indices of broiler chickens observed in this study. However, the experimental birds had their haematological values ranged as follow; packed cell volume (23.67 – 26.00%), haemoglobin (7.50 – 8.27 g/dL), red blood cell (1.92 - 2.42 × 10^6 µL), white blood cell (12.75 – 13.45×10^9 µL), platelet (10.78 – 11.47× 10^4 µL), mean corpuscular volume (103.20 – 133.44 fl), mean corpuscular heamoglobin concentration (31.20 – 32.18%-) and mean corpuscular haemoglobin (32.26- 42.90 pg). Also the white blood differential count such as lymphocytes, heterophils, monocytes, eosinophil and basophils ranged from 52.67 – 59.67%, 34.25 – 41.33%, 2.67 – 4.67%, and 0.00 – 0.33%, respectively.

Table 4 shows the effect of dietary bovine rumen filtrate fermented cocoa bean shells on the serum biochemical indices of broiler chickens. Dietary BRFCBS had no effect on the serum biochemical indices of broiler chicken except the alanine transaminase (ALT), alkaline phosphatase (ALP), and cholesterol. The ALT (25.33 – 37.33 iu/L) values observed in this study increased across the dietary treatment as the inclusion level of BRFCBS increased. The ALP values obtained in this study varied significantly across the dietary treatments in which birds on 0% BRFCBS (361.00 iu/L) recorded the highest values while the lowest value was obtained in birds fed 5% (205.00 iu/L), 10% (200.67 iu/L) and 15 % (231.00 iu/L) BRFCBS having statistically similar values. The cholesterol (146.00 – 192.67 mg/dL) values of the experimental birds observed varied significantly ($p<0.05$) across the dietary treatment in which birds on the highest inclusion level of BRFCBS had the highest cholesterol values (192.67 mg/dL) while the lowest value was obtained in those fed 5% (146.00 mg/dL) BRFCBS.

DISCUSSION

Erythrogram is one of the metrics used to assess an animal's health and nutritional status, and studies show that diet has a major impact on haematological profile (Oloruntola *et al.,* 2018). Tvedten (2010) posits that Hb, MCH, and MCHC are essential blood parameters used to determine the presence and severity of anaemia. A decline in these metrics is thought to indicate that the birds are stressed and not handling stress properly.

The non-significant values recorded for packed cell volume, haemoglobin concentration and red blood cells do not deviate from normal reference values for the experimental birds fed FCBS based diet and indicated that the dietary treatment used in this study did not have negative effects on the normal blood-forming processes in the experimental birds. This result agrees with Adeyeye *et al.* (2017), who reported similar haematological indices values among experimental rabbits fed processed cocoa pod husk meal-inclusive diets.

Another crucial technique for evaluating the health of animals is the measurement of serum biochemical markers (Milner *et al*., 2003). In this investigation, there were notable variations in the levels of alanine aminotransferase (ALT) between the other treatment groups and the control. The ALT concentration in the blood profile was probably elevated by the presence of phytochemicals found in FCBS, which may have contributed to the experimental birds' elevated ALT across dietary regimens. It should be emphasized that the liver is the centre of various digestive, metabolic, and productive activities, and so is vulnerable to varying degrees of chemical and biological damage. Serum levels of various liver enzymes make such damage visible. These enzymes, depending on their quantities, can alter biological activities, resulting in poor health and production performance. Alkaline phosphatase (ALP) is an enzyme produced primarily by the intestinal mucosa, liver, bone, kidney, and placenta; however, intestinal ALP does not contribute significantly to blood ALP levels (Hoffman and Solter, 2008). The ALP values obtained in this study varied significantly across the dietary treatments in which birds on control had the highest value while the lowest values were obtained in birds fed 5, 10, and 15% FCBS respectively. Reduced activity of ALP observed in birds fed dietary FCBS may be an indication of a slowdown of bone growth (Szabo *et al.,* 2005). The highest serum ALP levels seen in birds fed a control diet (0% FCBS) could be attributed to increased osteoblastic activity, which involves bone production and mineralization and is associated with higher skeletal growth (Lumeij, 2008). The ALP values obtained in this study were slightly higher than the referenced values (167 – 305 µL) reported by Oleforuh-okoleh *et al.* (2015) for poultry birds. The activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), in the blood are bioindicators of liver function and damage (Yildirim *et al.,* 2011). Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress (Lumeij *et al.,* 2008). The values of these enzymes in the present study showed significant differences across the dietary

Table 3. Effect of dietary bovine rumen filtrate fermented cocoa bean shell on the haematological indices of broiler chickens.

Parameters	Inclusion levels of BRFFCBS					SEM
	0	5	10	15	20	
Packed Cell Volume (%)	24.75	23.67	26.00	26.00	24.67	0.52
Haemoglobin (g/dL)	7.98	7.50	8.27	8.27	7.70	0.18
RBC (× 10^6µL)	1.92	2.02	2.41	2.39	2.42	0.13
White Blood Cell (×10^9µL)	12.81	13.32	13.45	13.02	12.75	0.21
Platelet (× 10^4 µL)	10.78	12.90	11.03	11.47	11.33	0.24
Lymphocytes (%)	57.75	56.00	59.67	58.00	52.67	1.03
Heterophils (%)	34.25	36.00	36.33	35.33	41.33	1.11
Monocytes (%)	3.25	3.00	4.33	2.67	2.67	0.28
Eosinophil (%)	4.00	4.67	2.67	4.00	3.33	0.39
Basophils (%)	0.25	0.33	0.33	0.00	0.33	0.11
MCV (fl)	133.44	122.68	110.86	111.22	103.20	5.24
MCHC (%)	32.18	31.66	31.83	31.79	31.20	0.20
MCH (pg)	42.90	38.75	35.32	35.35	32.26	1.70

MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin.

Table 4. Dietary effect of bovine rumen filtrate fermented cocoa bean shell on the Serum biochemical indices of broiler chickens.

Parameters	Inclusion levels of BRFFCBS					SEM
	0	5	10	15	20	
Total Protein (g/dL)	4.83	3.50	3.70	4.60	4.30	0.25
Albumin (g/dL)	0.97	0.67	0.70	1.03	0.83	0.07
Globulin (g/dL)	3.87	2.83	3.00	3.57	3.47	0.18
Alb/Glob	0.25	0.23	0.23	0.29	0.24	0.01
AST (i.u/L)	224.00	188.67	194.33	215.00	227.67	7.02
ALT (i.u/L)	30.33^{ab}	25.33^{b}	29.33^{b}	37.33^{a}	35.00^{a}	1.55
ALP (i.u/L)	361.00^{a}	205.00^{b}	200.67^{b}	231.00^{b}	319.67^{ab}	22.26
Creatinine (mg/dL)	0.67	0.50	0.50	0.60	0.57	0.03
Glucose (mg/dL)	363.67	250.67	273.33	341.33	360.00	22.24
Cholesterol (mg/dL)	166.67^{ab}	146.00^{b}	165.67^{ab}	184.00^{ab}	192.67^{a}	6.42
HDL (mg/dL)	149.67	154.67	136.67	158.33	168.33	10.42
LDL (mg/dL)	75.67	75.00	106.33	137.67	146.00	12.07
Na (Meg/L)	145.33	136.33	139.67	147.33	137.33	2.27
K (Meg/L)	2.90	2.80	3.03	3.10	2.53	0.13

^{abc} Means along the same row with different superscripts are significantly different ($p<0.05$), AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline Phosphatase, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein.

treatments. The increased glucose levels in the control diets may have resulted from naturally stress-induced activation of cortisol release, which in turn stimulated gluconeogenesis (Marai *et al*., 2007). The range of blood glucose measured in this study is greater than that of exotic rabbits (Jimoh and Ewuola, 2019) and heat-stressed exotic rabbits (Jimoh *et al.,* 2017), with the former having values between 60 and 75.65. This raises the possibility that heat stress may stimulate gluconeogenesis.

As the FCBS increased, the cholesterol levels found in this study increased in all treatment groups. The results of this finding are in tandem with the investigation reported by Olumide *et al.* (2017), who recorded that saponins, an anti-nutritional factor, have a physiological effect on reducing the content of plasma cholesterol in experimental mice. This discrepancy can be the result of the fermentation process used on the CBS in this particular investigation. The cholesterol concentrations (146.00 – 192.67 mg/dL) found in this investigation were more than the 135.33 ± 11.22 – 149.00 ± 3.79 mg/dL for hens recorded by Akinola and Abiola (1999).

Conclusion

This study reveals that hematological indices of Ross 308

broiler chickens genotype fed dietary FCBS were not distorted as the values obtained are within the normal range. Dietary FCBS influenced the serum biochemical indices of broiler chicken as it improved the High Density Lipoprotein (HDL) which is a good cholesterol observed across the treatment. This is an indication that the broiler chickens utilized in the present study was safe for consumption and therefore, poultry farmers can take advantage of availability of cocoa bean shells and include in the diets of the animals at 5% where high HDL and lowest Low Density Lipoprotein (LDL) were recorded to produce healthy carcasses for the teeming population.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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