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Original Research Article

Haematological and serum biochemical indices of broiler chicks fed cooked and fermented shea butter *Vitelleria paradoxa* cake meal

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ABSTRACT

A 28-day feeding trial was conducted to evaluate the haematological and biochemical response of broiler chicks fed Cooked and Fermented Shea Butter Cake Meal (CFSBCM). Shea butter cakes were processed by adding the cakes into water already boiled to 100^0C and were boiled for thirty (30) minutes after which they were later fermented for different periods of 3, 6 and 9 days. Thereafter, processed seeds were air-dried and milled to obtain fine particles of the test ingredient. Using a Completely Randomized Design (CRD), ninety-six (96) unsexed Ross 308 strains of broiler chicks were allocated to four (4) dietary treatments containing twenty-four (24) birds in each treatment and each treatment replicated four times with six (6) birds per replicate. The control diet (T_1) had only maize meal without CFSBCM while the experimental diets contained 20% of CFSBCM fermented for 3, 6 and 9 days and were labelled T_2, T_3 and T_4 respectively. The birds were fed all the experimental diets for four (4) weeks and on completion of the experiment on the 28th day, blood samples were collected and taken to laboratory for the analysis of haematological and serum biochemical indices. The results showed that of all the haematological parameters measured, significant difference ($P<0.05$) only exist in the values of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) while values of other parameters showed no significant difference ($P>0.05$). Values obtained from the serum biochemistry analysis of experimental birds showed that significant difference ($p<0.05$) exist in the values of the serum glucose, total protein, uric acid, creatinine and triglycerides while no significant differences ($p>0.05$) were recorded on serum albumin, alanine transaminase (ALT) and aspartate transaminase (AST). The results showed that the longer the fermentation days, the better the haematological and serum biochemical indices of broiler chicks.

Keywords: Anti-Coagulant, haematology, fermentation, serum biochemistry, Shea butter cake.

INTRODUCTION

Feed accounts for 70-80% of the total cost of broiler production in Nigeria (Ademola & Farinu, 2006*).* Hence, high cost of feeding poultry has necessitated the need to look for alternative energy feed source for poultry in order to reduce cost and limit dependence on maize (Oladunjoye *et al*., 2014; Adamu *et al*., 2015.).In view of this high cost of grain (maize) in poultry production, the use of Agro-industrial by-products that are not consumed by man and are available in cheap cost as substitute for maize in poultry diet is worthy of consideration. Therefore, there is an urgent need for an alternative in livestock feeds, to reduce the current pressure on maize as staple food for man, (Zulkifili *et al*., 2000; Alu *et al*., 2015). One of such alternatives for replacement of maize is the processed Shea butter cake which is an agro-forestry by-product obtained from the processing of nuts of the Shea butter tree *(Vitellaria paradoxa)* for fat with no economic value and environmental issue (Dei, *et al.*, 2008*;* Zanu, *et al*, 2012). Abdul-Mumeen, *et al., (*2013*)* investigated Shea butter cake for proximate quality, and reported its overall nutritional value to be high, containing 13.03, 23.38, 4.25, 8.71, 59.37% and 4485.86kcal ME/kg of crude protein, crude fat, ash, crude fiber, carbohydrates and metabolizable energy respectively as well as rich in minerals like calcium, potassium and magnesium. Based on its composition, Shea butter cake has been sampled as potential feed stuff as replacement for dietary maize in poultry ration (Dei *et al*., 2007 and 2008; Zanu *et al*., 2012, Orogun *et al*., 2015; Matthew *et al*., 2017). However, the major nutritional setback of Shea butter cake utilization for chicken is poor digestibility possibly due to the presence of anti-nutritional factors (ANF's) like saponins and most

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importantly tannins (Annongu *et al.*, 2006) and theobromine (Oddoye *et al.,* 2012; Abdul-Mumeen *et al.*, 2013). Nutritionists and researchers have used fermentation as a method of reducing the level of the ANF's in Shea butter cake in order to improve its utilization as alternative energy source in poultry diet. The process does not require the use of chemicals and can be easily managed in a local condition or on an industrial scale (Yamamoto, *et al.,* 2007). The characteristics of the fermented products include their acceptability by birds and nutrient availability (Hong, *et al.*, 2004). However, fermentation process can produce organic acids that break down saponins and tannins or create condition for the growth of native microbes that detoxify these components (Reddy and Pierson, 1994). Fermentative microbes have been used extensively in the improvement of agricultural by-products through its action on substrates such as non-starch polysaccharides and proteins (Ong *et al*., 2007; Aderemi & Nworgu, 2007) or structurally modifying anti-nutritive factors (Hong *et al*., 2004). The need to scientifically investigate the implication of cooked-and-fermented Shea butter cake meal (CFSBCM) on blood parameters of broiler chickens is necessary because the physiological and pathological state of the animal can be determined through observation of blood and its components (Sola-Ojo *et al*., 2016). Thus, this study was undertaken to determine the haematological and serum biochemical indices of broiler chicks fed CFSBCM.

MATERIALS AND METHODS

STUDY SITE

The study was conducted in the Poultry and Research Unit of Federal College of Wildlife Management, New Bussa, Niger State, Nigeria.

SOURCE AND PROCESSING OF TEST INGREDIENT

The Shea butter cakes for this study were obtained from the local Shea butter processing factories in Koro village, Borgu Local Government Area of Niger State, Nigeria. The Shea butter cakes were cooked in water already boiled to100^{0}C for thirty (30) minutes and the cooking temperature was monitored using thermometer. The boiled cakes were thereafter divided into three batches and fermented differently in air-tight containers for 3, 6, and 9 days respectively. These cakes were properly air-dried for another 5 days after fermentation to prevent the growth of moulds and aflatoxin production. Samples of the dried processed Shea butter cakes were taken to the laboratory after cooking and after fermentation for proximate analysis as shown in Table II before milling into smaller particles and incorporation into the experimental diets.

HOUSING OF BIRDS AND MANAGEMENT

On arrival of the chicks, they were housed in a well-lit and heated brooding pen and anti-stress (Vitalyte®) soluble powder was administered in their drinking water to ease the transit stress. The chicks were brooded together for the first week of life and were fed with a brand of commercial feed (Top feed®) and clean water *ad-libitum* in order to boost their immunity before the introduction of the experimental diets. They were managed in an open-sided constructed poultry facility and raised in a deep litter system, using wood shavings as litter material and 200Watt bulb lowered to their level as the source of heat. Other prophylactic measures as recommended by Oluyemi & Robert (2000) were strictly adhered to.

EXPERIMENTAL BIRDS AND DESIGN

A total of ninety-six (96) unsexed Ross 308 strains of day-old broiler chicks were used for this study. The birds were allocated to four experimental treatments designated as T_1, T_2, T_3 and T_4 each containing twenty-four (24) birds, (i.e six (6) birds in four (4) replicates) in a Complete Randomized Design (CRD)

EXPERIMENTAL DIETARY TREATMENT

Four (4) iso-nitrogenous diets were formulated to provide 23% Crude Protein (CP) requirement of broiler chicks. Diet 1 (T_1) was made to contain Maize-Soybean meal based diets as the control treatment. The CFSBCM were then used to replace 20% corn in T_1 to give diets T_2, T_3 and T_4 respectively as shown in Table 1.

BLOOD SAMPLING

On the termination of the feeding trial on day 28, thirty-two (32) chicks were randomly selected (eight chicks per treatment using two chicks per replicate) and blood samples were collected from each through the wing vein. Thereafter, 2.5ml of the blood were poured into differently labeled sterile bottles containing Ethylene Di-amine Tetra Acetic acid (EDTA) for determination of hematological parameters and another 2.5ml into differently labeled plain sterile bottles without anti-coagulant for serological indices respectively. The hematological indices were determined with the use of Winrobe hematocrit®, improved Neubauer haemocytometer® as described by Dacie & Lewis (1991), Mean corpuscular volume (MCV), Mean corpuscular heamoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined according to the method of Jain (1986). Blood glucose was determined using Hexokinase method; total protein and albumin were determined by Biuret and Bromocresol Green method, respectively (Kohn & Allen, 1995). The creatinine and other liver enzymes were determined using the standard enzymatic method described by Bush (1991).

Table I. Ingredient Composition of Experimental Diet

Ingredients/g	(Control) T_1	(C3-d FSBCM) T_1	(C6-d FSBCM) T_1	(C9-d FSBCM) T_1
Maize	55.00	44	44	44
Soya beans meal	33.75	33.75	33.75	33.75
Shea butter Cake	0.00	11.00	11.00	11.00
Fish meal	3.00	3.00	3.00	3.00
Soya Oil	2.00	2.00	2.00	2.00
DCP	1.50	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50
Limestone	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Vitamin Premix	0.50	0.50	0.50	0.50
Methionine	0.25	0.25	0.25	0.25
	100.00	100.00	100.00	100.00
ME Kcal/Kg	3232.28	3257.32	3257.32	3257.32
CP (g/Kg)	23.81	24.61	24.61	24.61
Calcium (g/Kg)	1.66	1.65	1.65	1.65
Phosphorous	7.17	6.86	6.86	6.86

PROXIMATE ANALYSIS

The proximate composition of the raw and CFSBCM were analyzed according to the procedures of AOAC (2006) and results are provided in Table II The moisture content in the five preparation ranged from 5.67 in *Raw Shea Butter Cake Meal to 8.50% in BSBCM= Boiled Shea Butter Cake Meal.*

STATISTICAL ANALYSIS

All blood data determined were analyzed using analyses of variance (ANOVA) using the Completely Randomized Design (CRD) according to GLM model of SAS statistical package (SAS, 2012) and the significant differences were /established using Turkey test at P>0.05

Table II. Proximate analysis of the raw and processed Shea Butter Cake Meal (SBCM)

Components	RSB CM	BSBCM	C3-d FSBCM	C6-d FSBCM	C9-d FSBCM
Moisture content %	5.67	8.50	8.32	8.19	8.01
Ash %	20.61	29.94	30.02	30.23	30.48
Crude fiber %	4.18	3.89	3.96	3.99	4.12
Crude protein %	15.85	13.71	13.89	14.46	15.04
Crude fat %	10.67	9.66	9.78	9.97	10.08
NFE %	45.41	32.61	31.56	31.23	30.96
Tannin (g/kg)	0.22	0.17	0.11	0.08	0.04

RESULTS

HAEMATOLOGICAL INDICES

Table III shows the results of replacing maize with 20% inclusion level of CFSBCM of broiler chicks on haematological parameters.

The PCV values ranged between 22.27% in T_4 to 25.51% in T_1. PCV for T_2 and T_3 were 23.40 and 22.70% respectively with no significant (P>0.05) difference between the PCV values across the treatment groups.

There was no significant (P>0.05) difference in the values of Hb concentration as well. Haemoglobin concentration value was highest (5.1 g/dl) in T_1 and minimum (4.09 g/dl) in $T_{4.}$ The Hb concentrations of T_2 and T_3 were 4.47 and 4.23 g/dl respectively.

The WBC values in T_1 was 9.65 ($\times10^3$/mm3) and a progressive reduction was observed from T_2 to T_4, although these changes were not significant (P>0.05). There was no significant changes in the RBC values across the four treatment groups despite their range in values between 1.12 in T_1 to 0.92 ($\times10^3 mm^3$) in T_4

The MCV in T_1 (139.06 μ^3) though higher but was not significantly (P>0.05) different from MCV (125.77 μ^3) for T_2 while these two were significantly (P>0.05) higher than 121.06 and 122.28 μ^3 obtained for T_3 and T_4 respectively.

The MCHC values of T_1, T_2 and T_4 were the same (33.34 %) while that of T3 was 33.00%. There was no significant difference in the MCHC values across the treatment groups.

Table IV revealed the serum biochemical indices of the birds fed the experimental diets containing different periods of fermentation on cooked Shea Butter Cake Meal.

SERUM BIOCHEMICAL INDICES

The values of AST, ALP, Creatinine, Albumin increased progressively from the initial values at T_1, to T_2, T_3 and T_4 (Table IV), however, these increments were not significant (P>0.05)

The glucose vales was 85.145 for $T_{1,}$ which reduced significantly (P<0.05) to 75.019 g/dl in T_2 and increased again to 77.303 and 81.19 g/dl in T_3 and T_4 respectively. Uric acid values also significantly changed across the treatment groups with maximum (1.18 Mmol/l) and minimum (0.67 Mmol/l) values obtained at T_1 and T_2 groups respectively. As per Triglycerides, the changes were also significant with T_1 and T_2 having the minimum (141.41 mg/dl) and maximum (148.77 mg/dl) values respectively. Triglycerides value for T_3 was 145.55 and T4 was 143.19 mg/dl.

Table III. Haematological indices of birds fed experimental diets containing cooked and differently fermented Shea butter cake meal

Parameters	T_1	T_2	T_3	T_4	SEM
PCV (%)	25.51	23.40	22.70	22.27	2.08
Hb (g/dl)	5.17	4.47	4.23	4.09	1.20
WBC ($x10^3/mm^3$)	9.65	9.75	9.40	9.20	0.79
RBC ($x10^3 mm^3$)	1.12	1.07	1.05	1.01	0.92
MCV (μ^3)	139.06^a	125.77^{ab}	121.06^b	122.28^b	2.11
MCH (*fl*)	46.38^a	41.93^{ab}	40.36^b	40.765^b	1.45
MCHC (%)	33.34	33.34	33.00	33.34	0.24

ab: same row with different superscript are significantly different at P<0.05.
T_1: Corn-soybean based control diet,
T_2: 3-days fermentation period of cooked Shea butter cake
T_3: 6-days fermentation period of cooked Shea butter cake
T_4: 9-days fermentation period of cooked Shea butter cake
Hb: Haemoglobin Concentration, MCH: Mean Corpuscular MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean Corpuscular Volume, RBC:Red Blood Cell, PCV: Packed Cell Volume, WBC: White Blood Cell, SEM: Standard error of mean

Table IV. Serum biochemical indices of birds fed experimental diets containing different periods of fermentation on cooked Shea Butter Cake Meal

Parameters	T_1	T_2	T_3	T_4	SEM
Glucose (g/dl)	85.145^a	75.019^c	77.303^{bc}	81.19^{ab}	2.01
Albumin (g/l)	13.45	14.98	14.43	14.04	1.14
Total protein (g/dl)	4.27^a	3.961^b	4.09^{ab}	4.18^a	0.85
Uric acid (Mmol/l)	0.67^c	1.18^a	0.96^{ab}	0.79^{bc}	0.12
Creatinine (Mmol/l)	48.12	52.25	49.50	48.87	1.21
Triglycerides (mg/dl)	141.41^c	148.77^a	145.55^{ab}	143.19^{bc}	1.77
AST (iµ/l)	23.10	24.44	24.01	23.90	0.81
ALP (iµ/l)	28.10	30.15	30.07	29.35	2.10

abc: same row with different superscript are significantly different at P<0.05.
T1: Corn-soybean based control diet,
T2: 3-days fermentation period of cooked shea butter cake
T3: 6-days fermentation period of cooked shea butter cake
T4: 9-days fermentation period of cooked shea butter cake
SEM: Standard Error Mean

DISCUSSION

The results in Table II above revealed that fermentation enhanced the nutrient profile of SBCM especially with respect to crude protein and crude fiber compared to raw SBCM. The crude protein content of the SBCM increased gradually as the duration of fermentation increased from 13.89% to 15.04% as compared to that of the CSBCM (13.71%). The reduction in the protein content of the CSBCM (13.71%) as compared to the raw SBCM (15.85%) might be connected with the effect of heat on protein as it has been established that heat denatures protein. The level of the crude fibre also increased gradually as the fermentation period increased from 3.16% to 4.12%. This report is in agreement with the report of Mutayoba *et al.*, (2011), that fermentation aids in improving nutrient composition of feedstuffs. Tannin which is an anti-nutritive factor found in a number of feedstuffs and it is capable of hindering availability of the nutrients in such feedstuff to the animals. The results of the present study showed that the concentration of tannin reduced from 0.11 to 0.04 as the period of fermentation increased, indicating that the concentration of tannin can be reduced by longer fermentation period. Cooking of RSBCM also help in reducing the level of tannin (from 0.22 to 0.17) as shown in Table II. This is in line with the findings of Reddy and Pierson (1994) who reported that fermentation process produces organic acids that break down tannins. The observed crude protein (12.85%) was higher than those reported by Ugese *et al*. (2010) and Orogun *et al*. (2015) who observed 9.2% and 12.70% respectively and lower than value reported by Abdul-Muumeen *et al*. (2013), Atuahene (1998) and Zanu *et al.* (2012) who reported 13.03%, 16.24% and 17.31% respectively. The crude fiber, nitrogen free extract and ash content obtained in the present study were lower compared to the values 16.57, 59.37 and 18.83 observed by Abdul-mumeen, *et al*. (2013) respectively. The variation in the nutrient composition could be attributed to differences in location, varieties and efficiency of oil extraction of the cake.

The results shown in Table III revealed that there is no significant difference (P>0.05) in all haematological parameters measured except for mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH).. All the haematological values obtained in this study were within the normal range and correlated with those reported by previous researchers who observed the normal haematological values for birds (Maxwell *et al*., 1990; Nse Abasi *et al*., 2014).

The results on Table IV showed that feeding of CSBCM fermented for 3 and 6 days significantly decreased total protein values (compared to the control diet) as evident in T_2 and T_3. Only the T_3 fed CSBCM fermented for 9 days is statistically similar to the control (T_1). This may imply that fermentation of CSBCM for a longer period than those considered in this study. It is possible that fermentation of CSBCM at longer period of time improved the protein quality of the diet and might have enhanced normal protein synthesis. Changes in the nutritional status of an animal are easily detected in the albumin because they are about two-thirds of total protein (Mitruka & Rawnsey, 1977). Broiler chicks fed CFSBCM did not have a reduced serum albumin which is usually said to be as a result of protein malnutrition owing to decreased synthesis. The pattern of the result on serum glucose is similar to that of total serum protein. This observation probably means that enhanced energy utilization of SBCM-containing diet will require longer period (above 9 days) of fermentation. The values of serum triglycerides have an inverse relationship with the number of fermentation days (it significantly decreased (P<0.05) as the fermentation days increased. The results suggested that increase in period of fermentation caused a reduction in the triglyceride biosynthesis and favoured the re-distribution of cholesterol among the lipoprotein molecules (Sola-Ojo *et al*., 2016, Zhai *et al*., 2019). Serum uric acid had its highest (P>0.05) value recorded in birds fed T_2 diet and the blood uric acid level of chicks decreased with increasing fermentation period of CFSBCM which indicates a higher utilization of protein according to Cetin (2002), because uric acid is reported to be a product of protein, non-protein nitrogen and purines (Champe, 2008). A significant variation (P<0.05) was observed in the creatinine level between the control and CFSBCM based diets; birds fed at T_2 diet showed the higher value while those on control diet had the lowest value but did not differ (p>0.05) with those on T_3 and T_4 groups. Serum creatinine is a measure of muscle mass which shows the level of degradation of tissue creatinine phosphate and is also regarded to be a measure of amino acid economy in vivo (Balogun & Otchere, 1995). Alanine transaminase (ALT) and Aspartate transaminase (AST) were observed to be decreasing (p>0.05) as the period of fermentation of Shea butter cake in the diet increased, indicating no toxic effect within the liver parenchyma of the experimental birds. Moreover, in the present study, all the blood parameters fell within the normal range as reported by Mitruka and Rawnsley (1977), an indication that the health of the birds were not compromised.

CONCLUSION AND RECOMMENDATION

The results showed that cooking and fermentation could improve the nutrient value and decrease the tannin level in Shea butter cake, thus making it a potentially valuable energy feedstuff. Incorporation of cooked Shea butter cake at longer period (9 days) of fermentation in the diet of broiler chickens showed more promising influence on their blood composition without any adverse effect and is thereby recommended for poultry farmers. Further investigation should pay attention to the mechanism by which period of fermentation affects the blood profile of poultry birds and the effects of increasing the number of days of fermentation of SBCM on nutrient availability in SBCM.

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