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EFFECT OF FERMENTATION PERIOD OF HEAT-TREATED SHEA BUTTER CAKE IN DIETS OF BROILER CHICKENS ON BLOOD COMPOSITIONS

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ABSTRACT

Shea butter cake is an agro-forestry by-product from shea butter production with high nutritive value as potential feed ingredient but its utilization is limited due to presence of anti-nutritional factors. A 28-d feeding trial was conducted to evaluate the effect of different fermentation period on heat-treated shea butter cake meal (SBCM) in diet of broiler chickens on haematological and serum biochemical parameters. A total of 208 *Arbor Acre*s broiler chicks were randomly allocated to 4 dietary treatments with 4 replicates of 13 birds each in a completely randomized design. The control diet (T_1) is maize-soybean based without fermented SBCM, while T_2, T_3 and T_4 contained 2, 3 and 4 days fermented SBCM, respectively, replacing 20% of maize in T_1 diet. Experimental diet and water were provided *ad-libitum.* At day 28, 3 birds per treatment were selected for haematological and serum biochemical evaluation. The results showed that the haematological parameters indicated no significant difference (P>0.05) among treatment groups for PCV, Hb, RBC, WBC and MCHC. However, MCV concentrations was higher (p<0.05) in birds fed control (139.06u^3 and T_4 (125.77 u^3) diets than in birds fed T_2 (121.06 u^3) and T_3 (122.06 u^3) diets, while birds fed control diet and T4 diet recorded higher (p<0.05) concentration of MCH of 46.38fi and 44.93fi respectively, than birds fed T_2 (40.36fi) and T_3 (40.77fi) diets. Serum glucose and total protein were significantly higher (p<0.05) in birds fed control (185.15 and 4.38 g/dL, respectively) and T_4 (183.19 and 4.28 g/dL, respectively) than in other treatment groups. Serum uric acid of the birds decreased significantly (p<0.05) in control (3.02 mg/dL) and T_4 (3.22mg/ dL) diet group but higher (p<0.05) in those fed T_2 (4.85mg/dL) and T3 (3.22mg/ dL) diet. However, these values were within the normal range for broiler chickens. It can be concluded that longer fermentation period of heat-treated SBCM is adequate in the diets of broiler chickens without any adverse effect on their blood composition.

Keywords: Shea butter cake, fermentation, haematology, serum biochemistry, broiler

Introduction

The rapid growth of human population has intensified the competition between humans and livestock for grains such as maize which is the major source of energy in poultry feeds. Grains such as maize constitute more than one-third of the poultry feed and due to its high price is becoming more expensive to use at high level of inclusion in poultry nutrition (Ojebiyi *et al.*, 2006; Colette *et al.*, 2013; Muhammad *et al.*, 2015). Hence, high cost of feeding poultry has necessitated the need to search for alternative energy source in order to reduce limit dependence on maize and feed cost (Farrell, 2005; Alsaffar *et al.*, 2013; Al-Harthi and Attia, 2015; Al-Harthi *et al.*, 2018). The ever increasing cost of poultry feed with an attendant increase in the cost of poultry products such as meat and eggs shows that there is need to adopt the principle of waste – to – wealth in having an environmental-friendly poultry production. In view of this high cost of grain (maize) in poultry production, the use of agro forestry by-products that are not consumed by man, create environmental waste, and are available at cheap cost can be used as substitute for maize in poultry diet.

One of such alternatives for replacement is the 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. The V*itellaria paradoxa* is a widely distributed plant in arid and semi-arid areas of Nigeria and tolerates harsh agronomic and environmental conditions, with a high resistance to diseases and pests (Annongu *et al.*, 2006; Ugese *et al*., 2010; Garba *et al*., 2011). The shea butter cake is of no economic value and constitute environmental nuisance as waste but has been reported to possess adequate nutritional characteristics of higher protein and energy value compare to maize (Dei, *et al.*, 2007*;* Abdulmumeen, *et al.*, 2013; Agbo and Prah, 2014). Based on its composition, shea butter cake has been sampled as potential feed ingredient as replacement for dietary maize in poultry ration (Dei *et al*., 2008; Zanu *et al*., 2012, Orogun *et al*., 2015; Aguihe *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 especially tannin (Oddoye, *et al*., 2012; Abdulmumeen *et al.*, 2013). Thus, the use of wet heat, however, has been reported to inactivate and reduce the level of anti-nutritional factors contained in it as well as improve the nutritive value of shea butter cake (Oddoye *et al*., 2012).

Furthermore, fermentation is a unique process with great potential for recycling some agro-industrial by-products into useful animal feeds in developing countries; thus, it does not require the use of chemicals and is easy to manage in a farmstead environment (Yamamoto *et al.*, 2007). The characteristics of the fermented products include their acceptability by animals and nutrient availability (Hong, *et al.,* 2004). Fermentative microbes have been used extensively in the improvement of agroforestry by-products through its action on substrates such as non-starch polysaccharides and proteins or structurally modifying anti-nutritive factors (Hong *et al*., 2004; Ong *et al*., 2007; Aderemi and Nworgu, 2007). Moreover, blood compositions are primary variables of physiological, pathological and nutritional state of an animal; and variations in the constituent compounds of blood when compared to reference values could be used to explain the metabolic stage of an animal as well as quality of feed (Muhammad *et al*., 2015). Changes in blood compositions have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on animals (Colette *et al*., 2013; NseAbasi *et al*., 2014) Therefore, this study was carried out to evaluate the effects of fermentation of heat-treated shea butter cake meal in the diets of broiler chickens on haematological and serum biochemical indices.

Materials and Methods

Study Site: The study was conducted in the Poultry Research Unit of Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located at a longitude 4^{o} 31^{o}E and 4^{o} 33^{o}E and latitude 7.3^{o}N and 10.00^{o}N in the Savanna Areas of Niger Basin (Abu, 2003).

Source and Processing of test ingredient: The shea butter cake used for this study was obtained fresh from the local shea butter processing factories in Kainji, Borgu Local Government Area of Niger State, Nigeria. The fresh shea butter cake was heated in boiling water for 30 minutes, thereafter divided into three batches and fermented differently at 2, 4 and 6 days and were all properly air-dried for 5 days. Thereafter, the processed shea butter cake was milled using a hammer mill to obtain fermented heat-treated shea butter cake meal (FHSBCM) before incorporation as an ingredient in the formulation of experimental diets.

Housing of birds and management: The birds were reared on a deep litter system, using wood shavings as litter material in a standard open-sided poultry facility. Birds were fed daily and given access to clean water at *ad libitum* throughout the experimental period of 28 days. Medication, vaccination and other prophylactic measures were appropriately observed.

Experimental birds and design: A total of 208 *Arbor Acre*s broiler chicks aged 7 days were used in this study. The birds were randomly allotted to four treatment groups with four replications of 13 birds per replicate pen in a completely randomized design (CRD).

Experimental dietary treatment: The five treatment diets were formulated to be isonitrogenous and isocaloric, where T_1 contained corn-soybean meal basal without FHSBCM (control); whereas T_2, T_3 and T_4 consisted 2, 3 and 4 days FHSBCM, respectively (Table 1).

Table 1: Gross composition of experimental diets

Ingredients	Control Diet	2d-FSBC Diet	4d-FSBC Diet	6d-FSBC Diet
Maize	51.00	40.80	40.80	40.80
Soya beans meal	35.75	35.75	35.75	35.75
Shea butter cake	0.00	10.20	10.20	10.20
Fish meal	4.00	4.00	4.00	4.00
Soya oil	3.00	3.00	3.00	3.00
DCP	1.50	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50
Lime stone	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
DL-Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Threonine	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Nutrients Composition				
ME kcal/kg	3000.20	3020.66	3020.66	3020.66
CP (g/kg)	22.08	22.82	22.82	22.82
Calcium (g/kg)	1.54	1.53	1.53	1.53
Phosphorus	6.65	6.36	6.36	6.36

* = Bio Mix Broiler Chicken Premix supplying the following per Kg of feed: Vitamin A = 3,400,000IU, Vitamin D3 = 600,000IU, Vitamin E = 4,000mg,Vitamin K3 = 600mg, Vitamin B1 =6 40mg, Vitamin B2 = 1600mg, Niacin = 8,000mg, Pantothenic = 2000mg, Vitamin B6 = 600mg, Vitamin B12 = 4mg, Folic acid = 200mg, Biotin H2 = 300mg, Choline, Chioride = 70,000mg, Cobalt = 80mg, Copper = 1200mg, Iodine = 400mg, Iron = 8,000mg, Manganese = 16,000mg, selenium = 80mg, Zinc = 12,000mg and Antioxidant=500mg.

Blood Collection and response criteria: At the end of the experiment, 3 per replicate making a total of 12 birds from each treatment were selected for evaluation of blood composition. The birds were starved for 24 hours before slaughtering by severing the jugular vein and blood samples were collected into sample bottles containing Ethylene Di-amine Tetra Acetic Acid (EDTA) for haematological determinations. The sample bottles were gently shaken to mix up the blood with EDTA to prevent clotting. Packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) were quantitatively determined using improved Neubar's haemacytometer after dilution, while haemoglobin level was analyzed using cyanomethaemoglobin method as described by Dacie and Lewis (1991). The standard ratios of the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the procedure of Stockham and Scott (2002) as shown below:

MCV (fl) = PCV x 10/RBCX10…….1
MCH (pg) = Hb x 10/RBC (10^6)..….2
MCHC (%) = Hb x 100/PCV……….3

Blood for clinical chemistry determinations was collected into clean test tubes without anticoagulant. Serum was obtained by allowing the blood sample to clot at room temperature for 30 minutes, after which it was centrifuged for ten minutes at 3,000 revolutions per minute using a table micro-centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle. Serum samples were analyzed for glucose, total protein, uric acid and triglyceride. The serum biochemical indices were done using the clinical routine procedures outlined by Olorede *et al*. (1996).

Proximate Analysis: The proximate composition of the raw and FHSBCM was analyzed according to (AOAC, 2006).

Statistical Analysis: All data collected were subjected to Analysis of Variance (ANOVA) by GLM model of completely randomized design of the SAS package (SAS, 2012) and where treatment effect differed significantly, were compared using the least significant difference (LSD). All statements of significance were based on 5% level.

Results and Discussion

Proximate and tannin composition

The proximate and tannin composition of raw and cooked SBCM is presented in Table 2. The result revealed that raw shea butter cake meal contains 5.67% moisture content (MC), 20.61% ash, 4.18% crude fiber (CF), 12.85% crude protein (CP), 10.67% crude fat (CF), 45.41% nitrogen free extract (NFE), and 0.22(g/kg) tannin. The above proximate composition is slightly at variance with the reports of previous authors (Ugese *et al*., 2010; Zanu *et al*., 2012; Abdulmumeen *et al*., 2013; Orogun *et al*., 2015). The variation in the nutrient composition could be attributed to differences in various factors ranging from location, varieties, efficiency of oil extraction of the cake and soil type on which the plant is grown (Ojewola *et al*., 2005). However, the processed shea butter cake meal contain 8.50% moisture content (MC), 29.94% ash, 3.89% crude fiber (CF), 15.71% crude protein (CP), 5.36% crude fat (CF), 32.61% nitrogen free extract (NFE), and 0.04(g/kg) tannin. This shows that cooking and fermentation enhanced the nutrient profile of SBCM, especially with respect to increased crude protein as well as decreased crude fiber and tannin content of the processed SBCM compared to raw SBCM. This is in accordance with the reports of Mutayoba *et al.,* (2011), that fermentation aids in improving nutrient composition of feed stuffs. The increase in crude protein in this present study could be as a result of bioconversion ability of microorganisms on some of the soluble carbohydrates into single cell protein and could also be due to the release of polysaccharide bound protein which makes the cake nutritional better

(Iyayi and Aderolu, 2004; Agbo and Prah, 2014). The low crude fiber (CF) content of the processed SBCM may be an indication of degradation of polymeric lignocelluloses of the SBCM (Alemawor *et al*., 2009). This is similar to earlier findings that reported a general reduction in CF content of wheat bran, wheat straw, palm kernel cake and saw dust (Kutlu *et al*., 2000; Akinyele *et al*., 2011). Moreover, microorganisms are able to synthesize enzymes such as ß-glucanase that digests cellulose and hemicellulose which constitutes the components of CF of fibrous feeds. The nitrogenous component and non-protein nitrogen hitherto locked up within the cell became available in the FSBCM after cellolytic action by microbes. This may explain the increase in the CP content and a decrease in CF of FCSM, thus, indicating that the longer the fermentation period, the higher the CP and lower the CF due to more microbes being produced. In addition, the microbes involved in fermentation are proteinous in nature, therefore, contributed (as single cell proteins) to the overall protein concentration of FCSM (Kanyinji and Sichangwa, 2014).

The tannin content of the SBCM after processing showed a reduction from 0.22g/kg to 0.04g/kg depicting a reduction of 81.82%. Dei *et al*. (2008) also reported lower tannin content in fermented shea nut meal than in unfermented meal. The reduction of tannin obtained in this study is higher than the value reported by Agbo and Prah (2014) that recorded a significant 72.98% reduction in tannin between the unfermented and fermented shea nut meal. This variation could be as a result of cooking of the cake which was done prior to fermentation and reports from earlier studies has proven that wet heat treatment decreases anti-nutrients, especially heat labile anti-nutritional factors such as tannin and saponins (Udedibie and Carlini, 1998; Akinmutimi, 2004; Akinmutimi and Okwu, 2006). Early studies that has shown that fermentation process can create conditions for the growth of microbes (*Bacillus, Corynebacterium, Klebsiella, Aspergillus, Penicillium, Fusarium, and Candida*) which possess the ability produce organic acids that break down tannin (Reddy and Pierson, 1994).

Table 2: Proximate analysis of the raw and fermented-cooked shea butter cake meal

Component	Raw SBCM	Processed SBCM
Moisture content %	5.67	8.50
Ash %	20.61	29.94
Crude fiber %	4.18	3.89
Crude protein %	12.85	15.71
Crude fat %	10.67	5.36
NFE %	45.41	32.61
Tannin (g/kg)	0.22	0.04

Response of broiler chickens to experimental diets on hematological indices

Table 3 shows the results of haematological response of broiler chickens on experimental diets. The result revealed that the birds did not differ significantly ($p>0.05$) in their PCV, WBC, RBC and MCHC values. The similar values obtained in this study are indication of the quality of the test diets because haematological parameters are reflection of the animal responsiveness to both external and internal factors which include feed and its good health. Also, this implies that, irrespective of the processing effect, the diets were nutritionally adequate in providing a sound plane of nutrition with high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al*., 2011; Iwuji and Herbert, 2012; Isaac *et al*., 2013; Soetan *et al*., 2013). The impact of the processing techniques of cooking and fermentation on residue anti-nutritional factors is reflected on the values of the blood haematology of broilers fed with the processed based diets, as they were relatively the same as that of broilers fed control diet. This is in accordance with the report of Oloyede *et al*. (2004) who observed no significant (p>0.05) difference in the PCV, Hb, RBC and WBC of broilers fed cooked and fermented melon seed meal. A similar trend has been reported for rats and rabbits fed on some raw and processed tropical legumes, pueraria seeds and thevetica cake based diets (Apata, 1990; Awosanya *et al*., 1990; Atteh *et al*., 1995). There were significant differences (P<0.05) among treatment groups with respect to mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The result of MCH, which is the average amount of Hb in each RBC measured, followed the same pattern as MCV. Birds fed control 6-d FHSBCM diet had similar MCV and MCH concentrations with those on control diet, and both were significantly (p<0.05) higher from those fed 2-d and 4-d FHSBCM diets. The significant differences noticed in MCV and MCH in this study could be attributed to effect of processing on the test material in the experimental diets. Mean corpuscular volume and mean corpuscular haemoglobin indicate blood level conditions and low level is an indication of anaemia (Aster, 2004; NseAbasi *et al*., 2014). It could be inferred from this that longer period of fermentation of the SBCM did not suppress intake and impair dietary nutrient availability that could cause anemic conditions (Adejumo and Ologhobo, 2012). This finding is in line with earlier report by Tuleun *et al*. (2007) in broiler chickens and Adenkola *et al*. (2009) in rabbits, who indicated that nutrient is an important factor in haemopoesis. All the haematological values obtained in this study were within the normal range and correlated with those reported by researchers who observed the normal haematological values for bird (Mitruka and Rawsley, 1977; Wikivet, 2013; NseAbasi *et al*., 2014).

Table 3: Haematological indices of birds fed experimental diets containing different periods of fermentation on cooked shea butter cake (FSBC) meal

Parameters	Control	2d_FSBC	4d_FSBC	6d_FSBC	SEM	LOS
PCV (%)	25.51	22.70	22.27	23.40	2.08	NS
Hb (g/dl)	5.17	4.23	4.09	4.47	1.20	NS
WBC ($x10^3/mm^3$)	9.65	9.40	9.20	9.75	0.79	NS
RBC ($x10^3mm^3$)	1.12	1.05	1.01	1.07	0.92	NS
MCV (u^3)	139.06[a]	121.06[c]	122.28[bc]	125.77[ab]	2.11	*
MCH (Fi)	46.38[a]	40.36[b]	40.765[b]	44.93[b]	1.45	*
MCHC (%).	33.34	33.00	33.34	33.34	0.24	NS

[ab] means on the same row with different superscript are significant (P>0.05) different

NS: Not significant; *: Significant

Response of broiler chickens to experimental diets on serum biochemistry

Effect of experimental diets on the serum glucose, total protein, uric acid, triglyceride and liver enzymes are presented in Table 4. The results showed significant (p<0.05) effect of the dietary treatments on serum glucose, total protein and uric acid. The serum glucose was significantly (P< 0.05) influenced by the incorporation of the processed SBCM diets; the values ranged from 185.15g/dl in control diet to 173.02g/dl in diet T_2. This shows that birds fed diet T_2 and T_3 having significant (p<0.05) low blood glucose could be an indication of inadequate intake or incipient problem with ketosis (Melluzzi *et al*., 1991). The pattern recorded among the treatments on glucose and total protein followed the same trend, having higher (p<0.05) concentration in birds fed control and 6d-FSBCM diets than the birds on 2d- and 4d-FSBCM diets. The highest serum protein of 4.38g/dL was recorded in birds on diet T_1 (control) but similar with those fed T_4 (4.28g/dL) and T_3 (4.10g/dL) group while the lowest of 3.06g/dL was observed in diet T_2. It has been reported that serum biochemical constituents positively correlate with the quality of the diet (Adeyemi *et al*., 2000). This observation has revealed the efficacy of fermentation in the enhancement of energy and protein bioavailability and depicted a direct correlation between length of fermentation of FSBC and these serum variables. This implies that as the length of fermentation of SBC increases, the diet is likely to possess identical dietary qualities with the control diet. Information regarding nutritional status and malnutrition is often obtained from the total protein (Allison, 1995). Also, an increase in serum total protein as observed in birds fed 6d-FSBCM diet probably was indicative of the improvement in protein synthesis in the liver and this shows a potential pointer to enhancing growth rate. Uric acid was significantly higher (p<0.05) in the T_2 group than control, T_3 and T_4 treatment groups, an indication of inefficient utilization of protein in birds fed T_2 diet. Serum uric gives an indication of the quality of protein fed and originates from tissue deamination of proteins (Ewuola and Egbunike, 2008); and high levels in the serum occur when energy deficiency and or diseases prevent the efficient utilization of protein (Oloyede *et al*., 2004). Moreover, the higher uric acid value obtained in T_2 diet could be as a result of residue anti-nutrients such as tannins that were not completely remove which was also corroborated by the low serum total protein observed in these groups of birds. Several studies have shown pronounced negative effect of residue tannin on protein and energy digestibility (Smulikowska *et al*., 2001; Iji *et al*., 2004) due to its ability to inhibit *in-vivo* activities of trypsin and a-amylase (Longstaff and McNab, 1991). The values of Aspatate amino tranferase (AST) and Alanine amino tranferase (ALT) recorded during this study ranged between 9.28 – 10.61 iµ/L and 9.67 – 10.80 iµ/L, respectively. The enzymes assay showed that ALT and AST did not differ (P > 0.05) among the treatment groups. These are liver enzymes that have linkages between the liver and the blood. Since there was no significant difference in both ALT and AST in all treatment groups, this could suggest that dietary treatment did not adversely affect the functions of the liver (Emenalon *et al*., 2012; Muhammad *et al*., 2015). The range of values for all serum variables evaluated in this study are in agreement with the normal range reported in previous studies (Mitruka and Rawnsley, 1977; Stockham and Scott, 2002). This observation suggests that the health of the birds were not compromised and could be related to the nutritional adequacy and safety of the diets.

Table 4: Serum biochemical indices of birds fed experimental diets containing different periods of fermentation on cooked shea butter cake (FSBC) meal

Parameters	Control	2d-FSBCM	4d-FSBCM	6d-FSBCM	SEM	LOS
Glucose (g/dL)	185.15[a]	173.02[b]	175.30[b]	183.19[a]	2.01	*
Total protein (g/dL)	4.38[a]	3.06[b]	4.10[ab]	4.28[a]	0.55	NS
Uric acid (mg/dL)	4.85[a]	3.02[b]	3.22[b]	4.28[a]	0.24	*
Triglycerides (mg/dL)	141.41[b]	148.77[a]	148.55[a]	143.19[b]	1.77	*
AST (IU/L)	10.61	9.28	10.19	10.32	2.53	NS
ALT (IU/L)	10.80	9.91	9.67	10.19	1.49	NS

[ab] means on the same row with different superscript are significantly (P>0.05) different.

AST: Aspartate amino transferase; ALT: Alanine amino transferase

Conclusion and Recommendation

Results obtained from this study revealed that boiling in combination with longer period of fermentation as a processing method, is the better for improving the nutritional value of shea butter cake meal as no deleterious effects has been elicited on broiler chickens as evidenced by the haematological (MCV and MCH) and serum biochemical (glucose, total protein and uric acid) indices in grower phase. This establishes its potential usefulness as an alternative feed ingredient in the diet of broiler chickens thereby reducing/minimizing competition between human being and livestock for conventional energy sources like maize and sorghum. Therefore, fermentation of the cake at longer periods for over a week should be further investigated at level exceeding 20% inclusion to ascertain its utilization in poultry diet, while more forest agro by-products should be subjected to this type of treatment to enhance their utilization for animal nutrition and safe environment promotion.

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