EFECTS OF FEEDING DIETS CONTAINING *Aspergillus niger* FERMENTED SHEA BUTTER CAKE ON THE HAEMATOLOGICAL PARAMETERS OF WEANER RABBITS

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ABSTRACT
Shea butter cake (SM), obtained from shea nut processing factories in Bida, Niger State, was subjected to fermentation for different hours of 0, 72, 144, 216 and 288 hours, using solid state fermentation technique, Aspergillus niger as the fermenting organism. The fermentation was stopped at the end of each time and dried in the oven at 105°C for five (5) hours, and packaged in plastic containers. A total of 72 rabbits were used for the study, and were allotted to six treatments, with two (2) rabbits per replicate of six replicates per treatments. The rabbits were fed the experimental diets for 12weeks ad-libitum. The diets were: T1 (maize GNC base control diet,), T2 (10% unfermented SM), T3 (10% SM fermented for 72 hours), T4 (10% SM fermented for 144 hours), T5 (10% SM fermented for 216 hours) and T6 (10% SM fermented for 288 hours). After the feeding trial, blood samples were collected from the rabbits for the determination of haematological and serum biochemical parameters. Results obtained revealed that the PCV and MCHC of T5 had superior values (30.67% and 29.33 % respectively). However, no significant difference (p>0.05) was observed for WBC, NUE, LYM and EOS across the treatments. While for serum biochemical indices only the alkaline phosphatase for T2 (298.87 UI/mol) was observed to differ from the remaining treatments. Hence, fermentation of shea butter cake for 216 hours with Aspergillus Niger improved most of the haematological parameters of weaner rabbits. It is an agro-waste material that can be a good substitute for the conventional groundnut cake for feeding rabbits when processed (fermented).
Keywords: *Aspergilus niger,* Fermented shea butter cake, Haematological parameters, Weaner rabbits.

INTRODUCTION

The astronomical increase in world population has led to a high demand in animal protein consumption from beef, poultry, rabbit, and other sources, especially in the developing countries of the world (Mohammed and Agwunobi, 2009). Rabbits have been identified to have the potential for filling the gap between the demand and supply of high-quality protein in developing countries like Nigeria (Wafar *et al*., 2018). Rabbits have high genetic potential, short generation interval, high fecundity, and high meat quality with low cholesterol. They are also reported to be efficient converters of fibrous feed ingredients and agro-industrial by-products than other livestock species (Bassey *et al*., 2008).

However, the feeding and nutrition of rabbits requires adequate supply of feed, in quantity and quality, for optimal growth (Abonyi *et al.,* 2012). In developing countries, rabbit production is based primarily on grasses and legumes whose availability during the rainy season and growth during dry season cannot sustain rabbit production (Olomu *et al.,* 2012). Thus, the search for cheaper and available feedstuffs that can sustain all year-round rabbit production has been the focus of animal nutritionist in recent years. The use of agro-by products in rabbit nutrition has been documented (Okorie, 2003 and Odeyinka, *et al.,* 2007). One of such agro-by products with potentials for feeding rabbits is the shea butter cake. Shea butter cake is a by-product obtained during processing of Shea nut to produce Shea butter, the Shea butter cake is receiving increase attention as a potential feed ingredient due to its availability and in most cases very cheap, and hence its suitability.

MATERIALS AND METHODS

Location of the study area

The study was conducted at the Rabbitry Unit of the Animal Production Teaching and Research Farm, Federal University of Technology Minna Niger State (Figure 1). Minna lies within the latitude $9^0$39´11´´N and Longitude $60^0$30´57´´E (Yahaya *et al*, 2020).

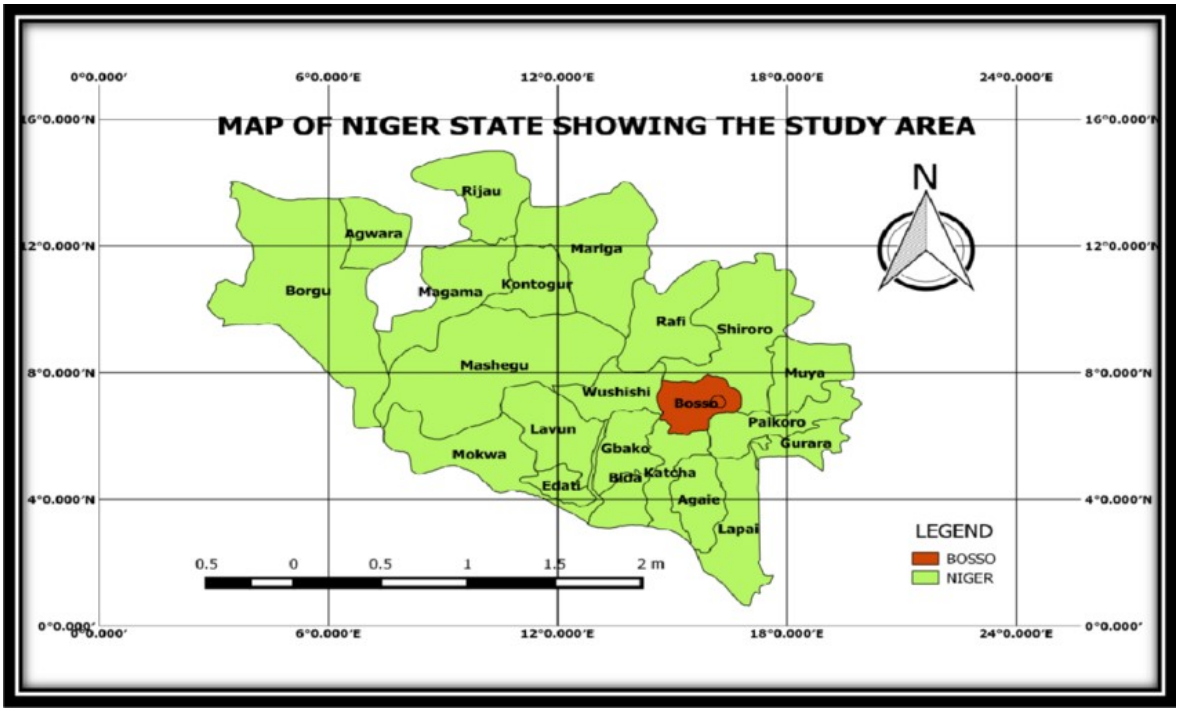

Figure 1**:** Map of Niger State showing the study area (Bosso)
Source: Yahaya *et al*. (2020)

Source of experimental materials

A total of 72 weaned rabbits were used for this study The Rabbits were purchased from the Rabbitry Unit of the National Veterinary Research Institute, Vom, Plateau State. Shea butter cake was sourced from villages around Bida Local Government Area in Niger State.

Preparation of shea butter cake and fermentation procedures

The Shea butter cake was air dried for seven days, and winnowed to remove impurities, then pulverized into powdery form. It was then mixed with water in the ratio 1:2 (1kg of shea butter cake: 2 litres of water) after which the spores of *Aspergillus niger* from potatoes Dextrose Agar (PDA) broth (2.5g/kg) were mixed properly with it for homogeneity. The mixture was packed into a plastic container, gently firmed, and sealed with adhesive film to provide anaerobic condition before being kept in a container at an ambient temperature of 28ºC, and allowed to undergo solid state fermentation. The Shea butter cake was allowed to ferment for 72hours, 144hours, 216hours and 288 hours respectively. The *Aspergillus niger* strain that was used for this study was obtained from the Microbiology Department Laboratory of the Federal University of Technology, Minna.

Experimental design and the experimental diets

A total of 72 weaner rabbits were allocated into six treatments; with six replicates of two rabbits per replicate. The completely randomized design model was adopted. The feeding trial was carried out for 12weeks and the rabbits were fed *ad-libitum*. The experimental diets were formulated to be isocaloric and isonitrogenous (16% CP and 2500 kcal/kg ME). Diet 1 was a maize-groundnut cake-based control diet having 0% Shea butter cake inclusion; Diet 2 contained 10% unfermented shea cake; while Diets 3, 4, 5 and 6 contained 3, 6, 9 and 12-days fermented Shea butter cake respectively, replacing maize at 10% inclusion level (Table 1).

Haematology and serum biochemistry

At the end of 12th week of the experiment, blood samples were collected from one rabbit per replicate in the morning at 8:00 am, for the determination of the haematological and serum biochemical parameters. A 5mls syringe and needle and bottles containing EDTA was used for the collection of the blood from the ear vein of each rabbit; 2mls blood was collected into plain bottles without anti-coagulant and then centrifuged for 10 mins at 1500 rpm and stored for serum biochemical indices.

Data analysis
Data collected were subjected to analysis of variance (ANOVA) using statistical analytical system package (SAS, 2014). Variations in means were separated using the Duncan's Multiple Range Test as illustrated by Steel and Torrie (1980).

Table 1: Ingredient composition of the experimental diets

Parameters	Experimental Diets					
	T1	T2	T3	T4	T5	T6
Maize	36.00	36.00	36.00	36.00	36.00	36.00
Rice husk	27.75	27.75	27.75	27.75	27.75	27.75
Shea butter cake	0.00	0.55	0.55	0.55	0.55	0.55
Groundnut cake	5.50	4.95	4.95	4.95	4.95	4.95
Full fat soya	26.00	26.00	26.00	26.00	26.00	26.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Values						
Crude protein	16.22	16.07	16.08	16.08	16.08	16.08
Metabolizable energy (kcal/kg)	2513.29	2508.45	2503.61	2503.61	2503.61	2503.61
Ether extract (%)	6.66	6.68	6.64	6.64	6.64	6.64
Crude fibre (%)	11.89	11.92	11.87	11.87	11.87	11.87
Calcium (%)	1.33	1.33	1.33	1.33	1.33	1.33
Available phosphorus (%)	0.68	0.68	0.68	0.68	0.68	0.68
Lysine (%)	1.11	1.11	1.11	1.11	1.11	1.11
Methionine+Cystine (%)	0.71	0.71	0.71	0.71	0.71	0.71

T1 = Control diet containing no shea nut cake
T2 = Diet containing SM fermented for 0 hours, replacing 10 % GNC
T3 = Diet containing SM fermented for 72 hours, replacing 10 % GNC
T4 = Diet containing SM fermented for 144 hours, replacing 10 % GNC
T5 = Diet containing SM fermented for 216 hours, replacing 10 % GNC
T6 = Diet containing SM fermented for 288 hours, replacing 10 % GNC

RESULTS AND DISCUSSION
Table 2 shows the haematological parameters of rabbits fed fermented shea nut meal replacing 10 % groundnut cake in the diets. The haemoglobin and the Pack cell volume (PCV) of the blood of the rabbit all increased with increase in the fermentation period. Hb and PCV are usually indicators of blood levels in animals. The result obtained are slightly at per with the one reported by Ansah *et al*. (2011) who reported a higher value for rabbit fed fermented shea nut cake. The increase in the Hb and PCV could be attributed to the increase in protein content of the shea nut due to fermentation, as protein is known to play a role in blood and tissue production. The MCV,MCHC and MCH results all showed Diet 4(T4) to be significantly higher when compared to the rest diets.

RBC, which is the erythrocyte concentration, showed an increase with increase in the fermentation period. These results obtained are in line with the normal range for rabbits as reported by Igwebuike *et al.* (2008). There was no significant difference ($p>0.05$) in the WBC, NEU, LYM and EOS values across all the diets for weaner rabbits. This outcome indicates that the shea butter cake has no detrimental effect on the immune system of the rabbit. These findings are in agreement with the results of Butcher and Mile (2002) who reported that most changes in immunological indices seen in malnutrition are usually aligned after nutritional rehabilitation. The mesophilvalues increased, however, with increase in fermentation period.

Table2: Haematological parameters of rabbits fed diets containing shea butter cake fermented with *Aspergillus niger* for different time periods

PARAMETER	Experimental Diets						SEM	LSD
	T1	T2	T3	T4	T5	T6		
HB g/dL	9.21[b]	7.87[c]	8.03[c]	8.63[bc]	9.27[b]	10.40[a]	0.24	*
PCV %	27.67[ab]	23.67[b]	25.00[b]	28.67[a]	30.67[a]	27.67[ab]	0.69	*
MCV ft	86.33[c]	86.67[c]	88.00[bc]	94.33[a]	91.67[ab]	86.00[c]	0.90	*
MCHC %	29.33[a]	27.00[b]	29.67[a]	30.00[a]	30.00[a]	29.67[a]	0.30	*
MCH pg	28.67[bc]	28.00[c]	29.67[ab]	30.67[a]	30.67[a]	29.00[bc]	0.29	*
RBC $x10^6$/L	3.23[a]	2.50[b]	3.60[a]	3.10[ab]	3.03[ab]	3.60[a]	0.11	*
WBC $x10^{12}$/L	13.67	12.23	13.47	14.27	9.50	14.77	0.75	NS
NEU %	35.66	36.33	36.00	38.67	39.00	31.33	1.22	NS
LYM %	57.67	56.67[a]	56.67	56.33	55.33	63.00	1.15	NS
MES %	2.67	2.33	1.33	2.00	2.33	3.00	0.15	*
EOS %	4.00	3.33	3.67	3.00	3.00	2.67	0.17	NS

[abc]Means on the same row with different superscripts are significantly($p<0.05$) different.
T1 = Control diet containing no shea nut cake
T2 = Diet containing SM fermented for 0 hours, replacing 10 % GNC
T3 = Diet containing SM fermented for 72 hours, replacing 10 % GNC
T4 = Diet containing SM fermented for 144 hours, replacing 10 % GNC
T5 = Diet containing SM fermented for 216 hours, replacing 10 % GNC
T6 = Diet containing SM fermented for 288 hours, replacing 10 % GNC

The serum biochemical results of rabbit fed 10% inclusion level of fermented shea butter cake is shown in Table 3. The parameters measured showed that the alkaline phosphatase values varied significantly ($p<0.05$) across all the diets with T2 having significantly higher value (298.87 U/L) than the other treatments. Alkaline phosphate is an indicator of the functional ability of the liver. Alkaline phosphate in high amount is an indication of liver disorder or bone enlargement (Lowe *et al*, 2021) while for AST, ALT and total protein all were not significantly ($p>0.05$) influenced by fermentation period of the shea butter cake.

Table 3: Serum biochemistry of rabbits fed diets containing shea butter cake fermented with *Aspergillus niger* for different time periods

PARAMETER	Experimental Diets						SEM	LSD
	T1	T2	T3	T4	T5	T6		
ALP (UI/mol)	160.20[b]	298.87[a]	161.70[b]	182.03[b]	192.50[b]	181.17[b]	14.61	*
AST (UI/mol)	70.87[a]	70.01[a]	66.87[a]	80.27[a]	69.80[a]	76.76[a]	1.74	NS
ALT (UI/mol)	31.66[a]	38.70[a]	39.23[a]	53.73[a]	50.17[a]	38.80[a]	3.41	NS
TP (g/dL)	5.30[a]	6.33[a]	5.80[a]	5.37[a]	6.63[a]	5.76[a]	0.25	NS

[ab:] means in the same row with different superscripts are significantly ($p<0.05$) different.
T1 = Control diet containing no shea nut meal
T2 = Diet containing SM fermented for 0 hours, replacing 10 % GNC
T3 = Diet containing SM fermented for 72 hours, replacing 10 % GNC
T4 = Diet containing SM fermented for 144 hours, replacing 10 % GNC
T5 = Diet containing SM fermented for 216 hours, replacing 10 % GNC
T6 = Diet containing SM fermented for 288 hours, replacing 10 % GNC

CONCLUSION

The result obtained in this study revealed that fermented shea butter cake (an agricultural waste) can be incorporated in to the feed of rabbit up to 10%. It is readily available and cheap and can compete favourably with groundnut cake. When fermented for 216 hours (9 days), it produced optimum haematological values for Hb, PCV, MCHC and WBC (9.27, 30.67, 29.67, 14.77) in weaner rabbits.

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