

## Haematobiochemical indices of broiler chickens fed probiotic supplemented shea kernel cake meal based diet

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### Abstract

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A 49-day experiment was conducted with a total of 240 Arbor acre broiler chicks, to determine the effect of probiotic supplementation on graded levels of shea kernel cake meal (SKCM) on their haematological and serum biochemical indices. The birds were randomly grouped into four dietary treatments comprising of 0%, 5%, 10% and 15% inclusion levels of SKCM as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively in a completely randomized design having 5 replicates with 12 chicks each. Diet T<sub>1</sub> served as the control, without SKCM and probiotic addition, while diet T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were supplemented with 0.5g/kg Biovet-YC® at both starter and finisher phases. Feed and water were given ad-libitum and routine management were strictly observed. At the end of the feeding trial, three broilers per replicate were randomly selected and blood collected using hypodermal syringe into two different labeled bottles with or without an anti-coagulant (EDTA) for haematology and serum biochemistry investigation respectively. The results showed that there were significant ( $P < 0.05$ ) increase in the white blood cell count and corresponding decrease ( $p < 0.05$ ) in serum triglyceride and cholesterol concentration of birds fed probiotic supplemented SKCM diets. However, other blood parameters measured were not affected ( $p > 0.05$ ) by the probiotic supplementation. Therefore, it can be concluded that incorporation of probiotic supplemented SKCM up to 15% inclusion level did not have any deleterious impact on blood status of the broiler chickens.

**Keywords:** Shea kernel cake, probiotics, broilers, hematology, serum biochemistry.

### Introduction

Poultry production is an important part of farming in many parts of the world especially the developing countries including Nigeria. The major attracting factor in poultry production is probably the tendency of providing a fairly rapid return on capital and the demand for poultry products for human consumption has been fairly high (Abdulkadir, 2002). In Nigeria, the poultry feed industry is heavily dependent on grains such as maize, millet and sorghum; and oilseed resources such as groundnut cake, soybean cake, and palm kernel meal (RMRDC, 2003). It has therefore become imperative to explore other alternatives for the feed industry in order to reduce the current stress on human

food supply. Research on the use of unconventional feedstuff for poultry production is on the increase and this encourages incorporation of agro-industrial by-products in poultry feed which reduce cost of production and maximize profit (Aduku, 1993; Esonu *et al.*, 2003; Ekenyem, 2007; Mmereole, 2008). One of the key agro-forestry species in Africa, particularly Nigeria is the shea butter tree, *Vitellaria paradoxa*. The shea tree produces fruits which is cherished and eaten by humans and animals; the nut of this fruit is processed to give shea butter, while the residue is the shea nut cake. Thus, shea nut cake is a by-product obtained during the processing of shea (*Butyrospermum parkii*) nuts to produce shea butter. (Okai and

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Bonsi, 1989; Dei *et al.*, 2008). Chemical analysis of the shea nut cake has shown that its overall nutritional value to be high but the major nutritional limitation of shea nut meal for poultry is the presence of anti-nutritive factors, particularly tannins that are in the range of 98.7 to 156.4 g/ kg (Annongu *et al.*, 1996). The biological significance of tannins in poultry nutrition is related to their characteristic adverse effects on feed intake (Armstrong *et al.*, 1974) and nutrient utilization (Smulikowska *et al.*, 2001).

With the advent of biotechnology, there are opportunities for economic utilization of agro-industrial residues such as shea nut cake as potential feed ingredient by poultry (Obadina *et al.*, 2006). Currently, in many parts of the world, feed additives such as probiotics, are being introduced to alleviate the problems associated with the ban and withdrawal of antibiotics from feed. This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers (European Commission, 2001). Probiotics are defined as feed additives that contain live microorganisms and promote beneficial effects to the host by favoring the balance of the intestinal microbiota (Fuller, 1989; Kabir, 2009). The potential health benefits associated with using a probiotics include improved digestion, stimulation of gastrointestinal immunity and increased natural resistance to enteric disease (Tellez *et al.*, 2001). Modes of action that have been suggested include increasing the number of beneficial bacteria in the intestine and therefore, improving the ratio of beneficial bacteria to pathogens (Simon *et al.*, 2001). When a higher number of beneficial bacteria are present they are more likely to outcompete the pathogens for both nutrients and adhesion sites on the gut wall, a process known as competitive exclusion. Beneficial

bacteria, such as *Lactobacillus*, are also known to release short chain fatty acids, bacteriocins and hydrogen peroxide, which have antagonistic effects on pathogenic bacteria (Patterson and Burkholder, 2003). Supplementing broilers with microbial cultures provides beneficial bacteria to aid in nutrient absorption normalize gut activity by enhancing the microbial balance in the avian digestive tract (Erdogan, 1999; Kutlu and Görgülü, 2001). Madubuike and Ekenyem (2006) stated that haematology and serum chemistry assay of livestock suggest the physiological disposition to their nutrition. Therefore, the present study was conducted with the aim of evaluating blood parameters of broilers chickens fed graded level of shea kernel cake meal with probiotics supplementation.

## **Materials and methods**

### ***Study site***

This study was conducted at the Poultry Unit of Teaching and Research Farm, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located at a longitude 4°31'E and 4°33'E and latitude 7.3°N and 10.00°N.

### ***Collection and processing of shea nut cake***

The shea nut cake (SNC) used for this experiment was collected from local shea butter processing centers at New Bussa, Niger State. The SNC was sundried for three days and then milled with a hammer mill to obtain the shea nut cake meal (SNCM).

### ***Experimental diets***

Four experimental diets were formulated with SNCM replacing dietary maize at 0%, 5%, 10% and 15% as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively for both starter and finisher phase (Tables 1 and 2). The T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> SNCM based diets were supplemented with probiotic at recommended rate of 0.5g/kg feed.

**Experimental birds, design and management**

Two hundred and forty (240) day-old broiler chicks were used for this experiment. The birds were allocated to four experimental treatments, replicated five times with 12 birds each in a

completely randomized design (CRD). The birds were raised in a deep litter system, using wood shavings as litter material in an open sided poultry house. Feed and water were supplied *ad-libitum*. Other routine management practices were strictly observed.

**Table 1: Gross composition of the experimental broiler starter diets**

<b>Ingredients</b>	<b>T<sub>1</sub> (0% SNCM)</b>	<b>T<sub>2</sub> (5% SNCM)</b>	<b>T<sub>3</sub> (10% SNCM)</b>	<b>T<sub>4</sub> (15% SNCM)</b>
Maize	61.45	56.50	52.00	47.15
SNCM	0.00	5.00	10.00	15.00
Soya beans meal	32.05	32.00	31.00	30.85
Fish meal	3.00	3.00	3.00	3.00
DCP	2.00	2.00	2.00	2.00
Oyster shell	0.50	0.50	0.50	0.50
Premix	0.40	0.40	0.40	0.40
Salt	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Probiotic	0.00	0.50	0.50	0.50
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Calculated Analysis</b>				
ME kcal/kg	3000.14	3013.39	3031.28	3050.71
CP%	22.72	22.94	22.98	22.96

**Table 2: Gross composition of the experimental broiler finisher diets**

<b>Ingredients</b>	<b>T<sub>1</sub> (0% SNCM)</b>	<b>T<sub>2</sub> (5% SNCM)</b>	<b>T<sub>3</sub> (10% SNCM)</b>	<b>T<sub>4</sub> (15% SNCM)</b>
Maize	68.10	61.50	56.80	50.65
SNCM	0.00	5.00	10.00	15.00
Soya beans meal	22.90	22.15	21.85	21.00
Fish meal	3.00	3.00	3.00	3.00
Wheat offal	2.00	4.00	4.00	6.00
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00
Premix	0.40	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Probiotic	0.00	0.50	0.50	0.50
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
<b>Calculated Analysis</b>				
ME kcal/kg	3045.03	3038.70	3054.53	3044.02
CP %	19.19	19.31	19.43	19.50

***Haematological and serum biochemical evaluation***

At the end of 49 days feeding trial, three birds were randomly selected from each replicate of each treatment group and blood samples were collected from the bronchial vein during slaughter. The collected blood samples were centrifuged at 2000 rpm for 10 min and the sera were decanted into aseptically treated vials and stored at -20 °C until further analysis of haematological parameters (white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH)) and serum biochemical indices (total protein, albumin, globulin, glucose, total cholesterol and triglycerides) with commercial kit.

***Chemical analysis***

The shea kernel cake meal and experimental diets were analyzed for proximate composition using the procedures of AOAC (2006).

***Statistical analysis***

All data on haematology and serum biochemistry of the experimental birds were subjected to one-way analysis of variance procedures of SAS Institute Inc (SAS, 2006). The treatment means were compared using the Duncan's procedures of the same software.

**Result and discussion**

***Proximate composition***

The proximate composition of shea nut cake meal (SNCM) is presented in Table 3. The result shows that SNCM contains 93.3 dry matter (DM), 13.30% crude protein (CP), 10% crude fiber (CF), 21% ether extract (EE), 6.7% ash, 42.7% nitrogen free extract (NFE) and 3725.25kcal/kg ME. The observed CP in this study was higher than the values of 12.70% and 13.03% reported by Orogun et al. (2015) and Abdumumeen

et al. (2013), respectively but lower than value 14.26% and 16.24% reported by Dei et al. (2008) and Atuahene (1998) respectively. The ME was slightly lower than the value (4502.23kcal/kg) reported by Abdumumeen et al. (2013) and higher than 3406.68kcal/kg reported by Atuahene (1998). The CP and ME value obtained in this present study suggested that SNCM is a potential alternative to dietary maize with 9.0% and 3434kcal/kg CP and ME respectively. The crude fiber, ether extract, NFE and ash content was higher than the values reported by Atuahene (1998) but the EE and NFE was lower compared to the value (23.38 and 59.37%) observed by Abdumumeen et al. (2013) respectively. The variation in the nutrient composition could be attributed to differences in shea nut variety, soil condition of the parent plant and the processing method employed and efficiency of oil extraction (Ugese et al., 2010).

The proximate composition of the experimental starter and finisher diets are shown in Tables 4 and 5, respectively. The diets were almost isocaloric and isonitrogenous. The CP was in the range of 22.88 - 23.07% and 21.22 - 21.98% for starter and finisher phase, respectively. The energy was recorded within the range of 3120.37 - 3020.74 kcal/kg ME and 3069.45 - 3037.87 kcal/kg ME for both starter and finisher, respectively. There was increase in the composition of ether extract with increasing level of shea kernel cake in diets as 4.2 - 6.11% and 8.97 - 10.68% for starter and finisher diets, respectively. The level of crude fiber also increases with increasing level of shea kernel cake as 3.45 - 4.89% and 5.97 - 15.23% for starter and finisher diets, respectively. The highest ash content was observed in T<sub>3</sub> for both starter and finisher as 10.64 and 15.65%, respectively. Moreover, the experimental diets were however formulated to meet the nutrient

and energy requirements of broiler starter and finishers under the tropical environmental conditions as recommended by Olomu (1995).

**Table 3: Proximate composition of shea kernel cake meal**

Constituents	% Composition
Dry matter (DM)	93.30
Crude protein (CP)	13.30
Ether extract (EE)	21.00
Crude Fibre (CF)	10.00
Ash	6.30
Nitrogen Free Extract (NFE)	42.70
Metabolizable Energy (Kcal/kg)	3725.75

**Table 4: Proximate composition of experimental starter diet**

Parameter	T1	T2	T3	T4
Dry matter (%)	94.2	92.01	92.25	91.87
Crude protein (%)	23.07	22.88	22.96	22.92
Ether extract (%)	4.02	5.55	5.23	6.11
Crude fibre (%)	3.45	3.62	4.31	4.81
Ash (%)	9.07	10.58	10.64	10.42
Nitrogen free extract (%)	54.59	49.38	49.11	47.61
Metabolizable Energy (kcal/kg)	3120.37	3053.54	3020.74	3037.99

**Table 5: Proximate composition of experimental finisher diet**

Parameters	T1	T2	T3	T4
Dry Matter (%)	93.96	93.24	93.2	93.55
Crude Protein (%)	21.22	21.98	21.23	21.42
Crude Fiber (%)	5.97	6.35	6.03	7.2
Ether Extract (%)	8.97	9.57	10.08	10.68
Ash (%)	14.08	14.67	15.65	14.87
Nitrogen Free extract (%)	44.72	40.67	40.21	39.38
Metabolizable Energy (kcal/kg)	3069.446	3039.871	3037.509	3064.154

**Haematological and serum biochemical indices**

The haematological response of broiler chicken fed graded levels of probiotic supplemented shea kernel cake meal is shown in Table 6. There was no significant difference ( $P>0.05$ ) in the Pack cell volume, Red blood cell count, Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH). These results are in agreement with the reports of Dimcho et al (2005), Alkhalf et al. (2010) and Mokhtar (2013), who found that the probiotic supplementation did not affect blood constituents comprising PCV and

hemoglobin concentrations. In contrast, the findings disagree with Cetin *et al.* (2005) reports, who observed that the probiotic supplementation caused statistically significant increase in hematological parameters. The difference may be attributed to type and number of species of bacteria present in probiotics. There was a significant ( $p<0.05$ ) difference in WBC count among the dietary treatments. The higher ( $p<0.05$ ) WBC counts obtained in birds fed probiotic supplemented SNCM diets could be explained by an increased need to challenge the foreign bodies in form of anti-nutritional factors in SNCM based diets by the action of probiotic addition.

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The WBCs are involved in antibody formation and cell mediated immunity (Iheukwemere and Odinamuo, 2009) and also plays major role in defending the body against disease-producing microbes. This observation could be attributed to the evidence that probiotic had positive effects and improved the immune system by producing high level of blood antibody via increasing the health status of the birds (Taklimi *et al.*, 2012; Altaf-Ur-Rahman *et al.*, 2009). The increased WBC count observed in birds fed probiotic supplemented SNCM diets is in line with the report of Khaksefidi and Ghoorchi (2006) who reported that when poultry diet is supplemented with probiotic, haematological profiles showed an increase in total erythrocyte and leukocyte cell count and marked increase in percentage of lymphocytes. This is probably due to the fact that probiotic stimulated immune system of birds by increasing the lymphocyte proliferation (Aathouri *et al.* 2001). This can help the birds to increase their resistance to infection.

The serum biochemical indices of broiler chicken fed graded levels of probiotics supplemented shea kernel cake meal based diets are presented in Table 7. There were non-significant ( $p>0.05$ ) differences for the serum metabolites measured except cholesterol and triglyceride values that differ significantly ( $P>0.05$ ) across the treatment groups. The non-significant values of total protein, albumin, globulin and glucose was an indication that probiotic supplementation suppress the effect of the anti-nutritional contents of SNCM diets from precipitating inadequacy of nutrients which could create an anemic condition (Tellez *et al.*, 2001). The serum cholesterol and triglyceride values obtained in this study significantly ( $P<0.05$ ) decreased with increasing inclusion level of probiotic supplemented SNCM. The lower values of

cholesterol and triglyceride observed in broilers fed probiotic- SNCM based diets agreed with the findings of previous researchers (Onifade *et al.*, 1999; Islam *et al.*, 2004; Jouybari *et al.*, 2010; Owosibo *et al.*, 2013; Mohammad *et al.*, 2014), who reported a reduced serum cholesterol and triglycerides in broilers fed diets containing probiotic supplementation. The negative effect of probiotic supplemented diets on broiler blood cholesterol and triglyceride content is well-known (El-Baky 2013, Kalavathy *et al.* 2003, Mansoub 2010, Panda *et al.* 2006, Santoso *et al.* 1995). The mechanism behind this observation could be explain by the fact that some bacterial probiotic strains such as *Lactobacillus acidophilus* are able to incorporate cholesterol into the bacterial cells (Mohan *et al.*, 1995, 1996), hydrolyze bile salts in the intestine, thereby preventing them from acting as precursors in lipid synthesis (Klaver and Van der Meer, 1993; Abdulrahim *et al.*, 1996) or inhibit hydroxyme thylglutaryl-CoA, the rate limiting enzyme of cholesterol synthesis in the body pool (Fukushima and Nakamo, 1995; Kalavathy *et al.* 2003).

### **Conclusion**

Based on the findings of the present study, probiotic supplementation improved the nutritional potential of SNCM when included up to 15% in broiler ration without compromising the health of the birds. Therefore, our current findings suggested and strengthened the previous findings that probiotics supplementation helps in sustaining normal hematology and reduces the concentration of blood cholesterol and triglyceride and elevated WBC concentration in broiler chickens. Further research is also needed to determine the optimum higher inclusion level of the probiotic supplemented SNCM used in this study for broiler chickens.

**Table 6: Effect of graded levels of probiotic supplemented shea kernel cake meal (SKCM) on hematological indices on broiler chickens.**

Parameters	T1	T2	T3	T4	SEM
PVC (%)	24.25	25.74	25.59	25.86	1.13
Hb (g/dl)	8.03	8.58	8.61	8.29	1.10
WBC (10 <sup>12</sup> /mm <sup>3</sup> )	20.53 <sup>c</sup>	21.95 <sup>bc</sup>	24.67 <sup>a</sup>	23.56 <sup>ab</sup>	0.83
RCB (10 <sup>9</sup> /mm <sup>3</sup> )	3.23	3.78	3.70	3.50	0.74
MCH (pg)	34.95	32.94	37.92	35.43	1.95
MCHC (mg/dl)	49.71	43.35	43.11	45.15	3.72

<sup>ab</sup> means on the same row with different superscript are significantly (P<0.05) different

**Table 7: Effect of graded levels of probiotic supplemented shea kernel cake (SKCM) on serum biochemistry of broiler chickens**

Parameters	T1	T2	T3	T4	SEM
Total protein (mg/dl)	5.82	6.16	5.12	5.46	0.35
Albumin (g/dl)	2.24	2.01	2.10	2.24	0.48
Globulin (mg/dl)	3.58	4.15	3.01	3.26	0.54
Glucose (mg/dl)	150.33	176.33	145.67	118.67	23.45
Cholesterol (mg/dl)	138.00 <sup>a</sup>	124.33 <sup>b</sup>	129.12 <sup>b</sup>	131.00 <sup>ab</sup>	4.80
Triglyceride (mg/dl)	94.58 <sup>a</sup>	83.67 <sup>c</sup>	78.17 <sup>c</sup>	88.03 <sup>b</sup>	2.23

<sup>abc</sup> Means on the same row with different superscript are significantly (P<0.05) different

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