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# SUPPLEMENTATION OF SHEABUTTER (*Vitellaria paradoxa,* Gaertn*)* CAKE BASED DIETS FED HELMETED GUINEA FOWLS (*Numida meleagris,* *galeata* Pallas**) ON BLOOD METABOLITES**

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## ABSTRACT

The recent high cost of feed arising from the growing competition between humans and animals for conventional feedstuffs like maize has compelled researchers in exploring some agro-forestry byproducts, such as shea butter cake as alternative energy source in poultry diet with multienzymes and probiotic supplementation to enhance efficiency of feed utilization and animal health. A 56-day study was conducted to evaluate the haemato-biochemical indices of guinea fowls (*Numidia meleagris)* fed fermented shea butter cake meal (FSBCM) based diets with mixture of multienzymes + *Bacillus* based-probiotic supplementation (Fullzyme®). Eighty, six-week old guinea fowl birds were weighed and assigned into four treatments with five replicates of five birds per pen in a completely randomized design. Four isocaloric and isonitrogenous diets were formulated to contain a positive control with maize-soybean based diet as diet 1 a negative control containing FSBCM replacing maize at 25% inclusion level as diet 2, while diet 3 and diet 4 contained Fullzyme® supplementation to diet 2 at 25 and 50 g/kg inclusion levels, respectively. Feed and water were supplied *ad libitum*. On day 56, blood samples were collected from 8 randomly selected guinea fowls per treatment (2 guinea fowls/pen) via the branchial vein by using a heparinized and non-heparinized tubes for determination of blood haematological and biochemical parameters. Data collected were subjected to analysis of variance (ANOVA) and differences among the treatment means were separated using Tukey test. The results showed that guinea fowls fed control and FSBCM diet with Fullyzme®supplementation recorded higher ($P<0.05$) white blood cell count and serum total protein; and significantly decreased ($P<0.05$) serum cholesterol, Aspirate aminotransferase (AST), Alanine aminotransferase (ALT) and uric acid compared to the FSBCM diet group. Therefore, it is concluded that Fullzyme® can be included at the minimum rate of 25 g/kg to diet of guinea fowls containing FSBCM replacing 25% maize without any detrimental health impact.

**Keywords:** Multi-enzymes, probiotic, shea butter cake, guinea fowl, blood

## Introduction

Guinea fowl is an indigenous species of African birds, although their adaptability has contributed to the spread of their breeding worldwide (Agwunobi and Ekpenyong, 1990). Variability of phenotypic features, availability of different color varieties, in addition to attractiveness of guinea fowl in terms of appearance andbehavior made themvery popular among small-scale farmers (Moreki and Seabo, 2012; Kyere *et al*., 2020). A recent study showed that guinea fowl is an important source of animal protein due to their high availability and relatively low market price (Obike *et al*., 2011).

The production of guinea fowls at the subsistence level is supported by a number of features such as attractive plumage, high adaptability, good forager, hardy disease-resistant nature, general acceptance, limited cultural barriers and good egg qualities that help address the issue of low protein intake in developing nations (Oke *et al*., 2015, Yamak *et al*., 2018). It has been reported that guinea fowl has a high socio-economic value in the rural society where they are mainly kept to supply meat and eggs and as a source of income for the rural farmers (Ayorinde, 1991; Oke *et al*., 2020). Additionally, the nutritional value of guinea fowl meat distinguishes it from other poultry species due to higher protein and lower fat content (Ayorinde, 1991). However, production results of these birds are constantly influenced by the quality and nutritional value of the feed, which indicates their adaptation to various sources of protein and energy (Batkowska *et al*., 2021).

The growth of Nigerian poultry industry has always been limited by feed which accounts for about 70 – 80% of the total cost of production intensively (Kehinde *et al*.,2006; Onu *et al*., 2011). The target of an animal nutritionist is to fully utilize locally available ingredients and produced feeds that supply basic nutrients to the animal at a very low and affordable cost while reducing the dependence of conventional ingredients such as maize and soybean.It has therefore become imperative to explore other alternatives such as shea butter cake (SBC),which has always been unusable by man and any conventional industry; thus, discarded as waste causing huge environmental burden. The SBC as an agroforestry by-product obtained during the processing of shea nuts to produce shea butter,has been identified to offer a cheaper source of energy required in poultry diet with respect to its promising nutrient profile composition (Dei *et al*., 2008; Oddoye *et al*., 2012). Previous reports on SBC nutrient composition indicated its overall nutritional value to be high but its inclusion in poultry diet has been limited due to the present of some anti-nutritional factors (ANFs) such as tannin, saponin and bromine that can hamper the animal's health and performance (Annongu *et al*., 1996; Orogun *et al*., 2015). Consequently, adopting a processing technique like fermentation has been considered to be an ideal approach that reduces the adverse effects of these ANFs and equally improves the nutritive value of SBC (Agbo and Prah, 2014; Aguihe and Kehinde, 2019).

However, preliminary studies showed that the nutrient digestibility and utilization of SBC is still poor due to its high fibrous nature leading to reduced feed efficiency and performance of birds (Dei *et al*., 2008; Oddoye *et al*., 2012; Matthew *et al*., 2017). Thus, the present study believes that the sub- optimal performance of the animals fed SBC based diets could be overcome by application of appropriate supplemental feed additives such as enzymes cocktail and probiotics in the diets. Exogenous multienzymes have been shown to increase the digestibility of fibrous feed ingredients by disrupting the plant cell walls, and by enhancing nutrients absorption (Sunmola *et al*., 2019; Attia *et al*., 2020). Probiotic is a culture of live microorganisms that can manipulate and maintain a beneficial gut microflora, and its use has been shown to improve the digestion and absorption of nutrients bound by antinutrients in the gut of poultry birds (Wealleans*et al*., 2017;Jha *et al*., 2020; Assan *et al*., 2022). Although, the aforementioned feed additives have gainedincreasing attention but there is scanty information onevaluation of their possible

additive or synergistic effects on performance of monogastric animals such as guinea fowls. It is hypothesized that if dietary exogenous multi-enzyme and probiotics are supplemented in combination in the diets, a synergistic effect would be shown in guinea fowls.Therefore, this study was designed to investigate the effect of supplementation of SBC based diets with mixture of enzymes cocktail and probiotic on haematological and serum metabolites of guinea fowls at growth phasewith view to ascertaining its nutritional potentials for enhanced nutrient utilization without any detrimental health implication.

## Materials and Methods

**Experimental site and preparation of test ingredient**:

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production Technology, Federal College of Wildlife Management (FCWM), New Bussa, Niger State. The FCWM - New Bussa is located at longitude 9° 81' 95" N and 9° 49' 10" N and latitude4° 58' 05" N and 4° 34' 49" N in the Savanna Areas of Niger Basin, North Central Zone of Nigeria. The SBC were obtained from some local shea butter processing factories in New Bussa, thoroughly mixed and subjected to fermentation under anaerobic conditions for five days. Thereafter, the fermented SBC were properly air dried for 3 days and then pulverized into finer particles to produce fermented shea butter cake meal (FSBCM), and thereafter subjected to proximate analysis using the procedure of AOAC (2006) as shown in Table 1.

**Table 1: Proximate composition of fermented shea butter cake meal (FSBCM)**

| Nutrients (%) | Dry matter | Ash | Crude fiber | Crude protein | Crude fat | NFE |
|---|---|---|---|---|---|---|
| FSBCM | 95.61 | 6.13 | 3.36 | 15.85 | 24.25 | 46.03 |

*NFE: Nitrogen Free Extract

**Experimental birds, design and management:**

A total of 80, six-week old helmeted guinea fowl growers were weighed individually and allocated to four dietary treatments with four replications of five birds each in a completely randomized design (CRD). Feed and water were given *ad-libitum*. Data on feed intake and body weight were collected on weekly basis. The birds were raised for a period of 56 days in a deep litter system using wood shavings as litter material and kept in an open sided poultry pen facility.

**Experimental Diets**:

Four iso-nitrogenous and iso-caloric diets for guinea fowl growers were formulated to meet or exceed their nutrient recommendation (NRC 1994). Diet1served as positive control containing corn-soybean based diet without incorporation of FSBCM and Fullzyme®. Diets 2 served as negative control diet formulated to incorporate FSBCM replacing maize at 25% inclusion level in positive control. Diet3 and 4were formulated to incorporate supplemental Fullzyme® to negative control diet at 25 and 50 g/kg respectively. The ingredients composition of

the experiment basal diets and the proximate composition of the experimental diets which was analyzed according to AOAC (2006) procedure are displayed in Table 2.

**Table 2: Ingredient composition (kg) of experimental diets**

| Ingredients | Diet 1 (Positive Control) | Diet 2 (Negative control) | Diet 3 (FSBCM + 0.25% Fullzyme®) | Diet 4 (FSBCM + 0.50% Fullzyme®) |
|---|---|---|---|---|
| Corn | 50.55 | 37.91 | 37.91 | 38.41 |
| Soybean meal | 37.00 | 28.00 | 28.00 | 28.00 |
| Fishmeal | 0.00 | 5.00 | 5.00 | 5.00 |
| Wheat offal | 5.00 | 9.00 | 8.75 | 8.00 |
| Groundnut oil | 4.00 | 3.00 | 3.00 | 3.00 |
| FSBCM | 0.00 | 12.64 | 12.64 | 12.64 |
| DCP | 1.00 | 2.00 | 2.00 | 2.00 |
| Limestone | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 |
| Methionine | 0.30 | 0.30 | 0.30 | 0.30 |
| Lysine | 0.30 | 0.30 | 0.30 | 0.30 |
| Premix* | 0.50 | 0.50 | 0.50 | 0.50 |
| Fullzyme® | 0.00 | 0.00 | 0.25 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | | | | |
| CrudeProtein% | 21.21 | 21.91 | 21.87 | 21.81 |
| ME kcal/kg | 3147.20 | 3105.74 | 3101.06 | 3104.04 |
| **Analyzed composition %** | | | | |
| Dry matter | 96.89 | 98.07 | 97.78 | 97.60 |
| Ash | 5.26 | 7.98 | 6.53 | 6.38 |
| Crude fiber | 4.78 | 7.08 | 4.70 | 6.30 |
| Crude protein | 21.89 | 21.28 | 21.67 | 21.21 |
| Crude fat | 4.71 | 5.76 | 5.91 | 5.76 |
| NFE | 63.36 | 57.90 | 61.19 | 60.35 |

*__Premix:__ To provide the following per kg of diet; Vit A 12500 I.U., Vit D3 2500 I.U., Vit E 50 mg, Vit K3 2.5mg, Vit B1 3.0mg, Vit B2 6.0mg, Vit B6 6.0mg, niacin 40.0mg, calcium pantothenote 10mg, Biotin 0.80mg, Vit B12 0.25mg, folic acid 1.0mg, choline chloride 300mg, manganese 100mg, iron 50mg, zinc 45mg, cobalt 0.25mg, iodine 1.55mg, selenium 0.1mg.

**Blood collection and analysis:**

Blood samples were collected from 4 randomly selected guinea fowls per treatment (1 guinea fowl/pen) at 56th day of the study to determine the blood haematology and serum chemistry. Blood samples were withdrawn from the branchial vein by using a vacutainer tube using heparinized and non-heparinized tubes. The blood samples collected in heparinized tubes were used for the determination of haemoglobin, packed cell volume, red and white blood cells using the procedure as described by Mitruka and Rawnsley (1977). The samples collected using non-heparinized tubes were separated by centrifugation at 3000 rpm for 15 min at 4°C. Commercial kits produced by Randox$^{R}$ diagnostic were used to measure the some biochemical traits of the blood (total protein, glucose, cholesterol, asparate aminotransferase (AST), Alanine aminotransferase (ALT) and uric acid) according to the method of Jain (1986).

**Statistical Analysis**:

Data generated was subjected to analysis of variance (ANOVA). Existence of significant differences among the treatment means were separated using Tukey test and level of significance was adopted at probability level of 5%.

**Results and Discussion**

The result of hematological and serum biochemical parameters of guinea fowls fed diets containing graded levels of FSBCM based diet with Fullzyme® supplementation is presented in Table 3. The evaluated hematological indices revealed no significant differences (P>0.05) among the guinea fowls fed FSBCM with graded levels of Fullyzme except for the white blood cells count (WBCs). Higher (P<0.05) WBC was recorded in birds fed Fullzyme® supplemented FSBCM diets than the group fed FSBCM diet, but no significant difference (P>0.05) was observed among the control diet and Fullzyme®-treated FSBCM diet groups. This result is in line with the findings of Attia *et al.* (2020) who reported an increased WBC in broilers due to multi-enzymes supplementation in the diet. Also, markedly increased WBCs was noticed in Nile tilapia fed protease and *Bacillus* based-probiotics and was suggested to be an indication of improved immunity to counteract infection with bacterial pathogens (Hassaan *et al*., 2014; Akbari *et al*., 2021).

The result of serum biochemical metabolites showed that all variables were significantly influenced (P<0.05) by Fullzyme® addition to FSBCM diets except for serum glucose. Birds fed Fullzyme® treated-FSBCM diets had higher (P<0.05) total protein concentration than those fed untreated FSBCM diet. A significant (P<0.05) reduction in serum cholesterol, aspirate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid were noticed in birds fed control diet and Fullzyme treated FSBCM diet compared to those offered FSBCM diet without supplemental Fullzyme. Thus, significant reduction of serum total protein in birds fed FSBCM diets indicate poor protein utilization due to the presence of tannin in FSBCM which has the ability to complex with protein, including enzymes in the gastro intestinal tract and thereby negatively inhibits the digestibility of protein and other essential nutrients (Annongu *et al*., 1996; Dei *et al*., 2008). However, higher (p<0.05) serum protein obtained in birds fed Fullyzme supplemented FSBCM diets attest to the nutritional adequacy of this diet in meeting the protein needs of the birds via increased protein digestibility due to synergetic influence of multi-enzymes and *Bacillus* based-probiotic supplementation (Flores *et*

*al*., 2016; Kim *et al*., 2021). In addition, a decreased (P<0.05) serum cholesterol concentration was observed in birds fed Fullzyme® supplemented FSBCM diets. A reduction in blood cholesterol is a reflection of the absence of stress, kidney, heart and liver failure as well as decreased lipid peroxidation (Adawi *et al*., 2001; Gidado *et al*., 2020). In agreement with our findings, Santose *et al*. (1995) have suggested that supplementation of *Bacillus subtilis* to broiler chickens diet reduces the serum cholesterol concentration, which is an indication that this bacterium can be effective in reducing the activity of acetyl coenzyme A carboxylase (the enzyme limiting the rate of synthesis of fatty acids). Furthermore, the influence of Fullzyme® on cholesterol concentrations observed in the present study is consistent with precedent reports suggesting that multienzymes reduces lipid profile properties, through which probiotics ferment to decrease short-chain fatty acids in the gut and then lower the systemic blood lipids (Abdulwahid *et al*., 2022). The decreased serum AST and ALT obtained in guinea fowls fed Fullzyme treated diets could be associated with hepatho-protective effects of the probiotics supplemented in the diet (Aluwong *et al*., 2013; Oke *et al*., 2014), any exaggerated increase in serum AST and ALT levels could be an indication of liver damage and necrosis (Yalcin *et al*., 2012). In addition, reduced serum uric acid was recorded for guinea fowls fed FSBCM diets Fullzyme supplementation informs efficient amino acid utilization by the birds. Blood uric acid contents could be used as influential criteria to reflect nitrogen utilization because uric acid is known to be the end product of protein catabolism in poultry (Donsbough *et al*., 2010).

**Table 3: Blood metabolites of guinea fowls growers fed FSBCM based diets containing graded levels of Fullzyme® supplementation**

| Indices | Positive control | Negative control | FSBCM + 0.25% Fullzyme® | FSBCM + 0.50% Fullzyme® | SEM | P-values |
|---|---|---|---|---|---|---|
| **Haematology** | | | | | | |
| PCV % | 36.50 | 38.50 | 35.50 | 37.00 | 1.45 | 0.077 |
| Hb g/dL | 10.50 | 11.00 | 11.25 | 10.80 | 0.88 | 0.101 |
| RBC $10^6$/L | 4.07 | 3.86 | 4.15 | 4.29 | 0.18 | 0.161 |
| WBC $10^3$/L | 24.63$^a$ | 21.14$^b$ | 23.33$^a$ | 24.04$^a$ | 0.73 | 0.007 |
| **Serum chemistry** | | | | | | |
| GLU mg/dL | 189.50 | 185.05 | 184.75 | 185.30 | 0.87 | 0.172 |
| TP g/dL | 6.08$^a$ | 3.69$^b$ | 5.85$^a$ | 5.91$^a$ | 0.42 | 0.001 |
| CHO mg/dL | 143.27$^b$ | 159.44$^a$ | 139.56$^b$ | 137.08$^b$ | 2.45 | 0.024 |
| AST (iu/L) | 69.25$^{ab}$ | 73.65$^a$ | 66.25$^b$ | 63.00$^b$ | 3.19 | 0.027 |
| ALT (iu/L) | 5.10$^b$ | 7.30$^a$ | 5.23$^b$ | 5.15$^a$ | 0.74 | 0.003 |
| Uric acid (mg/dl) | 2.83$^b$ | 4.08$^a$ | 3.64$^a$ | 3.10$^b$ | 0.22 | 0.031 |

$^{ab}$ Treatment means in column with common superscripts does not differ significantly (p<0.05). FSBCM: Fermented shea butter cake meal; PCV: Packed cell volume; Hb: Haemaglobin; RBC: Red blood cell; WBC: White blood cell; GLU: Glucose, TP: Total protein; CHO: Cholesterol; AST: Aspirate aminotransferase; ALT: Alanine aminotransferase.

## Conclusion

The result of this study has shown that FSBCM has good nutrient attributes that warrant its usefulness as a potential alternative energy source in poultry feed. Inclusion of FSBCM at 25% replacement for maize in diet of guinea fowls with a minimum of 25g/kg Fullzyme supplementation enhanced nutrient utilization without any negative impact on their blood composition. The study suggests that incorporation of enzymes cocktail + probiotic mixture in corn-soybean-FSBCM based diet may derive health advantages in guinea fowl growers.

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