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Effect of multienzymes + probiotic supplementation in fermented shea butter cake based diets on the carcass traits and the biochemistry of broiler chickens

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Abstract. *This study aimed to evaluate the effect of multienzyme+probiotic (MEP) supplementation on carcass characteristics and serum biochemistry of broiler chickens fed fermented shea butter cake meal (FSBCM) based diets. A total of two hundred and sixty (260), 28-day-old broiler chicks were distributed into four treatments, with five replicates of 13 birds each using a completely randomized design and reared on deep litter system from 28 to 56 days of age. The four treatments were: positive control - T_1 (corn-soybean based diet); negative control - T_2 (20% FSBCM replacement for corn in T_1); diet 3 - T_3 (T_2 diet + 0.05% MEP) and diet 4 - T_4 (T_2 diet + 0.10% MEP). On day 56, two birds per each pen were selected for carcass and organ evaluation and other two birds per replicate were selected for serum biochemical analysis. The results indicated that addition of MEP to FSBCM diet caused a significant ($p<0.05$) increase in live weight, dressing percentage and breast meat yield than FSBCM diet without MEP and did not differ from the T_1 diet. The abdominal fat in birds fed MEP treated diets were significantly ($p<0.05$) lower than those groups fed PC and FSBCM diets. Serum glucose was similar ($p>0.05$) between birds fed PC and MEP treated diets but significantly ($p<0.05$) higher than those on FSBCM diet, whereas serum cholesterol, alanine aminotransferase and aspartate aminotransferase concentrations reduced significantly ($p<0.05$) in groups fed MEP supplemented FSBCM diets. All other parameters were not significantly ($p>0.05$) affected by the MEP supplementation. Therefore, MEP supplementation in FSBCM based diet improved live weights and carcass attributes without any detrimental effect on blood composition of the broiler chickens.*

Keywords: carcass yield, chickens, feed additives, fermented shea butter cake, serum biochemistry,

Abbreviations: SBC- shea butter cake, FSBCM- fermented shea butter cake meal, AOAC- Association of Official Analytical Chemists, CP- crude protein, ME- metabolizable energy, NRC- National Research Council, MEP- multienzymes+probiotic, ALAT- alanine aminotransferase, ASAT- aspartate aminotransferase, ANOVA- analysis of variance, GLM- general linear model, SAS- Statistical Analysis Software, SBC- shea butter cake.

Introduction

In developing countries, the recent increase in the growth of human and livestock population has created higher demands for food and feed from traditional feedstuffs such as maize and soybean, resulting in their current exorbitant prices as well as increased cost of animal production. More than 70% of the total cost of operation in animal husbandry is dependent on feed availability especially in poultry production (Oloyede et al., 2004; Onu and Aniebo, 2011; Sunmola et al., 2019). Consequently, this has challenged animal nutritionists to seek ways of sustaining productivity by incorporating alternative local feed resources that possess comparative nutritive values to replace the traditional feedstuffs in feed formulation. One of such cheap and readily available untraditional feed materials is an agro forestry and industrial by-product known as shea butter cake (SBC) with promising nutritional value (Dei et al., 2007; Zanu et al., 2012; Abdul-Mumeen et al., 2013; Aguihe et al., 2019). The SBC is regarded as a by-product obtained from shea butter production with no economic value and its increasing output of late has become an environmental problem in developing countries, especially in most parts of West Africa like Nigeria. However,

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utilization of SBC in poultry diets is limited due to its fibrous nature and presence of possible nutrient limiting factors particularly tannins in the feed that can cause poor performance and health of birds (Annongu et al., 1996; Oddoye et al, 2012; Orogun et al., 2015). Consequently, adopting a processing technique like fermentation has been considered to be an ideal approach that reduces the tannin and saponin contents and equally improves the nutritive value of such feed (Reddy and Pierson, 1994; Dei et al., 2008; Agbo and Prah, 2014; Matthew et al., 2018). Moreover, application of appropriate supplemental feed additives such as enzymes and probiotics in feed formulation has been documented to enhance efficient utilization of poor quality feed or agro-industrial residues (Nawaz et al., 2016; Abd El-Hack et al., 2017; Kirkpinar et al., 2018; Sunmola et al., 2019).

Previous studies have indicated a beneficial effect of dietary inclusion of multi-enzymes and probiotics in improving live weight, feed efficiency, carcass yield and normalize gut activity by improving the microbial balance in the digestive tract of chickens (Mountzouris et al. 2010; Khan et al., 2011; Seifi, 2013; Wealleans et al., 2017; He et al., 2019). Although the application of enzymes and probiotic in poultry ration has gained wide attention, until recently, there is dearth information on combination of cocktail of enzymes and probiotic addition in diet of broiler chickens containing agroforestry by-product such as SBC that has been processed through fermentation technique. Therefore, the present work was conducted with the aim to evaluate the combined effects of multi-enzymes and *Lactobacillus acidophilus* probiotic supplementation on carcass evaluation, prime-cuts yield and serum biochemistry of broiler finisher chickens fed fermented shea butter cake meal based diets.

Material and methods

Study area

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production Technology, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located at longitude 9° 81' 95'' N and 9° 49' 10'' N and latitude 4° 58' 05'' N and 4° 34' 49'' N in the Savanna Areas of Niger Basin, North Central Zone of Nigeria.

Source and processing of test ingredient

The shea butter cake (SBC) was collected fresh from shea butter processing factories in New Bussa and transferred into a jute bag, which was further wrapped in large black polythene sacks to exclude air and fermented for seven days according to the procedures of Achi (1992). Thereafter, the fermented SBC were sun-dried to a constant weight, milled using hammer mill to produce FSBCM. The sample of fresh and FSBCM were subjected to proximate analysis according to the method of AOAC (2010) and the tannin composition was determined as outline by the procedure of Orogun et al. (2015) (Table 1).

Table 1. Proximate composition of fresh and fermented shea butter cake

Nutrients	Fresh SBC	Fermented SBC
Dry matter, %	94.80	95.04
Ash, %	6.20	5.51
Crude fiber, %	10.99	7.96
Crude protein, %	13.28	15.78
Crude fat, %	14.25	7.51
Nitrogen free extract, %	55.28	59.94
Tannin, mg/g	0.16	0.08

*SBC- Shea butter cake

Experimental birds, design and management

A total of two hundred and sixty (260), 28-day-old broiler chicks were distributed into four treatments, with five replicates of 13 birds per replicate using a completely randomized design. The birds were reared in a standard tropical open-sided and well-ventilated poultry facility under deep litter system using wood shaving as litter material. The standard routine management, vaccinations and medications were observed accordingly; feed in mash form and clean water were offered *ad-libitum* throughout the experiment. The experimental procedures of this current study complied with the provisions of the Ethical Committee on the use of animals of the Federal College of Wildlife Management for biometric research.

Experimental diets

Four experimental diets were formulated to meet the recommended requirements for nutrients of the birds during the finisher (d 28 to 56) phases (NRC, 1994; Table 2). Diet 1 (T_1) served as the positive control containing maize-soybean meal based, diet 2 (T_2) was made to be negative control containing fermented shea butter cake meal (FSBCM) replacing 20% of maize in T_1 diet, while diets 3 (T_3) and 4 (T_4) contained multienzymes+probiotic (MEP) supplemented to the T_2 at 0.05% and 0.10%, respectively. The compositions of MEP are protease, xylanase, lipase, and phytase with *Lactobacillus acidophilus* probiotic.

Data collection

At 56 day of age, two birds were randomly selected from each replicate on weight equalization basis and fasted overnight prior to collection of blood. Blood samples were collected from each of them through the jugular vein (*v. jugularis*) into specimen bottles without anti-coagulant (EDTA) for serum indices determination. Thereafter, the serum obtained after the blood was allowed to stand for 45 min at room temperature was centrifuged at 3.000 revolutions per minute (rpm) for 10 min to separate the cells from the serum. Blood samples placed in the specimen bottles were used to analyze the serum biochemical parameters such as total protein, albumin, glucose, cholesterol, creatinine, urea and the activity of ALAT and ASAT as outlined by Ochie and Kolhatkar (2000). Blood plasma globulin was calculated by subtracting the level of blood plasma albumin from that of total protein. Additional two birds with body

weights representing average pen body weight were selected, slaughtered and hand eviscerated. After the evisceration, the carcass yield and dress percentage were obtained in relation to the live body weight, whereas the yield for the prime cut-parts and internal organs were calculated in function of the carcass weight according to the procedures of Dalólio et al. (2016).

Table 2. Gross composition of experimental diets containing MEP supplemented fermented shea butter cake meal (FSBCM) based diets

Ingredients	T1	T2	T3	T4
Maize, %	58.85	46.85	46.80	46.75
Soybean meal, %	31.00	30.00	30.00	30.00
Fish meal, %	3.00	2.70	2.70	2.70
FSBCM, %	0.00	12.00	12.00	12.00
Vegetable oil, %	2.70	4.00	4.00	4.00
Di-calcium phosphate, %	2.00	2.00	2.00	2.00
Limestone, %	1.00	1.00	1.00	1.00
Salt, %	0.25	0.25	0.25	0.25
Vitamin-Premix, %	0.25	0.25	0.25	0.25
Mineral-Premix, %	0.25	0.25	0.25	0.25
DL-Methionine, %	0.30	0.30	0.30	0.30
Lysine, %	0.20	0.20	0.20	0.20
Threonine, %	0.20	0.20	0.20	0.20
MEP, %	0.00	0.00	0.05	0.10
Total, %	100	100	100	100
Calculated Nutrients				
Crude protein, %	20.23	20.43	20.43	20.43
Crude fiber, %	6.98	5.77	5.76	5.74
Crude fat, %	3.44	4.63	4.65	4.67
Energy, kcal/kg	3089.88	3060.54	3060.53	3060.52

FSBCM: Fermented shea butter cake meal; MEP: Multienzymes+probiotic;
T_1: Positive control containing maize-soybean meal based;
T_2: Negative control containing FSBCM replacing 20% of maize in T_1 diet;
T_3 and T_4 contained MEP supplemented to the T_2 at 0.05% and 0.10% respectively.

Statistical analysis

All data were statistically subjected to one-way ANOVA using the GLM procedure of SAS software package (SAS, 2012). The difference between means was separated using Duncan's multiple range test. The level of significance was considered at $p<0.05$.

Results and discussion

Proximate composition

The proximate and tannin compositions of fresh and fermented SBC are shown in Table 1. The result showed that subjecting the fresh SBC through fermentation treatment, the proximate analysis revealed an increase in CP and nitrogen free extract with corresponding decrease in crude fiber, ash and crude fat content. The tannin composition was observed to decrease up to 50% in the fresh SBC after being subjected to a seven-day fermentation process. This result is in accordance with the report of previous researchers who claimed that fermentation could improve the nutritional potential of SBC and enhance its in-vivo bioavailability in poultry (Dei et al., 2008; Agbo and Prah, 2014; Matthew et al., 2017).

Carcass traits, prime cut-parts and organ qualities of experimental birds

The result of carcass and organs evaluation of birds fed FSBCM diets with MEP supplementation is presented in Table 3. Live weight and dressing percentage of birds fed MEP supplemented FSBCM based diets were similar with those offered T_1 diet but significantly higher ($p<0.05$) than the group fed FSBCM diet (T_2). Eviscerated carcass of birds fed control diet were higher ($p<0.05$) compared to birds fed MEP supplemented diets at 0.05 and 0.10% while the lowest ($p<0.05$) mean values were recorded in those groups fed FSBCM diet. Also, results revealed that the breast meat yield in broilers fed control and FSBCM diet with supplemental MEP were higher ($p<0.05$) than those groups fed un-supplemented MEP, FSBCM diets. Broilers fed control and FSBCM diets showed higher ($p<0.05$) abdominal fat yield when compared to those groups fed MEP supplemented diets. The result of the carcass traits in the present study depicted that birds on MEP supplemented FSBCM based diets had comparable live weight and carcass yield with those on the control compared to those on FSBCM diet without supplemental MEP. This observation concurs with previous findings which showed that addition of feed additives containing exogenous enzymes and probiotics had consistently increased the live weight and carcass attributes of birds (Altaf et al., 2009; Karaoglu et al., 2014; Nawaz et al., 2016). The improvements in carcass characteristics may be attributed to the beneficial impact of exogenous amylase, protease, and lipase as well as *Lactobacillus acidophilus* probiotics, which have the potential to enhance the activities of the endogenous

enzymes and promote beneficial intestinal microbiota to liberate more energy nutrients in the feed (Shim et al., 2010; Sen et al., 2012; Aguihe et al., 2017; Kırkpınar et al., 2018). Among the cut-up-parts, breast and drumstick yields were similar among the groups on control and MEP supplemented diets and significantly ($p<0.05$) higher than the group fed FSBCM based diet without MEP. In line with the present result, previous studies (Brzóska et al., 2012; Sangilimadan et al., 2014; Ebu et al., 2019) reported increased slaughter weight, dressing percentage, breast and drumstick yield of broilers fed diet with the enzymes and probiotics supplementation. The possible mechanism through which MEP achieved this improvements are demonstrated by their ability to enhance synthesis and bioavailability of nutrients (Koop-Hoolihan, 2001; Karaoğlu et al., 2014), and accompanying with positive effects of increasing digestive enzymes on intestine activity, thereby promoting growth of muscle tissues (Endens, 2003; Santos et al., 2017). Supplementing MEP to the diets of broilers did not affect the weights of organs except for abdominal fat. These results agreed with the findings of previous workers (Salarmoini and Fooladi, 2011; Nawaz et al., 2016; Kirkpinar et al., 2018) who observed that relative weight of organs was not influenced by birds fed multi-enzymes and probiotic supplemented diets. The present study showed that abdominal fat was decreased on inclusion of FSBCM based diet containing supplemental MEP. This result may be attributed to the reducing effect of probiotics on fat deposition (Mohan et al., 1996; Kalavathy et al., 2003; Priya and Babu, 2013). According to Corcoran et al. (2005), fat digestion rate is linked to the rate of gallbladder acids in digestion latex and subsequently the lipid concentration. It has also been documented that *Lactobacillus acidophilus* in diets causes a reduction in gallbladder acids and this resulted in a decrease of fat digestion (Corcoran et al., 2005; Getachew, 2016), and, therefore, could be responsible for decreased abdominal fat deposition in the carcass. The addition of exogenous enzymes in the feed of broilers probably improves nutrient digestibility efficiency and promotes greater carcass yield and meat deposition (Allouche et al., 2015).

Table 3. Carcass and organ characteristics of broiler finisher chickens fed experimental diets

Parameters	T1	T2	T3	T4	SEM	P-values
Carcass traits						
Live weight, g	2100.50^{a}	1750.22^{c}	1975.76^{b}	1995.54^{ab}	28.74	0.042
Eviscerated carcass, g	1798.64^{a}	1456.25^{c}	1665.12^{b}	1698^{b}	12.09	0.137
Carcass yield, %	85.63	83.20	84.28	85.10	2.09	0.301
Dress percentage, %	95.14^{a}	85.08^{b}	93.52^{a}	94.11^{a}	1.80	0.026
Prime-cut parts (% of live weight)						
Breast	20.53^{a}	15.07^{b}	19.69^{a}	19.90^{a}	0.55	0.026
Thigh	10.57	10.65	11.08	10.47	1.68	0.697
Wing	8.65	8.94	8.74	9.08	3.61	0.987
Drumstick	16.93	15.80	16.55	17.38	1.73	0.050
Back	13.02	14.16	13.89	14.46	3.19	0.508
Relative weight of internal organs (% of live weight)						
Heart	0.52	0.45	0.38	0.34	0.45	0.604
Kidney	0.12	0.17	0.16	0.14	0.23	0.227
Liver	1.31	1.43	1.63	1.31	0.68	0.315
Lung	0.50	0.54	0.45	0.44	0.19	0.152
Bile	0.10	0.10	0.12	0.11	0.10	0.923
Abdominal fat	3.30^{a}	3.34^{a}	2.55^{b}	2.32^{b}	0.19	0.019
Spleen	0.03	0.07	0.05	0.07	0.07	0.269
Intestine	3.68	3.88	3.61	4.53	2.27	0.569
Pancreas	0.48	0.39	0.41	0.39	0.29	0.073
Gizzard	2.69	2.12	2.23	1.80	1.95	0.553

^{abc} Means on the same row with different superscripts are significantly different ($p<0.05$);
T_1: Positive control containing maize-soybean meal based;
T_2: Negative control containing FSBCM replacing 20% of maize in T_1 diet;
T_3 and T_4 contained MEP supplemented to the T_2 at 0.05% and 0.10%, respectively.

Serum biochemical indices of experimental birds

The blood biochemical indices of the broiler finishers fed experimental diets are presented in Table 4. The results showed that serum glucose, cholesterol, ASAT and ALAT were significantly ($p<0.05$) influenced by the dietary supplementation of MEP while other parameters did not show any significant ($p>0.05$) difference among the treatments. Serum glucose was higher ($p<0.05$) in birds fed control diet

but did not differ from those fed MEP supplemented diets while those birds fed FSBCM diet without MEP supplementation recorded the lowest (p<0.05) concentration of serum glucose. The higher serum glucose in the MEP supplemented groups could be explained by a higher absorptive capacity of the intestinal mucosa due to histomorphological changes and more effective digestion of the diet due to a higher intestinal enzyme activity, thus increasing the energy available to the animals (Wang and Gu, 2010; Aliakbarpour et al., 2012). Birds fed FSBCM diet recorded the lowest (p<0.05) serum cholesterol, followed by those on the control diet while those on FSBCM diets supplemented with MEP had lower (p<0.05) concentration of serum cholesterol. The significant decrease in blood cholesterol of broilers fed MEP diets could be attributed to lowered absorption of cholesterol in the gastro-intestinal tract due to enzyme and probiotic fortification (Mohan et al., 1996; Mansoub, 2010; Fathi, 2013; Chuka, 2014). In addition, previous studies have demonstrated the potential of *Lactobacillus acidophilus* in reducing serum cholesterol levels by de-conjugating bile salts in the intestine, thereby hindering them from acting as precursors in the cholesterol synthesis (Abdulrahim et al., 1996; Surono, 2003; Alkhalf et al., 2010). Another mechanism explaining the depressing effect of probiotic microbes on serum cholesterol is linked to the inhibition of hydroxymethyl-glutaryl-coenzyme A, the rate-limiting enzyme of cholesterogenesis, thus reducing the synthesis of cholesterol in the blood pool (Kalavathy et al. 2003; Pourakbari et al., 2016).

Table 4. Serum biochemistry of broiler finisher chickens fed experimental diets

Indices	T1	T2	T3	T4	SEM	P-values
Glucose, mg/dl	120.30^{a}	113.68^{b}	125.73^{a}	122.39^{a}	3.81	0.045
Total Protein, g/dl	5.81	7.66	6.49	6.90	1.83	0.541
Albumin, g/dl	3.48	4.56	3.79	3.03	1.75	0.669
Cholesterol, mg/dL	145.45^{b}	158.89^{a}	129.61^{c}	124.03^{c}	16.66	0.015
Globulin g/dL	2.33	3.10	2.70	3.86	1.21	0.321
Creatinine, mg/dL	1.63	1.43	1.59	1.65	1.81	0.532
Urea mg/dl	80.09	88.16	82.75	80.62	7.80	0.233
ASAT, iu/L	272.28^{b}	308.30^{a}	243.88^{c}	247.19^{c}	0.39	0.011
ALAT, iu/L	75.00^{ab}	88.00^{a}	60.00^{bc}	65.50^{c}	6.73	0.049

^{abc}: mean with difference superscript are significantly different (P<0.05);
T_1: Positive control containing maize-soybean meal based;
T_2: Negative control containing FSBCM replacing 20% of maize in T_1 diet;
T_3 and T_4 contained MEP supplemented to the T_2 at 0.05% and 0.10%, respectively;
ALAT- Alanine aminotransferase; ASAT- Aspartate aminotransferase.

The ASAT and ALAT concentrations in the blood were lower (p<0.05) in birds fed FSBCM based diet with MEP supplementation and were similar to those in the control group but differed (p<0.05) from those fed FSBCM based diet. The decrease in these liver enzymes in the blood in the current study is in agreement with earlier reports that supplementation of probiotics significantly reduced ASAT and ALAT concentrations in the blood of broilers (Yalcinkayal et al., 2008; Salarmoini and Fooladi, 2011; Fathi, 2013; He et al., 2019). According to Fathi (2013), ASAT and ALAT activities of the serum may indicate the state of liver performance and reduction in their levels is an indication of non-pathological metabolism of the liver. The non-significant influence of MEP supplementation on total protein, albumin, creatinine and urea is in accordance with the report of Sarcia et al. (2005) and Sherif (2009) who found that these blood parameters were not affected by adding probiotic and enzyme preparation in the diets of broilers.

Conclusion

It was evident from this study that fermentation improved the nutritive value of shea butter cake by increasing its crude protein content and reducing its crude fiber level in the raw cake, thereby making it a potential and valuable feed ingredient in poultry rations. Moreover, supplementation of the shea butter cake meal based diet with cocktail of enzymes and probiotics showed an improved live body weight and carcass trait without any detrimental impact on blood composition of broiler finisher chickens.

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