

## Effects of Treatment Methods on the Nutritional Value of Cotton Seed Cake for Laying Hens

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**Abstract:** The effects of treatment methods on the nutritive value of Cotton Seed Cake (CSC) for laying hens were examined. Olympia laying hens (n = 96) were assigned randomly to a 2×3 factorial combination of fermented or unfermented CSC supplemented with enzyme, Vitamin E and ferrous sulphate over a 4 week feeding period. CSC was incorporated at 15% in the diets. Results showed no significant differences (p>0.05) in feed intake, body weight gain and egg weight due to treatments. However, hen day egg production was significantly reduced (p<0.05) among dietary treatments compared with the control. Layers fed on unfermented CSC+Vitamin E and unfermented CSC+FeSO<sub>4</sub> had the lowest (p<0.05) values for packed cell volume and haemoglobin. Treatment differences in red blood cell and white blood cell were not significant (p>0.05). Serum concentration of total protein among dietary treatments was lower (p<0.05) than the control while albumin and cholesterol values were similar (p>0.05) for all treatments. Generally, activities of alanine and aspartate aminotransferases increased (p<0.05) among CSC dietary treatments compared with the control diet. The interaction between CSC form and additives had no significant effect on any of the parameters. The results of this study indicate that the treatment methods employed for CSC could not significantly improve its nutritive value for egg production at 15% level in the diet of laying hen.

**Key words:** Cotton seed cake, haematology, serum constituents, laying hen, enzyme, Nigeria

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### INTRODUCTION

One major limiting factor in the expansion of egg production enterprise is the high cost of feedstuffs such as soybean meal, groundnut cake and fish meal. This has made egg production expensive, thereby worsening the intake of protein sources by the populace of developing countries. One approach to reduce pressure on conventional protein ingredients is greater utilization of alternative sources of dietary protein alongside other strategies.

Cotton Seed Cake (CSC) is a by-product of the cotton processing industry. The cake is rich in protein (42%) but it contains gossypol which has been recognized since the turn of the century to be toxic to animals (Morgan, 1989; Risco and Chase, 1997). In monogastric animals, gossypol interfered with protein digestion, bind lysine (making it unavailable) and reduced growth rate and productivity (Ikurior and Fetuga, 1984; Calhoun *et al.*, 1990).

There is another problem as the cake contains a high level of fibre, perhaps 23% because undelinted seeds are used in the processing (Rinchard *et al.*, 2003). This high fibre content has limited the extent to which

it can be used in the diets of poultry which lack the appropriate enzymes capable of degrading fibre (Danicke *et al.* 1999; Babalola *et al.*, 2006).

Currently, CSC is used by feed millers in Nigeria at 7.5% inclusion level with ferrous sulphate supplement to inactivate gossypol in balanced layers feed. This chemical is usually not available to local farmers at farm sites. It becomes imperative therefore that simple treatment method be found that will overcome the gossypol and degrade the high fibre in CSC so that its nutritive value could be enhanced. Vitamin E is an antioxidant that prevents the damage of free radicals at the cellular level (Risco *et al.*, 2002).

This study was designed to investigate the effectiveness of enzyme, vitamin E and ferrous sulphate treatments in improving the nutritive value of CSC for laying hens.

### MATERIALS AND METHODS

Cotton seed cake was obtained from a feed miller in Ilorin. Samples (20 kg) of the cake were placed in a double layer polythene bag, tied up and placed in an empty plastic drum, sealed and fermented for a period of 7 days.

**Table 1: Composition of the experimental diets (%)**

Ingredient	Unfermented cotton seed cake			Fermented cotton seed cake			Control
	1	2	3	4	5	6	7
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat bran	12.64	12.62	12.45	13.14	13.12	12.95	15.85
Soybean meal	2.50	12.50	12.50	12.50	12.50	12.50	27.50
Cotton seed cake	15.00	15.00	15.00	15.00	15.00	15.00	0.00
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Blood meal	3.50	3.50	3.50	3.00	3.00	3.00	0.30
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Enzyme	0.01	-	-	0.01	-	-	-
Vitamin E	-	0.03	-	-	0.03	-	-
FeSO <sub>4</sub>	-	-	0.20	-	-	0.20	-
<sup>b</sup> Chemical composition (%)							
Crude protein	18.08	18.10	18.30	18.07	18.05	18.20	18.00
Crude fibre	5.36	5.31	5.32	5.34	5.30	5.34	4.71
Ether extract	4.01	4.01	4.01	3.92	3.92	3.92	3.89
ME (Kcal kg <sup>-1</sup> )	2623.00	2623.00	2623.00	2625.00	2625.00	2625.00	2624.00
Lysine	1.17	1.17	1.17	1.19	1.19	1.19	1.22
Methionine	0.52	0.52	0.52	0.53	0.53	0.53	0.49

\*Provided 1.5 mg retinol, 25 mg cholecalciferol, 16 mg α-tocopherol, 1 mg menadione, 0.8 mg thiamine, 2.4 mg riboflavin, 14 mg nicotinic acid, 4 mg calcium D-pantothenate, 1.4 mg pyridoxine, 10 mg cyanocobalamin, 0.4 mg folic acid, 0.02 mg biotin, 120 mg choline chloride, 0.62 mg zinc bacitracin, 36 mg avaten, 40 mg Mn, 18 mg Zn, 0.8 mg Cu, 0.09 mg Co, 20 mg Fe and 0.04 mg Se kg<sup>-1</sup> diet. <sup>b</sup>Determined values except for Metabolisable Energy (ME), lysine and methionine which were calculated from the published (NRC, 1995) compositions of the ingredients used

Thereafter, the fermented cake was removed and sundried for 3 days. The fermented and a batch of the unfermented cake (20 kg) were used in preparation of the diets described below.

Seven isonitrogenous (18% crude protein) and isoaloric (2623 kcal kg<sup>-1</sup> ME) experimental diets (Table 1) were formulated by incorporating fermented or unfermented CSC at 15% and supplemented with enzyme, vitamin E and Ferrous Sulphate (FeSO<sub>4</sub>) at 0.01, 0.03 and 0.20%, respectively in a 2×3 factorial design. Maize soybean meal-based diet was used as control. The enzyme preparation (Allzyme) derived from *Aspergillus niger* contains amylase, β-glucanase, cellulase, pectinase, phytase, protease and xylanase activities. The level of enzyme supplementation was chosen according to manufacturer's recommendation.

About 96 hens of the Olympia strain that had been in lay for 10 weeks were purchased from a commercial poultry farm in Ilorin. The birds were individually caged in a poultry pen which was illuminated at night and were randomly assigned to the dietary treatments. The trial lasted for 4 weeks. Feed intake was measured daily and body weight weekly.

Records of egg production per cage were kept daily throughout the experimental period. All eggs laid each day were weighed individually. At the end of the experimental period, 4 hens were randomly selected from

each dietary treatment, fasted overnight and blood samples collected from the wing vein. Blood was collected into bottles containing EDTA anticoagulant for haematology and clean dry bottles for serum constituent analysis. Serum samples were separated by centrifugation at 1600×g for 15 min and stored at -20°C. The whole blood was analysed for Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell count (RBC) and White Blood Cell count (WBC) using standard haematological techniques (Lewis *et al.*, 2001). Serum total protein, albumin, cholesterol, alanine amino transferase (ALT, EC 2.6.1.1) and aspartate amino transferase (AST, EC 2.6.1.1) activities were measured using commercial clinical investigation kits (Wako, Osaka, Japan).

**Statistical analysis:** Data collected were subjected to a two-way factorial analysis and Duncan's multiple range test to determine the significant difference at the 0.05 level (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

Laying performance of hens was affected by dietary CSC treatments at the end of the feeding trial (Table 2). There were no significant differences (p>0.05) in feed intake, weight gain and egg weight due to treatments. However, hen-day egg production was significantly

**Table 2: Performance of laying hens fed diets containing different treatments of unfermented or fermented Cotton Seed Cake (CSC)**

Dietary treatments	Feed intake (g per hen day <sup>-1</sup> )	Weight gain (g per hen day <sup>-1</sup> )	Hen-day egg production (%)	Egg weight (g)
Unfermented CSC+enzyme	83.0 <sup>a</sup>	3.4 <sup>a</sup>	45.7 <sup>b</sup>	54.6 <sup>b</sup>
Unfermented CSC+Vit. E	86.7 <sup>a</sup>	3.5 <sup>a</sup>	44.3 <sup>b</sup>	54.4 <sup>b</sup>
Unfermented CSC+FeSO <sub>4</sub>	81.0 <sup>a</sup>	2.9 <sup>a</sup>	43.5 <sup>b</sup>	53.2 <sup>b</sup>
Fermented CSC+enzyme	85.8 <sup>a</sup>	3.9 <sup>a</sup>	56.7 <sup>ab</sup>	56.7 <sup>b</sup>
Fermented CSC+Vit. E	82.5 <sup>a</sup>	3.1 <sup>a</sup>	53.1 <sup>b</sup>	56.4 <sup>b</sup>
Fermented CSC+FeSO <sub>4</sub>	78.6 <sup>a</sup>	3.4 <sup>a</sup>	52.8 <sup>b</sup>	55.0 <sup>b</sup>
Control	106.5 <sup>b</sup>	5.6 <sup>b</sup>	67.0 <sup>a</sup>	60.1 <sup>a</sup>
F×A	NS	NS	NS	NS
SEM	7.2	1.1	6.4	3.8

Values in a column with the same letter do not differ significantly at  $p < 0.05$ . NS = Not Significant; SEM = Standard Error of Means; F = Test ingredient form; A = additives

**Table 3: Haematological values of laying hens fed diets containing different treatments of unfermented or fermented Cotton Seed Cake (CSC)**

Dietary treatments	PCV (%)	Hb (g dL <sup>-1</sup> )	RBC ( $\times 10^{12}$ L <sup>-1</sup> )	WBC ( $\times 10^9$ L <sup>-1</sup> )
Unfermented CSC+enzyme	24.0 <sup>ab</sup>	8.1 <sup>ab</sup>	4.37	9.67
Unfermented CSC+Vit. E	18.0 <sup>b</sup>	4.3 <sup>a</sup>	3.50	11.30
Unfermented CSC+FeSO <sub>4</sub>	18.5 <sup>b</sup>	5.1 <sup>a</sup>	4.09	10.85
Fermented CSC+enzyme	27.0 <sup>a</sup>	7.8 <sup>ab</sup>	4.00	12.20
Fermented CSC+Vit. E	20.0 <sup>b</sup>	6.0 <sup>ab</sup>	4.03	9.97
Fermented CSC+FeSO <sub>4</sub>	22.3 <sup>b</sup>	7.1 <sup>ab</sup>	4.10	9.60
Control	30.0 <sup>a</sup>	9.1 <sup>b</sup>	4.90	9.10
F×A	NS	NS	NS	NS
SEM	1.6	0.41	0.19	1.02

Values in a column with the same letter do not differ significantly at  $p < 0.05$ . PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell. NS = Not Significant; F = tests ingredient form; A = Additives, SEM = Standard Error of Means

reduced ( $p < 0.05$ ) among dietary treatments compared with the control. The highest egg production (56.7%) in CSC diets achieved by hens fed fermented CSC+enzyme was lower ( $p < 0.05$ ) than the control (67.00%). Layers feed consumption and body weight gain were unaffected by treatment methods, probably because the fermentation process and the additives used in the diets were unable to cause appreciable reduction in the gossypol level. The feed consumed by the layers appeared to be principally used for body maintenance. This view is similar to that reported by Degen; Oloredo and Longe (2000) that hens can use feed or metabolizable energy intake to fulfill maintenance requirement, thereby leaving little for egg production.

In general, the decrease in egg production observed with dietary treatments of CSC might be the consequence of residual effect of gossypol that binds with the cake's protein thereby making it unavailable to the hens for egg production. The marginal improvement found with the fermented cake+enzyme may be attributed to the combination of fermentation process and enzyme degradation of the fibre that made nutrients available to the birds. This is in agreement with Babalola *et al.* (2006) who reported improved nutrient availability to pullet chicks fed  $\beta$ -xylanase supplemented castor seed meal diet.

The haematological values are shown in Table 3. For all treatments, PCV and Hb were lower than the control with the exception of fermented CSC+enzyme which had

similar PCV value with the control. No significant differences ( $p > 0.05$ ) were observed for RBC and WBC. The decreases in PCV and Hb values in hens fed diets containing unfermented CSC or fermented CSC+Vitamin E or ferrous sulphate, suggest inadequate nutrient utilisation in the hens. This may be partly due to the residual effect of gossypol on blood variables. A reduction in haematological parameter has been reported by Apata (2004) and Rinchar *et al.* (2003) in chicks and fish fed diets containing high levels of legume seed meal and cotton seed meal, respectively. However, Mitruka and Unfermentednsley (1977) reported 7.00-18.6 g dL<sup>-1</sup> as normal values of Hb in chickens. Thus, a range of 4.3-5.1 g dL<sup>-1</sup> obtained in this study for unfermented CSC+Vitamin E or FeSO<sub>4</sub> fell short of the range consistent with good layers health.

Of the serum biochemical constituents, total protein among dietary treatments was lower ( $p < 0.05$ ) than the control, whereas the ALT and AST were higher ( $p < 0.05$ ) than the control (Table 4) with no significant differences ( $p > 0.05$ ) observed in albumin and cholesterol values for all treatments. Decrease in serum total protein concentration irrespective of the treatment method corroborates the inadequacy of these simple technologies in improving the nutritional quality of this feedstuff especially at the level of inclusion. The increase in serum ALT and AST activities indicate release of the aminotransferases from cytoplasm to blood stream which is probably due to liver

Table 4: Serum biochemical constituents of laying hens fed diets containing different treatments of unfermented or fermented Cotton Seed Cake (CSC)

Dietary treatments	Total protein (mmol L <sup>-1</sup> )	Albumin (mmol L <sup>-1</sup> )	(Cholesterol mmol L <sup>-1</sup> )	ALT (IU L <sup>-1</sup> )	AST (IU L <sup>-1</sup> )
Unfermented CSC+enzyme	23.00 <sup>b</sup>	5.30	1.80	8.67 <sup>b</sup>	118.00 <sup>b</sup>
Unfermented CSC+Vit. E	26.00 <sup>b</sup>	6.00	2.50	12.00 <sup>b</sup>	116.00 <sup>b</sup>
Unfermented CSC+FeSO <sub>4</sub>	22.00 <sup>b</sup>	5.50	1.50	20.00 <sup>c</sup>	216.00 <sup>d</sup>
Fermented CSC+enzyme	24.00 <sup>b</sup>	5.00	1.50	19.33 <sup>c</sup>	163.00 <sup>c</sup>
Fermented CSC+Vit. E	27.00 <sup>b</sup>	5.30	1.50	19.33 <sup>c</sup>	163.00 <sup>c</sup>
Fermented CSC+FeSO <sub>4</sub>	26.00 <sup>b</sup>	5.30	2.10	13.67 <sup>b</sup>	134.00 <sup>c</sup>
Control	31.00 <sup>a</sup>	4.00	1.10	4.00 <sup>a</sup>	90.00 <sup>a</sup>
F×A	NS	NS	NS	NS	NS
SEM	1.08	0.67	0.90	1.06	13.86

Values in a column with the same letter do not differ significantly at p<0.05. AST = Aspartate aminotransferase activity; ALT = Alanine aminotransferase activity, NS = Not Significant; F = Tests ingredient form; A = Additives, SEM = Standard Error of Means

and/or other tissues damage. A similar observation has been reported by Muhammad and Oloyede (2010) for chicks fed fermented *Terminalia catappa* seed meal based diet.

### CONCLUSION

In this study, the results show that the different treatment methods employed for CSC could not significantly improve its nutritive value for egg production at 15% level in the diet of laying hen.

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