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Effects of Graded Replacement of Soyabean cake by *Aspergillus niger* Treated Jatropha Seed cake on Performance and Egg Quality in Laying Hen Diets

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Abstract

Poultry feed ingredients are becoming more scarce and expensive globally. Additionally, Soyabean cake which has been recognised as source of protein in poultry diet is also becoming very expensive due to the competition for it between humans and farm animals. Hence, there is the need to look for an economic alternative protein sources which do not attract such competition. This study was conducted to evaluate the effects of graded replacement of soyabean cake by *Aspergillus niger* treated Jatropha seed cake on performance, egg quality, weight gain, feed intake, nutrient retention and Feed Conversion Ratio (FCR) in Harco Black hens. Fifty point of lay hens were randomly allocated to 5 groups of 10 birds. Each group was made up of 2 replicates subgroup with 2 birds' each in a completely randomised design model. The *Aspergillus niger* treated Jatropha seed cake was included in pullet diet at 0 (A, control), 25 (B), 50 (C), 75 (D) and 100% (E) respectively *in lieu* of soyabean cake. Feed and water were offered *ad-libitum* for a 100 day period Feed intake, weight gain, nutrient retention and egg analysis were recorded on weekly basis. After three weeks of feeding experimental diet, 3 eggs per treatment were randomly selected on weekly basis to examine the egg quality traits of the bird. Result of the study showed that birds on Diet B (5% Aspergillus Treated Jatropha Seed cake) recorded the highest feed intake and weight gain followed by Diet A (Control diet) . The least feed intake and weight gain was reported on animals fed Diet E. The Feed Conversion Ratio (FCR) was better for birds on fungus treated diets compared to the Control Diets ith Diet E recorded the best performance in terms of FCR. Birds on Diet B had the highest Haugh Unit and yolk height while the yolk colour also improved slightly in Diet B compared with other Diets. This is a merit to consumer due to its rich xanthophylls. Thus, it could be feasible to use fungus treated Jatropha seed cake in pullets diet as an alternative ingredient replacing soyabean cake without affecting performance or egg quality while reducing the production cost and enhancing FCR.

Key words: Fungus treated Jatropha seed cake, pullet, feed intake, weight gain, nutrient retention, egg quality**.**

Introduction

Commercial poultry production is becoming more popular with developing countries while protein is one of the very expensive components of poultry diets (Velmurugu, 2015) . Soyabean cake is the preferred protein source due to its nutritional value but it cotinuous utilization is threatened by its cost per kilogram as well as competition between humans and farm animals. The best alternative ingredient today which has potential as low- cost protein source is Jatropha seed cake due to its high protein content (Belewu and Sam, 2010). *Jatropha curcas* is an underutilised plant while the cake from the seed is a good source of inexpensive protein for poultry due to the protein content and high amino acids content. However, there is paucity of information on its utilization in poultry diet. While its inclusion in ruminant animal diets are well documented in literature (Belewu and Akande, 2010., Belewu *et al*., 2011., Belewu *et al*., 2013., Belewu et al., 2014) .

Additionally, there is a need to explore the use of non-conventional feed sources that have the capacity to yield the same output as conventional feedstuff and perhaps at a cheaper cost. The strategy is to identify locally available feed resources to formulate diets that are as balanced as possible (Gueye and Branckaert, 2002) with nutrient. This will help to reduce the cost of feed and also minimize the direct competition between humans and farm animals for the availability of conventional feedstuffs.

Fungi treated *Jatropha curcas* kernel cake contains the following chemical composition: Crude protein 48.18% (*Penicillium spp*), 49.22%(*Rhizopus oligosporum), 52.49% (Rhizopus. nigians*), 65.75% (*Aspergillus niger*) , 63.06% *(T. Longibrachitum); Ether*

extract 12% ((Penicillium spp),12.55% (*Rhizopus oligosporum),16.20%(Rhizopus. nigicans*), 10.70% (*Aspergillus niger*),11.75% *(T. Longibrachitum)* (Belewu and Sam., 2010).

The anti-nutritional factors present in the cake includes saponins, tannins, lectins, curcin, trypsin inhibitors, phytates and phorbolester (Aregheore et al., 1998). The most difficult among the anti-nutrients is the phorbolester which could withstand higher temperature and which hinders its inclusion in livestock feed (Aderibigbe *et al*., 1997., Belewu *et al*., 2010). Diarrhoea, reduced water intake , dehydration, sunken eyes, skin irritation, loss of appetite and loss in condition are vital signs of phorbolester poisoning in small ruminant (Ahmad and Adams, 1979). Therefore, there is the need to find methods of removing these substances fro the seeds /cake .

Some workers (Adolf *et al.,* 1984. Becker, 1996. Aderibigbe *et al*., 1997. Aregheore *et al* 1998) used various methods (heat, chemical, physical and mechanical) to detoxified the cake but with no encouraging results..

Belewu (2008) , Belewu and Akande (2010)., Belewu *et al* . (2010, 2011 and 2013) reported on the use of biological method for detoxifying the cake with encouraging results.

The use of the fungus treated diets on the performance of ruminant animals is well documented (Belewu *et al*., 2013; Belewu *et al.,* 2014). Additionally, Oyebode (2015) reported the inclusion of biochemically treated Jatropha kernel cake in the pelleted diets of cat fish (*Claris gariepinus*) with encouraging results and without recording any mortality nor affecting the health of the fish. However, there is paucity of information on the inclusion of Jatropha seed cake in the diet of poultry.

Therefore, the thrust of this study was to determine the performance characteristics of pullet fed *Aspergillus niger* treated *Jatropha curcas* seed cake diet.

MATERIALS AND METHODS

SITE OF EXPERIMENT

The experiment was conducted at University of Ilorin Teaching and Research Farm, Kwara State, Ilorin. University of Ilorin is located at latitude $8^0.48''$ longitude 4.32 and elevation of 330m above sea level.

COLLECTION OF *JATROPHA CURCAS* SEED

Dried matured seeds of *Jatropha curcas* were bought from Jatropha plantation, the University of Ilorin, main campus, Ilorin, Kwara State, Nigeria. The seeds were screened to remove the dirt, dried and milled using a mechanical grinder (model 1107). Later pressed to expel the oil so as to obtain the cake.

PREPARATION OF SEEDS:

The seed cake was soaked in petroleum ether as a solvent (till submerged) inside an air tight container to avoid evaporation of the reagent for 24 hours and to remove remaining oil that might still be present in the cake after pressing. After 24 hours, the solvent containing the oil was then separated from the seed cake with the aid of muslin cloth. The defatted seed cake was kept in polythene bags and later autoclaved at 121^0C, 15psi for 30 minutes .

FUNGUS USED

The fungus used was *Aspergillus niger* which was cultured in the laboratory at the Department of Animal Production, Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria. The fungus was maintained on otato dextrose agar contained in petri dishes and kept in an incubator (37^{OC}) and in about 7 days the fungus enveloped the petri dishes and ready to be used.

INOCULATION AND INCUBATION:

After autoclaving the *Jatropha curcas* seed cake, it was cooled (20^{OC}) and then inoculated with the fungus (*Aspergillus niger)* 10^6 spore / ml and later incubated at (37^{OC}) room temperature. The fungus colonized the substrate after 7 days and the growth was terminated by oven drying the cake at 70^0C for 24 hours.

ANIMAL MANAGEMENT:

Fifty (50) Harco black Pullets (20weeks old) used for this study were bought from AFCOM FARMS, Ilorin, Kwara State, Nigeria. Before the start of the experiment, the cages, drinkers and feeders were cleaned, washed and disinfected using detergent for washing and Dettol as well as Morigad as disinfectants. The pullets were randomized against five (5) experimental Treatments containing Fungus treated Jatropha seed cake at 0(A), 25(B), 50(C), 75(D), 100%(E) with each having ten (10) pullets replicated five times. The cage, feeding and drinking troughs were cleaned on a daily basis while feed and water were supplied *ad-libitum* for 100days. Birds were housed in individual cages of dimension of 36 x 40cm providing 1,440cm^2/bird. The chemical composition of the fungus treated and untreated *Jatropha curcas* seed cake is shown on Table 1 and the composition of the experimental diets is presented in Table 2.

Sample Collection and Analytical Evaluation

The pullets were weighed before the commencement of the experiment and thereafter, weekly to determine the body weight changes during the experimental period.. Feed intake was recorded on a replicate basis at weekly intervals while egg production was collected daily during the study. Egg roduction was expressed as mean hen-day production, calculated from total eggs divided by the total number of hen days. Feed conversion ratio was expressed as grams of feed consumed per gram of egg produced. Eggs were evaluated for both the internal and external qualities.

EGG QUALITY MEASUREMENT

EGG WEIGHT

Egg production was determined by collecting egg laid per treatment every day. All eggs were sampled for weight by collecting the fresh egg per reatment per day, thoroughly

cleaned with a damp cloth to remove the faecal materials and feed smear which might affect the accuracy of the readings and it was then weighed using a sensitive scale.

ALBUMEN HEIGHT

Determination of internal egg quality was carried out on weighed egg. Three egg samples were randomly picked per treatment. The fresh eggs were gently cracked out on a flat non absorbent surface and the thick albumen was measured as it widest part as a position half between the yolk and the outer margin using a spyrometer. Albumen height was converted to Haugh units based on the calculation of Haugh (1937) thus:

$$H.U. = 100 \text{ Log } (H - \sqrt{G}\,(30w^{0.37} - 100 + 1.9))$$

100

H.U = Haugh unit

H = Albumen height in mm

G = 32.2

W = Weight of the whole egg in grams.

GG SHAPE INDEX

Egg Shape Index = Height/width x 100

SHELL THICKNESS

The two egg membranes were pulled off the shells immediately after being broken and emptying the content of the egg. The shell thickness was determined using micrometer screw gauge calibrated in millimetres.

YOLK HEIGHT

This was determined with the use of a spyrometer by placing the instrument gently on top of the yolk and the height read off in mm.

YOLK WIDTH

This was determined by placing a Vernier calliper at both edge of the yolk and the diameter read as width.

YOLK INDEX

Yolk index was calculated by dividing the yolk height with the yolk width.

$$\text{Yolk Index} = \frac{\text{Height of Yolk}}{\text{Width of Yolk}}$$

EGG YOLK COLOUR:

This was measured by using La Roche colour Yolk fan Scale

(Figure 1): **A Roche Color Yolk Fan**

ALBUMEN WIDTH

This was determined by placing a transparent ruler on the albumen and the diameter read off as a width.

ALBUMEN WEIGHT

The albumen was separated from the yolks and was measured on a scale and the weight was read in grams.

NUTRIENT RETENTION

At 22 weeks of age, the birds were subjected to a nutrient retention trial. Weighed quantities of feed was supplied and excreta samples were collected over a period of 72 hrs using the daily total collection method. The wet faecal weight was determined by weighing the faeces on a measuring scale. A portion was placed in the oven at 100^0C to determined the dry matter percentages after which the remaining faecal samples were oven dried at a temperature of 70^{0C}, weighed, milled and subjected to proximate analysis.

Nutrient retention (NR) was calculated as follows:

$$\text{Nutrient retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100\%$$

CHEMICAL ANALYSIS

The Proximate analysis of the diets and the faeces were determined using AOAC (1990) while the metabolizable energy was

determined using Carpenter and Clegg (1956) formular.

STATISTICAL ANALYSIS

Data collected was subjected to analysis of variance (ANOVA) using a Completely Randomized Design model (CRD). Means were separated using Duncan Multiple Range Test (1955) (DMRT).

RESULTS AND DISCUSSION

Performance

The production performance of the hens is presented on Table 4. There was no significant difference in body weight gain. Diets A (Control) and C recorded higher egg production compared to Diets B, D and E which are similar ($p>0.05$). Similar results were reported by Iyayi and Aderolu (2003) who fed biodegraded Brewer's Dried grain and Rice bran to laying hens. The body weight gain of the hens on the fungus treated diets was comparable to that in Diet A (Control). On the contrary, Belewu *et al*. (2011) attributed an increase feed consumption to the reduction in the anti-nutrient factors of the fungus treated diets (B –E) as well as the presence of microbial protein of these diets. Iyayi (2003) observed higher feed consumption of the commercial (Control diet) compared to the fungus treated diets. The same trend was observed in this study. The feed conversion reported in this study was better in Diets C, D and E compared to Diets A and B. However, Shi *et al. (2011)* reported similarity in the feed conversion ratio of all diets when sunflower was used to replace soyabean meal in the diet of laying hens. Shi *et al*. (2011) noted a reduced laying rate when laying hens were fed sunflower seed meal compared to the results reported herein. Shi *et al* .(2011) reported a reduction in the production and weight of eggs by hen fed diets containing sunflower seed meal . Nevertheless, egg production and egg weight were higher than previous reports (Shi *et al.,* 2011).

Egg Quality

Feeding diets supplemented with fungus-treated Jatropha seed cake did not influence mean egg weight, egg length, and yolk height and

Haugh unit. Similarly, Tsuzuki *et al* (2003) and Shi *et al.* (2011) noted no effect of the inclusion of graded levels of sunflower on Haugh unit. The albumen height was similar across all Diets of the present study. This showed that the anti-nutritional factors in the Fungus-treated Jatropha seed cake was reduced to tolerable and hence did not cause any effects on the albumen quality. The egg shell weight, egg shell thickness of pullet provided Diets C, D, and E were comparable to that in Diet A (Control).

Consumers perception of egg yolk colour is mostly linked to the geographical location , culture and tradition. Beardsworth and Hernandez (2004) in line with Hernandez *et al* .(2005) reported that in most parts of the world and specifically in European countries, consumers value a number of tangible characteristics of the egg (egg shell strength, albumen consistency and yolk colour). Hence, egg colour is a perception of its quality. However, it was noted from this study that birds fed Diet B had highest yolk colour and yolk width . This colour score indicated improved yolk colouration due to its rich xanthophylls. This visual assessment gives prompt unmistakable information and this corresponds better with the sensorial perception of egg yolk colour (Bovskova *et al*., 2014).

Nutrient Retention

The incorporation of the fungus treated Jatropha seed cake in the diets of laying hens had no significant effect on the results of nutrient retention (Table 5) . Similar results were reported by Simon *et al*. (1997) who noted no effect of the inclusion of 10% glycerol into low protein diets of broiler in an earlier experiment. The results of this study showed that fungus treated Jatropha seed cake included at up to 100% in the diet of layer had no effect on the nutrient retention. Finally, nutrient retention observed in this study was higher than those reported earlier in common carp (Kumar *et al.,* 2008). This shows that the inclusion of fungus treated *Jatropha* seed cake in diet of pullet is highly acceptable and maximized by the birds.

CONCLUSION AND IMPLICATIONS

In conclusion, the results of this study demonstrated that fungus treated Jatropha seed cake is a relatively rich source of protein for laying hens and could be included at the 100% level in the diet of laying hens without any detrimental effect on egg performance, egg quality parameters and nutrient retention.

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Table 1: Chemical Composition of Fungus treated and Untreated *Jatropha curcas* seed cake (%DM basis)

Parameters	Untreated seed cake	Fungus treated Seed cake
Dry matter	92.04	92.17
Crude fibre	40.76	23.07
Crude protein	15.59	20.36
Ether extract	46.44	33.62
Ash	5.49	8.03

Table 2: Ingredients Composition of the *Aspergillus niger* treated J*atropha curcas* Seed cake Based Diets

Ingredients	A	B	C	D	E
	0%	25%	50%	75%	100%
Maize	45.0	45.0	45.00	45.00	45.00
Corn Bran	10.0	10.0	10.00	10.00	10.00
Brewer's dried grains (BDG)	10.0	10.0	10.00	10.00	10.00
Wheat Offal	4.50	4.50	4.50	4.50	4.50
Fish meal (72%)	1.44	1.44	1.44	1.44	1.44
Soybean Cake	20.0	15.0	10.00	5.00	0.00
Fungus treated Jatropha	0.00	5.00	10.00	15.00	20.00
Bone meal	0.26	0.26	0.26	0.26	0.26
Oyster shell	8.00	8.00	8.00	8.00	8.00
*Vitamin / Mineral Premix	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.15	0.15	0.15	0.15	0.15
TableSalt	0.30	0.30	0.30	0.30	0.30
Total %	**100**	**100**	**100**	**100**	**100**

Table 3: Chemical Composition of the Experimental Diet

PARAMETERS %	DIETARY TREATMENT				
	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
Dry matter	92.13	91.08	90.25	90.99	90.98
Ash content	19.85	17.68	13.55	15.31	14.97
Ether extract	10.70	9.24	8.58	13.46	15.95
Protein	18.76	17.12	16.17	15.75	16.95
Crude fibre	12.02	14.32	18.08	21.76	20.32
Metabolizable energy(Kcal/Kg)	2942.17	2867.49	2848.64	2815.84	3061.12

Table 4: Effect of Graded Replacement of Soyabean cake by Fungus treated Jatropha Seed Cake on the Performance of Laying Hens

Parameters %	Dietary Treatment					
	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)	±SEM
Body weight gain (g)	115.30	114.50	110.52	111.22	114.73	6.74
Egg Production (%)	94.0^{a}	87.15^{b}	92.70^{a}	84.30^{b}	85.10^{b}	1.27
Daily egg weight (g/bird)	51.53^{b}	49.25	47.51^{a}	47.38^{a}	48.03^{a}	0.96
Daily Feed intake (g/bird/)	123.61	120.20	113.55	115.82	110.74	6.95
Feed Conversion Ratio (FCR)	1.64	1.75	1.26	0.70	0.61	

Means having similar superscript are not significant from each other (P>0.05)

Table 5 : Nutrient Retention of the Experimental Animals

Parameters (%)	Dietary Treatment					
	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)	±SEM
Dry matter	92.10	91.29	90.39	90.78	90.98	0.16
Crude protein	97.75	94.58	92.18	95.56	96.93	3.88
Ether extract	97.35	97.64	98.00	96.79	98.13	0.38
Ash	93.15	92.87	95.04	93.78	95.19	0.43
Crude fibre	97.97	98.23	98.92	97.47	98.51	0.28

Table 6: Effect of Different Inclusion levels of Fungus-treated *Jatropha curcas* Seed cake Based Diets on Egg quality Traits.

Dietary treatments	Parameters								
	Egg weight (g)	Egg shell weight (g)	Egg length (cm)	York height (mm)	Albumen height (mm)	Shell thickness (mm)	Yolk colour	Yolk width (mm)	Haugh unit
A (0%)	51.53	3.98^{a}	5.41	15.14	6.35^{a}	0.24^{a}	5.00^{a}	3.73^{a}	73.99
B (25%)	49.25	3.78^{c}	5.32	14.91	7.33^{a}	0.18^{b}	5.18^{a}	3.45^{b}	82.21
C (50%)	47.51	3.47^{b}	5.25	14.76	5.74^{a}	0.22^{ab}	3.85^{b}	3.49^{ab}	67.68
D (75%)	47.38	3.63^{a}	5.29	14.84	6.54^{a}	0.20^{a}	4.50^{a}	3.47^{a}	74.95
E (100%)	48.03	3.60^{a}	5.10	14.10	5.20^{a}	6.19^{a}	4.22^{a}	3.25^{a}	73.53
±SEM	0.97	0.06	0.04	0.26	0.37	0.009	0.22	0.05	3.48