Egg Quality Characteristics of Layers Fed Raw, Fermented, and Enzyme-Treated Cocoa Bean Shell-Based Diets.

Martha D. Olumide, Ph.D.[1]; Prof. A.O. Akinsoyinu[1]; and Prof. Rasheed A. Hamzat[*2,3]

[1]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.
[2]Crop Utilization Research Group, Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria (former).
[3]Department of Animal Production & Health, Federal University Dutsin-Ma, Katsina State, Nigeria.

E-mail: rahamzat6nov@yahoo.com*
Telephone: *+2348129236840*

ABSTRACT

One hundred and forty (140) six-week-in-lay hens were used for this research. The birds were randomly divided into seven treatment groups of 20 birds per treatment in a 2 x 3 factorial arrangement. These diets were: A {0% Cocoa Bean Shell (CBS) control}. B (5% raw CBS); C (10% raw CBS); D (5% CBS with enzyme); E (10% CBS with enzyme); F (5% fermented CBS); G (10% fermented CBS). The birds were offered feed and water *ad-libitum* throughout the experimental period. Production, feed efficiency, egg weight, egg shell thickness, Haugh Unit, yolk color, egg length, egg width, egg shape index, shell weight and shell surface area, yolk weight, yolk height, yolk width, albumen weight, albumen height, yolk color, and yolk index were determined. There were no significant differences (P > 0.05) in the values obtained for egg shape index, shell weight, shell thickness, albumen height, yolk color and Haugh Unit. Differences in egg quality parameters were not significant (P > 0.05). This research effort revealed that 10% enzyme-treated CBS could effectively, be included in layers' diet without any adverse effect on the performance and egg quality characteristics.

(Keywords: Cocoa bean shell; Feed resource; Layers; egg quality)

INTRODUCTION

Cocoa bean shell is a potential feed recourse and its utilization by animals will greatly reduce disposal problem facing cocoa processing industries. However, the occurrence of anti-nutrients, theobromine, and caffeine in plant parts of cocoa has limited the direct use of most of these products in livestock rations (Abiola and Tewe,1991). Cocoa bean shell contains 1.3 - 2.0% theobromine, which limit its use as animal feed ingredient (Abiola and Tewe,1991). Cocoa by products must be processed to reduce the theobromine content before they can be satisfactorily offered to livestock. The different methods of processing include boiling, drying, alkali, and urea treatment. Odunsi and Longe (1999) reported the effectiveness of alkaline and boiling treatment of cocoa bean cake in reducing theobromine level and improving performance of broilers. The exploitation of the potentials of cocoa bean shell appears rational and more economical than their disposal problems.

There is limited documented work on the potential use of cocoa bean shell as feed resource for broilers and layers. Olubamiwa *et al*, 2006, revealed that 15 minute boiling duration on cocoa bean shell is best for optimal and profitable utilization in layer's mash. The present study is focused on evaluating the egg quality characteristics of layers fed differently treated cocoa bean shell based diets.

MATERIALS AND METHODS

Experimental Birds: A total of one hundred and forty (140), six-week –in- lay Bovan Nera hens were used for this study. The study was carried out at the Teaching and Research Farm of the University of Ibadan, Nigeria, for a period of six weeks.

Management of Birds and Experimental Layout; The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected two tier cage system of 32 x 38 x 42 cm dimension. The birds were caged individually with 20 birds per treatment in a 2 x 3 factorial arrangement. The cocoa bean shell was sourced from the Cocoa Industries, Ikeja, Lagos, Nigeria, while other feed ingredients were purchased from a commercial feed miller in Ibadan, Nigeria. Seven diets were formulated as shown in Table 1. Diet A, (control diet) contained no test ingredient (CBS). Diets B and C contained raw cocoa bean shell (RCBS) with a 5% and 10% maize replacement respectively. Diet D and E contained 5 and 10% CBS with the inclusion of Rovabio enzyme (ECBS) while diet F and G also contained 5 and 10% fermented cocoa bean shell (FCBS).

The experimental birds were raised to point-of-lay on commercial diet, until when they were 6 weeks in-lay. The birds were given adequate medication and vaccination before the commencement of the experiment. The birds were weighed individually at the beginning of the experiment before they were placed on experimental diets. Feed and water were supplied adequately.

Data Collected and Analysis: Data were collected on performance parameters like average daily feed intake, feed conversion ratio, hen day production and egg weight. The internal and external egg quality that were monitored included egg length, egg width, egg shape index, shell weight, shell thickness, shell percentage, shell surface area, yolk weight, yolk height, yolk width, albumen weight, albumen height, yolk color, Haugh Unit, and yolk index. Egg collection was carried out thrice per day. Feed were weighed at the beginning of the week and left over (refusals) at the end of each week were monitored for the computation of average daily feed intake per bird. Proximate analysis and theobromine content of the diets were determined (AOAC, 1990).

Data collected were analyzed using descriptive statistics and analysis of variance (ANOVA) according to the Statistical Analysis software (SAS, 1999). The basal composition of the experimental diets is shown in Table 1.

Table 1: Gross Composition (g/100gDM) of Layers' Diets using Cocoa Bean Shell.

Ingredients	A (0%)	B (5%)	C(10%)	D (5%)	E (10%)	F (5%)	G(10%)
Maize	55.54	52.76	49.98	52.76	49.98	52.76	49.98
RCBS	-	2.78	5.55	-	-	-	-
ECBS	-	-	-	2.78	5.55	-	-
FCBS	-	-	-	-	-	2.78	5.55
SBM	12.13	12.13	12.13	12.13	12.13	12.13	12.13
GNC	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Wheat offal	12.11	12.11	12.11	12.11	12.11	12.11	12.11
Bone Meal	2.55	2.55	2.55	2.55	2.55	2.55	2.55
Oy. Shell	8.67	8.67	8.67	8.67	8.67	8.67	8.67
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Rovabio	-	-	-	0.05	0.05	-	-
Total	**100.00**	**100.00**	**100.00**	**100.05**	**100.05**	**100.00**	**100.00**

Calculated composition:	A (0%)	B (5%)	C(10%)	D (5%)	E (10%)	F (5%)	G(10%)
Met. Energy	2631.06	2602.82	2574.57	2602.82	2574.57	2602.38	2585.09
Cr.Protein %	16.05	16.17	16.34	16.17	16.34	16.20	16.40
Cr. Fibre %	4.34	4.72	5.09	472	5.09	4.55	4.75

**Composition Vitamin/Mineral Mix 1 kg (layers):Vitamin A 1000000IU,Biotin 40g,Vitamin B12 10mg.Folic acid 500mg,Manganese 4800MG,Zinc 58mg,Iron 5800mg,Selenium 120mg,Iodine 60mg, Cobalt 300mg.Composition of methonine 20,000mg, Butylated hydroxytolerance BHT 50,000mg.

RESULTS

The result of proximate composition of the variously treated CBS-based diets is as presented in Table 2. The crude protein values ranged between 17.00 (diet A) – 17.40% (diet E) and Crude fiber increased from 4.10% (diet A) to 4.8% (diet G). Ether extract values decreased from 3.49% (diet A) to 3.39% (diet G). The values obtained for ash ranged from 5.16 (diet G) – 10.00 (diet C). Metabolisable energy decreased from 2609.96 kcal ME/kg in diet A (control diet) to 2566.39 kcal ME/kg in diet G (10% FCBS). The analyzed proximate composition (g/100DM) of the experimental diets revealed that the percentage crude protein increases as the level of inclusion of various CBS increases in the diet.

The crude fiber level in the diet also increased with increase in the level of CBS in the diet while the ether extract values reduced accordingly. No particular trend was observed in the ash values obtained. Nitrogen free extract did not follow any particular trend but the values obtained were similar for all the dietary treatments. The highest values for crude protein were obtained for diet E (17.40%) while the least values were obtained for the control diet (17.00). The control diet (A) has the lowest crude fibre value (4.10%) while the highest value was obtained with diet C (4.82%). The highest ether extract value of 3.49% was obtained in the control diet. The metabolizable energy (kcal/kg) values of all the diets met the levels recommended by Olubamiwa et al., (2006).

The feed intake of the birds varied from 89.40–98.80 g/bird/day for variously treated CBS-based diets. (Table 3). Treatment effects on Hen day productions were significant, values for birds on diet A (control), D and E (5% and 10% ECBS) ware similar. Egg weight value varied from 60.06 (5% RCBS) for diet B, to the highest value of 66.56 in diet D (5% ECBS). While the FCR of birds on the control diet compared with the birds on diet C (10%RCBS) and E (10%ECBS).The values of feed conversion ratio obtained were not significant (P>0.05). However, significant differences (P<0.05) occurred when 10% of the RCBS, ECBS and FCBS diet were fed to the birds. The feed intake using 10% of the various treated CBS based diet ranged from 89.40 – 98.79 with the birds on diet G (10%FCBS) having the lowest feed intake of 89.40. Although, birds on diet C (10%RCBS) and E (10%ECBS) compared favorably with the birds on the control diets (A).

The cost of feed per kilogram reduces as the level of substitution of CBS with maize increases. Variations observed in the feed intake (FI), hen day production (HDP) and Egg Weight (EW) were significant (P<0.05) , except for feed conversion ratio (FCR). The feed intake of the birds on the control diet were higher and similar to those on diet D and E (5 and 10%ECBS). The average daily feed intake of the birds on the RCBS were lower than those on the control diet (A) and diets D and E. This could be as a result of the anti-nutritional factor (theobromine) in the diet. As the level of CBS in all the diet increased the feed intake declined. Several reports (Olubamiwa *et al,* 2000) depicted reduced feed intake by laying birds fed CBS and cocoa bean cake based-diets due to the theobromine content of the diet.

Table 2: Proximate Composition of treated Cocoa Bean Shell-Based Diets.

Parameters	A 0%	B 5%	C 10%	D 5%	E 10%	F 5%	G 10%
Crude Protein (%)	17.00	17.15	17.30	17.20	17.40	17.18	17.38
Crude Fibre (%)	4.10	4.46	4.82	4.44	4.76	4.51	4.80
Ether Extract (%)	3.49	3.46	3.40	3.46	3.44	3.44	3.39
Ash (%)	5.48	8.05	10.00	4.89	5.77	5.32	5.16
*NFE (%)	69.93	68.88	64.48	70.01	68.63	69.55	69.27
*ME (Kcal/kg/DM)	2620.1	2601.0	2573.8	2610.0	2590.0	2608.5	2589.3
Theobromine (%)	0.00	0.03	0.06	0.02	0.04	0.01	0.03

*NFE (Nitrogen Free Extract), ME (Metabolisable Energy)

Table 3: Performance Characteristics of Layers Fed Treated CBS-Based Diets.

Parameters	A 0%	B 5%	C 10%	D 5%	E 10%	F 5%	G 10%	SEM
FI (g)	98.80[a]	95.38[bc]	93.96[cd]	98.00[a]	97.06[ab]	92.16[d]	89.40[e]	0.46
HDP (%)	85.56[ab]	83.01b	81.51[c]	86.68[a]	85.27[ab]	81.01[c]	78.17[d]	0.53
EW (g)	61.37[bc]	60.06[c]	60.49[c]	65.56[a]	64.61[a]	63.24[b]	62.26[b]	3.19
FCR	2.44	2.47	2.55	2.43	2.48	2.53	2.65	0.05
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-

a, b, c: MEEeans along the same row with identical superscripts are not significantly different (P > 0.05)

Reduced feed intake is believed to be due to destruction of the intestinal lining and severe indigestion in the birds (Olubamiwa *et al, 2000)*. Although, differences in HDP of birds on diet D and E (ECBS) were not significantly (P>0.05) compared with control, the birds on diet D had the highest hen day production followed by those on diets A and E. This apparent increase in egg production could be due to the enzyme added to diets D and E. This could be due to the fact that the protein requirement of these birds was met at lower level of 10% CBS. The slight decrease in metabolizable energy, ether extract and crude fiber with increased levels of substitution of CBS for maize could be responsible for the slight numerical decreases in HDP.

As metabolizable energy increased from 2.42 to 2.64 and from 2.68 to 3.08 kcal ME/g diet, there was a 2.25% increase in egg production for every 0.22 kcal ME/g increase in the diet. The values of Average Daily Feed Intake (ADFI) (87.00–98.80g/bird/day) and HDP (78.17–86.68%) obtained in this study were lower than ADFI obtained for birds fed kola pod-based meal by Olubamiwa *et al.,* (2000).

The egg weight of birds on the RCBS was lower than all other treatments-this could be attributed to the presence of theobromine. Previous studies have shown that theobromine reduces feed intake which leads to lower laying rate and egg size (Odunsi and Longe, 1995, Olubamiwa *et al*., 2002). Egg weight of birds on diet with enzyme CBS diets D and E (5 and 10%) has higher egg weight compared with the control diet. This showed that addition of enzyme Rovabio reduced the effect of the anti-nutritional factor in cocoa bean shell. This improved performance of birds on enzyme-supplemented diets may be due to improved absorption of fats and fatty acids as well as fat-soluble micronutrients contained in the diet**.**

The absence of mortality throughout the period of this experiment further attested to the suitability of CBS as a substitute for maize in layers diet. This also indicates that layers can tolerate CBS based diet than broilers. When all the performance of the laying birds fed 5% of the various forms of CBS based diet was compared, there were no significant difference (P > 0.05) in values of ADFI, HDP, EW and FCR but when compared at 10%, significant differences (P < 0.05) were observed in the ADFI, HDP and FCR.

Variation observed in the egg length and width was significant (P > 0.05). The egg length varied from 5.54 – 5.77cm while that of the width varied from 4.25 – 4.33cm. Although the egg length of the bird fed with the control diet A is not significantly different from the eggs of those fed diet B and C. Also the egg width of the birds in the control diet A was not significantly different from those on diets B, C, D, E, and F except for those on diet G.

There were no significant differences in the values observed for egg shape index, shell weight and shell thickness (P > 0.05). The shell percentage ranged between 10.70 and 11.77. The lowest value (10.70) was obtained from the birds fed diet D while the highest was obtained from bird fed diet B. The value obtained for the shell surface area for the birds on the control diet A was not significantly different from those obtained for diet C and A, but differ from those obtained from diet B and F.

The mean values of egg shape index, shell weight, shell thickness, albumen height, yolk color score and Haugh Unit of eggs laid by birds fed all levels of variously treated CBS based diets were not different (P > 0.05) from the control.

The egg length, egg width, shell percentage, shell surface area, yolk weight, yolk height, yolk width, albumen weight, yolk percentage, albumen percentage and yolk index were, however, significantly ($P < 0.05$) affected by dietary treatments. The egg length and width of birds on diet D and G were higher than those of others, although the egg length of the birds on the control diet was comparable with those on diet B and C (5 and 10% RCBS). Egg shape index is important when eggs are packed in specialized containers. Abnormally shaped eggs do not fit into trays and get broken in handling process.

Dietary treatment did not have any effect on shell weight. This showed that shell deposition was similar in all dietary treatments. The variations observed in values of the egg shell thickness were not significant ($P > 0.05$).

There were no significant variations observed in the shell percentage. The shell thickness in the present study were not significantly different ($P>0.05$) from the control, this is in line with the findings of Olubamiwa *et al.,* (2000) that reported reduced egg and delay in egg production and depressed weight gain, egg quality content and thin shelled eggs on birds fed 20% CBS based diets. The shell surface area of the eggs on diet C, and D were comparable to those of eggs of diet A (the control), although, significant variations exist within the diets - as the level of the CBS in the diet increases, the shell surface area increase as well.

The values obtained for yolk weight were significantly different. Although the eggs of the birds fed with the control diet (A) were not significantly different from those on C (10% RCBS). Variations also occur in the values of yolk height, width and albumen weight. This could be due to the effect of the diet although no specific trend was shown with the yolk width and albumen weight. Albumen height of the eggs laid by birds fed CBS based diets reduces as the percentage inclusion of CBS (RCBS and EBBS) increases but reduces with increase in the level of FCBS for maize in the diets. Increase with no significant variations among all the dietary treatments including the control.

The yolk color score was the same for average (1.0) obtained was because white maize was used in formulating the diets. White maize and other ingredients used lack coloring pigments which would have impacted color to the yolk. Carotenoid pigments are usually transferred to the yolk from Carotenoid rich plants consumed by the hen since birds and animals generally do not have ability to synthesize Carotenoid.

Odunsi and Longe (1995) found yolk color was significantly ($P < 0.05$) enhanced by sun-flower leaf meal at all level over either the control or commercial layer mash. The generally accepted measurement of albumen is the Haugh Unit (HU) value and higher HU value is desirable. The HU value did not follow a particular trend with the increase of CBS in the diets. Eggs laid by birds fed the control diet A had the highest ($P<0.05$) average HU value followed by with 5% RCBS and then 5% ECBS based diets. Although, the HU values were not significantly different ($P>0.05$) from each other. The result indicated that the dietary treatments did not affect internal quality of eggs. The HU values fell within the range acceptable for good quality eggs.

The yolk and albumen percentage were significantly different ($P<0.05$) from each other. The yolk index value varies from 0.32–0.34. Variations observed in the feed intake (FI), hen day production (HDP) and egg weight were significant except feed conversion ratio.

The feed intake of the birds on the control diet were higher and similar to those on diet D and E (5 and 10%ECBS). However, the average daily feed intakes of the birds on the RCBS were lower than those on the control diets (A) and diets D and E (ECBS). This could be as a result of the anti-nutritional factor theobromine in the diet. As the level of CBS in all the diet increased the feed intake declined. Several reports (Olubamiwa *et al,* 2006) depicted reduced feed intake by laying birds fed CBS and cocoa bean cake based-diets due to the theobromine content of the diet. Reduced feed intake is believed to be due to destruction of the intestinal lining and severe indigestion in the birds (Olubamiwa *et al, 2000)*. Although, differences in HDP of birds on diet D and E (ECBS) were not significantly ($P>0.05$) compared with control, the birds on diet D had the highest hen day production followed by those on diets A and E. This apparent increase in egg production could be due to the enzyme added to diets D and E.

The slight decrease in metabolisable energy/ (kcal/kg) and ether extract, and increase in crude fiber with increased levels of substitution could be responsible for slight numerical decreases in HDP obtained with increased levels of substitution of CBS for maize. As metabolizable energy increased from 2.42 to 2.64 and from 2.68 to 3.08 kcal ME/g diet there was a 2.25% increase in egg production for every 0.22 kcal ME/g increase in the diet. The average egg weight and HDP obtained in this study were higher than 57g and 68.5% respectively recommended by McDonald *et al.*, (1995) for good flocks of layers. However, while higher ADFI was obtained for birds fed kola pod-based meal by Olubamiwa *et al.,* (2000), the percentage HDP (64.5–71.3) recorded by these authors were far below the values obtained in the present study.

The egg weight of birds on the RCBS was lower than all other treatments; this could be attributed to the presence of theobromine. Previous studies have shown that theobromine reduces feed intake which leads to lower laying rate and egg size (Olubamiwa *et al.,* 2006). Egg weight of birds on diet with enzyme CBS diets D and E (5 and 10%) has higher egg weight compressed with the control diet. This shows that addition of enzyme Rovabio effectively reduce the anti-nutritional factor in cocoa bean shell. The absence of mortality throughout the period of this experiment further attested to the suitability of CBS as a substitute for maize in layers diet. This also indicates that layer can tolerate CBS based diet than broilers. When all the performance of the laying birds fed 5% of the various forms of CBS based diet was compared, there were no significance difference in value of ADFI, HDP, egg with and FCR but when compared at 10%, significant difference ($P > 0.05$) was observed in the ADFI, HDP, and FCR.

The mean values of egg shape index, shell weight, shell thickness, albumen height, yolk color score and Haugh Unit of eggs laid by birds fed all levels of variously treated CBS based diets were not different ($P>0.05$) from the control. The egg length, egg width, shell percentage, shell surface area, yolk weight, yolk height, yolk width, albumen weight, yolk percentage, albumen percentage and yolk index were, however, significantly ($P< 0.05$) affected by dietary treatments. The egg length and width of birds on diet D and G were higher than those of others, although the egg length of the birds on the control diet was comparable with those on diet B and C (5 and 10% RCBS). Egg shape index is important when eggs are packed in specialized containers. Abnormally shaped eggs do not fit into trays and get broken in handling process.

Dietary treatment did not have any effect on shell weight. This showed that shell deposition was similar in all dietary treatments. Egg shell thickness values were very close and the variations observed were not significant ($P>0.05$).

The shell thickness in the present study were not significantly different ($P>0.05$) from the control, this is in line with the findings of Olubamiwa *et al.,* (2000) that reported reduced egg and delay in egg production and depressed weight gain, egg quality content and thin shelled eggs on birds fed 20% CBS based diets.

The shell surface area of the egg of birds on diet C, and D were comparable to those of eggs of diet A (the control), although, significant variation exist within the diets. As the level of the CBS in the diet increases, the shell surface area increase as well.

The values obtained for yolk weight were significantly different. Although the eggs of the birds fed with the control diet (A) were not significantly different ($P>0.05$) from those on C (10% RCBS). Variations also occur in the values of yolk height, width and albumen weight. This could be due to the effect of the diet although no specific trend was shown with the yolk width and albumen weight. Albumen height of the eggs laid by birds fed CBS based diets reduces as the percentage inclusion of CBS (RCBS and EBBS) increases but reduces with increase in the level of FCBS for maize in the diets. Increase with no significant variations among all the dietary treatments including the control.

The yolk color score was the same for average (1.0) obtained was because white maize was used in formulating the diets. White maize and other ingredients used lack coloring pigments which would have impacted color to the yolk. Carotenoid pigments are usually transferred to the yolk from Carotenoid rich plants consumed by the hen since birds and animals generally do not have ability to synthesize Carotenoid.

The generally accepted measurement of albumen is the Haugh Unit (HU) value and higher HU value is desirable. The HU value did not follow a particular trend with the increase of CBS in the diets. Eggs laid by birds fed the control diet A had the highest ($P<0.05$) average HU value followed by with 5% RCBS and then 5% ECBS based diets. The HU value was almost similar for all the dietary treatments, since Haugh Unit is the measurement most commonly used in measuring internal quality of eggs (Haugh, 1937). The result indicated that the dietary treatments did not affect internal quality of eggs. The HU values fell within the range acceptable for good quality eggs.

The yolk and albumen percentage were significantly different (P>0.05) from each other. The yolk index value varies from 0.32–0.34. The variations observed in the internal egg quality are as shown in Table 4. The values obtained from the yolk weight, yolk height, yolk width, yolk percentage and the yolk index were significantly different (P > 0.05). Although the value obtained for egg yolk weight on the control were not significantly different from those obtained in diet C. On the yolk height, the birds from diets B, C, D, and G were comparable with those of the control. The eggs of the birds in the control were comparable in the egg width value with those from other treatments. Variations exist in the values obtained for albumen weight. The value ranges from 33.02 (5% FCBS) and 37.92 (5%ECBS). Birds on diet A (control i.e. 0% CBS) and diet E (10% ECBS) gave the highest value of albumin height (0.72cm) while birds on diet G (10%FCBS) gave the lowest value of 0.69cm.

The yolk color score was the same, 1.0 across the dietary treatments. Haugh Unit value were (p<0.05) not significantly different across the dietary treatment and the values ranges from 80.70-85.10. The yolk percentage ranges from 24.67–8.23. The albumen percentage ranged from 52-56 to 63.32 with the highest value obtained from birds on diet C (10% RCBS) and least value obtained from birds on diet F (5% FCBS). The yolk index was similar across the dietary treatments with yolk from eggs laid by birds on diet F having a value of 0.34. Yolk from eggs from other dietary treatments had yolk index value of 0.33 with the exception of yolk from birds on diet E with 0.32.

CONCLUSION

Cocoa bean shell could replace 10% maize in the diets of layers effectively without any adverse effect on the performance and egg quality characteristics when supplemented with enzyme.

Table 4: Egg Quality Characteristics (External and Internal) of Laying Birds Fed Treated Cocoa Bean Shell-Based Diets.

Parameters	TREATMENTS							
		RCBS		ECBS	FCBS			
	A 0%	B 5%	C 10%	D 5%	E 10%	F 5%	G 10%	SEM
Egg Length (cm)	5.61^{b}	5.69^{b}	5.66^{b}	5.77^{a}	5.54^{c}	5.56^{c}	5.73^{a}	0.01
Egg Width (cm)	4.29^{a}	4.33^{a}	4.29^{a}	4.28^{a}	4.28^{a}	4.29^{a}	4.25^{b}	0.06
Egg shape index	1.09	1.06	1.01	1.03	1.01	1.05	1.03	0.02
Shell Weight (g)	7.18	7.15	7.12	7.12	7.12	7.12	7.13	0.05
Shell Thickness (mm)	0.33	0.32	0.32	0.31	0.31	0.29	0.28	0.01
Shell %	11.71^{ab}	11.91^{a}	11.77^{ab}	10.70^{e}	11.01^{de}	11.26^{dc}	11.44^{bc}	0.08
Shell Surface Area	69.80^{ab}	68.00^{c}	69.70^{ab}	69.25^{b}	70.19^{a}	68.15^{c}	69.50^{b}	2.51
Yolk Weight (g)	15.97^{b}	16.37^{a}	16.25^{ab}	16.40^{a}	15.50^{d}	15.42^{d}	15.80^{c}	0.31
Yolk Height(cm)	1.30^{b}	1.29^{b}	1.30^{b}	1.28^{b}	1.25^{c}	1.34^{a}	1.28^{b}	0.01`
Yolk Width(cm)	3.86^{b}	3.92^{ab}	3.88^{ab}	3.91^{ab}	3.92^{ab}	3.88^{ab}	3.94^{a}	0.05
Albumen Weight (g)	36.40a	37.67a	36.45a	37.92a	36.37a	33.02b	34.85b	0.37
Yolk Color	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
Yolk %	26.02^{b}	27.05^{b}	28.23^{a}	25.34^{c}	24.67^{c}	24.79	25.67	0.01
Albumen %	59.31^{c}	62.26^{b}	63.32^{a}	58.60^{bc}	57.89^{c}	52.56^{a}	56.63	3.8
Yolk Index	0.33^{b}	0.33^{b}	0.33^{b}	0.33^{b}	0.33^{b}	0.33^{c}	0.34^{a}	0.33^{b}

a, b, c, d, e: Means along the same row with any identical superscripts are not significant (P > 0.05).

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ABOUT THE AUTHORS

Martha Olumide, is a Monogastric Nutrionist. He completed his Ph.D. work recently at the Department of Animal Science. He is the Production Manager of Bronco Farms, Ibadan, Nigeria.

O. Akinsoyinu, is a Professor of Ruminant Nutrition formerly with the University o0f Ibadan and present with the Babcock University, Nigeria.

Rasheed A. Hamzat, is a Professor at the Department of Animal Production & Health, Federal University, Dutsin-Ma, Katsina State, Nigeria.

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