

## Evaluation of calcium and vitamin D<sub>3</sub> in cassava-based diets on internal and external qualities of chicken eggs

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### Abstract

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The aim of this study was to evaluate the effect of calcium and vitamin D<sub>3</sub> in cassava-based layers' diet on the external and internal qualities of egg. One hundred and thirty-five, ISA brown layers aged 54 weeks were assigned randomly to 9 treatment (T) combinations with three replicates in a 3×3 factorial arrangement in a completely randomized design. Each replicate had five hens while the diets were: T<sub>1</sub> = 3.5% Ca and 1000 IU vitamin D<sub>3</sub>, T<sub>2</sub> = 3.5% Ca and 2000 IU vitamin D<sub>3</sub>, T<sub>3</sub> = 3.5% Ca and 3000 IU vitamin D<sub>3</sub>, T<sub>4</sub> = 4.5% Ca and 1000 IU vitamin D<sub>3</sub>, T<sub>5</sub> = 4.5% Ca and 2000 IU vitamin D<sub>3</sub>, T<sub>6</sub> = 4.5% Ca and 3000 IU vitamin D<sub>3</sub>, T<sub>7</sub> = 5.5% Ca and 1000 IU vitamin D<sub>3</sub>, T<sub>8</sub> = 5.5% Ca and 2000 IU vitamin D<sub>3</sub>, and T<sub>9</sub> = 5.5% Ca and 3000 IU vitamin D<sub>3</sub>. After 12 weeks, two eggs were collected per replicate to assess the external and internal egg qualities. The increasing levels of calcium and vitamin D<sub>3</sub> had no effect on the external and internal qualities of the eggs. From the result, hens that were fed 4.5% and 5.5% Ca recorded highest shell thickness. The interaction of calcium and vitamin D<sub>3</sub> affected the shell thickness with hens fed diet T<sub>5</sub> (4.5% Ca with 2000 IU) recording the thickest egg shell while T<sub>2</sub> (3.5% Ca with 2000 IU) recorded the lowest. It was therefore concluded that 4.5% and 5.5% dietary calcium, 1000 IU and 3000 IU vitamin D<sub>3</sub> were best when used individually in the diet for hens while T<sub>5</sub> (4.5% calcium with 2000 IU) favoured eggs shell thickness for hens aged 54 -66 weeks.

**Keywords:** Calcium, Cassava diet, Egg quality, Laying hens, Vitamin D<sub>3</sub>

## Une Évaluation du calcium et de la vitamine D3 dans les régimes à base de manioc sur les qualités internes et externes des œufs de poulet



### Résumé

Le but de cette étude était d'évaluer l'effet du calcium et de la vitamine D3 dans le régime alimentaire des couches nourries à base de manioc sur les qualités externes et internes de l'œuf. Cent trente-cinq couches brunes 'ISA' âgées de 54 semaines ont été assignées au hasard à 9 combinaisons de traitement (T) avec trois répliques dans un arrangement factoriel de 3×3 dans une conception complètement randomisée. Chaque réplique avait cinq poules pendant que les régimes étaient : T1 = 3,5% Ca et 1000 UI vitamine D3, T2 = 3,5% Ca et 2000 UI vitamine D3, T3 = 3,5% Ca et 3000 UI vitamine D3, T4 = 4,5% Ca et 1000 UI vitamine D3, T5 = 4,5% Ca et 2000 UI vitamine D3, T6 = 4,5% Ca et 3000 UI vitamine D3, T7 = 5,5% Ca et 1000 UI vitamine D3, T8 = 5,5% Ca et 2000 UI vitamine D3, et T9 = 5,5% Ca et 3000 UI vitamine D3. Après 12 semaines, deux oeufs ont été recueillis par répétition pour évaluer les qualités externes et internes d'oeuf. Les niveaux croissants de calcium et de vitamine D3 n'ont eu aucun effet sur les qualités externes et internes des oeufs. Par conséquent, les poules nourries à 4,5 % et à 5,5 % ca ont enregistré l'épaisseur la plus élevée de la coquille. L'interaction du calcium et de la vitamine D3 a affecté l'épaisseur de la coquille avec les poules nourries au régime T5 (4,5 % ca avec 2000 UI) enregistrant la coquille d'œuf la plus épaisse tandis que T2 (3,5 % Ca avec 2000 UI) a enregistré la plus

*faible. On a donc conclu que 4,5 % et 5,5 % de calcium alimentaire, 1 000 UI et 3 000 UI de vitamine D<sub>3</sub> étaient les meilleurs lorsqu'ils étaient utilisés individuellement dans le régime alimentaire pour les poules, tandis que le T5 (4,5 % de calcium avec 2000 UI) favorisait l'épaisseur de la coquille des œufs pour les poules âgées de 54 à 66 semaines.*

**Mots-clés :** Calcium, Régime manioc, Qualité des œufs, Poules pondeuses, Vitamine D<sub>3</sub>

## **Introduction**

Calcium is a necessary cation in the diet of animals especially laying hens and regulates several functions of birds (Nunes *et al.*, 2006). It is also needed in the egg shell formation (Costa *et al.*, 2008). Laying hens require high levels of calcium during egg production (Leeson and Summers, 2005). The shell of an egg contains 90% mineral and 98% of it is made up of calcium carbonate (Mendonca –Junior, 1993). Egg shell breakage is still a major source of economic loss to the poultry industry. According to Roland (1988), about 13-20% of total egg production was estimated to crack before they reach the final destination. Clunies *et al.*, (1992) suggested that increased level of calcium in the diet of laying hens resulted in increase in shell thickness, weight and resistance to cracking and breakage.

Vitamin D<sub>3</sub> (cholecalciferol) improves egg yolk quality and does not alter the fatty acid composition of the sensory and functional properties of the egg (Mattila *et al.*, 2003). The functions of vitamin D<sub>3</sub> are associated with calcium and essential for intestinal uptake and subsequent calcium metabolism. NRC (1994) reported that vitamin D<sub>3</sub> is the only form in which vitamin D can appear in poultry and suggested that pre-lay hens require higher levels of vitamin D<sub>3</sub> per kg feed. Vitamin D<sub>3</sub> in its active form enhances the synthesis of calcium binding protein involved in the gut and uterus. The need for vitamin D<sub>3</sub> is increased if calcium supply is not optimum (Sreenivasaiah, 2006). McDowell (1989) reported that if there is enough sunlight dietary supplementation of vitamin D<sub>3</sub> will not be necessary. In the Humid tropics,

supplementation of dietary vitamin D<sub>3</sub> is required because the birds are reared under the intensive production system in covered facilities due to the regular rainfall and as such the birds are protected from direct sunlight which is the natural form of vitamin D<sub>3</sub>. The level of vitamin D<sub>3</sub> required has been suggested by many literatures. Among such are 300 IU (NRC1994), 500 IU (De Blas and Mateos, 1991), 750 IU (Rostagno *et al.*, 1985), 1000 IU (Scott *et al.*, 1982), and 2500 IU (Leeson and Summers, 1997). Cassava (*Manihot esculenta*) is a woody shrub and the third largest source of dietary carbohydrate after rice and maize. Raw cassava contains about 60% water, 38% carbohydrates and 1% crude protein. Studies have shown that cassava can be used as alternative source of energy for laying hens (Stupak *et al.*, 2006). Maize has been a common feedstuff in monogastric diet. Sixty percent (60%) of maize is used in poultry ration, which increases the cost of maize due to high consumption rates by both man and livestock and low production rate which invariably leads to high cost of production of animal feeds (Myanmar Statistical Year Book, 2011). Thus, there is need to reduce the competition between man and livestock for feedstuffs by incorporating unconventional feedstuff, and the use of cassava as alternative for maize would go a long way in reducing the cost of producing animal feed (Anaeto and Adighibe, 2011). The most limiting factor in the use of cassava in poultry diet is its low protein content, deficiency in essential vitamin and minerals (Chauynarong *et al.*, 2009). However, Oruwari *et al.* (2003) postulated that with proper protein balance cassava

could completely replace maize in poultry diets. Gomez and Valdivieso (1985) reported that the problem of hydrocyanic acid content of cassava can be eliminated with proper processing technique. Aderemi *et al.* (2006) observed that cassava meal could replace 25% of maize in the diets of laying hens without any adverse effect on the laying performance, Haugh unit, yolk colour, albumen weight, yolk weight and egg shell thickness but at 12.5% there was a decrease in yolk colour. The shell thickness and Haugh unit were the same in the cassava and maize diet as observed by Saparattan *et al.* (2005). Aderemi *et al.* (2012) reported increased egg shell thickness when cassava whole meal and cassava grit meal were included in the diet of layer hen. Akinola and Oruwari *et al.* (2007) reported best performance from laying hens fed 48% (120% replacement) of cassava meal diet. Akinola and Oruwari (2007) had advocated that farmers anywhere in Nigeria can completely and profitably replace maize with cassava in diet for laying hens. Since the aim of every farmer is to produce more eggs with good quality at reduced cost, this study was conducted to ascertain the best level of combination of calcium and vitamin D<sub>3</sub> in cassava-based diet that will give the best egg quality to recommend to farmers in the humid tropics, since diet with calcium, vitamin D<sub>3</sub> and cassava-based individually have been reported to be good for egg production.

## Materials and methods

### Experimental location

This experiment was carried out at the Poultry Unit of the University of Port Harcourt Teaching and Research Farm, Choba, Port Harcourt, Rivers State. It is situated on latitude 4°53'14" North and longitude 6°54'00" East of the equator (Ijeomah *et al.*, 2013), with an average of 2293.6mm rainfall annually, temperature range of 25°C-28°C and a very high relative

humidity (above 80%).

### Experimental animal, management and design

One hundred and thirty five, ISA brown laying hens of 54 weeks old were used to carry out this study. The birds were housed in a battery cage system in an open sided building that was well demarcated into replicates and the birds were assigned randomly to nine treatment combinations in a 3×3 factorial arrangement in completely randomized design with each treatment having 3 replicates of 5 birds each. Daily routine of cleaning, feeding and sanitation were carried out.

### Experimental feed and duration

The experimental diets were compounded with calcium levels of 3.5%, 4.5% and 5.5% (obtained from bone meal and limestone) while the vitamin D<sub>3</sub> inclusion were 1000 IU, 2000 IU and 3000 IU to produce nine treatments (T<sub>1</sub>- T<sub>9</sub>) as shown in Table 1. The calcium sources were from limestone and bone-meal while cassava root meal was the major energy source. This experiment lasted 12 weeks.

### Data collection and analysis

#### External and internal egg qualities

At the end of the experiment two eggs were collected from each replicate making a total of 6 eggs from each treatment and were used to obtain data for the external parameters (egg weight, egg width, shell weight, egg shape index, eggshell index, egg length and shell thickness) and internal parameters (albumen weight, albumen pH, albumen index, albumen height, yolk weight, yolk diameter, yolk height, yolk index, yolk: albumen ratio and Haugh unit).

The egg weights were measured using an electronic weighing balance and recorded to the nearest 0.01g while the lengths were measured using Vernier caliper by taking the longitudinal distance between the narrow and broad ends of the egg while the egg widths were obtained from the diameter of the widest cross-sectional region. The

values obtained were used to calculate the egg shape index (width of egg ÷ length of egg x 100 %).

Each egg was broken at the middle to keep the yolk intact and emptied into a clean petri-dish, the yolk was then removed into another petri-dish and all internal parameters were measured. The yolk heights were measured using a micro-meter screw gauge and recorded to the nearest 0.1cm. The yolk diameters were measured using a pair of Vernier calipers. The values obtained were used to calculate the yolk index (yolk diameter ÷ height of yolk x 100 %). The albumen heights were measured using a tripod micrometer calibrated in 0.1mm and the measurement taken between the yolk edge and the external edge of the thick albumen. The values obtained together with the egg weight were used to calculate the Haugh Unit (HU) using the method described by Haugh (1937) expressed mathematically as:

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}).$$

Where, H =Height of thick albumen in mm

W = weight of egg in grams.

The yolk was separated from the albumen and both were weighed individually and recorded in grams. The values obtained were used to calculate the yolk-albumen ratio (yolk weight ÷ albumen weight). The albumen and yolk pH were determined using the universal pH indicator. The eggshell was washed with flowing water to clean them properly and left to dry at room temperature along with the thin membrane for 24 hours, after which they were weighed with a sensitive electronic weighing scale. The shell thickness along with the membrane was recorded as the average value of the thickness taken from the broad end, narrow and the equatorial part of the egg with the aid of micrometer screw gauge and expressed in millimeter. The shell index was calculated using the shell weight and egg weight (as shell weight ÷ egg weight x

100 %).

#### **Statistical analysis**

All data collected were analyzed using the Statistical Package of Social Science (SPSS). Analysis of Variance ANOVA was used for treatment effect and General Linear Model (GLM) for the separate effect of calcium and vitamin D<sub>3</sub> while the significant differences in the mean were separated using Duncan Multiple Range Test (DMRT) of same software.

#### **Results**

##### ***Effect of calcium on external and internal qualities of eggs***

Table 2 shows the effect of different calcium levels on the external and internal qualities of eggs. It was observed that the diets did not significantly ( $P > 0.05$ ) affect the external and internal qualities of the eggs except the shell thickness which showed significant ( $P < 0.05$ ) difference with the highest shell thickness recorded at 4.5% Ca which was not different from 5.5% Ca and the lowest recorded at 3.5% Ca inclusion.

##### ***Effect of vitamin D<sub>3</sub> on the external and internal qualities of eggs***

The effect of different levels of vitamin D<sub>3</sub> on the external and internal qualities of eggs (Table 3) showed that there was no significant ( $P > 0.05$ ) effect of the diets on the external and internal qualities of eggs.

##### ***Interactive effect of calcium and vitamin D<sub>3</sub> on external and internal qualities of eggs***

The interactive effect of different levels of calcium (3.5, 4.5, and 5.5%) and vitamin D<sub>3</sub> (1000, 2000 and 3000 IU) on external and internal qualities of eggs is shown in Table 4. There were no significant differences ( $P > 0.05$ ) in the egg weight, egg length, egg width, egg shape index and all the internal egg qualities. However, there were significant differences ( $P < 0.05$ ) in the shell weight, shell thickness and shell index with the highest weight recorded at T<sub>7</sub> and T<sub>9</sub>, which was not different from T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>,

Table 1: Composition of experimental layers' diets

Ingredient (%)	T <sub>1</sub> 3.5% Ca, 1000 IU Vit. D <sub>3</sub> )	T <sub>2</sub> 3.5% Ca, 2000 IU Vit. D <sub>3</sub> )	T <sub>3</sub> 3.5% Ca, 3000 IU Vit. D <sub>3</sub> )	T <sub>4</sub> 4.5% Ca, 1000 IU Vit. D <sub>3</sub> )	T <sub>5</sub> 4.5% Ca, 2000 IU Vit. D <sub>3</sub> )	T <sub>6</sub> 4.5% Ca, 3000 IU Vit. D <sub>3</sub> )	T <sub>7</sub> 5.5% Ca, 1000 IU Vit D <sub>3</sub> )	T <sub>8</sub> 5.5% Ca, 2000 IU Vit D <sub>3</sub> )	T <sub>9</sub> 5.5% Ca, 3000 IU Vit D <sub>3</sub> )
Palm kernel cake	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Cassava Root meal	37.25	37.25	37.25	37.25	37.25	37.25	37.25	37.25	37.25
Soya bean meal	17.00	17.00	17.00	17.20	17.20	17.20	17.00	17.00	17.00
Groundnut cake	10.00	10.00	10.00	9.00	9.00	9.00	10.00	10.00	10.00
Fish meal	2.00	2.00	2.00	3.60	3.60	3.60	4.00	4.00	4.00
Wheat bran	7.20	7.20	7.20	3.10	3.10	3.10	2.8	2.80	2.80
Soya oil	2.00	2.00	2.00	2.80	2.80	2.80	2.00	2.00	2.00
Limestone	5.00	5.00	5.00	6.40	6.40	6.40	6.20	6.20	6.20
Bone meal	3.80	3.80	3.80	4.90	4.90	4.90	5.00	5.00	5.00
DL-Methionine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vit/TM Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100	100	100	100
<b>Nutrient Composition</b>									
Crude Protein	18.08	18.08	18.08	18.08	18.08	18.08	18.01	18.01	18.01
ME kcal/kg	2527.93	2527.93	2527.93	2534.77	2534.77	2534.77	2525.47	2525.47	2525.47
Ether extracts	3.55	3.55	3.55	4.25	4.25	4.25	4.39	4.39	4.39
Crude fibre	6.99	6.99	6.99	6.58	6.58	6.58	6.27	6.27	6.27
Lysine	0.89	0.89	0.89	0.93	0.93	0.93	0.97	0.97	0.97
Methionine	0.29	0.29	0.29	0.31	0.31	0.31	0.32	0.32	0.32
Calcium	3.50	3.50	3.50	4.52	4.52	4.52	5.53	5.53	5.53
Avail. Phosphorus	1.02	1.02	1.02	1.22	1.22	1.22	1.42	1.42	1.42
Ca: P	3:1	3:1	3:1	3.7:1	3.7:1	3.7:1	3.9:1	3.9:1	3.9:1

\*Vitamin and Trace minerals for each kg contained: Mn 40g, Fe 20g, Zn 18g, Cu 0.8g, I 0.62g, Co 0.09g and Se 0.04g, Pantothenic acid 1,000,000 IU, Cholecalciferol 1000 IU, Quinines 0.8g, Thiamine 0.6g and Riboflavin 2.4g, Folic Acid 0.4g, Biotin 0.02g, Ascorbic acid 10.0g,



### *Calcium and vitamin D<sub>3</sub> in cassava feed for layers*

**Table 2: Effect of calcium on external and internal qualities of eggs**

Parameters	3.5% Ca	4.5% Ca	5.5% Ca	SEM
<b>External quality</b>				
Egg weight (g)	58.01	58.51	60.03	1.64
Egg length (cm)	5.71	5.82	5.86	0.09
Egg width (cm)	4.30	4.26	4.39	0.04
Shell weight (g)	5.19	5.35	5.75	0.19
Shell thickness (mm)	0.48 <sup>b</sup>	0.59 <sup>a</sup>	0.53 <sup>ab</sup>	0.04
Shell index	8.93	9.17	9.62	0.03
Egg shape index	75.52	73.25	75.12	1.17
<b>Internal quality</b>				
Albumen weight (g)	35.05	35.44	35.41	1.15
Albumen height (mm)	3.65	3.64	3.87	0.15
Albumen pH	7.00	7.00	7.00	0.00
Yolk weight (g)	15.18	14.66	14.19	0.43
Yolk height (cm)	0.72	0.73	0.75	0.04
Yolk diameter (cm)	3.91	4.07	4.13	0.11
Yolk pH	5.00	5.00	5.00	0.00
Yolk:Albumen ratio	0.44	0.41	0.39	0.02
Yolk index	18.55	17.96	18.15	0.70
Haugh unit	76.24	75.99	77.33	0.72

<sup>a,b</sup>= Means within each row with different superscripts are significantly different (P<0.05)

**Table 3: Effect of vitamin D<sub>3</sub> on external and internal qualities of eggs**

Parameters	1000IU Vit. D <sub>3</sub>	2000IU Vit. D <sub>3</sub>	3000IU Vit. D <sub>3</sub>	SEM
<b>External quality</b>				
Egg weight (g)	59.27	59.16	58.12	1.64
Egg length (cm)	5.84	5.74	5.79	0.09
Egg width (cm)	4.35	4.32	4.27	0.04
Shell weight (g)	5.52	5.23	5.53	0.19
Shell thickness (mm)	0.51	0.59	0.51	0.04
Shell index	9.35	8.85	9.52	0.30
Egg shape index	74.71	75.42	73.77	1.17
<b>Internal quality</b>				
Albumen weight (g)	36.04	35.45	34.41	1.15
Albumen height (mm)	3.77	3.78	3.61	0.15
Albumen pH	7.00	7.00	7.00	0.00
Yolk weight (g)	14.61	14.74	14.69	0.43
Yolk height (cm)	0.73	0.74	0.74	0.04
Yolk diameter (cm)	4.00	4.13	3.97	0.11
Yolk pH	5.00	5.00	5.00	0.00
Yolk:Albumen ratio	0.41	0.42	0.42	0.02
Yolk index	18.36	17.81	18.48	0.70
Haugh unit	76.75	76.92	75.89	0.72

T<sub>6</sub>, and T<sub>8</sub> but the lowest was recorded at T<sub>2</sub>. Eggs from diet T<sub>5</sub> recorded the highest shell thickness which was not different from T<sub>8</sub>. Lower levels of shell thickness were

recorded at T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>9</sub>. The shell index had significantly highest level at T<sub>9</sub> but was not different from T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> while the lowest was recorded at T<sub>2</sub>.

Table 4: Interactive effect of calcium and vitamin D<sub>3</sub> on external and internal qualities of eggs

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>	SEM
<b>External Quality</b>										
Egg weight (g)	61.34	54.87	57.81	55.87	59.09	60.58	60.58	63.53	55.98	0.95
Egg length (cm)	5.79	5.65	5.67	5.68	5.70	6.07	6.05	5.88	5.64	0.06
Egg width (cm)	4.41	4.22	4.28	4.24	4.27	4.27	4.41	4.48	4.27	0.03
Shell weight (g)	5.62 <sup>ab</sup>	4.63 <sup>b</sup>	5.32 <sup>ab</sup>	5.08 <sup>ab</sup>	5.48 <sup>ab</sup>	5.48 <sup>ab</sup>	5.87 <sup>a</sup>	5.59 <sup>ab</sup>	5.79 <sup>a</sup>	0.12
Shell thickness (mm)	0.51 <sup>b</sup>	0.43 <sup>b</sup>	0.49 <sup>b</sup>	0.53 <sup>b</sup>	0.74 <sup>a</sup>	0.51 <sup>b</sup>	0.49 <sup>b</sup>	0.59 <sup>ab</sup>	0.51 <sup>b</sup>	0.02
Shell index	9.20 <sup>ab</sup>	8.42 <sup>b</sup>	9.18 <sup>ab</sup>	9.13 <sup>ab</sup>	9.34 <sup>ab</sup>	9.04 <sup>ab</sup>	9.71 <sup>ab</sup>	8.80 <sup>ab</sup>	10.35 <sup>a</sup>	0.18
Egg shape index	76.32	74.77	75.47	74.63	75.10	70.03	73.16	76.39	75.82	0.67
<b>Internal quality</b>										
Albumen weight (g)	37.42	32.46	35.25	33.11	36.23	36.97	37.59	37.65	30.99	0.72
Albumen height (mm)	3.91	3.43	3.62	3.44	3.74	3.73	3.94	4.19	3.49	0.07
Albumen Ph	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	0.00
Yolk weight (g)	15.78	14.69	15.08	14.24	14.43	15.32	13.81	15.09	13.66	0.25
Yolk height (cm)	0.79	0.67	0.70	0.68	0.73	0.79	0.72	0.81	0.72	0.02
Yolk diameter (cm)	3.91	4.01	3.81	3.98	4.08	4.16	4.12	4.31	3.95	0.06
Yolk pH	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.00
Yolk:Albumen ratio	0.42	0.46	0.43	0.43	0.40	0.41	0.37	0.40	0.43	0.01
Yolk index	20.42	16.95	18.27	17.01	17.89	18.97	17.65	18.59	18.20	0.39
Haugh unit	77.32	75.38	76.02	75.21	76.62	76.16	77.73	78.77	75.48	0.41

<sup>ab</sup>= Means within each row with different superscripts are significantly different (P<0.05)

## Discussion

### *Effect of calcium on the external and internal qualities of eggs*

The similarity in egg weight across treatments with increasing levels of calcium (3.5, 4.5, and 5.5%) was in line with the reports of Cufadar *et al.* (2011) who also did not find any difference in egg parameters of aged hens fed dietary calcium levels 3.0, 3.6, and 4.0%. Similarly, Keshavarz and Scott (1993) reported that there was no difference in egg weight from hens aged 38-62 weeks when dietary calcium was increased from 3.5 to 4.0%. This result however, was contrary to the findings of Bar *et al.* (2002) who observed decreased egg weight when dietary calcium was increased from 3.5 to 4.8% in aged hens of 57-64 weeks. Rao and Roland (1990) also observed no significant effect on eggshell thickness when dietary Ca was increased from 3.2 to 4.5%. Castillo *et al.* (2004) in another study, reported that there was no improvement in the internal quality of eggs when dietary calcium levels were increased. From this study, with increased calcium

intake from 3.5%, there was significant increase in shell thickness at 4.5% and 5.5% Ca levels, implying that an increase in dietary calcium level for aging hens (54-66 weeks) from 3.5% to 4.5% - 5.5% will improve shell quality, since the shell thickness improved considerably when dietary calcium was increased from 3.5% to 4.5% and 5.5%.

### *Effect of vitamin D<sub>3</sub> on the external and internal qualities of eggs*

The result which showed no effect of the vitamin D<sub>3</sub> in diets on the external and internal qualities of the egg was in agreement with Browning and Cowieson (2014) who reported no difference in egg quality with higher levels of vitamin D<sub>3</sub> up to 10,000 IU/Kg feed. Also, inclusion of vitamin D<sub>3</sub> in the diet up to 12,000 IU/kg feed was not harmful to birds throughout an entire production period (Mattila *et al.*, 2003). However, earlier report by Frost *et al.* (1990), recorded increased egg quality with high supplementation of Vitamin D<sub>3</sub>. There was also an increase in egg shell quality when vitamin D<sub>3</sub> was supplemented

in layers' diets (Tsang and Grunder, 1993). From this study, farmers can include vitamin D<sub>3</sub> in diets up to 3000 IU to support good quality egg in the humid tropics.

***Interactive effect of calcium and vitamin D<sub>3</sub> on the external and internal qualities of egg***

The result which showed that increasing the levels of calcium (3.5, 4.5 and 5.5%) and vitamin D<sub>3</sub> (1000, 2000, and 3000 IU) had no effect on the egg weight, egg length, egg width, egg shape index and the internal quality of the egg agreed with the report of Castillo *et al.* (2004) who reported that no additional improvement in the internal egg quality were obtained when dietary calcium was increased to more than 3.5%. The result also agreed with Chowdhury and Smith (2002) who reported that dietary calcium level had no significant effect on the yolk weight, albumen weight and Haugh units. The variation in the egg shell weight across all treatment groups was contrary to the report of William *et al.* (2006) who reported only linear increase in shell weight with increasing levels of dietary calcium up to 4%. The difference observed in shell thickness was contrary to the report of Mattila *et al.* (2004) who observed no significant difference in egg specific gravity and shell thickness with diets containing 1500, 2500 and 6000 IU vitamin D<sub>3</sub>/kg feed when hens were fed 3.6% to 3.87% calcium. However, the result from this study agreed with the report by Abdel-Azeem and El-Shafai (2006) that shell quality was maximized when laying quails' diet contained 3000 IU/kg feed. From this study, it was observed that at inclusion level of 4.5% Ca and 2000 IU and at 5.5% Ca and 2000 IU there was increase in shell thickness. This eliminated the fear of calcium deficiency which is usually accompanied by thin egg shell and reduced egg production. It implied that the 4.5% Ca and 2000 IU and 5.5% 2000 IU vitamin D<sub>3</sub> gave good quality egg shell which can lead

to less breakage of shells. Since eggshell breakage constitutes a major loss to the poultry industry and eggs with inferior quality stands as a major economic loss (Roberts, 2004) with an estimation by Roland (1988) that 13-20% of the total egg produced cracked before they reach their final destination, this study stands out, as a breakthrough for commercial egg production in the humid tropics of Nigeria where heavy rains do not allow commercial birds to have access to sunlight for production of vitamin D<sub>3</sub>.

**Conclusion**

Based on the result from the experiment it was observed that inclusion of dietary calcium at 4.5% and 5.5% improved shell thickness, thus supporting better egg quality. At 4.5% Ca and 2000 IU vitamin D<sub>3</sub> and 5.5% Ca and 2000 IU vitamin D<sub>3</sub> interactive levels, the best egg shell thickness was also recorded, thus, farmers are advised to use 4.5% or 5.5% calcium singly or 4.5% Ca and 2000 IU vitamin D<sub>3</sub> and 5.5% Ca and 2000 IU vitamin D<sub>3</sub> when used together in cassava-based diet for aged hens.

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