Research Article
Volume 13:02, 2024

Vitamins & Minerals

ISSN: 2376-1318

Open Access

Quality Attributes of Eggs from Laying Hens Fed Diets Supplemented with Varying Inclusion Levels of Ascorbic Acid and Cholecalciferol

Bamidele Samuel Adedeji[1], Folasade O. Jemiseye[2], Adeyemi Ayodeji Afolabi[1], Sabur O. Oladimeji[3], Funmilayo Grace Adebiyi[1], Bukola Christiana Majekodunmi[4,5], Babatunde F. Adebayo[5], T. V. Abokede[1], Sherifat Omowunmi Olufeko[6] and Olugbenga Adeniran Ogunwole[1]*

[1]Agricultural Biochemistry & Nutrition Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria
[2]Quality Control Laboratory, Agro Bar- Magen, Apata, Ibadan, Nigeria
[3]Department of Nutrition, Research and Development, Pandagric Novum Limited, Karu Local Government, Nasarawa, Nigeria
[4]Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[5]Department of Training and Internship, Nigeria Institute of Animal Science, Cadastral Zone C13, Kubusa, Abuja, Nigeria
[6]Animal Products and Processing Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

Abstract

Vitamin supplements like most feed additives are used to promote performance and wellbeing of chickens. Often times, the effect of these supplements are only monitored on performance attributes with little efforts on their impact on quality of produce from poultry. This study was therefore designed to assess the quality attributes of eggs produced by hens fed diets supplemented with Ascorbic Acid (AA) and cholecalciferol (D_3). The experiment was laid out in a 3 × 4 factorial arrangement in a completely randomized design. Eggs (n=288) obtained from hens fed three levels of supplemental AA (0, 300 and 600 mg/kg) and four levels of D_3 (0, 1000, 2000 and 3000 IU/kg) at the early laying (week 22-38) and mid laying (week 39-55) phases were monitored for external and internal egg quality traits. Neither dietary supplement of AA nor D_3 had influence (p>0.05) on all egg quality parameters monitored at the early laying phase except for yolk weight and yolk %. At the mid-laying phase, shell thickness improved with supplemental AA and cholecalciferol while 2000 IU/kg cholecalciferol increased Haugh unit (90.27). Effects of interaction of AA and cholecalciferol was only significant (p<0.05) on yolk % and diameter. In conclusion, supplemental AA and D_3 improved eggshell thickness while 2000 IU/kg supplemental D_3 increased Haugh unit. Combined dietary supplement of AA and D_3 for the hens enhanced egg yolk percentage and diameter.

Keywords: Supplemental vitamins • Egg quality characteristics • Shell thickness • Haugh unit • Albumen height

Introduction

Vitamins are previously believed to be useful only for the treatment and correction of deficiency diseases in livestock. According to report [1], dietary recommendation of vitamins and minerals in livestock was with respect to age, sex, breed, environment, season, health among others. These are however, now outdated and inadequate for the present day more highly productive commercial stock. The importance of vitamins is presently beyond prophylactic and curative treatments of diseases; rather, it has become an essential production tool in livestock management.

More recently, the diets of livestock, especially, poultry are fortified with supplemental vitamins to promote their wellbeing. The use of Ascorbic Acid (AA) also known as vitamin C, for example, has been prominent for stress management [2,3], performance [3-8], immunity, while vitamin D_3 has mostly been implicated in the improvement of immunity [9-12], optimization of skeletal integrity [13-17], egg nutrient deposition [18] and eggshell strength [19,20] in chickens.

In spite of these reports on the use of AA and D_3 in poultry, attention so far has been so much on performance attributes without similar emphases on the quality attributes of poultry produce. Few reports on egg quality attributes [4,21] from hens fed diets supplemented with combined AA and D_3, were inconsistent. This study was therefore aimed at assessing the implications of the sole and combined dietary supplement of AA and D_3 on hen egg quality characteristics.

Materials and Methods

Experimental location

The experimental site was the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The study area lies between longitude 7°27.05 north and 3°53.74 of the Greenwich Meridian east at an altitude 200 m above sea level. Average temperature and relative humidity of the location is between 23-42 °C and 60-80%, respectively. Laboratory procedures were conducted at the Animal Products and Processing Laboratory and the Nutritional Biochemistry Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental pullets and management

Bovan Brown pullets (n=384) with a track record of medication, vaccination schedule and productive performance from one day-old were used for this experiment. The pullets were raised in a conventional battery house. Each

***Address for Correspondence:** Olugbenga Adeniran Ogunwole, Agricultural Biochemistry & Nutrition Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria, E-mail: oa.ogunwole@ui.edu.ng*

Received: 23 February, 2024, Manuscript No. VTE-24-128100; **Editor Assigned:** 26 February, 2024, PreQC No. P-128100; **Reviewed:** 18 March, 2024, QC No. Q-128100; **Revised:** 23 March, 2024, Manuscript No. R-128100; **Published:** 30 March, 2024, DOI: 10.37421/2376-1318.2024.13.299

cage in the three-tier cage which measured 40 × 41 × 32 cm. The pullets were allotted to an isonitrogenous and isocaloric basal diet supplemented with three levels of AA (0, 300 and 600 mg) and four levels of D_3 (0 IU, 1000 IU, 2000 IU and 300 IU) in a 3 × 4 factorial arrangement of a completely randomized design to produce 12 treatments. Each treatment was replicated four times. Details of dietary experimental layout have been published [17].

Sample collection

Freshly laid eggs (n=96) (2 per replicate and 8 per treatment) were each collected at weeks 26, 30 and 34 in the early laying period (week 22-38) as well as weeks 43, 47 and 51 in mid laying phase (week 39-55). The eggs collected were assessed for internal and external egg characteristics.

Parameters monitored

Egg weight (g) was measured with a sensitive scale. Egg length and diameter (mm) were obtained using digital Vernier caliper. Egg shell thickness was assessed at three respective locations (broad, middle and small ends) with a micrometer screw gauge and the average taken [22] while, shell weight was measured with a sensitive scale after drying the shell at room temperature [23]. Each egg was broken on a flat surface for internal egg quality indices measurement. Yolk was carefully separated from the albumen for measurement. Yolk diameter and albumen diameter were monitored using a Vernier caliper. Yolk was placed on a petri-dish of known weight and measured using electronic digital scale. Yolk height was measured by dipping the pointed tip of the Vernier caliper through the middle of the yolk while for albumen height it was measured 1 cm away from the edge of the yolk and the height of the sticky fluid on the tip was adjusted to read the height on the digital screen. Yolk and shell weights were deducted from the weight of the egg to obtain albumen weight. The relationship between albumen height and egg weight was used to calculate the Haugh unit (Hu) as described by Haugh RR from the equation [24];

$Hu = 100 \log_{10} (h - 1.7W^{0.37} + 7.6)$; where

Hu = Haugh unit;

h = height of the albumen (mm)

W = egg weight (g)

Data were pooled for each monitored parameter in this study and the mean from the pool was further processed statistically.

Experimental design

The experiment was a factorial arrangement in a completely randomized design with the experimental model is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + e_{ijk}$$

Where;

Y_{ijk} = Observation k^{th} in i^{th} level of factor A and j^{th} level of factor B

μ = Overall population mean

α_i = Effect of i^{th} level of ascorbic acid supplementation

β_j = Effect of j^{th} level of cholecalciferol supplementation

$\alpha_i\beta_j$ = Interaction effect of ascorbic acid and cholecalciferol supplementation

e_{ijk} = random error with mean 0 and variance σ^2

Statistical analysis

Data were subjected to two-way ANOVA of the GLM procedure of SAS [25] and the means separated using Tukey's HSD option of the same software at $\alpha_{0.05}$.

Results

Egg quality attributes of laying hens at the early laying phase (22-38 weeks)

The external characteristics of eggs from hens fed supplemental AA and D_3 at the early phase of laying are shown in Table 1. The influence of AA was not observed on all external egg parameters monitored at this phase (p>0.05). Egg weight (55.85-57.10 g), egg length (54.26-56.20 mm), egg diameter (42.80-43.04 mm), shell thickness (0.37-0.40 mm), shell weight (5.13-5.59 g) and egg shell percentage (9.18-9.82%) were not significantly affected (p>0.05) by AA supplementation. Similarly, dietary D_3 supplementation had no significant influence (p>0.05) on egg weight (55.04-56.88 g), egg length (54.21-56.05 mm), egg diameter (42.34-43.43 mm), shell thickness (0.37-0.38 mm), shell weight (5.17-5.55 g) and shell percentage (9.06-9.61%).

The internal characteristics of eggs from hens fed supplemental AA and D_3 at the early laying phase are shown in Table 2. Supplemental AA had no significant effect (p>0.05) on all monitored internal egg parameters except yolk weight and yolk %. Albumen weight, albumen length, albumen diameter, albumen height, yolk diameter ranged from 32.75 to 34.94 g, 93.54 to 100.92 mm, 70.20 to 79.90 mm, 6.28 to 6.79 mm and 37.72 to 40.45 mm, respectively. Others were 13.50 to 13.98, 1.10 to 1.36 mm, 58.67 to 61.63% and 78.73 to 82.96, respectively for yolk colour, yolk height, albumen % and Hu. Yolk weight of eggs from pullets on 0 mg/kg AA (13.56) was lower (p<0.05) than 14.79 and

Table 1. External characteristics of eggs from hens fed diets supplemented with varying inclusion levels of ascorbic acid and cholecalciferol at the early phase of laying.

	Egg			Shell		
	Weight (g)	Length (mm)	Diameter (mm)	Thickness (mm)	Weight (g)	%
			Ascorbic Acid (mg/Kg)			
0	56.66	54.26	43.04	0.37	5.32	9.38
300	57.1	56.2	42.86	0.4	5.59	9.82
600	55.85	55.45	42.8	0.37	5.13	9.18
SEM	1.59	0.91	1.03	0.03	0.33	0.48
			Cholecalciferol (IU/Kg)			
0	56.88	54.21	42.92	0.37	5.31	9.36
1000	57.72	56.05	43.18	0.38	5.55	9.6
2000	55.04	55.58	42.34	0.38	5.29	9.61
3000	56.83	55.33	43.43	0.38	5.17	9.06
SEM	1.77	1.06	0.94	0.02	0.38	0.53
			P-Value			
Ascorbic acid	0.84	0.53	0.99	0.58	0.82	0.77
Cholecalciferol	0.45	0.47	0.71	0.99	0.84	0.48
Ascorb. × Chole.	0.78	0.83	0.98	0.52	0.69	0.62

Means with similar superscripts along the same column are not significantly different (P>0.05)

Ascorb.: Ascorbic Acid; Chole: Cholecalciferol; SEM: Standard Error of Mean

14.58 g in those on 300 and 600 mg/kg supplemental AA, while yolk (%) of pullets on 0 mg/kg (24.78%) was lower than 26.12% in those on 300 and 600 mg/kg supplemental AA, respectively.

The D_3 had no influence (p>0.05) on all internal egg parameters observed at this phase. Albumen weight, albumen length, albumen diameter, albumen height and albumen % ranged from 33.32 to 34.34, 81.87 to 105.26, 71.64 to 78.25, 6.01 to 7.44 and 59.08 to 60.28, respectively. Yolk weight ranged from 12.93 to 14.19, yolk diameter from 37.45 to 40.58, yolk colour from 12.93 to 14.19, yolk height from 1.00 to 1.33, yolk % from 25.06 to 25.93 and Hu from 77.95 to 86.75. Effect of interaction of ascorbic acid and cholecalciferol were not significant (p>0.05) except for the yolk %.

Effects of dietary supplement of AA and D_3 on egg quality attributes of hens at the mid-laying phase (week 39-55)

The external characteristics of egg from hens given diets supplemented with AA and D_3 at the mid phase of lay are shown in Table 3. The dietary supplemental AA had no significant effect (p>0.05) on EW (63.0 to 65.22), EL (56.66 to 57.46), ED (44.14 to 44.60), SW (6.30 to 6.84) and S% (10.04 to 10.48). Shell thickness of eggs from hens given 600 mg/kg supplemental AA (0.41) was similar (p>0.05) to those on 300 mg/kg AA (0.39) but thicker (p<0.05) than 0.37 in those on 0 mg/kg AA. Similarly, supplemental D_3 resulted in significantly thicker (p<0.05) egg shell in hens on 3000 IU/kg (0.42) compared to 0.37 in those on 0 IU/kg supplemental D_3. However, shell thickness of eggs from hens on 3000 IU/kg was similar (p>0.05) to 0.39 in hens fed 1000 and 2000 IU/kg D_3.

The internal characteristics of eggs from hens fed supplemental AA and D_3 at the mid phase of lay are shown in Table 4. Supplemental AA had no influence on albumen weight (39.60 to 39.74), albumen length (85.06 to 90.79), albumen diameter (70.98 to 75.51), albumen height (7.44 to 7.94) and albumen % (62.51 to 63.00). Yolk weight (14.88 to 15.22), yolk diameter (38.96 to 39.39), yolk height (13.16 to 14.89), yolk colour (5.90 to 6.50), yolk % (22.92 to 24.03) and Hu (84.54 to 88.03) also followed the same pattern. However, dietary supplement of D_3 on the other hand, had no significant influence (p>0.05) on monitored parameters except for albumen height, yolk diameter, yolk weight and HU. Albumen height of eggs from hens on 2000 IU/kg D_3 (8.37) was similar (p>0.05) to those on 1000 IU/kg (7.55) and 3000 IU/kg D_3 (7.78)

Table 2. Internal characteristics of eggs from laying hens fed diets supplemented with varying inclusion levels of ascorbic acid and cholecalciferol at the early phase of lay.

	Albumen					Yolk					
	Weight (g)	Length (mm)	Diam. (mm)	Height (mm)	%	Weight (g)	Diam. (mm)	Colour	Height (mm)	%	HU
Ascorbic Acid (mg/Kg)											
0	34.94	93.54	70.2	6.79	61.63	13.56[b]	37.72	13.54	1.1	24.78[b]	82.96
300	34.29	100.92	79.9	6.57	58.67	14.79[a]	40.45	13.98	1.13	26.12[a]	78.73
600	32.75	95.03	73.39	6.28	58.67	14.58[a]	40.28	13.5	1.36	26.12[a]	78.73
SEM	1.56	7.6	4.66	0.65	1.56	0.41	1.32	0.67	0.24	0.63	4.29
Cholecalciferol (IU/Kg)											
0	34.34	96.12	73.39	6.01	60.28	14.36	37.45	14.19	1.13	25.28	77.75
1000	34.15	105.26	78.25	7.44	59.08	14.92	40.16	14.12	1	25.93	86.57
2000	33.32	99.59	71.64	6.31	60.48	13.77	39.99	12.93	1.33	25.06	79.17
3000	34.08	81.87	74.51	6.67	59.89	14.33	40.58	13.52	1.33	25.26	82.04
SEM	1.6	10.04	5.18	0.8	1.77	0.47	1.61	0.89	0.3	0.71	5.67
P-Value											
Ascorbic acid	0.55	0.44	0.65	0.81	0.3	0.04	0.44	0.39	0.89	0.04	0.78
Cholecalciferol	0.74	0.3	0.71	0.36	0.75	0.11	0.21	0.32	0.67	0.48	0.4
Ascorb. × Chol.	0.37	0.53	0.75	0.86	0.22	0.18	0.99	0.27	0.9	0.01	0.84

[abc] Means with similar superscripts along the same column are not significantly different (P>0.05)
SEM: Standard Error of Mean; Diam.: Diameter; HU: Haugh Unit; Ascorb.: Ascorbic Acid; Chol: Cholecalciferol

Table 3. External characteristics of eggs from hens fed diets supplemented with varying inclusion levels of ascorbic acid and cholecalciferol at the mid phase of laying.

	Egg			Shell		
	Weight (g)	Length (mm)	Diameter(mm)	Thickness (mm)	Weight (g)	%
Ascorbic Acid (mg/Kg)						
0	65.22	57.46	44.6	0.37[b]	6.84	10.48
300	63	56.66	44.24	0.39[ab]	6.3	10.04
600	63.5	57.2	44.14	0.41[a]	6.84	10.19
SEM	1.93	0.79	0.45	0.01	0.25	0.3
Cholecalciferol (IU/Kg)						
0	63.79	57.1	44.27	0.37[b]	6.54	10.27
1000	62.77	56.78	44.03	0.39[ab]	6.67	10.63
2000	64.77	57.34	44.73	0.39[ab]	6.43	9.91
3000	64.58	57.24	44.04	0.42[a]	6.49	10.07
SEM	2.23	0.92	0.52	0.13	0.3	0.35
P-Value						
Ascorbic acid	0.53	0.58	0.7	0.01	0.12	0.32
Cholecalciferol	0.79	0.92	0.65	0.04	0.92	0.29
Ascorb.× Chole.	0.34	0.78	0.2	0.87	0.54	0.47

[ab] Means with similar superscripts along the same column are not significantly different (P>0.05)
Ascorb.: Ascorbic Acid; Chole: Cholecalciferol; SEM: Standard Error of Mean

Table 4. Internal characteristics of eggs from hens fed diets supplemented with varying inclusion levels of ascorbic acid and cholecalciferol at the mid laying phase.

	Albumen					Yolk					
	Weight (g)	Length (mm)	Diam (mm)	Height (mm)	%	Weight(g)	Diam (mm)	Height (mm)	Colour	%	HU
Ascorbic Acid (mg/Kg)											
0	41.13	85.49	72.33	7.44	63	14.91	38.96	13.16	6.25	22.92	84.54
300	39.6	85.06	70.98	7.94	62.88	14.88	39.17	14.89	6.5	23.61	88.03
600	39.74	90.79	75.51	7.57	62.51	15.22	39.39	14.25	5.9	24.03	85.78
SEM	1.38	2.76	2	0.38	0.66	0.43	0.52	1.02	0.52	0.56	2.29
Cholecalciferol (IU/Kg)											
0	39.7	90.68	75.04	7.07[b]	62.22	15.09[ab]	39.45[ab]	12.63	6.89	23.69	82.50[b]
1000	39.79	82.53	70.76	7.55[ab]	63.33	14.37[b]	38.19[b]	14.01	6.44	22.98	86.11[b]
2000	40.14	86.88	69.89	8.37[a]	61.98	15.09[a]	40.64[a]	14.24	6	24.46	90.27[a]
3000	41.08	87.49	74.83	7.78[ab]	63.56	14.87[ab]	38.68[b]	15.51	5.55	23.06	86.58[b]
SEM	1.6	3.18	2.31	0.44	0.77	0.5	0.6	0.6	1.18	0.65	2.64
P-Value											
Ascorbic acid	0.55	0.07	0.3	0.43	0.98	0.88	0.91	0.26	0.45	0.36	0.33
Cholecalciferol	0.79	0.09	0.1	0.04	0.3	0.02	0.05	0.15	0.14	0.48	0.04
Ascorb. × Chole	0.21	0.14	0.23	0.17	0.06	0.08	0.01	0.21	0.5	0.03	0.19

[ab]Means with similar superscripts along the same column are not significantly different (P>0.05)

Diam; Diameter; HU: Haugh Unit; Ascorb.: Ascorbic acid; Chole: Cholecalciferol; SEM: Standard Error of Mean

but significantly higher (p<0.05) than 7.07 in those on 0 IU/kg D_3. Yolk weight of eggs from hens offered 2000 IU/kg (15.09) was significantly higher (p<0.05) than 14.37 in those on 1000 IU/kg but similar (p>0.05) to 15.09 and 14.87 in those on 0 and 3000 IU/kg D_3 supplementation. The Hu of eggs from hens on 2000 IU/kg (90.27) was higher than 85.50, 86.11 and 86.58 in eggs from hens on 0, 1000 and 3000 IU/kg D_3, respectively. Effects of interaction of AA and D_3 dietary supplementation was however significant (p<0.05) for yolk % and diameter.

Discussion

Early laying phase

Egg Weight (EW) is a parameter that could be determined without breaking an egg [26,27] and a vital egg characteristic which impacts highly on quality and grading [27,28]. Egg weight from this study was similar irrespective of whether they were obtained from hens fed vitamin supplemented diets individually or in combination. The eggs were however, within medium to large sized category (55.04-57.10 g) in line with Canadian egg size standards [29,30]. It was earlier noted that egg weight is directly proportional to the size of the albumen, yolk and shell [31]. Since egg weight is similar across treatments, it is expected that quality parameters for albumen, yolk and shell be the same.

The similarity in egg weight may also be an indication that the dietary supplementations did not confer any negative influence on the productivity of the hens in this study. Earlier reports showed improvement in production parameters with supplemental AA or D_3 in poultry [3,6,8,9,15-17,20]. Other reports also showed that AA did not proffer any beneficial influence on egg production parameters in chickens under normal environmental conditions [20,32,33]. Conversely, dietary AA supplementation did not influence observation in this study, in spite the hens were raised under temperature above thermoneutrality. The plausible reason for the insignificant effect of dietary supplement of AA on measured egg parameters would be that the effects of the vitamins on physical egg qualities at this stage were not pronounced as the stress of laying was mitigated with the adequate dietary D_3 levels. Since egg weight was reported to be directly proportional to albumen, yolk and shell [32,34], the similarity in albumen and yolk attributes monitored in vitamin treated and non-treated groups was unexpected. Yolk weight and yolk percentage showed a different trend as they were influenced by AA supplementation. Similarly, Adesola AA, et al. observed changes in albumen, yolk and shell weight in Venda hens fed AA supplemented diets [35].

Though, eggshell thickness at this phase of the study was similar across treatments, the values were higher than approximated 0.33 mm tagged suitable to withstand normal handling chance of conditions for at least 50% without breakage [36]. This suggests that the eggs were strong enough to withstand considerable handling or transportation pressures, contrary to observed low egg HU when diets of hens had no supplemental AA [37]. At week 28 of the birds' age, authors observed that albumen index and Hu increased with AA supplementation, with noticeable changes in HU values at week 35. Yolk weight and % were the only internal egg parameters which improved with dietary supplement of AA in this study.

Dietary AA supplement has been implicated in the uptake of nutrients especially non-heme iron in plant-based feed to a more absorbable form for use by animals [38]. This attribute of supplemental AA in the feeds of animals may account for the observed increase in yolk weight and yolk percentage. The observed improvement in some yolk indices due to AA supplementation conformed to earlier reports on improved yolk attributes [39,40] though this did not translate to increase in deposition of calcium, iron and phosphorus as earlier reported by OA Ogunwole [18]. The scanty reports by Saki AA, et al. [41] on supplemental AA effect on egg yolk were inconsistent with earlier study of Çıftçı M, et al. [39] that hens offered supplemental AA, α-tocopherol or their combination had higher deposition of yolk in the egg compared to those on the control diet. Other authors [33,37] however, did not report such deposition in the egg.

Mid-laying phase

Although the influence of dietary vitamin supplements was not observed in the early laying phase of the hens. Howbeit, their roles were obvious at this stage as shown in Table 3. Amidst all egg assessed external parameters, egg shell thickness was the only parameter that was improved at this stage [42]. The influence of hen age on the observed increase in eggshell thickness compared to the early laying phase may not be totally erased as there has been documentation on changes in egg shell weight with the age of laying flock [43]. Shell thickness in this study (0.37-0.42 mm) were higher than 0.29 to 0.34 mm reported [44]; and 0.354 mm [45] in 20-24 weeks old hen but similar to 0.372 mm in 56-60 weeks old hens [45]. Another author [46] observed decreased shell thickness with increased hen age. Observed improvement in eggshell thickness at this phase of study compared to the early stage of lay could be that the hens had developed or improved in their efficient use and mobilization of calcium and phosphorus for shell production. This further explained the importance of vitamin C and D on egg shell calcification.

According to Animal Nutrition (7th edition) [47] improved shell thickness in hens given AA could be adduced to importance of the vitamin in collagen metabolism sine qua non for the normal structure of egg shells. Also, dietary AA has the capacity to maintain dietary metallic ions in reduced state for enhance rapid absorption and utilization [48]. Thus, AA perhaps stimulated the mobilizing potential of D_3 for calcium and phosphorus in egg shell calcification. Also, the cofactor D_3, is imperative for the synthesis of calbindin required for active calcium uptake across intestinal membrane may facilitate Ca availability for shell synthesis [49].

Similarly, studies [4] and [50], shown that AA improved eggshell thickness while other reports [4, 50,51] showed improved shell quality in hens given vitamin D_3 compared to the control group. Other authors [33,39.41.51], conversely, reported no effect of AA and D_3 [52-58] on monitored egg shell quality parameters.

The nutrition of the hen does not appear to have any great effect on albumen quality [59,60]. Authors [61] ascertained earlier that any nutrient which improves the rate of production would affect the quality of the eggs. Supplemental dietary AA had no influence on albumen, yolk and Hu at this phase. The Hu, yolk and albumen are vital indices of egg quality [41]. Haugh unit is a measure of the integrity or protein quality in the egg [24] and is a numerical expression of the quality of egg albumen [41]. Contrary to observations in this study, albumen quality and HU, were reportedly improved with AA supplementation [20,38]. Also, albumen height or HU or both were reportedly improved by adding AA to the diet of laying hens [59,60]. Other reports on effects of AA on albumen quality were inconsistent [33,39-41,60,61]. However, in this study, supplemental D_3 at 2000 IU/kg was observed to increase albumen height, yolk weight and diameter and HU. This suggests that supplemental vitamin D_3 increased the quality of protein in eggs of the hens when diets were supplemented with D3. There was progressive increase in HU with higher dietary D_3 supplementation. Beyond 2000 IU/kg dietary supplementation with D_3, the HU diminished, thus suggesting the quality of protein may be affected by higher supplemental levels of D_3. Contrary to findings in this study, Observations [62] did not find any effect of D_3 intake on internal egg quality of second productive cycle hens which allied with other findings [63] on non-observable effect of D_3 administration in the diets of hens on yolk parameters and HU. Interaction effect of AA and D_3 was consistently positive on egg yolk % at the early and mid-laying stages. This suggests that both vitamins had synergistic effect and would positively enhance yolk percentage when combined in the diets of hens. Previous study had earlier demonstrated the synergistic relationship between AA and D_3 on increased phosphorus deposition in the yolk as reported [18]. Perhaps the increase in yolk percentage observed in this study may be due to increased nutrient deposition in the egg.

Conclusion

The influence of ascorbic acid and cholecalciferol on egg quality attributes of hens may be age related. Supplemental ascorbic acid and cholecalciferol improved eggshell thickness while supplemental cholecalciferol beyond 2000 IU/kg in hens' diets lowered the quality of egg albumen and Haugh unit. Interaction of dietary supplement of ascorbic acid and cholecalciferol in the diets of laying hens enhanced egg yolk percentage and diameter.

It is recommended that further studies be conducted on the relationship between vitamin D and internal egg parameters.

Acknowledgement

None.

Funding Statement

Authors hereby express our unflinching appreciation to the Tertiary Education Funds (TETFUND) for the institutional grant availed to Prof. O. A. Ogunwole from which the research was funded.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Dale, Nick. "National research council nutrient requirements of poultry–ninth revised edition (1994)." *J Appl Poult Res* 3 (1994): 101.
2. McKee, J. S. and P. C. Harrison. "Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors." *Poult Sci* 74 (1995): 1772-1785.
3. Adedeji, B. S. and O. A. Ogunwole. "Performance and physiological response of heat stressed growing pullets fed diets supplemented with ascorbic acid." *Niger J Anim Prod* 44 (2017): 167-176.
4. Faria, D. E., O. M. Junqueira, P. A. Souza and E. A. L. Titto. "Performance, body temperature and egg quality of laying hens fed vitamins D and C under three environmental temperatures." *Braz J Poultry Sci* 3 (2001): 49-56.
5. Sahin, Kazim, Osman Kucuk, Nurhan Sahin and Mustafa Sari. "Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite and mineral concentrations of Japanese quails reared under heat stress (34 °C)." *Int J Vitam Nutr Res* 72 (2002): 91-100.
6. Sahin, Kazim, Nurhan Sahin, Muhittin Onderci and Mehmet F. Gursu, et al. "Vitamin C and E can alleviate negative effects of heat stress in Japanese quails." *Food Agric Environ* 2 (2003): 244-249.
7. Oso, A. O., O. E. Oke, M. O. Abioja and J. A. Abiona, et al. "Growth and physiological response of local turkey (*Meleagris gallopavo*) offered dietary vitamin C." *Pac J Sci Technol* 14 (2013): 441-447.
8. Ogunwole, O. A., Akinola Yinka Paul Ojelade, M. O. Oyewo and E. A. Essien. "Proximate composition and physical characteristics of eggs from laying chickens fed different proprietary vitamin-mineral premixes under two rearing systems during storage." *Int J Food Sci Nutr Eng* 5 (2015): 59-67.
9. Adedeji, B. S., O. A. Ogunwole, M. D. Olumide and Mosuro AO. "Performance and blood profile of growing pullets fed diets supplemented with cholecalciferol." *Int J Agric For* 8 (2018): 213-219.
10. Aslam, S. M., J. D. Garlich and M. A. Qureshi. "Vitamin D deficiency alters the immune responses of broiler chicks." *Poult Sci* 77 (1998): 842-849.
11. Reinhardt, Timothy A. and Frank G. Hustmyer. "Role of vitamin D in the immune system." *J Dairy Sci* 70 (1987): 952-962.
12. DeLuca, Hector F. and Claudia Zierold. "Mechanisms and functions of vitamin D." *Nutr Rev* 56 (1998): S4-S10.
13. Frost, T. J., D. A. Roland Sr and G. G. Untawale. "Influence of vitamin D_3, 1α-Hydroxyvitamin D_3 and 1, 25-Dihydroxyvitamin D_3 on eggshell quality, tibia strength and various production parameters in commercial laying hens." *Poult Sci* 69 (1990): 2008-2016.
14. Hustmyer, Frank G., Munro Peacock, Siu Hui and C. Conrad Johnston, et al. "Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus." *J Clin Invest* 94 (1994): 2130-2134.
15. Van Leeuwen, Johannes PTM, Gert-Jan CM van den Bemd, Marjolein van Driel and Cok J. Buurman, et al. "24, 25-Dihydroxyvitamin D_3 and bone metabolism." *Steroids* 66 (2001): 375-380.
16. Fritts, C. A. and P. W. Waldroup. "Effect of source and level of vitamin D on live performance and bone development in growing broilers." *J Appl Poult Res* 12 (2003): 45-52.
17. Ogunwole, O. A., B. S. Adedeji, M. D. Olumide and A. O. Mosuro, et al. "Effects of dietary supplemental ascorbic acid and cholecalciferol on bone characteristics of hens at the late laying stage." *Int J Food Sci Nutr Eng* 8 (2018): 142-150.
18. Ogunwole, O. A., A. Adeeko and B. S. Adedeji. "Vitamin C, iron and phosphorous deposition in eggs of pullets fed dietary supplement of vitamin C and D at the mid laying phase."
19. Shen, Huile, J. D. Summers and S. Leeson. "Egg production and shell quality of layers fed various levels of vitamin D_3." *Poult Sci* 60 (1981): 1485-1490.
20. Keshavarz, K. "The effect of different levels of vitamin C and cholecalciferol with

adequate or marginal levels of dietary calcium on performance and eggshell quality of laying hens." *Poult Sci* 75 (1996): 1227-1235.

21. Keshavarz, K. "The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens." *Poult Sci* 82 (2003): 71-91.
22. Tyler, C. "Shell strength: Its measurement and its relationship to other factors." *Br Poult Sci* 2 (1961): 3-19.
23. Scott, T. A. and F. Gi Silversides. "The effect of storage and strain of hen on egg quality." *Poult Sci* 79 (2000): 1725-1729.
24. Haugh, R. R. "The Haugh unit for measuring egg quality." United States Egg Poultry Magazine 43 (1937): 552-555.
25. https://support.sas.com/documentation/onlinedoc/91pdf/sasdoc_91/stat_ug_7313.pdf
26. Coutts, Jeffrey A. and Graham C. Wilson. "Egg quality handbook." Queensland Department of Primary Industries (1991).
27. Farooq, M., M. A. Mian, Murad Ali Murad Ali and F. R. Durrani, et al. "Egg traits of Fayumi birds under subtropical conditions." (2001): 141-145.
28. Khurshid, A., M. Farooq, F. R. Durrani and K. Sarbiland, et al. "Predicting egg weight, shell weight, shell thickness and hatching chick weight of Japanese quails using various egg traits as regressors." (2003): 164-167.
29. https://www.ams.usda.gov/sites/default/files/media/Egg%20Grading%20Manual.pdf
30. Seidler, E. S. "Egg marketing. A guide for the production and sale of eggs." FAO Agricultural Services Bulletin (FAO) (2003).
31. Shi, S. R., K. H. Wang, T. C. Dou and H. M. Yang. "Egg weight affects some quality traits of chicken eggs." *J Food Agric Environ* 7 (2009): 432-434.
32. Kechik, Ishak T. and A. H. Sykes. "The effect of intestinal coccidiosis (*Eimeria acervulina*) on blood and tissue ascorbic acid concentrations." *Br J Nutr* 42 (1979): 97-103.
33. Amaefule, K. U., G. S. Ojewola and E. C. Uchegbu. "The effect of methionine, lysine and/or vitamin C (ascorbic acid) supplementation on egg production and egg quality characteristics of layers in the humid tropics." *Livest Res Rural Dev* 16 (2004): 2004.
34. Pandey, N. K., C. M. Mahapatra, S. S. Verma and D. C. Johari. "Effect of strain on physical egg quality characteristics in White Leghorn chickens." (1986): 304-307.
35. Adesola, Adejoke Abidemi, Jones Wilfred Ng'ambi and David Norris. "Effect of ascorbic acid supplementation level to diets of indigenous Venda hens on egg production, hatchability and subsequent productivity of chicks." *Afr J Biotechnol* 11 (2012): 12606-12611.
36. Stadelman, W. J. "Quality identification of shell eggs in egg science and technology." Ed. WJ Stadelman and Cotterill DJ AVI Publishing company Inc. Westport, Connecticut 33 (1977).
37. Souza, Pedro Alves De, Hirasilva Borba Alves De Souza, Alexandre OBA and Claudio Henrique Cruz Gardini. "Influence of ascorbic acid on egg quality." *Food Sci Technol* 21 (2001): 273-275.
38. Gershoff, Stanley N. "Vitamin C (ascorbic acid): New roles, new requirements?." *Nutr Rev* 51 (1993): 313-326.
39. Çıftçı, M., O. Nihat Ertas and T. Güler. "Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress." (2005): 107-111.
40. Asli, Maziar Mohiti, Seyed Abdollah Hosseini, Houshang Lotfollahian and Farid Shariatmadari. "Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature." *Int J Poult Sci* 6 (2007): 895-900.
41. Saki, A. A., M. Momeni, M. M. Tabatabaei and A. Ahmadi, et al. "Effect of feeding programs on broilers Cobb and Arbor Acres plus performance." *Int J Poult Sci* 9 (2010): 795-800.
42. Ajuwon, Kolapo M., Opes Matanmi and Olajumoke C. Daniyan. "Effect of water sources and ascorbic acid supplementation on egg quality and production parameters of laying hens." *Livest Res Rural Dev* 14 (2002).
43. Silversides, F. G. "The Haugh unit correction for egg weight is not adequate for comparing eggs from chickens of different lines and ages." *J Appl Poult Res* 3 (1994): 120-126.
44. Van Den Brand, H., H. K. Parmentier and and B. Kemp. "Effects of housing system (outdoor *vs*. cages) and age of laying hens on egg characteristics." *Br Poult Sci* 45 (2004): 745-752.
45. Tůmová, E. and Z. Ledvinka. "The effect of time of oviposition and age on egg weight, egg components weight and eggshell quality." *Arch Poult Sci* 73 (2009): 110-115.
46. Bozkurt, Zehra and Mustafa Tekerli. "The effects of hen age, genotype, period and temperature of storage on egg quality." *Kafkas Univ Vet Fak Derg* 15 (2009): 517-524.
47. https://eliasnutri.files.wordpress.com/2020/07/animal-nutrition-7th-edition.pdf
48. Olivares, Manuel, Fernando Pizarro, Oscar Pineda and José J. Name, et al. "Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans." *J Nutr* 127 (1997): 1407-1411.
49. Bar, A., E. Vax and S. Striem. "Relationships between calbindin (Mr 28,000) and calcium transport by the eggshell gland." *Comp Biochem Physiol A Mol Integr Physiol* 101 (1992): 845-848.
50. Silva, Man, Iran José Oliveira da Silva, Sônia Maria de Stefano Piedade and E. Martins, et al. "Resistance to heat stress in naked-neck broilers." *Braz J Poult Sci* 3 (2001): 27-33.
51. Mirabdollbaghi, J., S. A. Hosseini, H. Lotfollahian and M. Ali, et al. "Effects of ascorbic acid on egg production and egg shell quality in laying hens drinking saline water." (2006): 2897-2900.
52. Abdulrahim, S. M., M. B. Patel and J. McGinnis. "Effects of vitamin D_3 and D_3 metabolites on production parameters and hatchability of eggs." *Poult Sci* 58 (1979): 858-863.
53. Panda, A. K., S. V. Rama Rao, M. V. L. N. Raju and M. Niranjan, et al. "Influence of supplemental vitamin D_3 on production performance of aged white Leghorn layer breeders and their progeny." *Asian-Australas J Anim Sci* 19 (2006): 1638-1642.
54. Persia, M. E., M. Higgins, T. Wang and D. Trample, et al. "Effects of long-term supplementation of laying hens with high concentrations of cholecalciferol on performance and egg quality." *Poult Sci* 92 (2013): 2930-2937.
55. Saunders-Blades, J. L. and D. R. Korver. "Effect of hen age and maternal vitamin D source on performance, hatchability, bone mineral density and progeny *in vitro* early innate immune function." *Poult Sci* 94 (2015): 1233-1246.
56. Wells, R. G. "Egg shell strength: 2. The relationship between egg specific gravity and egg shell deformation and their reliability as indicators of shell strength." *Br Poult Sci* 8 (1967): 193-199.
57. Naber, E. C. "The effect of nutrition on the composition of eggs." *Poult Sci* 58 (1979): 518-528.
58. Williams, K. C. "Some factors affecting albumen quality with particular reference to Haugh unit score." *Worlds Poult Sci J* 48 (1992): 5-16.
59. Cheng, Thim K., Craig N. Coon and Melvin l. Hamre. "Effect of environmental stress on the ascorbic acid requirement of laying hens." *Poult Sci* 69 (1990): 774-780.
60. Kucuk, O., N. Sahin, K. Sahin and M. F. Gursu, et al. "Egg production, egg quality, and lipid peroxidation status in laying hens maintained at a low ambient temperature (6 °C) and fed a vitamin C and vitamin E-supplemented diet." *Vet Med* 48 (2003): 33.
61. Njoku, P. C. and Adeline OU Nwazota. "Effect of dietary inclusion of ascorbic acid and palm oil on the performance of laying hens in a hot tropical environment." *Br Poult Sci* 30 (1989): 831-840.
62. Plaimast, Hatairat, Suwanna Kijparkorn and Punyaphat Ittitanawong. "Effects of vitamin D_3 and calcium on productive performance, egg quality and vitamin D_3 content in egg of second production cycle hens." *Thai J Vet Med* 45 (2015): 189-195.
63. Geng, Yanqiang, Qiugang Ma, Zhong Wang and Yuming Guo. "Dietary vitamin D_3 supplementation protects laying hens against lipopolysaccharide-induced immunological stress." *Nutr Metab* 15 (2018): 1-14.

How to cite this article: Adedeji, Bamidele Samuel, Folasade O. Jemiseye, Adeyemi Ayodeji Afolabi and Sabur O. Oladimeji, et al. "Quality Attributes of Eggs from Laying Hens Fed Diets Supplemented with Varying Inclusion Levels of Ascorbic Acid and Cholecalciferol." *Vitam Miner* 13 (2024): 299.