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Effects of two supplemental proprietary vitamin-mineral premixes, blend of phyto-additives and storage duration on egg quality of Isa Brown layer chickens

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Target Audience: Egg suppliers, Food processors, Poultry farmers, Researchers

Abstract

Egg quality has been found to be influenced by diet variation and storage duration, hence the need to further explore the role of phyto-additives in enhancing egg quality. Two supplemental proprietary vitamin-mineral premixes, blend of phyto-additives (moringa+turmeric) with or without vitamin C and days of storage (DOS) on egg quality were assessed. Moringa-Turmeric leaf powder (MTLP) contained (g/kg): 914.5 dry matter, 122.4 crude protein, 31.5 ether extract, 105.4 ash and 46.4 crude fibre. Seventy-two (72) Isa Brown layers (22 weeks in lay) were divided into four treatments and six replicates each with three layers per replicate. Four maizesoybean meal basal diets were formulated such that diet A contained a proprietary vitamin/mineral premix. Diet B contained yolk colorant vitamin/mineral premix. Diet C had MTLP at 5g/kg diet while diet D contained MTLP (5g/kg) + Vit C (0.5g/kg diet). The diets were fed for eight weeks after which 45 eggs per treatment were picked and stored under room temperature. Nine eggs per treatment were evaluated on day 1 for egg quality parameters and subsequently after 7, 14, 21 and 28 DOS. Data were subjected to two-way analysis of variance using completely randomized design. As DOS increased, egg weight (EW), Haugh unit (HU), yolk index (YI), yolk color (YC) and shell thickness (ST) significantly (P < 0.05) decreased. HU and YI were not significantly affected (P>0.05) up to day 21 in layers fed diets C and D. Reductions in HU and EW were 33.69 vs 27.43 and 9.49 vs 6.18% in eggs from layers fed diets A and D, respectively after 28 DOS. MTLP had no adverse effect on quality of stored eggs at the level studied.

Keywords: Egg quality, Layers, Phyto-additives, Yolk pigmentation.

Description of Problem

The primary product of commercial layer production in Nigeria is table egg and, the movement of this product from farms to end users usually take a period of time. This situation could be more apparent during a period of lockdown as experienced with the Novel Coronavirus disease (COVID-19) pandemic that is currently ravaging the whole world (1). The transition period revealed that eggs were stored longer than necessary which may impact negatively on egg quality (2). Egg quality can also be influenced bv environmental conditions (temperature and humidity) and gaseous exchange (3; 4). Various research efforts have been made to improve the quality and shelf life of edible eggs through application of oil coatings (5), dietary nutrient interventions (6: 7). application of different vitamin/ mineral premixes (8; 9, 10) and use of phyto-additives (11; 12) which is gaining more ground. Moringa oleifera belongs to the family

Moringa oleffera belongs to the family Moringaceae and is known as a miracle tree because of its wealthy resource of various nutrients with high biological values. Various parts of the plant have been utilized in growth promoting, immune enhancer and antioxidant with hypo-cholesterol effect in chickens (13; 14). Turmeric (Curcuma longa), a leafy plant in the family of herbs, the Zigiberaceae is widely grown both as kitchen spice and for its medicinal uses. It is a natural source of yelloworange pigment, antioxidant, antimicrobial, anti-viral, anti-inflammatory and antitumor compounds (15). Both turmeric and moringa have been individually evaluated in egg production and egg quality studies with varying outcomes (16;17). It is hypothesized that their synergistic effect in poultry diets could help in stability and shelf life of poultry products.

Vitamins and minerals are added as premix in layer diets and are important micro-nutrients for egg production (8). Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin and a potent natural anti-oxidant that has been reported to improve egg production and quality (18;19) whereas (20) did not find any relationship between supplemental dietary ascorbic acid and qualitative parameters of the produced eggs. Vitamin C is needed to maintain normal body metabolic activities poultry especially for under stressful conditions (21).

The essence of this study is to see how dietary intervention may impact on the quality of stored eggs over a period of time. Therefore, the present study was conceived to ascertain the effects of two different vitamin / mineral premixes, blend of phyto-additives (moringa + turmeric) with or without ascorbic acid on egg quality parameters in laying hens during different storage durations.

Material and Methods Experimental site

The study was carried out at the Poultry Unit of Teaching and Research Farm of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The evaluation of stored eggs was done at the Animal Biosciences Unit, Department of Nutrition Biotechnology, Animal and LAUTECH, Ogbomoso. The study site is located in the derived savannah which lies on longitude 4⁰ 27¹ East of Greenwich meridian and Latitude $8^0 17^1$ North of the equator. The altitude is between 300m and 600m above sea level while the mean temperature and annual rainfalls are 27 °C and 1247 mm, respectively (22).

Sources and Preparation of Test ingredients

from *Moringa* Leaves oleifera and Curcuma longa plants were harvested at the flowering stage from the Teaching and Research Farm, LAUTECH, Ogbomoso. They were separated from the stems, air dried for 5 days until a crispy greenish colour was attained. The dried leaves were separately milled using an electric grinding machine (Master mixer grinder with 3 S.S jars, Excella model), sieved with 0.5mm mesh and mixed together (ratio 1:1) to form moringa-turmeric leaf powder (MTLP) and stored in an airtight until container needed. Two different proprietary vitamin-mineral premixes were purchased from a commercial feed milling factory in Ogbomoso and the compositions as displayed on the labels are shown in Table 1.

Experimental diets

Four isonitrogenous (18%CP) and isocaloric (2550 ME kcal/kg) maize/soyabean meal-based diets were formulated such that diet A contained a proprietary vitamin/mineral premix. Diet B contained yolk colorant vitamin/mineral premix. Diet C had MTLP at 5g/kg diet while diet D contained MTLP (5g/kg) + Vitamin C (0.5g/kg diet). The gross and the proximate compositions of the diets are shown in Table 1.

	Die	ets			
Ingredients (g/kg)	А	В	С	D	
White maize	500.0	500.0	500.0	500.0	
Soybean meal	203.0	203.0	203.0	203.0	
Wheat offal	150.0	150.0	150.0	150.0	
Fish meal	30.0	30.0	30.0	30.0	
Oyster shell	70.0	70.0	70.0	70.0	
Di calcium phosphate	40.0	40.0	40.0	40.0	
Methionine	2.5	2.5	2.5	2.5	
Salt	2.0	2.0	2.0	2.0	
MTLP*	-	-	+	+	
Vitamin C**	-	-	-	+	
Premix***	2.5	-	2.5	2.5	
Premix****	-	2.5	-		
Total	1000	1000	1000	1000	
Determined chemical composit	tion (g/kg diet)				
Crude protein	178.5	177.2	181.4	176.9	
Crude fibre	53.1	43.8	51.7	55.6	
Ether extract	54.2	45.9	55.9	48.2	
Ash	141.2	149.1	142.8	138.9	
Dry matter	909.4	910.8	912.5	921.4	
Nitrogen free extracts	482.4	494.8	480.7	501.8	
^a ME (kcal/kg)	2563.8	2555.4	2553.5	2553.5	

Table 1: Gross composition of diets

*MTLP = Moringa/turmeric leaf powder at ratio 1:1 (5g/kg),

**Vitamin C included at 0.5g/kg diet.

***Premix supplied per kg of layer diet: Vit. A 10,000,000i.u., Vit D₃ 2,200,000i.u., Vit E 10, 000mg, Vit K3, 2, 000mg, Vit B1, 1,500 mg; Vit B2 5, 000mg; Niacin 15, 000mg; Calpan, 5, 000mg; Vit B6, 1, 500mg; Vit. B12 10mg; Folic acid, 5, 000mg; Biotin, 20mcg; Choline chloride, 150,000mg, cobalt 500mg, copper 4,000mg, iodine, 1000mg; iron 10,000mg; Manganese, 70,000mg, Selenium, 200 mg, Zinc, 50,000mg and anti-oxidant 125,000mg.

****Premix supplied per kg of layer diet: Vit. A 10,000,000i.u., Vit D₃ 2,200,000i.u., Vit E 10, 000mg, Vit K3, 2, 000mg, Vit B1, 1,500 mg; Vit B2 5, 000mg; Niacin 15, 000mg; Calpan, 5, 000mg; Vit B6, 1, 500mg; Vit. B12 10mg; Folic acid, 5, 000mg; Biotin, 20mcg; Choline chloride, 150,000mg, cobalt 500mg, copper 4,000mg, iodine, 1000mg; iron 10,000mg; Manganese, 70,000mg, Selenium, 200 mg, Zinc, 50,000mg and anti-oxidant 125,000mg + Yolk colorant.

^aME (kcal/kg): calculated

Experimental laver chickens and management

Seventy-two Isa Brown layers (42 weeks old and 22 weeks in lay) were individually weighed and randomly allotted into four treatment groups. Each treatment group was further subdivided into six replicates with each replicate having 3 hens making a total of 18 layers per treatment. Each cage measuring 50 x 45 x 40cm³ accommodated three layers. Feed and fresh drinking water were made available ad libitum throughout the 8 weeks feeding trial.

Treatment SEM А В С P. value D Egg Weight (g) Day 1 59.77±2.01× 60.34±4.77× 60.97±2.25× 61.68±2.44× 1.14 0.24 0.98 Day 7 57.70±2.81bx 58.38±5.59ax 59.92±2.9ax 60.33±1.38axy 0.02 Day 14 57.08±3.21xy 57.92±1.18^y 58.27±3.94 xy 60.07±1.97^y 1.32 0.16 Day 21 54.97±5.28^z 56.68±2.12^z 57.08±2.27 ×y 58.05±2.89yz 1.02 0.95 Dav 28 54.10±2.71^z 55.40±3.87^z 56.57±4.82^z 57.87±0.4z 1.07 0.37 % EWL. 9.49 8.32 7.22 6.18 Shell thickness (mm) 0.44±2.71^z 0.01 0.13 Dav 1 0.42±2.71^z 0.43±2.71^z 0.42 ± 2.71^{z} Day 7 0.38±2.71^z 0.39±2.71z 0.40±2.71^z 0.40±2.71^z 0.01 0.06 Day 14 0.35±2.71zyb 0.39±2.7ax 0.38±2.7ª 0.40±2.7ª 0.01 0.17 Day 21 0.35±2.7by 0.41±2.7^{az} 0.41±2.7^{az} 0.01 0.41 0.34±2.7^{by} Day 28 0.35+2.7^{by} 0.36+2.7^{by} 0.39+2.7^a 0.39+2.7^a 0.00 0.79 % Change 16.67 16.27 7.14 11.36

 Table 2: Effects of two proprietary vitamin-mineral premixes and blend of phyto-additives

 with or without vitamin C on egg weight and shell thickness during storage

^{a,b} Means across a row with different superscripts indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

^{x,y,z} Means with different superscripts along each column indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

Data Collection

At day 56 of feeding, 45 eggs were collected from each treatment (eggs pooled per treatment), labelled and individually weighed. From the first day of collection, 9 eggs from each treatment were analyzed for egg quality parameters. The remaining eggs were stored under room temperature $(28.4\pm3.1^{\circ}C)$ and 64±7.2%RH) which is the normal mode of storing eggs in Nigeria. Subsequently, nine eggs per treatment were evaluated on 7, 14, 21 and 28 days of storage (DOS). For egg quality parameters, the egg weight (EW) was determined with a sensitive electronic digital scale model 60332 (1 kg max, d 0.1g) in grams. The eggs were broken on to a flat surface where the height of the albumen was measured, halfway between yolk and edge of the inner thick albumen by using a tripod spherometer. The volk was separated from the albumen and weighed. Yolk width was measured the widest horizontal as circumference with a Vernier caliper while the height was measured as the height of the yolk at the mid-point with a tripod micrometer. The membrane was carefully removed from the shell and both were air dried at room temperature for 24 hours and weighed. Shell thickness was measured using micrometer screw gauge (Pittsburg^R Digital thickness gauge model 66319). The mean of three points (broad, middle and narrow) was taken as shell thickness. The Haugh unit, yolk index (YI) and egg weight loss were calculated as follows: Haugh unit (HU) = 100log (H+7.57 – 1.7W^{0.37}) where H is albumen height and W is egg weight (23)

Yolk index (YI) = Yolk height (mm))/ Yok diameter (mm).

Egg weight loss (EWL) = [(Egg weight - weight of stored egg) / Egg weight] x 100

The yolk colour (YC) was assessed with DSM yolk colour fan. For egg proportions, the weight of the albumen was calculated as the difference between the weight of the egg and weight of yolk plus shell plus membrane. The individual weights of the albumen, yolk, shell and membrane were expressed over egg weight and multiplied with 100.

storage						
Treatment	А	В	С	D	SEM	P. value
Haugh unit, %						
Day 1	91.77± 5.85 [×]	93.83±3.81×	91.01±5.61×	93.11±4.29 [×]	0.98	0.64
Day 7	82.10± 5.76 ^y	84.29±7.75 ^y	85.19±6.13×	83.22±7.13s	1.38	0.39
Day 14	72.50± 10.17 ^y	73.52±6.20 ^y	74.35±9.66×	75.66±5.14×	1.61	0.59
Day 21	68.58± 9.91 ^z	69.49±8.15 ^z	70.17±12.84 [×]	73.84±2.68 ^x	3.45	0.16
Day 28	60.85±7.97 ^{bz}	58.02±3.32 ^{bz}	65.60±3.15 ^{ay}	67.57±3.38 ^{ay}	5.49	0.92
% Change	33.69	38.17	27.92	27.43		
Yolk index						
Day 1	0.35±0.03×	0.37±0.04×	0.35±0.05×	0.39±0.04×	0.01	0.20
Day 7	0.30±0.04×	0.31±0.04×	0.28±0.03 ^{xy}	0.26±0.06 ^y	0.01	0.22
Day 14	0.23±0.05 ^y	0.25±0.04 ^y	0.22±0.04 ^{xy}	0.24±0.04 ^y	0.01	0.53
Day 21	0.20±0.03 ^{yz}	0.16±0.08 ^z	0.19±0.05 ^{xy}	0.18±0.09y ^z	0.02	0.14
Day 28	0.15±0.08 ^z	0.15± 0.08 ^z	0.16±0.09 ^z	0.17±0.12 ^z	0.02	0.94
% Change	57.14	59.46	54.29	56.41		
Yolk color						
Day 1	1.00±0.00 ^b	4.67±0.52ax	3.71±0.98 ^{ax}	3.83±1.17 ^{ax}	0.35	0.00
Day 7	1.00±0.00 ^b	3.33±0.52 ^{ax}	3.17±0.63 ^{ax}	3.33±0.82 ^{ax}	0.21	0.00
Day 14	1.00±0.00 ^b	3.17±2.14 ^{ax}	3.10±0.41 ^{ax}	3.33±0.52 ^{ax}	0.22	0.00
Day 21	1.00±0.00 ^b	2.83±0.41 ^{ay}	2.00±0.63 ^{ay}	2.50±0.55 ^{ax}	0.30	0.01
Day 28	1.00±0.00 ^b	2.67±0.52 ^{ay}	1.67±0.82 ^{bz}	2.17±1.17 ^{ay}	0.19	0.01
% Change	0.00	42.83	54.99	43.34		

Table 3: Effects of two proprietary vitamin-mineral premixes and blend of phyto-additives with or without vitamin C on Haugh unit, yolk index and yolk color at different days of storage

^{a,b} Means across a row with different superscripts indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

^{x,y,z} Means with different superscripts along each column indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

Chemical Analysis

The experimental diets and mixture of moringa+turmeric leaf powder was analyzed for proximate contents using the method of AOAC (24).

Statistical Analysis

Data were subjected to two-way analysis of variance using the completely randomized design (25). Significant means were separated by Duncan's multiple range test of the same software at P<0.05.

Results and Discussion

The resultant mixture of moringa-turmeric

leaf powder (MTLP) at ratio 1:1 contained 914.5g/kg dry matter, 122.4g/kg crude protein (CP), 31.5g/kg ether extract (EE), 105.4g/kg ash, 46.4g/kg crude fibre (CF) and 608.8 g/kg nitrogen free extracts (NFE). This revealed a product of medium protein, low fat, low fibre and high ash content. The CP and EE values were lower than 182.9-295.0g/kg and 43.8-171.1g/kg, respectively reported for *Moringa oleifera* leaf meal by (26; 27; 28). On the other hand, the CP of the mixture was higher than 63.0-98.9g/kg reported by other authors (15; 29) for turmeric powder.

Treatment*	A	B	C	D	SEM	P. value
Yolk %						
Day 1	26.92± 1.88	26.21±2.11	26.18±2.63	25.36±0.81×	0.42	0.17
Day 7	29.68±2.70	29.24±1.82	28.48±1.02	30.23±2.11×	0.40	0.50
Day 14	30.50±2.40	27.80±2.23	30.15±4.41	29.43±1.36×	0.58	0.38
Day 21	30.23±1.13 ^a	27.53±12.1 ^b	31.66±2.90 ^a	30.84±15.2 ^{ax}	2.37	0.21
Day 28	34.37±13.9	31.33±13.1	32.51±12.7	32.69±13.3 ^y	0.43	0.69
% Change	21.68	16.34	19.47	22.42		
Albumen %						
Day 1	58.58±2.87 ^{by}	64.02±3.55 ^{ax}	64.33±3.12 ^{ax}	64.27±1.04 ^{ax}	0.80	0.00
Day 7	62.35±2.72 ^y	61.77±2.10 ^y	63.70±1.10 [×]	61.05±2.10 ^y	0.44	0.19
Day 14	59.45±2.70 ^y	60.51±2.40 ^y	59.08±4.47 ^y	60.27±1.74 ^y	0.46	0.23
Day 21	58.71±0.83 ^{by}	62.18±11.3 ^{ay}	58.54±3.25 ^{by}	62.56±15.1 ^{ay}	2.32	0.19
Day 28	54.25±13.7 ^z	58.10±13.2 ^z	57.28±12.7 ^y	58.47±13.7 ^z	0.45	0.98
% Change	7.39	9.25	10.96	9.02		
Shell (%)						
Day 1	12.02±3.66 ^{ax}	9.29±1.73 ^b	8.78±0.78 ^b	8.98±0.72 ^b	0.60	0.00
Day 7	11.53±0.73×	10.59±0.68	9.35±0.44	10.31±0.72	0.17	0.01
Day 14	9.61±0.59 ^y	9.25±0.93	10.20±1.10	9.86±0.66	0.18	0.28
Day 21	10.52±1.50×	9.85±1.47	9.40±1.09×	10.10±0.86×	0.25	0.49
Day 28	10.10±0.84×	10.20±0.31	10.04±0.98	9.51±0.77	0.16	0.42
Membrane (%)						
Day 1	0.49±0.18	0.48±0.18	0.51±0.17	0.38±0.07	0.04	0.01
Day 7	0.45±0.12	0.40±0.10	0.47±0.14	0.42±0.19	0.03	0.40
Day 14	0.45±0.12	0.45±0.10	0.49±0.26	0.44±0.09	0.03	0.55
Day 21	0.42±0.19 ^y	0.44±0.07	0.41±0.13	0.40±0.05	0.03	0.31
Day 28	0.39±0.11	0.37±0.19	0.34±0.02	0.34±0.14	0.03	0.09

Table 4: Effects of two proprietary vitamin-mineral premixes and blend of phyto-additives with or without vitamin C on egg proportions at different days of storage

^{a,b} Means across a row with different superscripts indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

^{x,y,z} Means with different superscripts along each column indicate significant differences (p<0.05) while same or no superscript indicate no significant difference (p>0.05).

Effect of diet and DOS on egg weight and shell thickness are shown in Table 2. Egg weight was not significantly influenced (P>0.05) by dietary treatments in days 1, 14, 21 and 28 except on day 7 where the lowest (P<0.05) value of 57.7g was recorded for layers on diet A. The egg weights were numerically higher in layers fed diets C and D compared to those fed diets A and B irrespective of DOS. Earlier report (30) showed no significant difference in egg weights of hens fed 5-15% moringa leaf meal contrary to other report (31) with layers fed 0.2-0.8% moringa leaf meal. Average egg weight loss during storage was 9.49, 8.32, 7.22

and 6.18% in layers fed diets A, B, C and D respectively. The values were higher than the 2.08% and 3.11% for eggs stored within 5 and 10 DOS respectively (3) probably due to shorter time and cold storage condition employed. As storage duration increased, evaporation of water from eggs through eggshell pores rose, resulting in decreased egg weights (4, 6). There was no effect of diet on shell thickness between day 1 and 14. However, thicker shells were recorded in layers fed diets C and D compared to layers fed diets A and B by day 28. The higher egg weight and shell thickness underscored the

importance and sensitivity of supplemental vitamins and minerals, and the probable effectiveness of moringa-turmeric leaf powder in egg shell deposition. The DOS did not affect egg shell thickness of layers fed diet C whereas layers on diet A had significantly values (P<0.05). The reduced percent reduction observed in egg shell thickness was of the order 16.67, 16.27, 7.14 and 11.36 for layers on diets A, B, C and D respectively. Report by (32) showed a decrease in egg shell weight and shell thickness with extended storage time.

The effect of proprietary vitamin/mineral premixes and MTLP with or without vitamin C on Haugh unit (HU), yolk index (YI) and yolk color (YC) are shown in Table 3. Values for HU and YI were not influenced (P>0.05) by dietary treatments up to day 21, however by day 28, layers fed diets C and D had higher (p<0.05) HU values than those from diets A and B. Supplemental vitamin C caused no significant effect which was similar to findings by (33). HU and YI decreased (P<0.05) with increased DOS, similar to reports by (3, 34). HU is a measure of albumen quality and egg freshness. Changes in HU have a direct relationship with moisture loss through shell pores by evaporation and also escape of carbon dioxide from egg albumen (16). The loss of carbon dioxide and increase in pH caused the mucin fibers which gives albumen its gel structure to lose strength thereby becoming watery resulting to reduced HU of eggs during storage. In addition, as DOS increases, water migrates from albumen to the yolk through the vitelline membrane in an attempt to equalize the pressure between the albumen and yolk which leads to the swelling of the yolk and consequently exertion of pressure on the vitelline membrane (11). This pressure eventually causes the yolk to change from a spheroid shape to a round flattened mass thereby causing reduction in YI (33, 35). The HU decreased from 91.77 to 60.85 in layers

fed diet A (a loss of 33.7%) while it decreased from 93.1 to 67.6 (a loss of 27.4%) in layers fed diet D. The addition of yolk colorant to the premix in diet B did not exert any positive influence on HU and YI. It was reported that egg weight, shell weight, HU and YI were significantly higher when a mixture of five vegetable leaf meals totally replaced conventional premix in layer diets while shell thickness was not (P>0.05) affected (8). The higher the HU and YI, the more desirable is the egg quality (12) and inclusion of MTLP with or without vitamin C only caused numerical increase (P>0.05) on the eggs stored at Day 28. Yolk color was highest in layers fed diet B (4.67) followed by diet D (3.83). The yolk color values are generally low because white maize was used in all diets. With DOS, there was a decrease in egg yolk color similar to earlier findings (32). Addition of turmeric and moringa leaf powders separately in layer diets reportedly had positive effects on egg volk color (36). Turmeric contains curcuminoid, a non-volatile coloring agent that has a phenolic pigment called curcumin (37). Moringa leaf meal provides dietary protein, vitamins, minerals and oxy-carotenoids which elevates egg yolk coloration (12; 13; 38). The increased egg yolk coloration thus indicated that layers could absorb and utilize the carotene in MTLP.

Effect of dietary treatments and DOS on egg proportion is shown in Table 4. Percent yolk was not significantly influenced (P>0.05) by DOS and diet type except at Day 21. Percent albumen was influenced by diet on days 1 and 21 while diets impacted no effect at day 7, 14 and 28. Percent shell was influenced (P<0.05) by diets on day 1. Percent membrane was not influenced (P> 0.05) by diets and DOS. The observed linear increase in percent yolk and decreased proportion of albumen is a function of water or solvent diffusion from albumen to yolk with DOS which concurred with previous observations (4, 6).

Conclusion and Applications

- 1. The internal egg quality attributes viz a viz percent albumen, Haugh unit and yolk index decreased with DOS while percent yolk increased.
- 2. Egg weight and shell thickness were not affected by diet but by duration of storage.
- 3. Dietary supplementation with moringaturmeric leaf powder and vitamin C did not negatively influence quality of stored eggs.

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