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Housing systems and type of supplemental vitamin-mineral premix in hens' diets influenced deposition of vitamin in eggs at the late laying phase

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Target Audience: *Animal Nutritionists, Poultry Farmers, Poultry Scientists*

Abstract

Effects of five different proprietary vitamin-mineral premix (VMP) and two housing systems (HS) on deposition of vitamin B_1 (thiamine), B_6 (pyridoxine), D_3 (cholecalciferol), and folic acid in eggs of hens at the late laying stage were investigated. Black Bovan Nera hen strain (n=480) aged 59 weeks were randomly allotted to five dietary treatments; each treatment was replicated six times with eight hens per replicate. The hens were raised in a conventional cage and an open-sided deep litter (DPL) HS. Five isocaloric and isonitrogenous diets were formulated and each was supplemented with 0.25% proprietary VMP 1, 2, 3, 4 and 5 for the hens in cage and DL in 2x5 factorial arrangement and completely randomized design. At age 71 weeks, six randomly selected eggs per treatment (n=60) were processed and assayed for vitamin B_1, folate, B_6 and D_3. Significantly higher ($p<0.05$) thiamine deposition (0.063 mg/100g) was in the eggs of hens on VMP5 with similar ($p>0.05$) content of pyridoxine, folic acid and vitamin D. The HS had significant impact on vitamin D (93.969 IU) and foliate content (51.909 mg/100g) of the eggs in the DPL. Effect of interactions of HS x VMP on pyridoxine, folic acid and vitamin D_3 deposition in egg were similar ($p>0.05$) but was significantly higher ($p<0.05$) for thiamine deposition. Hens from DPL had higher vitamins deposition in egg than cage, while vitamin depositions in eggs were influenced by different the proprietary VMP and HS.

Keywords: *Deep litter, Battery, Proprietary vitamin-mineral premixes, Vitamin composition*

Description of Problem

Vitamins are essential nutrients contained in food, which are required in small quantity by humans. They participate in vital metabolic processes imperative for health and adequate body functions (1). The approach to ensure adequate vitamin intake in a population could be to produce vitamin-fortified foods such as eggs and encourage their intake by the populace. Intake of an egg per day will have positive impact in meeting human daily vitamin requirements. Studies have shown that vitamins in hen diets are absorbed into the blood stream and transferred to egg yolk via the ovary (2, 3). Thus, appropriate vitamins and minerals inclusion in the feed was reported to be crucial for chickens to achieve their full genetic potentials and enhance health, welfare and productivity (4, 5).

Housing system (HS) and nutrition are non-genetic factors that can improve the well-being of animals to obtain better quality products (6). An understanding of how different HS and commercial brands of vitamin-mineral premixes (VMP) can influence the vitamin B1, folate, B6 and D content of eggs will be of great importance first to the animal nutritionists and poultry farmers. Enrichment of eggs with vitamins can be achieved by manipulating the diets of laying hens. Eggs enriched with certain trace minerals, vitamins and a variety of bioactive substances can be an excellent source of vitamin in human diets (7). Studies (8, 9, 10) have confirmed the likelihood of novel functional eggs production with enhanced levels of important trace minerals and vitamins.

However, increasing all dietary vitamins concurrently may have little impact on egg vitamin status (11). High supplementation of certain vitamins in layer diets may negatively influence egg quality and vitamin deposition (3). Report (2) suggests that 102,000 IU dietary vitamin D would be toxic to hens and cause mal-metabolism of D vitamin as the hens tried to reduce vitamin D exposure. Therefore, optimum level of vitamins and minerals in feed is essential for birds' better performance and productivity (12). It is important to note that the minimum dietary vitamin levels, required to prevent clinical deficiencies may not support optimum health, performance, and welfare of poultry under current production systems. It is therefore recommended to include a safety margin to the vitamin requirements of laying hens (13). The brands of commercial VMP available to farmers in Nigeria varied considerably in their composition (5) and may therefore alter production differently.

However, the combined effects of supplementing laying hen diets with different proprietary VMP and HS on egg properties and shelf life stability (17), performance characteristics and haematology of pullets from weeks 10 to 21 (18) and deposition of pantothenic acid, riboflavin, tocopherol and selenium in eggs (19) have been reported. Also, there have been reports on impacts of HS on egg quality attributes of the laying chickens (21, 22). However, there is dearth of information on the deposition of vitamin B1, folate, B6 and D in eggs as influenced by the housing type and the different dietary VMP. Therefore, this study was aimed at evaluating the effect of the different proprietary VMP on the deposition of vitamins B1, B6, D3 and folic acid in chicken eggs under two HS.

Materials and Methods

This experiment was conducted at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The study area lies between latitude 7° 23' 28.19" N and Longitude: 3° 54' 59.99" E (23).

Animals and Housing

Bovan Nera black hens (n=480) at the late stage of laying (week 59*)* with records of vaccination schedule, medication, and production performance from one-da*y* old were used for this study. Hens were randomly allotted to ten dietary treatments of 48 chicks per treatment. Each treatment comprised six replicates with eight birds per replicate. The hens were raised under two HS (conventional cage and a carefully partitioned deep litter). Feed and water were provided *ad libitum* throughout the feeding trial. Hens were exposed to the same management system all through the trial period.

Experimental design and diets

The experimental design was a 2 x 5 factorial arrangement in a completely randomised desig*n.* There were two HS (battery and deep litter (DPL)) and five different VMP per HS. The five dietary VMP were purchased from a commercial feed milling factory in Ibadan. The VMP were Nutripoult premix, Hi-nutrient premix, Agrimix premix, Daramvita premix and Micro-mix premix. Each diet was supplemented with 0.25% of a VMP as previously reported (14, 15, 16, 17, 18) and shown in Table 1.

Data collection

At week 59, the feeding of experimental diets to the respective hens commenced. At week 71, six freshly laid eggs were collected per replicate of each treatment in both management systems and labelled appropriately. Six eggs were randomly selected per treatment, broken, whipped, together and freeze-dried. Egg samples were analysed for vitamin B1, B6, D3 and folate deposition.

Chemical Analyses

Vitamin B1 (Thiamine) Analysis

One gram of egg sample was weighed into 100 mL volumetric flask; 25 mL of 0.1M H_2SO_4 was added and mixed by careful swirling. Additional 25 mL of 0.1M H_2S0_4, was added to rinse any adhering sample particles in the flask. The flask was set in a boiling water bath to ensure a complete dissolution of the sample in the acid. The flask was shaken frequently in the first five minutes and subsequently for 3 minutes. Precisely, 5mL of taka-diastase in 0.5M sodium-acetate solution was added and flask set in cold water to cool content below 50 °C. The flask was stoppered and kept at 45-50 °C for 2 hours and thereafter made up to 100 mL in flask after mixing thoroughly. Ten (10) mL of the mixture filtrate was pipetted into a 50 mL volumetric flask and 5 mL of acid potassium chloride solution was added, shaken thoroughly to mix well. Standard thiamine solutions of range 10 mg/mL to 50 mg/mL were prepared from 100 mg/mL stock and treated same way as prepared from sample above. The absorbance of the sample as well as that of standards were read on a fluorescent UV spectrometer (Cecil A20 model) at a wavelength of 285 nm (24)**.**

Vitamin B1, in mg/100g was calculated using the formula:

$$\frac{Absorbance\ x\ Ave.Gradient\ x\ Dilution\ Factor}{Weight\ of\ sample}$$

Folic Acid Analysis

One gram of each sample was weighed into a 250 mL volumetric flask, 100 mL of distilled water was added and spin or shaken for 45 mins and made up to mark with distilled water. The sample mixture was filtered into another 250 mL beaker. Another 20mL filtrate was collected. To the filtrate, 5mL of 1% sodium dithionite solution was added to decolorize the yellow colour. Standard folic acid of range 0-10 µg/mL was prepared from stocked folic acid. A sample blank made of distilled water was also prepared. The absorbance of sample as well as standard were read at a wavelength of 445 nm on a 21D spectrophotometer (25).

Folic acid in mg/100g was calculated using the formula:

$$\frac{Absorbance\ of\ sample\ x\ Gradient\ Factor\ x\ Dil.Factor}{weight\ of\ sample}$$

Table 1: Gross composition (%) of experimental layer diets

Ingredients (%)	T1	T2	T3	T4	T5
Maize	59.00	59.00	59.00	59.00	59.00
Soyabean	24.37	24.37	24.37	24.37	24.37
Wheat bran	3.00	3.00	3.00	3.00	3.00
Palm kernel cake	3.00	3.00	3.00	3.00	3.00
Table salt	0.30	0.30	0.30	0.30	0.30
Di-calcium phosphate	0.11	0.11	0.11	0.11	0.11
Limestone	9.30	9.30	9.30	9.30	9.30
Biotronics	0.30	0.30	0.30	0.30	0.30
Mycofix	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.12	0.12	0.12	0.12	0.12
Premix 1	0.25				
Premix 2	-	0.25	-	-	-
Premix 3	-	-	0.25	-	-
Premix 4	-	-	-	0.25	-
Premix 5	-	-	-	-	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients					
ME (Kcal/kg)	2,687.56	2,687.56	2,687.56	2,687.56	2,687.56
Crude protein (%)	17.00	17.00	17.00	17.00	17.00
Crude fibre (%)	3.80	3.80	3.80	3.80	3.80
Fat	3.59	3.59	3.59	3.59	3.59
Lysine (%)	0.97	0.97	0.97	0.97	0.97
Meth + Cyst (%)	0.71	0.71	0.71	0.71	0.71
Calcium (%)	3.68	3.68	3.68	3.68	3.68
Av. Phosphorus (%)	0.40	0.40	0.40	0.40	0.40

Av - Available Phosphorus

Pyridoxine (Vitamin B6) Analysis

One gram of sample was weighed into a 100 mL beaker, 0.5 g of ammonium chloride, 45 mL of chloroform and 5 mL absolute alcohol were added to extract all the pyridoxine. The mixture was thoroughly mixed in a separatory funnel by shaking for 30mins. 5 mL of distilled water was added to the mixture in the separatory funnel to distinguish the aqueous layer from the chloroform layer.

The chloroform layer containing the pyridoxine was filtered into a 100 mL volumetric flask and made up to mark with chloroform. 0-10 ppm of vitamin pyridoxine standard was prepared from 100 ppm stock standard solution of pyridoxine and treated in a similar way as sample to obtain the gradient factor. The absorbance of a yellowish colour solution developed was measured on a Cecil 505E Spectrophotometer at a wavelength of 415 nm (26).

Vitamin B6, in mg/100g was calculated using the formula:

$$\frac{\text{Absorbance of sample x Gradient Factor x Dilution Factor}}{\text{Weight of sample x 100}}$$

Vitamin D analysis

Five grams of sample was weighed into a 250 mL beaker. One hundred (100) mL absolute alcohol was added to extract D vitamin. The mixture was filtered through a Whatman No 1 filter paper. Five (5) drops of 0.1% Pyrogallol solution was added to the filtrate and evaporated to about 1mL. Three (3) drops of 10% aluminium chloride solution was added and heated in a water bath until a red violet colour crust developed in the test tube. Ten (10) mL of absolute alcohol was added to the crust in the tube to dissolve it and made ready for reading of absorbance at a wavelength of 625 nm. Standards of vitamin D of range 0-5 µg/mL was also prepared. The absorbance was read on a spectronic 21D Spectrophotometer at a wavelength of 625nm (26).

Vitamin D ug/100g

$$= \frac{Absorbance\ of\ sample\ x\ Gradient\ factor\ x\ Dil\ Factor}{Weight\ of\ sample}$$

The compositions of the five proprietary VMP included in diets of hens raised in the two HS were previously documented (14, 15, 16, 17, 18, 19, 20) and shown in Table 2.

Table 2: Composition of **2.5 kg of test ingredients (Layer vitamin-mineral premixes) as shown on their labels**

Vitamins and minerals	Premix 1	Premix 2	Premix 3	Premix 4	Premix 5
Vit. A (IU)	10, 000,000	10, 000,000	10, 000,000	12,000,000	10, 000,000
Vit. D3 (IU)	2,000,000	2,000,000	2,000,000	2,400,000	2,000,000
Vit. E (IU)	12,000	12,000	12,000	12,000	23,000
Vit. K3 (mg)	2,000	2,000	2,000	2,000	2,000
Vit. B1 (mg)	1,500	1,500	1,500	1,500	3,000
Vit. B2 (mg)	5,000	4,000	5,000	4,000	6,000
Vit. B6 (mg)	1,500	1,500	1,500	1,800	5,000
Vit. B12 (meg)	10	10	10	10	25
Niacin (mg)	15,000	15,000	15,000	25,000	50,000
Ca Pantothenate (mg)	5,000	5,000	5,000	5,000	10,000
Folic acid (mg)	600	500	600	500	1,000
Biotin (mcg)	20	20	20	25	50
Choline chloride (mg)	150,000	100,000	150,000	240	400,000
Manganese (mg)	80,000	75,000	75,000	80,000	120,000
Zinc (mg)	60,000	50,000	50,000	50,000	80,000
Iron (mg)	40,000	20,000	25,000	20,000	100,000
Copper (mg)	8,000	5,000	5,000	5,000	8,500
Iodine (mg)	1,000	1,000	1,000	1,200	1,500
Selenium (mg)	150	200	100	200	120
Cobalt (mg)	250	500	400	200	300
Antioxidant (mg)	100,000	125,000	125,000	125,000	120,000

Premix 1-Nutripoult premix, Premix 2- Hi-nutrient premix, Premix 3-Agrimix premix, Premix 4-Daramvita premix and Premix 5- Micro-mix premix.

Statistical analyses

Data were subjected to descriptive statistics and analysis of variance using the General Linear Model Procedure of SAS (27). Means were separated by Duncan's multiple range test option of the same software at $\alpha_{0.05}$.

Results

The main effects of five proprietary VMP and HS on vitamin deposition in egg of laying he*n*s are shown in Table 3. The combined effect of HS and VMP on vitamin B_1 deposition in eggs was significant ($p<0.05$*)*. Eggs from T5 had the highest vitamin B_1 content of 0.063 mg/100g which was significantly higher ($p<0.05$) than 0.043, 0.045 and 0.053mg/100g in Treatments 1, 2 and 3, respectively but statistically similar ($p>0.05$) to 0.060mg/100g in Treatment 4. Also, vitamin B1 deposition level of 0.061 mg/100g in eggs of hens in cage was significantly higher ($p<0.05$) than 0.045 mg/100g for those on DPL.

Neither VMP nor the HS had significant influence ($p<0.05$) on the deposition of pyridoxine in eggs as their concentration in eggs were similar ($p>0.05$). Likewise, there was no significant effect ($p>0.05$) of VMP on the deposition of vitamin D. However, the HS had effects, as the eggs from hens on DPL had higher ($p<0.05$) vitamin D deposition of 93.969 IU/100g compared to those from cage with 87.630 IU/100g. The HS also had effect on folic acid deposition in egg. Deposition of folic acid in eggs from DPL (51.909 m*g/*10*0*g) was significantly ($p<0.05$) higher than that recorded in eggs collected from cage with deposition level of 49.164mg/100g. However, there was no significant effect ($p>0.05$) of VMP on deposition of folic acid in egg among the treatments.

Table 3: Main effects of supplemental proprietary vitamin-mineral premixes and housing system on vitamin deposition in eggs of hens

Experimental treatments	Vitamin B1 (mg/100g)	Vitamin B6 (mg/100g)	Vitamin D (IU)	Folic acid (mg/100g)
Main effect of VMP				
VMP 1(T1)	0.043[c]	0.062	93.596	51.537
VMP 2(T2)	0.045[c]	0.059	92.053	51.468
VMP 3(T3)	0.053[b]	0.056	90.708	50.228
VMP 4(T4)	0.060[ab]	0.053	89.037	49.530
VMP 5(T5)	0.063[a]	0.052	88.603	49.920
SEM	0.002	0.002	0.960	0.417
Main effect of HS				
Cage	0.061[a]	0.053	87.630[b]	49.164[b]
DPL	0.045[b]	0.059	93.969[a]	51.909[a]
SEM	0.002	0.002	0.960	0.417

[a,b,c,d] means within the same column with different superscript differs significantly ($p<0.05$). VMP 1,2…= VMP 1= Nutripoult, VMP 2= Hinutrient, VMP 3= Agrimix, VMP 4= Daram-vita and VMP 5= Micro-mix, SEM= standard error of Mean, DPL= Deep litter. HS= housing system, T1, T2, T3 T4 and T5 + Treatments 1, 2 3, 4 and 5

Effect of interaction of HS and dietary VMP was not significant on the deposition of vitamins B_6, D and folic acid in eggs. Treatment 5 in the cage had the highest B_1 deposition of 0.070 mg/100g which was similar ($p>0.05$) to those on treatments 3 and 4 in the same cage system. Likewise, treatment 5 on DPL recorded the highest vitamin B_1 deposition of 0.067 mg/100g which was significantly higher ($p< 0.05$) than in treatments 1(0.037), 2 (0.033), 3 (0.040) and 4 (0.050 mg/100g) in the DPL. However, similar values of vitamin B_1 were recorded in eggs from the combination of cage x VMP 3, 4, 5, and DPL x VMP 5. Eggs from DPL x VMP 2 had the lowest B1 of 0.033 mg/100g which did not differ significantly ($p>0.05$) from 0.037 mg/100g in DPL x VMP 1 and 0.040 mg/100g in DPL x VMP 3. However, effects of interactions of VMP and HS on vitamin B_6, D and folic acid deposition in egg were not significant ($p>0.05$).

Table 4: Effect of interaction of housing system and vitamin-mineral premix on vitamin deposition in eggs

Treatment	Vitamin B_1 (mg/100g)	Vitamin B_6 (mg/100g)	Vitamin D (IU)	Folic acid (mg/100g)
Cage x VMP 1	0.050^{cd}	0.063	92.393	51.113
Cage x VMP 2	0.057^{bc}	0.058	89.893	51.007
Cage x VMP 3	0.067^{ab}	0.051	87.137	48.727
Cage x VMP 4	0.060^{abc}	0.041	82.583	47.163
Cage x VMP 5	0.070^{a}	0.050	86.143	47.750
DPL x VMP 1	0.037^{e}	0.060	94.800	51.960
DPL x VMP 2	0.033^{e}	0.061	94.213	51.870
DPL x VMP 3	0.040^{de}	0.060	94.280	51.730
DPL x VMP 4	0.050^{cd}	0.064	95.490	51.897
DPL x VMP 5	0.067^{ab}	0.054	91.063	52.090
SEM	0.002	0.002	0.960	0.417

^{a,b,c,d,e} means within the same column with different superscripts differs significantly ($p<0.05$). DPL = Deep litter, VMP = Vitamin- mineral premix, SEM -= Standard Error of Mean, Cage x VMP 1, 2….5 = Interaction between Cage and VMP 2….5, DPL X VMP = Interaction between DPL and VMP

Discussion

Vitamin deposition as influenced by VMP

Thiamine deposition in eggs ranged from 0.033 to 0.070 mg/100g. This result was within 0.02 to 0.08 reported (28) The high level of thiamine deposition in the eggs from treatment 5 in both HS compared with other treatments could be due to high level of vitamin B_1, in VMP 5 compared with the VMP brands used in the other treatments. Reports showed that the level of a particular VMP incorporated into the feed directly influences the deposition of vitamin in the egg (11; 29). Some reports indicated increased deposition of thiamine in eggs as a result of higher dietary supplementation of thiamine for laying hens (9, 12, 30). In another study, Lima and Souza (10) reported that the concentration of vitamin A in egg yolk was a direct reflection of its inclusion in the diet of laying hens.

The egg concentration of vitamin D obtained in this study (82.583 – 95.490 1U/100g) was within the reported range (29). The different proprietary VMP had no influence on vitamin D deposition in this study. This may be as a result of the similarity in the vitamin D composition of the test five-proprietary VMP. However, this phenomenon differed from earlier report (3) that the transfer efficiency of vitamin D_3 to eggs was improved when dietary vitamin D3 of laying hens was increased up to 35,014 IU by 12.42%. This implies that vitamin D_3 concentrations of egg may be considerably manipulated through diets of laying hens if its concentration is increased.

Vitamin B_6 and folate content in eggs were also not affected in spite that treatments 4 and 5 had higher composition of vitamin B_6 and folic acid. This findings agreed with those of (11). In their study, dietary folic acid and vitamin B_6 were increased by 200%, without record of any appreciable deposition of the vitamins in eggs. It was therefore surmised that concurrent increment of all dietary vitamins had little impact on egg vitamin status

Effect of housing system on vitamins deposition in egg

The effect of HS on deposition of vitamins in eggs was significant. There were higher depositions of vitamins B_1, D_3 and folic acid in eggs from DPL than those from the cages. This was adduced to the ability of hens to pick additional nutrients from the litter which was consequently deposited in the eggs. Thiamine deposition however was more favourable in the cage in this study, which conformed to earlier observations (31) for better performance of birds and egg quality reared in cages compared to DPL. Tumová *et al*. (32) conversely, reported no significant difference in the content of thiamine in both HS. The HS, however, did not influence the deposition of vitamin B_6 in eggs as observed in this study.

The effect of interaction of HS and VMP was not significant on the deposition of Vitamin D, Vitamin B_1, and folic acid in eggs. This is an indication that HS and not in conjunction with VMP was able to significantly influence the deposition of vitamin B_6, D_3 and foliate in eggs. However, the effects of interaction of HS x VMP was positive for B_1, indicating that HS and dietary VMP supplement increased the deposition of thiamine in the eggs of chickens. The effect of interaction of cage and VMP consistently enhanced the level of vitamin B1 deposition compared to those of DPL x VMP, except for the interaction between DPL x VMP 5 which had a similar value. The effect of interaction of VMP 5 x cage led to the highest B_1 deposition in eggs, which could be due to synergistic effect of seeming better health condition of hens in the cage (33) and higher level of thiamine composition in the VMP.

Conclusion and Applications

1. Increased dietary supplementation of a particular vitamin could be used to fortify its deposition in the egg as obtained for thiamine in this study.
2. Eggs from hens in DPL had relatively higher concentration of vitamins compared to those from the cage.
3. The combination of DPL and VMP 5 resulted in higher deposition of vitamin B_1.

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