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Effect of five different dietary vitamin-mineral premixes and two rearing systems on selected vitamin and iron deposition in eggs at the late laying stage

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Abstract

Effects of different commercial Vitamin-Mineral Premixes (VMP) and Housing Systems (HS) on selected vitamin and iron deposition in eggs at the late laying stage were evaluated. In a 2×5 factorial arrangement and completely randomized design, Bovan Nera layers (n=480) aged 60 were allotted to five treatments of 48 hens per treatment in both HS, each replicated six times comprising eight birds per replicate. Formulated basal diet was supplemented with 0.25% of five different VMP to obtain corresponding treatments 1- 5, Supplemental VMP had no effect (P>0.05) on vitamin A and biotin deposition contrary to niacin and iron (P<0.05). Vitamin A (690.68 IU/retinol equivallent) and biotin (21.46mg/100g) depositions were higher in DL eggs contrary to higher niacin (0.39mg/100g) (P<0.05) in BC. Iron deposition was independent (P>0.05) of HS, so also, was interaction of VMP and HS (P>0.05) on egg vitamin A and biotin. Hence, vitamin A and biotin in egg were not altered by VMP supplementation. Iron deposition was however, influenced by HS, while interactions of HS and VMP enhanced deposition of niacin and iron.

Keywords: Dietary vitamin-mineral premixes, Nutrients deposition, Laying hens, Housing systems, Egg composition

1. Introduction

Nutrition is a key factor in efficient poultry production [2]. Among the dietary factors affecting the nutritional value of egg and keeping quality includes vitamin, minerals and fat. *Jones (2006)* [7] stated that when a hen is nutritionally compromised, the body begins to shut down unnecessary metabolic processes and laying hens will not efficiently produce eggs and the eggs laid will be of inferior quality. The hen's egg possesses an excellent nutritive value and constitutes a traditional food used in many basic and formulated preparations. Eggs contain several nutrients such as proteins, lipids, vitamins and minerals, and also other substances with important biological functions like essential amino acids. Improvements in egg nutritional value may have direct positive implications on daily nutrient intake and consequently for human health [17, 26].

Vitamin-mineral premix is the combination of vitamins and minerals added to meet the nutrient requirements of vitamins and minerals that are deficient in the formulated diet of a particular animal [37]. Inclusion of vitamin-mineral premix in formulated rations has become indispensable because feed ingredients do not contain all the essential vitamins and minerals in the right amounts needed by chickens [11]. Vitamins and minerals are required in small quantities compared to other nutrients; the actual amounts depend on diet, growth rate, egg production, size of birds and climate [11]. Vitamins and minerals are essential for physiological functions and to provide the nutrients for growth and repair of bones, teeth, skin and organs. Eggs are natural sources of vitamins and

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minerals. However, vitamin deposition in eggs is low since poultry ingredients are low in some vitamins, thus the addition of premix to poultry diet is therefore a good insurance to protect birds from diseases, avoidable stress and disorders [19].

Quality of eggs may be affected through changes in the integrity of the shell, yolk, or albumen along with changes in function, composition or nutrition. Season, hen breed, flock age, and flock disease-vaccination status also interact to affect egg safety and quality and must be taken into account. An understanding of these different effects is prudent before any large-scale move to an alternative housing system is undertaken. On the other hand, the management system of layers has also been observed to contribute to the chickens performance, egg quality and shelf-life [23]. There is insufficient experimental evidence on the effects of different vitamin-mineral premixes and rearing systems on vitamin and mineral deposition in eggs with different trade names available in Nigeria.

According to *Zang et al. (2011)* [38] effects of different dietary vitamin combinations on egg quality and vitamin deposition in whole egg of laying hens should be considered under which rearing systems could engender substantial deposition of selected vitamins. There have been reports on effect of dietary VMP on broiler performance [19, 20], eggs chemical composition [12], lipid profile [13], mitigating effect of VMP on heat stress in broilers [20]. The scanty report [3] in laying chickens considered caged laying pullets without emphasis on layers in the DL which remained the main alternative rearing system. Therefore, the present study was aimed at investigating the effect of supplementing diets with five different VMP and two RS on selected vitamin and iron depositions in eggs at the late laying phase.

2. Materials and Methods

***2.1 Experimental Site*:** The experiment was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

The site was within the geographical location of longitude 7.27° and latitude 53.74° North.

2.2 Experimental Birds and Management: Black Bovan Nera (n=480) laying hens were assigned to five dietary treatments in six replicates of eight birds per replicate making a total of 48 birds per treatment. Birds (n=240) were housed in both rearing systems (battery cage and deep litter, respectively) in a completely randomized design.

2.3 Experimental Diets: A basal isocaloric and isonitrogenious diet was formulated and supplemented with 0.25 % of the different VMP 1, 2, 3, 4 and 5 to obtain treatments 1, 2, 3, 4 and 5, respectively. Detailed composition/2.5kg of test ingredients (Layer vitamin-mineral premixes) and gross composition (%) of experimental diets have been documented [12,13] and are respectively presented, in Tables 1 and 2. The experiment was a 2 x 5 factorial arrangement in a completely randomized design which comprised two management systems (BC and DL) and five different VMP.

2.4 Data Collection: A total of six eggs were randomly collected from each treatment by sampling one egg from a replicate in each HS at week 60 of laying hen life. Thirty eggs were therefore, pooled from each HS. Eggs were broken, and entire contents homogenized. Samples were thereafter, assayed for vitamin A, niacin, biotin and iron.

Vitamin A extraction in whole egg was according to *Rutkowski et al*. (2007) [25, 30] with some modifications. Eggs were broken into a flat plate, homogenized and 1g of the homogenized sample was weighed into a test tube with a tight stopper. 5 mL of potassium hydroxide was added to saponify the sample and was thoroughly mixed for one minute. The mixture in the test tube was heated in water bath for 20 minutes at 60˚C and allowed to cool. One mL of xylene was added for the extraction process and was mixed to homogenize.

The sample in the test tube was centrifuged at 1500 revolution for 10 minutes and the supernatant was collected, later transferred into cuvette to read the absorbance of the extract at 335 nm against xylene. The extract was irradiated by ultra violet light for 30 minutes to measure the second absorbance. All readings were in triplicates and estimated in IU/Retinol equivalence.

***2.5 Determination of niacin*:** Niacin was analyzed according to *AOAC (2005)*. 1g of sample was weighed, mixed with 10 mL of sulphuric acid to homogenize. A drop of ammonia was added into the mixture and filtered. 2 mL of the filtrate and 1 mL of potassium cyanide was added. 0.2 mL of H_2SO_4 was added. 10mL of Niacin stock solutions were also prepared.

The absorbance of the diluted stock solutions and sample extract were measured at a wavelength of 385 nm on a UV spectrophotometer.

2.6 Determination of biotin: 1g of sample was weighed; 7 mL of sulphuric acid and 10 mL of methanol were added. Extraction was done according to *AOAC (2005)* [1]. 2 mL of 0.5M NaOH was added to the solution in the test tube and made ready for reading on a spectrophotometer, 1mL of biotin was prepared and treated as sample. Absorbance of sample and standard were read on spectrophotometer at the wavelength of 418 nm.

2.7 Determination of iron: 1g of sample was oven died to reduce the moisture content of raw egg. It was dry ashed and analyzed according to *Okwu, (2005)* [21] using atomic absorption spectrophotometer. 5 mL HNO_3 was added to the ashed content to dissolve in a beaker. The mixture was heated at low temperature for 10 minutes at 35 ^{0}C on hot plate to evaporate the acid.

The remaining residue was dissolved by adding 2 mL of HCl. The mineral solution was transferred into 100 mL volumetric flask and marked with deionized water.10mL of the sample solution was pipetted into 100 mL of volumetric flask and 1 mL of hydroxylamine hydrochloric solution was added, mixed well and allowed to cool.10 mL of standard solution was pipetted into another 100 mL volumetric flask,.5 mL of acetate buffer and 4 mL of 1,10 phenantholine were added to the two solutions in the flask to develop colour, allowed to cool and the reading was taken on atomic absorption spectrophotometer at wavelength of 510 nm.

***2.8. Statistical Analysis*:** Data were subjected to one way analysis of variance (ANOVA) using the GLM procedure of SAS [27] and means were separated using Duncan's multiple range test of the same package. Statistical significance was established at $p<0.05$.

Table 1. Composition/2.5kg of test ingredients (Layer vitamin-mineral premixes, VMP)

Type of experiment	Solvent type	Ratio (sample/solvent)	Time (min)	Temperature (°C)	Betalain yield (mg/g sample)
Solvent					
	Water	1:5	10	25	71.4 ± 0.2
	1% CA	1:5	10	25	88.6 ± 0.7
	0.5% CA	1:5	10	25	92.3 ± 0.4
	0.2% CA	1:5	10	25	95.1 ± 0.6
	0.1% AsA	1:5	10	25	63.9 ± 0.6
	50% EtOH	1:5	10	25	72.7 ± 0.3
	20% EtOH	1:5	10	25	75.4 ± 0.6
	0.5% CA + 0.1% AsA	1:5	10	25	98.2 ± 0.5
	0.2% CA + 0.1% AsA	1:5	10	25	101.0 ± 0.4
	20% EtOH + 0.1% CA	1:5	10	25	91.7 ± 0.4
	20% EtOH + 0.5% CA	1:5	10	25	106.3 ± 0.3
Sample/solvent ratio					
	20% EtOH + 0.5 %CA	1:10	10	25	110.7 ± 0.3
	20% EtOH + 0.5% CA	1:15	10	25	112.4 ± 0.3
	20% EtOH + 0.5% CA	1:20	10	25	114.0 ± 0.5
Extraction time					
	20% EtOH + 0.5% CA	1:20	15	25	116.3 ± 0.3
	20% EtOH + 0.5% CA	1:20	20	25	117.5 ± 0.4
	20% EtOH + 0.5% CA	1:20	25	25	118.9 ± 0.4
	20% EtOH + 0.5% CA	1:20	30	25	119.0 ± 0.3
Temperature					
	20% EtOH + 0.5% CA	1:20	30	30	121.2 ± 0.3
	20% EtOH + 0.5% CA	1:20	30	35	124.5 ± 0.2
	20% EtOH + 0.5% CA	1:20	30	40	129.9 ± 0.3

Citric acid (CA), ascorbic acid (AsA), and ethanol (EtOH) was dissolved in distilled water.

Table 2. Gross composition (%) of experimental diets

Ingredients	T1	T2	T3	T4	T5	T6
Maize	59.00	59.00	59.00	59.00	59.00	59.00
Soybean meal	24.37	24.37	24.37	24.37	24.37	24.37
Wheat bran	3.00	3.00	3.00	3.00	3.00	3.00
Palm kernel cake	3.25	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Di-calcium phosphate	0.11	0.11	0.11	0.11	0.11	0.11
Limestone	9.30	9.30	9.30	9.30	9.30	9.30
Biotronics	0.30	0.30	0.30	0.30	0.30	0.30
Mycofix	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.12	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**	**100.00**
Calculated nutrients						
ME (Kcal/kg)	2,692.94	2,687.56	2,687.56	2,687.56	2,687.56	2,687.56
Crude protein (%)	17.05	17.00	17.00	17.00	17.00	17.00
Crude fibre (%)	3.83	3.80	3.80	3.80	3.80	3.80
Fat	3.61	3.59	3.59	3.59	3.59	3.59
Lysine (%)	0.97	0.97	0.97	0.97	0.97	0.97
Meth + Cyst (%)	0.71	0.71	0.71	0.71	0.71	0.71
Calcium (%)	3.68	3.68	3.68	3.68	3.68	3.68
Ave. Phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40

3. Results

The effect of VMP on deposition of vitamins and iron in egg at the late laying stage is shown in Table 3. Vitamin A deposition in eggs was not significantly different (P>0.05) across the treatments. However, the deposition ranged from 679.8 to 689.8 (IU/Ret.eq.) with eggs collected from treatment 1 having the highest value and those from treatment 5, the least. Niacin deposition was significantly different (P<0.05) across the treatments. Niacin deposition ranged from 0.23 to 0.46mg/100g with eggs collected from treatment 3 having the least deposition while egg of birds on treatment 2 having higher Niacin deposition. However, values obtained for egg in treatment 5 was not statistically different (P>0.05) from those in treatments 1 and 4 but different from those obtained from treatments 2 and 3. Iron deposition ranged from 2.88 to 3.22mg/100g. Treatment 4 was not significantly different (P>0.05) from treatments 3 and 5 but statistically different (P<0.05) from treatments 1 and 2. Mean values for biotin ranged from 20.53 to 21.50mg/100g, however, the deposition was statistically similar (P>0.05) across the treatments.

Table 3. Main effect of vitamin-mineral premix on deposition of vitamin and iron in eggs at the late laying stage

Parameters	Vit A(IU) Ret.eq.	Niacin mg/100g	Biotin mg/100g	Iron mg/100g
VMP T1	689.80	0.35 ^{c}	21.47	3.14 ^{b}
VMP T2	686.96	0.46 ^{a}	21.50	2.88 ^{c}
VMP T3	685.50	0.23 ^{d}	21.07	3.21^{ab}
VMPT4	683.40	0.39 ^{b}	20.74	3.23^{a}
VMPT5	679.76	0.36^{bc}	20.53	3.18^{ab}
SEM	1.84	0.02	0.19	0.03

^{a,b,c}Mean values along same row with different superscript are significantly different (p<0.05)
SEM-Standard error of mean

The effect of HS on vitamin and iron deposition in eggs is presented in Table 4. Vitamin and iron deposition in eggs collected from the two HS were significantly different (P<0.05). Vitamin A, niacin and biotin deposition were significantly affected (P<0.05) by HS. Vitamin A (IU) and niacin (mg/100g) deposition were significantly higher (P<0.05) in the DL system (690.68 and 21.46), respectively compared with (679.48 and 20.66, respectively in eggs collected from the) BC system. Biotin deposition was significantly higher (P<0.05) for the BC eggs, while iron deposition was not influenced by the different HS.

Table 4. Main effect of housing system on deposition of some vitamins and iron in eggs at the late laying stage

Parameters	Vitamin A (IU) Retinol equivalent	Niacin mg/100g	Biotin mg/100g	Iron mg/100g
Battery cage system	679.48[b]	0.39[a]	20.66[b]	3.14
Deep litter system	690.68[a]	0.32[b]	21.46[a]	3.12
SEM	1.84	0.02	0.19	0.03

[a, b] Mean values along same row with different superscript are significantly different (p<0.05)

Effect of interaction of different VMP and housing systems on vitamins and iron deposition is shown in Table 5. Interaction of housing systems and VMP was not significant (P>0.05) for Vitamin A and Biotin depositions across the treatments. However, egg collected from hens in treatments 1, 4 and 5 in BC system recorded statistically (P<0.05) similar depositions and the values were also not statistically different (P>0.05) from those in treatments 2 and 4 in the DL system. Eggs collected from treatment 1 and 5 in the DL system were not significantly different (P>0.05) from those on treatment 3 in the BC system. Also, eggs from hens fed treatment 4 in the BC system was statistically the same as eggs on treatments 1 and 3 and was also not significantly different (P>0.05) from those on treatments 4 and 5 in the DL system. Eggs collected from treatment 2 in the BC and DL systems were statistically same (P<0.05) while those collected from treatments 1 and 3 in the DL system were not significantly different (P>0.05) compared with those from treatment 5 in BC system.

Table 5. Interactive effect of vitamin-mineral premixes and housing system on deposition of vitamins and iron at late laying stage

	Battery Cage					Deep Litter					
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	SEM
Vitamin A	687.32	682.68	678.90	672.63	675.87	692.28	691.23	692.10		683.65	1.84
NIACIN	0.40[b]	0.54[a]	0.29[c]	0.37[b]	0.37[b]	0.29[c]	0.37[b]	0.17[d]	0.40[b]	0.34[c]	0.02
BIOTIN	21.31	21.36	20.44	19.88	20.31	21.62	21.63	21.70	21.59	20.75	0.19
IRON	3.21[b]	2.83[d]	3.33[a]	3.22[ab]	3.09[c]	3.08[c]	2.92[d]	3.09[c]	3.23[ab]	3.28[ab]	0.03

[a,b,c] Mean values across same row with different superscript are significantly different (p<0.05)
SEM-Standard error of mean

4. Discussion

Deposition of vitamin as influenced by supplemental dietary vitamin-mineral premix: Some promising results have been highlighted by *Lesson and Caston (2004)* [9,10] for the potential transfer efficiency of some vitamins from hen diets to the egg. Vitamin concentration in hen diet was the most important factor determining vitamin content in egg [38].The different dietary VMP did not show any significant difference (P>0.05) on vitamin A and biotin deposition in chicken eggs at the late laying stage. In the current study, 10,000 IU/kg was supplemented to the laying hen diet and deposition of vitamin A in the egg ranged from 679.76-689.80 IU/100g. This by far was below the reported values by earlier authors [6, 32, 34]. *Mori et al. (2003)* [14,15] reported yolk retinol concentration was enhanced by supplemental vitamin A, from 24.6 IU/g for eggs from control group, to 33.6 and 37.7 IU/g of yolk of hens when given diet supplemented with 15,000 and 30,000 IU/kg of the diet contrary to observations in this study. The efficiency of dietary vitamin transference to egg was considered very high for vitamin A [32]. However, the values were within the range of 5905-6222IU/kg deposition of vitamin A reported by *Perez-Vendrell (2003)* [22] in egg when fed with diets supplemented with 8110- 12000IU/kg of vitamin A.

Water-soluble vitamins plays major role in energy and protein metabolism. According to *Simmins and Dussert, (1998)* [29], B-vitamins are key elements in protein accretion rates potentially resulting in greater dietary vitamin requirements. Biotin deposition in eggs were significantly different (p<0.05) with the type of supplemental VMP in the study. Biotin (mg/100g) deposition ranged from 19.88 - 21.70 similar to the reported 19.5mg/100g biotin deposited in whole egg) [24]. Also, consistent with these findings are reports of *Briggs and Wahlqvist, (1988)* [4,5] and *Staggs et al. (2004)* [33] that biotin deposition in eggs ranged from 13-35ug.

Significant variation (P<0.05) exists in the values recorded among various treatments for niacin and iron deposition. Niacin deposition in egg from this study ranged from 0.23 – 0.46mg/100g. This value was higher when compared with those reported [16, 24, 35] that niacin deposition was noted to be generally low in eggs. The lower deposition was adduced to the fact that niacin being water soluble vitamin and as such could be easily excreted.

The various treatments applied in this study effectively enhanced a considerable deposition of niacin into the eggs. The high content of niacin in the eggs might be due to increased inclusion in the diets of hen. NRC (1994) [16], summarized that economic benefits may be achieved if higher doses of niacin are included in the diets of birds. Also, there might be an increased level of tryptophan in the diets of birds as increased level of tryptophan is needed to synthesize a significant amount of niacin [18]. The least iron deposition (2.88mg/100g) recorded in eggs of birds fed dietary supplement of VMP2 was higher than 1.72mg/100g reported [24].

***Effect of housing system in vitamins and iron deposition in egg*:** Housing systems (BC and DL) had significant effect on the deposition of vitamin A, biotin and niacin in egg at the late laying stage. Vitamin A deposition in egg under DL system was significantly different from that of the BC system. This may be due to the fact that hens had access to ingestion of some additional nutrients from the litter that was not directly from the feed supplied. This concurred with the report of Karadas *et al*. (2005) [8] for free range hens. The author reported higher retinol levels in the eggs from the DL compared with intensively housed hens. However, *Sekeroglu et al.* (2010) [28] noted that the yolk colour produced by hens raised in the DL was lighter than those from free range indicating that birds on free range had improved deposition of retinol. *Van Der Brand et al*. (2004) [36] found significant interactions between housing and layer age for egg weight, egg shell weight and albumen height. Furthermore, *Singh et al*. (2009) [31], also documented similar interactions between housing and layer age and egg shell weight.

***Effect of interaction of VMP × HS on vitamins and iron deposition in chicken egg*:** Interactive effect of VMP and housing system was not significant (P>0.05) for vitamin A and biotin deposition in egg. Vitamin A and biotin as observed were not significantly affected by dietary treatments (Table 3). Also, housing system and not VMP influenced the deposition of the studied vitamins in eggs. However, interaction effect observed for niacin and iron showed that both HS and supplemental dietary VMP influenced their depositions in eggs.

5. Conclusion

Vitamin A and biotin depositions in egg were not altered appreciably by the type of supplemental dietary VMP while iron deposition was influenced by the HS. However, interactive effect of housing and VMP enhanced the deposition of niacin and iron.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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