Vitamin C, Iron and Phosphorous Deposition in Eggs of Pullets Fed Dietary Supplement of Vitamin C and D at the Mid Laying Phase

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Abstract

Deposition of vitamin C (VC), iron and phosphorous in eggs of hens fed supplemental VC and vitamin D (VD) at the mid laying phase was assessed in this study. Bovans Brown pullets (n=576) aged 40 weeks were allotted to 12 treatments each replicated six times. A replicate comprised eight pullets. A basal diet (isonitrogenous and isocaloric) was formulated and supplemented with three levels of VC (0, 300 and 600mg) and four levels of VD (0, 1000, 2000 and 3000iu) in a 3 x 4 factorial arrangement and completely randomized design. At week 46 (sixth week of feeding experimental diets), two eggs were randomly sampled from each replicate and immediately assayed for phosphorus, iron and VC. There was no significant effect ($P>0.05$) of supplemental VC on the deposition of phosphorus, iron and VC in eggs. Similarly, phosphorus, iron and VC deposition in eggs of pullets on supplemental vitamin D were not significantly different ($P>0.05$). Effect of Interaction of supplemental VC and VD was not significant ($P>0.05$) on VC and iron deposition. However, phosphorus deposition in the egg differed significantly ($P<0.05$) with combinations of VC and VD. Pullets on combined 600mg VC+3000iu VD, 600mg VC+1000iu VD and 300mg VC+0iu VD supplementation recorded similar levels ($P>0.05$) of 180.50, 180.60 and 178.67mg/100g phosphorous, respectively which were significantly higher ($P<0.05$) than other combinations. In conclusion, supplemental dietary VC and VD had no effect on VC and iron depositions but enhanced phosphorus deposition in egg at the mid laying stage.

Keywords: Supplemental vitamin, Nutrients deposition in egg, Eggs biofortification, Bovans Brown pullets.

Introduction

Egg constitutes a rich array of nutrients and has long been promoted for its nutrients (Brufau and Tacon, 1999; Gray and Graffin, 2009). Despite the rich array of nutrients present in egg, it is still deficient in certain nutrients particularly, vitamin C (VC) and calcium (Wardlaw, 2003). Some aspects of nutritional qualities of egg could be modified by feeding hens with special diets (Naber and Squires, 1991). The quantity of any nutrient deposited in the egg could however be increased through dietary supplementation.

Avian species have the inherent ability to synthesize VC (Keshavarz, 1996). However, the requirement of VC during heat stress in poultry is greater than the amount synthesised by normal tissues (Balogun *et al.*, 1990). Stress has been reported to interfere with nutrient uptake and metabolism at physiological and cellular levels in animals. According to Schola and Gillani (1995) free radicals are generated in the body in large quantities during heat

stress thereby overwhelming the body systems. The VC is a water-soluble vitamin required to maintain normal metabolic functions (Lesson, 2001). Yigit *et al*. (2002) in their report, surmised that dietary VC only increased the plasma concentration of VC, improve shell quality, albumen quality, egg weight and egg production.

The roles of VC in the modulation of iron homeostasis have been well documented (Bhattacharya, 2001; Cheeke and Dierenfeld, 2010; Vasudevan *et al*., 2011). Vitamin C is a cofactor of aconitase enzyme system which prevents oxidation of Fe^{2+} to Fe^{3+} thereby preserving the digestible iron in the absorbable form within the intestinal lumen (Mckie *et al*., 2001; Mayes, 2002; Kathryn *et al*., 2014). Dietary supplement of VC heightens plasma and liver concentration of iron (DSM, 2018) and by extension could increase its deposition in the laying hens' egg.

The metabolically active form of Vitamin D (VD) in poultry (cholecalciferol-VD_3) is another very important dietary factor responsible for normal growth, egg production, shell quality and reproduction in fowls (Ameenuddin *et al.,* 1982). It is also a required component of the endocrine system of pullets and regulates calcium and phosphorous homeostasis and bone mineralization. Eggs are among the few potent natural sources of VD for humans. Pullets can synthesize cholecalciferol from cholesterol when they receive adequate sunlight. The VD_3 insufficiency may be a common problem when pullets are reared in environmentally controlled house (Panda *et al*., 2006). Mattilda *et al*. (1999) showed that cholecalciferol content of eggs can be further increased by supplementing hen's feed with VD_3. Inclusion of VD_3 up to 12,000 IU/kg feed used does not appear to be harmful to hens (Mattila *et al*., 1999; 2003). Naber and Squires (1991) reported a linear relationship of dietary VD_3 to the deposition of VD_3 in egg yolk. In other experiments, the concentration of VD_3 in the egg yolk was 14.5 IU/g when the concentration in the diet was 20 IU/g (Kawazoe *et al*., 1996).

The likely metabolic relationship among VC and VD_3 and utilization of Fe and P has been properly documented (Vannucchi, 1991). Also, a sufficient VC status is the prerequisite for C-1 hydroxylation of VD to its storage form 25-hydroxy cholcalciferol (25(OH)D) in the liver which on further hydroxylation in the kidney becomes 1, 25-dihydroxy cholecalciferol and eventually the highly metabolically active calcitriol 1,25(OH)2D in the kidney (Cheeke and Dierenfeld, 2010; Vasudevan *et al*., 2011).

Calcitriol stimulates the intestinal mucosa secretion of calbindin (Cheeke and Dierenfeld, 2010; Vasudevan *et al*., 2011) associated with rapid calcium and phosphorous absorption from the intestine. The VC, VD_3, iron and phosphorous homeostasis are therefore highly intricately and intrinsically connected.

There have been few reports on VC, iron and phosphorus composition of eggs (Ahn, 2014; UIE, 2019). Most reports have delved mainly on their composition in the shell (DSM, 2018; Yigit *et al.*, 2002; Wardlaw, 2003; UIE, 2019). Mason *et al.* (1993) reported that the variation in concentration of minerals in egg yolk ranged from 4 to 12% which also was analogous to Revell and Hughes (2005) reported concentration range of between 4 and 18%. Both studies affirmed that the iron composition of egg yolk was most highly varied in the eggs collected from different pullets. There has been dearth of documentation of the effects of dietary supplement of VC and VD on the deposition

of VC, iron and phosphorous in the eggs of pullets. Therefore, this study was aimed at assessing the deposition of VC, iron and phosphorous in eggs of pullets fed varying dietary supplements of VC and VD at the mid laying stage (from week 40 to 46 of life).

Materials and Methods

Experimental location

The experiment was carried out at the Poultry Unit of the 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 (SMUI, 2018).

Experimental Design

A total of 576 pullets were randomly allotted to twelve treatments each replicated six times. A replicate comprised eight pullets each. The experiment was a 3 x 4 factorial arrangement in a completely randomized design.

Experimental Pullets and Management

Bovans Brown pullets with a track record of medication, vaccination schedule and productive performance from one day old through point of lay to peak of laying at week 36 were used for the experiment. The pullets were raised in a conventional cage house. Each cage in the three-tier cage measured 40 x 41 x 32 cm. At week 40 of age, the pullets were allotted to a basal diet (isonitrogenous and isocaloric) supplemented with three levels of vitamin C (0, 300 and 600mg) and four levels of vitamin D (0, 2000 and 300 IU) and their combinations to produce 12 treatments as follows: Treatment 1 _Basal diet + no supplements; Treatment 2 – Basal diet + 300mg VC; Treatment 3 _Basal diet + 600mg VC; Treatment 4_ Basal diet + 1000 IU VD Treatment 5 – Basal diet + 1000 IU VD + 300 mg VC; Treatment 6 – Basal diet + 1000 IU VD + 600 mg VC; Treatment 7 – Basal diet + 2000 IU VD + 0 mg VC; Treatment 8 – Basal diet + 2000 IU VD + 300 mg VC; Treatment 9 – Basal diet + 2000 IU VD + 600 mg VC; Treatment 10 – Basal diet + 3000 IU VD + 0 mg vitamin C; Treatment 11 – Basal diet + 3000 IU VD + 300 mg VC; Treatment 12– Basal diet + 3000 IU VD + 600 mg VC. The laying pullets were fed the experimental diets for six weeks. At week 46, two eggs were randomly selected from each replicate and used for the chemical analyses of VC, iron and phosphorous.

Table 1: Composition of basal diet fed to laying pullets

Ingredients	Percentage (%)
Maize	50.00
Soya bean meal	22.00
Wheat offal	11.08
Di-calcium phosphate	1.34
Oyster shell	3.00
Palm kernel cake	11.30
Table Salt	0.30
Mycofix	0.15
Biotronics SE	0.30
Layer premix	0.30
DL-Methionine	0.15
L-Lysine	0.15
Total	100

Determination of Vitamin C in egg

One percent solution of VC was made with 1.0 g of egg in 100 cm^3 distilled water. Egg solution of 2 cm^3 was put into a test tube. Using graduated pipette, 1% of 2, 6-Dichlorophenol indophenols (DCPIP) was added drop by drop to the egg solution and shaken gently after adding each drop until the blue colour of the final drop did not disappear and the exact amount of DCPIP solution added was recorded (AOAC, 2005).

Determination of Iron in egg

Five gram (5g) of egg sample was ashed in a muffle furnace at 550 °C. A standard of 10, 8, 6, 4, 2, and 0 µg Iron/mL from a stock solution of 10 µg/mL was prepared. Dilutions were made using Ca 0.1N/HCL. The ash was dissolved in small amount of 1NHCL, and diluted to 50 mL with 0.1NHCL. Precisely, 0.50 mL of appropriately diluted samples and standards were transferred into 10 mL test tubes, 1.25 mL ascorbic acid was added (0.02% in 0.2NHCL, made fresh daily) and the solution agitated for 10 minutes. Precisely, 2.00 mL 30% Ammonium acetate (to adjust pH to >3 for colour development) and 1.25 mL Ferrozine (1m M in water) were added and left in a dark chamber for 15 min. The measurement was taken using a spectrophotometer (752W UV-VIS Grating, Easy Way Medical, England) at 562 nm. A standard curve was plotted and the content of iron (µg/mL) in the dissolved ash solution was determined (AOAC, 2005). The iron (µg/g) in the sample was calculated using the formula:

$$\frac{\mu g \text{ iron}}{mL \text{ ash solution}} \text{ X 50 mL ash solution}$$

Determination of Phosphorus

Phosphorous was determined by Vanado-molybdate spectrophotometric procedure. The ash sample obtained, as previously described, was digested by adding 5 mL of 2M HCL to ash in the crucible and heated to dryness in heating mantle, 5 mL of 2M HCL was added again and heated to boil and filtered through a Whatman No.1 filter paper. The filtrate solution (10 mL) was pipetted into 50 mL standard flask and 10 mL of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 min for full colour development. The concentration of the phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a spectrophotometer (752W UV-VIS Grating, Easy Way Medical, England) at a wavelength of 470 nm (AOAC, 2005). The percentage phosphorus was calculated from using this formula;

$$\% \text{ Phosphorus} = \text{Absorbance x slope x } \frac{\text{Dilution factor}}{1000}$$

Statistical analysis

Data were subjected to descriptive statistics, analysis of variance (ANOVA) using the General Linear Model (GLM) procedures (SAS, 2003). The treatment means were separated using Tukey's HSD option of the same software at $\alpha_{0.05}$.

Results

The deposition of VC, iron and phosphorus in eggs of pullets fed diets supplemented with varying levels of VC are shown in Table 2. There was no significant difference

(P>0.05) in the phosphorus content of eggs across the treatments. Phosphorus content in the eggs ranged from 174.19 to 176.57 mg/100g in eggs of pullets fed diets supplemented with 300 and 0 mg vitamin C, respectively. Similarly, iron deposition in the eggs was not significantly affected (P>0.05) by the dietary supplement of VC as iron deposition ranged from 1.32 (0 mg) to 1.33mg/100g (300 and 600mg). Also, VC composition in egg ranged from 0.096 (0 mg supplementation) to 0.099 mg/100g (300mg). The deposition in the eggs however, was similar (P>0.05) across the treatments.

Effect of diets supplemented with VC and VD on VC, iron and Phosphorous deposition in eggs of pullets at the mid laying stage is shown in Table 3. Phosphorus, iron and VC deposition in eggs of pullets fed supplemental VD were not significantly affected (P>0.05) by the treatments. Phosphorus in the eggs ranged from 173.17 in pullets on 2000 IU/kg VD_3 to 176.7mg/100g in those on 1000 IU/kg. Egg iron deposition ranged from 1.31 (1000 IU/kg VD_3) to 1.35mg/100g (2000 IU/kg VD). Also, VC deposition content of eggs ranged from 0.093 (0 IU/kg VD) to 0.106 mg/100g (2000 IU/kg VD).

Effects of the combination of dietary supplement of VC and VD on deposition of VC iron and phosphorus in egg of pullets at the mid laying stage is shown in Table 4. Dietary combination of both vitamin supplements in the diets of pullets had no significant effect on (P>0.05) on VC and iron deposition across the treatments. Iron content of the eggs ranged from 1.28 mg/100g in those on 0 mg VC x 3000 iu VD and 300 mg VC x 3000 iu VD combinations to 1.34 mg/100g in treatments without VC and VD (0 mg VC x 0 iu VD) and 600 mg x

Table 2: Phosphorous, iron and ascorbic acid deposition in chicken eggs as influenced by graded dietary supplements of vitamin C

	Vitamin C (mg/kg)			
Parameter	**0**	**300**	**600**	**SEM**
Phosphorus	174.19	174.53	176.57	2.54
Iron	1.32	1.33	1.33	0.05
Vitamin C	0.096	0.099	0.098	0.09

SEM- Standard error of means compares values in each row

Table 3: Phosphorous, iron and ascorbic acid deposition in chicken's eggs as influenced by graded dietary supplements of vitamin D

	Supplemental vitamin D (IU/kg)				
Parameter	**0**	**1000**	**2000**	**3000**	**SEM**
Phosphorous	176.60	176.71	173.17	176.58	2.93
Iron	1.34	1.31	1.35	1.30	0.05
Vitamin C	0.09	0.09	0.10	0.09	0.01

SEM- Standard error of mean compares values in each row

Table 4: Effect of Interaction of dietary supplement of vitamin C and D on the deposition of vitamin C, iron and phosphorous in chicken eggs

Interaction Vitamin C	Vitamin D	Phosphorus mg/100g	Iron (mg/kg)	Vitamin C (mg/kg)
0	0	175.70b	1.34	0.09
	1000	172.67b	1.31	0.08
	2000	174.27b	1.38	0.11
	3000	174.13b	1.28	0.09
300	0	178.67ab	1.32	0.09
	1000	176.87b	1.33	0.09
	2000	175.47b	1.37	0.12
	3000	175.10b	1.28	0.09
600	0	175.43b	1.35	0.09
	1000	180.60a	1.29	0.11
	2000	169.77c	1.30	0.09
	3000	180.50a	1.34	0.10
SEM		5.07	0.09	0.01

SEM- Standard Error of Mean , Vit C- vitamin C, Fe- Iron
[a, b, c]Means in the same column with different superscripts are significantly different ($p<0.05$)

3000VD combinations. The VC ranged from 0.088 mg/100g in pullets on combined 0 mg VC x 1000 iu VD to 0.107 mg/100g in eggs of those on 300 mg x 2000 iu VD combinations.

However, phosphorus deposition in the egg was significantly affected ($P<0.05$) across the treatments as pullets on combined 600 mg VC x 1000 iu VD had similar ($P>0.05$) phosphorous level (180.60 mg/100g) with eggs of pullets on combined 300 mg VC x 0 iu VD (178.67 mg/100g) and 600 mg VC x 3000 iu VD (180.50 mg/100g) which were significantly higher ($P<0.0$) than the values recorded for eggs of pullets on other treatments.

Discussion

The internal components of eggs can be transformed through dietary manipulation (Singh *et al*., 2010). Leeson and Caston (2004) highlighted the potential transfer efficiency of some nutrients from hens' diets to egg as the concentration of vitamins in the diet play important roles in achieving this (Ahmed and Abdelati, 2009; Singh and Sachan, 2010).

Dietary iron is predominantly non-heme iron and must be reduced from its ferric state to ferrous iron by dietary reducing agents (Mckie *et al*., 2001). Absorption of non-heme iron is much more variable and significantly affected by other components of the diets (Kathryn *et al*., 2014). Ascorbic acid is the most effective enhancer of non-heme iron absorption among other dietary factors (Bender and Mayes, 2018). Though, the concentration of iron in eggs of pullets on dietary supplement of VC as observed in this study was not affected across the treatments. The values obtained however were slightly higher than 0.72 mg/100 g reported in unfortified eggs (Meister, 2002). This observation may be linked to the roles of VC as earlier enunciated (Mckie *et al*., 2001;

Kathryn *et al*., 2014; Bender and Mayes, 2018) in the absorption of dietary iron.

Wardlaw (2003) remarked that in spite of the high-quality protein and balanced distribution of minerals and vitamins in eggs, VC was absent. Observations from this study contradicted this assertion as VC, though in trace amount was found in the eggs. Supplementation of the diets at different levels of VC however did not improve the deposition of VC in the eggs. Similar observation was reported for effect of supplemental VD on VC depositions in the eggs.

Phosphorus plays an important role in the formation of eggshell (Ahmad and Balander, 2004) but a significant amount is deposited in the yolk. Intestinal phosphate absorption has been reported to be mediated by both transcellular and paracellular routes (Fukumoto, 2014). The VD increases intestinal transcellular phosphate absorption in part by enhancing expression of type 2b sodium- phosphate co-transporter (Xu *et al*., 2002). Despite the role of VD in phosphorus absorption, increasing its level in diet of laying hens did not influence the deposition of phosphorus in the egg. Egg phosphorus composition (173.17 to 176.71 mg) in this study was however higher than the earlier reported 89 mg (Meister, 2002) but compared favourably with the latter documented 180 mg (Miranda *et al*., 2015).

There have been reported improvement in weight gain, toe bone weight and ash weight (Weiser *et al*., 1990; Fukumoto, 2014) as well as performance and bone characterization (Lohakare *et al*., 2005) of pullets due to interaction of dietary supplement of VC and VD. Effect of Interaction of VC and VD_3 in this study did not improve the deposition of VC and iron in the eggs. The sole dietary supplementation of either VC or VD did not have any influence on egg phosphorus deposition. However, interaction of both nutrients affected the deposition of phosphorus in the eggs as egg phosphorus composition increased with combined higher dietary levels of VC and VD_3. This cross over effect as observed in this study suggests the synergistic role of both vitamins in enhancing phosphorus deposition in the egg of pullets.

Conclusion

Dietary supplementation of vitamin C and D had no effect on vitamin C and iron composition of eggs but enhanced the deposition of phosphorus at the mid laying stage.

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Trend Analysis of Rice Production, Import and Consumption in Nigeria (1970–2016): Comparative Assessment of Three Economic Reforms Periods (Pre-SAP, SAP and Post SAP)

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Abstract

This study was carried out to provide empirical evidence on the growth rates of rice production in three sub – periods in Nigeria namely; pre – Structural Adjustment Programme period (1970 - 1985), Structural Adjustment Program period (1986 – 1994) and post – Structural Adjustment Programme period (1995 -2016). Secondary data were used for the study. Both instantaneous and compound growth rate models were used to estimate the growth rates in the three sub– periods. The result shows that time trend variable was a major factor in determining quantity of rice production, import and consumption in Nigeria during the three periods. The results of the analysis show that the compound growth rates of rice production were 5.79%:9.64% and 2.43% for the periods, respectively.: Rice imports shows 59.36%, -3.63% and 8.22% compound growth rate for the pre-SAP, SAP and post-SAP periods, respectively, while the annual compound growth rate of rice consumption shows increase of 13.20%, 11.18% and 4.6 during Pre-SAP, SAP and Post Sap periods, respectively. There was a significant difference among the mean quantity of rice production, imports and consumption in Nigeria across the three economic periods (Pre-SAP, SAP, and Post-SAP) under study. The study recommends that research be intensified in order to improve rice production technologies significantly in a way that the rate of growth will achieve the needed self- sufficiency in domestic rice production and thereby reduce the amount of money spent in rice imports in this country.

Keywords: Growth rate, Rice consumption, Rice import, Rice Production, Trend.

Introduction

Rice is the second most important cereal in the world after wheat in terms of production. Nigeria ranks the highest as both producer and consumer of rice in the West Africa sub-region (Goni and Amaza, 2006).In fact, the government recognized the unhealthy condition of the Nigerian agricultural sector since 1970, and has formulated and introduced a number of programmes and strategies aimed to remedy this situation. In a bid to increase food production in Nigeria over the years, several policy reforms have been put in place by successive governments and one of such policy reforms in time past is the Structural Adjustment Programme (SAP) introduced in July 1986.

By the end of the second half of 1986 it was clear that Nigeria had fully adopted the International Monetary Fund (IMF) induced structural economic reforms whose main focus is liberalization among others. The adoption was premised on the belief that the weaknesses of the economics of control trade will prevent the enjoyment of the benefit of