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Effects of Vitamin C Administration on Laying Performance, Egg Quality, Serum and Egg Cholesterol in Two Layer Strains

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ABSTRACT

A 2x4 factorial randomized complete block experimental layout was used to allot 80 pullets belonging to two breeds (Harco Black and Isa Brown) to four levels (0.000%, 0.001%, 0.002%, 0.003%) of vitamin C supplemented diets. The parameters considered were Feed Intake (FI), Hen Body Weight (BW), Hen-Day production, Egg Weight (EW), Egg Length (EL), Egg Diameter (ED), Yolk Height (YH), Albumen Height (AH), Yolk Weight (YW), Albumen Weight (AW), Shell Thickness (ST), Serum and Egg Yolk Cholesterol. Vitamin C supplementation in diets resulted in a significant decline ($P<0.05$) in feed intake and a significant increase ($P<0.05$) in albumen weight. Birds fed with Vitamin C supplemented diets also had better Hen-Day production. Birds fed diet with 0.003% Vitamin C

supplementation produced the best result in term of EW, EL, EW, YH, YW and AW. Supplementation caused a decline of 13 to 37% in the serum cholesterol and a reduction of 29.68 to 49.94% in egg yolk cholesterol. The interaction between genotypes x Vitamin C level did not significantly influence ($P>0.05$) the egg weight, external egg quality indices, serum cholesterol and egg yolk cholesterol.

The present result established the potential of Vitamin C supplementation in reducing serum and egg yolk cholesterols and improving some egg characteristics like the egg and albumen weight.

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Key words: Vitamin C, Cholesterol, Isa Brown, Harco Black, Egg quality.

INTRODUCTION

Egg yolk is considered to be one of the richest sources of cholesterol and fats in the human diet (Bartov *et al.*, 1971, Djoussé and Gaziano, 2008). Although cholesterol is important and necessary for human health, high levels of cholesterol in the blood (or *hypercholesterolemia*) have been linked to damage of arteries and cardiovascular diseases (Menotti *et al.*, 1985, Weggemens *et al.*, 2001). The need to curb hypercholesterolemia is responsible for the increasing advocacy against excessive egg consumption (Kritchevsky, 2004). Dietary approaches aimed at reducing the level of cholesterol in eggs includes supplementation of standard ration with 0.5% carboxymethylcellulose and 3H-sitosterol emulsion (Clarenburg *et al.*, 1971) and supplementation of standard ration with fermented red mold rice (Pan and Wang, 2004).

According to Kutlu and Forbes (1993), Murray (1996) and Aengwanich *et al.* (2003), Vitamin C is an antistress and an immunomodulator which enhances white blood cell function and activity. It also increases the interferon levels, antibody responses, formation of antibodies and secretion of thymic hormones. Although biosynthesis of Vitamin C occurs in poultry, the need for supplementation may be occasioned by environmental stressors (Pardue and Tharxon, 1986) such as the high ambient temperature in the tropics. Aside its high content of cholesterol, egg is a highly nutritious, low price animal product with great propensity to alleviate nutritional problems in low income populations (Souza *et al.*, 2001). Producers of eggs must therefore ensure increased quality indexes related to egg preservation and accessibility to the populace. Previous studies in other countries showed that feeding of laying birds with diets containing supplemental vitamin C helped to increase shell weight per unit surface area, Haugh Unit, Feed Efficiency and Live Weight Change, and reduce mortality in laying hen under heat or cold stress (Chen *et al.*, 1990, Puthongsiriporn *et al.*, 2001, Sahin *et al.*, 2002). Studies on supplemental feeding of Vitamin C to bird in Nigeria are of interest to consumers who are willing to consume low cholesterol eggs and to poultry farmers who are willing to improve the efficiency of egg production. The objectives of the present study were to investigate the effects of graded levels of vitamin C in diets of Harco Black and Isa Brown layers on their laying performance, egg indices, serum and egg yolk cholesterol.

MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research farm of the Faculty of Agriculture, University of Ilorin, Ilorin, Kwara state. Ilorin is located between the tropical rainforest of the Southwest and Savannah grassland of Northern Nigeria. It bears the co-ordinates of 8° 30' 0" North, 4° 33' 0" East and lies on an altitude of 305m, 1001' above sea level. It has a tropical

climate with annual rainfall, relative humidity and day temperature of 600-1200 mm, 65-80% and 33-37⁰ C, respectively (Ojo *et al.*, 2014).

A 2x4 factorial randomized complete block experimental layout was used to allot 80 twenty weeks old pullets, belonging to two breeds (40 Harco Black and 40 Isa Brown) to four levels (0.000%, 0.001%, 0.002%, 0.003%) of vitamin C supplemented diets. The four diets were diet 1 or control (basal diet with 0.000% Vitamin C), diet 2 (basal diet with 0.001% Vitamin C), diet 3 (basal diet with 0.002% Vitamin C) and diet 4 (basal diet with 0.003% Vitamin C) (Table 1). The birds were given feed and water *ad-libitum*. Routine prophylaxis was also provided for the birds. The parameters considered were Feed Intake (FI), Hen Body Weight (BW), Hen-Day production (HD), Egg Weight (EW), Egg Length (EL), Egg Diameter (ED), Yolk Height (YH), Albumen Height (AH), Yolk Weight (YW), Albumen Weight (AW), Shell Thickness (ST), Serum and Egg Yolk Cholesterol. FI was taken as the difference between the feed supplied and unconsumed feed. Egg quality parameters were determined using 4 eggs picked at random from each treatment group. Each egg was cleaned before weighing on electronic weighing scale. EL was taken as the distance between the broad and narrow ends of each egg. ED was taken as the maximum (central) cross-sectional diameter of each egg. ST was measured by the use of Micrometer screw gauge calibrated in millimeter (mm). The YH (mm) and AH (mm) were taken as the readings obtained from spherometer for yolk and albumen placed in a dry clean petridish, respectively. Each of the eggs used for the evaluation of egg internal quality was broken and poured into a dry clean petridish and its yolk was separated into another petridish of known mass using a spatula. The weight of the petridish containing the yolk or albumen was then measured on electronic scale. Both YW and AW were obtained by weight difference.

Egg yolks were collected for egg cholesterol analysis at the 3rd and 6th weeks of the experiment. 2ml of blood was collected from the wing vein of two randomly selected birds per treatment at the 3rd and 6th week. The blood was collected using sterilized syringe with needle into a plain bottle and then centrifuged at 400rpm for 5 minutes to separate serum sample. The centrifuged sample was then transferred into a lithium heparin bottle before storage at about -2°C. Eggs for cholesterol analysis were stored at temperatures between 4 and 8°C. Egg and serum cholesterols were analysed at the University of Ilorin Teaching Hospital (UIITH), Oke-oyi.

The data collected was subjected to analysis of variance (ANOVA) appropriate for a 2x4 factorial design. Differences between treatment means were determined by Duncan Multiple Range Test (Duncan, 1955).

RESULTS

Body weight and laying indices: Effects of Vitamin C supplementation on hen weight, feed intake and hen-day production were presented in Table 2. Supplementation resulted in a significant decline ($P<0.05$) in feed intake and but an increase in Hen-Day production from 57.9 to 75.7 %. Isa Brown consumed a significantly ($P<0.05$) less feed but produced a slightly higher percent Hen-day than Harco Black layers. Effects of Vitamin C supplementation on egg weight and egg quality indices were presented in Table 3. Supplementation resulted in a significant increase in albumen weight ($P<0.05$). Birds fed diet with 0.003% Vitamin C supplementation produced bigger eggs than those fed on diet with 0.001% Vitamin C supplementation (48.12 vs 42.00 grams). Birds fed diet with 0.003% Vitamin C supplementation also produced eggs with nominally higher EL, ED, YH, AH, YW, and AW than those fed on diet with 0.001% Vitamin C supplementation. The differences between Isa Brown and Black Harco in egg weight and egg

quality indices were not significant ($P < 0.05$). Genotype x Vitamin C level interaction did not significantly influence ($P > 0.05$) the egg weight and egg quality indices in the present study.

Serum and Egg Yolk Cholesterol: Results showing the effects of Vitamin C supplementation on serum and egg yolk cholesterol were presented in Table 4. Un-supplemented diet (i.e. 0.000% Vitamin C diet) had the highest total serum cholesterol. Birds fed on diets supplemented with Vitamin C had lower serum cholesterol (13 to 37% reduction). The serum cholesterol decreased as the level of Vitamin C supplementation increases from 0.001% to 0.003%. The differences between Isa Brown and Black Harco in serum cholesterol levels were not significant ($P < 0.05$). Genotype x Vitamin C level interaction did not significantly influence ($P > 0.05$) serum cholesterol levels. Birds fed with un-supplemented diet (i.e. control) had the highest total yolk cholesterol in 3 weeks (45.0 mm/mol or 14.07% increase). Supplementation caused a decline of 29.68 to 49.94% in egg yolk cholesterol within the same period. Yolk cholesterol decreased as the level of Vitamin C supplementation increases from 0.001% to 0.003%. Vitamin C supplementation tends to give higher reduction in egg yolk cholesterol in Isa Brown than in Harco Black layers, although the percent reduction in egg yolk cholesterol between the two breeds was not significantly different ($P > 0.05$). The interaction between genotypes and levels of Vitamin C supplementation was not significant ($P > 0.05$) for both serum and egg yolk cholesterols.

DISCUSSION

Feed intakes for Black Harco and Isa Brown in the present study were lower than that reported for Harco layers in another hot-humid climate by Houndonougbo *et al.* (2012). Such variation may be due to such factors as the differences in the nutritional environment of the bird (Houndonougbo *et al.*, 2012). The hen-day production reported for Isa Brown and Harco layers

in this study were comparable to that reported for Lohmann layer in South-West Nigeria by Olatunde (2000). However, the Average Hen-day production was higher than the level recorded by Houndonougbo *et al.* (2012) for Harco layers and Al-Harhi (2006) for Hy'line layers. The effect of diets on laying performance of birds in this study confirm earlier report (Houndonougbo *et al.*, 2012) that the nutritional environment play a significant role in birds such as the Harco strains. The decline in shell thickness with Vitamin C supplementation in this study support earlier report by Perek and Kendler (1963) and Mirabdollahi (2006) that ascorbic acid supplementation did not improve shell thickness of heat or nutritionally stressed birds. The nominally higher egg weight and albumen weight obtained in hens fed with ascorbic acid supplemented diets suggest the possibility of improving some egg quality parameters with ascorbic acid supplementation. Perek and Kendler (1962) reported that supplementation with vitamin C led to production of heavier eggs.

The result in the present study agrees with that of al-Janabi *et al.* (1988) who reported that vitamin C supplementation significantly decreased egg yolk cholesterol content. The lower serum cholesterol among hens fed with ascorbic acid supplemented diets agrees with previous report in man (Ginter *et al.*, 1970) and experimental animals (Fedorova, 1960).

CONCLUSION

It can be concluded from the present study that Vitamin C supplementation helped to reduce both serum and egg yolk cholesterols of laying hens. It also helped to improve hen-day production and the albumen weight of eggs. Feeding of 0.003% Vitamin C supplemented diet produced the best result in term of EW, EL, EW, YH, YW and AW.

REFERENCES

Aengwanich, W., Sridama, P., Phasuk, Y. Vongpralab, T.E., Pakdee, P., Katawatin, S. and

Simaraks, S. (2003). Effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress

Songklanakarinn Journal of Science and Technology, 25:297-305.

Al-Harhi, M. A. (2006). Utilisation of treated mangrove leaves meal in pullet diets and its

effects on sub-sequent hens performance. *International Journal of Poultry Science*, 5 (7): 598-606.

Al-Janabi, A. S., al-Kattib, S. R. and Taha, Z. D. (1988). Effect of vitamin C administration on

serum and egg-yolk cholesterol level of the chicken. *Australian Journal of Biological Sciences*, 41(4):403-7.

Bartov, I., Borntein S., and Budowski, P. (1971). Variability of cholesterol concentration in

plasma and egg yolk of hens and evaluation of the effect of some dietary oils.

Poultry Science, 50: 1357-64.

Chen, T.K., Coon, C.N. and Hamre, M. L. (1990). Effect of environmental stress on the ascorbic

acid requirement of laying birds. *Poultry Science*, 69 (5): 774-80.

Clarenburg, R., Chung, I A. K, and Wakefield, L. M.(1971). Reducing the egg cholesterol level

by including emulsified sitosterol in standard chicken diet. *J. Nutr.* 101: 289-298.

Djousse, L. and Gaziano, J.M. (2008). Egg consumption in relation to cardiovascular disease and

- mortality: the Physicians' Health Study. *Am. J. Clin. Nutr.* 87:964-969.
- Duncan, D.R. (1955). Multiple range and multiple F-Test. *Biometrics*, 11:1-42 (paper5).
- Fedorova, E. R. (1960). The long range treatment of coronary atherosclerosis with ascorbic acid. *Sov. Med* 25: 56.
- Ginter, E., Kajaba, I., and Nizner, O. (1970). The effect of ascorbic acid on cholesterolaemia in healthy subjects with seasonal deficit of vitamin C. *Nutrition and Metabolism*, 12: 76
- Houndonougbo, M. F., Chrysostome, C. A. A. M., DadJo, F. D. and Adjaho, S. L. (2012). Bioeconomic performance of pullets and layerhens fed soybean grains-based diets in hot and humid climate. *IRN Vet. Sci.* 2012:812564. www.ncbi.nlm.nih.gov/pmc/articles/PMC/365694/
- Kritchevsky, S.B. (2004). A review of scientific research and recommendations regarding eggs
Journal of American College Nutrition, 23:596-600
- Kutlu H.R. and Harrison P.C (1993). Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. *Livestock production Sci.* 36:335-350.
- Menotti A, Conti S, Dima F, Giampaoli S, Giuli B, Matano M, Seccareccia F. (1985). Incidence of Coronary heart disease in the two generations of men-exposed to different levels of risk factors. *Acta cardiologica*, 40(3): 307-14.
- Mirabdollahi, J., Hosseini, S. A., Lotfollahian, H., Ali, M. and Mehdi, K. (2006). Effects of Ascorbic Acid on Egg Production and Egg Shell Quality in Laying Hens Drinking Saline Water. *Pakistan Journal of Biological Sciences*, 9: 2897-2900
- Murray, M. T. (1996). The encyclopedia of nutritional supplements. Rocklin, C.A: Prima

publishing p.59, p.61.

Olatunde, S. I. (2000). Growth, short-term hen-day production performance of three strains of chicken. M.Sc. Thesis, Federal University of Agriculture, Abeokuta.

Pan, T. and Wang, C. (2004). Fermented red mold rice is added to hens diet to reduce cholesterol level of egg yolks. *US 7157107 B2*. <http://www.google.com/patents/US7157107>.

Pardue S.L. and Thaxton, J. P. (1986). Enhanced livability and improved immunological responsiveness in ascorbic acid supplemented chicks during acute heat stress- Abstract of papers *Poultry sci.* 61:522.

Perek, M. and Kendler, J. (1962). Vitamin C supplementation to hens in a hot climate. *Poultry Science*, 41: 677-678.

Perek M. and Kindler, J. (1963). Ascorbic acid as a dietary supplement for white leghorn hens under condition of climate stress. *British Poultry Science*, 41:191-200.

Puthongsiriporn, U., Sheideler, S. E., Sell, J. L. and Beck, M. M. (2001). Effect of vitamin E and C Supplementation on Performance in vitro lymphocyte proliferation and anti-oxidant status of laying hens during heat stress. *Poultry Science*, 80: 1190-1200

Sahin, K., Sahin, N. and Onderci, M. (2002). Vitamin E supplementation can alleviate negative effects of heat stress on egg production, egg quality, and digestibility of nutrients and egg yolk mineral concentrations of Japanese quails. *Research in Veterinary Science*, 73(3):307-312.

Souza, P. A., Souza, H. B. A., Oba, A. and Gardini, C. H. C. (2001). Influence of ascorbic acid on

egg quality . *Ciênc. Tecnol. Aliment.* vol.21 no.3. <http://dx.doi.org/10.1590/S0101->

2061200100-0300004 .

Weggemans, R. M., Zock, P. L. and Katan, M. B. (2001). Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol on humans; A meta-analysis. *The American Journal of Clinical Nutrition*, 73:885-891.

Table 1: Composition of experimental diet (%)

Ingredients	Diet 1(control)	Diet 2	Diet 3	Diet 4
Maize	40	40	40	40
Cornbran	14	14	14	14
Soybean	16	16	16	16
Wheat offal	13	13	13	13
Palm kernel cake	6	6	6	6
Bone meal	2	2	2	2
Oyster shell	8.5	8.5	8.5	8.5
Salt	0.4	0.4	0.4	0.4
Methionine	0.2	0.2	0.2	0.2
Layer's premix	0.246	0.246	0.246	0.246
Vitamin C	0.000	0.001	0.002	0.003
Silica	0.004	0.003	0.002	0.001
Total	100	100	100	100

Table 2: Effect of vitamin C supplementation on body weight and laying performance

Factors	Body weight (g)	Feed intake (g/bird/day)	Hen day production (%)
Strain Harco Black	1534	99.39 ^b	70.4
Isa Brown	1709	97.55 ^a	71.0
Diet (% Vit. C) 0.000	1625	109.68 ^d	57.9
0.001	1650	88.22 ^a	75.7
0.002	1619	99.61 ^c	73.6
0.003	1594	96.39 ^b	75.6
S.E.M	81	0.310	6.77
Interaction (genotype×diet)	ns	ns	ns

^{a,b,c} indicate significant difference in mean value ($P < 0.05$). ns = non-significant difference ($P > 0.05$).

Table 3: Effect of vitamin C supplementation on egg weight and egg quality indices

Factors		EW	EL	ED	YH	AH	YW	AW	ST
Strain	Harco Black	42.25	4.58	3.52	13.00	3.92	12.00	29.44	0.30
	Isa Brown	46.62	4.69	3.61	13.11	4.19	12.75	31.19	0.32
Diet (% Vit. C)	0.000	44.88	4.54	3.63	13.19	3.78	12.62	26.38 ^a	0.37
		42.00	4.56	3.49	12.32	4.06	11.88	31.00 ^{ab}	0.31
	0.001	42.75	4.64	3.45	12.95	3.68	11.38	29.75 ^a	0.27
	0.002	48.12	4.81	3.69	13.76	4.70	13.62	34.12 ^c	0.30
	0.003								
S.E.M.		2.57	0.11	0.07	0.53	0.49	0.79	1.47	0.02
Interaction (genotype×Vit. C)		ns	ns	ns	ns	ns	ns	ns	ns

^{a,b,c} indicate significant difference in mean value ($P < 0.05$). EW, EL, ED, YH, AH, YW, AW, and ST represents the Egg Weight, Egg Length, Egg Width, Yolk Height, Albumen Height, Yolk Weight, Albumen Weight and Shell Thickness, respectively. ns= non-significant difference ($P > 0.05$). ns = non-significant difference ($P > 0.05$).

Table 4: Effects of vitamin C supplementation on Serum and Egg yolk cholesterol

Factors	Serum cholesterol mm/mol			Egg yolk cholesterol mm/mol		
	week 3	week 6	% reduction	week 3	week 6	% reduction
Strain Harco Black	3.50	2.92	16.57	43.05	30.9	28.22
Isa Brown	3.58	2.96	17.32	46.01	31.7	31.10

% Vit. C)	0.000	3.00	3.73	24.33*	39.45	45.0	14.07*
	0.001	3.60	3.12	13.33	46.93	33.0	29.68
	0.002	3.47	2.38	31.41	47.40	25.0	47.26
	0.003	4.07	2.55	37.35	44.35	22.2	49.94
	S.E.M	0.391	0.330		1.966	4.90	
Genotype× Vit. C levels		ns	ns		ns	ns	

*% Increase in cholesterol level. Ns = non-significant difference ($P>0.05$).