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Effect of vitamin C supplementation on some productive and physiological parameters in laying hens

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ABSTRACT

Two hundred and sixteen Hy-Line laying hens were used to study the effect of vitamin C supplementation during summer months on some productive traits, egg quality, plasma and yolk total lipids and cholesterol.

The results showed that pullets treated with vit. C had lower ($P<0.001$) egg production rate and significantly higher ($P<0.001$) egg weight and egg mass. In addition, their eggs were characterized with significantly higher ($P<0.001$) absolute and proportional weights of albumen and yolk. Significant improvements ($P<0.05$) in shell quality estimated as weight and shell weight per unit surface area (SWUSA) was attained with vit. C supplementation. Dietary vit. C supplementation showed highly significant effects on feed consumption and conversion. Pullets of control group had the better feed conversion when compared with those supplemented with vit. C.

Plasma calcium and inorganic phosphorus increased in vit. C supplemented birds. Plasma calcium significantly increased ($P<0.01$) as the time passed reaching its maximum value at the end of the experiment. Plasma total lipids content was significantly lower ($P<0.001$) in controls and in pullets fed 400 g/ton vit. C, the latter had significantly the lowest value of plasma cholesterol content. Dietary vit. C supplementation decreased yolk total lipids

and cholesterol, the rate of decrease was positively correlated with the level of vit. C supplemented.

Significant (P<0.01) positive correlation was noted between egg production and each of plasma calcium and cholesterol. Inverse relationships were found between yolk cholesterol and each of plasma total lipids and cholesterol. Positive highly significant (P<0.01) regression coefficients were found for most studied traits on plasma calcium. Egg production, egg weight, shell, albumen and yolk weights increased by 2.9 egg, 0.89, 0.15, 0.42 and 0.30 g, respectively for unit change in plasma calcium. The results obtained demonstrate that ascorbic acid supplementation can be effective in reducing egg cholesterol and has influences on egg quality.

INTRODUCTION

Increasing attention has been focused on the potential role of vitamin C supplementation in preventing over-reaction to heat stressful stimulation to help chickens and other animals to cope with such challenges (Jones et al., 1996).

Earlier studies in poultry have shown that exogenous ascorbic acid supplemented in feed or drinking water or by injection improved performance of chickens during heat stress (Pardue and Thaxton, 1982 and Pardue et al., 1984).

In laying hens, there are inconsistent reports concerning the influence of ascorbic acid supplementation on egg production, egg weight and shell quality. Some reports indicated an improvement in the previously mentioned parameters as a result of vit. C supplementation (Bell and Marion, 1990; Orban et al., 1993; and Zapata and Gernat, 1995), whereas others, Ahmed et al., (1967) and Rowland et al., (1973) showed insignificant effect.

This study was planned to determine the effect of dietary vit. C supplementation with different levels on egg productive traits and some physiological parameters related to egg quality in laying pullets.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Farm, Department of Animal Production, Faculty of Agriculture, Zagazig University, Benha Branch.

A total number of 216 White Leghorn (Hy-Line) pullets aged 22 weeks were randomly selected and kept in the floor laying houses. Pullets were randomly divided into four groups each of 54 pullets.

The experimental treatments were based on layer diet (Table 1) as a control to be supplemented with vitamin C (ascorbic acid) at levels of 200, 300 and 400 g/ton ration. The trial was initiated in May 1997 and continued after sexual maturity for seven 15-day periods that extended. Average of ambient temperature (^{o}C) through the experimental period was 22.41±0.76 and 33.86±0.92 at 8 am and 2 pm, respectively.

Pullets were exposed to the natural day light (16 hours per day). Feed and water were provided ad libitum. Diets were mixed every 2 weeks.

Parameters refering to productive performance were egg production, egg weight, egg mass, feed intake and conversion (recorded for six days biweekly intervals). Whereas, absolute and relative weights of albumen, yolk, shell and shell weight per unit surface area (SWUSA) were considered as parameters of egg quality. Eggs laid during two days per two consecutive weeks were weighed, broken and yolk was completely separated from albumen. Yolks were then frozen at -10 ^{o}C until the chemical analysis to determine cholesterol according to Zlatkis et al. (1953) and total lipids content according to the official methods of A.O.A.C. (1990).

Heparinized blood samples were withdrawn from the wing vein of the five hens randomly selected per each group at sexual maturity, (50% egg production) and at the end of the experimental period. Plasma samples were prepared and stored at – 20 ^{o}C till the time of chemical analysis. Plasma total

lipids, cholesterol, calcium and inorganic phosphorus were colorimetrically estimated using commercial kits purchased from Bio-Merieux (Morcyl Etiols Charbon mierels Rains/ France).

The calculations of analysis of variance using ANOVA Procedure were carried out using SAS Procedure Guide 1996 under Windos 95 (SAS, 1996) using the following linear moder:

$$\mathbf{Y_{ijk} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + e_{ijk}}$$

where:

Y_{ijk} = The observation on k^{th} hen or egg.

μ = The common mean.

α_i = The fixed effect of the i^{th} treatment.

B_j = The fixed effect of the j^{th} interval.

$(\alpha B)_{ij}$ = The fixed effect of treatment × interval interaction.

e_{ijk} = The random error assumed to be independently randomly distributed $(0, \delta^2 e)$.

Means were compared by the "Duncan" multiple comparison option in Base SAS software (SAS, 1996).

Table (1): Composition and calculated analysis of experimental basic laying diet.

Ingredients	%
Yellow corn	65.0
Soybean meal	10.0
Layer concentrate (48%)	10.0
Wheat bran	8.0
Limestone	6.0
Bone meal	1.0
Calculated analysis:	
ME Kcal/kg	2725
Crude protein %	16
Crude fiber %	3.44
Calcium %	3.38
Available phosphorus %	0.56

RESULTS AND DISCUSSION

1- Rate of Egg production, egg weight and egg mass:

From results obtained (Table 2) it could be concluded that pullets of the control group had significantly higher ($P<0.001$) egg production rate (64.59%/hen/day) followed by those fed diet supplemented with 300 g (62.26%/hen/day) and 400 g (61.91%/hen/day) vitamin C per ton ration, respectively. These results agreed with those of Bell and Marion (1990) who reported that, egg production was slightly higher for the control than for vitamin C supplemented birds.

Pullets received 300 g/ton vitamin C had significantly higher ($P<0.001$) egg weight (53.21 g) and egg mass (33.91g/hen/day) when compared with the control and those received either 200 or 400 g vitamin C/ton ration. There are inconsistent reports regarding the effect of vit.C supplementation on egg production and egg weight. Some reports have shown remarkable improvement (Bell and Marion, 1990) while others showed an opposite trend (Kechik and Sykes, 1974 and El-Fiky, 1998).

Highly significant ($P<0.001$) variations in the rate of egg production, egg weight and egg mass were found due to dietary vit. C supplementation.

The average of egg production, egg weight as well as egg mass increased as pullets grew older reaching its maximum magnitude during the period from 10-12 wk after sexual maturity then decreased toward the end of the experimental period. Variations observed in egg production, egg weight and egg mass due to experimental intervals and the interaction effect between dietary treatment and experimental intervals were highly significant ($P<0.001$).

2- Absolute and proportional weights of albumen and yolk:

Pullets fed diet supplemented with 300 g/ton vitamin C had almost the highest ($P<0.01$) absolute and proportional egg albumen weight. In addition,

absolute and proportional yolk weight was significantly decreased (P<0.001) in the control group than in the vitamin C supplemented birds. These findings are in agreement with those reported by Abdelhamied et al. (1995), Keshavarz (1996) and El-Fiky (1998) who found that vitamin C supplementation significantly increased albumen quality, albumen and yolk weights in egg layers.

Treatments applied showed highly significant effect on absolute and proportional weights of albumen and yolk (Table, 3) . The positive effects of vitamin C on egg quality are confirmed because of its physiological functions related to minerals, amino acids and polysaccharides metabolism as well as to adrenal cortex, pituitary, ovary and liver as target organs (Tillman, 1993 and Abdelhamid et al., 1995).

Absolute and proportional weights of albumen and yolk increased as pullets grew older (Table, 3) reaching its maximum magnitude at the 8^{th} and 12^{th} wks after sexual maturity for albumen and yolk, respectively. Variation found in albumen weight due to treatments applied and experimental intervals may be attributed to the significant effect of these factors on average egg weight. It is well known that there is positive correlation between albumen weight and egg size (EL-Aggoury, 1974).

3- Absolute and proportional egg shell weight and shell weight per unit surface area (SWUSA):

Absolute and proportional egg shell weight and SWUSA were significantly (P<0.05&P<0.01)) influenced by dietary vitamin C level (Table, 4). Absolute shell weight was negatively correlated with the level of vit. C supplementation. Hashish (1992) and El-Fiky (1998) reported that vit. C supplemented layer diet or drinking water with vitamin C did not improve shell weight. Significant improvement in shell quality estimated as the SWUSA was attained in vit.C supplemented groups of pullets. SWUSA increased (P<0.05) as the level of vitamin C supplementation increased. Similar conclusion was

postulated by Balnave et al., (1991) and El-Fiky (1998). Orban et al., (1993) demonstrated that ascorbic acid supplementation increased blood calcium concentration, which could be associated with the improvement in egg shell thickness and breaking strength. Other studies reported that ascorbic acid may be involved in the hydroxylation of 25-hydroxycholecalciferol to 1.25-dihydroxycholecaliferol, as well as hydroxylation of proline and lysine that are amino acid involved in collagen biosynthesis (Volker andWeiser, 1993).

4. Feed consumption and conversion:

Dietary treatments applied showed highly significant ($P<0.001$) effects on feed consumption and conversion (Table 5). Pullets fed 300 g/ton vitamin C had the highest average of feed consumption (114.2 g/hen/day) throughout the experimental period. However, pullets of control group had the better feed conversion when compared with vitamin C supplemented birds. Relative greater daily feed intake was observed in pullets fed diet supplemented with 300 g/ton vitamin C as compared to the control. This may be due to the role of ascorbic acid to this level in activating thyroid gland which influence the feed intake (El-Fiky, 1998). Variation in average feed conversion due to treatments applied may be attributed to the effect of vit. C supplementation on the rate of production and the amount of feed consumed.

Variations in feed consumption and conversion within intervals and the interaction between dietary treatment by intervals were found to be of highly significant value ($P<0.001$).

5. Plasma calcium and inorganic phosphorus:

Data presented in Table (6) showed that the plasma calcium and inorganic phosphorus insignificantly increased by the addition of vitamin C to the laying diet. The increase in plasma calcium level of layers provided with dietary ascorbic acid may indicate that this vitamin is involved in calcium mobilization

by enhancing its absorption through intestine (Orban et al. 1993). Other reports indicated that ascorbic acid promotes mineral mobilization resulting in increased plasma calcium (Dorr and Balloun, 1976).

Plasma calcium and phosphorus increased as pullets grew older. The rate of increase mounted 3.24 and 0.53 mg/100 ml for plasma calcium and phosphorus, respectively. Highly significant ($P<0.01$) effect was found in plasma calcium level only due to experimental intervals (Table, 6).

6. <u>Plasma total lipids and cholesterol:</u>

Data in Table (6) revealed that plasma total lipids were significantly lower ($P<0.001$) in pullets of the control group (14.91) and those fed vitamin C at a level of 400 g/ton vitamin C (15.05 g/100 ml) as compared to the groups fed 200 or 300 g/ton of vit.C. However, plasma cholesterol significantly decreased ($P<0.01$) in pullets fed diet supplemented with 400 g/ton vitamin C (168.0 g/100 ml) when compared with the control and pullets treated with other levels of vitamin C. Significant higher values of plasma total lipids and cholesterol were observed in pullets received dietary vitamin C is probably due to the effect of the vitamin on fat metabolism. The increase occured in plasma lipids might cause plasma cholesterol elevation.

Plasma total lipids mounted 15.46; 15.57 and 15.57 g/100 ml at sexual maturity, at 50% production and at the end of the experiment. The corresponding values for plasma cholesterol were 175.0; 182.4 and 193.2 g/100 ml, respectively. The average of plasma total lipids as well as the average of cholesterol increased as pullets grew older. The rate of increase was 0.11 g/100 ml and 18.2 g/100 ml for plasma total lipids and cholesterol, respectively.

7. <u>Yolk total lipids and cholesterol:</u>

Dietary supplementation of vitamin C decreased yolk total lipids and cholesterol, the rate of decrease was possitively correlated with the rate of vit. C

supplementation (Table, 7). These results agree with those of Abdelhamied et al. (1995) who reported that vitamin C supplementation significantly decreased yolk cholesterol content. This may be due to a physiological relationship between yolk cholesterol concentration and rate of egg production but not between yolk cholesterol concentration and yolk weight (Hall and Mckay, 1992). Highly significant ($P<0.001$) effect was found on yolk cholesterol only due to treatments applied.

Yolk total lipids significantly increased ($P<0.01$) at sexual maturity (39.00) and at the end of the experiment (38.81 g/100 g) when compared with the corresponding values obtained at 50% egg production (38.31 g/100 g). However, yolk cholesterol differed according to the time of estimation. Similar results were observed by Ingr et al. (1987) and Hall and Mckay (1992) who reported that yolk cholesterol concentration was affected by season and variation in the rate of cholesterol biosynthesis.

8- Correlation coefficient values among some traits of egg yolk, albumen and shell quality and some blood plasma physiological parameters.

Egg production and weight of the different egg components (albumen, yolk and shell) were found to be positively and significantly correlated with plasma calcium content; values ranged from 0.344 to 0.448 (Table, 8). This may be attributed to the fact that ionic calcium in blood plasma may of great biological importance as it plays as a second messenger for most of the biological reactions related to egg formation. Total lipids in plasma was positively and significantly correlated with yolk weight only. This is scientifically logic since most lipids of the egg exists in egg yolk. Similarly positive and significant correlation coefficient was found between plasma cholesterol and total lipids which is normally expected. On the other hand, no significant correlation coefficients were found between other studied variables.

9- Multivariate regression coefficients among plasma contents and each of egg traits and yolk total lipids and cholesterol:

Table (9) show the multivariate regression coefficients of egg weight, egg production, egg quality, yolk total lipids and cholesterol and plasma calcium, inorganic phosphorus and cholesterol. It was found that regression coefficients for most studied traits on plasma calcium are positively and highly significant ($P<0.01$). Thus, it could be stated that most of variation in egg production, egg weight, shell albumen and yolk weights may be attributed to plasma calcium which may play an important role in various biological reaction on second messenger. Egg production increased by 2.9 egg and the other traits increased by 0.89, 0.15, 0.42 and 0.30 g, respectively for each unit change occur in plasma calcium. The regression coefficients for egg production on plasma cholesterol and yolk weight on plasma total lipids are also positive and significant ($P<0.05$). Most Y-intercept for all traits are positive and significant ($P<0.05$ & $P<0.01$) with different values ranged from – 41.41 for egg production to 53.26 for SWUSA.

In general, most of studied traits are negatively regressed on plasma total lipids. Regression coefficient for all traits on plasma inorganic phosphorus were very low and non-significant.

10. Economic efficiency:

Results of economic efficiency from pullets of different experimental groups are summarized in Table (10). The results indicate that all diets supplemented with vit. C gave economic efficiency lower than the control. This may be due to the higher cost per kg of vit. C containing diets and the lower egg production rate compared to control pullets.

Table (10): Economical evaluation of applying vitamin C (g/ton) in ration of laying hens.

Item	0	200	300	400
Fixed cost/hen L.E	12.00	12.00	12.00	12.00
Managment/hen L.E[1]	1.50	1.50	1.50	1.50
Total feed cost/hen L.E	5.22	5.42	5.71	5.54
Total costs /hen L.E.	18.72	18.92	19.21	19.04
Total No. of egg/hen	67.82	62.93	65.37	65.01
Total egg price/hen L.E.[2]	14.24	13.22	13.73	13.65
Price of sold bird L.E.	9.00	9.00	9.00	9.00
Total revenue/hen L.E.	23.24	22.22	22.73	22.65
Net revenue/hen L.E.	4.52	3.30	3.52	3.61
Economic efficiency[3]	0.241	0.174	0.183	0.190
Relative E. E.	100	72.20	75.93	78.84

1- Include medication, vaccines and sanitation.
2- The price of an egg at the time of the experiment = 21 P.T.
3- Net revenue per unit of total costs.

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