Asian J. Med. Biol. Res. 2015, 1 (2), 158-162; doi: 10.3329/ajmbr.v1i2.25605

**Asian Journal of**
**Medical and Biological Research**
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

*Article*

# Effects of graded levels of vitamin C supplemented feed of layers on serum concentrations of vitamin A and vitamin C

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Received: 14 July 2015/Accepted: 05 August 2015/ Published: 30 September 2015

**Abstract:** The study was conducted to determine the effects of layers' diets containing 0, 100, 150 and 200 mg/kg vitamin C on vitamin A and C concentrations in serum of layers. A total of 80 Isa brown layers (28 weeks of age) were used in this study. There were a control and three treatment groups each containing 20 hens. The experimental period lasted 8 weeks. Serum vitamin A and vitamin C concentrations were determined twice at 32 and 36 weeks of age. Serum vitamin A and serum vitamin C concentrations increased as vitamin C supplementation increased in the diet. During the study, serum concentration of vitamin C was increased significantly ($P<0.05$) by the addition of 150 and 250 mg/kg vitamin C and serum concentration of vitamin A was increased significantly ($P<0.01$) in all treatment groups in increasing order as vitamin C supplementation increases in the diet. It has been concluded that vitamin C protected vitamin A against oxidation and increased serum vitamin A levels in parallel with increased serum vitamin C.

**Keywords:** layers; serum concentrations; supplementation; vitamin A; vitamin C

## 1. Introduction

Poultry have the ability to biosynthesize ascorbic acid within their body. However, biosynthesis of vitamin C is limited in very young birds and increases with age up to about 60 days of age (Leeson and Summers, 2001). Strains of birds differ in ascorbic acid synthesis as measured by L-gulonolactone oxidase activity and tissue ascorbic acid concentration (Maurice *et al.*, 2004). Nevertheless, it was reported that usage of this vitamin far outweighs production under praxis condition (Ajakaiye *et al.*, 2011).

Although vitamin C can be synthesized by poultry, the biosynthesis may be reduced or the requirement for vitamin C increased during times of stress (Whitehead and Keller, 2003). During stresses, either nutritional, environmental, or pathological, the inclusion of vitamin C in birds feed or drinking water appears to alleviate many of the undesirable physical consequences (Whitehead and Keller, 2003; Roussan *et al.*, 2008).

Several researchers reported improvement in egg production (Haazele *et al.*, 1991; Fayeye *et al.*, 2013), egg quality (Bell and Marion, 1990), albumen weight (Cheng *et al.*, 1990; Fayeye *et al.*, 2013), egg weight (El-boushy and Van Albada, 1970) and reduction in body temperature (Pardue *et al*., 1985; Orban *et al*., 1993) due to supplementing the layers diet with vitamin C under high environmental temperatures.

There are reports that 200ppm vitamin C supplementation in feed of poultry raised under heat stress causes significant improvements in live performance, digestibility of nutrients and immune response of the birds (Sahin and Kucuk, 2001; Lohakare *et al*., 2005). Furthermore, some studies reported significant decreases in blood serum cholesterol (Yigit *et al*., 2000; Yigit *et al*., 2002) and yolk cholesterol (Al-Janabi *et al*., 1998) by the inclusion of vitamin C in diets.

It is believed that generally, vitamin C plays a central role in the bird's ability to cope with stress as it is involved in the synthesis of adrenaline and corticosteroid which are responsible hormones for the mobilization of energy for the essential functions such as maintenance of body temperature, respiration, blood flow, heat dissipation etc. (Pardue *et al.,* 1993). As long as vitamin C is not depleted, adrenaline and later corticosteroid can be synthesized and released thereby allowing the birds to survive and remain productive (Siegel and Morton, 1977).However, the greater the depletion of vitamin C, the least is the ability of the birds to synthesize these hormones (Siegel and Morton, 1977).

Ascorbic acid (vitamin C) has been found to be involved in a number of biochemical processes. The function of vitamin C is related to its reversible oxidation and reduction characteristics (Franceschi, 1992).Vitamin A is one of the most important cellular antioxidants like vitamin C. Poultry are capable of transforming carotene in diets to vitamin A. Deficiency ofvitamin A includes, weakness, retarded growth, poor feathering, high mortality, xerophthalmia, ataxia and night blindness (Merck, 2010). Reduction in egg production and hatchability are seen in layers (Merck, 2010). Other vitamin A deficiency syndromes include eyelids stuck shut with thick exudate, nasal and ocular discharge, drowsiness, pale comb and wattles (Mcmullin, 2014). The synergistic relationship of vitamins A and C have been reported (Surai *et al*., 2000). Vitamin C is a chain breaking antioxidant that protects fat soluble vitamins especially vitamin A from rancidity and degradation (Patrick and Schaible, 1980). There are several studies establishing the beneficial effects of large amount of vitamin C (Marks, 1975; Yigit, 2002) studied the effect of vitamin C on serum vitamin A and serum vitamin C in Europe. No such research on the Isa brown breed of layers in Nigeria has been documented.

The objectives of this study therefore were to determine the effects of large doses of vitamin C in layers' feed on serum, albumin and yolk concentrations of vitamin C and A.

## 2. Materials and Methods

This study was carried out at the poultry unit of the Livestock Teaching and Research Farm of Kogi State University, Anyigba, Kogi State, Nigeria.

Eighty (80) Isa brown layers at the age of 28 weeks were raised on deep litter system. The experimental period lasted 8weeks. The layers were fed with a commercial layers mash (Topfeed®) for an adjustment period of two weeks and were then allotted to four treatment groups with two replicates per treatment and 10 birds per replicate in a completely randomized design. Each of the four diet treatments were labelled as $T_1$, $T_2$, $T_3$ and $T_4$ with their diets containing 0, 100, 150 and 200 mg/kg vitamin C respectively. Feed and water were supplied *ad libitum*. Pure Vitamin C used was the crystalline type (a product of Shandong Luwel Pharm Company Limited, India).

Blood samples were taken from the wing vein at 32 and 36 weeks of age. Sera samples were obtained after 8 hours by slanting the blood sample bottles and the sera decanted. Sera samples were analyzed for vitamin C and vitamin A concentrations spectrophotometrically as described by Kway (1978) and Zee *et al*. (1991).

Data obtained were evaluated using one way Analysis of Variance (ANOVA) procedure of Statistical Package for the Social Sciences (SPSS, 2013). Means were compared using Duncan's Multiple Range Test.

## 3. Results and Discussion

The effects of graded levels of vitamin C in feed of layers on serum vitamins A and C are shown in Table 1 and Serum concentrations of Vitamin A in parallel to graded levels of Vitamin C supplementation is shown in Figure 1.

In this study, it was observed that both serum vitamin C and vitamin A concentrations increased in parallel with vitamin C supplementation in feed ($P<0.05$ and $P<0.01$ respectively). The results agree with the findings of Yigit *et al*. (2002) who reported an increase in serum vitamin A with increasing vitamin C supplementation and thus agreed with the report that vitamin A protects lipids from oxidation and therefore fat soluble vitamin A from destruction against peroxidative damage. The result also agrees with that of Khan and Sadar (2005) who used three breeds of chickens that were exposed to heat stress and observed that the supplementation with vitamin C protected vitamin A against oxidation.

Vitamin C was however not detected in the albumen and yolk of the eggs laid by hens fed vitamin C fortified feed. Though vitamin A was detected in the albumen and yolk of the eggs, there was no significant difference ($P>0.05$) in the values obtained and the levels were rather low ranging from 0.10 to 0.13 and 0.06 to 0.24 for albumen and the yolk respectively.

Vitamin C is very low in the predominant feedstuffs for poultry (i.e. grains and plant protein supplements) (Zee *et al*., 1991).

It was surmised in the work of Khan and Sadar (2005) that the effect of vitamin C supplementation on the performance of desi, fayoumi and commercial leghorn chickens exposed to heat stress that the mean egg production, egg weight and egg shell thickness improved (P<0.05) with supplementation of vitamin C in all layers. Also the blood parameters showed that the concentration of ascorbic acid (Vitamin C) was higher (P<0.05) in groups supplemented with vitamin C. However, the concentrations of blood enzymes (alkaline phosphate, aspartate and alanine aminotransferase) were lowered (P<0.01) with vitamin C supplementation in all types of layers used.

Effects of vitamin C on some vitamins and minerals have also been studied, ascorbic acid promotes non-heme iron absorption from food (Olivares *et al*., 1997), and a sufficient vitamin C status is necessary for the C-1 hydroxylation of vitamin D3.

There is also inter-relationships between vitamin C and the B vitamins; such that tissue levels and urinary excretion of vitamin C are affected in animals with deficiencies of thiamine, riboflavin, pantothenic acid, folic acid and biotin. Vitamin C is very important in several biochemical functions. In general, research studies have shown that healthy animals under normal conditions do not respond to supplemental vitamin C (Newman and Leeson, 1999). However, it was suggested that while plasma ascorbic acid levels may change precipitously, tissue uptake may be limited by a ‘protective’ mechanism that prevents excessive and potentially detrimental cellular concentrations of ascorbic acid (Marks, 1975).

As shown in the Table 1, serum vitamin C concentration was increased in all treatment groups during supplementation and there was significant difference (P<0.05) between the treatment groups and the control with $T_4$ (200mg vitamin C) having the highest value of 2.63mg/100mL. The result revealed an increasing trend of serum vitamin C as supplemental vitamin C increased.

Likewise, serum vitamin A concentration was also increased significantly (P<0.01) in parallel with increasing supplementation of vitamin C in feed as shown in Table 1. Vitamin C caused an increase in serum vitamin A concentration in parallel with increases in serum vitamin C concentrations. High correlations (P<0.05) between vitamin A and vitamin C were found in control and treatment groups at 32 and 36 weeks respectively as can be observed in Figure 1.

It has been reported that vitamin C protected vitamin A against oxidation and increased serum vitamin A levels in parallel with increased serum vitamin C (Yigit *et al.*, 2002; Khan and Sadar, 2005). Vitamin C helps to protect phagocytic cells from oxidative damage (McDowell, 2006). Also, supplemental vitamin C could significantly reduce body temperature (Pardue *et al*., 1985; Orban *et al*., 1993).

Ascorbic acid supplementation has been shown to have positive effect on mean egg production, egg weight and egg shell thickness (Khan and Sardar, 2005) and on albumen weight, egg length, yolk height and yolk weight (Fayeye *et al*., 2013) under elevated environmental temperature. This will therefore suggest that supplementing layers feed with ascorbic acid (vitamin C) prior to the onset of environmental stressors would be beneficial to the laying hens.

**Table 1. Effects of Vitamin C supplemented diets on serum, albumen and yolk concentrations of Vitamin A and Vitamin C.**

| **Traits** | **$T_1$ (0mg)** | **$T_2$ (100mg)** | **$T_3$ (150mg)** | **$T_4$ (200mg)** | **SEM** | **LOS** |
|---|---|---|---|---|---|---|
| Serum Vitamin A (µg/dL) | $75.00^{d}$ | $91.14^{c}$ | $140.34^{b}$ | $232.23^{a}$ | 0.10 | ** |
| Serum Vitamin C (mg/100mL) | $1.03^{c}$ | $1.32^{bc}$ | $1.53^{b}$ | $2.63^{a}$ | 0.23 | * |
| Albumen Vitamin A | 0.13 | 0.12 | 0.10 | 0.08 | 0.03 | NS |
| Albumen Vitamin C | ND | ND | ND | ND | ND | ND |
| Yolk Vitamin A | 0.24 | 0.23 | 0.17 | 0.15 | 0.06 | NS |
| Yolk Vitamin C | ND | ND | ND | ND | ND | ND |

NS = not significant, ND = not detected, * = p<0.05 **= p<0.01

a, b, c, d = Means with different superscripts across the same row are significantly different

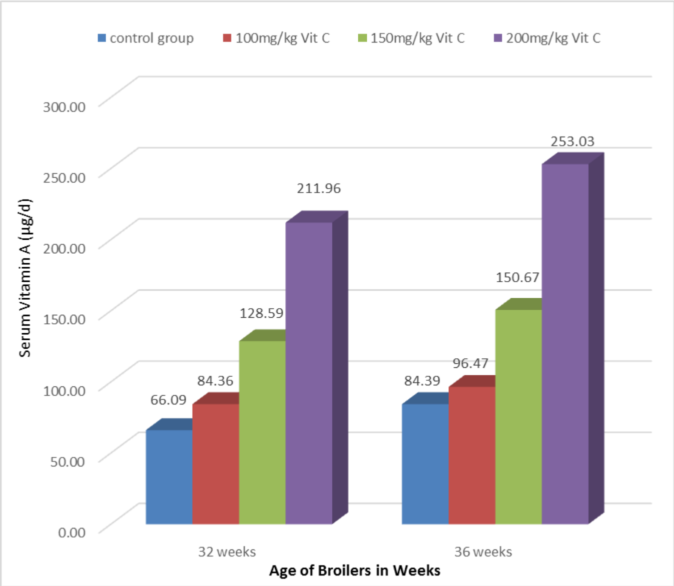


**Figure 1. Serum concentrations of Vitamin A in parallel to graded levels of Vitamin C supplementation.**

## 4. Conclusions

Supplementation of vitamin C increased serum vitamin A concentration and therefore, maintaining vitamin C concentration in diet is highly important in protecting vitamin A against oxidation. Study on the effect of vitamin C on concentration of vitamin C level in body organs like the liver is recommended.

## Conflict of interests

None to declare.

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