Effect of higher levels of dietary vitamin E on humoral immune response, water holding capacity and oxidative stability of meat in growing Japanese quail (Coturnix coturnix japonica)

Einfluss höherer Gehalte an Vitamin E im Futter auf die humorale Immunantwort und auf das Safthaltevermögen sowie auf die Oxidationsstabilität von Fleisch wachsender Japanischer Wachteln (Coturnix coturnix japonica)

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Introduction

Intensive poultry production makes use of fast growing strains that are usually maintained at high stocking densities. However, poultry flocks may be more susceptible to infectious agents, either as a result of reduced immune competence possibly due to genetic selection for growth, the ready transmission of pathogens between birds and/or poor environmental hygiene (Friedman et al., 1998). It therefore seems highly desirable to improve immune responses by nutritional manipulation. Vitamin E functions in biological systems primarily as an agent that provides protection against free radicals. Apart from its protective effect on lipid peroxidation, the immunoregulatory effects of dietary vitamin E on humoral and cell mediated immunity are well known. Vitamin E also improves the action of phagocytic cells and passive antibody transfer (Franchini et al., 1995; Raza et al., 1997). Vitamin E accumulated to some extent in the liver and adipose tissue, however, this deposition is not sufficient to maintain physiological requirements for a long time and it is not toxic (Surai, 2002). Supplementing quail diets with vitamin E at levels higher than basal requirements has been shown to enhance primary immune responses by increasing number of antibody forming cells and antibody response to inoculated sheep red blood cells (SRBC) (Biswas et al., 2008).

The ability of meat to retain inherent water, also defined as water holding capacity (WHC), is an essential quality parameter for both the industry and the consumer. For the meat industry, low WHC implies increased economical losses, and consequently there is a strong interest in optimizing this parameter (Huff-LonerGAN and LonerGAN, 2005). The WHC of meat is also known to influence its technological stability of meats, with the oxidative potential increasing as the degree of unsaturation of the lipids in the meat increases. Vitamin E cannot be synthesized by animals, and therefore the presence of the vitamin in animal tissues reflects the dietary availability (Zouari et al., 2010). Membrane lipids are protected against oxidative attack by a number of natural occurring antioxidants, including the chain breaking antioxidants ascorbic acid, a-tocopherol and plant extracts. Of all them, a-tocopherol has demonstrated the highest biological efficiency in preventing the lipid oxidation in vivo. Supplementation of poultry diets with vitamin E can achieve different objectives. In the first place, it prevents nutritional deficiencies. Secondly, it improves the oxidative stability of poultry meat products. Finally, it produces a highly nutritional food supplementary source of vitamin E for the human consumer (Barroeta, 2006).

The objective of this present study was to evaluate the effects of feeding supplemental vitamin E at concentrations significantly above the NRC requirements on humoral immune response, water holding capacity and oxidative stability of meat in growing Japanese quail.

Materials and Methods

Birds and Diets

A total of 240 one-day-old Japanese quails (Coturnix coturnix japonica) were used in this study. The birds were randomly assigned, according to their initial body weights, into 3 groups with 4 replicates of 20 birds. The birds were reared in cages of identical size (100 × 100 cm floor area and 80 cm in height) for 42 days of experimental period. All the groups were subjected to similar management practices (brooding, lighting, feeding and watering) through-
out the experiment except the diets offered. Ingredients and chemical compositions of the basal diet are shown in Table 1. The representative samples of feed ingredients (corn and soybean meal) were analyzed for crude protein (AOAC, 2005). The diets were formulated to be isocaloric and isonitrogenous. Three isocaloric (12.1 MJ/kg) and isonitrogenous (24% CP) CSM-based diets based on NRC (1994) poultry recommendations were formulated to contain 3 levels of vitamin E (18, 90 and 180 mg/kg diet from DL α-Tocopheryl acetate) in a completely randomized desiging. Basal diet contained 12.7 mg vitamin E/kg diet. Quails were provided with feed and fresh water for ad libitum consumption.

Lymphoid organ and humoral immune response
At the end of experiment, three male birds from each replicate of the treatment (12 birds per each dietary treatment and 36 in total) were selected randomly and killed to determined the relative weight of the lymphoid organs (bursa and spleen). Organ weights were measured to the nearest 0.001 g then their relative weight to body weight at 42 day was calculated. To investigate the effect on the humoral immune response, two male birds were selected from each of the replicated groups (that is, 8 birds/dietary treatment providing 24 birds in all) at 28 days of age and were immunized intramuscularly with 0.2ml of a 5% suspension of SRBC. On day 35 (7 days after primary immunization), blood samples were collected from the branchial vein and previously immunized birds were again injected with the same dose of SRBC. Blood for secondary antibody titres was collected 7 days after secondary immunization (on day 42 of age). The antibody titre against SRBC was determined by haemagglutination assay (HA) and expressed as log2 values for the reciprocal of the highest titre where complete agglutination was observed (MUNNS and LAMONT, 1991).

Storage of samples and meat quality parameters
At the end of the experiment, three male birds from each of replicates were slaughtered and the abdominal fat pads removed immediately. The thigh muscle were packed in ziploc plastic bags and stored for 1, 90 and 180 day at −20°C. At the time of the analysis, frozen quail thigh samples were thawed and extent of lipid oxidation was evaluated as thiobarbituric acid reacting substances (TBARS) by the modified method of Ke et al. (1977). Ten grams of minced thigh samples were homogenized for 2 min with 95.7 ml of distilled water and 2.5 ml of 4N HCl. The mixture was distilled until 50 ml was obtained. Then, 5 ml of the distillate and 5 ml of TBA reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid) were heated in a boiling water bath for 35 min. After cooling under running tap water for 10 min, the absorbance was measured at 538 nm against a blank. TBARS values were obtained by multiplying optical density by 7.843. Oxidation products were quantified as malondialdehyde equivalents (mg MDA kg−1 muscle). The water holding capacity (WHC) was estimated (BOUTON et al., 1971) by centrifuging 1 g of the muscles placed on tissue paper inside a tube for 4 min at 1500 × g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as: (weight after centrifugation − weight after drying)/initial weight × 100.

Statistical analysis
The experiment was conducted as a completely randomized design with 3 levels of vitamin E (18, 90 and 180 mg/kg diet). The obtained data humoral immune response and lymphoid organs were subjected to statistical analysis using the general linear model (GLM) procedures of the SAS software (SAS INSTITUTE, 2001). Data lipid oxidation and WHC were analyzed using GLM procedures with main effects (vitamin E and time storage) and the interaction between vitamin E × time storage. Significant differences among the means of treatments were determined by using Tukey test.

Result and discussion
Lymphoid organ and humoral immune response
The results of the present study showed secondary antibody titres against SRBC at 42 day of age were significantly greater in quails given dietary supplements of 90 and 180 mg/kg vitamin E but primary antibody titres against SRBC at 35 day of age were not affected by the levels of vitamin E (Table 2). These results were in agreement with previous reports (BISWAS et al., 2008; ABDUKALYKOVA and RUIZ-FERIA, 2006; BISWAS et al., 2005; LESCHINSKY and KLASING, 2001). BISWAS et al. (2008) reported that vitamin E supplementation (150 or 300 mg/kg of diet) improved humoral immune response in growing Japanese quail. ABDUKALYKOVA

Table 1. Composition and main characteristics of basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (CP = 7.89%)</td>
<td>505</td>
</tr>
<tr>
<td>Soybean meal (CP = 43.68%)</td>
<td>420</td>
</tr>
<tr>
<td>Fish meal (CP = 55.32%)</td>
<td>30.0</td>
</tr>
<tr>
<td>Soy oil</td>
<td>20.7</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.2</td>
</tr>
<tr>
<td>Limestone</td>
<td>11.6</td>
</tr>
<tr>
<td>Salt</td>
<td>3.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>2.5</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Calculated chemical component</td>
<td></td>
</tr>
<tr>
<td>Metabolisable energy, MJ/kg</td>
<td>12.1</td>
</tr>
<tr>
<td>Crude protein</td>
<td>24.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.0</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>13.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.0</td>
</tr>
<tr>
<td>Methionine + cystine</td>
<td>8.8</td>
</tr>
</tbody>
</table>

2 Experimental diets were supplemented with vitamin E at 18, 90 and 180 mg/kg.
3 Each kg of vitamin/mineral premix contained: vitamin A, 3,600,000 IU; vitamin D3, 800,000 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B2, 3,300 mg; vitamin B3, 4,000 mg; vitamin B5, 12,000 mg; vitamin B6, 1,200 mg; vitamin B9, 500 mg; vitamin B12, 6,000 mg; vitamin H2, 2,000 mg; choline chloride, 200,000 mg; Mn, 66,140 mg; Fe, 100,000 mg; Zn, 117,600 mg; Cu, 16,000 mg; I, 640 mg and choline chloride, 133,340 mg.
and Ruiz-Feria (2006) showed that addition of 80 mg/kg vitamin E significantly increased antibody response to SRBC measured at 4, 8 and 16 day after injection. Biswas et al. (2005) studied the effect of antioxidants on immune response in Japanese quail and reported that, significantly higher humoral immune response was observed with diets having 0.5 mg/kg selenium and 300 mg vitamin E/kg diet. In contrast, no significant effect on antibody titres against SRBC in birds was observed in broilers fed vitamin E (10 and 300 mg/kg) and concluded that vitamin E supplementation increases heterophil/lymphocyte ratio, indicating that vitamin E improved the phagocytic capacity of the immune system, promoting the birds against the invasion of pathogenic microorganisms (Boa-Amponsen et al., 2000). The reason for the contradiction among the results might be the result of the difference in the species used, age of animals and the amount of vitamin E and selenium supplemented to the diet. The exact mechanism of interaction for vitamin E with the immune system is not known, vitamin E has been reported to influence T-helper cell activity (Tanaka et al., 1979), phagocytic activity (Boa-Amponsen et al., 2000) and the rate of prostaglandin synthesis in lymphoid organs (Chen et al., 1980). Vitamin E concentrations can deeply influence the eicosanoid profile. As such, tocopherol is reviewed as a bioregulator, modulating both the lipoxigenase and the cyclooxygenase pathways at the level of arachidonate oxidation (Chen, 1993). Eicosanoids regulate immune responses at many different levels and tocopherol could, therefore, mediate direct effects on immunity (Brigham, 1989).

There were no significant differences (P > 0.05) in final body weight (Table 2). Lymphoid organ weights were expressed as a g/kg of body weight (Table 2). The results of the present study showed that none of the lymphoid organ weights (bursa and spleen) were significantly influenced by dietary vitamin E. These results consistent with those of Niu et al. (2009) and Akbari et al. (2008). In contrast, Singh et al. (2006) reported that the weights of spleen and bursa were significantly greater when chicks were given dietary supplements of 0.2 mg/kg selenium and 200 mg/kg vitamin E. Abdukalykova and Ruiz-Feria (2006) reported that the relative weight of bursa was reduced with higher levels of vitamin E (400 IU) compared with bursa of birds fed 40 and 80 IU of vitamin E but the relative weight of spleen was not affected by dietary vitamin E. Marsh et al. (1986) reported that vitamin E deficiency had adverse effects on the development of lymphoid organs and also resulted in impaired function of these organs.

### Water holding capacity (WHC)

The results of the present study showed WHC was affected by level of vitamin E and it was significantly (P < 0.05) increased, with increasing dietary vitamin E supplementation (Table 3), but the difference between 90 and 180 mg/kg vitamin E was not significant (P > 0.05). WHC levels decreased for all dietary treatment with increasing time of frozen storage. These results were in agreement with previous reports (Jensen et al., 1998; Creah et al., 1995; Morrissey et al., 1994). Vitamin E (α-tocopherol) is a membrane associated antioxidant, capable of quenching highly reactive free radicals, which initiate and propagate oxidation of unsaturated fatty acids within the membrane (Buckley et al., 1989). This oxidation leads to a decrease in

<table>
<thead>
<tr>
<th>Vitamin E (mg/kg)</th>
<th>Body weight (g)</th>
<th>HA titre (log$_2$) against sheep red blood cells (SRBC) Bursa of Fabricius (g/kg BW)</th>
<th>Spleen (g/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary response</td>
<td>Secondary response</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>259 ± 1.24</td>
<td>1.87 ± 0.225</td>
<td>0.230 ± 0.022</td>
</tr>
<tr>
<td>90</td>
<td>262 ± 3.11</td>
<td>2.62 ± 0.263</td>
<td>3.62 ± 0.323 a</td>
</tr>
<tr>
<td>180</td>
<td>260 ± 2.29</td>
<td>2.50 ± 0.375</td>
<td>3.12 ± 0.295 a</td>
</tr>
<tr>
<td>Probability</td>
<td>0.814</td>
<td>0.221</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different (P < 0.01)

1 Each value is the mean ± SEM
2 The haemagglutinin assay (HA) titre was determined 7 days after primary and secondary immunization against SRBC.
3 BW = Body Weight

### Table 3. Effect of supplementing dietary vitamin E on TBARS value (mg malonaldehyde/kg muscle) and WHC (percent) of quail thigh meat kept under frozen (−20°C) storage (mean ± SEM, n = 36)

<table>
<thead>
<tr>
<th>Vitamin E (mg/kg)</th>
<th>TBARS value</th>
<th>WHC (percent)</th>
<th>Storage time (day)</th>
<th>TBARS value</th>
<th>WHC (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2.57 ± 0.33 a</td>
<td>61.6 ± 1.29 b</td>
<td>1</td>
<td>1.23 ± 0.25 c</td>
<td>67.9 ± 0.57 a</td>
</tr>
<tr>
<td>90</td>
<td>1.77 ± 0.27 b</td>
<td>62.1 ± 0.87 ab</td>
<td>90</td>
<td>1.88 ± 0.16 b</td>
<td>60.2 ± 0.77 b</td>
</tr>
<tr>
<td>180</td>
<td>1.41 ± 0.21 b</td>
<td>63.7 ± 1.27 a</td>
<td>180</td>
<td>2.64 ± 0.33 a</td>
<td>59.1 ± 0.78 b</td>
</tr>
<tr>
<td>Probability</td>
<td>0.003</td>
<td>0.08</td>
<td>0.006</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different (P < 0.01)

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fluidity and disruption of normal membrane structure and function, and may affect the ability of the membrane to act as a semi-permeable barrier. The exact mechanism of interaction for vitamin E with the WHC is not known. Ghah et al. (1995) suggested that vitamin E inhibited phospholipase A2 resulting in prolonged stability of mitochondria membranes and hindering leakage of Ca²⁺ from the sarcoplasmatic reticulum. Release of Ca²⁺ ions from the sarcoplasmatic reticulum induces shortening and may influence shortening of sarcomeres. The relationship between endogenous vitamin E and drip loss reduction deserves further investigation to explain the contradictory results and the mechanism behind this. The integrity of the cell membrane is thought to influence drip loss and protection of membranal lipids against lipid oxidation by endogenous vitamin E has been suggested to be the mechanism responsible for the positive influence of dietary vitamin E on the water holding capacity (Asghar et al., 1991).

**Oxidative stability**

The influence of vitamin E and storage time on lipid oxidation in the thigh muscle of quails is shown in Table 3. TBARS values increased (P < 0.05) for all dietary treatment with increasing time of frozen storage. The interaction between vitamin E × time were not significant (data not shown). TBARS value in thigh muscle was affected by different levels of vitamin E. The thigh samples taken from birds fed 90 and 180 mg/kg vitamin E compared to birds supplemented with 18 mg/kg vitamin E, showed the lowest TBARS values and thus greater oxidative stability during storage.

Some studies have shown that dietary vitamin E supplementation significantly increased the α-tocopherol content of muscle membranes and functions as a lipid antioxidant and free radical scavenger (Guo et al., 2001; O’Neill et al., 1998; Jensen et al., 1998). Hooda et al. (2009) reported that higher dietary vitamin E levels at 225 and 300 mg/kg vitamin E were more effective in retarding oxidative deterioration of quail meat samples stored at –18°C for two and three months, respectively. Li et al. (2009) reported TBARS values decreased with increase in dietary vitamin E, and the addition of 100 mg/kg or more vitamin E had a beneficial effect on oxidative stability as indicated by TBARS values during storage time. Zouari et al. (2010) reported that Vitamin E dietary supplementation with 200 mg/kg of feed or 300 mg/kg of feed for 20 days before slaughtering has been found to be effective in reducing lipid oxidation in meat during refrigerated storage. O’Neill et al. (1998) reported a reduction in muscle oxidation of chickens supplemented with vitamin E at 150 mg/kg. Such decrease was independent of part, supplementation period, and storage conditions, suggesting that vitamin E supplementation could be an alternative to increase the shelf life of chicken products.

The presence or absence of vitamin E in animal tissues has a critical influence on the stability of lipids during the storage of meat. By trapping free radicals, Vitamin E protects fatty acids from oxidation. In this process, vitamin E releases a hydrogen atom, which is captured by a peroxyl radical which is thereby reduced to form a hydroperoxide. Vitamin E radicals are extremely stable and do not react with polyunsaturated fatty acids. They can also react with other radicals and thus terminate the chain of reactions of lipid oxidation at this point. Vitamin E is highly efficient as an antioxidant. From chemical and biochemical studies it is known that after being oxidized and before undergoing decomposition, vitamin E can be re-reduced. Ascorbic acid and the enzyme glutathione are the principal water soluble intracellular antioxidants (reductants) that generate reduced vitamin E. This reaction depends on the concentration of these substances and/or of the enzymes that maintain them in their reduced form (Berges, 1999).

**Conclusion**

In conclusion, supplementing quail diets with vitamin E at levels higher than NRC requirements had a beneficial effect on immune response, water holding capacity and improves oxidative stability of thigh meat during frozen storage but does not affect lymphoid organs in Japanese quail.

**Summary**

This experiment was conducted to evaluate the effect of different levels of vitamin E on humoral immune response, water holding capacity and oxidative stability in growing Japanese quail (Coturnix coturnix japonica). This experiment was carried out using 240 quails in a completely randomized design with three levels of vitamin E (18, 90 and 180 mg/kg). Four replicates of 20 quails were allocated to each experimental treatment and the birds were reared for 42 days. Quails were immunized against sheep red blood cell (SRBC) at 28 and 35 days of age. Hemagglutination assay (HA) titres were determined 7 days after an intramuscular immunization. At the end of experiment, three birds from each of replicates were slaughtered and thigh muscles were stored for 1, 90 and 180 day at −20°C to assess meat quality parameters.

The results showed that primary antibody titres against SRBC at 35 day of age were not affected by the levels of vitamin E but secondary antibody titres against SRBC at 42 day of age were significantly greater in quails given dietary supplements of 90 and 180 mg/kg vitamin E. There were no significant differences in relative weight of the lymphoid organs (bursa and spleen). Water holding capacity (WHC) was affected by level of vitamin E and it was significantly increased, with increasing dietary vitamin E supplementation (P < 0.05). But the difference between 90 and 180 mg/kg vitamin E was not significant. Oxidative stability of meat from quails fed 90 and 180 mg/kg vitamin E was greater than that of quails fed 18 mg/kg vitamin E. It was concluded that supplementing diet with vitamin E had a beneficial effect on immune response, water holding capacity and oxidative stability of thigh meat during frozen storage but did not affect lymphoid organs in Japanese quail.

**Key words**

Quail, nutrition, vitamin E, humoral immune response, water holding capacity, oxidative stability

**Zusammenfassung**

Einfluss höherer Gehalte an Vitamin E im Futter auf die humorale Immunantwort und auf das Safthaltevermögen sowie auf die Oxidationsstabilität von Fleisch wachsender Japanischer Wachteln (Coturnix coturnix japonica).

Der Versuch wurde durchgeführt, um die Auswirkung von unterschiedlichen Zulagen von Vitamin E zum Futter auf die humorale Immunantwort und das Safthaltvermö-

Die Ergebnisse zeigen, dass zwar die primären Anti- körpertiter gegen SRBC am 35. Lebenstag nicht durch die Zulagen an Vitamin E zum Futter beeinflusst wurden, dass aber die sekundären Antikörpertiter gegen SRBC am 42. Lebenstag bei den Zulagen von 90 und 180 mg Vitamin/kg Futter signifikant höher waren. Dagegen haben sich die Behandlungen nicht auf die relativen Gewichte der lymphoiden Organe (Bursa fabrizius und Milz) ausgewirkt. Das Safthaltevermögen (WHC) wurde durch die Vitamin E-Zulage signifikant beeinflusst. Mit zunehmender Vitamin E-Zulage verbesserte sich das Safthaltevermögen signifi- kant (P < 0,05). Allerdings unterschieden sich die Zulagen 90 und 180 mg/kg nicht signifikant. Die Oxidationsstabi- tät des Fleischs war bei den Zulagen 90 und 180 mg Vita- min E/kg Futter höher als bei der Zulage von 18 mg/kg Vitamin E. Es wurde der Schluss gezogen, dass Zulagen von Vitamin E zum Futter von Wachteln zwar die Immunant- wort und das Safthaltevermögen sowie die Oxidationssta- bilität des Schenkelfleischs günstig beeinflussten, dass sie sich aber nicht auf die lymphoiden Organe auswirkten.

**Stichworte**

Wachtel, Fütterung Vitamin E, humorale Immunität, Safthaltevermögen, Oxidationsstabilität

**References**


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