Effect of Feeding Rosemary and α-Tocopheryl Acetate on Hen Performance and Egg Quality

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The goal of this study was to determine the effect of feeding rosemary and α-tocopheryl acetate on hen performance and egg quality. Ninety-six Lohmann laying hens, 32-week-old, were allocated into four groups. One of the groups was given a control diet (CONT), two groups were given diets supplemented with ground rosemary at 1/2 and 1 g/kg (ROS-1/2 and ROS-1, respectively), whereas the other group a diet supplemented with 200 mg/kg α-tocopheryl acetate (VIT-E). Following 60 days feeding, hen performance and some egg quality characteristics were determined, whereas the oxidative stability of the refrigerated stored eggs and liquid yolks was also examined. Results showed that there were no significant (P>0.05) differences in egg production, feed consumption, feed conversion ratio, egg weight and shape, yolk diameter, height and color, Haugh units, and shell thickness, among the dietary treatments. The extent of lipid oxidation in eggs differed (P<0.05) between the dietary treatments, but did not change with the storage time. In liquid yolks, lipid oxidation was lower (P<0.05) in the ROS-1 group compared to the CONT group. The ROS-1 group, in turn, exhibited lower (P<0.05) oxidation rate than the ROS-5 group, a finding suggesting that rosemary exerted a dose dependent antioxidative activity. The VIT-E group presented lower (P<0.05) lipid oxidation rate compared to all other groups.

Key words: egg quality, hen performance, lipid oxidation, rosemary, α-tocopherol

Introduction

The oxidative stability of eggs has not been an area of major concern since their built-in antioxidanit characteristics maintain the flavour during extended storage. Phosvitin, a yolk protein, and conalbumin, an albumen protein, have both been shown to exert antioxidant activity by inhibiting Fe³⁺ and Cu²⁺ catalyzed reactions, whereas other egg yolk constituents including α-tocopherol, xanthophylls and lecithin, have all been shown to be very effective in preventing oxidation of yolk lipids (Cuppert, 2001).

Although shell eggs are relatively stable against oxidation, processed eggs are readily subject to lipid oxidation, particularly at low pH values (Pike and Peng, 1988; Botsoglou et al., 1998). The susceptibility of these eggs to lipid oxidation is of further concern as novel feeding strategies have been adopted by the Agrifood industry to alter the lipid composition of eggs. However, increasing polyunsaturated fatty acid content of eggs increase the
degree of unsaturation and could actuate their potency to lipid oxidation, leading to losses in quality characteristics and nutritional value, and lower consumer acceptability (Cherian et al., 1996).

Use of aromatic plant extracts including thyme (Botsoglou et al., 1997) and rosemary (Galobart et al., 2001) have been demonstrated to delay lipid oxidation in eggs when used in hen feeding. Rosemary extracts, in particular, reportedly reduced lipid oxidation in eggs when used in hen feeding. Rosemary leaves on eggs, the objective of this study was to compare it with that of rosemary accepts (Cherian et al., 1996). Rosemary extracts contain a wide range of different phenolic compounds with varying biological activity. Carnosic acid is the major phenolic constituent present in rosemary leaves with an antioxidant activity approximately three times higher than carnosol and seven times higher than the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole (Richheimer et al., 1996). Rosemary contains also lesser amounts of carnosol, rosmarinol, epirosmaranol, rosmarinic acid, and their methoxy-derivatives (Cuvelier et al., 1996; Offord et al., 1997).

In addition, use of α-tocopherol, an efficient free radical scavenger, in hen feeding seems to be another efficient means for improving the oxidative stability of eggs. Antioxidative effects of dietary supplemented α-tocopherol on eggs are well established (Wahle et al., 1993; Cherian et al., 1996; Qi and Sim, 1998), although prooxidant effects on egg yolk at high supplementation levels have also been reported (Gebert et al., 1998; Chen et al., 1998).

Since there has not been yet any report dealing with the antioxidant effect of dietary supplemented rosemary leaves on eggs, the objective of this study was to evaluate the use of ground rosemary leaves and α-tocopherol acetate in hen feeding to promote performance and delay lipid oxidation in eggs, and to compare it with that of α-tocopherol.

Materials and Methods

Animals and Diets

Ninety-six Lohmann Brown-Classic laying hens, 32-week-old, were used in this study. The birds were assigned into four dietary treatments replicated four times with six hens per replicate. Dietary treatments included a corn-soybean-based typical layer diet (Table 1) that served as the control (CONT), two diets based on the typical diet further enriched with 5 g/kg and 10 g/kg ground rosemary (ROS-5, ROS-10), respectively, and another diet based on the same typical diet further enriched with 200 mg/kg α-tocopherol acetate (VIT-E). Ground rosemary was from stems and leaves of Rosmarinus officinalis L. plants that were first dried at 42°C for 48 h and then ground to pass 2 mm screen, whereas α-tocopherol acetate was from Roche Products Ltd. (Hertfordshire, UK). Diets were formulated to meet the requirements for nutrient and energy content for laying hens (NRC, 1994) and stored in airtight containers. During the feeding period that lasted 60 days, diets and water were provided ad libitum, whereas the lighting regimen was 15 h of continuous light per day.

All birds were weighed at the start of the experiment and at intervals of 15 days until the end of the experiment. Feed consumption, egg production and total egg weight were recorded daily. Egg quality characteristics including egg weight, egg shape

<table>
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<th>Table 1. Composition of the basal hen diet</th>
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<td>Vitamin premix</td>
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1 Provided per kg of diet: 12500 IU vit. A, 1250 IU vit. D3, 30 mg vit. E, 2 mg vit. B12, 4 mg vit. B6, 3 mg vit. B1, 0.02 mg vit. B12, 2 mg vit. K3, 20 mg nicotinic acid, 10 mg pantothenic acid, 1 mg folic acid, 0.08 mg biotin, 50 mg vit. C, 300 mg choline.
2 Provided per kg of diet: 80 mg Zn, 40 mg Mn, 160 mg Fe, 7 mg Cu, 0.2 mg Co, 1 mg I, 0.2 mg Se.
3 According to AOAC, 1990.
index, yolk diameter, yolk height, yolk color, Haugh units and shell thickness, were measured weekly using 8 eggs from each dietary treatment.

In addition, 10 eggs from each replicate totaling 40 eggs from each dietary treatment were used for lipid oxidation studies. Egg collection for this purpose commenced 45 days after feeding the dietary treatments and lasted 2 weeks. All eggs collected during that period, were stored at 4°C, pending further handling.

**Determination of Total Phenolics in Rosemary**

The amount of total phenolics in rosemary was determined with the Folin-Ciocalteau reagent according to a slightly modified method (Singleton et al., 1999). Ground rosemary (0.5 g) was extracted with 25 ml 80% aqueous methanol using an Ultra-Turrax homogenizer at high speed for 30 s. The homogenate was filtered and 0.05 ml from the filtrate was mixed with 6.45 ml distilled water and 0.5 ml of the Folin-Ciocalteau reagent. The mixture was allowed to stand for 3 min, and then, 3 ml of a 7.5% aqueous Na₂CO₃ solution was added. The absorbance of the mixture was measured at 760 nm after 60 min of incubation at room temperature and centrifugation at 2000 g for 5 min. The amount of total phenolics was expressed as gallic acid equivalents/g herb, using a gallic acid calibration curve.

**Lipid Oxidation Studies**

To investigate the effect of diet on lipid oxidation of shell eggs during refrigerated storage, 4 freshly collected eggs from each subgroup totaling 16 eggs from each dietary treatment were placed in a refrigerated cabinet at 4°C to be analyzed for malondialdehyde (MDA) levels in yolk in sets of 4 eggs at 0, 20, 40 and 60 days of storage. To investigate further the effect of diet on the oxidative stability of liquid yolk, 6 eggs from each subgroup totalling 24 eggs from each dietary treatment were broken, yolks were separated, and adhering albumen was removed by rolling on a paper towel. Yolk pools were prepared from each subgroup, and the contents were mixed with a wire whisk.

For studying the effect of diet on the oxidative stability of liquid yolk (Botsoglou et al., 1997), two 9-g portions were taken from each pool and transferred into a 100-ml flask where 16 ml of water were also added. The pH of flask content was left at the value of 6.2. Water addition was indispensable for decreasing yolk viscosity and providing rapid dispersion of 50 μl of sodium azide (Sigma-Aldrich Co., St. Louis, MO) solution (60 mg/ml) added thereafter to prevent microbial growth. All flasks were then covered with air-permeable film to retain moisture yet not to exclude air, and submitted to medium agitation on a temperature-controlled shaker bath (GFL, Gmbh, Hannover, Germany) at 20°C in daylight. For ensuring even exposure of samples to air during the 15-days period that lasted the agitation, all flasks were inspected daily for film cracking. The first day of agitation, and at time intervals of 5 days thereafter, 2-g samples were removed from each flask and directly analyzed for MDA concentration.

**Determination of MDA in Yolk**

Lipid oxidation was assessed on the basis of the MDA formed during refrigerated storage. MDA, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method (Botsoglou et al., 1994). In brief, yolk samples were homogenized (Polytron homogenizer, PCU, Switzerland) to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, Japan) in the range of 400-650 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of MDA in analyzed samples (ng/g yolk) was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane tetraethoxypropane (Sigma Chemical, Co., St. Louis, MO), the precursor of MDA.

**Statistical Analysis**

All data were computerized using the SPSS 12.00 statistical package (SPSS Ltd., Woking, Surrey, UK). Before statistical analysis, the Levene’s test was applied to test the homogeneity of the variances.
Both performance and lipid oxidation data were analyzed by analysis of variance using the completely randomized design. When the effect of factors was significant, the Tukey’s test was applied to test the statistical significance of data at the probability level of $P < 0.05$.

**Results**

The effect of the dietary treatment on final body weight, egg production, feed consumption and feed conversion ratio values, are presented in Table 2. None of these performance parameters was significantly ($P > 0.05$) changed with the dietary treatment.

The effect of dietary ground rosemary and $\alpha$-tocopheryl acetate on some egg quality characteristics is shown in Table 3. There were no significant ($P > 0.05$) differences in egg weight, egg shape, yolk diameter and height, yolk color, Haugh units, and shell thickness, among the dietary treatments.

The effect of dietary treatments on lipid oxidation of eggs refrigerated stored for 60 days is shown in Figure 1. The extent of lipid oxidation, as measured by MDA formation, differed ($P < 0.05$) between the dietary treatments but did not change with the storage time. The ROS-10 group exhibited MDA values lower ($P < 0.05$) than the ROS-5 group, which in turn presented MDA values lower ($P < 0.05$) than the CONT group. The VIT-E group exhibited the lowest ($P < 0.05$) MDA values among the treatments.

The effect of dietary treatments on the oxidative stability of yolks agitated for 15 days in presence of light was also investigated. Figure 2 shows the susceptibility of yolks to lipid oxidation during agitation as a function of the dietary supplementation...
Lipid oxidation in liquid yolks at pH 6.2 from control chickens (CONT), and chickens dietary supplemented with 0.5% (ROS-5) or 1% (ROS-10) rosemary, or 200 mg/kg α-tocopheryl acetate (VIT-E). All data points represent mean MDA values of four samples and their standard deviations, some of which however lie within the data points.

Fig. 2. Lipid oxidation in liquid yolks at pH 6.2 from control chickens (CONT), and chickens dietary supplemented with 0.5% (ROS-5) or 1% (ROS-10) rosemary, or 200 mg/kg α-tocopheryl acetate (VIT-E). All data points represent mean MDA values of four samples and their standard deviations, some of which however lie within the data points.

with ground rosemary or α-tocopheryl acetate. The extent of lipid oxidation, as measured by MDA formation, differed (P < 0.05) between the dietary treatments at all time points. Yolks from the ROS-5 group presented mean MDA values that were higher (P < 0.05) than those of the ROS-10 group, but lower (P < 0.05) than those of the CONT group. Determination of total phenolics in the rosemary used in the present study showed that it contained an equivalent of 29 mg gallic acid/g of ground herb. The VIT-E treatment presented MDA values that were the lowest (P < 0.05) among all other treatments at all time points.

Discussion

We are reporting that neither rosemary nor α-tocopherol feeding could influence body weight, egg production, feed consumption and feed conversion ratio values. These findings agree well with previous works as Jiang et al. (1994), and Qi and Sim (1998) showed no significant differences in egg production and feed consumption when laying hens were fed a basal diet supplemented with up to 200 mg α-tocopheryl acetate/kg. However, Jiang et al. (1994) reported a decreased feed consumption when hens were fed 400 mg α-tocopheryl acetate/kg, whereas Qi and Sim (1998) did not notice such an effect even when hens were fed a basal diet supplemented with up to 800 mg α-tocopheryl acetate/kg. As far as the effect of dietary supplementation with ground rosemary on layers performance is concerned, there were no other pertinent studies in literature to compare with.

We are also reporting that there were no significant (P > 0.05) differences in egg weight, egg shape, yolk diameter and height, yolk color, Haugh units, and shell thickness, among the dietary treatments. Previous reports on α-tocopheryl acetate use in hen diets at the level of 200 mg/kg showed no significant effect on egg weight, yolk weight and Haugh units (Jiang et al., 1994; Qi and Sim, 1998). Previous reports on the effect of dietary ground rosemary on egg quality characteristics could not be found in literature.

The MDA found in the yolk of fresh eggs refrigerated stored for 60 days might be due to either the consumption and subsequent deposition of MDA that was already present in the diets or to in vivo production of MDA by the hens fed the diets. The former possibility appears unlikely because in that case the levels of MDA in yolks should have been equal among the groups. However, MDA analysis revealed not differing (P > 0.05) values among diets. The latter one seems to reasonably explain the lower values of MDA found in eggs from the hens fed rosemary or α-tocopherol as compared to controls. Possible transfer of the antioxidant constituents of rosemary or α-tocopherol into hen organism through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk. Determination of total phenolics in the rosemary used in the present study showed that it contained an equivalent of 29 mg gallic acid/g of ground herb.

Consistently with these results, other workers (Marshall et al., 1994; Aymond and Van Elswyk, 1995) also reported that MDA was found in fresh eggs although it could not be produced during shell egg storage; however, much higher MDA concentrations (180 ng/g) were reported than those found in the present study. This discrepancy is most likely to reflect a difference in the methods applied to detect lipid oxidation. The extent of lipid oxidation is commonly determined by an assay based on reaction between 2-thiobarbituric acid (TBA) and MDA during heating at acidic pH. Experienced investigators have cautioned that this assay may give misleading results due to the contribution of other compounds in addition to the TBA-MDA complex formed and, hence, the term “thiobarbituric acid reactive..."
substances” (TBARS) is frequently used (Tarladgis et al., 1964). TBA reactivity can be influenced by several factors including the formation of MDA as an artifact in the analysis during the assay itself and the occurrence of MDA in various bound forms (Raharjo et al., 1993). The derivative spectrophotometric method (Botsoglou et al., 1994) used in the present study substantially improves the reliability of the measurements because the applied third-order derivative spectral analysis of the TBA-MDA complex eliminates potential interferences from other reactive compounds, whereas the sample preparation procedure inhibits lipid oxidation occurring in vitro during the assay itself. When the derivative spectrophotometric method applied in this study was used in a previous research by some of the authors (Botsoglou et al., 1997), the mean level of MDA found for the control eggs (40 ng/g) was close to that (32 ng/g) of the present study.

As far as the antioxidant effect of rosemary on egg yolk is concerned, direct comparison of our results with other studies is difficult since there have been no reports on dietary use on laying hens. However, Botsoglou et al. (1997) have found a protective effect on yolk lipids for eggs refrigerated stored for 60 days when a thyme extract was fed to laying hens. Some authors have also reported that the in vitro antioxidant activity of rosemary extracts was higher or comparable to that of α-tocopherol (Kuzmenko et al., 1999), whereas others lower than α-tocopherol in vitro (Wong et al., 1995) or in vivo (Lopez-Bote et al., 1998).

Since eggs were inherently resistant to oxidative deterioration upon refrigerated storage, additional experiments were carried out in order to evaluate yolk lipid stability under conditions that could promote lipid oxidation. Yolks from the ROS-5 group presented mean MDA values that were higher (P < 0.05) than those of the ROS-10 group, but lower (P < 0.05) than those of the CONT group, a finding suggesting that dietary rosemary exerted a dose dependent antioxidative activity. The VIT-E treatment presented MDA values that were the lowest (P < 0.05) among all other treatments at all time points.

In pertinent studies with a rosemary extract, Galobart et al. (2001) found that the dietary supplementation to laying hens had no effect on lipid oxidation of eggs, using an iron-induced model of yolk lipid oxidation. However, these authors used a much lower supplementation level than those tested in the present study (0.1% rosemary extract versus 0.5–1.0% ground rosemary, respectively). In contrast, Krause and Ternes (2000) observed an improvement of lipid oxidation in egg yolk when carnosic acid, the main antioxidant constituent of rosemary, was used a dietary supplement in laying hens.

Considering that the egg yolks from the rosemary supplemented groups exhibited increased resistance to lipid oxidation compared to control, one could say that antioxidant constituents of rosemary had passed through feeding into the developing yolk, thus providing egg with antioxidant properties. By now, no method is available for the determination of antioxidant constituents of rosemary passed into egg yolk. Additional research is needed towards developing such a method, which could identify and quantify each of the main antioxidant constituents of rosemary deposited into egg yolk.

Recent consumer concerns on the nutritive value of foods have precipitated interest in both the vitamin and the antioxidant composition of eggs. The interest in modifying the level of vitamins and antioxidant substances in eggs now extends beyond production consideration to designing a high-quality food for consumption by health-conscious humans. Results showed that there were no significant differences in egg production, feed consumption, feed conversion ratio and egg quality characteristics. The extent of lipid oxidation in eggs differed (P < 0.05) between the dietary treatments, but did not change with the storage time. In liquid yolks, lipid oxidation was lower (P < 0.05) in the ROS-5 group compared to the CONT group. The ROS-10 group, in turn, exhibited lower (P < 0.05) oxidation rate than the ROS-5 group, a finding suggesting that rosemary exerted a dose dependent antioxidative activity. The VIT-E group presented lower (P < 0.05) lipid oxidation rate compared to all other groups. The present study gives evidence that not only α-tocopherol but antioxidant constituents of rosemary could pass into egg yolk and efficiently prevent lipid oxidation.

References

AOAC Official Methods of Analysis of the Association of Official Analytical Chemists (Herlich K ed.). A.O.A.C.
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