The potency of α- and γ-tocopherol, and their combination, in reducing dietary induced oxidative stress in vivo and improving meat lipid stability in broilers

Potential von α- und γ-Tocopherol und deren Kombination zur Verminderung von über das Futter ausgelöstem oxidativem Stress in vivo und zur Verbesserung der Stabilität von Fleischlipiden beim Broiler

Urška Tomažin, Tamara Frankič, Mojca Voljč, Vida Rezar, Alenka Levart and J. Salobir

Manuscript received: 23 November 2012, accepted 12 January 2013

Introduction

Vitamin E is a generic term for a family of eight natural, structurally related compounds: α-, β-, γ- and δ-tocopherols and tocotrienols. Of all the compounds that possess vitamin E activity, α- and γ-tocopherols are the principal ones found in human and animal diets and constitute most of the vitamin E content of tissues (TOMASCH et al., 2001). α-Tocopherol is a highly efficient antioxidant that reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation, and intercepts the chain reaction (BURTON and INGOLD, 1981). It is highly efficient because of its preferential retention in the organism and its low metabolic degradation. Although all forms are absorbed in the intestines without discrimination, in the liver only α-tocopherol is singled out by the α-tocopherol transfer protein for incorporation into VLDL and subsequent distribution to peripheral tissues (WAGNER et al., 2004). The activity of γ-tocopherol relative to α-tocopherol was investigated in an in vivo study from BIERI and POUKKA EVARTS (1974). γ-Tocopherol was shown to be 10% as effective as α-tocopherol in the prevention of liver necrosis or hemorrhage in rats and in the prevention of exudative diathesis and muscle dystrophy in chicks. However, its role in the prevention of oxidative stress in chicken has not been well described. Even though γ-tocopherol is not retained and distributed in the body in the same way as α-tocopherol, its biological effects in other species then poultry, especially the detoxification of reactive nitrogen species (MORTON et al., 2002), its anti-inflammatory activity (JIANG and AMES, 2003) and ability to reduce the risks of cardiovascular diseases (SALDEEN et al., 1999) and cancer (BARVE et al., 2009) have been shown. In addition, its performance in protecting foods is proven to be superior to that of α-tocopherol, thus making it responsible for increased food stability and thereby reducing the intake of free radicals in the human diet (WAGNER et al., 2004). Importantly, it has also been shown that the efficiency of the combination of α- and γ-tocopherol is superior to that of either tocopherol alone, i.e. in preventing platelet aggregation (LIU et al., 2003) and reducing biomarkers of oxidative stress and inflammation in patients with metabolic syndrome (DEVARAJ et al., 2008). Despite the fact that research on γ-tocopherol in poultry is scarce, the reduced levels of α- and γ-tocopherol in the liver and lungs of chickens suffering from pulmonary hypertension syndrome indicate its role in antioxidant protection and metabolic disorders (BOTTEJ et al., 1995).

Vitamin E protects animals from various diseases and affects the quality of meat and meat products. It is especially important in the prevention of oxidative stress caused by feeding animals high amounts of polyunsaturated fatty acids (PUFA) (GRAY et al., 1996). These are used in order to create functional food by improving the fatty acid composition of meat, but such products are susceptible to lipid oxidation and can have a reduced shelf life (VOLJČ et al., 2011). The addition of α-tocopherol protects chicken meat and meat products from lipid deterioration and prolongs their stability (NAM et al., 1997), while the role of γ-tocopherol in this respect is in poultry and also in other species unknown.

The fact that γ-tocopherol is the most abundant form in some crops used in poultry nutrition (corn, soy) and because its performance is in poultry poorly studied, encouraged us to investigate if, and to what extent, this form of vitamin E contributes to the prevention of oxidative stress when fed either alone or together with the α-homologue. Assuming its efficiency, γ-tocopherol present in feed could be considered in meeting vitamin E requirements, consequently enabling to lower the amount of α-tocopherol added to the feed.

The aim of our study was to investigate the effects of α- and γ-tocopherol, and their combination, on the prevention of oxidative stress in vivo, the retention of both tocopherols in breast and thigh muscle and the oxidative stability of these muscles under different storage conditions in broilers exposed to high oxidative stress. Oxidative stress was induced by the diets high in vitamin E-free linseed oil.

Materials and methods

The experiment protocol was approved by the Animal Ethics Committee of Veterinary Administration of the Republic of Slovenia and performed in the experimental facilities of the Animal Science Department of the Biotechnical Faculty, University of Ljubljana.
Animals and dietary treatments

Forty-six one-day-old male broiler chickens ROSS 308 were randomly divided into five groups and were housed in floor pens on litter at a temperature of 30°C (gradually decreasing as the animals grew) in conditions consisting of 16 h of light and 8 h of dark for 30 days. The diets were formulated according to broiler nutrition specifications for Ross 308 (AVIANET, 2007). The negative control (Cont-, n = 10) received a diet with 5% palm fat, rich in saturated fatty acids (SFA). Four groups received feed with 5% linseed oil rich in n-3 PUFA. Group Cont+ was used in order to evaluate the extent of oxidative stress caused by feeding linseed oil instead of palm oil. The positive control (Cont+, n = 10) was used as a reference to evaluate the effect of possible vitamin E deficiency for normal development. The remaining three groups were supplemented with either 67 mg/kg of RRR-α-tocopherol (TOCa, n = 10), which represents 100 IU of vitamin E, 67 mg/kg of RRR-γ-tocopherol (TOCy, n = 8) or their combination – 33.5 mg/kg of RRR-α-tocopherol plus 33.5 mg/kg of RRR-γ-tocopherol (TOCaγ, n = 8). Feed mixtures were prepared fresh every 10 days at our department’s feed mill, stored at 0°C and thawed on the day of feeding. Samples of every batch of feed mixed for each group were obtained for chemical analysis, the determination of fatty acid composition and vitamin E-deficiency for normal development. The remaining three groups were supplemented with either 67 mg/kg of RRR-α-tocopherol (TOCa, n = 10), which represents 100 IU of vitamin E, 67 mg/kg of RRR-γ-tocopherol (TOCy, n = 8) or their combination – 33.5 mg/kg of RRR-α-tocopherol plus 33.5 mg/kg of RRR-γ-tocopherol (TOCaγ, n = 8). Feed mixtures were prepared fresh every 10 days at our department’s feed mill, stored at 0°C and thawed on the day of feeding. Samples of every batch of feed mixed for each group were obtained for chemical analysis, the determination of fatty acid composition and α- and γ-tocopherol content (Tables 1, 2). The chemical analyses of feed mixtures were conducted according to official VDLUFA methods (NAUMANN and BASSLER, 1997) (Table 2). Vitamin E from linseed oil was removed by means of a deodorization process for the purpose of knowing and maintaining its exact concentrations. The oil was mixed with 15% diatomaceous solum, shaken for 3 hours, centrifuged for 5 min and stored at 0°C. The exact amounts of RRR-α-tocopherol (T3634, Sigma-Aldrich, Seelze, Germany) and RRR-γ-tocopherol (T1782, Sigma-Aldrich, Seelze, Germany) were dissolved in linseed oil and then added to the feed.

Water and feed were provided ad libitum. Live weight gain and feed consumption were recorded weekly and just before sacrifice. After 30 days of treatment, the animals were sacrificed by cervical dislocation and exsanguination. Whole blood, thigh and breast muscles were collected for analyses.

Sample collection

On day 20 of the experiment, 2 ml of blood were collected from the wing vein into EDTA tubes for the determination of DNA fragmentation in blood lymphocytes with Comet Assay. Blood samples were kept in dark and on ice for a maximum of 1 hour prior to the cell isolation procedure. At the end of the experiment blood samples for the determination of malondialdehyde (MDA), ferric reducing capacity (FRAP) and vitamin E were collected into EDTA tubes, while samples for measuring the antioxidant capacity of the lipid soluble compounds (ACL) were collected into Li heparin tubes. Plasma was obtained by centrifugation, transferred to Eppendorf tubes and stored at –70°C. Individual chicken breast and thigh muscles were sampled for fatty acid composition and α- and γ-tocopherol concentration. Malondialdehyde was determined in both muscles, which were either fresh, stored at 4°C for 6 days or frozen at –20°C for 2 and 4 months.

Lymphocyte nuclear DNA fragmentation (Comet Assay)

Lymphocytes were isolated from fresh blood samples as described in detail in Volč et al. (2011), in accordance with a modified procedure as used by Singh (1997). The Comet Assay (Single cell gel electrophoresis) was performed in line with Singh et al. (1998), with slight modifications as described by Rezar et al. (2003). An Olympus CH 50 epifluorescent microscope (Olympus, Tokyo, Japan) at 200× magnification was used to examine the lymphocyte nuclei (100-W Hg lamp, excitation filter of 480–550 nm and barrier filter of 590 nm). The images were captured by a Hamamatsu Orca 1 CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan). The nuclear DNA damage was estimated using Comet 5 computer software (Single Cell Gel Electrophoresis, Kineting Imaging Ltd., Liverpool, UK). The results are presented as the percentage of DNA in the tail of the comet and as the Olive tail moment (OTM). It is calculated as the product of the tail length and the fraction of total DNA in the tail (Oliva et al., 1992).

Table 1. Feed composition and vitamin E supplementation of finisher diets

<table>
<thead>
<tr>
<th>Composition of feed mixtures (g/kg)</th>
<th>Cont–</th>
<th>Cont+</th>
<th>TOCa</th>
<th>TOCy</th>
<th>TOCaγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>658</td>
<td>658</td>
<td>658</td>
<td>658</td>
<td>658</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>252</td>
<td>252</td>
<td>252</td>
<td>252</td>
<td>252</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>/</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Palm fat</td>
<td>50</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Mineral – vitamin – amino acid supplement 1</td>
<td>40.1</td>
<td>40.1</td>
<td>40.1</td>
<td>40.1</td>
<td>40.1</td>
</tr>
<tr>
<td>Vitamin E supplementation (mg/kg):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopheryl acetate</td>
<td>10</td>
<td>10</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>RRR-α-tocopherol</td>
<td>/</td>
<td>/</td>
<td>67.0</td>
<td>33.5</td>
<td>33.5</td>
</tr>
<tr>
<td>RRR-γ-tocopherol</td>
<td>/</td>
<td>/</td>
<td>67.0</td>
<td>33.5</td>
<td>33.5</td>
</tr>
</tbody>
</table>

1 Calculated to meet the mineral and vitamin requirements for the Ross 308: NaCl (3.2 g), CaCO3 (13.3 g), MCAp (13.7 g), L-lysine 78.8% (1.5 g), DL-methionine 98% (2.3 g), threonine 98% (0.5 g), Cu (16 mg), I (1.25 mg), Fe (40 mg), Mn (120 mg), Se (0.3 mg), Zn (100 mg); vitamins: A (10,000 IU), D3 (5,000 IU), K (3 mg), biotin H (0.2 mg), choline (1,500 mg), folic acid (1.75 mg), B(12 mg), B2 (6 mg), B3 (55 mg), B5 (13 mg), B6 (4 mg), B12 (0.02 mg), monesin sodium – coccidiostatic (0.6 g).
Antioxidant capacity of the lipid soluble compounds (ACL) and FRAP in plasma

The antioxidant capacity of the lipid-soluble compounds in blood plasma was measured using a photochemiluminescence method by PhotoChem® (Analytik Jena, Jena, Germany) and is presented as Trolox equivalent. This procedure ran as follows: 200 μl of plasma and 200 μl of methanol (Sigma-Aldrich Chemie GmbH, Munich, Germany) were mixed on a vortex and centrifuged (15000×g, 10 min, 4°C). The supernatants were analysed in accordance with ACL-Kit protocol (Analytik Jena, Jena, Germany). The FRAP was performed following the procedure used by BENZIE and STRAIN (1996). The kinetics of reduction of the Fe3+ to Fe2+ was measured spectrophotometrically ( Cary 50 UV-Visible Spectrophotometer, Agilent, Santa Clara, CA, USA) in an acidic medium at a temperature of 37°C, wavelength 593 nm and at the end point of 4.2 min. FeSO4 ×7 H2O was used as a standard.

Determination of vitamin E (α-, β-, γ- and δ- tocopherol)

Concentrations of vitamin E in feed, plasma and meat were measured in accordance with the methodology of ABDI and MOUNTS (1997) and RÜPÉREZ et al. (2001). Put briefly, the samples were treated with ethanol and the tocopherols were extracted from the samples using hexane. Hexane phases were transferred into fresh tubes and carefully evaporated under a gentle stream of nitrogen; the residues were subsequently dissolved in ethanol. The samples were transferred to vials and analysed by a reverse-phase HPLC, using a Luna 5u PFP(2) column (100A 250 × 4.6 mm; Phenomenex Inc., Torrance, CA, USA). The mobile phase rate was 1.2 ml/min and it consisted of 95% methanol and 5% water. The results of the analysis were evaluated using the Waters Millenium32 Chromatography Manager (Waters, Milford, MA, USA) program. Only α- and γ-tocopherol were of interest in the results.

Malondialdehyde determination

The methodology of WONG et al. (1987), as modified by CHIRICO (1994) and FUKUNAGA et al. (1995), was implemented to determine the concentrations of MDA in blood plasma and a modified protocol of the methodology of VILÀ et al. (2002) was used to determine the MDA concentrations in meat samples (breast and thigh). Put briefly, meat samples were homogenized (Grindomix homogenizer, Retsch GmbH & Co, Haan, Germany), 0.3 g of the sample was mixed with 1.5 ml of 2.5% trichloroacetic acid, left for ten minutes and centrifuged (15000×g, 15 min, 4°C). The supernatant (1 ml) was mixed with 1.5 ml of 0.6% thiobarbituric acid and heated at 90°C for 60 minutes. After cooling, the samples were filtered through Millipore filters.

Table 2. Basic analysis of feed mixtures, vitamin E concentration and fatty acid composition in finisher diets

<table>
<thead>
<tr>
<th>Chemical analyses of feed mixtures (g/kg)</th>
<th>Cont−</th>
<th>Cont+</th>
<th>TOCα</th>
<th>TOCγ</th>
<th>TOCαγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>860</td>
<td>891</td>
<td>888</td>
<td>889</td>
<td>889</td>
</tr>
<tr>
<td>Crude protein</td>
<td>188</td>
<td>194</td>
<td>198</td>
<td>198</td>
<td>196</td>
</tr>
<tr>
<td>Crude fat</td>
<td>63.2</td>
<td>60.6</td>
<td>62.4</td>
<td>61.7</td>
<td>61.9</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>33.1</td>
<td>32.3</td>
<td>33.7</td>
<td>35.7</td>
<td>34.2</td>
</tr>
<tr>
<td>Crude ash</td>
<td>51.9</td>
<td>53.5</td>
<td>50.2</td>
<td>51.5</td>
<td>50.9</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>525</td>
<td>550</td>
<td>544</td>
<td>543</td>
<td>546</td>
</tr>
<tr>
<td>Vitamin E concentration (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>16.9</td>
<td>8.48</td>
<td>73.8</td>
<td>6.84</td>
<td>39.9</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>10.2</td>
<td>4.18</td>
<td>4.47</td>
<td>58.8</td>
<td>35.6</td>
</tr>
</tbody>
</table>

The predominant fatty acids (wt % of total fatty acids)

| C16:0 | 37.7 | 8.92 | 8.69 | 8.77 | 8.74 |
| C18:0 | 42.7 | 3.96 | 3.79 | 3.77 | 3.77 |
| C18:1 | 3.63 | 18.9 | 19.0 | 19.0 | 19.0 |
| C18:2 ω-6 | 12.5 | 26.5 | 26.3 | 26.5 | 26.5 |
| C18:3 ω-3 | 1.29 | 40.5 | 41.0 | 40.8 | 40.8 |
| Σ SFA | 82.4 | 13.6 | 13.2 | 13.2 | 13.2 |
| Σ MUFA | 3.81 | 19.3 | 19.5 | 19.4 | 19.5 |
| Σ PUFA | 13.8 | 67.1 | 67.4 | 67.4 | 67.3 |
| n-3 PUFA | 1.29 | 40.5 | 41.0 | 40.8 | 40.8 |
| n-6 PUFA | 12.5 | 26.5 | 26.3 | 26.6 | 26.5 |
| n-6/n-3 PUFA | 9.70 | 0.654 | 0.641 | 0.652 | 0.650 |

Values are the means of 10 (Cont+, Cont−, TOCα) or 8 (TOCγ, TOCαγ) animals per group. Cont− = low oxidation; 5% of palm fat high in saturated fatty acids + 10 mg/kg α-tocopherol acetate; Cont+ = high oxidation; 5% of linseed oil high in polyunsaturated fatty acids (PUFA) + 10 mg/kg α-tocopherol acetate; TOCα = high oxidation; 5% of linseed oil high in PUFA + 67 mg/kg RRR-α-tocopherol; TOCγ = high oxidation; 5% of linseed oil high in PUFA + 33.5 mg/kg RRR-γ-tocopherol + 33.5 mg/kg RRR-γ-tocopherol. Only predominant fatty acids are listed, but the sums of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are computed from all fatty acids analysed.
Results

Body weight gain did not differ among treatment groups, but feed consumption of the group fed palm oil was higher than of the groups fed linseed oil. Feed efficiency in Cont– was consequently worse than in other treatments (Table 3).

### Fatty acid composition

Fatty acids in feed and meat samples were transmethylated in situ, in accordance with PARK and GOINS (1994), and FAMEs (fatty acid methyl esters) were extracted using hexane. Prior to the esterification of samples, the C19:0 was added as an internal standard. For FAME separation, an Agilent 6890 GC (Agilent, Santa Clara, CA, USA), equipped with Omegawax 320 (30 m × 0.32 mm i.d. × 0.25 μm, Supelco Bellefonte, PA, USA) and flame-ionization detector was used. Analytical results are expressed in wt % of total fatty acids.

### Statistical analysis

The data were analysed using the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA). For statistical analysis of fatty acid percentages, the raw data were transformed with arcus sinus of the square root for normalization. For the analysis of MDA in breast and thigh samples stored under different storage conditions, the MIXED procedure, with group and storage duration, and the associated interaction, as fixed and animal as a random effect, was used. The analyses of all other parameters were performed by the one-way ANOVA, using the General Linear Models (GLM) procedure, with group as the main effect. Differences between groups were determined using orthogonal contrasts. Significance was considered to be established at P < 0.05.

### Results

The extent of DNA fragmentation in blood lymphocytes presented as Olive Tail Moment (OTM) showed lower values in Cont– and in all three groups receiving vitamin E supplementation in comparison with Cont+, which demonstrates less DNA damage (Table 4). Results presented as a percentage of the tail DNA showed the same pattern but the differences were not significant.

The substitution of palm fat with linseed oil in the Cont+ group resulted in a 2.6-fold increase in plasma MDA concentration. The addition of α-tocopherol alone and its half amount in combination with γ-tocopherol lowered the MDA concentration, but not to the level of Cont–. γ-Tocopherol alone showed no such an effect (Table 4).

The composition of plasma ACL, but it increased in group TOCγ by 31%, relative to Cont+. A similar trend was shown in the TOCαγ group, while γ-tocopherol had no effect on ACL. The same trend as for ACL was observed for FRAP (Table 4).

### α- and γ-tocopherol determination in plasma, breast and thigh

The plasma α- and γ-tocopherol concentrations reflected the supplementation regimen, but the retention of α-tocopherol was much higher than that of γ-tocopherol (Table 5). The concentration of plasma γ-tocopherol in group TOCαγ was the same as in group TOCγ. Group Cont–, which received palm fat instead of linseed oil, had higher concentrations of γ-tocopherol than Cont+. As concerns the muscles, a similar trend to that noted in plasma α-tocopherol concentration was observed. The concentration of both tocopherols was higher in thigh muscles than in those of the breast (Table 5). With respect to γ-tocopherol, the difference in breast and thigh concentration was observed between the groups supplemented with γ-tocopherol. Group TOCγ had higher concentrations than TOCαγ (Table 5).

### Fatty acid profile of breast and thigh muscle

The fatty acid profile of both muscles reflected the composition of the diet (Table 6). There were no differences in fatty acid proportions among linseed oil fed groups in the breast muscle, but proportions of n-3 very long-chain
Lipid stability of meat

The lipid stability of breast and thigh muscles stored under different conditions was evaluated by MDA determination. Divergences between control groups that had been fed different sources of fat were observed in both muscles. In most cases, α-tocopherol reduced MDA, in contrast to γ-tocopherol, which was not efficient. The efficiency of the combination was lower as α-tocopherol’s, but higher than of γ-tocopherol except for the fresh meat. In breast, there was an interaction found between the treatment group and the storage (Table 7).

Table 5. Effect of dietary α- and γ-tocopherol on concentrations of α- and γ-tocopherol in plasma, breast and thigh

<table>
<thead>
<tr>
<th></th>
<th>Cont–</th>
<th>Cont+</th>
<th>TOCα</th>
<th>TOCγ</th>
<th>TOCγ</th>
<th>p-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (μg/ml)</td>
<td>8.19a</td>
<td>5.79a</td>
<td>39.9c</td>
<td>3.62a</td>
<td>19.9b</td>
<td>&lt; 0.001</td>
<td>1.80</td>
</tr>
<tr>
<td>γ-tocopherol (μg/ml)</td>
<td>0.501b</td>
<td>0.290a</td>
<td>0.314a</td>
<td>1.05c</td>
<td>1.07c</td>
<td>&lt; 0.001</td>
<td>0.062</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (μg/g)</td>
<td>3.06a</td>
<td>3.50a</td>
<td>14.92c</td>
<td>1.95a</td>
<td>7.38b</td>
<td>&lt; 0.001</td>
<td>0.67</td>
</tr>
<tr>
<td>γ-tocopherol (μg/g)</td>
<td>0.241a</td>
<td>0.205a</td>
<td>0.258b</td>
<td>1.00c</td>
<td>0.848b</td>
<td>&lt; 0.001</td>
<td>0.028</td>
</tr>
<tr>
<td>Thigh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (μg/g)</td>
<td>9.94a</td>
<td>13.48a</td>
<td>48.89c</td>
<td>6.79a</td>
<td>26.29b</td>
<td>&lt; 0.001</td>
<td>2.33</td>
</tr>
<tr>
<td>γ-tocopherol (μg/g)</td>
<td>0.841a</td>
<td>0.925a</td>
<td>0.875c</td>
<td>3.46c</td>
<td>3.06b</td>
<td>&lt; 0.001</td>
<td>0.125</td>
</tr>
</tbody>
</table>

abc Least square means within a line with unlike superscripts differ significantly (P < 0.05).

PUFA (C20:5n-3 – EPA and C22:6n-3 – DHA) in the thigh muscle were higher in the group supplemented with γ-tocopherol than in Cont+.

Lipid stability of meat

The lipid stability of breast and thigh muscles stored under different conditions was evaluated by MDA determination. Divergences between control groups that had been fed different sources of fat were observed in both muscles. In most cases, α-tocopherol reduced MDA, in contrast to γ-tocopherol, which was not efficient. The efficiency of the combination was lower as α-tocopherol’s, but higher than of γ-tocopherol except for the fresh meat. In breast, there was an interaction found between the treatment group and the storage (Table 7).

Discussion

A trend to produce functional foods of animal origin by appropriate changes in animal nutrition involves increasing or changing the level of some unsaturated fatty acids in feed in order to produce animal products with a functional fatty acid composition. According to LAURIDSEN et al. (1997) PUFAs are also more effectively absorbed than the SFA and in our case the chickens from Cont– group were able to compensate it by consuming larger amounts of feed. Because consuming high level of PUFA increases the risk of oxidative stress in the organism (LAURIDSEN and HØJSGAARD, 1999), various antioxidants are added to the feed. Vitamin E in the form of α-tocopherol is one of the most commonly used, while γ-tocopherol is not considered into calculations.
The inclusion of linseed oil increased the oxidation of body lipids as shown by higher MDA concentrations and also by the lymphocyte DNA damage. When presented as OTM, all three vitamin E supplemented groups showed the same extent of DNA damage as in Cont–. When presented as the percentage of tail DNA the differences were not significant. Nevertheless, OTM is considered to be a better indicator of oxidative stress than the percentage of DNA in head or tail (OLIVE et al., 1992). The lower DNA fragmentation in group TOC– in accordance with improved prevention of oxidative stress measured as MDA, and also higher antioxidative capacity of the blood. The same amount of γ-tocopherol showed no such link; MDA, ACL and FRAP are not supporting the reduced degree of DNA damage. This could be attributed to the higher retention of γ-tocopherol in the lymphocytes than in other tissues. JIANG and AMES’ study in rats (2003) showed a role of γ-tocopherol in the inhibition of inflammation and its 20-fold increase in an exudate fluid from the site of inflammation relative to the serum of rats treated with 100 mg/kg α-tocopherol. The combination of both tocopherols also showed lower plasma MDA, but ACL and FRAP values were not as high as in group TOCα. Combining these results with concentrations of α-tocopherol, ACL and FRAP are most likely reflecting the amounts of α-tocopherol in plasma. α-Tocopherol is effective in the prevention of oxidative stress and its superior performance can be attributed to its broader spectrum of action and thus its higher biological value. According to BEHRENS and MADÈRE (1983) the mechanisms regulating absorption, transport and tissue uptake are so specific for α-tocopherol that a relatively small amount of it is sufficient to displace γ-tocopherol. In their study, an increased plasma level of γ-tocopherol was found only when the level of dietary α-tocopherol was low. The results of our study are similar, plasma and muscle γ-tocopherol concentrations were very low when compared to α-tocopherol. In all treatment groups, the accumulation of both tocopherols was greater in thigh than in breast muscle, an observation similar to that already described in a study by LANARI et al. (2004). This observation could be explained by the difference in oxidative capacity and fat content between both muscle types. The concentrations of α-tocopherol in plasma and both muscles reflected the amount of α-tocopherol present in feed. Surprisingly, the concentration of plasma and muscle α-tocopherol in group TOCγ was higher than the concentration of γ-tocopherol. We did not expect such results since group TOCγ hasn’t been provided with α-tocopherol, and vitamin E free (purified) linseed oil was used. However, a similar observation was presented by CLEMENT and BOURRE (1997) where α-tocopherol...
TOC

γ

TOC

γ

TOC

γ

Table 7. Effect of dietary α- and γ-tocopherol on malondialdehyde concentration (nmol/g) in breast and thigh muscle under different storage conditions

<table>
<thead>
<tr>
<th></th>
<th>Cont–</th>
<th>Cont+</th>
<th>TOCα</th>
<th>TOCγ</th>
<th>TOCαγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>0.339a</td>
<td>2.74b</td>
<td>0.775ab</td>
<td>2.17ab,x</td>
<td>1.32ab</td>
</tr>
<tr>
<td>6 days</td>
<td>0.403a</td>
<td>3.22c</td>
<td>1.38ab</td>
<td>5.63c,y</td>
<td>1.67ab</td>
</tr>
<tr>
<td>2 months</td>
<td>0.339a</td>
<td>2.52c</td>
<td>1.08ab</td>
<td>2.80h,xz</td>
<td>1.22ab</td>
</tr>
<tr>
<td>4 months</td>
<td>0.536a</td>
<td>3.08b</td>
<td>1.52ab</td>
<td>4.59h,yz</td>
<td>2.60ab</td>
</tr>
</tbody>
</table>

Significance – group < 0.001
Significance – storage < 0.001
Significance – group * storage 0.005
SEM 0.475

Thigh

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.534a</td>
<td>4.73c</td>
<td>x,y</td>
<td>1.61ab,x</td>
<td>4.63c</td>
</tr>
<tr>
<td>6 days</td>
<td>1.14a</td>
<td>3.43bc,x</td>
<td>1.80a,x</td>
<td>5.41c</td>
<td>2.77ab</td>
</tr>
<tr>
<td>2 months</td>
<td>0.923a</td>
<td>4.65b,c,x,xy</td>
<td>2.10ab,xy</td>
<td>6.04c</td>
<td>3.06abc</td>
</tr>
<tr>
<td>4 months</td>
<td>0.783a</td>
<td>7.18b,h,y</td>
<td>2.81b,h,y</td>
<td>8.66b</td>
<td>3.79a</td>
</tr>
</tbody>
</table>

Significance – group < 0.001
Significance – storage < 0.001
Significance – group * storage 0.088
SEM 0.682

abc Least square means within a row with unlike superscripts differ significantly (P < 0.05).
xyz Least square means within a column with unlike superscripts differ significantly (P < 0.05).

Values are the means of 10 (Cont+, Cont–, TOCα) or 8 (TOCγ, TOCαγ) animals per group. Cont– = low oxidation, 5% of palm fat high in saturated fatty acids + 10 mg/kg α-tocopherol acetate; Cont+ = high oxidation, 5% of linseed oil high in polyunsaturated fatty acids (PUFA) + 10 mg/kg α-tocopherol acetate; TOCα = high oxidation, 5% of linseed oil high in PUFA + 67 mg/kg RRR-α-tocopherol; TOCγ = high oxidation, 5% of linseed oil high in PUFA + 33.5 mg/kg RRR-γ-tocopherol; TOCαγ = high oxidation, 5% of linseed oil high in PUFA + 33.5 mg/kg RRR-α- and γ-tocopherol.

Storage: 6 days = 6 days at 4°C; 2 months = 2 months at –20°C; 4 months = 4 months at –20°C.

Conclusions

In the light of all the data presented, it can be concluded that α-tocopherol and the combination of α- and γ-tocopherol in

Arch.Geflügelk. 4/2013

very long-chain n-3 PUFA (EPA, DHA) was greater in breast than in thigh muscles, but thigh muscles from the γ-tocopherol-supplemented group contained greater proportions of these fatty acids than the Cont+ group. γ-Tocopherol did not possess any antioxidant activity as evaluated by MDA measurements, while α-tocopherol was able to reduce lipid oxidation in muscles, although not to the level of the negative control. The combination of both tocopherols was as efficient in the prevention of meat lipid oxidation under most of the storage conditions as α-tocopherol alone, while γ-tocopherol alone was efficient only in fresh breast. Because the groups with half amount of each tocopherol alone were not included in the trial, the synergistic action of both tocopherols can’t be suggested. Though, since α-tocopherol level in TOCαγ group was too low to meet high requirements induced by high PUFA as reviewed by Volić et al. (2011), the same level of oxidative stress in most in vivo parameters and also parameters of lipid stability of meat in TOCαγ can give a hint about possible synergistic action between both isomers also in poultry as it was already established in humans by Tomasch et al. (2001).

Animals that are fed diets rich in unsaturated fats have increased PUFAs in meat. This modification is nutritionally desirable but increases the susceptibility of meat to lipid oxidation. It is a major cause of quality deterioration in meat and meat products (Gray et al., 1996). Besides this, it causes the formation of potentially toxic compounds (such as MDA) that compromise meat quality and reduce its shelf life. Linseed oil contains high levels of α-linolenic acid, consequently reflected in higher PUFAs percentages in breast and thigh muscles. This is in agreement with other studies where the fatty acid composition of chicken muscle reflected those from the dietary oils (Nam et al., 1997). The percentage incorporation of very long-chain n-3 PUFA (EPA, DHA) was greater in breast than in thigh muscles, but thigh muscles from the γ-tocopherol-supplemented group contained greater proportions of these fatty acids than the Cont+ group. γ-Tocopherol did not possess any antioxidant activity as evaluated by MDA measurements, while α-tocopherol was able to reduce lipid oxidation in muscles, although not to the level of the negative control. The combination of both tocopherols was as efficient in the prevention of meat lipid oxidation under most of the storage conditions as α-tocopherol alone, while γ-tocopherol alone was efficient only in fresh breast. Because the groups with half amount of each tocopherol alone were not included in the trial, the synergistic action of both tocopherols can’t be suggested. Though, since α-tocopherol level in TOCαγ group was too low to meet high requirements induced by high PUFA as reviewed by Volić et al. (2011), the same level of oxidative stress in most in vivo parameters and also parameters of lipid stability of meat in TOCαγ can give a hint about possible synergistic action between both isomers also in poultry as it was already established in humans by Tomasch et al. (2001).

Conclusions

In the light of all the data presented, it can be concluded that α-tocopherol and the combination of α- and γ-tocopherol in

Arch.Geflügelk. 4/2013
half concentration are both efficient in the reduction of oxidative stress in vivo and in improving the oxidative stability of meat, while γ-tocopherol alone is efficient only in the reduction of the lymphocyte DNA damage, which has not been well described in poultry and may indicate its role in immune functions. In order to clarify the role of each form and to elucidate a possible synergy between α- and γ-tocopherol a further research with graduate increase in concentrations of both isomers and their combination is needed.

Summary

The objective of this study was to investigate the roles of α- and γ-tocopherol, and their combination, in the prevention of oxidative stress in vivo and on lipid oxidation of meat in broiler chickens. One day-old chicks were included in a 30 day trial and divided into five experimental groups, the negative control (Cont–, n = 10) received 5% palm fat, while other four groups (positive control, Cont+, n = 10; TOCa, n = 10; TOCy, n = 8 and TOCγy, n = 8) received 5% linseed oil that was vitamin E-stripped. Cont + and Cont– were given 10 mg/kg α-tocopheryl acetate to cover basic needs, group TOCa 67 mg/kg RRR-α-tocopherol, group TOCy 67 mg/kg RRR-γ-tocopherol and group TOCγy 33.5 mg/kg each of tocopherols. Oxidative stress in vivo was evaluated by means of a Comet Assay to measure lymphocyte DNA damage, by plasma, breast and thigh malondialdehyde (MDA) formation, and by measuring antioxidant capacity of plasma. The tocopherols in plasma, breast and thigh muscles, the fatty acid composition of both muscles and their lipid stability under different storage conditions (6 days at 4°C, 2 and 4 months at –20°C) were also analysed.

Both forms of vitamin E were able to reduce the lymphocyte DNA damage, while the results of other analyses evaluating oxidative stress indicated that only α-tocopherol and the combination of both tocopherols are effective in its prevention. This seems to be a consequence of α-tocopherol being preferentially retained in the organism. Its concentrations in plasma and muscles reflected the feeding regimen, while γ-tocopherol's concentrations were only slightly elevated in both groups supplemented with γ-tocopherol. The fatty acid composition of the diet was reflected in the fatty acid composition of both muscles. The lipid stability of muscles in groups receiving linseed oil was compromised, but lower MDA values in groups TOCa and TOCγy under some of the storage conditions proved the ability of α-tocopherol and the combination of both tocopherols (TOCγy) to improve it.

Key words

Broilers, α-tocopherol, γ-tocopherol, oxidative stress

Zusammenfassung

Potential von α- und γ-Tocopherol und deren Kombination zur Verminderung von über das Futter ausgeleistem oxidativem Stress in vivo und zur Verbesserung der Stabilität von Fleischlipiden beim Broiler

Das Ziel der Studie war die Untersuchung der Rolle von α- und γ-Tocopherol und ihrer Kombination bei der Verhinderung von oxidativem Stress in vivo und bei der Lipidoxidation bei Broilern. Eintagsküken wurden auf 5 Versuchsgruppen verteilt: negative Kontrolle mit 5% Palmfett (Cont–, n = 10), 4 Versuchsgruppen mit 5% Leinöl, dem Vitamin E entzogen worden war (positive Kontrolle Cont+, n = 10; TOCa, n = 10; TOCy, n = 8; TOCγy, n = 8). Cont + und Cont– wurde 10 mg α-Tocopherol-Acetat/kg zur Deckung des Grundbedarfs zugegeben. Der Futterration TOCa wurde 67 mg RRR-α-Tocopherol/kg, der Ration TOCy 67 mg RRR-γ-Tocopherol/kg und der Ration TOCγy wurde von jeder Tocopherolform 33.5 mg/kg zugegeben. Der oxidative Stress in vivo wurde mittels Comet Assay bestimmt, indem folgende Parameter gemessen wurden: DNA-Schädigung der Lymphozyten, Bildung von Malondialdehyde (MDA) in Plasma, Brust- und Schenkel Muskeln, die Fett säuremuster sowie die Lipidstabilität der beiden Muskeln bei verschiedenen Lagerbedingungen (6 Tage bei 4°C, 2 und 4 Monate bei –20°C) bestimmt.


Stichworte

Broiler, α-Tocopherol, γ-Tocopherol, oxidativer Stress

References

LANARI, M.C., A.K. HEWAVITHARANA, C. BECU, S. DE JONG, 2004: 

DEVARAJ, S., S. LEONARD, M.G. TRABER, I. JIALAL, 2008: 

GRAY, J.I., E.A. GOMAA, D.J. BUCKLEY, 1996: 

LIU, M., A. WALLMON, C. OLSON-MORTLOCK, R. WALLIN, T. SALDEEN, 2003: 

MORTON, L.W., N.C. WARD, K.D. CROFT, I.B. PUDDEY, 2002: 

FUKUNAGA, K., K. TAKAMA, T. SUZUKI, 1995: 

LAURIDSEN, C., D.J. BUCKLEY, P.A. MORRISSEY, 1997: 

NAM, K.-T., H.-A. LEE, B.-S. MIN, C.-W. KANG, 1997: 

NAUMANN, C., R. BASSLER, 1997: 

CHRICO, S., 1994: 

Clémont, M., J.-M. Bourre, 1997: 

Devaraj, S., M.G. Traber, I. Jalal, 2008: 


Correspondence: Janez Salobir, Chair of Nutrition, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia; E-Mail: janez.salobir@gbf.uni-lj.si