Influence of the Dietary Polyunsaturation Level on Chicken Meat Quality: Lipid Oxidation

L. Cortinas,* A. Barroeta,* 1 C. Villaverde,* J. Galobart,* F. Guardiola,† and M. D. Baucells*

*Department of Animal and Food Science, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain; †Department of Nutrition and Food Science-CeRTA, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII, E-08028 Barcelona, Spain

ABSTRACT The present study was carried out to evaluate the influence of increasing amounts of dietary polyunsaturated fatty acids (PUFA) and α-tocopheryl acetate (α-TA) supplementation on lipid oxidation of raw and cooked thigh meat stored under refrigeration. One hundred ninety-two female, 1-d-old, broiler chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary PUFA (15, 34, 45, and 61 g/kg) and 4 levels of supplementation with α-TA (0, 100, 200, and 400 mg/kg). Thiobarbituric acid reactive substance (TBARS) values in cooked meat and cooked refrigerated meat were 12- and 24-fold higher, respectively, than in raw meat. Dietary polyunsaturation and α-TA supplementation affected lipid oxidation more markedly in cooked meat and cooked refrigerated meat than in raw meat and raw refrigerated meat. Lipid oxidation in cooked meat showed a significant linear increase as the concentration of PUFA in raw meat increased. The oxidative stability of meat was not affected by an increase in the dietary α-TA level from 200 to 400 mg/kg. Nonlinear relationship between TBARS values in cooked meat and α-tocopherol content of raw meat showed saturation in the antioxidant effect of α-Toc. The equation y = x (11.88 + 63.38e−0.007z) was calculated to predict the minimum inclusion of α-tocopherol to diets (z) of chickens with certain dietary PUFA content (x) to assure a certain TBARS value (y).

(Key words: lipid oxidation, polyunsaturation, α-tocopherol, thigh and breast meat, cooked meat and meat storage)

INTRODUCTION

There is interest in foods containing higher levels of polyunsaturated fatty acids (PUFA) because of their beneficial effects on human health, mainly in the prevention of cardiovascular disease (Krauss et al., 2001). For this reason, there are several studies concerning the enrichment of chicken meat with PUFA by the addition of polyunsaturated fats to the diet (Lin et al., 1989; Ajuyah et al., 1993; López-Ferrer et al., 1999, 2001). However, chicken meat enriched with PUFA contains longer fatty acids (FA) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life. One such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among all the methods proposed for assessing MDA, the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. In practice, meat is stored and cooked for consumption. These processes of cooking and storage of meat promote degradation of its lipid fraction (Pikul et al., 1984; Lin et al., 1989; Jensen et al., 1997; Ruiz et al., 1999; Grau et al., 2001a,b).

The negative consequences of lipid oxidation can be overcome by the use of antioxidants in the diet, such as α-tocopherol (α-Toc). α-Toc supplementation prevents lipid oxidation and, therefore, increases the shelf life of meat (Lin et al., 1989; Ahn et al., 1995; De Winne and Dirinck, 1996; Bou et al., 2001; Grau et al., 2001a,b). Thus, it is of great commercial interest to assess the protective effect of α-Toc during storage and cooking processes of poultry meat (Ahn et al., 1995; King et al., 1995; Ruiz et al., 1999; Grau et al., 2001a,b). Second, dietary supplementation with α-Toc permits the enrichment of chicken meat in this antioxidant vitamin (Miller and Huang, 1993; O’Neill et al., 1998; Grau et al., 2001a,b; Bou et al., 2004).

Abbreviation key: C = cooked; CF = cooked refrigerated; MDA = malondialdehyde; PUFA = polyunsaturated fatty acids; R = raw; RF = refrigerated raw; α-TA = α-tocopheryl acetate; TBARS = thiobarbituric acid reactive substances; α-Toc = α-tocopherol.
The extent of lipid oxidation in R, RF, C, and CF thighs was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Grau et al. (2000), using third derivative spectrophotometry. The height of the third-order derivative peak that appeared at approximately 521.5 nm was used for calculation of the MDA concentration in the samples. Tetraethoxypropane7 was used as an MDA precursor in the standard curve. TBARS was expressed as micrograms of MDA per kilogram of sample.
**Fatty Acid Content**

Fatty acid content of the feeds was determined by gas chromatography following the method described by Sukhija and Palmquist (1988). Thigh samples were analyzed as described previously by Carrapiso et al. (2000). Nonadecanoic acid6 was used as internal standard. The FA content was determined using a gas chromatograph (HP68906) equipped with a flame ionization detector and an HP capillary column9 (60 m × 0.25 mm i.d.) with a 0.25 μm film thickness of stationary phase. Helium was used as carrier gas. Oven temperature was programmed as follows: increasing from 140 to 160°C at 1.50°C/min; from 160 to 180°C at 0.50°C/min; and from 180 to 230°C at 2.50°C/min. The other chromatographic conditions were injector and detector temperatures, 280°C; and sample volume injected, 1 μL. FA were identified by matching their retention times with those of their relative standards, as well as by mass spectrometry (HP597310) of each peak.

**α-Toc Analysis**

Tocopherol content in feeds and thighs were extracted as previously described by Jensen et al. (1999) starting from 2 g of feed and 100 mg of freeze-dried thigh meat. Chromatographic separation was achieved on a HS-5-Silica column11 (125 × 4 mm). Heptane modified with 2-propanol (99.5:0.5 vol/vol) and degassed with helium constituted the mobile phase. HPLC determination was performed according to the conditions described by Jensen et al. (1999). Fluorescence detection was performed with an excitation wavelength of 290 nm and an emission wavelength of 327 nm.

**Statistics**

Multifactorial ANOVA with repeated measures (n = 384) was performed to determine whether processing, dietary PUFA, and α-TA supplementation affected TBARS values in thigh meat. Data were treated using the proc mixed procedure of SAS software (SAS Institute, 2000). Differences among treatment means were tested using Tukey’s test correction for multiple comparisons. The relationship between TBARS values in cooked thigh meat and PUFA content of raw meat was fitted by linear regression analysis. The comparative response of TBARS values depending on the variation in dietary PUFA content was assessed by the likelihood ratio test (n = 96). The evolution of TBARS values (n = 96) in cooked thigh due to variation in dietary α-Toc and PUFA was fitted by using nonlinear regression by means of the NLIN procedure of SAS software. This data analysis was represented using SigmaPlot 8.02 (2002). In all cases, P ≤ 0.05 was considered significant.

**RESULTS AND DISCUSSION**

The fatty acid content of the experimental diets is shown in Table 2. Fatty acid content of diets and thigh meat were discussed in Cortinas et al. (2004). Supplementation with 0, 100, 200 and 400 mg α-TA/kg of feed resulted in dietary α-Toc content of 6 ± 0.6 g/kg, 136 ± 1.5 g/kg, 236 ± 14.5 g/kg, and 451 ± 18.1 g/kg for E0, E1, E2, and E4 treatments, respectively.

**Lipid Oxidation in Thigh Meat**

The TBARS values in R, RF, C, and CF thigh meat were expressed as micrograms of MDA per kilogram of solids and were compared (Table 3). TBARS values in R meat were low, but during cooking and refrigeration they significantly increased. Thus, lipid oxidation in C and CF thigh meat was 12- and 24-fold higher, respectively, than in R thigh meat. An increase of the lipid oxidation in cooked thigh meat (Ang, 1988; Sheehy et al., 1993; Galvin et al., 1997; Jensen et al., 1997; Maraschakello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b) and cooked thigh meat stored in various conditions for different periods (Pikul et al., 1984; Lin et al., 1989; Adjuyah et al., 1993; Galvin et al., 1997; Jensen et al., 1997; Ruiz et al., 1999) has

---

6Agilent, D-76337 Waldbronn, Germany.
8Perkin Elmer, D-88662 Überlingen, Germany.
9Perkin Elmer, D-88662 Überlingen, Germany.
10HP19091-136 Hewlett Packard, Newtown, PA.
11Perkin Elmer, D-88662 Überlingen, Germany.
TABLE 3. Effect of dietary polyunsaturation, α-tocopheryl acetate (α-TA) supplementation and processing on thiobarbituric acid reactive substances values in thigh meat (µg of malondialdehyde/kg on a dry-matter basis)1

<table>
<thead>
<tr>
<th>Process2</th>
<th>Global Means</th>
<th>PU15</th>
<th>PU34</th>
<th>PU45</th>
<th>PU61</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>338p</td>
<td>88p</td>
<td>159p</td>
<td>287p</td>
<td>816p</td>
</tr>
<tr>
<td>RF</td>
<td>946p</td>
<td>146p</td>
<td>339p</td>
<td>775p</td>
<td>2526p</td>
</tr>
<tr>
<td>C</td>
<td>3870p</td>
<td>1141p</td>
<td>3274p</td>
<td>4644p</td>
<td>6421p</td>
</tr>
<tr>
<td>CF</td>
<td>7939p</td>
<td>4366p</td>
<td>8466p</td>
<td>10126p</td>
<td>8798p</td>
</tr>
<tr>
<td>Global Means</td>
<td></td>
<td>1435</td>
<td>3059</td>
<td>3958</td>
<td>4640</td>
</tr>
</tbody>
</table>

Supplementation with α-TA4

<table>
<thead>
<tr>
<th>E0</th>
<th>E1</th>
<th>E2</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>901p</td>
<td>164p</td>
<td>144.0p</td>
</tr>
<tr>
<td>RF</td>
<td>2654p</td>
<td>682p</td>
<td>251.2p</td>
</tr>
<tr>
<td>C</td>
<td>7860p</td>
<td>3884p</td>
<td>2195.9p</td>
</tr>
<tr>
<td>CF</td>
<td>9298p</td>
<td>9433p</td>
<td>6688.4p</td>
</tr>
<tr>
<td>PU15</td>
<td>2448p</td>
<td>1438p</td>
<td>1081</td>
</tr>
<tr>
<td>PU34</td>
<td>4419p</td>
<td>3980p</td>
<td>1760p</td>
</tr>
<tr>
<td>PU45</td>
<td>5653p</td>
<td>3736p</td>
<td>3322p</td>
</tr>
<tr>
<td>PU61</td>
<td>8153p</td>
<td>5009p</td>
<td>3116p</td>
</tr>
<tr>
<td>Global Means</td>
<td>5168p</td>
<td>3540p</td>
<td>2320p</td>
</tr>
</tbody>
</table>

P values

Process *** Process × PUFA ***
α-TA level *** Process × α-TA level ***
PUFA *** PUFA × α-TA level **

A–CGrand means within the same column or row with different superscripts are significantly different.

***Different superscripts indicate significant differences within the same column.
**Different superscripts indicate significant differences within the same row.

1Values given in this table correspondence to least squares means obtained from multifactor ANOVA (n = 384) and their pooled SE.

2R = raw thigh meat; RF = raw refrigerated thigh meat; C = cooked thigh meat; CF = cooked refrigerated thigh meat.

3PU15 = 15 g of polyunsaturated fatty acids per kilogram of feed; PU34 = 34 g of polyunsaturated fatty acids per kilogram of feed; PU45 = 45 g of polyunsaturated fatty acids per kilogram of feed; PU61 = 61 g of polyunsaturated fatty acids per kilogram of feed.

4E0 = without supplementation with α-tocopheryl acetate; E1, E2 and E4 = supplemented with 100, 200, or 400 mg/kg α-tocopheryl acetate, respectively.

have suggested that reduction in TBARS values observed as a function of storage time is probably associated with increased concentrations of highly polar products, probably resulting from polymerization of secondary oxidation products. It has been reported that MDA reacts with a wide range of compounds or can form dimers or trimers of MDA, which decreases the amount of MDA available to react with TBA and, as a result, decreases the TBARS values (Gutteridge, 1975; Esterbauer et al., 1991; Aubourg, 1993). The present data indicate that TBARS numbers may not be a good method for determining the oxidative stability of meat during extended storage. In this sense, some authors who observed a decrease in TBARS values did not find a reduction in total volatile compounds (Aju-yah et al., 1993) and lipid hydroperoxides (Grau et al., 2001a).

The design of the present study permitted the observation that TBARS values in thigh meat depended on dietary polyunsaturation (P ≤ 0.001). An interaction between dietary polyunsaturation level and processing of meat showed that dietary polyunsaturation significantly affected TBARS values in C and CF thigh meat (Table 3). Hence, TBARS values in C thighs from PU45 and PU61 treatments were 4.1 and 5.6% higher, respectively, than in those from PU15 treatments (P ≤ 0.001). A similar interaction showing higher TBARS values for cooked and stored thighs of chickens fed polyunsaturated fat sources has been reported (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). At the same time, α-TA supplementation resulted in a significant decrease of oxidation for RF, C, and CF thigh meat (Table 3). An interaction between dietary α-TA supplementation and processing of meat indicated that the antioxidant effect of α-Toc increased as the oxidative tendency increased, i.e., cooking and refrigeration. Reduction of TBARS values as a consequence of dietary tocopherols has been reported by several authors and has been attributed to the accumulation of the α-Toc in thigh meat (Lin et al., 1989; Sheehy et al., 1993; Ahn et al., 1995; King et al., 1995; De Winne and Dirinck, 1996; Wen et al., 1996; Galvin et al., 1997;
Lauridsen et al., 1997; O’Neill et al., 1998; Ruiz et al., 1999; Grau et al., 2001a,b).

The lower TBARS values in cooked meat for treatments supplemented with α-Toc indicated that this antioxidant remained active after processing at high temperatures (80°C). Hence, α-Toc is an effective antioxidant for preventing lipid oxidation in meat subjected to heat processes. Obviously, the protective effect of α-Toc against lipid oxidation depended on the level of dietary polyunsaturation. Thigh meat from chickens fed the more polyunsaturated diets and, therefore, containing more PUFA was more protected by α-TA supplementation. Thus, α-Toc protected thigh meat against lipid oxidation in diets containing more PUFA, whereas this effect was not significant in thighs from more saturated treatments (PU15). This finding could be attributed to the higher PUFA content of meat from the more polyunsaturated treatments, which involved greater vulnerability of its lipid fraction to free radical attack. Other studies have also reported an interaction between dietary fat source and α-TA supplementation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b).

It is well established that PUFA contents of thigh meat increase with their levels in diets (Lin et al., 1989; Ajuyah et al., 1993; López-Ferrer et al., 1999, 2001). Table 4 shows linear regressions between PUFA content of raw thigh meat and TBARS values in cooked meat for different levels of supplementation with α-TA. Lipid oxidation in cooked thigh meat increased linearly when the concentration of PUFA in raw thigh increased, but this increase was lower as the dietary α-TA supplementation increased. However, the response was similar for the treatments supplemented with 200 and 400 mg of α-TA/kg. These results indicated that α-TA inclusion at levels higher than 200 mg/kg did not further improve lipid stability of thigh meat in terms of TBARS values. These results are in agreement with those of Jensen et al. (1995), who found that the reduction in TBARS values of thigh meat was similar for dietary α-TA levels of 100 and 500 mg/kg. Bou et al. (2004) showed that cooked dark meat in chickens fed α-TA levels of 70 and 140 mg/kg of feed did not have significant differences in TBARS values. Similarly, Ahn et al. (1998) did not find significant differences between TBARS of aerobically packaged cooked thighs from turkeys fed 200, 400, or 600 mg α-TA/kg. Sheldon et al. (1997) showed that breast meat of turkeys fed α-TA at 120 and 300 mg/kg of feed did not have significant differences in TBARS values.

In relation to α-Toc content of raw thigh meat, an increase of 10 mg of α-Toc/kg in the broiler diets caused an increase from 0.71 to 1.14 mg of α-Toc/kg of thigh meat depending on dietary polyunsaturation level (data not shown). The relationship between TBARS values of cooked thighs and α-Toc content of raw thighs for the different polyunsaturation levels showed a nonlinear relation. We established equations of the type \( y = a + be^{\alpha x} \), where \( y \) is TBARS values in cooked thigh, and \( x \) is α-Toc content in raw thigh. As an example, the correlation between TBARS and α-Toc content in thigh meat from PU61 treatment is represented in Figure 2. It can be observed that within the range of α-TA doses used, there was saturation in the antioxidant effect of α-Toc. That is, an increase in α-Toc content of thigh meat did not always imply a reduction in lipid oxidation. Thus, at low α-Toc content in thigh, a marginal increase of α-Toc sharply reduced lipid oxidation, whereas at high α-Toc content in thigh, a large α-Toc increase in thigh only caused slight or no improvement of oxidative stability. Except for TBARS values from PU45 treatments that presented higher variability, in general, maximum and minimum TBARS values obtained through α-TA supplementation increased as dietary polyunsaturation increased. For all levels of dietary PUFA, supplementation with α-TA prevented 84 to 88% of the maximum lipid oxidation in terms of TBARS values. The fractional rate of reduction of the TBARS values was 10.6% in all cases. Despite the fact that α-Toc antioxidant effectiveness was the same for all dietary polyunsaturation levels, in the most polyunsaturated treatments the effectiveness was much more evident because maximum TBARS values were 4-fold higher than

**Table 4. Equations: \( y = \text{thiobarbituric acid values (µg/kg of malondialdehyde on a wet-weight basis)} \) of cooked thigh meat, \( x = \text{PUFA content of raw thigh meat (mg/kg)} \), and \( z = \text{α-tocopherol content (mg/kg)} \) of raw thigh meat**

<table>
<thead>
<tr>
<th>Independent variable (mg/kg)</th>
<th>Dietary treatments(^1,2)</th>
<th>Equation</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA content of raw thigh meat</td>
<td>E0</td>
<td>( y = 69.67x )</td>
<td>0.92</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>( y = 35.62x )</td>
<td>0.82</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>E2, E4</td>
<td>( y = 18.13x )</td>
<td>0.73</td>
<td>***</td>
</tr>
<tr>
<td>α-Tocopherol content of raw thigh meat</td>
<td>PU15</td>
<td>( y = 170.7 + 1,133.5e^{-0.106z} )</td>
<td>0.75</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>PU34</td>
<td>( y = 434.5 + 2,285.1e^{-0.106z} )</td>
<td>0.90</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>PU45</td>
<td>( y = 1138.7 + 1,616.5e^{-0.106z} )</td>
<td>0.92</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>PU61</td>
<td>( y = 580.5 + 4,419.7e^{-0.106z} )</td>
<td>0.92</td>
<td>***</td>
</tr>
</tbody>
</table>

\(^1\)E0 = without supplementation with α-tocopheryl acetate; E1, E2, and E4 = supplemented with 100, 200, or 400 mg/kg α-tocopheryl acetate, respectively.

\(^2\)PU15 = 15 g of polyunsaturated fatty acids per kilogram of feed; PU34 = 34 g of polyunsaturated fatty acids per kilogram of feed; PU45 = 45 g of polyunsaturated fatty acids per kilogram of feed; PU61 = 61 g of polyunsaturated fatty acids per kilogram of feed.

***\( P \leq 0.001. ***
those in the least saturated treatments. Other authors have reported different relationships between α-Toc concentration and TBARS values in tissues: inverse linear (Bartov and Bornstein, 1978; Bartov and Frigg, 1992), inverse logarithmic (Mercier et al., 1998), potential (Ruiz et al., 1999), and binomial (Yamauchi et al., 1982). Contrary to our results, the prediction equations from all of these authors did not find saturation in the antioxidant effect of α-Toc. However, these authors worked with lower concentrations of α-Toc in thighs of poultry than those used in our study.

Considering the different factors which affect lipid oxidation, the dietary supplementation with α-TA should be adjusted depending on dietary polyunsaturation level and on the processing and storage conditions of thigh meat, as well as the objective of this supplementation: either to prevent lipid oxidation or to enrich poultry meat with vitamin E. To predict the minimal dietary supplementation with α-TA in meat enriched with PUFA to minimize lipid oxidation, we performed a regression analysis of the evolution of TBARS values in cooked thigh in response to variation in dietary PUFA and α-Toc content. Therefore, the following equation was obtained (Figure 3): y = x(11.88 + 63.38e^{−0.007z}) (P ≤ 0.001), where y is TBARS value in cooked thigh (µg of MDA/kg), x is dietary PUFA (g/kg), and z is dietary α-Toc (mg/kg). A TBARS value ≥ 800 µg of MDA/kg has been considered as threshold for warmed-over flavor detection in cooked dark chicken meat (O’Neill et al., 1998; Bou et al., 2001). Thus, when the polyunsaturation level of PUFA is low in a diet (15 g of PUFA/kg) only 60 mg of dietary α-Toc per kilogram of feed is necessary to assure TBARS values below 800 µg of MDA/kg, whereas at high level of dietary PUFA (30 g of PUFA/kg), 200 mg of dietary α-Toc per kilogram of feed are necessary. Moreover, supplementation with α-Toc greater than 200 mg/kg did not improve lipid stability (see Table 4). Therefore, dietary PUFA level should not be greater than 30 g PUFA/kg of feed to avoid appearance of warmed-over flavor in cooked chicken meat.

In conclusion, dietary polyunsaturation and α-tocopheryl acetate supplementation affected lipid oxidation more markedly in cooked meat and cooked refrigerated meat, with higher TBARS values, than in raw meat and raw refrigerated meat. The experimental design used permitted the study of lipid oxidation pattern in function of polyunsaturated fatty acids and α-Toc content in the diet and thigh. Lipid oxidation increased linearly as the concentration of polyunsaturated fatty acids in raw meat increased, but this increase was lower with greater dietary α-TA supplementation. In fact, the nonlinear relationship between TBARS values in cooked meat and α-Toc content of raw meat showed saturation in the antioxidant effect of α-tocopherol. These results indicate that α-TA inclusion at levels higher than 200 mg/kg did not further improve lipid stability of thigh meat in terms of TBARS values, and, therefore, it was necessary to limit dietary polyunsaturated fatty acids content to minimize lipid oxidation. The equation calculated in the present work allowed prediction of the minimal dietary supplementation with α-TA to minimize lipid oxidation of cooked thigh meat in response to variation in dietary PUFA.

**ACKNOWLEDGMENTS**

The authors are grateful to F. Hoffmann-La Roche Ltd. for the donation of the α-tocopheryl acetate used and to Agrupación de Fabricantes de Aceites Marinos, S.A., for the donation of the fish oil used. This work was supported, in part, by research grants from the Ministerio de...
Educazione, Cultura y Deporte, and the Comisión Interministerial de Ciencia y Tecnología (CICYT).

REFERENCES


