# Capítulo 7

**Influencia del Nivel Dietético de Poliinsaturación y Vitamina E sobre la Calidad de la Carne de Pollo: Oxidación Lipídica**

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

### 7.1. Summary

The present study was carried out to evaluate the influence of diets containing increasing amounts of PUFA at different levels of supplementation with $\alpha-$TA on the lipid oxidation of raw and cooked thigh meat stored under refrigeration for different periods. One hundred and ninety-two female broiler one-day-old chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary PUFA (15, 34, 45 and 61g/kg) and 4 levels of supplementation with $\alpha-$TA (0, 100, 200 and 400mg/kg). TBA values in cooked meat (C) and cooked refrigerated (CR) meat were 12 and 24 fold higher, respectively, than in raw meat (R). Dietary polyunsaturation and $\alpha-$TA supplementation affected lipid oxidation more markedly in cooked meat and cooked refrigerated meat than in raw meat and raw refrigerated meat (RR). Lipid oxidation in cooked meat increased linearly as the concentration of PUFA in raw meat increased. The oxidative stability of meat was not affected by an increase in the dietary $\alpha-$TA level from 200 to 400mg/kg. Non-linear relationship between TBA values in cooked meat and $\alpha-$Toc content of raw meat showed saturation in the antioxidant effect of $\alpha-$Toc. The equation y $= \times 1(11.88 + 63.38e^{(-0.007 \times 2)})$ was calculated to predict the minimum inclusion of $\alpha-$Toc to diets ($\times$2) of chickens with certain dietary PUFA content ($\times$1) to assure a certain TBA value (y).

## 7.2. Results and Discussion

### 7.2.1. Diet Composition

The different blend of fat and oils added to diets resulted in PUFA levels of $15 \pm 0.4$, $34 \pm 0.8$, $45 \pm 1.5$ and $61 \pm 1.3$ g/kg of feed for PU15, PU34, PU45 and PU61, respectively (Chapter 4). Supplementation with 100, 200 and 400 mg $\alpha-$TA/kg of feed resulted in dietary $\alpha-$Toc content of $6 \pm 0.6$ g/kg, $136 \pm 1.5$ g/kg, $236 \pm 14.5$ g/kg and $451 \pm 18.1$ g/kg for E0, E1, E2 and E4 treatments, respectively (Chapter 5).

### 7.2.2. Lipid Oxidation in Thigh Meat

TBA values in R, RR, C and CR thigh meat were expressed as μg of MDA per kg of solids and were compared (**Table 7.1**). TBA values in R meat were low but during cooking and refrigeration they significantly increased. Thus, lipid oxidation in C and CR thigh meat were 12 fold and 24 fold higher, respectively, than in R thigh meat. An increase of the lipid oxidation in cooked thigh meat (Ang et al., 1988; Sheehy et al., 1993; Galvin et al., 1997; Jensen et al., 1997; Maraschiello 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., 1989a,b; Ajuyah et al., 1993a; Galvin et al., 1997; Jensen et al., 1997; Ruiz et al., 1999) has been reported. However, the magnitude of the increase in the TBA values after cooking and storage of thigh meat differs between authors. Generally, it is difficult to make comparisons of TBA values between studies because differences in the magnitude of this increase could be attributed to different factors such as the analytical method used, cooking and storage conditions (time, temperature and packaging), and vitamin E content and fatty acid profile of meat.

Despite the fact that CR thigh meat usually showed TBA values higher than C thigh meat (Table 7.1), this was not observed in thigh meat from the more polyunsaturated treatment non-supplemented with $\alpha-$TA (PU61+E0) (**Figure 7.1**). A decrease in TBA values has been previously observed in thigh meat after prolonged storage (King et al., 1995; Wen et al., 1996; Grau et al., 2001a). Several authors have suggested that reduction in TBA values observed as a function of storage time is probably associated with increased concentrations of highly polar products, probably resulting from polymerisation 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 TBA values (Gutteridge, 1975; Esterbauer et al., 1991; Aubourg, 1993). The present data indicate that TBA 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 TBA values did not find a reduction in total volatile compounds (Ajuyah et al., 1993a) and lipid hidroperoxides (Grau et al., 2001a).

**Table 7.1.** Effect of dietary polyunsaturation, α−tocopheryl acetate (α−TA) supplementation and processing on TBA values in thigh meat (μg malondialdehyde/kg on a dry matter basis)[1].

| Process[2] | Global Means | Dietary polyunsaturation level[3] | | | |
|---|---|---|---|---|---|
| | | **PU15** | PU34 | **PU45** | **PU61** |
| R | 338$^C$ | 88$^b$ | 159$^c$ | 287$^c$ | 816$^c$ |
| RR | 946$^C$ | 146$^b$ | 339$^c$ | 775$^c$ | 2526$^c$ |
| C | 3870$^B$ | 1141$^{bz}$ | 3274$^{byz}$ | 4644$^{byz}$ | 6421$^{by}$ |
| CR | 7939$^A$ | 4366$^{az}$ | 8466$^{ay}$ | 10126$^{ay}$ | 8798$^{ay}$ |
| | | | | | |
| Global Means | | 1435$^C$ | 3059$^B$ | 3958$^{AB}$ | 4640$^A$ |

| | Supplementation with α−TA[4] | | | |
|---|---|---|---|---|
| | **E0** | E1 | **E2** | **E4** |
| R | 901$^c$ | 164$^c$ | 144.0$^b$ | 142$^b$ |
| RR | 2654$^{by}$ | 682$^{cyz}$ | 251.2$^{byz}$ | 199$^{bz}$ |
| C | 7860$^{ax}$ | 3884$^{by}$ | 2195.9$^{byz}$ | 1540$^{bz}$ |
| CR | 9258$^{ay}$ | 9433$^{ay}$ | 6688.4$^{az}$ | 6377$^{az}$ |
| | | | | |
| PU15 | 2448$^c$ | 1438$^b$ | 1081 | 774 |
| PU34 | 4419$^{bcy}$ | 3980$^{abyz}$ | 1760$^z$ | 2078$^{yz}$ |
| PU45 | 5653$^{aby}$ | 3736$^{abyz}$ | 3322$^{yz}$ | 3123b$^z$ |
| PU61 | 8153$^{ax}$ | 5009$^{ay}$ | 3116$^{yz}$ | 2282$^z$ |
| | | | | |
| **Global** | 5168$^A$ | 3540$^B$ | 2320$^C$ | 2064$^C$ |
| | | | | |
| **SE** | **501** | | | |

| *P* values | | | |
|---|---|---|---|
| Process | *** | Process × PUFA | *** |
| α−TA level | *** | Process × α−TA level | *** |
| PUFA | *** | PUFA × α−TA level | ** |

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

[2] R: Raw thigh meat; RR: Raw Refrigerated thigh meat; C: Cooked thigh meat; CR: Cooked Refrigerated thigh meat.

[3] PU15: 15 g polyunsaturated fatty acids /kg of feed; PU34: 34 g polyunsaturated fatty acids /kg of feed; PU45: 45 g polyunsaturated fatty acids /kg of feed; PU61: 61 g polyunsaturated fatty acids /kg of feed.

[4] E0: without supplementation with α−tocopheryl acetate; E1, E2 and E4: supplemented with 100, 200 and 400 mg/kg α−tocopheryl acetate, respectively.

A,B,C Grand means in the same column/row with different superscript were significantly different.

a,b,c Different superscripts indicate significant differences in the same column.

x,y,z Different superscripts indicate significant differences in the same row.

**$P \leq 0.01$, ***$P \leq 0.001$.

**Figure 7.1.** Influence of processing on TBA values in thigh meat ($\mu$g malondialdehyde (MDA)/kg on a dry matter basis) from the dietary treatment with 61 g PUFA/kg of feed and non-supplemented with $\alpha$−tocopheryl acetate[1].
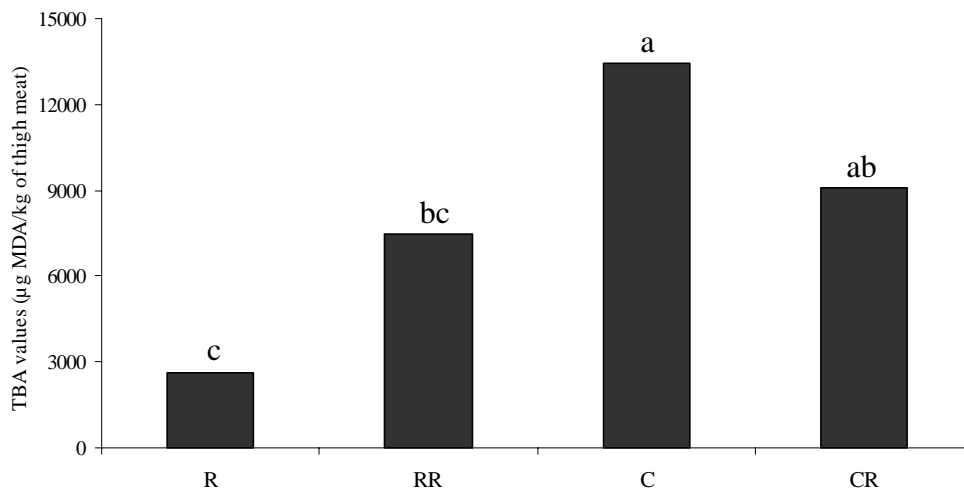


[1] R: Raw thigh meat; RR: Raw refrigerated thigh meat; C: Cooked thigh meat; CR: Cooked refrigerated thigh meat.
[a,b,c] Different superscripts indicate significant differences.

The design of the present study permitted the observation of the fact that TBA values in thigh meat depended on dietary polyunsaturation ($P \le 0.001$). An interaction between dietary polyunsaturation level and processing of meat showed that dietary polyunsaturation affected significantly TBA values in C and CR thigh meat (Table 7.1). Hence, TBA values in C thighs from PU45 and PU61 treatments were 4.1% and 5.6% higher, respectively, than in those from PU15 treatments ($P \le 0.001$). A similar interaction has been reported in literature, showing higher TBA values for cooked and stored thighs of chickens fed polyunsaturated fat sources (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b).

At the same time, $\alpha$−TA supplementation resulted in a significant protection against oxidation for RR, C and CR thigh meat (Table 7.1). An interaction between dietary $\alpha$−TA supplementation and processing of meat indicated that the antioxidant effect of $\alpha$−Toc increased as the oxidative pressure increased, i.e. cooking and refrigeration. Reduction of TBA values as a consequence of dietary tocopherols has been reported by several authors and has been attributed to the accumulation of the $\alpha$−Toc in thigh meat (Lin et al., 1989a,b; Sheehy et al., 1993; Ahn et al., 1995; King e t al., 1995; De Winne and Dirinck, 1996; Wen et al., 1996; Galvin et al., 1997; Lauridsen et al., 1997; O'Neill et al., 1998a; Ruiz et al., 1999; Grau et al., 2001a,b).

The strong antioxidative activity observed for $\alpha$−Toc in cooked meats indicated that this antioxidant still 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 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 thighs against lipid oxidation in more polyunsaturated treatments whereas this effect was not significant in thighs from more saturated treatments (PU15). This could be attributed to their higher PUFA content, which involved higher vulnerability of its lipid fraction to free radical attack. Some 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).

**Table 7.2.** Equations: y = TBA values (μg/kg malondialdehyde on a wet weight basis) of cooked thigh meat; $x_1$ = PUFA content of raw thigh meat (mg/kg) and $x_2$ = α–tocopherol content (mg/kg) of raw thigh meat.

| Independent variable (mg/kg) | Dietary treatments[1] | Equation | $r^2$ | $P$ |
|---|---|---|---|---|
| PUFA content of raw thigh meat[2] | E0 | $y = 69.67x_1$ | 0.92 | *** |
| | E1 | $y = 35.62x_1$ | 0.82 | *** |
| | E2, E4 | $y = 18.13x_1$ | 0.73 | *** |
| α–Tocopherol content of raw thigh meat | PU15 | $y = 170.7 + 1133.5e^{-0.106x_2}$ | - | *** |
| | PU34 | $y = 434.5 + 2285.1e^{-0.106x_2}$ | | |
| | PU45 | $y = 1138.7 + 1616.5e^{-0.106x_2}$ | | |
| | PU61 | $y = 580.5 + 4419.7e^{-0.106x_2}$ | | |

[1] E0: without supplementation with α–tocopheryl acetate; E1, E2 and E4: supplemented with 100, 200 and 400 mg/kg α–tocopheryl acetate, respectively; PU15: 15 g polyunsaturated fatty acids /kg of feed; PU34: 34 g polyunsaturated fatty acids /kg of feed; PU45: 45 g polyunsaturated fatty acids /kg of feed; PU61: 61 g polyunsaturated fatty acids /kg of feed.
[2] Equations fitted for the range of PUFA content of cooked thigh meat studied.
***$P \leq 0.001$.

It is well-established that PUFA content of thigh meat increases with their level in the diet (Chapter 4). **Table 7.2** shows linear regressions between PUFA content and TBA values in cooked thigh meat for different levels of supplementation with α–TA. Lipid oxidation in cooked thigh meat increased linearly when the concentration of PUFA in cooked 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 α–TA/kg. These results indicated that α–TA inclusion at levels higher than

200 mg/kg did not improve lipid stability of thigh meat, in terms of TBA values. These results are in agreement with Jensen et al. (1995) who found that the reduction in TBA 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 no have significant differences in TBA values. Similarly, Ahn et al. (1998) did not find significant differences between TBA of aerobic-packaged cooked thighs from turkeys fed 200, 400 or 600 mg α−TA/kg. Sheldon et al. (1997) showed that breast meat in turkeys fed α−TA levels of 120 and 300 mg α−TA/kg of feed did not have significant differences in TBA values.

**Figure 7.2.** Relationship between α−tocopherol content (mg/kg) in raw thigh and TBA values (μg malondialdehyde (MDA)/kg on a wet weight basis) in cooked thigh meat from chickens fed diets containing 61 g polyunsaturated fatty acids per kg of feed.
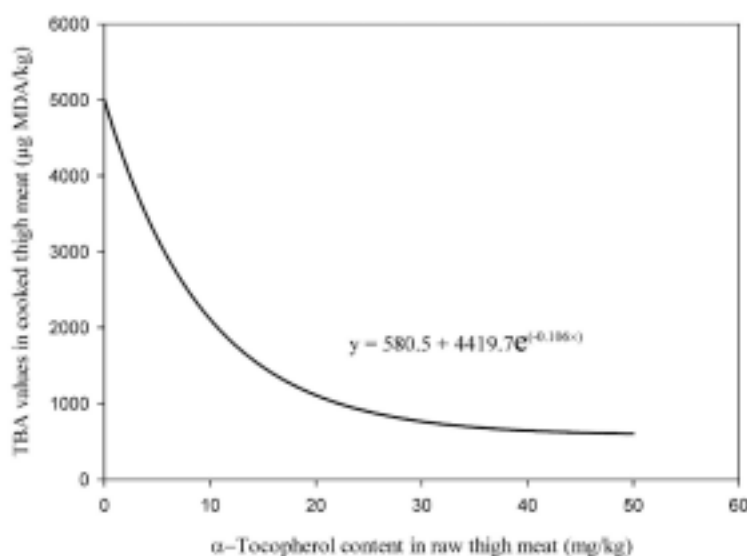


In relation to α−Toc content of raw thigh meat, an increase of 10 mg α−Toc/kg in the broiler diets caused an increase from 0.71 to 1.14 mg α−Toc/kg of thigh meat depending on dietary polyunsaturation level (Chapter 5). Relationship between TBA values of cooked thighs and α−Toc content of raw thighs for the different polyunsaturation levels showed a non-linear relation. Equations of the type: $y = a + be^{(-cx)}$ were established, where y is TBA values in cooked thigh and x is α−Toc content in raw thigh. As an example, the correlation between TBA and α−Toc content in thigh meat from PU61 treatment is represented in **Figure 7.2**. 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 none improvement of oxidative stability. Except for TBA values from PU45 treatments which presented higher variability, in general, both maximum and minimum TBA values obtained through α–TA supplementation increased as dietary polyunsaturation increased. Supplementation with α–TA prevented 84-88% of the maximum lipid oxidation, in terms of TBA values. The decrease in the fractional rate of TBA 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 TBA values were 4 fold higher than those in the least saturated treatments. Other authors have reported an inverse linear (Bartov and Bornstein, 1978; Bartov and Frigg, 1992) and inverse logarithmic (Mercier et al., 1998) relationship between α–Toc concentration and TBA values in tissues. Similarly, Ruiz et al. (1999) and Yamauchi et al. (1982) found a relationship following potential and binomial equations. Contrary to our results, 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 that those in our study.

**Figure 7.3.** Estimated response surface for TBA values (μg malondialdehyde (MDA)/kg on a wet weight basis) in cooked thigh to variation in dietary content of α–tocopherol (mg/kg) and polyunsaturated fatty acids (g/kg).
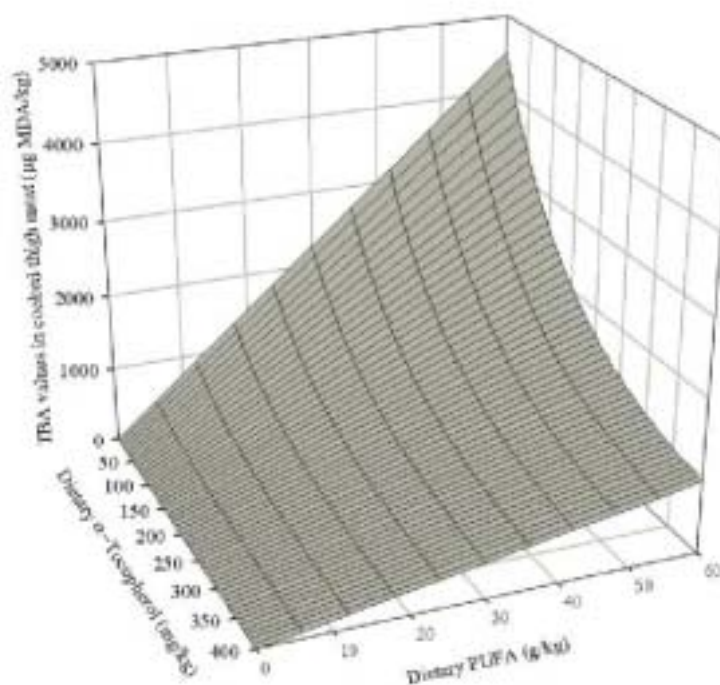


Considering the different factors which affect lipid oxidation, the dietary supplementation with α–TA should be adjusted depending on dietary polyunsaturation

level and on the processing and storage conditions of thigh meat, as well as the objective of this supplementation: to prevent lipid oxidation or enrich poultry meat with vitamin E. In order to predict the minimal dietary supplementation with $\alpha$−TA in meat enriched with PUFA (by means of different dietary fat sources) minimizing lipid oxidation, the evolution of TBA values in cooked thigh to variation in dietary PUFA and $\alpha$−Toc contents were calculated. Therefore, the following equation was obtained (**Figure 7.3**): y = ×1(11.88 + 63.38e$^{(-0.007 \times 2)}$) (*P* ≤ 0.001), where y is TBA value in cooked thigh ($\mu$g MDA/kg), ×1 is dietary PUFA (g/kg) and ×2 is dietary $\alpha$−Toc (mg/kg). A TBA value ≥ 800 $\mu$g MDA/kg of meat has been considered as threshold for warmed-over flavor detection in cooked dark chicken meat (O'Neill et al., 1998a; Bou et al., 2001). Thus, when polyunsaturation level of PUFA is low in diet (15 g PUFA/kg) only 60 mg of dietary $\alpha$−Toc per kg of feed is necessary to assure TBA values below 800 $\mu$g MDA/kg, whereas at high level of dietary PUFA (60 g PUFA/kg), 540 mg of dietary $\alpha$−Toc per kg of feed is necessary. However, at dietary PUFA level higher than 67 g PUFA/kg of feed, the equation predicts that dietary supplementation with $\alpha$−Toc would be not enough to avoid appearance of warmed-over flavor in cooked chicken meat.

In conclusion, cooking and refrigeration of thigh meat increases its lipid oxidation. Dietary PUFA also increases susceptibility of meat to oxidation due to PUFA deposition in meat, which leads to higher oxidative pressure. Contrarily, dietary $\alpha$−TA supplementation prevents lipid oxidation through the accumulation of the $\alpha$−Toc in thigh meat. The antioxidant activity of $\alpha$−Toc remains after heat process. Furthermore, there is saturation in the antioxidant effect of $\alpha$−Toc, so it is necessary to limit dietary PUFA to minimize lipid oxidation.

# Capítulo 8

## Influencia del Nivel Dietético de Aceite Poliinsaturado y Vitamina E sobre el Contenido de Ácidos Grasos en la Carne de Pollo

# 8. Fatty Acid Content in Chicken Meat as Affected by Dietary Polyunsaturated Oils and Vitamin E Levels

## 8.1. Summary

The present study was carried out to evaluate the influence of diets containing increasing levels of polyunsaturated oils (2, 4, 6 and 8%; PUFA: 65.2%) and different levels of supplementation with $\alpha-$TA (0, 100, 200 and 400 mg/kg) on the modifications of the amount and type of FA deposited in marketable raw and cooked chicken meat. At 40 days, quantified FA of raw thigh and breast meat were not affected by the dietary supplementation with $\alpha-$TA. Total FA content of thigh was not modified by level of added oil, whereas increasing added oil of diet by 60 g, from 20 to 80 g/kg, increased total FA content of breast 30%. Relationship between PUFA content of feed and meat was exponential suggesting that the origin of these FA is preferentially the diet. A linear relationship between the level of added oil ($\times$) and total MUFA ($y_1$) and SFA ($y_2$) content in thigh meat ($y_1 = 38.42 - 0.15\times$; $y_2 = 28.07 - 0.10\times$) showed that only 20% of the variability in their content can be attributed to changes in the level of dietary fat. Furthermore, total MUFA and SFA content of breast meat did not depend on the level of added oil. The behaviour of the deposit of SFA and MUFA content in chicken meat suggested that these FA depend more on the endogenous fat synthesis than on direct deposition from diet. Cooking of thigh meat led to a reduction of 12.6% for SFA, 13.3% for MUFA and 14.8% for PUFA, and most MUFA and PUFA were modified by dietary $\alpha-$TA supplementation.

## 8.2. RESULTS

### 8.2.1. Diet Composition

The FA composition of the experimental diets is shown in **Table 8.1**. The oil added to the diets had a PUFA content of 65.2%. Increasing the amount of oil added to the diet increased the PUFA concentration of the feeds, and the same was true, although to a lesser extent for MUFA and SFA. Therefore, dietary PUFA to SFA ratio increased with dietary oil level.

**Table 8.1.** Fatty acid composition of the experimental diets, expressed as g per kg.[1]

| Fatty Acid[3] | Dietary treatments[2] | | | |
|---|---|---|---|---|
| | **O2** | **O4** | **O6** | **O8** |
| **Total FA** | 45.37 | 60.29 | 75.70 | 91.63 |
| **Total SFA** | 7.89 | 9.79 | 11.65 | 13.66 |
| C 10:0 | 0.08 | 0.08 | 0.08 | 0.08 |
| C 14:0 | 0.30 | 0.53 | 0.76 | 0.99 |
| C 15:0 | 0.03 | 0.05 | 0.07 | 0.09 |
| C 16:0 | 5.82 | 6.84 | 7.84 | 8.96 |
| C 17:0 | 0.06 | 0.08 | 0.09 | 0.11 |
| C 18:0 | 1.39 | 1.92 | 2.44 | 2.98 |
| C 20:0 | 0.16 | 0.19 | 0.22 | 0.26 |
| **Total MUFA** | 9.91 | 12.83 | 15.90 | 19.07 |
| C 16:1 ω7t | 0.02 | 0.03 | 0.03 | 0.04 |
| C 16:1 ω7 | 0.40 | 0.71 | 1.01 | 1.32 |
| C 18:1 ω9[4] | 8.67 | 10.85 | 13.21 | 15.67 |
| C 18:1 ω7t | 0.49 | 0.67 | 0.86 | 1.05 |
| C 20:1 ω9 | 0.15 | 0.23 | 0.30 | 0.38 |
| C 21:1 ω9 | ND | 0.09 | 0.15 | 0.20 |
| C 24:1 ω9 | 0.10 | 0.11 | 0.12 | 0.14 |
| **Total PUFA** | 27.60 | 37.66 | 48.08 | 58.79 |
| C 18:2 ω6 | 17.79 | 18.72 | 19.87 | 21.31 |
| C 18:3 ω3 | 8.57 | 16.65 | 24.63 | 32.69 |
| C 18:4 ω3 | 0.14 | 0.27 | 0.41 | 0.53 |
| C 20:4 ω6 | ND | ND | 0.12 | 0.14 |
| C 20:5 ω3 | 0.72 | 1.38 | 2.07 | 2.75 |
| C 22:4 ω6 | ND | ND | 0.10 | 0.13 |
| C 22:6 ω3 | 0.28 | 0.53 | 0.76 | 1.01 |
| **PUFA:SFA** | 3.50 | 3.85 | 4.13 | 4.30 |

[1] Values are means of 4 dietary treatments with different level of supplementation with α−TA: 0, 100, 200 and 400 mg/kg.
[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil, respectively
[3] FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[4] C 18:1 ω9 includes sum of *cis* and *trans* forms.
ND: Not detected.

Supplementation with 100, 200 and 400 mg α−TA/kg of feed resulted in α−Toc dietary levels of 3 ± 0.4 g/kg, 99 ± 4.2 g/kg, 190 ± 12.2 g/kg and 339 ± 17.4 g/kg for E0, E1, E2 and E4 treatments, respectively.

### 8.2.2. Effect of oil level

Average daily feed intake (106.5 g/bird/day), daily weight gain (61.8 g/bird/day), feed efficiency (1.72 g:g), body weight (2,233 ± 208.1 g), dressing percentage (81 ± 1.3 %) and thigh (455 ± 47.0 g) and breast (409 ± 47.8g) weights were not modified by the level of added oil in the diet. Total FA content of thigh was not modified by dietary added oil level, whereas total FA content of breast was increased as added oil in diet increased (**Table 8.2** and **8.3**). Therefore, increasing added oil (65.2% PUFA) of diet by 60 g, from 20 to 80 g/kg increased total FA of breasts by 30%.

As expected, PUFA content of the studied tissues increased as the level of oil, and therefore PUFA, increased in the diet (Tables 8.2 and 8.3). An increase of 60g/kg in the level of added oil (from 20 to 80 g/kg) resulted in a 1.8- and 1.9 times increase in the PUFA level of thigh and breast meat, respectively. A similar response was observed in certain PUFA, mainly LNA, LA, EPA and DHA, as well as in long chain MUFA like C20:1ω9, C21:1ω9 and C24:1ω9. The relationship between level of oil in feeds and PUFA content of tissues was studied, and proved to be exponential (**Table 8.4**). Incorporation rates of PUFA in treatments supplemented with a level of added oil of 6% (O6) reached 87% of the maximum which was 8.44 g/kg in breast and 88% of the maximum of 52.91 g/kg in thigh. The accretion fractional rate of PUFA in both, breast and thigh, was 3.74%.

The total MUFA and total SFA content of thigh meat were influenced by the level of added oil (Table 8.2). Whereas PUFA content of thigh meat increased as the level of dietary oil increased, total MUFA and total SFA content were reduced. Therefore, the 60 g/kg increase in added oil resulted in a reduction of approximately 23% reduction of SFA and MUFA in thigh meat, Some FA like C18:0, C20:0 and C18:1ω7 were scarcely modified at all by dietary level of added oil. In breast meat, levels of C16:0, C18:1ω9 and C18:1ω7, and total SFA and MUFA were not affected by the level of added oil (Table 8.3), and, C18:0 content was only slightly modified.

A linear relationship was established between the level of added oil and total MUFA and SFA content of thigh meat (Table 8.4). Only about 20% of the variability in the content of total MUFA and total SFA in thigh can be attributed to changes in the level of dietary fat. Contrary to what happens with PUFA content, MUFA and SFA content of thigh meat was reduced with increasing levels of oil in the diet. Therefore, total MUFA and total SFA content in thigh decreased by 0.15 g/kg and 0.10 g/kg, respectively, when dietary

**Table 8.2.** Effect of the level of added oil and α–tocopheryl acetate (α–TA) supplementation on the fatty acid content of thighs meat with skin, expressed as g/kg.[1]

| Fatty Acid[3] | Dietary treatments[2] | | | | P Oil level | P α–TA | SE |
|---|---|---|---|---|---|---|---|
| | O2 | O4 | O6 | O8 | | | |
| **Total FA** | 91.23 | 96.45 | 97.26 | 99.92 | NS | NS | 3.322 |
| **Total SFA** | 26.04[a] | 24.22[b] | 22.13[bc] | 20.15[c] | *** | NS | 0.894 |
| C 10:0 | 0.012[ab] | 0.014[a] | 0.012[ab] | 0.011[b] | * | NS | 0.001 |
| C 12:0 | 0.02 | 0.03 | 0.03 | 0.03 | NS | NS | 0.001 |
| C 14:0 | 0.65[c] | 0.80[b] | 0.88[ab] | 0.96[a] | *** | NS | 0.030 |
| C 15:0 | 0.10[c] | 0.12[b] | 0.13[ab] | 0.14[a] | *** | NS | 0.003 |
| C 16:0 | 18.60[a] | 16.75[ab] | 14.62[bc] | 12.98[c] | *** | NS | 0.705 |
| C 17:0 | 0.09[c] | 0.16[b] | 0.21[a] | 0.23[a] | *** | NS | 0.010 |
| C 18:0 | 6.40[a] | 6.18[a] | 6.06[a] | 5.61[b] | * | NS | 0.173 |
| C 20:0 | 0.11[a] | 0.11[ab] | 0.11[ab] | 0.12[b] | * | NS | 0.003 |
| **Total** | 35.72[a] | 32.73[b] | 28.80[bc] | 27.57[c] | *** | † | 1.294 |
| C 14:1 ω5 | 0.11[a] | 0.10[ab] | 0.08[b] | 0.08[b] | *** | NS | 0.007 |
| C 16:1 ω7t | 0.45[a] | 0.43[ab] | 0.37[bc] | 0.32[c] | *** | † | 0.017 |
| C 16:1 ω7 | 3.82[a] | 3.25[ab] | 2.70[b] | 2.59[b] | *** | NS | 0.193 |
| C 18:1 ω9[4] | 28.84[a] | 26.32[ab] | 23.03[bc] | 21.88[c] | *** | † | 1.015 |
| C 18:1 ω7t | 1.56[a] | 1.46[ab] | 1.34[b] | 1.36[b] | * | NS | 0.051 |
| C 20:1 ω9 | 0.28[b] | 0.29[ab] | 0.29[ab] | 0.32[a] | † | † | 0.009 |
| C 21:1 ω9 | 0.01[b] | 0.02[b] | 0.02[b] | 0.04[a] | *** | NS | 0.003 |
| C 24:1 ω9 | 0.57[b] | 0.76[a] | 0.87[a] | 0.87[a] | *** | NS | 0.031 |
| **Total PUFA** | 29.47[d] | 39.50[c] | 46.34[b] | 52.19[a] | *** | NS | 1.281 |
| C 18:2tt | 0.03 | 0.04 | 0.03 | 0.04 | NS | NS | 0.003 |
| C 18:2 ω6 | 17.76[b] | 19.24[ab] | 19.48[ab] | 19.83[a] | * | † | 0.471 |
| C 18:3 ω6 | 0.13[a] | 0.11[b] | 0.10[b] | 0.09[b] | *** | * | 0.004 |
| C 18:3 ω3 | 8.62[d] | 16.44[c] | 22.59[b] | 27.96[a] | *** | NS | 0.680 |
| C 18:4 ω3 | 0.22[d] | 0.39[c] | 0.49[b] | 0.58[a] | *** | NS | 0.021 |
| C 20:2 ω6 | 0.14[a] | 0.14[ab] | 0.13[ab] | 0.12b | * | NS | 0.004 |
| C 20:3 ω6 | 0.23[a] | 0.21[b] | 0.18[c] | 0.16[c] | *** | NS | 0.005 |
| C 20:4 ω6 | 0.74[a] | 0.59[b] | 0.53[c] | 0.48[d] | *** | * | 0.013 |
| C 20:5 ω3 | 0.78[c] | 1.32[b] | 1.70[a] | 1.95[a] | *** | NS | 0.074 |
| C 22:4 ω6 | 0.07 | 0.06 | 0.06 | 0.05 | NS | NS | 0.005 |
| C 22:6 ω3 | 0.62[b] | 0.85[a] | 0.95[a] | 0.86[a] | *** | NS | 0.032 |
| **PUFA:SFA** | 1.15[d] | 1.65[c] | 2.10[b] | 2.59[a] | *** | NS | 0.028 |

[1] Values given in this table correspond to least-squares means obtained from ANOVA (n= 96) and their pooled SE. [1] $P$ values for the interaction between dietary level of added oil and α–TA supplementation in all cases were not significant.

[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil, respectively

[3] FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[4] C 18:1 ω9 includes sum of *cis* and *trans* forms.

* = significant effect at $P \leq 0.05$; *** = $P \leq 0.001$; † = $P \leq 0.10$.

level of added oil (65.2 % PUFA) increased 1 g/kg of feed. Nevertheless, total MUFA and total SFA content of breast meat did not depend on the level of added oil. The estimated equation of total SFA and total MUFA content in chicken thigh meat (Table 8.4) depending on added oil level in the diet revealed the maximum theoretical content of synthesized and deposited FA in thigh meat. The total SFA and total MUFA content of thigh coming from endogenous synthesis in liver when no fat is added to the diet is estimated to be 28.1 and 38.4 g/kg of thigh meat, respectively. In breast meat, the constant content of these FA, regardless of the level of added oil, suggested that the maximum content of synthesized or deposited SFA and MUFA is estimated to be 4.2 and 4.8 g/kg, respectively.

Some SFA and MUFA in thigh and breast meat presented higher values in broilers fed the lowest level of added oil despite the lower presence of these FA in the diet. When the proportion of a particular FA is compared between tissues and diet, values above 1 indicate enrichment in this particular FA or family, as described previously by Crespo and Esteve-Garcia (2002c). SFA (1.78 vs. 1.74 vs. 1.71 vs. 1.61 for O2, O4, O6 and O8 treatments, $P \leq 0.001$) and MUFA ratio (1.63 vs. 1.49 vs. 1.35 vs. 1.29 for O2, O4, O6 and O8 treatments, $P \leq 0.001$) showed values greater than 1 in all treatments, whereas the relation of tissue PUFA with respect to dietary PUFA (0.55 vs. 0.64 vs. 0.71 vs. 0.76 for O2, O4, O6 and O8 treatments, $P \leq 0.001$) was always below 1. Thigh SFA (1.64 vs. 1.54 vs. 1.78 vs. 1.35 for O2, O4, O6 and O8 treatments, $P \leq 0.001$), MUFA (1.78 vs. 1.58 vs. 1.40 vs. 1.32 for O2, O4, O6 and O8 treatments, $P \leq 0.001$) and PUFA (0.54 vs. 0.66 vs. 0.75 vs. 0.81 for O2, O4, O6 and O8 treatments, $P \leq 0.001$) to diet ratio showed a similar behaviour to breast tissue.

### 8.2.3. Effect of dietary α−tocopheryl acetate

Productive parameters, as well as carcass and breast and thigh yield were not affected by the level of supplementation with α−TA. Similarly the FA content of both thigh and breast meat were not modified by dietary α−TA level. However, total MUFA, C16:1ω7t, C20:1ω9 and oleic (C18:1ω9), linoleic (C18:2ω6) and arachidonic (C20:4ω6) acids in thigh meat showed a tendency ($P < 0.10$) to increase as dietary α−TA level increased (data not shown).

### 8.2.4. Effect of cooking process

The FA in raw and cooked thighs (expressed as g/kg on a dry matter basis; water content: 69.34% vs. 66.98% in raw and cooked thigh, respectively, $P \leq 0.001$) are shown in **Table 8.5**. Cooking of thigh meat led to a reduction in total FA content. This reduction was 12.6% for SFA, 13.3% for MUFA and 14.8% for PUFA. The greater reduction in

**Table 8.3.** Effect of level of added oil and α−tocopheryl acetate (α−TA) supplementation on the fatty acid content of breasts meat without skin, expressed as g/kg.[1]

| Fatty Acid[3] | Dietary treatments[2] | | | | P Oil Level | P α–TA | SE |
|---|---|---|---|---|---|---|---|
| | O2 | O4 | O6 | O8 | | | |
| **Total FA** | 13.57[c] | 15.20[bc] | 16.19[ab] | 17.59[a] | *** | NS | 1.521 |
| **Total SFA** | 4.21 | 4.29 | 4.24 | 4.20 | NS | NS | 0.353 |
| C 10:0 | 0.05 | 0.05 | 0.05 | 0.05 | NS | NS | 0.003 |
| C 12:0 | 0.01 | 0.01 | 0.01 | 0.01 | NS | NS | 0.001 |
| C 14:0 | 0.08[c] | 0.11[b] | 0.13[b] | 0.15[a] | *** | *** | 0.014 |
| C 15:0 | 0.014[c] | 0.017[b] | 0.020[b] | 0.023[a] | *** | NS | 0.002 |
| C 16:0 | 2.74 | 2.73 | 2.58 | 2.50 | NS | NS | 0.254 |
| C 17:0 | 0.029[c] | 0.035[b] | 0.041[a] | 0.044[a] | *** | NS | 0.003 |
| C 18:0 | 1.27[b] | 1.32[ab] | 1.39[a] | 1.40[a] | * | NS | 0.088 |
| C 20:0 | 0.019[c] | 0.020[bc] | 0.021[ab] | 0.023[a] | *** | NS | 0.001 |
| **Total MUFA** | 4.85 | 4.85 | 4.61 | 4.76 | NS | NS | 0.535 |
| C 14:1 ω5 | 0.01 | 0.01 | 0.01 | 0.01 | NS | NS | 0.002 |
| C 16:1 ω7t | 0.05 | 0.05 | 0.05 | 0.05 | NS | NS | 0.007 |
| C 16:1 ω7 | 0.42 | 0.39 | 0.34 | 0.36 | NS | NS | 0.065 |
| C 18:1 ω9[4] | 3.71 | 3.70 | 3.43 | 3.55 | NS | NS | 0.426 |
| C 18:1 ω7t | 0.28 | 0.27 | 0.27 | 0.29 | NS | NS | 0.023 |
| C 20:1 ω9 | 0.04[b] | 0.04[b] | 0.04[ab] | 0.05[a] | * | NS | 0.005 |
| C 24:1 ω9 | 0.33[c] | 0.38[b] | 0.43[a] | 0.42[a] | *** | NS | 0.026 |
| **Total PUFA** | 4.52[d] | 6.06[c] | 7.33[b] | 8.63[a] | *** | NS | 0.687 |
| C 18:2 ω6 | 2.42[c] | 2.80[bc] | 3.00[ab] | 3.27[a] | *** | NS | 0.272 |
| C 18:3 ω6 | 0.02 | 0.02 | 0.01 | 0.02 | NS | NS | 0.002 |
| C 18:3 ω3 | 0.97[d] | 2.03[c] | 2.99[b] | 4.01[a] | *** | NS | 0.361 |
| C 18:4 ω3 | 0.03[d] | 0.05[c] | 0.06[b] | 0.08[a] | *** | NS | 0.009 |
| C 20:2 ω6 | 0.050[a] | 0.043[b] | 0.040[bc] | 0.036[c] | *** | NS | 0.004 |
| C 20:3 ω6 | 0.096[a] | 0.084[b] | 0.077[c] | 0.071[c] | *** | NS | 0.004 |
| C 20:4 ω6 | 0.35[a] | 0.28[b] | 0.26[b] | 0.23[c] | *** | NS | 0.013 |
| C 20:5 ω3 | 0.21[d] | 0.32[c] | 0.40[b] | 0.47[a] | *** | NS | 0.034 |
| C 22:4 ω6 | 0.04[a] | 0.03[b] | 0.02[c] | 0.02[c] | *** | NS | 0.003 |
| C 22:6 ω3 | 0.31[c] | 0.39[b] | 0.44[a] | 0.41[b] | *** | NS | 0.022 |
| **PUFA:SFA** | 1.08[d] | 1.41[c] | 1.72[b] | 2.05[a] | *** | NS | 0.020 |

[1] Values given in this table correspond to least-squares means obtained from ANOVA (n= 96) and their SE.

*P* values for the interaction between dietary level of added oil and α−TA supplementation in all cases were not significant.

[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil, respectively.

[3] FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[4] C 18:1 ω9 includes sum of *cis* and *trans* forms.

* = significant effect at $P \leq 0.05$; *** = $P \leq 0.001$.

PUFA resulted in a reduction in PUFA to SFA ratio when thighs were cooked (P ≤ 0.01). Most MUFA and PUFA in thigh meat were modified by dietary α−TA supplementation (Table 8.5). In some of these FA, an interaction between dietary α−TA and added oil level showed that α−TA supplementation only affected FA content in O2 treatments. However, in more predominant PUFA like LNA, EPA and DHA, this interaction was not significant.

**Table 8.4.** Regression equations: y = Fatty acid content in skinless breast meat or thigh meat with skin (expressed as g/kg); × = level of added oil (expressed as g/kg).

| Dependent variable[1] | Independent variable[2] | Tissue | Equation | $R^2$ | *P* |
|---|---|---|---|---|---|
| Total PUFA | Added oil level | Breast | $y = 8.44(1 - e^{(-0.037 \times)})$ | - | *** |
| Total PUFA | Dietary added oil | Thigh | $y = 52.91(1 - e^{(-0.037 \times)})$ | - | *** |
| Total MUFA | Dietary added oil | Thigh | $y = 38.42 - 0.15 \times$ | 0.21 | *** |
| Total SFA | Dietary added oil | Thigh | $y = 28.07 - 0.10 \times$ | 0.25 | *** |

[1] SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids.
[2] Added oil contains 65.2% of PUFA.

*** = $P \le 0.001$.

## 8.3. DISCUSSION

### 8.3.1. Effect of oil level

Isocaloric diets containing graded levels of fat would be expected to produce variations in fat deposit of edible portions of chicken meat commensurate with the theorized increased utilization of calories derived from fat (Jensen et al., 1970). However, the results of the present study did not clearly show this effect of fat, given that only FA content of breast meat increased as level of dietary oil increased.

The effect of the level of added oil on lipid content of edible portions of chicken meat has scarcely been studied. Roth et al. (1993) showed that fat deposit, such as abdominal fat pad, and intermuscular fat in breast and thigh decreased and intramuscular fat increased in chicken with increasing levels of dietary rapeseed oil. Similarly, Ajuyah et al. (1991) studied thigh and breast muscles, which reflect mainly intramuscular fat, and observed an increase in lipid content of thigh and breast meat of 22-27% and 13-16%,,respectively, when full-fat flax seed and canola oil plus flax meal were increased in isocaloric diets from 10% to 20%. Contrarily, Keren-Zvi et al. (1990) observed that breast muscle fat was not modified by the level of soyabean oil in the diet. In the present study, breast meat reflects mainly intramuscular fat, whereas thigh meat with skin reflects, on one hand, fat depot as subcutaneous deposit, and on the other hand, intermuscular and intramuscular fat. The differences between the pattern of total FA deposit in thigh and breast may be due to different functions of these FA in these tissues: structural FA in

intramuscular fat or storage FA in fat deposit. The deposit of total FA in breast may show the increasing utilization of calories derived from fat. However, some authors have reported that PUFA are a less efficient energy substrate than SFA and MUFA (Leyton et al., 1987), therefore increasing levels of dietary polyunsaturated oils might not result in an increased utilization of calories derived from fat in thigh meat. Nevertheless, further studies are needed on why different classes of fat deposited in chicken tissues have a different behaviour as dietary added oil increases.

The FA composition in chicken tissues is a combination of endogenous fat synthesis and direct deposition from dietary FA. Endogenous synthesis of FA mainly produces SFA such as C16:0 and C18:0, and MUFA, such as C18:1ω9 (Ajuyah et al., 1991; Crespo and Esteve-Garcia, 2002b). The strong influence of the level of oil added on PUFA and some long-chain MUFA in thigh and breast meat suggests that the origin of these FA is principally the diet. However, the apparent independence of total MUFA and SFA content in breast meat  from the level of added oil and the low relationship between MUFA and SFA content in thigh meat and diet, suggest that MUFA and SFA content in breast and thigh depends more on the endogenous fat synthesis than on direct deposition from diet.

However, the critical aspect is to quantify the amount of fat which is synthesized or directly accumulated from the feed. The relative importance of synthesis and direct deposition depends on dietary fat level, given that fat incorporation as an ingredient in broilers diet produces a decrease in the endogenous fat synthesis (Tanaka et al., 1983b; Donaldson, 1985). The present study suggests that in broilers fed diets with the highest fat level (O8), the endogenous synthesis was lower and the composition of the tissues reflected to a greater extent the dietary FA composition. Therefore, FA tissue to diet ratio in treatments O8 were closer to 1 than treatments supplemented with lower fat level. Similarly, Crespo and Esteve-Garcia (2002b) observed that broilers fed a basal diet with the lowest amount of fat showed negative values of fat losses and a percentage of fat efficiency much higher than 100%, indicating higher endogenous fat synthesis than broilers fed diets with 6% of added fat. Donaldson (1985) observed that lipogenesis and activities of the lipogenic enzymes increased as the level of fat was reduced. Therefore, the maximum FA synthesis happens when no fat is added to the diet. The total SFA and total MUFA equations in the present study allowed the quantification of the maximum theoretical responsibility of endogenous synthesis in thigh and breast meat.

Furthermore, endogenous synthesis influenced breast meat more markedly, whereas thigh was more influenced by direct deposition from diet. Modification of FA

composition of intramuscular fat, as in the case of breast muscle, has been reported to be more limited (Pan and Storlien, 1993; López-Bote et al., 1997). The scarce content of SFA and MUFA in diets may cause higher synthesis of these FA to maintain physical characteristics of cellular membranes in this tissue. On the other hand, FA in thigh with skin have a storage function and, therefore, they may be more easily influenced by FA from the diet.

### 8.3.2. Effect of dietary α−TA

The FA content of breast and thigh were not modified by dietary α−TA level. In a previous work with similar dietary polyunsaturation levels to the present study but with a constant 9% of added oil, the FA content of breast and raw and cooked thigh meat were neither modified by dietary α−TA level (Chapter 4). These authors explained that in literature this effect is rather controversial and the expression of the results as a percentage makes comparison of the results difficult. Quantifying the different FA with the aid of an internal standard, it was concluded that, regardless of the type and concentration of dietary fat, the effect of supplementing broilers diets with dietary α−TA did not have any significant effect on content of the main FA in thigh and breast meat.

### 8.3.3. Effect of cooking process

Cooking of thigh meat samples led to a reduction in PUFA content that was higher than that of MUFA and SFA. There are few studies that have dealt with the variation of FA composition when chicken meat is cooked. Some authors reported a similar reduction in all FA families when chicken meat was cooked at mild temperatures (80-90°C). However, this reduction was of lower magnitude than in the present study (Myers and Harris, 1975; Chapter 4.). Lopez-Ferrer et al. (1999c) found a 12.4% reduction in PUFA content when thigh meat was processed at higher temperatures (200°C).

Furthermore, contrary to the present study Chapter 4 showed that dietary α−TA had no significant effect on FA content of cooked thighs. However, thighs in that study had a lower PUFA:SFA ratio (1.21) than in the present study. Therefore, the effect of dietary α−TA on FA content of thigh meat may be manifested in those meats processed under hot temperatures, and therefore, subjected to higher oxidative stress.

The reduction in FA content of cooked thigh meat is probably due to two different effects. First, due to the manipulation of the samples during cooking, there is exudate that probably contains all FA families in a similar proportion. Second, due to the prooxidant conditions during cooking, FA undergo lipid oxidation which may result in a higher reduction of unsaturated FA, mainly PUFA.

**Table 8.5.** Effect of processing, α−tocopheryl acetate (α−TA) supplementation and dietary added oil level on the fatty acid content of raw and cooked thigh meat with skin, expressed as g/kg of thigh solids.[1]

| Fatty Acid[2] | Process | | | Dietary α−TA (mg/kg) | | | | | Significance[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw | Cooked | P | 0 | 100 | 200 | 400 | P | P Oil level | P Oil × α−TA | SE |
| **Total FA** | 312.01 | 269.05 | *** | 281.82[b] | 285.96[b] | 287.96[b] | 306.40[a] | ** | † | † | 6.52 |
| **Total SFA** | 75.10 | 65.61 | *** | 68.69[b] | 69.03[b] | 69.71[ab] | 73.99[a] | * | *** | † | 1.851 |
| C 10:0 | 0.040 | 0.037 | * | 0.039 | 0.039 | 0.038 | 0.040 | NS | *** | NS | 0.002 |
| C 12:0 | 0.09 | 0.08 | *** | 0.080[ab] | 0.082[ab] | 0.079[b] | 0.087[a] | * | * | † | 0.002 |
| C 14:0 | 2.67 | 2.36 | *** | 2.44 | 2.52 | 2.48 | 2.60 | † | *** | NS | 0.076 |
| C 15:0 | 0.39 | 0.34 | *** | 0.36 | 0.37 | 0.36 | 0.38 | † | *** | NS | 0.011 |
| C 16:0 | 51.05 | 44.76 | *** | 46.56[b] | 46.73[ab] | 47.69[ab] | 50.65[a] | * | *** | * | 2.241 |
| C 17:0 | 0.56 | 0.30 | *** | 0.44 | 0.46 | 0.42 | 0.42 | NS | *** | NS | 0.009 |
| C 18:0 | 19.72 | 17.23 | *** | 18.23[b] | 18.30[ab] | 18.10[b] | 19.27[a] | *** | *** | NS | 0.614 |
| C 20:0 | 0.36 | 0.32 | *** | 0.35 | 0.34 | 0.34 | 0.34 | NS | *** | NS | 0.014 |
| **Total MUFA** | 101.22 | 87.80 | *** | 91.97[b] | 90.83[b] | 94.08[ab] | 101.17[a] | *** | *** | ** | 3.354 |
| C 14:1 ω5 | 0.31 | 0.27 | *** | 0.28 | 0.28 | 0.30 | 0.31 | NS | *** | NS | 0.011 |
| C 16:1 ω7t | 1.26 | 1.10 | *** | 1.16[b] | 1,15[b] | 1.14[b] | 1.28[a] | *** | *** | * | 0.029 |
| C 16:1 ω7 | 10.01 | 8.81 | *** | 9.16 | 9.02 | 9.57 | 9.89 | NS | *** | † | 0.413 |
| C 18:1 ω9[3] | 81.16 | 70.47 | *** | 73.78[b] | 72.69[b] | 75.34[ab] | 81.47[a] | *** | *** | *** | 2.876 |
| C 18:1 ω7t | 4.64 | 4.03 | *** | 4.23[b] | 4.21[b] | 4.34[ab] | 4.57[a] | *** | *** | *** | 0.299 |
| C 20:1 ω9 | 0.96 | 0.82 | *** | 0.88 | 0.87 | 0.88 | 0.93 | † | *** | NS | 0.044 |
| C 21:1 ω9 | 0.07 | 0.07 | NS | 0.06 | 0.07 | 0.07 | 0.08 | † | *** | NS | 0.003 |
| C 24:1 ω9 | 2.49 | 1.97 | *** | 2.14[b] | 2.25[ab] | 2.17[ab] | 2.35[a] | * | *** | NS | 0.079 |
| **Total PUFA** | 135.68 | 115.64 | *** | 121.15[b] | 126.09[a] | 124.17[a] | 131.23[a] | *** | *** | NS | 3.765 |
| C 18:2tt | 0.12 | 0.10 | *** | 0.10 | 0.11 | 0.10 | 0.12 | † | NS | † | 0.003 |
| C 18:2 ω6 | 61.98 | 53.25 | *** | 55.78[b] | 57.76[ab] | 57.09[ab] | 59.82[a] | *** | *** | * | 2.638 |
| C18:3 ω6 | 0.34 | 0.29 | *** | 0.30[b] | 0.31[b] | 0.31[b] | 0.34[a] | *** | *** | *** | 0.010 |
| C 18:3 ω3 | 61.08 | 51.85 | *** | 54.40[b] | 56.46[ab] | 55.72[ab] | 59.29[a] | * | *** | NS | 1.467 |
| C 18:4 ω3 | 1.35 | 1.14 | *** | 1.19[b] | 1.25[ab] | 1.20[b] | 1.34[a] | * | *** | † | 0.041 |
| C 20:2 ω6 | 0.44 | 0.37 | *** | 0.39 | 0.41 | 0.40 | 0.41 | NS | *** | *** | 0.009 |
| C 20:3 ω6 | 0.63 | 0.53 | *** | 0.56 | 0.59 | 0.57 | 0.59 | † | *** | *** | 0.012 |
| C 20:4 ω6 | 1.91 | 1.58 | *** | 1.67[b] | 1.79[a] | 1.71[ab] | 1.81[a] | *** | *** | *** | 0.052 |
| C 20:5 ω3 | 4.64 | 3.79 | *** | 4.01 | 4.23 | 4.14 | 4.48 | † | *** | NS | 0.258 |
| C 22:4 ω6 | 0.19 | 0.15 | *** | 0.17 | 0.18 | 0.17 | 0.16 | NS | *** | * | 0.004 |
| C 22:6 ω3 | 2.66 | 2.19 | *** | 2.26[b] | 2.61[a] | 2.39[b] | 2.44[ab] | *** | *** | NS | 0.077 |
| **PUFA:SFA** | 1.87 | 1.82 | ** | 1.81 | 1.87 | 1.86 | 1.85 | NS | *** | † | 0.049 |

[1] Values given in this table correspond to least-squares means obtained from ANOVA (n = 192) and their pooled SE.

[2] P values for the interactions between process and both dietary added oil level and α−tocopheryl acetate supplementation in all cases were not significant. Oil: Dietary added oil.

[3] FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[3] C 18:1 ω9 includes sum of *cis* and *trans* forms.

* = significant effect at $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; † = $P \leq 0.10$.

# Capítulo 9

## Influencia del Nivel Dietético de Aceite Poliinsaturado y Vitamina E sobre el Contenido de $\alpha$-Tocoferol y la Oxidación Lipídica en la Carne de Pollo

# 9. α−Tocopherol Content and Lipid Oxidation in Chicken Meat as Affected by Dietary Polyunsaturated Oils and Vitamin E Levels

## 9.1. SUMMARY

The present study was carried out to evaluate the influence of diets containing increasing levels of polyunsaturated oils (PUFA: 65.2%) and different levels of supplementation with α−TA on the modifications of the α−Toc content and lipid oxidation of marketable raw and cooked chicken meat. One hundred and ninety-two female broiler chickens were randomly distributed into 16 isocaloric experimental diets resulting from the combination of 4 levels of added oil (2, 4, 6 and 8%) and 4 levels of supplementation with α−TA (0, 100, 200 and 400 mg/kg). At 40 days, α−Toc content in thigh meat depended more on α−Toc and PUFA composition of the diet than their content in meat. Thus, it increased at a rate of 0.15, 0.11 and 0.09 mg/kg when α−Toc increased 1 mg/kg of feed containing 2, 4 and 6-8% of polyunsaturated oil, respectively. A similar response was observed in breast meat but with rates of α−Toc incorporation 1.8-2.0 times lower than in thigh meat. Lipid oxidation of thigh meat mainly depended on its composition, increasing linearly as the concentration of PUFA in raw meat increased, and decreasing non-linearly as its α−Toc content increased at a fractional rate of 48.9%. Non-linear relationship between TBA values in cooked thigh and α−Toc content in raw meat showed saturation in the antioxidant effect of α−Toc.

## 9.2. Diet composition

The α−Toc content of the experimental diets is shown in **Table 9.1**. Increasing the level of added oil which contained high concentration of PUFA, resulted in PUFA increased content, and to a lesser extent, in SFA and MUFA increased content. Therefore, PUFA content of diets was 28 ± 0.7 (O2), 38 ± 1.6 (O4), 48 ± 1.8 (O6) and 59 ± 1.9 (O8) g/kg of feed (Chapter 8).

**Table 9.1.** α−Tocopherol content of experimental diets, expressed as mg/kg[1].

| Level of added oil[2] | Dietary supplementation with α−tocopheryl acetate[3] | | | |
|:---:|:---:|:---:|:---:|:---:|
| | E0 | **E1** | **E2** | **E4** |
| **O2** | 4 ± 0.2 | 105 ± 8.1 | 173 ± 2.2 | 351 ± 7.6 |
| **O4** | 3 ± 0.2 | 98 ± 3.0 | 200 ± 7.6 | 354 ± 7.1 |
| **O6** | 3 ± 0.3 | 96 ± 1.2 | 198 ± 0.7 | 336 ± 16.5 |
| **O8** | 3 ± 0.2 | 96 ± 6.9 | 191 ± 14.1 | 316 ± 21.2 |

[1] These values correspond to means ± SE.
[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil (65.2% PUFA), respectively.
[3] E0: without supplementation with α−tocopheryl acetate; E1: supplemented with 100 mg/kg α−tocopheryl acetate; E2: supplemented with 200 mg/kg α−tocopheryl acetate; E4: supplemented with 400 mg/kg α−tocopheryl acetate.

## 9.3. α−Tocopherol content of thigh and breast meat

The α−Toc content of thigh and breast meat, expressed as mg/kg of tissue, is shown in **Table 9.2**. As can be observed there is a wide range of variability in the α−Toc content of chicken meat. Therefore, at dietary levels of 200 mg α−TA/kg of feed, α−Toc content ranged from 15.0 to 24.3 mg/kg in thigh meat and from 7.1 to 13.2 mg/kg in breast meat. Similar values have been reported in thigh meat with skin (Ruiz et al., 1999; Grau et al., 2001a,b), and breast meat (Galvin et al., 1993; Morrissey et al., 1997; O'Neill et al., 1998a) for similar levels of dietary α−TA supplementation.

The α−Toc content in thigh from treatments supplemented with α−TA was 1.5-2.3 times higher ($P \leq 0.001$) than those in breast. These results agree with other authors who observed α−Toc content of thighs was 1.1-2.2 times higher than those of breasts (Lin et al., 1989a; Ahn et al., 1995; Cherian et al., 1996; De Winne and Dirinck, 1996; Nam et al., 1997; O'Neill et al., 1998a; Malczyk et al., 1999). Some authors have suggested that higher content of PUFA may cause a greater need for α−Toc to prevent lipid oxidation (Jensen et al., 1997). Therefore, in the present study the absolute amount of PUFA in thigh meat was 6-7 times higher than in breast meat (Chapter 8). When the α−Toc in muscle was expressed based on the total PUFA content of meat, it revealed that the α−Toc concentration in breast meat (116.2, 171.6 and 335.3 mg/100g of total PUFA for E1, E2

and E4, respectively) was 3-4 times higher than thigh (27.9, 49.3 and 98.3 mg/100g of total PUFA for E1, E2 and E4, respectively). These results agree with those of Jensen et al. (1997) and Chapter 5 who also observed that α−Toc to PUFA ratio in breast meat was 2 and 3-4 times higher than thigh meat, respectively. Despite the fact that the latter authors observed a similar difference between α−Toc to PUFA ratio in thigh and breast meat, the magnitude of this ratio was higher than in the present study in concordance with the dietary α−Toc content. It seems that the α−Toc deposit in chicken tissues depends on studied tissue, as well as on the real α−Toc content supplemented in the diet. Thus, it is very important to quantify and not to estimate the α−Toc content of feed.

**Table 9.2.** Effect of dietary polyunsaturation and α−tocopheryl acetate supplementation on α−tocopherol content in thigh and breast meat (mg/kg)[1].

| Level of added oil[2] | Thigh meat | | | | | Breast meat | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0[3] | E1 | E2 | E4 | Global Mean | E0 | E1 | E2 | E4 | Global Mean |
| O2 | 0,33$^g$ | 12,57$^{ef}$ | 24,34$^{cd}$ | 55,46$^a$ | 23,17$^x$ | 0,41$^g$ | 8,54$^e$ | 13,19$^{cd}$ | 29,29$^a$ | 12,86$^x$ |
| O4 | 0,41$^g$ | 12,89$^{ef}$ | 19,05$^{de}$ | 41,56$^b$ | 18,48$^y$ | 0,52$^g$ | 7,33$^{ef}$ | 10,42$^{cde}$ | 20,94$^b$ | 9,80$^y$ |
| O6 | 0,10$^g$ | 10,34$^{ef}$ | 17,57$^{de}$ | 30,52$^c$ | 14,63$^z$ | 0,16$^g$ | 6,24$^{ef}$ | 9,84$^{de}$ | 13,44$^{cd}$ | 7,42$^z$ |
| O8 | 0,04$^g$ | 6,05$^{fg}$ | 14,98$^{ef}$ | 30,64$^c$ | 12,93$^z$ | 0,17$^g$ | 3,85$^{fg}$ | 7,10$^e$ | 15,04$^c$ | 6,54$^z$ |
| Global Mean | 0,22$^z$ | 10,46$^y$ | 18,98$^x$ | 39,55$^w$ | | 0,31$^z$ | 6,49$^y$ | 10,14$^x$ | 19,68$^w$ | |
| SE | 1.840 | | | | | 0.923 | | | | |
| P values | | | | | | | | | | |
| Fat level | 0.001 | | | | | 0.001 | | | | |
| α−TA level | 0.001 | | | | | 0.001 | | | | |
| Fat level × α−TA level | 0.001 | | | | | 0.001 | | | | |

[1] Values given in this table correspond to least-square means obtained from multifactorial ANOVA (n = 96) and their pooled SE.
[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil (65.2% PUFA), respectively.
[3] E0: without supplementation with α−tocopheryl acetate; E1: supplemented with 100 mg/kg α−tocopheryl acetate; E2: supplemented with 200 mg/kg α−tocopheryl acetate; E4: supplemented with 400 mg/kg α−tocopheryl acetate.
[a,b,c,d,e,f,g] Different superscripts indicate significant differences in the interaction between dietary polyunsaturation and α−TA supplementation.
[w,x,y,z] Grand means in the same column/row with different superscript were significantly different.

Relationships between α−Toc content in feed and tissues are shown in **Table 9.3**. As expected, α−Toc content of thigh and breast meat significantly increased as the dietary α−Toc supplementation increased. The incorporation rates of α−Toc content in thigh meat were 1.8 to 2.3 times higher than those from breast meat ($P \leq 0.001$), in concordance with other authors in the literature (Lin et al., 1989a,b; Chapter 5). However, incorporation rates of α−Toc were influenced by the level of added oil. Thus, linear regression analysis showed that, when 2% of polyunsaturated oil (O2) was added, α−Toc content in thigh and breast meat increased at a rate of 0.15 mg/kg and 0.08 mg/kg, respectively, for each mg

increase of α−Toc per kg feed, whereas in treatments supplemented with the highest levels of added oil (O6 and O8), this increase was 0.09 mg/kg and 0.04 mg/kg in thigh and breast meat, respectively. Thus, the α−Toc content of thigh and breast meat were reduced as the inclusion of dietary polyunsaturated oil increased (**Table 9.4**).

**Table 9.3.** Regression equations: $y = $ α−Tocopherol content in thigh and breast (mg/kg) and $\times = $ α−tocopherol content of feed (mg/kg).

| Independent variable[1] | Dietary treatments[2] | Thigh meat | | | Breast meat | | |
|---|---|---|---|---|---|---|---|
| | | Equation | $r^2$ | P | Equation | $r^2$ | P |
| α−Tocopherol | O2 | $y = 0.15\times$ | 0.98 | 0.001 | $y = 0.08\times$ | 0.98 | 0.001 |
| content of | O4 | $y = 0.11\times$ | 0.93 | 0.001 | $y = 0.06\times$ | 0.97 | 0.001 |
| feed [mg/kg] | O6, O8 | $y = 0.09\times$ | 0.95 | 0.001 | $y = 0.04\times$ | 0.93 | 0.001 |

[1] Values obtained by analysis of feeds.
[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil (65.2% PUFA), respectively.

The decrease rates of α−Toc in thigh meat when added oil was increased by 1% were 0.06 mg/kg, 1.06 mg/kg, 1.48 mg/kg and 4.48 mg/kg for E0, E1, E2 and E4, respectively. Decrease rates of α−Toc in breasts when added oil was increased by 1% were lower than thigh, and were 0.05 mg/kg, 0.75 mg/kg, 0.94 mg/kg and 2.66 mg/kg for E0, E1, E2 and E4, respectively. Similarly to our results, some authors found that α−Toc content in liver and serum of rats decreased as dietary level of DHA (C22:6ω3) increased (Saito et al., 1996; Kubo et al., 1997). This could be explained by the possibility that α−Toc is degraded in part by its activity in suppressing increased tissue lipid oxidation. However, the variability in α−Toc content of thigh and breast meat was caused more by the dietary level of polyunsaturated oil than by the PUFA content of meat (Table 9.4), which is poorly or not at all correlated ($r^2$ ranged from 0.15 to 0.50; data not shown). Therefore, as the level of oil in the diet increases, the deposition of α−Toc in thigh meat is reduced and this may be attributed to the consumption of α−Toc, not only in the muscles, but also during the digestion and absorption of lipids and their transport in the lipoproteins Furthermore, it can be observed that the effect of polyunsaturated added oil level was more evident in diets supplemented with high doses of α−TA. These results agree with other authors (Kubo et al., 1997; Chapter 5) who suggested that "in vivo" recycling mechanism of α−Toc may not be efficient enough under high oxidative status.

Since α−Toc content in thigh and breast meat depended mainly on α−Toc and PUFA content of the diet, increasing linearly with dietary α−TA supplementation and decreasing linearly with dietary polyunsaturated added fat, and in order to compare both variables, the following equations were calculated:

Thigh meat: $y = \times_1(0.166 - 0.011\times_2)$ ($P \leq 0.001$)

Breast meat: $y = x_1(0.090 - 0.007x_2)$ $(P \le 0.001)$

where y is $\alpha$−Toc concentration in the studied tissue (mg/kg), $x_1$ is $\alpha$−Toc content of the diet (mg/kg) and $x_2$ is the level of polyunsaturated added oil in the diet (g/100g). The estimated response surface for $\alpha$−Toc content in thigh and breast meat are shown in **Figure 9.1**.

**Table 9.4.** Regression equations: y = $\alpha$−Tocopherol content in thigh and breast meat (mg/kg); $x_1$ = dietary added oil level (g/100g).

| Independent variable | Dietary treatments[1] | Thigh | | | Breast | | |
|---|---|---|---|---|---|---|---|
| | | Equation | $r^2$ | P | Equation | $r^2$ | P |
| Level of added oil (g/100g) | E0 | $y = 0.51 - 0.06x_1$ | 0.22 | 0.020 | $y = 0.58 - 0.05x_1$ | 0.26 | 0.011 |
| | E1 | $y = 15.77 - 1.06x_1$ | 0.40 | 0.002 | $y = 10.23 - 0.75x_1$ | 0.53 | 0.001 |
| | E2 | $y = 26.37 - 1.48x_1$ | 0.40 | 0.001 | $y = 14.85 - 0.94x_1$ | 0.41 | 0.001 |
| | E4 | $y = 61.71 - 4.48x_1$ | 0.58 | 0.001 | $y = 32.82 - 2.66x_1$ | 0.70 | 0.001 |

[1] E1: supplemented with 100 mg/kg $\alpha$−tocopheryl acetate; E2: supplemented with 200 mg/kg $\alpha$−tocopheryl acetate; E4: supplemented with 400 mg/kg $\alpha$−tocopheryl acetate.

**Figure 9.1.** Estimated response surface for $\alpha$−tocopherol content in thigh (A) and breast (B) meat (mg/kg) to variation in dietary content of $\alpha$−tocopherol (mg/kg) and oil level (g/100g).

**A**                                                **B**
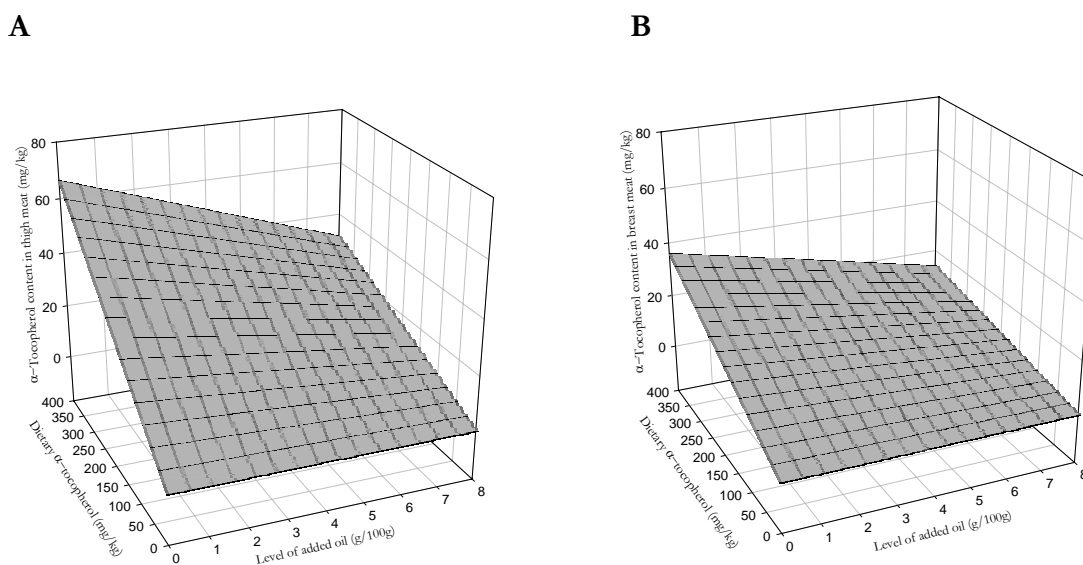


## 9.4. Lipid oxidation in cooked thigh meat

The TBA values (expressed as µg MDA/kg) for cooked thigh meat are shown in **Table 9.5.** TBA values in cooked thigh meat increased with the level of added oil. Lipid oxidation in thigh meat from O4, O6 and O8 treatments were 1.9, 2.7 and 3.9 times higher, respectively, than those from O2 treatments (P $\le$ 0.001). Contrarily to $\alpha$−Toc content, TBA values directly depended on the PUFA content of thigh meat. Therefore, **Table 9.6**

shows linear regressions between PUFA content and TBA values in cooked thigh meat for different levels of supplementation with $\alpha$−TA. Lipid oxidation in cooked thigh meat increased linearly as the concentration of PUFA in raw thigh increased, but this increase was lower as the dietary $\alpha$−TA supplementation increased. However, the comparative response for treatments supplemented with 200 and 400 mg $\alpha$−TA/kg was not significantly different.

**Table 9.5.** Effect of dietary fat level and $\alpha$−tocopheryl acetate supplementation on TBA values in cooked thighs (expressed as µg malondialdehyde/kg of thigh)[1].

| Level of added oil[2] | Global Mean | $\alpha$−Tocopheryl acetate (mg/kg)[3] | | | |
|---|---|---|---|---|---|
| | | E0 | E1 | E2 | E4 |
| **O2** | 390.4[c] | 1011.2 | 190.3 | 231.3 | 128.7 |
| **O4** | 745.1[bc] | 1740.6 | 677.5 | 355.8 | 206.5 |
| **O6** | 1055.2[b] | 2137.7 | 884.7 | 886.3 | 312.5 |
| **O8** | 1505.1[a] | 2545.7 | 1754.4 | 1129.7 | 590.5 |
| | | | | | |
| Global Mean | | 1858.8[a] | 876.7[b] | 650.8[bc] | 309.5[c] |
| **SE** | 115.62 | | | | |
| | | | | | |
| P values | | | | | |
| Fat level | 0.001 | | | | |
| $\alpha$−TA level | 0.001 | | | | |
| Fat level × $\alpha$−TA level | 0.338 | | | | |

[1] Values given in this table correspond to least-square means obtained from ANOVA (n = 96) and their pooled SE.
[2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil (65.2% PUFA), respectively.
[3] E0: without supplementation with $\alpha$−tocopheryl acetate; E1: supplemented with 100 mg/kg $\alpha$−tocopheryl acetate; E2: supplemented with 200 mg/kg $\alpha$−tocopheryl acetate; E4: supplemented with 400 mg/kg $\alpha$−tocopheryl acetate.
[a,b,c,d] Grand means in the same column/row with different superscript were significantly different.

The $\alpha$−TA supplementation resulted in a significant protection against oxidation in cooked thigh meat (Table 9.5). The reduction of TBA values with $\alpha$−TA, indicate that this antioxidant still remained active after processing at 80°C. Supplementation with 100, 200 and 400 mg $\alpha$−TA/kg of feed caused a reduction of TBA values in cooked thigh meat by 52.5, 54.0 and 83.4 %, respectively, than in thighs from non-supplemented treatments. However, the response was not significantly different for the treatments supplemented with either 200 or 400 mg $\alpha$−TA/kg. These results indicated that $\alpha$−TA inclusion at levels higher than 200 mg/kg did not improve lipid stability, in terms of TBA values, of thigh meat, in our experimental conditions. Reduction of TBA 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., 1989a,b; 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., 1998a; Ruiz et al., 1999; Grau et al., 2001a,b). Effectively, TBA values in thigh meat depended on the α–Toc content (Table 9.6).

**Table 9.6.** Multiple regression equations: $y$ = TBA values (μg malondialdehyde/kg); $x_1$ = PUFA content of raw thighs (g/kg); $x_1$ = α–tocopherol content of raw thigh (mg/kg).

| Independent variable | Dietary treatments[1] | Equation | $R^2$ | $P^2$ |
|---|---|---|---|---|
| PUFA content of raw thigh meat (g/kg) | E0 | $y = 46.42x_1$ | 0.88 | 0.001 |
| | E1 | $y = 21.75x_1$ | 0.78 | 0.001 |
| | E2, E4 | $y = 11.52x_1$ | 0.68 | 0.001 |
| α–Tocopherol content of raw thigh meat (mg/kg) | O2 | $y = 188.8 + 933.9e^{-0.489x_2}$ | - | 0.001 |
| | O4 | $y = 417.5 + 1506.1e^{-0.489x_2}$ | | |
| | O6 | $y = 729.7 + 1421.4e^{-0.489x_2}$ | | |
| | O8 | $y = 1146.4 + 1468.4e^{-0.489x_2}$ | | |

[1] E0: without supplementation with α–tocopheryl acetate; E1, E2 and E4: supplemented with 100, 200 and 400 mg/kg α–tocopheryl acetate, respectively; [2] O2, O4, O6, and O8: 2%, 4%, 6%, and 8% of added oil (65.2% PUFA), respectively.
[2] Significance of the model.

Relationship between TBA values of cooked thighs and α–Toc content of raw thighs for the different dietary levels of added oil (65.2% PUFA) showed a non-linear relation. Equations of the type: $y = a + be^{(-cx)}$ were established, where $y$ represents TBA values in cooked thigh and $x$ is α–Toc content in raw thigh. 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 raw thigh meat did not always imply a reduction in lipid oxidation. Thus, at low α–Toc content in raw thigh meat a marginal increase of α–Toc sharply reduced lipid oxidation, whereas a large α–Toc content in thigh only caused a slight improvement in lipid oxidation or none at all. In general, both maximum (a + b) and minimum (a) TBA values obtained through α–TA supplementation increased as dietary added oil level increased. Supplementation with α–TA, prevented 56%, 66%, 78% and 83% of the maximum lipid oxidation in O2, O4, O6 and O8, respectively. The decrease fractional rate of TBA values was 48.9% in all cases, indicating that α–Toc antioxidant effectiveness was not different for all dietary added oil levels. Other authors have reported 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) relationships between α–Toc concentration and TBA values in poultry tissues. Contrary to our results, prediction equations from all of these authors did not find

saturation in the antioxidant effect of α−Toc. However, most of these authors worked with lower concentrations of α−Toc in thigh meat of poultry (2-20 mg α−Toc/kg) than those in our study. Nevertheless, in Chapter 7 broiler diets were supplemented with similar α−TA doses than in the present chapter and saturation in the antioxidant effect of thigh meat was also observed.

No interaction between the level of added oil and dietary supplementation with α−TA was observed. That is, the protective effect of different levels of α−Toc against lipid oxidation did not depend on the level of added oil in spite of working with higher levels of dietary polyunsaturation. Similarly to our results, Bou et al. (2004) did not find an interaction between dietary levels of added fish oil and α−TA on TBA values in chicken meat. Nevertheless, some authors have reported an interaction when different sources of dietary fat source were used (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). These authors showed that thigh meat from chickens fed the more polyunsaturated diets, and therefore, containing more PUFA, were more influenced by α−TA supplementation than those fed the more saturated diets.

Since lipid oxidation in cooked thigh meat increased linearly with its PUFA content and decreased with its α−Toc content, the following equation were calculated:
$y = x_1(5.26 + 41.55e^{(-0.087 \times_2)})$ (P ≤ 0.001), where y is the TBA value in cooked thigh meat (μg MDA/kg), $x_1$ is PUFA content in cooked thigh meat (g/kg) and $x_2$ is α−Toc content in raw thigh meat (mg/kg). The estimated response surface for TBA values in cooked thigh meat is shown in **Figure 9.2**.

In conclusion, α−Toc content in chicken meat depends more on the α−Toc and PUFA composition of the diet than their content in meat. α−Toc content in thigh and breast meat increases as dietary α−Toc increases and decreases as dietary level of polyunsaturated oil increases. However, lipid oxidation of thigh meat mainly depends on its composition, increasing as its PUFA content increases and decreasing as its α−Toc content increases. Furthermore, the antioxidant effect of α−Toc presents saturation on lipid oxidation in cooked thigh meat.

**Figure 9.2.** Estimated response surface for TBA values (μg malondialdehyde (MDA)/kg) in cooked thigh meat to variation in dietary content of α–tocopherol (mg/kg) and oil level (g/100g).