Arch.Geflügelk., 70 (3). S. 98-105, 2006, ISSN 0003-9098. © Verlag Eugen Ulmer, Stuttgart

Influence of dietary polyunsaturation level on $\alpha\mbox{-tocopherol}$ content in chicken meat

Einfluss des Grades der Ungesättigtheit der Fettsäuren im Futter auf den Gehalt an α -Tocopherol im Hühnerfleisch

Lucia Cortinas*, Maria D. Baucells*, Cecilia Villaverde*, F. Guardiola**, S. K. Jensen*** and Ana C. Barroeta*

Manuskript eingegangen am 3. Dezember 2004, angenommen am 20. März 2005

Introduction

In recent years, human nutritionists have recommended an increase in the intake of polyunsaturated fatty acids (PUFA) because of their beneficial effects on health, mainly in the prevention of cardiovascular disease (KRAUSS et al., 2001). Some authors have increased the PUFA content of chicken meat by enriching animal diets in polyunsaturated fats (LIN et al., 1989; AJUYAH et al., 1993; LÓPEZ-FERRER et al., 1999, 2001; GONZALEZ-ESQUERRA and LEESON, 2000). However, a higher PUFA content in poultry meat increases its susceptibility to oxidation (MARASCHIELLO et al., 1999; RUIZ et al., 1999; GRAU et al., 2001a,b) and, as a consequence, also enhances the development of organoleptic problems compromising meat quality (MALCZYK et al., 1999; GONZALEZ-ES-QUERRA and LEESON, 2000; BOU et al., 2001).

In order to improve the oxidative stability and, thus, to increase the shelf life of meat, antioxidants have been successfully added to animal feeds. Among them, α -tocopheryl acetate (α -TA) has demonstrated the highest efficiency in preventing lipid oxidation (LIN et al., 1989; AHN et al., 1995; DE WINNE and DIRINCK, 1996; GRAU et al., 2001a,b), in improving the sensory quality of poultry meat (O'NEILL et al., 1998; MALCZYK et al., 1999; BOU et al., 2001), and in increasing the vitamin E content in meat (MILLER and HUANG, 1993; O'NEILL et al., 1998; GRAU et al., 2001a,b; FLA-CHOWSKY et al., 2002; BOU et al., 2004). Thus, supplementation with α -TA will often increase the quality and nutritive value of the food products obtained. However, from the literature it is obvious that supplementation of the feed with α -TA leads to different levels of vitamin E in the tissues investigated (O'NEILL et al., 1998, MARASCHIELLO et al., 1999; Ruiz et al., 1999). Part of this variability may be attributed to the analytical methods used to determine α -tocopherol (α -Toc) in feed and animal tissues (BALL, 1996). However, there is evidence that the deposition of α -Toc in chicken meat depends on the lipid profile of the meat, and ultimately of the diet (MILLER and HUANG, 1993). Most studies limit their research to different fat sources or different amounts of fat and do not take into account the actual degree of polyunsaturation of the dietary fat and how it may affect the deposition of α -Toc.

The aim of the present study was to determine the effects of increasing amounts of dietary PUFA and different levels of supplementation with α -TA on α -Toc content of broiler breast and thigh meat.

Materials and methods

Animals and diets

The experiment received prior approval from the Animal Protocol Review Committee of the Universitat Autònoma of Barcelona, housing and husbandry of all animals conformed to European Union guidelines.

One hundred and ninety-two Ross strain 308 female broilers at one day of age were randomly distributed into 16 dietary treatments. Three cages by four birds were used for each of 16 dietary treatments.. The animals were housed under standard conditions of temperature, humidity and ventilation. The diet was formulated to meet or exceed recommendations of NRC (1994) on the basis of 39% wheat, 34% soybean meal and 13% barley (Table 1). The experimental treatments resulted from the combination of 4 levels of supplementation with α-TA (Rovimix [®] E-50 Adsorbate. F. Hoffmann- LaRoche Ltd., Basel, Switzerland): 0 (E0), 100 (E1), 200 (E2), and 400 (E4) mg/kg of feed, and 4 levels of dietary PUFA. The dietary PUFA levels were achieved by blending different proportions of tallow (100, 60, 40 and 0%) and a mixture of linseed and fish oils (0, 40, 60 and 100%), keeping total added fat constant (9%).

Tallow and linseed oil were provided by Cailà-Parés S. A. (Barcelona, Spain) and fish oil was provided by Agrupación de Fabricantes de Aceites Marinos, S.A. (Vigo, Spain).

The fatty acid content of the experimental diets is shown in Table 2. More details about fatty acid content of these diets are found in CORTINAS et al. (2004). The increase in the polyunsaturation level of diets, while maintaining the total fatty acid content constant, was achieved at the expense of the saturated and monounsaturated fatty acids. Therefore the PUFA to SFA ratio of diets was 0.4, 1.0, 1.7 and 3.9 for PU15, PU34, PU45 and PU61, respectively.

Feed (all-mash) and water were provided *ad libitum* during the experimental period. Feed samples were taken during the experiment for α -Toc and fatty acid analysis.

Sample collection

At the end of the experimental period (44 days of age), two animals per cage were randomly selected and processed in a commercial slaughterhouse. The edible portion of thighs

^{*} Department of Animal and Food Science, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain ** Department of Nutrition and Food Science-CeRTA, Facultat de Farmàcia,

Universitat de Barcelona, Barcelona, Spain

^{***} Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark

Table 1. Composition and content of nutrients of basic diet (expressed as % of fresh matter) ¹
Zusammensetzung und Inhaltsstoffe der Grundration

Ingredients	%	Composition	%
Wheat	39.3	Dry matter	90.8
Soybean meal 48% CP	34.01	Crude protein	23.0
Barley	13.4	Crude fat	10,2
Added fat	9.0	Crude fibre	3.5
Bicalcium phosphate	2.2	Ash content	6.1
Calcium carbonate	1.0	Crude Energy (Kcal/kg)	4481
Salt	0.4	Metabolizable Energy (Kcal/kg) ³	3100
Vitamin-mineral mix ²	0.4		
DL-Methionine	0.28		
L-Lysine	0.04		

¹Values given in this table are means of 16 dietary treatments, result of a 4 × 4 factorial design with 4 different proportions of tallow, linseed and fish oil, and 4 different levels of dietary supplementation with α -tocopheryl acetate (0, 100, 200 and 400 mg/kg).

² Vitamin and mineral mix per kg of feed: Vitamin A: 12000 IU; Vitamin D₃: 2400 IU; Vitamin K₃: 3 mg; Vitamin B₁: 2.2 mg; Vitamin B₂: 8 mg; Vitamin B₆: 5 mg; Vitamin B₁₂: 11 μg; Folic acid: 1.5 mg; Biotin: 150 μg; Calcium pantotenate: 25 mg; Nicotinic acid : 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0.33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.15 mg.

³ Calculated value (FEDNA; http://www.etsia.upm.es/fedna/tables.htm)

Table 2. Fatty acid composition of the experimental diets, expressed as g per kg ¹
Fettsäuremuster der Versuchsrationen (g je kg Futter)

		Polyunsatu	ration level ²	
Fatty Acid ³	PU15	PU34	PU45	PU61
Total FA	100.45	00.01	00.57	06.80
TOTALEA	100.45	98.81	99.57	96.89
Total SFA	43.75	32.38	26.22	15.74
Total MUFA	41.30	32.55	28.32	20.31
Total PUFA	15.40	33.77	45.03	60.84
C 18:2 ω6	13.16	16.23	17.98	20.17
C 18:3 ω3	1.55	16.45	24.62	36.27
C 20:5 ω3	ND	0.81	1.77	3.35
C 22:6 ω3	ND	0.07	0.18	0.33
PUFA:SFA	0.35	1.04	1.72	3.87

¹ Values given in this table are means of 4 dietary treatments with different level of supplementation with α -tocopheryl acetate: 0, 100, 200 and 400 mg/kg.

² 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.

³ FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. ND: Not detected.

ND: Not detected

and breasts were removed and weighed individually. Thighs were deboned and ground with skin whereas breasts were ground without skin. Thighs were collected with skin because it is usual to commercialize them this way. Tissue samples were freeze-dried, ground and stored at -20° C until further analyses.

α -Tocopherol analysis

The α -Toc from feeds, thighs and breasts was extracted as described by JENSEN et al. (1999) starting from 2 g of feed sample and 100 mg of freeze-dried thigh and breast. α -Toc content was determined by normal-phase HPLC. The α -Toc recoveries were examined in order to observe if the

lipid polyunsaturation level of chicken meat affects the α -Toc determination. Two levels of α -Toc standard (10 and 20 µg) (Sigma, St. Louuis, MO 63103, USA) were added to 100-mg aliquots of tissue samples. α -Toc recovery for each α -Toc addition level in thigh and breast meat samples was determined using 4 aliquots.

Fatty acids analysis

Fatty acid content of the experimental diets was analyzed by gas-chromatography following the direct transesterification method described by SUKHIJA and PALMQUIST (1988). Nonadecanoic acid (Sigma, St. Louis MO, USA) was used as internal standard. Medium and long chain fatty acids

		Dietary supplementation v	with α -tocopheryl acetate ²	
Dietary polyunsaturation ¹	EO	E1	E2	E4
PU15	5 ± 0.3	135 ± 7.3	252 ± 13.3	441 ± 32.4
PU34	6 ± 0.7	136 ± 3.7	243 ± 15.3	452 ± 12.3
PU45	6 ± 0.8	138 ± 11.2	232 ± 5.7	477 ± 25.0
PU61	5 ± 0.6	135 ± 6.3	219 ± 7.9	436 ± 47.1

Table 3. Analysed α -Tocopherol values in experimental diets (mg/kg: mean ±SE). Analysierte α -Toc-Gehalte in den Versuchsrationen (mg/kg; Mittelwert ± Standardfehler)

¹ 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 ϵ 0: 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.

Table 4. Recovery of α -tocopherol (α -Toc; %) in meat from broilers fed diets with different degree of polyunsaturation Einlagerung von α -Tocopherol (α -Toc; %) in das Fleisch von Broilern, bei Fütterung mit Rationen mit unterschiedlichem Grad der Ungesättigtheit der Fettsäuren

			Recove	ry ¹			
Dietary		Thigh with ski	n	Breast			
	α–Toc standard		Global Recovery	α–Toc s	Global Recovery		
treatments ²	10 ^A	20 ^A	(n = 8)	10 ^A	20 ^A	(n = 8)	
	(n = 4)	(n = 4)		(n = 4)	(n =4)		
PU15	86.61	87.49	87.05ª	93.51	93.33	93.42 ^c	
PU34	78.41	78.77	78.59 ^b	94.89	93.48	94.18 ^{bc}	
PU45	73.78	76.52	75.15 ^c	95.23	94.42	94.82 ^b	
PU61	59.13	61.17	60.15 ^d	97.87	97.98	97.92 ^a	
<i>P</i> values			***			***	

¹ Total recovery values as the means of recoveries measured after two levels of α -tocopherol standard addition.

² 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.

^A Level of addition of α -tocopherol: 10 or 20 µg α -tocopherol/100 mg freeze-dried sample.

^{a,b,c,d} Values in the same column with different superscript are significantly different (p<0.001).

*** = *P*≤0.001.

were quantified, and the ones containing 2 or more double bonds were included in the group of PUFA.

Statistical analysis

The Mann-Whitney U test was used to determine whether the α -Toc recoveries in thigh (n = 4) and breast (n = 4) meat were affected by the α -Toc addition levels. One-way ANOVA was performed to determine whether the PUFA dietary levels affected α -Toc global recoveries in thigh (n = 8) and breast (n = 8) meat. Effect of a dietary treatment on α -Toc content in chicken thigh (n = 96) and breast (n = 96) meat was tested by multifactorial ANOVA with repeated measurements. Data were treated using the MIXED procedure of SAS package (SAS[®] Institute 2000). Differences between treatment means were tested using Tukey's multiple range test. The relationship between α -Toc content in thigh (n = 96) and breast (n = 96) meat and dietary α -Toc or PUFA concentration was fitted by linear regression equation of type $y = a + b \times$. When the slopes were not different among treatments, only one equation was presented. The effect of the interaction between dietary α -Toc and PUFA concentration on α -Toc content of thigh and breast meat was fitted using GLM (general linear model) procedure of SAS[®] (n = 96). The interaction allowed comparing the slopes of the equations. Relationship between α -Toc content in thigh (n = 96) and breast (n = 96) meat, respectively, on the one hand, and dietary α -Toc and PUFA on the other hand, was fitted by using non-linear regressions by means of the NLIN (non-linear regression) procedure of SAS[®]. The equation type was y = x₁(a - bx₂), where y is the α -Toc concentration in studied tissues, x₁ is α -Toc content of the diet and x₂ is PUFA content of the diet. This data analysis was represented using SigmaPlot 8.02 (2002). In all cases, $P \le 0.05$ were considered significant.

Results and discussion

The α -Toc content of the experimental diets is shown in Table 3. It can be observed that the analyzed dietary α -Toc content was approximately 20% higher than the theoretical value.

Recovery of α -tocopherol

As hypothesized, the level of dietary supplementation with α -TA did not affect α -Toc recoveries in thigh and breast

Table 5. Effect of dietary polyunsaturation and α -tocopheryl acetate supplementation on least-squares means and their pooled SE of α -tocopherol content (mg/kg) in thigh with skin and breast meat¹

Einfluss des Grades der Ungesättigtheit der Fettsäuren im Futter und der Zulage an α -Tocopheryl-Acetat zum Futter auf den α -Tocopherrol-Gehalt im Schenkel- (mit Haut) und im Brustfleisch von Broilern (LSQ-Mittelwerte und gepoolte Standardfehler)

			Thigh					Breast		
Dietary polyunsaturation ²	E0 ³	E1	E2	E4	Grand Mean	EO	E1	E2	E4	Grand Mean
PU15	2.6 ^z	20.6 ^y	37.3 ^{axy}	53.2 ^{ax}	28.4 ^A	1.3 ^z	9.0 ^y	17.3 ^{ax}	26.2 ^{aw}	13.4 ^A
PU34	0.7 ^z	17.1 ^{yz}	21.0 ^{aby}	46.9 ^{abx}	21.4 ^B	0.8 ^z	8.7 ^y	13.1 ^{aby}	25.1 ^{ax}	11.9 ^A
PU45	0.4 ^z	12.3 ^{yz}	19.5 ^{bxy}	34.3 ^{bcx}	16.6 ^{BC}	0.6 ^z	6.5 ^{yz}	12.5 ^{abxy}	16.2 ^{bx}	9.0 ^B
PU61	0.1 ^z	10.8 ^{yz}	14.9 ^{bxy}	29.3 ^{cx}	13.8 ^C	0.4 ^z	4.6 ^{yz}	9.0 ^{by}	13.5 ^{by}	6.9 ^B
Grand Mean	0.9 ^D	15.5 ^C	23.2 ^B	40.9 ^A		0.8 ^D	7.2 ^C	13.0 ^B	20.2 ^A	
SE	3.19					1.52				
<i>P</i> values										
PUFA		***				***				
α -TA level		***				***				
PUFA $\times \alpha$ -TA level		*				**				

¹ Values given in this table were obtained from multifactorial ANOVA (n = 96)

² 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.

³ ϵ 0: 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 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.

w.x.y.z Different superscripts indicate significant differences in the same row for thigh or breast samples.

* = significant effect at $P \le 0.05$; ** = $P \le 0.01$; *** = $P \le 0.001$.

Table 6. Dependance of α -Tocopherol content (y; mg/kg) in breast meat and thigh meat, respectively, on dietary α -tocopherol (x₁; mg/kg) and on polyunsaturated fatty acid content in feed (x₂; g/kg)

Abhängigkeit des α -Tocopherol-Gehaltes (y; mg/kg) im Brust- und im Schenkelfleisch vom α -Tocopherol-Gehalt (x₁; mg/kg) sowie dem Grad der Ungesättigtheit der Fettsäuren (x₂; mg/kg) im Futter

Independent		Thigh w	vith skin		Bre	ast	
variable ¹ Dietary treatments ²	Dietary treatments ²	Equation	r ²	Р	Equation	r ²	Р
α –Tocopherol	PU15, PU34	y = 0.114x ₁	0.93	***	y = 0.071x ₁	0.93	***
content in feed P [mg/kg]	PU45, PU61	y = 0.059x ₁	0.94	***	y = 0.035x ₁	0.93	***
PUFA content of feed	EO	y = 3.03-0.06x ₂	0.56	***	y = 1.52-0.02x ₂	0.49	***
[g/kg]	E1	y = 24.43-0.24x ₂	0.49	**	y = 11.28-0.11x ₂	0.56	***
	E2	y = 39.68-0.42x ₂	0.50	***	y = 19.51-0.17x ₂	0.47	**
	E4	y = 63.55-0.58x ₂	0.50	***	y = 32.68-0.32x ₂	0.49	**

¹ Values obtained by analysis of feeds.

² 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; 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.

** = $P \le 0.01$; *** = $P \le 0.001$.

meat samples (data not shown). In addition, the α -Toc recoveries in thigh and breast meat samples were not affected by the level of α -Toc standard addition. Therefore, the global percentages of recovery (Table 4), grouping the results from both levels of α -Toc addition, were used to calculate the α -Toc content in meat samples for the different polyunsaturation treatments. On the other hand, global recoveries of α -Toc in thigh meat samples decreased as the dietary polyunsaturation increased (Table 4). Thus, an increase of dietary PUFA from 15 to 61 g/kg, of dietary PUFA, significantly (p<0.001) reduced α -Toc recovery in thigh meat samples by 30.9%. In general, global recoveries in breast meat was higher than in thigh meat samples, and global recoveries in breast meat samples increased as die-

tary PUFA increased. The differences in the recoveries of α -Toc may be due to the different level of PUFA in the tissues. Increasing the level of dietary polyunsaturation caused an increase in the accumulation of PUFA in thigh (PU15: 17.91, PU34: 38.54, PU45: 47.70, PU61: 55.66 g/kg) and breast (PU15: 3.48, PU34: 5.39, PU45: 5.98, PU61: 8.48 g/kg). More details about fatty acid content of these tissues are found in CORTINAS et al. (2004). In fact, thigh meat had levels of PUFA that were 7 fold higher than those of breast meat. This is due to the fact that skin was included in the thigh meat samples but was excluded from the breast meat ones. The higher PUFA content of thigh meat, together with its higher oxidative potential corresponds with a higher susceptibility to oxidation, that under the prooxidant conditions during saponification may increase lipid oxidation rate, which is in turn, neutralized by the α -Toc present in the sample. The net result is a reduction of total α -Toc in the sample. Therefore, the degree of polyunsaturation, and in turn, the level of oxidation, may negatively affect the recovery of α -Toc from the samples during the analytical determination. A correct use of a combination of antioxidants (e.g., citric acid + pyrogallol + butylated hydroxytoluene) for the hydrophilic and lipophilic phases during the analysis of α -Toc in meat may be able to avoid or minimize differences in α-Toc recoveries (Bou et al., 2004).

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 5. At dietary levels of 200 mg α -TA/kg of feed, α -Toc content ranged from 14.9 to 37.3 mg/kg in thigh meat and from 9.0 to 17.3 mg/kg in breast meat. Similarly, at this dietary level of α -TA supplementation, some authors found similar α -Toc content in thigh meat with skin (RuIz et al., 1999; GRAU et al., 2001a,b), as well as in breast meat (GALVIN et al., 1993; MORRISSEY et al., 1997; O'NEILL et al., 1998).

Furthermore, α -Toc content in thigh meat was 1.8-2 fold higher ($P \le 0.001$) than in breast meat. Despite the fact that in our study thigh meat contained skin, these results agree with other authors who observed α -Toc content of thigh meat without skin were 1.1-2.2 fold higher than those of breast meat (LIN et al., 1989; AHN et al., 1995; CHERIAN et al., 1996; DE WINNE and DIRINCK, 1996; NAM et al., 1997; O'NEILL et al., 1998; MALCZYK et al., 1999). Differences among tissues may result from the different metabolic function of each tissue. So, thigh meat of broilers, apart from having a more developed vascular system (DE WINNE and DIRINCK, 1996; LIN et al., 1989), also have higher lipid content than breast meat (MALCZYK et al., 1999; CRESPO and Es-TEVE-GARCIA, 2001). In the animals from the present study, although PUFA expressed as percentage of total fat was higher in breast meat, the absolute amount was higher in thigh meat, since the total fat content of thigh meat was 6.5-7.8 times that of breast (total FA content in breast was 18.1 g/kg whilst in thigh meat ranged between 141.2 and 116.8 g/kg, as the dietary polyunsaturation level increased see CORTINAS et al., 2004). Therefore, this higher content of PUFA may cause a greater need for α -Toc to prevent lipid oxidation. When the protective potential of α -Toc in muscle was estimated based on the total PUFA content, it revealed that the α -Toc concentration in breast meat (139.5, 305.8 and 436.3 mg/100 g of total PUFA for E1, E2 and E4, respectively) was 3-4 fold higher than in thigh meat (51.5, 83.9 and 135.2 mg/100 g of total PUFA for E1, E2 and E4, respectively). These results agree with those of JENSEN et al. (1997) who observed that α -Toc to PUFA ratio in breast meat was 2 fold higher than in thigh meat.

Dietary α -Toc supplementation and polyunsaturation level influenced α -Toc content of thigh and breast meat. In relation to dietary α -Toc supplementation, several authors have reported that α -Toc content in chicken tissues is well correlated with its dietary supplementation (KLAUSS et al., 1995; JENSEN et al., 1999; FLACHOWSKY et al., 2002). Relationships between α -Toc content in feed (supplemented as α -TA) and tissues are shown in Table 6. The α -Toc content of thigh and breast meat significantly increased as the dietary α -Toc (values obtained by analysis) increased (p<.0.001) Furthermore, the rate of α -Toc deposition was influenced by dietary polyunsaturation level (p<0.01) However, comparing the slopes of the equations (PU15 and PU34 vs. PU45 and PU61), an interaction between the dietary polyunsaturation level and dietary supplementation with α -TA was observed (p<0.01). Thus, linear regression analysis showed that, in the more saturated treatments (PU15 and PU34), α -Toc content in thigh and breast meat increased at a rate of 0.114 mg/kg and 0.071 mg/kg, respectively, for each mg increase of α -Toc per kg feed, whereas in the more polyunsaturated treatments (PU45 and PU61), this increase was 0.059 mg/kg and 0.035 mg/kg in thigh and breast meat, respectively. Therefore, the rate of α -Toc deposition in thigh meat was 1.9 to 2.0 fold higher than that in breast meat ($P \le 0.001$). This agrees with the results of LIN et al. (1989) who observed that α -Toc deposition in thigh meat was approximately 50% higher than in breast meat. The different rate of deposition may result, as explained above, from the higher metabolic rate of thigh muscles.

There is a wide range of variability in the α -Toc content of chicken meat obtained by different authors with similar levels of α -TA supplementation. This variation can be due in part to the tissues studied and to the different analytical methods used by each author to determine α -Toc in tissues. Furthermore, most studies do not determine α -Toc in feed, and the real α -Toc content in diet does not always coincide with the estimated α -Toc from dietary supplementation. In addition, probably factors related to the PUFA composition of the diet cause important differences in α -Toc content of chicken meat.

In relation to dietary polyunsaturation level, α –Toc content of thigh and breast meat was reduced as the inclusion of dietary PUFA increased (Table 6). Thus, the decreased rates of α –Toc in thigh meat when PUFA/kg of feed was increased by 1 g were 0.06 mg/kg, 0.24 mg/kg, 0.42 mg/kg and 0.58 mg/kg for E0, E1, E2 and E4, respectively. Decrease deposition of α –Toc in breast meat when PUFA/kg of feed was increased by 1 g was lower in comparison with thigh meat, and were 0.02, 0.11 mg/kg, 0.17 mg/kg and 0.32 mg/kg for E0, E1, E2 and E4, respectively.

It has been shown that fat and oil sources varying in the polyunsaturation degree give variable results with respect to α -Toc accumulation in chicken meat. Dietary polyunsaturation entails higher content of PUFA in chicken meat, which is more susceptible to oxidation (GRAU et al., 2001a,b). Data concerning the oxidation level measured as TBARS values of thigh meat of the animals from this experiment are described in CORTINAS et al. (2005). The relative quantities of α -Toc required to protect a fatty acid are higher as the number of double bonds in the molecule increase (WITTING and HORWITT, 1964). For this reason, supplementing chicken diets with fish oil rich in long chain PUFA with a high number of double bonds, produces a reduction in the α -Toc content of chicken tissues (MILLER and HUANG, 1993; HUSVÉTH et al., 2000; SURAI and SPARKS, 2000; ZANINI et al., 2003). However, using vegetable oils with high levels of PUFA with a lower number of double bonds, did not affect α -Toc content of chicken tissues (Cherian et

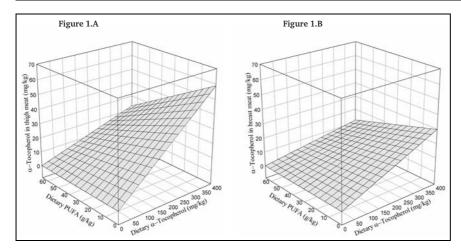


Figure 1. Estimated response surface for α -tocopherol content in thigh with skin (A) and breast (B) meat (expressed as mg/kg) to variation in diof α -tocopherol etarv content (expressed as mg/kg) and polyunsaturated fatty acids (expressed as g/kg) Geschätztes Reaktionsverhalten des α -Tocopherol-Gehaltes im Schenkel-(mit Haut; A) und im Brustfleisch (B) (mg/kg) auf die Variation des α -Tocopherol-Gehaltes (mg/kg) und des Grades der Ungesättigtheit der Fettsäuren (mg/kg) im Futter

al., 1996; LAURIDSEN et al., 1997; MALCZYK et al., 1999; RUIZ et al., 1999; GRAU et al., 2001a,b).

Since in the present experiment α -Toc content in thigh meat and breast meat, respectively (y; mg/kg) increased linearly with dietary α -Toc content (x₁; mg/kg) and simultaneously decreased linearly with dietary PUFA (x₂; g/kg), the multifactorial regressions were calculated for thigh meat with skin, y = ×₁(0.1473- 0.0014×₂), and for breast meat, y = x₁(0.0746- 0.0007x₂) ($P \le 0.001$). The estimated response surface for α -Toc content in thigh and breast meat is shown in Figure 1.

In conclusion, tissue retention of α -Toc varies considerably among tissues, being higher in thigh than in breast meat. α -Toc content in meat increases linearly as dietary α -Toc supplementation increases. Furthermore, as the dietary polyunsaturation level increases, α -Toc content of chicken meat decreases.

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 grant from the Ministerio de Educación, Cultura y Deporte, and the Comisión Interministerial de Ciencia y Tecnología (CICYT).

Summary

One hundred and ninety-two female broiler chickens were randomly distributed into 16 experimental treatments (three replicates each) as a result of the combination of 4 levels of dietary polyunsaturated fatty acids (PUFA: 15, 34, 45 and 61 g/kg) and 4 levels of supplementation with α -tocopheryl acetate (α -TA: 0, 100, 200 and 400 mg/kg), in order to determine the modification of the α -tocopherol $(\alpha$ -Toc) content of chicken thighs and breast meat. Dietary PUFA content influenced the α -Toc recoveries in thigh with skin and breast muscle tisssue. Dietary α -Toc and polyunsaturation level influenced (p<0.001) α -Toc content in meat of chickens at the age of 44 days. α -Toc content of thigh increased linearly as the dietary α -Toc supplementation increased. Thus, it increased at a rate of 0.114 mg/kg $(P \le 0.001)$ and 0.071 mg/kg $(P \le 0.001)$ when α -Toc increased 1 mg/kg of feed in the most and the least saturated treatments, respectively. Furthermore, α -Toc content of thigh decreased linearly to the inclusion of dietary PUFA. When PUFA content in feed was increased by 1 g/kg, α -Toc content in thigh meat decreased in a rate of 0.06 mg/kg, 0.24 mg/kg, 0.42 mg/kg and 0.58 mg/kg for 0, 100, 200 and 400 mg/kg of dietary supplementation with α -TA, respectively. A similar response was observed in breast meat but with rates of α -Toc incorporation 1.9-2.0 fold lower than in thighs.

Key words

Chicken, nutrition, α -tocopherol, dietary polyunsaturation, thigh meat, breast meat

Zusammenfassung

Einfluss des Grades der Ungesättigtheit der Fettsäuren im Futter auf den Gehalt an α -Tocopherol im Hühnerfleisch

Das Ziel der vorliegenden Untersuchung war, den Einfluss des Grades der Ungesättigtheit der Fettsäuren im Futter auf den α -Tocopherol-Gehalt von Schenkel- und Brustfleisch von Masthühnern zu bestimmen. Hierzu wurden 192 weibliche Broiler zufällig auf 16 Behandlungsgruppen mit je 3 Wiederholungen verteilt. Die Behandlungen waren: 4 Grade der Ungesättigtheit (PUFA: 15, 34, 45, 61 g/kg), 4 Zulagestufen für α -Tocopherol (α -TA: 0, 100, 200, 400 mg/kg).

Die PUFA-Gehalte im Futter beeinflussten die Aufnahme von α -Toc in das Schenkel- (mit Haut) und in das Brustfleisch. Der Gehalt an α-Toc im Futter und der Grad der Ungesättigtheit hatten einen hoch signifikanten Einfluss auf den α -Toc Gehalt im Fleisch der 44 Tage alten Broiler (P<0,001). Der α -Toc-Gehalt im Schenkelfleisch nahm mit der Zulage im Futter kontinuierlich zu. Beim geringsten und höchsten Grad der Ungesättigtheit des Futters erhöhte sich der Gehalt im Schenkelfleisch um 0,114 mg/kg (P≤0,001) bzw. 0,071 mg/kg (P \leq 0,001) je g α -Toc-Zulage zum Futter. Ferner ging der α -Toc-Gehalt im Schenkelfleisch linear mit zunehmendem PUFA-Gehalt im Futter zurück. Jede Erhöhung des PUFA-Gehaltes im Futter um 1 g/kg führte zu einem Rückgang im α -Toc-Gehalt des Schenkelfleischs in der Höhe von 0,06 mg, 0,24 mg, 0,42 mg und 0,58 mg/kg für die α -Toc-Zulagen von 0, 100, 200 und 400 mg/kg Futter. Für das Brustfleisch wurden ähnliche Beziehungen beobachtet, wobei die Einlagerungsrate von α -Toc 1,9 bis 2-fach geringer war.

Stichworte

Broiler, Fütterung, α –Tocopherol, PUFA, Schenkelfleisch, Brustfleisch

References

- AHN, D.U., F.H. WOLFE and J.S. SIM, 1995: Dietary α -linolenic acid and mixed tocopherols, and packaging influences on lipid stability in broiler chicken breast and leg muscle. J. Food Sci. 5, 1013-1018.
- AJUYAH, A.O., R.T. HARDIN and J.S. SIM, 1993: Effect of dietary full-fat flax seed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats. Poult. Sci. 72, 125-136.
- BALL, G.F.M., 1996: Determination of fat-soluble vitamins in foods by high performance liquid chromatography. Pages 601-647 in Handbook of Food Analysis. L. M. L. Nollet, ed. Marcel Decker Inc., New York.
- BOU, R., F. GUARDIOLA, A. GRAU, S. GRIMPA, A. MANICH, A. BAR-ROETA and R. CODONY, 2001: Influence of dietary fat source, α -tocopherol, and ascorbic acid supplementation on sensory quality of dark chicken meat. Poult. Sci. 80, 1-8.
- BOU, R., F. GUARDIOLA, A. TRES, A.C. BARROETA and R. CODONY, 2004: Effect of dietary fish oil, and α -tocopheryl acetate and zinc supplementation on the composition and consumer acceptability of chicken meat. Poult. Sci. 83, 282-292.
- CHERIAN, G., F.W. WOLFE and J.S. SIM, 1996: Dietary oils added tocopherols: Effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poult. Sci. 75, 423-431.
- CORTINAS, L., C. VILLAVERDE, J. GALOBART, M.D. BAUCELLS, R. CODONY and A.C. BARROETA, 2004: Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poult. Sci. 83, 1155-1164.
- CRESPO, N. and E. ESTEVE-GARCIA, 2001: Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. Poult. Sci. 80, 71-78.
- DE WINNE, A. and P. DIRINCK, 1996: Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. J. Agric. Food Chem. 44, 1691-1696.
- FLACHOWSKY, G., D. ENGELMAN, A. SÜNDER, I. HALLE and H.P. SALLMANN, 2002: Eggs and poultry meat as tocopherol sources in dependence on tocopherol supplementation of poultry diets. Food Res. Int. 35, 239-243.
- GALVIN, K., P.A. MORRISSEY, D.J. BUCKLEY and M. FRIGG, 1993: Influence of oil quality and α -tocopheryl acetate supplementation on α -tocopherol and lipid oxidation in chicken tissues. Pages 423-429 in Proceedings of the 11th European Symposium on the Quality of Poultry Meat. Tours, France.
- GONZALEZ-ESQUERRA, R. and S. LEESON, 2000: Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat. Br. Poult. Sci. 41, 481-488.
- GRAU, A., F. GUARDIOLA, S. GRIMPA, A.C. BARROETA and R. CODONY, 2001a: Oxidative stability of dark chicken meat through frozen storage: Influence of dietary fat and α -to-copherol and ascorbic acid supplementation. Poult. Sci. 80, 1630-1642.
- GRAU, A., R. CODONY, S. GRIMPA, M.D. BAUCELLS and F. GUARDI-OLA, 2001b: Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and α -tocopherol and ascorbic acid supplementation. Meat Sci. 57, 197-208.
- HUSVÉTH, F., H.A. MANILLA, T. GAÁL, P. VAJDOVICH, N. BALOGH, L. WÁGNER, I. LÓTH and K. NÉMETH, 2000: Effects of saturated and unsaturated fats with vitamin E supplementa-

tion on the antioxidant status of broiler tissues. Acta Vet. Hung. 48, 69-79.

- JENSEN, C., R. ENBERG, K. JAKOBSEN, L.H. SKIBSTED and G. BER-TELSEN, 1997: Influence of the oxidative quality of dietary oil on broiler meat storage stability. Meat Sci. 47, 211-222.
- JENSEN, S.K., R.M. ENGBERG and M.S. HEDERMANN, 1999: All-rac– α –tocopherol acetate is a better vitamin E source than all-rac– α –tocopherol succinate for broilers. J. Nutr. 129, 1355-1360.
- KLAUSS, A.M., H. FUHRMANN and H.P. SALLMANN, 1995: Peroxidative and antioxidative metabolism of the broiler chicken as influenced by dietary linoleic acid and vitamin E. Arch. Geflügelk. 59, 135-144.
- KRAUSS, R.M., R.H. ECKEL, B. HOWARD, L.J. APPEL, S.R. DANIELS, R.J. DECKELBAUM, J.W. ERDMAN, P. KRIS-ETHERTON, I.J. GOLDBERG, T.A. KOTCHEN, A.H. LICHTENSTEIN, W.E. MITCH, R. MULLIS, K. ROBINSON, J. WYLIE-ROSSETT, S.S. JEOR, J. SUTTIE, D.L. TRIBBLE and T.L. BAZZARE, 2001: Revision 2000: Statement for healthcare professionals from the nutrition committee of the american heart association. J. Nutr. 131, 132-146.
- KUBO, K., M. SAITO, T. TADOKORO and A. MAEKAWA, 1997: Changes in susceptibility of tissues to lipid peroxidation after ingestion of various levels of docosahexaenoic acid and vitamin E. Br. J. Nutr. 78, 655-669.
- LAURIDSEN, C., D.J. BUCKLEY and P.A. MORRISSEY, 1997: Influence of dietary fat and vitamin E supplementation on α -tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. Meat Sci. 46, 9-22.
- LIN, C.F., J.I. GRAY, A. ASHGAR, D.J. BUCKLEY, A.M. BOOREN and C.J. FLEGAL, 1989: Effects of dietary oils and α -tocopherol supplementation on lipid composition and stability of broiler meat. J. Food Sci. 54, 1457-1460.
- LÓPEZ-FERRER, S., M.D. BAUCELLS, A.C. BARROETA and M.A. GRASHORN, 1999: Influence of vegetable oil sources on quality parameters of broiler meat. Arch. Geflügelk. 63, 29-35.
- López-Ferrer, S., M.D. BAUCELLS, A.C. BARROETA and M.A. GRASHORN, 2001: N-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: Fish oil. Poult. Sci. 80, 741-752.
- MALCZYK, E., W. KOPEC and T. SMOLINSKA, 1999: Influence of oil and vitamin E (alfa-tocopherol) supplementation on lipid oxidation and flavor of poultry meat. Pages 167-172 in Proceedings of the XIV European Symposium on the quality of poultry meat. Bologna, Italy.
- Maraschiello, C., C. Sárraga and J.A. García Regueiro, 1999: Glutathione peroxidase activity, TBARs, and α–tocopherol in meat from chicken fed different diets. J. Agric. Food Chem. 47, 867-872.
- MILLER, E.L. and Y.X. HUANG, 1993: Improving the nutritional value of broiler meat through increased n-3 fatty acid and vitamin E content. Pages 404-411 in Proceedings of the 11th European Symposium on the Quality of Poultry Meat. Tours, France.
- MORRISSEY, P.A., S. BRANDON, D.J. BUCKLEY, P.J.A. SHEEHY and M. FRIGG, 1997: Tissue content of α–tocopherol and oxidative stability of broilers receiving dietary α–tocopheryl acetate supplement for various periods pre-slaughter. Br. Poult. Sci. 38, 84-88.
- NAM, K., H. LEE, B. MIN and C. KANG, 1997: Influence of dietary supplementation with linseed and vitamin E on fatty acids, α–tocopherol and lipid peroxidation in muscles of broiler chicks. Anim. Feed Sci. Technol. 66, 149-158.
- NATIONAL RESEARCH COUNCIL, 1994: Nutrient Requeriments of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
- O'NEILL, L.M., K. GALVIN, P.A. MORRISSEY and D.J. BUCKLEY,

1998: Comparison of effects of dietary olive oil, tallow and vitamin E on the quality of broiler meat and meat products. Br. Poult. Sci. 39, 365-371.

- RUIZ, J.A., A.M. PEREZ-VENDRELL and E. ESTEVE-GARCIA, 1999: Effect of β–carotene and vitamin E on peroxidative stability in leg meat of broilers fed different supplemental fats. J. Agric. Food Chem. 47, 448-454.
- SAITO, M., K. KUBO and S. IKEGAMI, 1996: An assessment of docosahexaenoic acid (DHA) intake with special reference to lipid metabolism in rats . J. Nutr. Sci. Vitaminol. 42, 195-207.

SAS INSTITUTE, 2000: SAS[®] 8.01. SAS institute Inc., Cary, NC. SIGMAPLOT, 2002: SigmaPlot 2002 for Windows version

8.02. Copyright c 1986-2001 SPSS Inc.

SUKHIJA, P.S. and D.L. PALMQUIST, 1988: Rapid method of determination of total fatty acid content and composition of feedstuffs and faeces. J. Agric. Food Chem. 36, 1202-1206.

- SURAI, P.F. and N.H.C. SPARKS, 2000: Tissue-specific fatty acid and α–tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci. 79, 1132-1142.
- WITTING, L.A. and M.K. HORWITT, 1964: Effect of degree of fatty acid unsaturation in tocopherol deficiency-induced creatinuria. J. Nutr. 82, 19-33.
- ZANINI, S.F., C.A.A. TORRES, N. BRAGAGNOLO, J.M. TURATTI, M.G. SILVA and M.S. ZANINI, 2003: Effect of oil sources and vitamin E levels in the diet on the concentration of total lipids, cholesterol, vitamin E in thigh and chest meat of cockerels. Pages 278-284 in Proceedings of the XVI European Symposium on the Quality of Poultry Meat. Saint Brieuc, France.

Correspondence: Prof. Dr. Ana C. Barroeta, Departament de Ciència Animal i dels Aliments. Facultat de Veterinària. Universitat Autònoma de Barcelona. Campus Bellaterra, 08193 Bellaterra, Spain; E-mail: Ana.Barroeta@uab.es