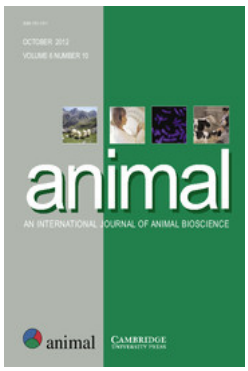

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Use of recovered frying oils in chicken and rabbit feeds: effect on the fatty acid and tocol composition and on the oxidation levels of meat, liver and plasma

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The addition of some fat co- and by-products to feeds is usual nowadays; however, the regulations of their use are not always clear and vary between countries. For instance, the use of recycled cooking oils is not allowed in the European Union, but they are used in other countries. However, oils recovered from industrial frying processes could show satisfactory quality for this purpose. Here we studied the effects of including oils recovered from the frying industry in rabbit and chicken feeds (at 30 and 60 g/kg, respectively) on the fatty acid (FA) and tocol (tocopherol + tocotrienol) compositon of meat, liver and plasma, and on their oxidative stability. Three dietary treatments (replicated eight times) were compared: fresh non-used oil (LOX); oil discarded from the frying industry, having a high content of secondary oxidation compounds (HOX); and an intermediate level (MOX) obtained by mixing 50 : 50 of LOX and HOX. The FA composition of oil diets and tissues was assessed by GC, their tocol content by HPLC, the thiobarbituric acid value was used to assess tissue oxidation status, and the ferrous oxidation-xylenol orange method was used to assess the susceptibility of tissues to oxidation. Our results indicate that FA composition of rabbit and chicken meat, liver and plasma was scarcely altered by the addition of recovered frying oils to feed. Differences were encountered in the FA composition between species, which might be attributed mainly to differences in the FA digestion, absorption and metabolism between species, and to some physiological dietary factors (i.e. coprophagy in rabbits that involves fermentation with FA structure modification). The α-tocopherol (αT) content of tissues was reduced in response to the lower αT content in the recovered frying oil. Differences in the content of other tocols were encountered between chickens and rabbits, which might be attributable to the different tocol composition of their feeds, as well as to species differences in the digestion and metabolism of tocols. Tissue oxidation and susceptibility to oxidation were in general low and were not greatly affected by the degree of oxidation of the oil added to the feeds. The relative content of polyunsaturated fatty acids/αT in these types of samples would explain the differences observed between species in the susceptibility of each tissue to oxidation. According to our results, oils recovered from the frying industry could be useful for feed uses.

Keywords: vegetable frying oils, tissue lipid composition, feed ingredients, oxidability, vitamin E

Implications

Various fat by-products of the food chain are included in feeds. Recycled cooking oils are not intended for feed uses in the European Union, although they might be used in other countries. However, oils recovered from industrial frying processes could show satisfactory quality for this purpose. Thus, here we studied how the addition of frying oils to feeds affects the lipid composition and the stability of meat and other animal tissues. Our results reveal an acceptable tissue oxidative stability, and slight effects on tissue composition. These findings may be useful for future regulatory policies regarding the quality and applications of frying oils.

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Introduction

Animal feeds are commonly supplemented with fats in order to increase their energetic value. The amount of fat supplied can vary depending on the animal species and rearing conditions involved. However, an increasing amount of fat is being added to feeds in recent years, reaching up to a maximum of 100 g/kg, with certain exceptions depending on the species. For instance, fish have high energetic requirements and fat added to feeds

can reach up to 400 g/kg (Wood *et al*., 2004). These fats also provide essential fatty acids (EFA) and liposoluble vitamins. Moreover, fats have several digestive functions, for example serving as solvents and emulsifiers for several nutrients.

There is a wide range of fat products available in the market as feed ingredients (European Commission, 2011). These products include not only fats with well-defined composition, but also several co- and by-products of the food chain. Their characteristics might differ substantially from the composition and quality of their corresponding raw materials. Their quality vary depending on the production process and handling and storage conditions. In certain cases (i.e. recycled oils, acid oils, fish oils), the levels of undesirable compounds, such as lipid oxidation compounds and persistent pollutants, could be high enough to be critical control parameters for feeds including these ingredients (Ábalos *et al*., 2008; Nuchi *et al*., 2009; Ubhayasekera and Dutta, 2009). The European Union (EU) has recently imposed stringent regulations on the manufacturing processes by which some of these fat materials (such as animal fats) are produced (European Commission, 2010a and 2010b). However, there is still no standardization procedure for others, which implies that little attention is paid to assessing the degradation of these materials. The control of lipid oxidation, particularly in polyunsaturated fats (PUFA) in animal feed, deserves special attention (Nuchi *et al*., 2009). Recycled cooking oils are not intended for feed uses according to the recent EU regulations (European Commission, 2003), but they are indeed currently used in some non-EU countries. However, oils recovered from industrial frying processes could show satisfactory quality for this purpose. In spite of the relevance of this subject, few studies have addressed their effects on animal performance, and meat composition and stability.

The fatty acid (FA) composition of feed influences the FA composition of meat and other animal products. In addition, the oxidative stability and shelf life of meat depend on the balance between certain FA (such as PUFA), pro-oxidants and antioxidants (Wood and Enser, 1997; Bou *et al*., 2009) and therefore can be also altered by the modification of the FA composition. Antioxidant vitamins, such as α-tocopherol (αT) and other tocols, contribute to decrease meat oxidability. Their contents in meat and other tissues depend on the balance between the tocol amount supplied by feed and the PUFA content of the same (Bou *et al*., 2009; Dalle Zotte and Szendro, 2011; Kouba and Mourot, 2011).

The study presented here forms part of the European Commission project *Feeding Fats Safety* (Feeding Fats Safety, 2008), which addresses the inclusion of fat co- and by-products from the food chain in animal feed. In a preliminary study, more than 120 commercial fat products were characterized (Gasperini *et al*., 2007; Ábalos *et al*., 2008; Abbas *et al*., 2009; Nuchi *et al*., 2009; Ubhayasekera and Dutta, 2009; Van Ruth *et al*., 2010). Those containing the highest concentrations of several pollutants and undesirable compounds, including oxidation products, were selected for a series of experimental chicken and rabbit trials. Here we examined whether the inclusion of recovered frying oils in feed affects the FA and tocol composition and the oxidation and oxidative stability of meat, liver and plasma. The effects of these experimental diets on animal performance and health and on cholesterol and cholesterol oxidation products have been published by other research groups collaborating as partners in this European Commission project (Blas *et al*., 2010; Ubhayasekera *et al*., 2010a and 2010b).

Material and methods

Feed formulation and manufacture

Experimental feeds were formulated following the corresponding nutritional requirements for chickens (National Research Council, 1994) and rabbits (De Blas and Wiseman, 1998). Chicken feed contained 60 g of added fat/kg; rabbit feed contained 30 g added fat/kg. The type of added fat varied depending on the dietary treatment. Feed ingredients and average nutrient performed compositions are given in Table 1. Chemical analysis of diets was as described in Blas *et al.* (2010). In the case of rabbit feed, robenidine was included as a coccidiostatic drug. Batches of rabbit feed without this drug were also prepared and distributed during the last fattening week. All feeds were manufactured at the feed plant of the *Universidad Politécnica de Valencia* (Spain). For rabbit feed, a 3-mm sieve was used for grinding, and steam was added for meal conditioning to 18% to 20% humidity and 75°C to 80°C before pelleting. Chicken feeds were manufactured following the same procedure but were not pelleted.

Experimental design

A previous study in the first part of the *Feeding Fats Safety* project addressed the composition and oxidation of commercial fat co- and by-products (Nuchi *et al*., 2009). The highest oxidation levels were found in a group of recycled cooking oil samples. Thus, a fat blend (sunflower oil/olive oil, 70 : 30, v/v) was collected at the end of a commercial potato chip frying process (at 170°C) and then heated again at 155 ± 10°C for 8 h. This second heating (at the same temperature as that used in frying) was conducted in order to reach a polymer value over 6%, approximately half of the limits established for discarding frying oils in most European countries (Firestone, 1996). This oil (HOX) was used to prepare the feed having the highest level of oxidation to be tested in the animal trials. An aliquot of the same fresh oil blend was taken before the commercial frying process to be used as a control treatment (low oxidized oil, LOX).

Chicken and rabbit trials included three dietary treatments: LOX, HOX and MOX. LOX treatment corresponded to the addition of LOX oil (fresh oil, low oxidation), at 30 g/kg (for rabbits) or 60 g/kg (for chickens). HOX treatment corresponded to the addition of HOX oil (see above, high oxidized oil), also at 30 g/kg for rabbit feed and 60 g/kg for rabbit and chicken feed. MOX treatment corresponded to an intermediate degree of oxidation (MOX) obtained by adding 15 g/kg of LOX oil + 15 g/kg of HOX oil for rabbit feeds, or 30 g/kg of LOX oil + 30 g/kg of HOX oil for chicken feed. The three treatments were replicated eight times. In the chicken trial, 96 7-day-old Ross 308 female broilers

Table 1 *Ingredients and average nutrient composition of the broiler chicken and rabbit diets*

Ingredient	g/kg	Nutrient composition as fed basis	
	Chicken diets		
Maize	527	Gross energy (MJ/kg)	20.80
Soya bean meal (47% of CP)	300	Dry matter (g/kg)	908
Full-fat soya bean	60	Ash (g/kg)	65
Added fat material (LOX, MOX or HOX)	60	CP (g/kg)	211
Dicalcium phosphate	25	Ether extract (g/kg)	95
Calcium carbonate	13	Crude fibre (g/kg)	38
Salt	5		
Vitamin and mineral premix[a]	5		
HCl L-lysine	3		
DL-Methionine (99%)	2		
	Rabbit diets		
Alfalfa hay	340	Gross energy (MJ/kg)	16.36
Beet pulp	300	Dry matter (%)	89.5
Sunflower meal (30% of CP)	200	Ash (%)	8.5
Barley	100	CP (%)	13.1
Added fat material (LOX, MOX or HOX)	30	Ether extract (%)	4.2
Dicalcium phosphate	13	Crude fibre (%)	20.1
Salt	5	NDF	35.4
Vitamin and mineral premix[b]	5	ADF	22.7
HCl L-lysine	3.5	ADL	4.5
DL-Methionine (99%)	2		
L-Threonine	1.5		

LOX = oil with a low degree of oxidation; MOX = oil with a medium degree of oxidation; HOX = oil with a high degree of oxidation.
[a]Composition of vitamin and mineral premix used in chicken feeds (1 kg of feed contained): Vitamin A, 6000 IU; Vitamin D_3, 1200 IU; Vitamin E, 10 mg; Vitamin K_3, 1.5 mg; Vitamin B_1, 1.1 mg; Vitamin B_2, 4 mg; Vitamin B_6, 1.5 mg; Vitamin B_{12}, 9 μg; Folic acid, 4 mg; Biotin, 50 μg; Pantothenic acid, 6 mg; Nicotinic acid, 21 mg; Choline, 360 mg; Mn, 75 mg; Zn, 50 mg; I, 0.18 mg; Fe, 30 mg; Cu, 6 mg; Se, 0.2 mg; Co, 0.2; Ethoxiquin, 16 mg. Addition of choline chloride 15 mg.
[b]Composition of vitamin and mineral premix used in rabbit feeds (1 kg of feed contained): Vitamin A, 8375 IU; Vitamin D_3, 750 IU; Vitamin E, 20 mg; Vitamin K_3, 1 mg; Vitamin B_1, 1 mg; Vitamin B_2, 2 mg; Vitamin B_6, 1 mg; Nicotinic acid, 20 mg; Choline chloride, 250 mg; Mg, 290 mg; Mn, 20 mg; Zn, 60 mg; I, 1.25 mg; Fe, 26 mg; Cu, 10 mg; Co, 0.7; Butylhydroxianisole + Ethoxiquin, 4 mg.

were randomly distributed into 24 experimental groups (3 dietary treatments × 8 replicates) under standard conditions of temperature, humidity and ventilation. The animals were housed in groups of four per cage, and feed and water were provided *ad libitum* during the study. When the broilers reached 47 days of age, they were slaughtered in a commercial abattoir. For the rabbit trial, a total of 144 rabbits (a cross of New Zealand and Californian rabbit) were housed in 24 collective cages (24 experimental groups, 3 treatments × 8 replicates × 6 animals per cage). In all cases, feed and water were provided *ad libitum*. At 63 days of age, rabbits were electrically stunned and killed by cutting carotids and jugulars. The experimental trials received prior approval from the Animal Protocol Review Committees of the *Universitat Autònoma de Barcelona* (Spain) and the *Universidad Politécnica de Valencia* (Spain). All animal housing and husbandry conformed to EU guidelines (Council European Communities, 1986).

Fat and feed sampling

The two oil samples (LOX and HOX) were taken as explained in the previous section and stored under N_2 in glass vials, capped with Teflon caps, at −25°C until analysis. Feed samples were taken at the beginning of the trial. A 500-g aliquot from each of the 10 sacks was collected, thus reaching a total of 5 kg, which was homogenized. A sample of 1 kg was then taken, packed in a hermetic plastic bag and kept at −25°C. Before analysis, feed samples were ground in a mill to a particle size of 1 mm.

Meat, liver and plasma sampling

Chicken and rabbit carcasses were refrigerated at 4°C for 24 h after death. From each cage, one leg of each animal was taken and hand-deboned. Meat was pooled and ground. Meat samples were vacuum-packed in high-barrier multilayer bags (Cryovac BB325; permeability to O_2, 25 cm^3/m^2 per day per bar at 23°C and 0% relative humidity, ASTMD-3985; Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain; ∼20 g meat/bag) and stored at −25°C until analysis. Chicken meat samples comprised dark meat with skin, whereas rabbit meat samples comprised leg meat only. The meat collection procedure differed for chickens and rabbits in order to simulate their usual edible portions. The main repercussion was that the lipid content was higher in chicken (mean value 10.4%) than in rabbit (mean value 2.8%).

Livers were removed from carcasses immediately after death. Livers from animals in each cage were pooled, ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; ~15 g liver/bag) and stored at −80°C until analysis.

Chicken plasma samples (around 5 ml) were obtained at day 37 from blood taken by syringes from jugular vein for the four birds in each cage. For rabbit plasma samples, around 20 ml of blood from carotids and jugulars was collected at the moment of sacrifice of four rabbits from each cage. All blood samples were collected in heparinized tubes and immediately centrifuged at 1450 **g** at 4°C for 10 min. Pooled plasma samples from the animals in each cage were homogenized and aliquots were transferred to plastic tubes (4.5 ml capacity) and stored at −25°C.

Reagents and standards

Butylated hydroxytoluene, αT, pyrogallol, thiobarbituric acid (TBA), *p*-anisidine and cumene hydroperoxide (CHP) were obtained from Sigma-Aldrich (St Louis, MO, USA). FA methyl esters were from Larodan Fine Chemicals AB (Malmo, Sweden) and Sigma-Aldrich. Tocopherol and tocotrienol standards were purchased from Calbiochem (La Jolla, CA, USA). Xylenol orange was from Scharlab (Barcelona, Spain). The methanol and ethanol used in the tocol analysis and in the ferrous oxidation-xylenol orange (FOX) method were of HPLC grade. Other reagents were of ACS grade.

Characterization of experimental fats

The two experimental oils (HOX and LOX) were characterized following Nuchi *et al.* (2009), including FA composition and tocol composition, Acid Value, Peroxide Value, *p*-Anisidine Value and Polymer Content. The values obtained are shown in Table 2.

FA composition

The FA composition of feed, meat, liver and plasma was determined by gas chromatography, following Tres *et al.* (2009a), adjusted to the required sample amount. FA methyl esters were prepared following Guardiola *et al.* (1994).

Tocol content

Tocopherols and tocotrienols from feed, meat and liver were extracted after saponification following Bou *et al.* (2004). Plasma tocopherols and tocotrienols were extracted (without saponification) as described by Tres *et al.* (2009a). Tocopherol and tocotrienol composition was determined by HPLC with fluorescence detection, following a procedure adapted from Hewavitharana *et al.* (2004).

Susceptibility to oxidation

The induced FOX method was used to assess susceptibility of meat and liver to oxidation, adapting the method of Grau *et al.* (2000a) to these samples. Plasma susceptibility to oxidation was also determined by the induced FOX method, as described in Tres *et al.* (2009a).

The measurement of the susceptibility to oxidation by means of induced methods, such as the FOX method applied in this study, is an estimation of the achievable oxidation of the sample (Tres *et al.*, 2009b). In the FOX-induced method, a methanolic extract of samples is mixed with the FOX reagents in glass cuvettes capped with Teflon caps. The mixture (the final volume of the reaction mixture was 2 ml) is then incubated in the dark to induce lipid hydroperoxide (LHP) formation until absorbance at 560 nm is steady. Absorbance is then measured with a spectrophotometer (Schimadzu UV-160A, Shimadzu, Kyoto, Japan) and the LHP value is determined by means of a calibration curve using CHP as standard.

TBA value

The TBA value of meats was determined by an acid aqueous extraction method with third derivative spectrometry, as

Table 2 *Chemical composition of the sunflower/olive oil blend (70 : 30, v/v) before (lox oxidized oil) and after (high oxidized oil) its use in a commercial frying process (and subsequent heating at 165°C to 170°C for 8 h)*

	Low oxidized oil		High oxidized oil	
	Mean[a]	s.e.m.	Mean	s.e.m.
FA composition (g/kg)				
C14:0	0.55	0.004	0.62	0.015
C16:0	69	0.6	79	1.2
C17:0	0.58	0.004	0.66	0.007
C18:0	26.7	0.24	25.2	0.33
C20:0	2.26	0.029	2.37	0.008
C22:0	4.6	0.07	4.0	0.02
C24:0	1.42	0.028	1.31	0.020
Total SFA	105	1.0	113	1.5
C16:1n-9	0.48	0.004	0.59	0.009
C18:1n-9	293	3.0	321	3.4
C20:1n-9	1.50	0.024	1.64	0.045
C16:1n-7	3.5	0.03	4.5	0.07
C18:1n-7	10.9	0.14	13.0	0.12
Total MUFA	309	3.2	341	3.7
C18:2n-6	372	3.7	302	2.8
C18:3n-3	1.74	0.017	1.96	0.026
Total PUFA	374	3.7	304	2.8
Trans-18:1	0.09	0.013	0.39	0.011
Tocol composition (mg/kg)				
α-tocopherol	461	4.1	297	2.9
β-tocopherol	23.1	0.41	21.2	0.34
γ-tocopherol	6.7	5.53	nd	
δ-tocopherol	nd		nd	
Total tocopherols	491	1.8	318	2.6
α-tocotrienol	nd		nd	
β-tocotrienol	11.5	1.01	nd	
γ-tocotrienol	nd		nd	
δ-tocotrienol	nd		nd	
Total tocotrienols	11.5	1.01	nd	
Acid Value (mg KOH/g)	0.27	0.002	1.15	0.004
Peroxide Value (mEq O_2/kg)	5.3	0.06	1.7	0.04
p-Anisidine Value	2.7	0.18	67.4	0.42
Polymer Content (%)	0.35	0.007	6.61	0.030

s.e.m. = standard error of the mean; FA = fatty acid; SFA = saturated FAs; MUFA = monounsaturated FAs; PUFA = polyunsaturated FAs.
[a]Values correspond to means ($n = 2$ for the FA composition, $n = 5$ for the tocol content, Acid Value, Peroxide Value, *p*-Anisidine Value and Polymer Content).

Table 3 *FA composition (mg/100 g feed) of the experimental feeds*[a]

	Chicken				Rabbit			
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.
C12:0	nd	nd	nd		nd	nd	nd	
C14:0	4.3	5.0	4.9	0.12	6.4	5.4	6.7	0.11
C15:0	nd	tr	nd		1.97	1.57	1.99	0.027
C16:0	591	692	670	19.9	286	251	320	2.9
C17:0	4.9	5.7	5.6	0.15	3.2	2.8	3.5	0.02
C18:0	174	194	179	5.4	87	73	87	1.57
C20:0	17.4	19.9	19.3	0.44	10.66	9.0	11.2	0.13
C22:0	26.2	28.7	26.5	0.59	17.7	14.3	16.5	0.27
C24:0	10.7	11.8	11.0	0.25	10.2	8.3	10.0	0.17
Total SFA	829	958	916	26.4	423	365	457	4.5
C16:1n-9	3.3	4.0	4.1	0.10	2.0	1.8	2.1	0.03
C18:1n-9	1763	2087	2051	54.7	801	712	871	6.7
C20:1n-9	11.6	13.6	13.1	0.40	6.3	5.5	6.6	0.09
C16:1n-7	17.9	23.2	24.2	0.51	9.9	9.3	12.3	0.08
C18:1n-7	63	73	76	1.3	32	29	37	0.3
Total MUFA	1858	2201	2168	56.4	850	758	928	7.2
C18:2n-6	2692	2900	2620	86.2	1186	958	1055	9.5
C18:3n-3	80	88	82	2.2	70	58	73	0.6
Total PUFA	2773	2989	2701	88.2	1255	1017	1128	10.0
Trans-18:1	0.06	0.19	0.24	0.062	0.36	0.40	1.30	0.165
*t*9, *t*12-18:2	1.13	1.50	1.73	0.053	nd	nd	0.58	0.019

FA = fatty acid; LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; tr = traces; SFA = saturated FAs; MUFA = monounsaturated FAs; PUFA = polyunsaturated FAs.
[a]Values correspond to means ($n = 2$); s.e.m., pooled standard error of the means for each animal species.

described by Grau *et al.* (2000b). The same procedure was used for liver and plasma but was adapted as in Tres *et al.* (2009a).

Statistics

Each cage was considered an experimental unit. One-way ANOVA was used to determine differences in FA and tocol composition, oxidation (TBA values) and susceptibility to oxidation (LHP values) of chicken ($n = 24$) and rabbit ($n = 24$) meat, liver and plasma caused by the factor 'degree of oxidation of oil added to the feeds'. Multifactor ANOVA ($n = 48$) was used to determine whether the factor 'degree of oxidation of oil added to the feeds' differentially affected the FA and tocol composition, the oxidation (TBA values) and the susceptibility to oxidation (LHP values) in chickens and rabbits (factor 'animal species'). In all cases, least-square means for the main factors that had a significant effect were separated by the Scheffe test, considering $\alpha \leqslant 0.05$ as significant. The software used was SPSS 15.0 (version 15.0.1, SPSS Inc., Chicago, IL, USA).

Results and discussion

Effect of frying on oil composition and its oxidation status

The heating of the oil caused a decrease in its αT, βT and γT content ($P < 0.05$; Table 2). Total tocopherol content decreased about 35%. In contrast, the FA composition did not differ greatly between LOX and HOX oils, as only a slight decrease in the content of linoleic acid was observed (Table 2). The Acid Value of the oil increased from 0.27 to 1.15 mg KOH/100 g during frying, as a result of the hydrolytic alteration, which usually occurs when food products containing a certain amount of water (such as potatoes) are fried. In fact, the Acid Value has traditionally been used by industry to monitor the continuous frying process (Navas *et al.*, 2007). Furthermore, during frying the Peroxide Value of the oil showed a decrease (as the high temperature catalyses their decomposition), whereas the content of secondary oxidation compounds increased, reaching a *p*-Anisidine Value of 67 (Table 2). In addition, heating caused an increase in the Polymer Content up to 6.6% (w/w). These three parameters evaluate the thermo-oxidative alteration of the oil during frying, and the *p*-Anisidine Value and the Polymer Content have been proposed, among others, as complementary parameters to the measurement of the Acid Value to monitor industrial frying processes (Navas *et al.*, 2007). Furthermore, the *p*-Ansidine Value and the Polymer Content are more suitable control parameters than the Peroxide Value and the Acid Value for routine monitoring of the degradation status of fat products added to feeds (Nuchi *et al.*, 2009).

Feed FA composition

The FA composition of the chicken and rabbit feeds differed (Table 3) because they had been formulated with distinct ingredients in order to meet the specific dietary requirement of these two species (Table 1). As the amount of fat added to the chicken feed (60 g/kg) was higher than that added to rabbit feed (30 g/kg), the content of the main FA in oil

Table 4 *FA composition of chicken and rabbit meat (mg/100 g of meat) in response to the level of oxidation (low, medium or high) of the oil added to feeds*

	Chicken[a]				Rabbit[a]				Species effect[b]	Species × level effect[b]
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.		
C10:0	nd	nd	nd		7.1	7.4	7.9	0.83	*	
C12:0	nd	nd	tr		4.1	4.3	4.8	0.67	**	
C14:0	35 y	29 x	32 xy	1.3	22.5	23.9	29.2	1.77	**	**
C15:0	6.0	5.3	5.8	0.25	10.9	10.7	12.4	0.64	**	
iso-16:0	nd	nd	nd		2.1	2.4	2.8	0.19	**	
C16:0	1583	1325	1480	71.3	337	346	410	21.7	**	*
C17:0	15.6	14.0	15.9	0.53	15.3	14.7	16.9	0.91		
C18:0	514 y	443 x	483 xy	16.7	133	128	149	8.3	**	*
C19:0	2.6	2.3	3.0	0.19	2.3	2.2	2.5	0.15	*	
C20:0	10.8 xy	10.0 x	11.5 y	0.36	2.8	2.8	3.1	0.14	**	
C22:0	4.8	4.4	5.1	0.22	1.30	1.23	1.35	0.087	**	
C24:0	1.70	1.47	1.79	0.090	0.66	0.59	0.66	0.044	**	
Total SFA	2174	1835	2040	89.6	539	544	641	34.0	**	*
C16:1n-9	36 y	30 x	35 xy	1.3	7.0	7.1	8.4	0.46	**	*
C18:1n-9	3165	2785	3253	150.9	461	476	573	30.1	**	
C20:1n-9	21.8	18.1	21.7	1.02	4.1	4.4	5.2	0.34	**	*
C16:1n-7	218	173	212	17.0	16.3 x	20.0 xy	25.1 y	2.09	**	
C18:1n-7	141	124	147	6.5	24	24	29	1.52	**	
Total MUFA	3584	3133	3672	175.8	515	534	643	34.1	**	
C18:2n-6	3209 y	2750 x	3007 xy	101.4	664	618	671	36.8	**	*
C18:3n-6	23.8	19.9	22.0	1.20	1.32	1.29	1.46	0.107	**	
C20:2n-6	26.3 y	21.5 x	23.7 xy	0.93	6.9	6.7	7.4	0.39	**	*
C20:3n-6	22.7	19.7	21.4	0.86	4.6	4.2	4.6	0.28	**	
C20:4n-6	91	83	90	3.6	41	37	39	2.43	**	
C22:4n-6	25.4	23.0	24.3	0.90	13.5	12.4	13.0	0.67	**	
C22:5n-6	6.5	6.1	7.0	0.49	3.9	3.6	4.0	0.24	**	
Total n-6 PUFA	3404 y	2923 x	3195 xy	105.7	735	684	740	40.3	**	*
C18:3n-3	90	79	88	3.7	35	35	41	2.2	**	
C20:3n-3	tr	tr	tr		tr	tr	tr			
C20:5n-3	nd	nd	nd		tr	tr	tr			
C22:5n-3	3.9	3.5	4.0	0.16	5.5	5.2	5.5	0.38	**	
C22:6n-3	3.6	3.7	4.4	0.37	3.7	2.8	3.0	0.50		
Total n-3 PUFA	97	86	97	3.7	44	43	50	2.6	*	
Total PUFA	3501 y	3009 x	3291 xy	109.4	779	726	790	42.8	**	*
Trans-18:1	2.05	1.71	2.15	0.200	3.0	3.2	3.4	0.37		
*t*9, *t*12-18:2	nd	nd	nd		1.28 x	1.46 xy	1.85 y	0.139	**	
Total TFA	2.05	1.71	2.15	0.200	4.3	4.7	5.2	0.46		

FA = fatty acid; LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; nd = not detected; tr = traces; SFA = saturated FAs; MUFA = monounsaturated FAs; PUFA = polyunsaturated FAs; TFA = *trans* FAs.

[a]Values correspond to means ($n = 8$); s.e.m., pooled standard error of the means of each animal species.

[b]Multifactor ANOVA ($n = 48$, chicken + rabbit) was conducted to study whether the factor 'level of oxidation of oil added to feed' led to different effects between animal species. *$P \leqslant 0.05$, **$P \leqslant 0.01$.

x, y, z: Values in the same row for a certain species bearing no common letters are statistically ($P \leqslant 0.05$) different according to one-way ANOVA conducted in each animal species ($n = 24$ for chicken, $n = 24$ for rabbit) to study the effects of the factor 'level of oxidation of oil added to feeds'. Letters were obtained by means of the Scheffé's test ($\alpha = 0.05$).

(i.e. linoleic and oleic acids) was much higher in the chicken feed. However, the type of oil added (LOX, MOX and HOX) to feeds did not greatly alter the FA composition of rabbit or chicken feed (Table 3).

Meat, liver and plasma FA composition

The overall results reveal that meat, liver and plasma FA composition varied between the chicken and rabbit (Tables 4, 5 and 6). The significant differences in meat FA composition between species (Table 4) were mainly due to the distinct fat content of chicken (10.4%, expressed as wet basis) and rabbit meat (2.8%, expressed as wet basis) samples, and to their tissue composition, as chicken samples consisted of a mix of muscle and adipose tissue (skin), whereas rabbit samples contained only muscle tissue. The different roles of muscular and adipose tissue in the FA metabolism might explain some of the different effects encountered between rabbit and chicken meat samples. For instance, in contrast to other monogastric

Table 5 *FA composition of chicken and rabbit liver (mg/100 g of liver) in response to the level of oxidation (low, medium or high) of the oil added to feeds*

	Chicken[a]				Rabbit[a]				Species effect[b]	Species × level effect[b]
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.		
C10:0	nd	nd	nd		nd	nd	tr			
C12:0	0.37	0.34	0.51	0.079	0.39	0.38	0.29	0.063		
C14:0	20.4	17.5	17.5	2.20	5.7	7.5	7.1	0.34	**	
C15:0	6.9	6.1	6.3	0.36	11.4	11.2	10.8	0.11	**	
iso-16:0	nd	nd	nd		0.57	0.85	0.79		**	
C16:0	1049	852	866	85.7	350	363	369	10.0	**	
C17:0	8.0	9.4	8.8	0.49	32.1	30.6	31.2	0.90	**	
C18:0	748	679	692	37.4	487	465	472	10.1	**	
C19:0	2.1	2.4	2.2	0.14	11.9	11.0	11.0	0.49	**	
C20:0	5.4	5.2	5.3	0.32	3.0	3.1	3.0	0.12	**	
C22:0	3.5	3.8	3.9	0.18	2.5	2.5	2.4	0.11	**	
C24:0	2.8	2.8	3.0	0.14	2.5	2.5	2.5	0.10	**	
Total SFA	1846	1578	1604	124.6	906	900	910	15.3	**	
C16:1n-9	12.0	14.1	13.9	1.20	5.1	5.7	5.5	0.29	**	
C18:1n-9	1260	964	1069	104.8	263	274	289	8.7	**	
C20:1n-9	12.1	11.3	12.3	0.70	6.5	7.5	7.3	0.39	**	
C16:1n-7	91	52	62	10.4	4.3	5.4	5.8	0.43	**	*
C18:1n-7	55	48	52	3.3	24	24	25	0.70	**	
Total MUFA	1438	1097	1217	119.0	302	317	333	9.9	**	
C18:2n-6	773	813	733	31.5	868 y	831 xy	816 x	13.7	**	
C18:3n-6	11.0	10.9	10.4	1.05	1.94	1.83	1.78	0.109	**	
C20:2n-6	20.2	22.3	19.2	1.11	22.1	24.2	22.2	1.31	*	
C20:3n-6	42	37	40	1.7	16.3	16.9	17.1	0.57	**	
C20:4n-6	237	268	250	9.2	178	171	174	4.8	**	
C22:4n-6	27	32	28	1.5	23	24	23	0.51	**	
C22:5n-6	28.7	34.5	31.0	2.50	14.0	13.6	13.9	0.47	**	
Total n-6 PUFA	1140	1218	1110	35.4	1123	1083	1069	18.3	**	
C18:3n-3	16.1	15.3	14.5	1.02	16.0	17.0	17.8	0.60	*	
C20:3n-3	tr	tr	tr		tr	tr	tr			
C20:5n-3	3.3	2.9	3.1	0.22	nd	nd	nd		**	
C22:5n-3	5.2	6.5	6.2	0.46	7.6	7.2	7.8	0.26	**	
C22:6n-3	17.7	23.3	22.4	1.85	4.8	4.4	4.1	0.47	**	
Total n-3 PUFA	42	48	46	2.2	28	29	30	1.0	**	
Total PUFA	1182	1266	1156	36.6	1152	1112	1098	19.1	**	
Trans-18:1	3.9	2.2	2.5	0.55	3.0	4.2	2.9	0.83		
*t*9, *t*12-18:2	nd	nd	nd		0.86	1.03	1.04	0.089	**	
Total TFA	3.9	2.2	2.5	0.55	3.8	5.2	3.8	0.89	*	

FA = fatty acid; LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; nd = not detected; tr = traces; SFA = saturated FAs; MUFA = monounsaturated FAs; PUFA = polyunsaturated FAs; TFA = *trans* FAs.
[a]Values correspond to means ($n = 8$); s.e.m., pooled standard error of the means of each animal species.
[b]Multifactor ANOVA ($n = 48$, chicken + rabbit) was conducted to study whether the factor 'level of oxidation of oil added to feeds' led to different effects between animal species. $*P \leqslant 0.05$, $**P \leqslant 0.01$
x, y, z: Values in the same row for a certain species bearing no common letters are statistically ($P \leqslant 0.05$) different according to one-way ANOVA conducted for each animal species to study the effects of the factor 'level of oxidation of oil added to feeds'. Letters were obtained by means of the Scheffé's test ($\alpha = 0.05$).

species, lipogenesis in chicken does not take place in adipose tissue. In addition, differences between the digestive systems, the dietary habits and FA metabolism may also have contributed to the differences in FA content between these two species (Kouba and Mourot, 2011). This fact was revealed by the higher presence of C15:0 and *iso*-16:0 in rabbit meat samples than in chicken meat samples, despite the higher fat content of the latter (Table 4), which led to a significant effect of the factor 'animal species' for these FAs in meat. This effect was also significant in liver and plasma, because odd-chain and branched-chain FAs were also higher in rabbit (Tables 5 and 6). Rabbit's dietary habits include coprophagy (Leiber *et al*., 2008). Rabbits produce a particular kind of soft faeces, the caecotrophs. The FA profile of the caecotrophs is rich in saturated FA (SFA) and odd-chain and branched-chain FAs because of the microbial fermentation that takes place in the caecum. Thus, by ingestion of the caecotrophs, these FAs might be reabsorbed and reach tissues, although the overall impact of coprophagy on the general tissue FA composition is not substantial (Leiber *et al*., 2008; Tres *et al*., 2008).

Table 6 *FA composition of chicken and rabbit plasma (mg/l of plasma) in response to the level of oxidation (low, medium or high) of oil added to the feeds*

	Chicken[a]				Rabbit[a]				Species effect[b]	Species × level effect[b]
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.		
C10:0	1.67	1.68	1.62	0.086	1.57	1.54	1.63	0.116		
C12:0	1.99	1.48	1.98	0.152	2.84	2.89	2.97	0.260	**	
C14:0	7.6 y	6.8 xy	6.5 x	0.30	9.8	10.4	10.0	0.80		
C15:0	2.16	2.11	1.96	0.093	10.3	9.7	9.9	0.77	**	
iso-16:0	nd	nd	nd		1.37	1.84	1.71	0.161	**	
C16:0	437	410	366	25.0	245	232	243	14.9	**	
C17:0	4.0	4.2	4.1	0.24	16.0	14.4	15.0	1.03	**	
C18:0	358 y	337 xy	296 x	16.9	143	137	142	6.9	**	*
C19:0	1.41	1.41	1.33	0.081	3.50	3.21	3.24	0.228	**	
C20:0	4.0 y	3.8 xy	3.4 x	0.15	2.84	2.64	2.68	0.147	**	
C22:0	4.4	4.7	4.2	0.15	4.4	4.2	3.9	0.22		
C24:0	2.68	2.77	2.41	0.163	2.16	2.08	1.93	0.110	**	
Total SFA	825	776	689	42.0	442	421	437	24.4	**	
C16:1n-9	6.3	6.3	6.4	0.33	6.2	6.0	6.5	0.34		
C18:1n-9	529	500	457	42.1	338	304	332	19.8	**	
C20:1n-9	5.7	5.0	4.5	0.34	3.3	3.4	4.0	0.41	**	
C16:1n-7	28.2	21.7	19.9	4.74	5.0	5.6	5.9	0.31	**	
C18:1n-7	30.0	29.0	26.7	1.92	14.5	13.5	14.9	0.91	**	
Total MUFA	671	641	585	50.0	407	373	406	22.8	**	
C18:2n-6	754 xy	779 y	664 x	29.6	626	536	539	38.2	**	
C18:3n-6	6.6	6.4	5.4	0.61	1.24	0.94	0.96	0.297	**	
C20:2n-6	13.2 y	12.1 xy	11.2 x	0.58	5.8	6.4	6.1	0.47	**	
C20:3n-6	34.8	31.0	28.2	2.75	3.1	3.4	3.5	0.13	**	
C20:4n-6	158	175	154	7.8	40	42	43	1.2	**	
C22:4n-6	23.6 xy	25.0 y	20.9 x	1.15	6.2	6.4	6.5	0.25	**	*
C22:5n-6	19.5	20.9	19.3	1.00	3.9	4.0	4.4	0.21	**	
Total n-6 PUFA	1010	1049	902	30.9	687	598	604	39.7	**	*
C18:3n-3	13.5	13.2	11.3	0.79	20.7	17.0	18.1	1.62	**	
C20:3n-3	tr	tr	tr		tr	tr	tr			
C20:5n-3	1.88	1.99	1.66	0.288	nd	nd	nd		**	
C22:5n-3	3.4	4.14	3.57	0.430	1.70	1.80	1.90	0.137	**	
C22:6n-3	1.47	2.7	3.1	0.88	nd	nd	nd		**	
Total n-3 PUFA	20.2	22.1	19.6	1.35	22.5	21.2	20.1	2.31		
Total PUFA	1030 xy	1071 y	922 x	31.489	709	620	624	41.5	**	
Trans-18:1	2.34	1.98	1.07	0.516	0.68	1.23	0.51	0.515	*	
*t*9, *t*12-18:2	nd	nd	nd		0.89	1.337	1.15	0.196		
Total TFA	2.34	1.98	1.07	0.516	1.57	2.56	1.68	0.596		

FA = fatty acid; LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; SFA = saturated FAs; MUFA = monounsaturated FAs; PUFA = polyunsaturated FAs; tr = traces; nd = not detected; TFA = *trans* FAs.
[a]Values correspond to means ($n = 8$); s.e.m., pooled standard error of the means of each animal species.
[b]Multifactor ANOVA ($n = 48$, chicken + rabbit) was conducted to study whether the factor 'level of oxidation of oil added to feeds' led to different effects between animal species. *$P \leqslant 0.05$, **$P \leqslant 0.01$.
x, y, z: Values in the same row for a certain species bearing no common letters are statistically ($P \leqslant 0.05$) different according to one-way ANOVA conducted for each animal species ($n = 24$ for chicken, $n = 24$ for rabbit) to study the effects of the factor 'level of oxidation of oil added to feed'. Letters were obtained by means of the Scheffé's test ($\alpha = 0.05$).

Liver and plasma FA composition also differed significantly between chickens and rabbits (Tables 5 and 6). Rabbits showed a higher tendency to incorporate n-6 PUFA in liver and plasma than chickens. Rabbit feed provided less n-6 PUFA than chicken feed (Table 3). However, in plasma (Table 6), and particularly in liver (Table 5), the difference in n-6 content between rabbit and chicken samples was less pronounced than in feeds ($P < 0.05$). Variations in the digestion process between species, their FA metabolic sites and in the activity of enzymes involved in FA metabolism between species might explain these differences (Benatmane *et al*., 2011; Kouba and Mourot, 2011). Furthermore, the tendency of rabbit liver to incorporate higher PUFA than chicken is consistent with our findings in animals receiving a diet that was richer in SFA, supplied through hydrogenated palm FA distillates (Tres *et al*., 2012).

With regard to the effects of the dietary treatments, the addition of oxidized oil to feeds did not lead to major

alterations in the FA composition of meat, liver or plasma (Tables 4, 5 and 6). The inclusion of oxidized oil in feed produced slight but significant effects on the content of C18:2n-6, C20:2n-6 and total n-6 PUFA content in chicken meat samples. MOX chicken meat samples presented the lowest content of these FA, and the same tendency, although non-significant, was observed for almost all the other FAs. However, this trend was not seen in feeds or in rabbit meat samples, which led to a significant interaction of the factors 'animal species × level' in meat (Table 4). Because of this, it is probable that this effect in chicken meat was related to a decrease in the overall FA content in MOX chicken meat. As no differences had been observed for digestibility between diets (Feeding Fats Safety, 2008; Blas *et al.*, 2010), it seems quite evident that the cause might be found in the post-absorptive steps of the FA metabolism, for instance a lower FA storage. Furthermore, this significant interaction was only encountered in chicken meat, but not in liver or plasma. But although this interaction was significant, the magnitude of the differences between treatments (in terms of FA amounts) was quite low for most FA. In liver (Table 5), only a decrease in the C18:2n-6 content in rabbit was observed in response to HOX oil. In chicken plasma (Table 6), decreases in the content of total SFA and some n-6 PUFA such as C18:2n-6, C20:2n-6 and C22:4n-6 were observed when oxidized oils were added to feeds. The trend followed by FA contents in plasma was intermediate between that of meat and liver.

Although some slight (but significant) differences were observed for the tissue FA composition, overall, these results indicate that the FA composition of rabbit and chicken (meat, liver and plasma) was not greatly affected by the addition of recovered frying oils to feeds. However, in some cases the addition of highly oxidized oils (Sheehy *et al.*, 1993) produced greater alterations in the FA composition of meat and liver. In contrast, other studies in which intermediate (more realistic) levels of feed oil oxidation were assayed, also showed none or very few effects on the FA composition of meat (Sheehy *et al.*, 1993; Jensen *et al.*, 1997; Bou *et al.*, 2005; Tres *et al.*, 2010a). In general, it appears that tissue FA composition is not affected by the addition of oxidized fats to feeds when these fats are used at suitable doses and when they are below the cut-off limit established for discarding frying oils (Firestone, 1996).

Feed tocol composition

Tocol (tocopherols + tocotrienols) content and composition in feed depended on the tocol content in the oil, on the mineral–vitamin premix added to feed formulation, and on the rest of the feed ingredients (Table 1). Thus, differences in tocol composition between feeds followed a different pattern depending on the tocol. The main tocopherol in the fat added to feed was αT, followed by βT (Table 2). As the fat added to feed was higher in chicken than in rabbit, the amount of αT supplied by the oil might be higher in chicken. However, owing to the different nutritional requirements of each species, the vitamin–mineral premix added to rabbit feeds supplied a higher α-T amount than in chicken feed. The combination of both tocol sources led to a higher αT content in rabbit feed than in chicken feed (Table 7).

On the other hand, αT and βT contents decreased in chicken and rabbit feeds supplemented with LOX to HOX oils (Table 7). This might be attributed to the decrease of these substances in the HOX oil as a result of heating. Furthermore, the αT decrease was more prominent in chicken feed because of its higher amount of added fat. Other tocols in feed, such as α-tocotrienol, were supplied by the rest of feed ingredients, and because of this they were similar among the three treatments for rabbit and chicken.

Effects on the tocopherol and tocotrienol composition of meat, liver and plasma

The incorporation of αT in chicken and rabbit meat, liver and plasma was much lower than that of tocotrienols and other tocopherols (Table 7). Tocotrienols are absorbed by intestinal cells faster than tocopherols (Tsuzuki *et al.*, 2007). However, in liver, αT is preferentially carried stereo-selectively by the cytosolic αT transfer protein to very-low-density lipoproteins and then released into the circulation (Schneider, 2005).

The inclusion of skin (adipose tissue) in chicken meat samples caused a higher tocol content than in rabbit meat samples. Nevertheless, the content of all tocols in plasma and liver was also higher in chicken than in rabbit (Table 7), although the fat content in these samples was similar for these two species. The higher content of βT, γT and tocotrienols in chicken feed explained the higher content of these compounds in chicken plasma and liver; however, the αT content in chicken feed was lower than in rabbit feed. Thus, chickens may have a greater capacity to absorb αT than rabbits. Indeed, the higher fat content added to chicken feed may have facilitated the absorption of liposoluble vitamins such as αT. Moreover, other unknown feed matrix effects may also contribute to these differences. Furthermore, differences in digestion processes and feed intake between chickens and rabbits might also influence the final αT content of their tissues.

The increase in the oxidative degree of the oils added to the feeds caused a reduction in the αT content of chicken plasma, liver and meat. In rabbit, the αT content of meat and liver also decreased as did that of plasma, but in this last case it was not significant, which led to a significant interaction of the factors 'animal species' × 'level of oxidation' for αT (and other tocols) in plasma, liver and meat (Table 7). Feed with MOX and HOX oils also presented a lower αT content than LOX feed, as a result of the loss of αT in the frying oil, which might contribute to the differences observed between tissues. However, the decrease in αT content observed in feed formulated with MOX and HOX oil was greater in tissues than in feeds, particularly in rabbit meat and in the liver and plasma of both animals (Table 7). Thus, we conclude that factors other than the amount of αT supplied by the feed affect the final tissue αT content. Previous studies attributed the reduction of tissue αT content also to a loss of this antioxidant in the gastrointestinal tract as a result of its reaction with radical species, thereby reducing the amount available for absorption (Sheehy *et al.*, 1993). However, this

Table 7 *Tocol composition of meat, liver and plasma of chicken and rabbit in response to the level of oxidation of the oil added to feeds*

	Chicken[a]				Rabbit[a]				Species effect[b]	Species × level effect[b]
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.		
Feed (mg/kg)										
α-tocopherol	50.78 z	46.42 y	40.92 x	0.836	54.84 y	54.51 xy	52.24 x	0.701	**	**
β-tocopherol	2.19 z	2.09 y	1.90 x	0.025	1.99 y	2.02 y	1.81 x	0.024	**	*
γ-tocopherol	28.54 x	29.70 xy	31.16 y	0.410	2.64	2.73	2.68	0.032	**	**
δ-tocopherol	6.91	6.77	7.14	0.302	0.55	0.64	0.65	0.033	*	
Total tocopherols	88.42 y	84.98 xy	81.11 x	1.151	60.03 y	59.90 y	57.37 x	0.729	**	**
α-tocotrienol	5.15	4.90	4.99	0.338	2.68	2.59	2.73	0.063	**	
β-tocotrienol	1.79	1.71	1.67	0.051	3.24	3.28	3.07	0.145	**	
γ-tocotrienol	6.88	6.33	5.56	0.833	0.94	1.03	0.93	0.037	**	
δ-tocotrienol	0.48	0.28	nd	0.251	nd	nd	nd			
Total tocotrienols	14.30	13.22	12.22	1.457	6.81	6.88	6.72	0.195	**	
Meat (mg/kg)										
α-tocopherol	17.73 y	15.67 xy	13.79 x	0.822	4.34 y	3.96 xy	3.69 x	0.166	**	*
β-tocopherol	0.44	0.42	0.42	0.030	0.19	0.19	0.19	0.007	**	
γ-tocopherol	3.16	1.97	1.93	0.483	0.25	0.23	0.25	0.007	**	
δ-tocopherol	0.96	0.44	0.53	0.192	nd	nd	nd		**	
Total tocopherols	22.10 y	18.49 xy	16.67 x	1.187	4.79 y	4.38 xy	4.13 x	0.170	**	*
α-tocotrienol	0.70	1.65	0.91	0.524	0.26	0.31	0.22	0.041	**	
β-tocotrienol	tr	tr	0.34	0.339	nd	nd	nd			
γ-tocotrienol	0.60	1.65	0.96	0.630	0.04 xy	0.28 y	nd	0.072	**	
δ-tocotrienol	nd	nd	nd		nd	nd	nd			
Total tocotrienols	1.29	3.31	1.87	1.211	0.30	0.48	0.22	0.093	**	
Liver (mg/kg)										
α-tocopherol	27.35 y	21.41 x	16.87 x	1.632	6.18 y	4.55 x	4.51 x	0.410	**	*
β-tocopherol	0.89 z	0.40 y	0.25 x	0.022	0.11	0.06	0.09	0.022	**	**
γ-tocopherol	3.38	2.92	3.25	0.243	0.15	0.14	0.16	0.008	**	
δ-tocopherol	nd	nd	nd		nd	nd	nd			
Total tocopherols	31.62 y	24.74 x	20.37 x	1.820	6.44 y	4.75 x	4.75 x	0.415	**	*
α-tocotrienol	0.28 xy	0.24 x	0.36 y	0.034	0.09	0.09	0.03	0.022	**	*
β-tocotrienol	nd	nd	nd		nd	nd	nd			
γ-tocotrienol	nd	nd	0.03	0.017	nd	nd	nd			
δ-tocotrienol	nd	nd	nd		nd	nd	nd			
Total tocotrienols	0.28 xy	0.24 x	0.39 y	0.043	0.09	0.09	0.03	0.022	**	*
Plasma (mg/l)										
α-tocopherol	15.54 z	12.11 y	8.71 x	0.647	3.66	3.50	3.20	0.305	**	**
β-tocopherol	0.28 z	0.22 y	0.17 x	0.010	nd	nd	nd		**	**
γ-tocopherol	1.21	1.05	1.00	0.086	0.14	0.14	0.14	0.006	**	**
δ-tocopherol	0.13	0.14	0.12	0.007	nd	nd	nd		**	
Total tocopherols	17.17 z	13.51 y	10.01 x	0.741	3.80	3.64	3.34	0.308	**	**
α-tocotrienol	0.18	0.16	0.16	0.009	nd	nd	nd		**	
β-tocotrienol	nd	nd	nd		nd	nd	nd			
γ-tocotrienol	0.03	0.05	0.04	0.026	tr	tr	tr			
δ-tocotrienol	nd	nd	nd		nd	nd	nd			
Total tocotrienols	0.20	0.20	0.20	0.029	tr	tr	tr			

LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; nd = not detected; tr = traces.

[a]Values correspond to means ($n = 8$); s.e.m., pooled standard error of the means of each animal species.

[b]Multifactor ANOVA ($n = 48$, chicken + rabbit) was conducted to study whether the factor 'level of oxidation of oil added to feed' led to different effects between animal species. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

x, y, z: Values in the same row for a certain tissue bearing no common letters are statistically different ($P \leq 0.05$) according to one-way ANOVA conducted for each species to study the effects of the factor 'level of oxidation of oil added to feed'. Letters were obtained by means of the Scheffé's test ($\alpha = 0.05$).

was more evident when fats presented a higher content of primary oxidation compounds than in our study (Sheehy *et al.*, 1994; Tres *et al.*, 2010b). These results highlight the relevance of a complete characterization of feed ingredients, including their composition and oxidation status, as these two parameters might affect the nutritional characteristics of meat and other animal products. Indeed, some studies have recommended supplementing diets with α-tocopheryl acetate to counteract

Table 8 *Oxidation, susceptibility to oxidation and relationship between the content of polyunsaturated FAs and α-tocopherol in meat, liver and plasma, depending on the level of oxidation of oil added to feeds*

	Chicken[a]				Rabbit[a]				Species effect[b]	Species × level effect[b]
	LOX	MOX	HOX	s.e.m.	LOX	MOX	HOX	s.e.m.		
Oxidation (TBA values)										
Meat (μg MDA/kg)	24	23	28	3.8	67	60	45	15.5	**	
Liver (μg MDA/kg)	80	82	69	9.6	172	153	165	13.9	**	
Plasma	nd	nd	nd		nd	nd	nd			
Susceptibility to oxidation (LHP values)										
Meat (mmol CHP eq/kg)	0.51	0.59	0.55	0.047	0.22	0.22	0.22	0.032	**	
Liver (mmol CHP eq/kg)	2.49 x	3.42 x	3.74 y	0.359	6.93	6.34	6.42	0.360	**	*
Plasma (mmol CHP eq/l)	0.010	0.012	0.010	0.0037	0.037 y	0.032 x	0.031 x	0.0017	**	
PUFA/α-tocopherol[c]										
Meat	194	195	230	22.7	178	181	215	13.1		
Liver	44 x	59 xy	75 y	5.9	189	243	251	19.8	*	
Plasma	6.8 x	9.2 y	10.7 y	0.63	18.4	17.7	19.5	1.28	*	

LOX = feed containing oil with a low degree of oxidation; MOX = feed containing oil with a medium degree of oxidation; HOX = feed containing oil with a high degree of oxidation; TBA = thiobarbituric acid; nd = not determined; LHP = lipid hydroperoxide; CHP = cumene hydroperoxide; PUFA = polyunsaturated FAs.
[a]Values correspond to means ($n = 8$); s.e.m., pooled standard error of the means of each animal species.
[b]Multifactor ANOVA ($n = 48$, chicken + rabbit) was conducted to study whether the factor 'level of oxidation of oil added to feed' led to different effects between animal species. *$P \leq 0.05$, **$P \leq 0.01$.
[c]PUFA/α-tocopherol values were calculated by dividing the total PUFA content of each sample (expressed as mg of FA per 100 mg or 100 ml of tissue) by its α-tocopherol content (expressed as mg of α-tocopherol per kg or litre of tissue).
x, y, z: Values in the same row for a certain tissue bearing no common letters are statistically different ($P \leq 0.05$) according to one-way ANOVA conducted for each species to study the effects of the factor 'level of oxidation of oil added to feed'. Letters were obtained by means of the Scheffé's test ($\alpha = 0.05$). For liver LHP value, significance was found by Duncan's test, but not by Scheffe's test.

the reduction of αT caused by the addition of oxidized oils to feeds (Bou *et al.*, 2009; Tres *et al.*, 2010b).

Effects on the oxidation levels and oxidability of meat, liver and plasma

Oxidation (TBA values) and susceptibility to oxidation (LHP values) of meat, liver and plasma from chicken and rabbits fed oxidized oils were low (Table 8), even in samples from animals on diets including HOX oil, and they were similar to values obtained in other studies on heated oils in feed (Bou *et al.*, 2005; Tres *et al.*, 2010b). The development of oxidation both *in vivo* and *post mortem* (meat) is related to a balance between substrates (such as PUFA), pro-oxidants (such as some inorganic elements) and antioxidants (such as αT; Erickson, 2007). In the present study, this balance led to low oxidation values in tissues. However, higher oxidation has been reported when animals receive a higher PUFA supply from the diet (Grau *et al.*, 2001; Bou *et al.*, 2004; Tres *et al.*, 2009a; Kouba and Mourot, 2011).

With regard to differences between species, rabbit meat and liver samples showed higher TBA values than the corresponding chicken samples (Table 8). This is in accordance with the lower αT content in rabbit than chicken samples (Table 7). The reduction of the formation of malondialdehyde is a well-known effect of αT (Frankel, 1991). Thus, compared with rabbit samples, the formation of malondialdehyde in chicken meat samples was retarded by the higher αT content, although chicken meat samples had a higher fat content. Nevertheless, as commented above, the TBA values found were low in all cases.

With regard to differences in the susceptibility to oxidation between species, chickens showed higher meat susceptibility to oxidation (LHP values) than rabbits (Table 8). However, their liver and plasma LHP values were lower than in rabbits (Table 8). The relative content of PUFA/αT in these types of samples would explain the differences observed between species in the susceptibility of each tissue to oxidation. This relative PUFA/αT was calculated by dividing the PUFA content in tissues (expressed as mg of FA per 100 mg or 100 ml of tissue/plasma) by the αT content (expressed as mg of αT per kg or litre of tissue/plasma). In liver and plasma, chicken presented lower PUFA/αT values than rabbit, and in meat samples chickens had higher (but non-significant) PUFA/αT values than rabbits (Table 8). An increase in the PUFA/αT value implies that there is less αT available to protect PUFA from oxidation and that, once started, oxidation might develop faster, reaching a higher final oxidation value. In our study, rabbit meat showed higher TBA values than chicken meat, but lower susceptibility to oxidation. Globally, this would indicate that because of the lower αT content the formation of malondialdehyde might have started before in rabbit meat. However, owing to the PUFA/αT balance, the final oxidation value that rabbit meat could potentially reach would be lower than that in chicken meat.

With regard to the effects of feed, the addition of LOX, MOX and HOX oils to feeds did not cause major changes in the oxidation degree of tissues (TBA values) or their susceptibility to oxidation (LHP values; Table 8). This finding thus indicates that raw meat from chickens and rabbits receiving feeds containing recovered frying oils showed acceptable oxidation status and susceptibility to oxidation. Only slight differences were observed for the LHP value of chicken liver (Table 8). These results are in agreement with previous studies in which the inclusion of oil heated at high

temperatures did not enhance the oxidation of chicken and rabbit tissues or their susceptibility to this process (Grau *et al.*, 2001; Bou *et al.*, 2005; Tres *et al.*, 2010b). Although the absorption of secondary oxidation compounds in the gastrointestinal tract has been reported (Kanazawa and Ashida, 1998; Marquez-Ruiz *et al.*, 2008), results from these and previous studies indicate that the effects on tissue oxidation are more related to the content of αT in tissues, which in turn may depend on its loss during oil heating and during digestion and on its antioxidant activity *in vivo* and *post mortem*. Indeed, most of the effects on tissue composition and oxidation described in previous studies on the addition of oxidized oils to feeds seemed to be explained by the use of highly oxidized fats, sometimes added at huge amounts to feed, which led to altered fat digestion and absorption, as well as to deficiencies in EFA and liposoluble vitamins, such as αT (Marquez-Ruiz *et al.*, 2008). In contrast, the oxidation level of the oils that we tested was much lower and did not alter fat digestibility (Blas *et al.*, 2010). However, results obtained in the same samples by another partner of the *Feeding Fats Safety* project showed significant increases in the content of cholesterol oxidation compounds in tissues as a result of the addition of HOX oil to feeds (Ubhayasekera *et al.*, 2010a and 2010b).

General conclusions

In summary, the inclusion of recovered frying oils in rabbit and chicken feeds did not lead to major changes in the FA composition, oxidation status or susceptibility to oxidation of the meat, liver and plasma of these animals. Thus, the addition of these oils to feeds, at the amounts usually added for each species, may lead to a similar FA composition, oxidation status and stability of the final products to that achieved with the addition of a similar non-oxidized oil. However, the use of oxidized oils may lead to a decrease in the αT content of tissues and thus alter the nutritional quality of meat and other animal products. This could be avoided by supplementing feeds with αT. Oils recovered from frying industries show better characteristics than 'recycled cooking oils', which present a more complex and variable composition, higher levels of impurities and higher levels of degradation. Therefore, and according to our results, recovered frying oils could be useful for feed uses and provide safer characteristics, although further studies are still necessary.

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