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ORIGINAL PAPER

Effect of Feed Fat By-Products with Trans Fatty Acids and Heated Oil on Cholesterol and Oxycholesterols in Chicken

Sarojini J. K. A. Ubhayasekera · Alba Tres · Rafael Codony · Paresh C. Dutta

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Abstract Chicken is the most widely consumed meat all over the world due to chickens being easy to rear, their fast growth rate and the meat having good nutritional characteristics. The main objective of this paper was to study the effects of dietary fatty by-products in low, medium and high levels of oxidized lipids and *trans* fatty acids (TFAs) on the contents of cholesterol and oxycholesterols in meat, liver, and plasma of chickens. A palm fatty acid distillate, before and after hydrogenation, and a sunflower–olive oil blend (70/30, v/v) before and after use in a commercial frying process were used in feeding trials after adding 6% of the fats to the feeds. Highly oxidized lipid and TFA feeds significantly increased the contents of cholesterol and oxycholesterols in all tissues of chicken ($0.01 < p \leq 0.05$). The contents of oxycholesterols in chicken meat, liver and plasma obtained from TFA feeding trials varied between 17 and 48 μg/100 g in meat, 19–42 μg/100 g in liver and 105–126 μg/dL in plasma. In contrast, in the oxidized lipid feeding trials, oxycholesterols varied between 13 and 75 μg/100 g in meat, 30–58 μg/100 g in liver and 66–209 μg/dL in plasma. Meat from chickens fed with feeds containing high levels of TFAs or oxidized lipids may contribute to higher ingestion of cholesterol and oxycholesterols by humans.

S. J. K. A. Ubhayasekera (✉) · P. C. Dutta
Division of Food Chemistry, Department of Food Science, Swedish University of Agricultural Sciences (SLU), Box 7051, 75007 Uppsala, Sweden
e-mail: Kumari.Ubhayasekera@lmv.slu.se

A. Tres · R. Codony
Nutrition and Food Science Department, XaRTA, INSA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

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Introduction

Thermally oxidized lipids are commonly considered to contain potentially toxic lipid oxidation products that are readily absorbed by animals and may induce oxidative stress [1]. Studies in rabbit, chicken, and other susceptible species showed that dietary cholesterol and oxycholesterols can lead to atherosclerosis [1–3]. Cholesterol and cholesterol oxidation products (COPs, oxycholesterols) are also known to be a risk factor for coronary heart disease (CHD) and COPs may have cytotoxic, mutagenic and atherogenic effects, and thus are harmful to humans [4].

Little is known about the effects of dietary TFA in formulated feed on animal fat deposition, and their subsequent effects on human health in quantitative terms. In some animals, dietary *trans* fatty acids (TFAs) increase the total cholesterol and LDL-cholesterol and tend to decrease HDL-cholesterol, possibly due to increased cholesteryl ester transfer protein (CETP) activity in the plasma [5, 6]. Recently, it has been shown that female broilers fed a diet containing TFA had similar body fat and protein contents and did not differ significantly from the control group except for body fatty acid composition. The authors showed that 50% of ingested TFA was incorporated into the body fat and that it could have a negative affect on the nutritional value of chicken meat [7]. As far as we know, there have been no previous reports on the content of COPs in tissues of chicken fed with different levels of TFA.

Chickens, like other animals, biosynthesize cholesterol or ingest it in their diet, and its level in body tissues can be modulated by feed lipids [8, 9]. This in vivo cholesterol

may undergo enzymatic oxidation, mainly during biosynthesis of bile acids and steroid hormones. Moreover, cholesterol can be oxidized to COPs in vivo by hydroxy radicals (OḢ), alkoxy radicals (RȮ) and peroxy radicals (ROȮ) formed during lipid peroxidation and COPs may also originate from the diet [10]. Grau et al. [11] have observed that the dietary lipid sources including beef tallow, fresh and oxidized sunflower oils did not have a considerable effect on the cholesterol content in raw chicken meat, whereas the content of COPs was significantly higher only in the chickens fed with sunflower oil [11]. In contrast, Bonoli et al. [12] have reported that chickens fed with animal fat had significantly higher contents of cholesterol and COPs in their meat compared with chickens fed with vegetable oil-based feeds [12].

To prepare animal feeds, fats and oils from various sources can be added, including used frying oil, by-products from edible fat and oil refining, or fatty acid distillates [13]. However, to our knowledge, their effects on the meat product quality, specifically in terms of cholesterol and COPs in chicken have not been investigated earlier. In the present study, we investigated the effect of heated (oxidized) oils (OXL) and TFAs in feeding fats obtained from by-products of the food chain on the levels of cholesterol and COPs in chicken meat, liver, and plasma.

Materials and Methods

Experimental Oils

In the TFA and the OXL trials, two types of fat by-products were used. These fats contained 'high' and 'low' levels of TFA and OXL, respectively, which were assessed in the trials. The selected fats were commercially available and similar in other characteristics to minimize the errors. The 'low' TFA fat (0.14% total TFA, α-tocopherol 16.43 mg/kg) was a palm fatty acid distillate; the 'high TFA' fat (10.01% total TFA, α-tocopherol 14.24 mg/kg) was a hydrogenated palm fatty acid distillate. A mix of sunflower and olive oil (70:30, v/v) before and after industrial frying was selected as 'low OXL' (*p*-anisidine = 2.74, α-tocopherol 52.23 mg/kg) and 'high OXL' (*p*-anisidine = 67.43, α-tocopherol 41.24 mg/kg), respectively, for the OXL trial. The high levels of TFA and OXL were selected based on the maximum values found in the samples of commercial feed fats during the first work program of the EU project [13], and do not reflect usual practices in feeding animals. The 'medium' levels for both TFA and OXL trials were prepared by mixing 'high' and 'low' levels 1:1. These three levels of the two experimental fats were added to the basal diets at 6% (w/w) to prepare the chicken feeds.

Animal Feeds and Experimental Protocol

The experimental feeds for the chickens were formulated according to their nutritional requirements [14]. The feed ingredients and nutrient composition of the chicken feed are given in Table 1. To reduce dietary variations other than the added fat, the following procedures were carried out: (a) the same batches of each one of the seven raw materials tested were used in all the experimental feeds, (b) batches of basal mix were prepared and then subdivided to obtain identical participation of each batch in each experimental feed. Samples of all raw materials were checked to confirm low levels of dioxin/polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs).

The protocols followed in the chicken trials were approved by the Animal Protocol Review Committee of the University Autonoma of Barcelona. The protocols, housing, husbandry and slaughtering conditions conformed to current European Union guidelines. For each trial (TFA and OXL), 96 female chickens (Ross 308, 7 days old) were randomly distributed into three different dietary treatment groups (high, medium, and low) with eight replicates per treatment and four chickens constituting each replicate. Standard temperature, humidity and ventilation conditions were provided. In all cases, feed and water were given

Table 1 Ingredients and nutrient composition of the basal chicken feed

Ingredient	(%)	Nutrient composition	(%)
Corn	52.7	Metabolizable energy (kcal/kg)	4,968
Soybean meal (47% of CP)	30.0	Dry matter	90.8
Added fat material	6.0	Crude protein	21.1
Full fat soyabean	6.0	Ether extract	9.5
HCl L-Lysine	0.3	Crude fiber	3.8
DL-Methionine (99%)	0.2	Ash	6.5
Dicalcium phosphate	2.5		
Calcium carbonate	1.3		
Salt	0.5		
Vitamin and mineral premix*	0.5		

* Composition of vitamin and mineral premix (1 kg of feed contained): Vitamin A: 6000 UI; vitamin D_3: 1200 UI; 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; Co: 0.2; ethoxyquin: 16 mg; addition of choline chloride 15 mg

ad libitum. After 40 days, chickens were sacrificed in a commercial slaughterhouse.

Preparation of Meat, Liver and Plasma Samples

Chicken meat samples consisting of de-boned dark leg meat with skin and each replicate were ground in a mixer until a suitable homogeneous sample was obtained. Representative sub-samples for each analysis (15–20 g) were vacuum packed, and stored at −20 °C until further analysis. Each replicate contained livers from four chickens and this mixture of livers was ground until it became homogenized, it was then vacuum packed in plastic bags and stored at −20 °C until further analysis. Chicken plasma samples were collected from four animals from each replicate by means of a syringe, placed in heparinized tubes, and centrifuged at 4 °C and 1,400*g* for 10 min. The plasma was separated, mixed well, and divided into aliquots that were transferred to plastic tubes (4.5 mL capacity) and stored at −20 °C.

Determination of Cholesterol in Meat, Liver and Plasma

The cholesterol content was determined by the direct saponification method as described previously with slight modification [15]. In brief, about 100 mg of chicken meat or liver and 5α-cholestane (10–20 μg) were saponified with 4 mL 2 M KOH in 95% ethanol in a glass tube at 100 °C for 15 min in a water bath. For chicken plasma, the same method was used except that each 100 μL sample was saponified with 1 mL of 2 M KOH. The extraction of sterol, preparation of trimethyl ester derivatives of cholesterols, and quantification by GC were done as described earlier [16].

Determination of Oxycholesterols (COPs) in Meat and Liver

Extraction of Lipids

A slightly modified method of lipid extraction was used [17]. In brief, approximately 10 g meat samples or 8 g liver samples were homogenized with 25 mL HIP (hexane/isopropanol; 3:2, v/v) using an Ultra-Turrax T_{25} homogenizer (S 25N-8G) (Jankel & Kunkel GmbH, Staufen, Germany). Then 10 mL of an aqueous 6.67% Na_2SO_4 was added and mixed thoroughly. The upper organic solvent phase was collected after centrifuging the samples and the remaining sample was re-extracted three times with 12.5 mL hexane. The solvent was evaporated using a rotary evaporator under vacuum at 30 °C and dry lipids were stored at −20 °C until further analysis.

Cold Saponification and Enrichment of COPs

The COPs were enriched by cold saponification of the extracted lipids (approximately 300 mg) from both meat and liver and then further enriched by solid phase extraction (SPE) to remove unoxidized cholesterol and derivatized to trimethylsilyl (TMS) ether prior to analysis by GC and GC–MS as described previously [18].

Determination of COPs by GC

Analysis of COPs was performed using an Agilent GC 6890N and ChemStation Rev. B.02.01 software (Agilent Technologies, Wilmington, DE, USA) equipped with an auto-sampler (CTC Analytics AG, Zwingen, Switzerland). Two fused silica capillary columns DB5-MS (15 m × 0.18 mm, 0.18 mm) and DB35-MS (10 m × 0.2 mm, 0.18 mm) (J & W Scientific, Folsom, CA, USA) connected by a universal pressfit connector in the order of decreasing polarity were used for separation and quantification of COPs. The initial oven temperature was 60 °C for 1 min, then increased to 290 °C at a rate of 50 °C/min and maintained for 10 min, then raised to 300 °C at a rate of 0.8 °C/min and maintained for another 7 min. The detector and injector were set at 320 °C and 260 °C, respectively. The TMS ether derivatives of the COPs were injected with a splitless mode of injection. Helium was used as the carrier gas and nitrogen as the make-up gas at a flow rate of 0.7 and 30 mL/min, respectively. Quantification of COPs was accomplished using 5α-cholestane as the internal standard. The retention times were compared against standard COPs for peak identification.

Identification of COPs by GC–MS

Further structural confirmation of COPs was done using a Voyager mass spectrometer with Xcalibur version 1.2 software (Finnigan, TheromQuest, Manchester, UK) coupled to a 8000 Top Series gas chromatograph and an AS800 autosampler (CE Instruments, ThermoQuest Italia S.p.A., MI, Italy). The COPs were separated on a similar column, except that the length of the DB5MS column was 20 m, as used in GC analysis. Helium was used as the carrier gas at an inlet pressure of 80 kPa. The injector temperature was 250 °C and the samples were injected using a splitless mode of injection. The oven temperature was 60 °C for 1 min, then it was raised to 290 °C at a rate of 50 °C/min for 10 min, and finally the temperature was raised to 300 °C at a rate of 0.8 °C/min for another 7 min. The mass spectra were recorded at an electron energy of 70 eV and the ion source temperature was 200 °C. The spectra were scanned in the range 50–600 *m/z*. Identification of the COPs was done by comparing mass spectra of

standard samples of COPs. A list of the COPs analyzed in this study along with retention times (RT), relative retention times (RRT) in relation to 5α-cholestane, a GC–MS chromatogram and the full scan mass spectra of the standard COPs, are shown in Table 2 and Figs. 1 and 2, respectively.

Determination of COPs in Plasma

Cold Saponification and Enrichment of COPs

COPs were enriched by saponification of 1 mL of plasma with 5 mL of 10% KOH in 95% EtOH at room temperature for 18 h in the dark, following a method described previously with some modifications [19]. The reaction was stopped by addition of 5 mL of saturated NaCl solution. The unsaponifiables were then extracted twice with 2 mL of hexane. The pooled hexane phase was washed with 5 mL of 5% NaOH solution and then the upper phase was washed with 5 mL saturated NaCl solution. The hexane phase was dried under nitrogen and dissolved in 500 μL of hexane: diethyl ether (75:25). The fraction containing COPs was enriched by SPE, except that 6 mL of hexane: diethyl ether (60:40, v/v) was used for a second wash of the SPE cartridge and quantified by GC as described above.

Statistical Analysis

Unless stated otherwise, all results are expressed as the mean of four replicates. Pearson's correlation and one-way ANOVA (analysis of variance) with TFA and oxidized lipid treatments at three levels were performed using the GLM option in Minitab. p-Values ≤ 0.05 were considered statistically significant.

Table 2 Retention times (RT) and relative retention times (RRT) of oxycholesterols (COPs) separated on two dimensional capillary GC[a]

COPs	Peak number	RT (min)	RRT
5α-cholestane (IS)	1	17.88	
7α-hydroxycholesterol	2	21.29	1.19
7β-hydroxycholesterol	3	25.36	1.42
7-ketocholesterol	9	36.70	2.05
20α-hydroxycholesterol	4	28.06	1.57
Cholesterol-5α,6α-epoxide	5	29.67	1.66
Cholesterol-5β,6β-epoxide	6	28.76	1.61
Cholestanetriol	7	30.41	1.70
25- hydroxycholesterol	8	32.41	1.81

[a] GC capillary columns were DB-35MS (10 m × 0.2 mm × 0.33μm) and DB-5MS (15 m × 0.18 mm × 0.18μm); RRT, relative to 5α-cholestane (IS, internal standard)

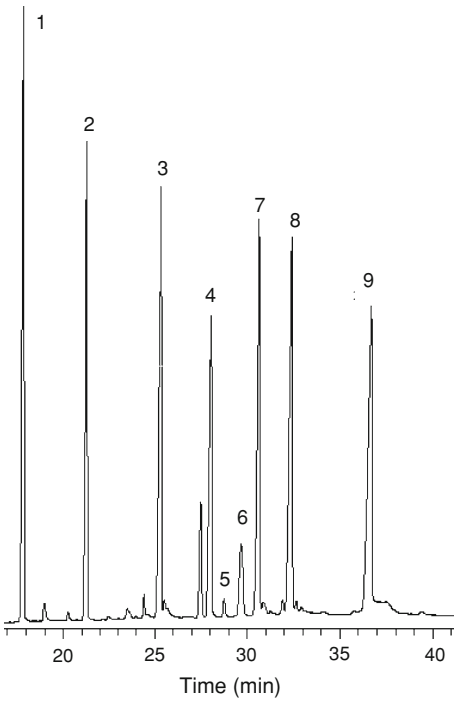


Fig. 1 GC–MS chromatogram showing resolution of standard samples of some common cholesterol oxidation products. GC–MS conditions are described in "Materials and methods". *1*, 5α-cholestane, (IS); *2*, 7α-hydroxycholesterol; *3*, 7β-hydroxycholesterol; *4*, 20α-hydroxycholesterol; *5*, Cholesterol-5β,6β-epoxide; *6*, Cholesterol-5α,6α-epoxide; *7*, cholestanetriol; *8*, 25-hydroxycholesterol; *9*, 7-ketocholesterol

Results and Discussion

The meat, liver and plasma samples of chickens from low, medium, and high levels of both the TFA (basal + *trans* fatty acid) and OXL (basal + oxidized lipid) experiments were assessed for total cholesterol and cholesterol oxidation products (COPs). Determination of total cholesterol was conducted by direct saponification of the meat, liver and plasma samples. The COPs in the extracted lipids from meat and liver were analyzed by direct saponification of the plasma samples at room temperature. The most common COPs were identified: i.e., β-ring oxidation products such as 7α-hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), 7-ketocholesterol (7-KC), cholesteroltriol (CT), and epoxy isomers (α-epoxycholesterol (α-CE), β-epoxycholesterol (β-CE)), and common side-chain COPs such as 20α-hydroxycholesterol (20α-HC) and 25-hydroxycholesterol (25-HC); however, none of the samples contained quantifiable amounts of 20α-HC (<0.1 μg/100 g sample). The purity of the identified peaks of the COPs in the tissue samples were checked by comparing their MS-data with standard COPs as shown in Figs. 1 and 2. The values reported are the means of analyses of four replicates from each treatment ($n = 4$).

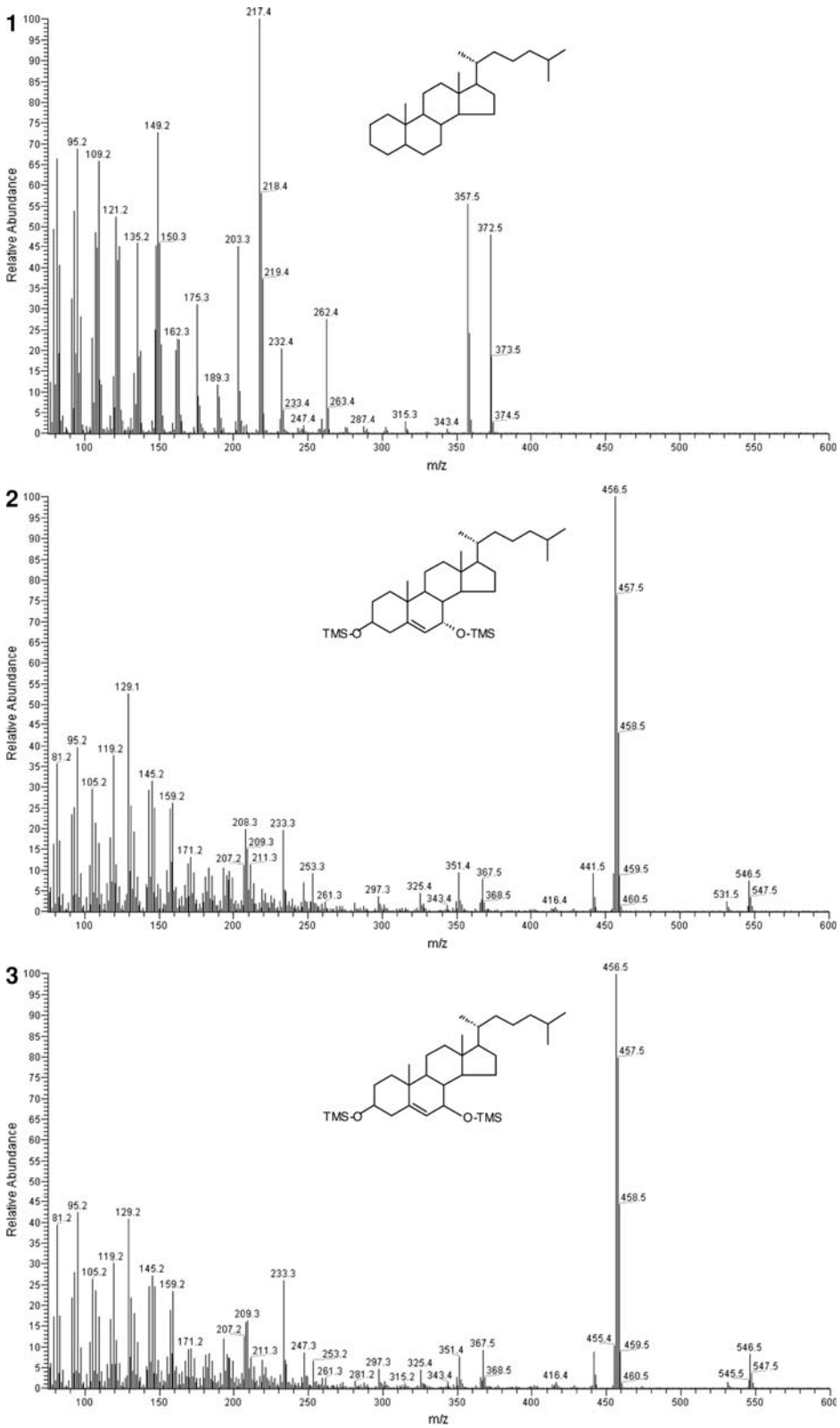


Fig. 2 Full scan MS spectra of 5α-cholestane (IS) and trimethylsilyl ether (TMS) derivatives of standard sample of oxycholesterols from the corresponding peaks 1–9, respectively, as shown in Fig. 1. A, 5α-cholestane, *m*/*z* 372 (**1**); B, 7α-hydroxycholesterol, *m*/*z* 546 (**2**); C, 7*β*-hydroxycholesterol, *m*/*z* 546 (**3**); D, 20α-hydroxycholesterol, *m*/*z* 546, 461, 201 (**4**); E, Cholesterol-5*β*,6*β*-epoxide, *m*/*z* 474 (**5**); F, Cholesterol-5α,6α-epoxide, *m*/*z* 474 (**6**); G, cholestanetriol, *m*/*z* 564 (**7**); H, 25-hydroxycholesterol, *m*/*z* 546, 131 (**8**); I, 7-ketocholesterol, *m*/*z* 472 (**9**)

Fig. 2 continued

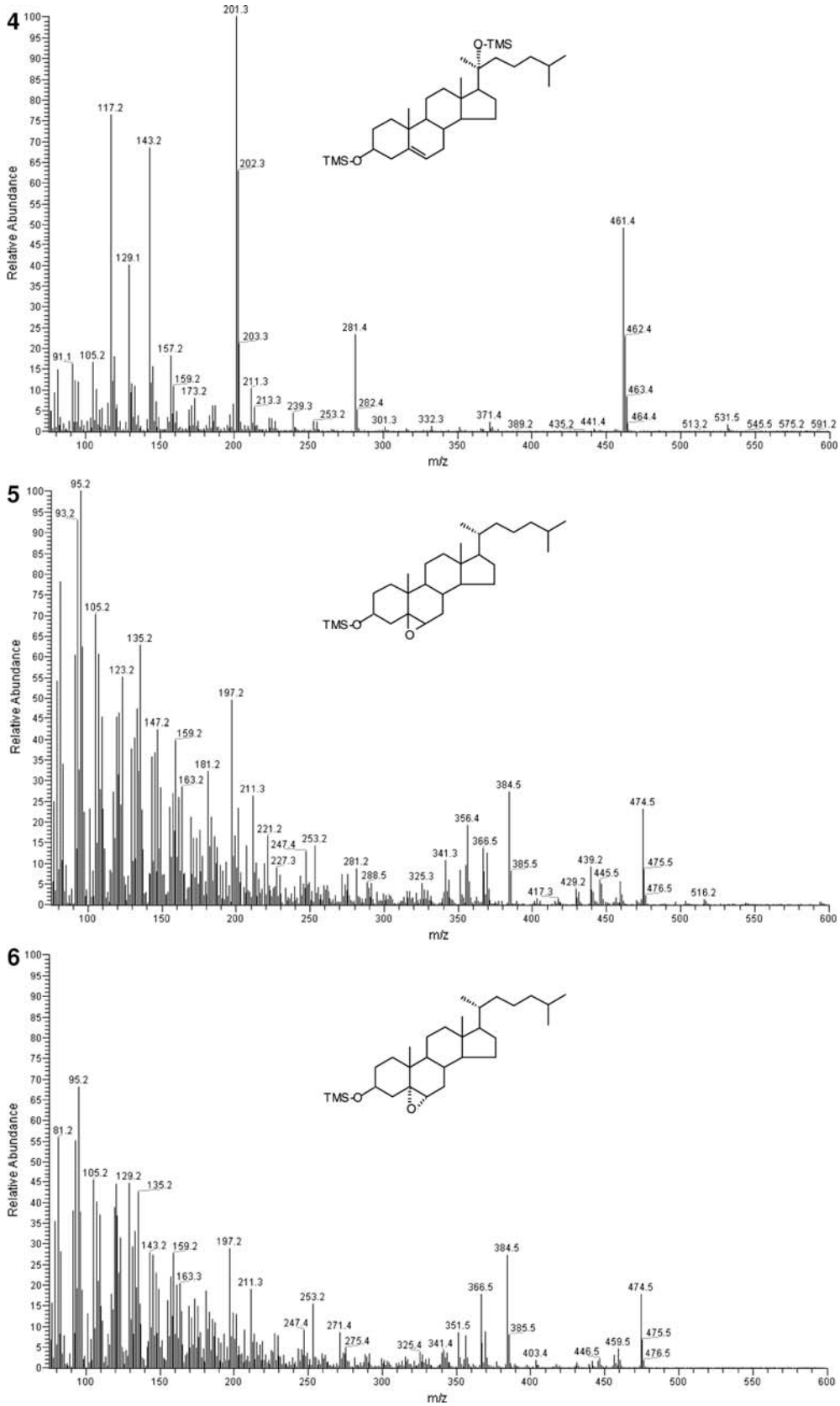
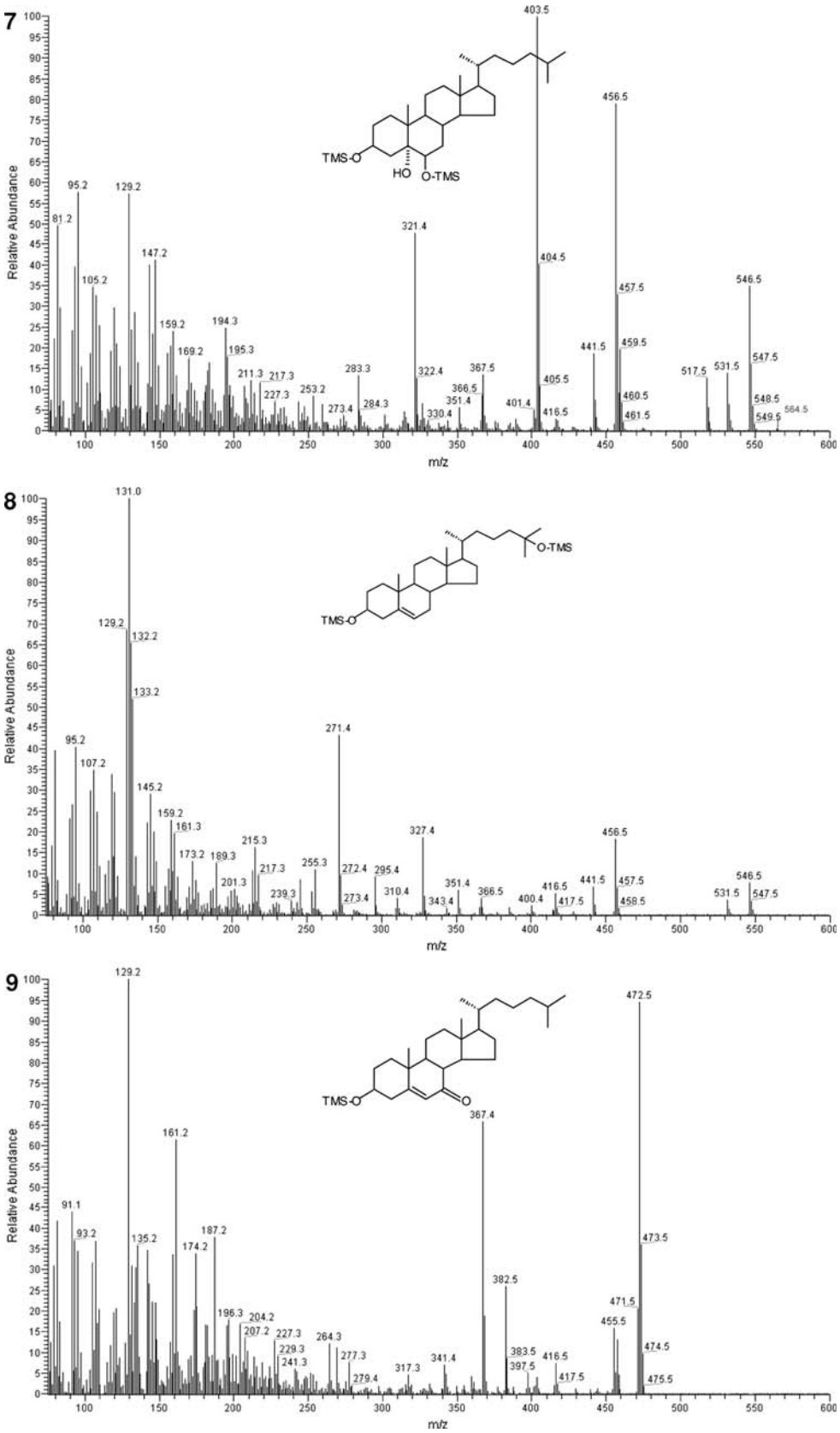


Fig. 2 continued



The inclusion of high TFA fat in feed increased the cholesterol level in chicken meat from 94 to 107 mg/100 g (Fig. 3a), and had a strong positive correlation and significant effect (0.912; $0.01 < p \leq 0.05$). Among the COPs, 7-HC isomers and 7-KC were prominent, which increased 2-fold and 7-fold, respectively, from low to high levels of TFA fed chicken. The total content of COPs increased from 17 to 48 μg/100 g from low to high TFA diet, and had a strong positive correlation and significant effect (0.905; $0.01 < p \leq 0.05$) in chicken meat (Table 3). The content of cholesterol in chicken meat increased from 84 to 104 mg/100 g (Fig. 3a) from low to high level of OXL, and had a strong positive correlation and significant effect (0.997; $0.01 < p \leq 0.05$). The total amount of COPs increased from 13 to 75 μg/100 g in chicken meat from low to high levels of OXL in feed, and had a strong positive correlation and significant effect (0.867; $0.01 < p \leq 0.05$). Among the quantified COPs, 7-HC isomers and 7-KC were the most frequent major ones (Table 3). In addition, considerable amounts of CT and 25-HC were observed only in the meat of chickens fed with high levels of OXL.

The cholesterol levels in chicken meat values presented in this paper are generally within the ranges of the published data [20–22]. In a study with dietary beef tallow, fresh and oxidized sunflower oil and linseed oil, no significant differences were observed in the cholesterol level (96–99 mg/100 g) in raw chicken meat [11]. On the other hand, Bonoli et al. [12] observed that fresh meat from chickens fed with animal fat had a significantly higher cholesterol level than that from chickens fed with vegetable oil in the feed. The levels of cholesterol were however quite low, 56 and 36 mg/100 g, in the meat of chicken fed with animal fat and vegetable oil, respectively. The level of cholesterol obtained was much lower than our results, possibly because meat samples were analyzed without skin in that study [12].

The content of COPs found in chicken meat was increased as a result of high TFA and OXL feeds. One of the reasons was that these increased COPs may have resulted from TFA and OXL feeds. Among the COPs, 7-KC, CT, and 25-HC were present in considerably large amounts, and all these COPs have been detected in animal tissues [23]. It has been suggested that 7-KC is formed both in vivo and in vitro through dehydration of the epimers of 7-HC, and CT is the hydration product of cholesterol epoxides [22]. The mechanism of the generation of the side-chain oxidation product of cholesterol (25-HC) in vivo is not yet clearly understood [24]. Whether the high amount of 25-HC in the present study in high OXL feed was the result of the high level of oxidized lipids in the diet, which facilitated oxidation of cholesterol at the tertiary carbon atom at position 25, remains to be explored.

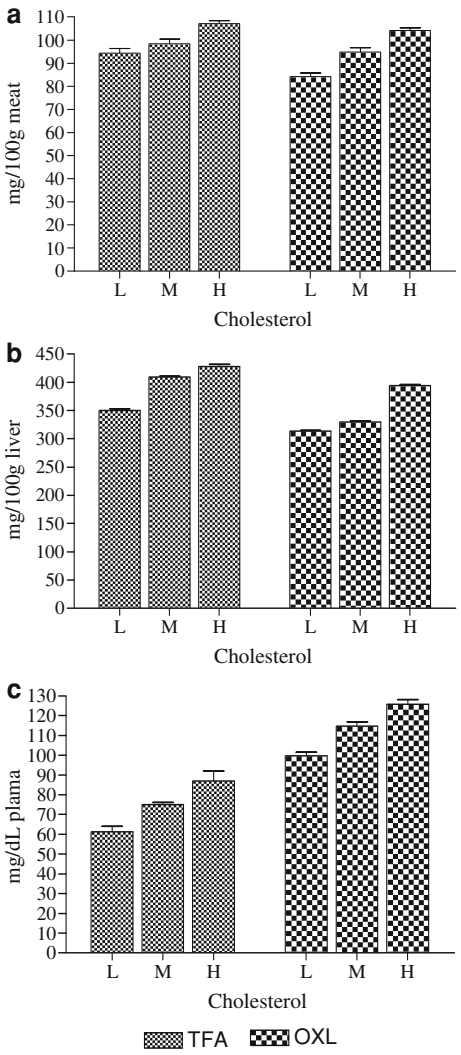


Fig. 3 **a** Contents of cholesterol in chicken meat at three levels of *trans* fatty acid (TFA) and oxidized lipid (OXL) in the feed. **b** Contents of cholesterol in chicken liver at three levels of TFA and OXL in feed. **c** Contents of cholesterol in chicken plasma at three levels of TFA and OXL in feed. *L*, low level; *M*, medium level; *H*, high level

To our knowledge, no study has been conducted previously on the effects of feed containing TFA on in vivo formation of COPs in chicken. The TFA are unsaturated fatty acids that contain at least one double bond and high level of TFA is considered to be associated with oxidative stress in vivo [23], and may be associated with the formation of COPs [25]. Other factors may affect cholesterol oxidation in meat from animals fed high TFA or high OXL feeds. The contents of tocopherols in feeds decreased to some extent from low to high TFA (16 to 14 mg α-tocopherol/kg feed), because of hydrogenation, and decreased also from low to high OXL (52 to 41 mg α-tocopherol/kg feed) (unpublished). In spite of higher amounts of α-tocopherol in the OXL feeds but resulted consistently in higher contents of

Table 3 Content of cholesterol oxidation products (COPs) in chicken meat at three different levels of *trans* fatty acid (TFA) and oxidized lipid (OXL) feeds (mean of 4 replicates per treatment)

COPs	Chicken meat (μg/100 g ± SD)					
	TFA			OXL		
	Low	Medium	High	Low	Medium	High
7α-HC	6.47 (±0.65)	8.13 (±0.53)	12.08 (±1,04)	2.94 (±0.37)	2.82 (±0.21)	5.88 (±0.75)
7β-HC	6.01 (±0.52)	8.56 (±0.37)	11.32 (±0.68)	2.48 (±0.37)	5.57 (±1.11)	4.22 (±1.82)
7-KC	3.03 (±1.45)	7.48 (±0.47)	20.77 (±2.63)	7.46 (±0.13)	13.44 (±0.9)	29.13 (±1.24)
α-CE	nd	tr	1.14 (±0.17)	nd	tr	6.37 (±1.36)
β-CE	1.94 (±0.64)	tr	2.70 (±1.7)	nd	1.07 (±0.09)	2.92 (±1.11)
CT	nd	nd	nd	nd	nd	5.13 (±0.63)
25-HC	nd	nd	nd	nd	tr	21.59 (±1.75)
Total	17.45[a]	24.17[a]	48.01[a]	12.88[b]	22.90[b]	75.24[b]

tr below the quantification level (>0.1μg/100 g), *nd* not detected, *7α-HC* 7α-hydroxycholesterol, *7β-HC* 7β-hydroxycholesterol, *β-CE* β-epoxycholesterol, *α-CE* α-epoxycholesterol, *CT* cholesteroltriol, *25-HC* 25-hydroxycholesterol, *7-KC* 7-ketocholesterol

[a] Significant increment of total and individual COPs at $0.01 < p \leq 0.05$ in chicken meat from low to high TFA feeding trials

[b] Significant increment of total and individual COPs at $0.01 < p \leq 0.05$ in chicken meat from low to high OXL feeding trials

Table 4 Content of cholesterol oxidation products (COPs) in chicken liver at three different levels of *trans* fatty acid (TFA) and oxidized lipid (OXL) feeds (mean of four replicates per treatment)

COPs	Chicken liver (μg/100 g ± SD)					
	TFA			OXL		
	Low	Medium	High	Low	Medium	High
7α-HC	4.50 (±0.24)	4.52 (±0.83)	6.18 (±0.63)	5.25 (±0.03)	12.36 (±0.33)	15.68 (±1.98)
7β-HC	5.06 (±0.48)	7.47 (±1.48)	5.26 (±1.18)	5.08 (±0.77)	7.53 (±1.49)	11.95 (±0.95)
7-KC	3.96 (±0.52)	4.47 (±1,18)	6.36 (±0.61)	4.67 (±0.56)	9.13 (±1.26)	11.87 (±0.5)
α-CE	2.19 (±0.14)	3.76 (±0.93)	6.77 (±0.57)	5.20 (±1.25)	6.80 (±1.27)	6.12 (±0.86)
β-CE	2.93 (±0.15)	7.94 (±1.43)	9.27 (±1.25)	5.24 (±0.95)	5.75 (±0.71)	5.88 (±1.17)
CT	nd	nd	4.54 (±1.49)	2.75 (±0.51)	4.14 (±0.93)	3.60 (±1.14)
25-HC	nd	nd	4.00 (±0.72)	2.14 (±0.51)	3.65 (±1.56)	3.26 (±0.58)
Total	18.64[a]	28.16[a]	42.38[a]	30.33[b]	49.36[b]	58.36[b]

tr below the quantification level (>0.1 μg/100 g), *nd* not detected, *7α-HC* 7α-hydroxycholesterol, *7β-HC* 7β-hydroxycholesterol, *β-CE* β-epoxycholesterol, *α-CE* α-epoxycholesterol, *CT* cholesteroltriol, *25-HC* 25-hydroxycholesterol, *7-KC* 7-ketocholesterol

[a] Significant increment of total and individual COPs at $0.01 < p \leq 0.05$ in chicken liver from low to high TFA feeding trials

[b] Significant increment of total COPs at $0.01 < p \leq 0.05$ in chicken liver from low to high OXL feeding trials

COPs in the tissues of chicken compared with the TFA feeds. This may be due to the destruction of α-tocopherol in the gastrointestinal tract by free radicals present in OXL feed or in the tissues by other oxidation products, which were absorbed from heated oils in the OXL feed [26]. However within TFA and OXL feeds, the differences in the α-tocopherol levels were minimal. Whether such low differences in tocopherol contents affect in vivo cholesterol oxidation needs to be studied.

It has been observed that fresh chicken meat contains very low amounts of COPs compared with processed chicken meat. And dietary supplementation of α-tocopherol can reduce the formation of COPs during processing [10, 21, 22, 27]. Tissue components in animals such as fat (mainly triacylglycerols), phospholipids, cholesterol and other polar compounds are susceptible to oxidation by molecular oxygen [10]. A high OXL diet in this study was enriched with large amounts of peroxyl and alkoxyl radicals, which may also facilitate COPs formation in vivo. A few previous reports have shown contradictory results on in vivo formation of COPs in chicken, e.g., it was shown that meat samples from oxidized and unoxidized sunflower oils and beef tallow fed chicken contained 1.42, 1.90 and 1.26 μg/g total COPs, respectively [11]. However, in

Table 5 Content of cholesterol oxidation products (COPs) in chicken plasma at three different levels of *trans* fatty acid (TFA) and oxidized lipid (OXL) feeds (mean of 4 replicates per treatment)

COPs	Chicken plasma (μg/dL ± SD)					
	TFA			OXL		
	Low	Medium	High	Low	Medium	High
7α-HC	15.32 (±2.67)	14.39 (±2.59)	14.63 (±1.52)	6.20 (±1.81)	16.89 (±1.36)	33.48 (±0.94)
7β-HC	13.77 (±0.42)	36.05 (±1.73)	24.49 (±2.43)	11.69 (±1.17)	29.50 (±3.56)	34.68 (±2.8)
7-KC	3.18 (±1.86)	2.55 (±0.61)	15.39 (±0.18)	5.31 (±0.69)	6.20 (±1.33)	33.59 (±2.09)
α-CE	7.53 (±1.40)	5.66 (±1.53)	11.84 (±2.34)	12.70 (±1.27)	15.02 (±3.59)	33.08 (±8.18)
β-CE	56.87 (±3.72)	47.52 (±1.36)	54.31 (±3.08)	24.37 (±1.94)	37.47 (±6.47)	46.05 (±8.42)
CT	1.15 (±0.15)	1.16 (±0.14)	1.36 (±0.25)	2.50 (±0.66)	1.67 (±0.41)	9.47 (±2.57)
25-HC	6.80 (±3.77)	2.07 (±1.74)	4.27 (±1.86)	2.82 (±0.84)	4.25 (±1.43)	18.16 (±7.12)
Total	104.62[a]	109.40[a]	126.29[a]	65.59[b]	111.00[b]	208.51[b]

tr below the quantification level (>0.1 μg/100 g), *nd* not detected, *7α-HC* 7α-hydroxycholesterol, *7β-HC* 7β-hydroxycholesterol, *β-CE* β-epoxycholesterol, *α-CE* α-epoxycholesterol, *CT* cholesteroltriol, *25-HC* 25-hydroxycholesterol, *7-KC* 7-ketocholesterol

[a] Significant increment of total and individual COPs at $0.01 < p \leq 0.05$ in chicken plasma from low to high TFA feeding trials

[b] Significant increment of total and individual COPs at $0.01 < p \leq 0.05$ in chicken plasma from low to high OXL feeding trials

another study animal fats and vegetable oil feeds generated 1.1 and 0.7 μg/g total COPs, respectively, in raw chicken meat [12]. These differences in the results may be due to other differences in the feeds such as fatty acid composition, natural oxidants, and pro-oxidant in the diet. These compounds may alter the levels of cholesterol and COPs in the body.

The high TFA fat in feed increased the amount of cholesterol from 350 to 428 mg/100 g in chicken liver (Fig. 3b) with respect to a low TFA feed, and showed a strong positive correlation and significant effect (0.919; $0.01 < p \leq 0.05$). Among the COPs, 7-HC isomers, CE isomers, and 7-KC were prominent in chicken liver at all TFA feed levels. Total content of COPs increased from 19 to 42 μg/100 g from low to high TFA feed, and had a strong positive correlation and significant effects (0.905; $0.01 < p \leq 0.05$), with the content of total COPs in chicken liver (Table 4).

The content of total cholesterol increased from 314 to 394 mg/100 g in liver from the low to high level of OXL fed chicken (Fig. 3b) and had a strong positive correlation and significant effect (0.822; $0.01 < p \leq 0.05$). The content of total COPs increased from 30 to 58 μg/100 g from low to high OXL in feed (Table 4). 7-HC isomers and 7-KC were the most commonly quantified COPs, where 7α-HC, 7β-HC, and 7-KC increased 3-fold, 2-fold, and 2.5-fold from the low to the high level of OXL in feed, respectively. Levels of OXL in feeds had strong positive correlation and significant effects (0.823; $0.01 < p \leq 0.05$) on total COPs in chicken liver.

In the present study, liver cholesterol levels in chicken were shown to be affected by both TFA and OXL feeds. High TFA and OXL levels in the diet caused higher cholesterol and COPs levels in chicken liver compared with low and medium diets. The COPs in liver can be formed via enzymic as well as non-enzymatic pathways. The non-enzymatic production COPs might be facilitated by high-energy free radicals from the high OXL diet. It has been suggested that the formation of COPs in vivo is affected by oxidized lipids in the diet [24].

The content of total cholesterol in plasma increased from 61 to 87 mg/dL ($0.01 < p \leq 0.05$) and from 100 to 126 mg/dL ($0.01 < p \leq 0.05$) in chicken, from low to high feed levels of TFA and OXL, respectively (Fig. 3c). The results also showed strong positive correlations (0.998 and 0.992, respectively). All the common COPs were observed in various quantities at all levels of TFA and OXL in the feed. The content of total COPs increased from 105 to 126 μg/dL in plasma of chicken from low to high levels of TFA feed and this increase was much more prominent (66–209 μg/dL) in OXL feed. These results had strong positive correlations and significant effects, (0.906; $0.01 < p \leq 0.05$) and (0.958; $0.01 < p \leq 0.05$), respectively (Table 5). Among the COPs, β-CE was prominent and was present in high amounts, whereas α-CE increased moderately in chicken plasma from the low to high level of TFA and about three times more in OXL trials. In plasma, 7β-HC and 7-KC increased 2-fold and 5-fold, respectively, and the contents of 7α-HC and CT were virtually unchanged in chicken meat from the TFA feeding experiments.

All major COPs were observed in comparatively high quantities with high OXL feed compared with TFA feed except for β-CE. The highest individual oxycholesterol increase rates were observed for 7KC and 25-HC ca. Six times for each, compared with low OXL feeds in chicken

plasma (Table 5). In addition, 7α-HC and CT and also increased to high amounts from low to high OXL in feed.

Contents of total COPs in plasma of chicken consistently increased with the increase of OXL in the feeds in contrast to TFA feeds, although the levels of total cholesterol were rather similar. Dietary TFA is known to increase total plasma cholesterol and LDL-cholesterol and reduce HDL-cholesterol in humans [28]. It has been reported that TFA activates CETP in rabbit and thereby increases the plasma LDL-cholesterol level [6]. Our results from chickens fed TFA agree with that observation, so we can conclude that chickens also adopt the same mechanism as rabbits, with CETP activity increasing plasma LDL-cholesterol levels due to increased levels of TFA in the feeds.

It has been reported that thermally oxidized fats in feeds reduce α-tocopherol in plasma and cause increased meat lipid oxidation [11]. In animals, the presence of COPs in the plasma depends on many factors e.g. rate of absorption from gut, transport to the specific tissues, elimination from the tissues by transport or metabolism, and the rate of their formation in vivo [24]. A diet high in oxidized lipids could increase endogenous oxidative stress, which may facilitate formation of COPs in vivo. One of the major factors influencing oxidative stress in plasma (in vivo) is antioxidant activity. The amount of dietary antioxidant is depleted in the oxidized lipid, resulting in increased in vivo lipid autoxidation and subsequently increased generation of COPs [11, 22, 26, 29].

A sunflower oil/olive oil blend (heated and fresh) was used for the OXL trials in this study. Sunflower oil is rich in 18:2n-6 and 18:3n-3, and olive oil is rich in 18:1n-9, and both oils contain large amounts of endogenous tocopherols. During heating of these oils, the amount of tocopherols in the oxidized lipid feed was reduced, resulting in final α-tocopherol levels in low and high OXL feeds of 52 and 41 mg/kg feed, respectively (unpublished results). This caused lower tocopherol intake by the chicken and subsequent higher oxidation of cholesterol in plasma. The presence of a large number of oxidized compounds in thermally abused oils could also play an important role in degrading α-tocopherol in plasma as well as in the gastrointestinal tract and thereby in increasing oxidation of cholesterol [30]. In our study, values of α-tocopherol in plasma were 15.5 and 8.7 mg/L plasma, in animals from low and high OXL diets, respectively (unpublished results). The influence of the lower intake and lower deposit of tocopherol in tissues can be clearly seen comparing the values found for COPs in TFA trials and in OXL trials. In the chicken plasma, the content of 7-KC was lower than that of the other C7 oxycholesterols in the low and medium TFA and OXL feeding trials. This may be due to the rate of 7-KC breakdown, which was possibly higher than formation [31].

Moderate amounts of TFA and OXL in the feeds used in this study were shown to enhance the levels of cholesterol and COPs in the chicken tissues. This is a new study on the effects of feed fats containing TFA on the levels of cholesterol COPs in chicken. Although COPs in foods are considered potential health risk but there is no regulation limiting their levels in foods. The levels shown in this paper are far below those in the literature (20 μg/100 g in fresh meat and 146 μg/100 g in cooked meat) and can be considered as safe [10]. More studies are necessary using wider ranges of TFA and OXL in feeds for their long-term effects in chicken both in terms of animal welfare and on the oxidative stability of its edible tissues for high quality chicken for human consumption.

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