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Effects of different levels of *trans* fatty acids and oxidised lipids in diet on cholesterol and cholesterol oxidation products formation in rabbit

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ABSTRACT

The objective was to assess the effects of *trans* fatty acids and oxidised lipids, present in dietary fat by-products used in feeds, on cholesterol and oxycholesterols in meat, liver and plasma of rabbits. A palm fatty acid distillate, before and after hydrogenation (*trans* fatty acid trial), and a sunflower–olive oil blend (70/30, v/v), before and after use in a commercial frying process (oxidised lipid trial), were used in experimental feeds (at 3%, w/w). High *trans* fatty acid and oxidised lipid diets caused significantly higher cholesterol and oxycholesterol levels in all tissues of rabbit ($0.01 < p \leq 0.05$). The content of oxycholesterols in rabbit meat, liver and plasma obtained from *trans* fatty acid experiments varied from 9 to 34 μg/100 g, 24 to 61 μg/100 g and 60 to 138 μg/dl, respectively, from low to high levels. In the oxidised lipid experiments, content of oxycholesterols varied from 16 to 52 μg/100 g, 14 to 108 μg/100 g and 52 to 269 μg/dl in meat, liver and plasma, respectively. As a consequence, meat products from rabbits fed a diet containing higher levels of *trans* fatty acids or oxidised lipids may result in higher intakes of cholesterol and oxycholesterols by humans.

1. Introduction

Lipid oxidation in foods results in the development of off-flavour, loss of nutritive value and generation of potentially toxic compounds (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid oxidation can occur in the living animal due to an imbalance between formation of reactive oxidants and the animal's defence mechanism arising from e.g. high intake of oxidised lipids and low intake of antioxidants. In addition to oxidation, factors such as saturated fats, *trans* fatty acids (TFA), cholesterol, food safety, animal welfare, and environmental issues have also become concerns for the general public regarding the quality of meat and meat products (Morin, 2005; Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006).

It is well-documented that higher intakes of TFA in humans increase total cholesterol level and LDL-cholesterol level compared with *cis* mono unsaturated (MUFA) and polyunsaturated (PUFA), and tend to decrease HDL-cholesterol compared with saturated fatty acids (SFA), *cis* MUFA and PUFA (Morin, 2005). Studies on the effects of TFA on cholesterol metabolism in rabbits have demonstrated that plasma LDL-cholesterol concentration is significantly higher in rabbits fed a TFA diet compared with a *cis* fatty acid diet (Gatto, Lyons, Brown, & Samman, 2001). Due to these adverse effects of TFA, different public health authorities recommend reducing dietary intake, as well as reducing TFA levels in the food supply (Gebauer, Psota, & Kris-Etherton, 2007; Mozaffarian et al., 2006). To our knowledge, there are no previous reports on the content of cholesterol oxidation products (COPs) in tissues of rabbits fed with different levels of TFA.

Oxidised lipids (OXL) present in diets and in tissues show harmful activities in modulation of lipid metabolism and various diseases, such as atherogenesis and cyto-function disturbances. Moreover thermally oxidised fat is generally considered to contain potentially toxic lipid peroxidation products that would increase oxidative stress *in vivo* (Osada, 2002; Staprans, Rapp, Pan, Hardman, & Feingold, 1996). Similarly, cholesterol can undergo both enzymatic and non-enzymatic oxidation in animals, forming various cholesterol oxidation products (oxycholesterols/COPs) (Diczfalusy, 2004; Kerry, Gilroy, & O'Brien, 2002). The genesis of COPs in meat and meat-related products by storage, cooking and processing is well documented (Kerry et al., 2002). It has been shown that dietary COPs are rapidly absorbed in humans and their levels were increased both in total plasma and in plasma chylomicron after consumption of a egg powder meal rich in COPs (Emanuel, Hassel, Addis, Bergman, & Zavoral, 1991). Some of the COPs have been implicated in a wide rage of adverse biological effects in animals, such as atherogenesis, cytotoxicity, mutagenesis carcinogenesis

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(Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002). In feeding studies with rabbits, it has been demonstrated that dietary oxidised lipids and COPs result in a 100% increase in fatty streak lesions in the aorta and cause a 100% increase in total cholesterol in the pulmonary artery of rabbits (Staprans, Pan, Rapp, & Feingold, 1998; Staprans et al., 1996).

Animal feeds often contain fats arising from the food chain, such as recycled frying oils or by-products from refining of edible fats and oils (e.g. distillates, acid oils and soap stocks) (Sheehy, Morrissey, & Flynn, 1993). In a previous paper, the effects of OXL and TFA in the feeds on chicken (meat, liver and plasma) were reported (Ubhayasekera, Tres, Codony, & Dutta, 2009). The main objective of this part of a multinational EU project (FOOD-CT2004-007020) was to investigate the effect of different levels of OXL and TFA in commercial feeding fats obtained from by-products (from the food chain), on the levels of cholesterol and COPs in meat and other tissues of rabbit.

2. Materials and methods

2.1. Feed formulation

Experimental feeds for rabbits were formulated according to their established nutritional needs (De Blas & Mateos, 1998). Feeds were prepared including 3% of the appropriate fat, as described under Section 2.2, with the rest of the feed being constituted of an identical basal mix (based on alfalfa, sugar beet pulp, barley and sunflower meal (see Table 1). Robenidine was included as a coccidiostatic agent. Batches of rabbit feeds without coccidiostatic were also prepared and distributed during the last fattening week in all cases. All feeds were manufactured at the feed plant of the Polytechnic University of Valencia, Barcelona, Spain.

2.2. Design of the experiment

Commercial fat by-products were selected, based on the results of the first phase of the EU-project (Nuchi et al., 2009). The design of the trials included six different dietary treatments, each one, corresponding to the respective addition of palm fatty acid distillate (PFAD) (low TFA; 0.41% in feed fat), the hydrogenated PFAD (high TAF; 6.27% in feed fat), and the fat blend 50:50 (intermediate TFA; 3.78% in feed fat). In the low TFA diet, total saturated fatty acid (SFA) and total polyunsaturated fatty acid (PUFA) contents were 45.6% and 23.5%, respectively. The corresponding values in high TFA diets were 66.6% and 17.4%, respectively. The final addition was an oil blend of olive/sunflower (30:70) before frying (low OXL; *p*-anisidine value 2.74) and the same blend after being used in industrial frying (high OXL; *p*-anisidine value 67.4). Total SFA and total PUFA contents in the low OXL diets were 16.8% and 49.6%, and the corresponding values in high OXL diets were 18.2% and 44.9%, respectively. The 'medium' level for the OXL trial was prepared as above by mixing 'high OXL' and 'low OXL' levels at 50:50 (Ubhayasekera et al., 2009). All treatments were replicated eight times. For the rabbit trial, 144 rabbits (three treatments × eight replicates × six animals) were housed in collective cages. 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 Polytechnic University of Valencia, Barcelona, Spain. All animal housing and husbandry conforms to the European Union guidelines.

Table 1
Ingredients and nutrient composition of the basal rabbit feed.

Ingredient (%)		Nutrient composition (%)	
Barley	10.0	Metabolizable energy (kcal/kg)	4.0
Beet pulp	30.0	Dry matter	89.5
Sunflower meal (30%)	20.0	Crude protein	13.1
Alfalfa hay	34.0	Ether extract	4.2
Added fat material	3.0	Crude fibre	20.1
HCl L-lysine	0.35	Available carbohydrates	43.6
DL-methionine (99%)	0.2	Ash	8.5
L-Threonine	0.15		
Dicalcium phosphate	1.3		
Salt	0.5		
Vitamin and mineral premix*	0.5		

* Composition of vitamin and mineral premix (1 kg of feed contained): vitamin A: 8375 UI; vitamin D_3: 750 UI; 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; BHA + ethoxyquin: 4 mg.

2.3. Samples

The same procedure was followed in order to prepare meat, liver and plasma samples from each group of animals. Thus, we obtained a sample of meat, one of liver and one of plasma per each replicate of each treatment: a total of 48 samples for each animal species (six treatments per eight replicates). Carcasses were refrigerated for 24 h after the sacrifice at 4 °C. From each group, one leg of each animal was taken and legs were hand-deboned, mixed and ground. Meat samples were vacuum-packed in high-barrier multilayer bags (Cryovac BB325; approximately 20 g meat/bag) and stored at −25 °C prior to analysis. Livers were immediately removed from carcasses at the sacrifice, and all livers from each group were ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; permeability to O_2 25 cm^3/m^2, 24 h, 23 °C, 1 bar, ASTMD-3985; Cryovac Europe, Sealed Air S.L., Sant Boi de Llobregat, Spain; approximately 15 g of liver/bag) and stored at −20 °C prior to analysis. Plasma samples were obtained from blood collected at the sacrifice. Around 20 ml of blood were collected from four rabbits in heparinized tubes and immediately centrifuged at 1450*g* at 4 °C for 10 min. Plasma samples from the animals in each group were homogenised and aliquots were transferred to plastic tubes (4.5 ml capacity) and stored at −20 °C. All the samples were packed in a dry-ice box and were transported from Barcelona, Spain, to Uppsala, Sweden by DHL air transport. After arrival, the samples were stored at −20 °C for further analyses.

2.4. Determination of cholesterol

A direct saponification and GC methods were used to determine cholesterol, as described previously, using about 100 mg of rabbit meat or liver and 100 μl of plasma (Rule, Broughton, Shellito, & Maiorano, 2002) with slight modification. Details of the methodologies have been described elsewhere (Ubhayasekera et al., 2009).

2.5. Determination of cholesterol oxidation products (COPs) in meat and liver

2.5.1. Extraction of lipids

A slightly modified method of lipid extraction by hexane and iso-propanol (HIP) was used (Hara & Radin, 1978). Approximately 10 g of meat samples or 8 g of liver samples were used for lipid extraction by a slightly modified method of Ubhayasekera et al. (2009).

2.5.2. Enrichment of COPs by cold saponification and SPE

Approximately 300 mg of lipids from both meat and liver were cold-saponified with 95% 1 M ethanolic KOH. The COP fraction was further enriched by solid phase extraction (SPE) to remove unoxidized cholesterol (Ubhayasekera, Verleyen, & Dutta, 2004).