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Enriching breakfast sausages by feeding pigs with CLA supplemented diets

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ABSTRACT

Approaches for improving the profile of functional unsaturated fatty acids in pork products include dietary supplementation of pigs with functional oils. Little information is available to indicate the benefit of this approach in a processed and cooked pork product such as breakfast sausages. Therefore, the aim of the present study is to examine the fatty acid profile and oxidation level in cooked pork sausages, produced following dietary supplementation with CLA compared to sunflower oil (SFO). Fat and moisture percentages, total fatty acid profiles and TBARS were analysed. Fatty acid profiles were altered in the sausages following all treatments. While a stronger effect was seen for CLA treatments, addition of SFO in the diet also resulted in linear increases of CLA in the sausages. CLA supplementation resulted in increased saturated fatty acid content; however, all treatments were within the recommended polyunsaturated/saturated fatty acid ratio of above 0.4. Improved oxidative stability was observed in sausages from CLA supplemented diets.

1. Introduction

Meeting consumer requirements for healthier meat products demands adoption of new strategies by the pork industry to improve the nutritional status of their products. Approaches for improving the nutritional profile of processed meat products include altering the fatty acid profile of the raw materials used through dietary intervention (Jiménez-Colmenero, Carballo, & Cofrades, 2001). In doing so, however, it is imperative that the alterations are evident after processing and cooking of the product.

Conjugated linoleic acid (CLA) is a collective term for a group of octadecadienoic acids that are geometric (*cis*, *cis*; *cis*, *trans*; *trans*, *cis*; and *trans*, *trans*) and positional (c8, c10; c9, c11; c10, c12, and c11, c13) isomers of linoleic acid (C18:2) (Pariza, Park, & Cook, 2001). CLA has been shown to have a variety of biological effects (Hur, Park, & Joo, 2007) and studies suggest that CLA health benefits may include anti-oxidation, anti-atherosclerosis, anticarcinogenic and improvements in immune-responses (Belury, Nickel, Bird, & Wu, 1996; Lee, Kritchevsky, & Pariza, 1994; Miller, Stanton, & Devery, 2001; Pariza & Hargraves, 1985; Park et al., 1999). These fatty acids have been found in the meat and milk of ruminants, where they are mainly formed by bio-hydrogenation of grass derived fatty acids. Pork, however, contains only small amounts of CLA as the pig is a mono-gastric animal (Chin, Liu, Storkson, Pariza, & Ha, 1992). Interest in dietary supplementation with CLA for pigs has increased during the last decade from an animal production perspective, due to its potential to improve productive carcass and meat quality traits and, at the same time, for obtaining meat and meat products enriched in CLA (Gatlin, See, Larick, Lin, & Odle, 2002; Martín, Antequera, Muriel, Andrés, & Ruiz, 2008; Raes, De Smet, & Demeyer, 2004; Schmid, Collomb, Sieber, & Bee, 2006; Wiegand, Sparks, Parrish, & Zimmerman, 2002). While some studies focused on the influence of processing and cooking on CLA content in meat products (Badiani et al., 2004; Ma, Wierzbicki, Field, & Clandinin, 1999; Shantha, Crum, & Decker, 1994), there has been little focus on pork products. In particular there is no information on the influence of dietary supplementation on the fatty acid profiles of processed and cooked pork sausages, which have been manufactured with lean and fat following dietary supplementation with CLA rich oil.

Therefore, the aim of the present study is to examine the fatty acid profile and oxidation level in cooked pork sausages, produced following dietary supplementation with CLA.

2. Materials and methods

2.1. Animal management

Female pigs of about 40 kg live weight were selected and formed into equal weight groups of 10 animals. The groups were isolated from each other in different pens and randomly assigned to six oil treatments, three different levels of SFO and CLA: low,

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medium and high sunflower oil (SFO) diets (0.9%, 1.8% and 3.6% SFO) and low, medium and high CLA diets (0.9%, 1.8% and 3.6% Luta-CLA 60) (Table 1). The CLA used to supplement the diets was Luta-CLA 60 (BASF, Germany) which consists of 56% (w/w) of the two main CLA isomers (*trans*-9, *cis*-11 and *cis*-10, *trans*-12) dissolved in a base of linoleic acid (C 18:2 *n*–6c). Pigs' regular diets contain 36–38% barley, 38% wheat, 20% solvent extracted soyabean meal with L-lysine, DL-methionine, L-threonine and a full range of minerals and vitamins, and were formulated to contain 13.6–14.3 MJ digestible energy/kg, 18% crude protein and 1.1% total lysine. The supplemental fat used in this study replaced barley in the diet. SFO treatments were used as a control to allow a valid comparison from a calorific perspective between the CLA supplemented diets and the control diets. Pigs were fed *ad libitum* from hopper feeders, with water available *ad libitum* from nipple-in-bowl drinkers. The trial lasted for 8 weeks, after which the pigs were slaughtered, having reached live weights of around 95 kg. Boston Butt (*M. infraspinatus*, *M. supraspinatus*, *M. subscapularis* and *M. serratus ventralis*) and back fat cuts were removed from the pigs 24 h after slaughter.

2.2. Breakfast sausage preparation and sampling

For each of the six dietary treatments (Table 1), breakfast sausages were produced using Boston Butt (see above) and back fat. Sausages were produced by combining, in percentage by weight, 44.25% of lean meat, 18.75% of back fat, 27.5% water, 7.0% rusk and 2.5% seasoning. The manufacturing process was standardised to ensure that all sausages were prepared in the same manner. Prior to the manufacture of the sausages, back fat was chopped whilst frozen for 1 min at chopping speed 2 and bowl speed 2 and refrigerated. Diced lean meat, seasoning, overnight-hydrated rusk and a third of the ice-water were then introduced into the bowl chopper (Fatosa C-35-2Z, Fatosa S.A., Sabadell, Spain) and blended at bowl speed 1 for 20 s. Finally, chopped fat and the remaining ice-water were added to the bowl chopper. All ingredients were then chopped for an additional 2 min at chopping and bowl speed 1 and the mix stuffed into collagen casings of 16 sausages to the lb. For each batch, the sausages were then vacuum packed in bags containing six sausages, and stored at −20 °C.

2.3. Sample preparation

Before analysis, for each batch, six bags were taken at random and a sample of two sausages extracted from each bag. The samples were grilled for 15 min at 200 °C on both sides using a domestic oven grill (B-AH51-7 SIEMENS-Electrogerate, GmbH Germany), then each sample was blended separately in a R301 Ultra Robot Coupe (Robot Coupe UK Ltd., Middlesex, UK) and vacuum packed in separate bags for subsequent chemical analyses (fat and moisture, TBARS and total fatty acid composition). All results are expressed as the mean and standard deviation of six replicates.

Table 1
Percentage and type of added oil added for each treatment.

Treatment	Added oil	% (oil/total feed)	CLA (%)
Low SFO[a]	SFO	0.9	–
Medium SFO	SFO	1.8	–
High SFO	SFO	3.6	–
Low CLA	Luta-CLA 60[b]	0.9	0.5
Medium CLA	Luta-CLA 60	1.8	1.0
High CLA	Luta-CLA 60	3.6	2.0

[a] SFO: Sunflower oil.
[b] Luta-CLA 60: 56% (w/w) of the two main CLA isomers (*t*9,*c*11 and *c*10,*t*12). SFO and Luta-CLA were included at the same oil level so that it can serve as a calorific control.

2.4. Chemical analysis (moisture, total fat and TBARS)

Moisture and total fat contents were determined, in percentage (w/w), by magnetic nuclear resonance using the CEM SMART Trac™ Fat and Moisture Analyzer (CEM Corporation, Matthews, USA).

Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Bruna, Ordóñez, Fernández, Herranz, and de la Hoz (2001), using trichloro acetic acid instead of perchloric acid as a solvent.

2.5. Fatty acid analysis

The total fatty acids (FA) were extracted, methylated and analysed by an adaptation of the method described by Aldai, Osoro, Barron, and Najera (2006), which has been reported to be highly effective for PUFA analysis (Juárez et al., 2008). Duplicate 1 g samples were hydrolysed in 5 M KOH in methanol:water (50:50) at 60 °C for 1 h. After dilution with 0.5% (w/v) NaCl in water, the non-saponifiable lipid fraction was removed by extraction with petroleum ether. Following protonation of the FA salts with glacial acetic acid, FAs were twice extracted in petroleum ether, dried under a stream of N_2 gas, dissolved in a methanol:toluene (2:1) mixture and methylated for 10 min at 40 °C with 2 M trimethylsilyldiazomethane in *n*-hexane (Supelco, Poole, UK).

Separation and quantification of the fatty acid methyl esters (FAME) was carried out using a gas chromatograph (GC, Varian 3400CX, Varian Associates Inc., CA, USA) equipped with a flame ionisation detector (FID) and fitted with a BPX-70 capillary column (120 m, 0.25 mm i.d., 0.2 μm film thickness, SGE, Australia). The injector was used in split mode with a ratio 1:30. The injector and the detector were set to 270 °C and 300 °C. The carrier gas used was hydrogen with a flow rate of 1.6 ml/min. FAME separation was made using a programmed temperature gradient. The oven was initially set to 50 °C and held for 1 min before the temperature was ramped at 20 °C/min up to 160 °C. The temperature was then ramped at 4 °C/min up to 220 °C held for 5 min and ramped again at 4 °C/min up to 240 °C and held at this temperature for 10 min.

Tricosanoic acid methyl ester (C23:0 ME) at 10 mg/ml was used as an internal standard. The individual FAMEs were identified by comparing their retention times with those of FAME standards (Sigma Chemical Co. Ltd., Poole, UK). Quantification was performed by calculating the response factors of each standard FAME with respect to the internal standard according to Alltech Associates Inc. (1997). All results are expressed as mg of FA/g of sausage.

2.6. Statistical analysis

All statistical analyses were performed using the statistic software Statgraphics Plus (v 5.1). Data were analysed using the multifactor ANOVA procedure to determine the significance of the type of oil added in the diet (SFO and CLA) and level (low, medium and high) as well as the interaction of both factors. In cases where the effects were significant, the measurements were compared using Fisher's least significant difference (LSD) test ($p < 0.05$). Moreover, the effect of the oil levels added to the diet on the FA profiles was examined by simple regression analysis. The results of this regression have not been included in the table but have been commented on the results section when the relationship was significant.

3. Results and discussion

Fat and moisture percentages and ratios, as measured using the SMART-Trac, are presented in Table 2. The effects of the type of oil and level, as well as the interaction were statistically significant for fat and moisture percentage ($p < 0.001$) and for the ratio Fat%/