Enriching breakfast sausages by feeding pigs with CLA supplemented diets

Aurora Marcoa, Manuel M. Juarez a, Nigel Brunton a, Przemyslaw D. Wasilewskia, Brendan Lynchnb, Sung-Sil Monona, Declan J. Troy a, Anne M. Mullena,*

a Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland
b Pig Development Unit, Teagasc, Moorepark, Co Cork, Ireland

ARTICLE INFO

Article history:
Received 27 August 2008
Received in revised form 9 September 2008
Accepted 27 October 2008

Keywords:
CLA
Fatty acid composition
Pork products
Sunflower oil
TBARS

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.

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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, & Corredes, 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; Martin, Antequera, Muriel, Andrés, & Ruiz, 2008; Raes, De Smet, & Demeyer, 2004; Schmid, Collomb, Sieber, & Bee, 2006; Wiegand, Sparks, Parrish, & Zimmermann, 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,
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 soybean meal with ω-linoleic, α-methionine, ω-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 20 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 and a sample of two sausages extracted from each bag. The sausages were produced using 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.

2.3. 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-AHS1-7 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 bags containing six sausages, and stored at –20 °C.

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, Ordónez, 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 N2 gas, dissolved in a methanol:toluene (2:1) mixture and methylated for 10 min at 40 °C with 2 M trimethylsilyl-diazomethane 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%
Moisture% (p < 0.01). All CLA treatments resulted in sausages with higher per cent of fat compared to SFO treatments and both Low and Medium CLA treatments produced product with lower moisture (Table 2). The fat percentage decreased linearly with the level of CLA oil added (r² = 88%). This was also true for the ratio Fat%/Moisture% (r² = 87%). This linearity was not observed in the sausages from SFO diets, which explains the significant interaction (p < 0.001) between both effects. Total fat levels (mg of FA/g of sausage) were also calculated following GC-fatty acid analysis (Table 3). For total extracted FAs, there was a significant effect of the type of oil and oil level (p < 0.001). However, the effect of the oil level was not significant (p > 0.05). Low CLA treatment resulted in higher fat levels but other treatments showed no significant effect. Differences between fat content values from SMART Trac™ and from GC-fatty acid analyses were due to differences in the fat analysed by each method. While SMART Trac™ measures total lipid content, GC analysis measures fatty acid content only.

In the saturated fatty acid (SFA) category, C16:0 (palmitic) followed by C18:0 (stearic) were the most abundant fatty acids. For the total amounts of SFA, the effects of the type of oil (p < 0.001) and oil level (p < 0.01) were significant (Table 3). Higher amounts of SFA were present in sausages from CLA supplemented diets compared to those from SFO diets at the same oil level. The same effect has been widely reported by several authors in carcas fat as a side effect of CLA supplementation (Eggert, Belury, & Schincel, 1998; Gatlin et al., 2002; Lauridsen, Mu, & Henckel, 2005; Raes et al., 2004; Smith et al., 2002; Wiegand et al., 2002). However, increases in the SFA content, especially increased levels of C14:0 and C16:0, are to be avoided due to the relation between high levels of SFA and an increased incidence of atherosclerosis in humans as a side effect of CLA supplementation. Type x level: effect of the interaction between oil type and oil level. **Significance p < 0.01; *** significance p < 0.001.

In general, mono-unsaturated fatty acid (MUFA) levels (Table 3) were not affected by the type of oil added in the diet (p > 0.05) but significant differences were found due to the oil level (p < 0.001). The highest levels of MUFA were found in sausages from low CLA diet and medium SFO diet. In general the C18:1 n-9c (oleic) isomer seemed to be the most abundant FA in this category and in the total FAs. A linear decrease related to the increase of added CLA was observed for C16:1 (palmitoleic; r² = 85%) and C 18:1 n-11t (trans-vaccenic; r² = 72%) FAs. The decrease of the MUFA content related to the CLA addition has been previously reported by Dugan, Aalhus, and Kramer (2004). The decrease in the deposition of C18:1 n-11t could be most likely due to a down-regulation of the Δ9-desaturase activity (Griinar et al., 2000).

Both the type of oil and the level supplemented impacted on the polysaturated (PUFA) content in the cooked sausages (p < 0.001) (Table 3). The highest and lowest levels of PUFA were found in the sausages from high and low SFO diets, respectively; whilst sausages from CLA diets had intermediate levels. C18:2 n-6c (linoleic) was the most abundant FA in this group. In the sausages from SFO supplemented diets, a linear increase of this FA was related to the increase of SFO added in the diet (r² = 80%), since SFO is rich in...
The two main CLA isomers (c9, t11; c10, c12) were found in all the treatments and their contents agree with those reported by Lauridsen et al. (2005) who performed a similar experiment feeding pigs with 0.5% SFO or CLA diets. Moreover, all sausages from CLA or SFO supplemented diets presented an increase in the CLA with respect to the levels commonly reported in pork meat (Schmid et al., 2006). The c9, t11 isomer was always in a higher proportion than the t10, c12, representing in the treatments with CLA and SFO approximately 60% and 40% of total CLA, respectively. These proportions agree with the proportion of isomers in the Luta-CLA product. A. Marco et al. / Food Chemistry 114 (2009) 984–988

From a nutritional perspective, the highest amount of CLA was obtained in the sausages from high CLA supplemented diets which resulted in a level of 12 mg of CLA/g of sausage. Considering that the weight of a sausage is around 20 g and that a normal meal will include at least two of them, the approximate intake would be 480 mg of CLA/meal. While this intake is below the level which has been shown to have significant effects in human diet, 3.4–6 g, to see changes in body composition according to previous studies (Gaullier, Berven, Blankson, & Gudmundsen, 2002), it is considerably higher than the normal intake in a human diet, which is between 150 and 200 mg/day (Ritzenthaler et al., 2001). It is interesting to note that other authors report that relative small amounts of CLA (0.1% by weight in the diet) (Ip, Singh, Thompson, & Scimeca, 1994) inhibit tumour development. The results from this study show that pork products can be altered to provide a significant increase in a functional lipid which can have positive influences on health.

Lower PUFA/SFA ratios (p < 0.001) were evident in cooked sausages from the CLA diets at all levels of inclusion, except at highest dose (Table 3). Other authors reported lower PUFA/SFA ratios in the fat from pigs fed with CLA compared to those fed SFO (Eggert, Belury, Kempa-Steczko, Mills, & Schinkel, 2001). However, in the present study, a linear increase of the PUFA/SFA ratio could be observed when increasing the levels of CLA added to the diet (r² = 97%). From a nutritional perspective, all the treatments meet the recommendations for the ratios between 0.4 (Department of Health, 1994), and the sausages with the highest PUFA/SFA ratios were those with high levels of both added oils (p < 0.001).

The n–6/n–3 ratio was not affected by the type of oil added in the diet (Table 3) but the level of addition of the oils resulted in a linear increase (r² = 92% and 94%) with increasing the amount of CLA and SFO supplemented in the diet, respectively. The recommendations for the ratios n–6/n–3 is for less than 4 (Enser et al., 2001), however, in the present study this ratio was greater than 10 for all samples. Nevertheless, it is interesting to note that the ratio is lower than the average consumed ratio in Western diets (15–16.7) as reported by Simopoulos (2002).

TBARS presented significant differences due to the type of oil and level (p < 0.001) (Fig. 2). In the low and high level of inclusion, sausages from CLA diets had lower TBARS levels than those from SFO diets; no difference was observed for the medium level of inclusion. Our results are supported by findings in studies focusing on hams and fresh meat. Corino, Magni, Pastorelli, Rossi, and Mourrot (2003) fed pigs with 0%, 0.25% and 0.5% of CLA, and used ham samples from these pigs to make “forced-oxidation studies” and TBARS analysis. These authors found that CLA increased the oxidative stability of their product. Joo, Lee, Ha, and Park (2002) also observed how TBARS of loins from CLA fed pigs were more stable than control loins which displayed a rapid increase of TBARS on storage. In other studies however, increases in TBARS levels in pork products with increased CLA content have been reported (Hur et al., 2007; Martín et al., 2008).

4. Conclusions

This study provides information on both fatty acid and oxidation levels in a cooked pork product, manufactured following dietary supplementation with CLA and SFO. A clear impact was observed in fatty acid levels with both treatments resulting in increased CLA levels in the product. All treatments met the recom-
mended PUFA/SFA ratios above 0.4. The n–6/n–3 ratio was affected only by the level of addition of the oil and while above the recommended n–6/n–3 ratio of less than 4 the ratio is lower than the average consumed ratio in Western diets.

Although not as efficient as CLA supplementation, SFA supplementation also demonstrated effectiveness at increasing the CLA levels. Coupled with the lowering of the SFA category of fatty acids, these results suggest that SFO holds potential in strategies for improving the lipid profile in pork products.

Acknowledgements

This research was carried out at Ashtown Food Research Centre (formerly known as the National Food Centre), Teagasc which was designated as a Marie Curie Training Site in Biochemistry of Meat Quality under the Improving Human Potential and Socio-economic Knowledge Base Programme of the Fifth Framework. The authors acknowledge funding support for Manuel Juarez, Aurora Marco and Przemyslaw Wasilewski under this Marie Curie Fellowship scheme.

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