EFFECT OF DIETARY CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON THE TECHNOLOGICAL PROPERTIES OF BACKFAT OF PIGS

C. Bothma[1], G. Osthoff[1], C.C. Joubert[3], A. van As[3], J.C. Swarts[3], H.L. de Kock[2], A. Hugo[1]

[1]Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa
[2]Department of Food Science, University of Pretoria, Pretoria, South Africa
[3]Department of Chemistry, University of the Free State, Bloemfontein, South Africa

Abstract - The purpose of this study was to determine the effect of dietary conjugated linoleic acid (CLA) supplementation on the technological properties of backfat of pigs. Pigs were fed diets containing 0, 0.25, 0.5 and 1% CLA. Compared to controls, backfat from CLA fed pigs was firmer and extracted lipid contained increasing amounts of CLA, but a ±11% overall decrease in unsaturated fatty acids and a ±5% overall increase in each of C16:0 and C18:0 saturated fatty acids. An increase in C18:0/C18:2 ratio and decrease in double bond index (DBI) was observed in backfat from CLA supplemented pigs compared to controls. Higher content of saturated fatty acids also resulted in a change in the melting properties of fat as demonstrated by differential scanning calorimetry (DSC). The onset setting temperature increased from ±13°C to ±18°C for lipid of backfat of pigs from the 0.25 and 0.5% CLA supplementation groups and to ±24°C for lipid from in the 1% CLA supplementation group. The final melting temperatures increased from ±37°C to ±43°C and ±45°C respectively. These findings explain the improvement in technological quality of backfat of pigs receiving CLA supplemented diets.

Key words - Conjugated linoleic acid, Improved firmness, Pork backfat

I. INTRODUCTION

Fat is an important part of the pig carcass for both the consumer and the meat processing industry [1]. Wood [2] defined good quality fat in pigs as firm and white, while poor quality fat is soft, oily, wet, grey and floppy. Meat products containing soft fat, show quality defects, such as insufficient drying, oily appearance, rancidity development and lack of cohesiveness between muscle and adipose tissue on cutting [3].. Meat with a more saturated fatty acid profile is therefore more suitable for the meat processing industry while fat with a lower degree of saturation is associated with superior health properties [4]. The nutritional and technological qualities of backfat are therefore inversely related [5].

A possible solution to this problem may be the supplementation of pig diets with CLA. It has been reported that dietary CLA supplementation increased the saturated/unsaturated fat ratio of fat tissue, and improved belly firmness [6]. The aim of this research was to determine how dietary CLA supplementation of pigs effect the physical properties, fatty acid composition and melting and crystallization behaviour of fat in an attempt to explain the effect of elevated CLA levels on technological properties of backfat.

II. MATERIALS AND METHODS

Animals and diets:
Fourty eight Large White x Duroc gilts weighing on average 35 kg, were divided into four groups of twelve pigs each. The groups were then assigned to four dietary treatments, that consisted of a control diet containing 1% sunflower oil (SFO), a diet containing 0.75% SFO + 0.25% CLA-60, a diet containing 0.5% SFO + 0.5% CLA-60 and a diet containing 1% CLA-60. Diets were formulated to provide similar energy (9.63 MJ/kgDE) and lysine levels (0.1 %). The pigs were individually penned and provided *ad libitum* access to feed and water

Slaughter and tissue sampling
At an average live weight of 95 kg, the pigs were slaughtered following commercial

procedures. Heads were removed and carcasses were split between the second and third last rib. The firmness of the subcutaneous fat was measured with a fat hardness meter MK2 (FHM) on the cross sectional surface, 4.5 cm off the carcass midline between the second and third last rib. A core (±1 g) sample of both layers of backfat was taken at the same point that FHM was measured.

Backfat quality

Extraction of lipid from the backfat was performed according to Folch *et al.* **[7]**, using chloroform and methanol in a ratio of 2:1. Total lipids from backfat were converted to methyl esters, with sodium methoxide (0.5 M solution in anhydrous methanol), during 2 h at 30°C **[8]**. Fatty acid methyl esters were quantified, using a flame ionization gas chromatograph. Fatty acid data were used to calculate C18:0/C18:2 ratio and DBI **[9]**. DSC, on extracted lipid was performed on a Mettler Toledo DSC 822e/700.

Statistical analysis

Differences between treatments were determined by using an analysis of variance (ANOVA) procedure. The Tukey-Kramer multiple comparison test (α=0.05) was used to determine differences between treatment means [10].

III. RESULTS AND DISCUSSION

Significant differences were observed in FHM measurements of backfat from the different dietary groups (Table 1). Backfat from the 1% CLA treatment had the hardest fat and differed significantly ($p<0.001$), with the 0.25 and 0.5% CLA treatments, from the control group.

Increased dietary CLA content resulted in a significant ($p<0.001$) increase in SFAs (C16:0 and C18:0) and a decrease in MUFA (C18:1c9) (Table 1). A similar increase in SFAs and decrease in MUFAs, due to dietary CLA supplementation, were observed by Han *et al.* [11], who attributed this phenomenon to the inhibition of $^{\Delta}9$ desaturase activity and mRNA expression by CLA. The levels of the two CLA isomers also increased significantly ($p<0.001$) in backfat with increased CLA content. (Table 1). Backfat from the control contained no CLA.

Increased dietary CLA level resulted in a significant ($p<0.001$) increase in C18:0/C18:2 ratios and decrease in double bond index (DBI) (Table 1). With C18:0/C18:2 ratios of > 1.2 and < 1.2, the sensory consistency of fresh subcutaneous fat was considered as firm and soft, respectively [12]. A significant ($p<0.001$) increase in C18:0/C18:2 ratio was observed in the backfat from the pigs that received CLA (Table 1). Backfat samples from the control could not conform to Honkavaara's [12] minimum proposed C18:0/C18:2 ratio of 1.2 while backfat from the CLA treatment groups did conform (Table 1). For good quality and consistency, subcutaneous fat should have a DBI of < 80 [13]. A significant ($p<0.001$) decrease in DBI was observed, with increased dietary CLA content (Table 1). Backfat from the control group had significantly ($p<0.001$) higher DBI's than backfat from the CLA treatment groups. Although backfat from all dietary treatments conformed to the DBI maximum of 80, all CLA treatment groups had better DBI values than the control group.

Table 1: Physical quality characteristics and fatty acid composition content (%) of the backfat from gilts from the four dietary treatments.

Dietary Treatment	Control (n=12)	0.25% CLA (n=12)	0.5% CLA (n=12)	1% CLA (n=12)	Sign. level
Physical properties:					
FHM	773.5^{a}	985.3^{b}	996.6^{b}	1026.1^{b}	$p < 0.001$
Major fatty acids:					
C16:0	24.9^{a}	27.8^{b}	28.4^{bc}	29.4^{c}	$p < 0.001$
C18:0	13.3^{a}	17.1^{b}	17.6^{bc}	18.9^{c}	$p < 0.001$
C18:1*c*9	39.2^{d}	32.3^{c}	30.4^{b}	27.9^{a}	$p < 0.001$
C18:2*c*9,12 (*n*-6)	14.0	13.8	13.7	13.1	NS
C18:2*c*9*t*11(CLA)	ND	0.3^{a}	0.6^{b}	1.3^{c}	$p < 0.001$
C18:2*t*10*c*12 (CLA)	ND	0.1^{a}	0.3^{b}	0.7^{c}	$p < 0.001$
Fatty acid ratios:					
C18:0/C18:2	1.0^{a}	1.2^{b}	1.2^{b}	1.3^{b}	$p < 0.01$
DBI	77.8^{c}	70.1^{b}	69.4^{ab}	66.4^{a}	$p < 0.001$

Means with different superscripts in the same row differ significantly ND = Not Detected

The setting and melting isotherms are shown in Figure 1, while the respective enthalpies (dH) are given in Table 2. The enthalpy (dH) of the setting exotherm between -20 and -30°C, with a peak at ca. -22°C was large for the controls, smaller for the fats of the 0.25 and 0.5% CLA fed pigs, and very small for the 1% CLA fed pigs. The difference in dH of this -22°C

endotherm was only significant ($p < 0,001$) between the fat from the 1% CLA fed pigs and the other three treatments. The setting isotherms of fats from the control pigs showed an exotherm at ca. 10°C, with an onset of ca. 13°C.

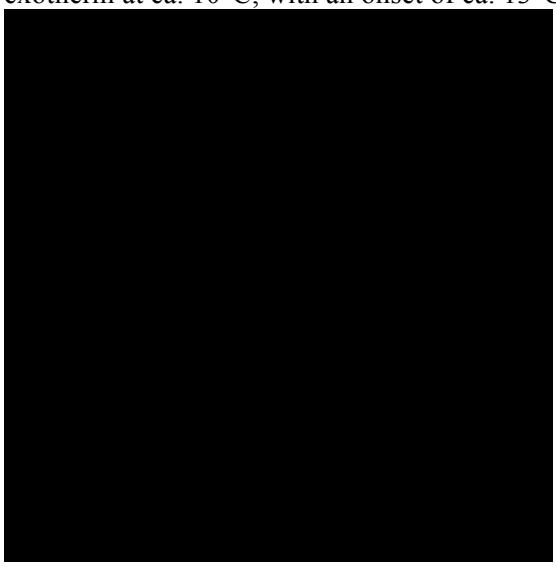

Figure 1. The setting (top) and melting (bottom) thermograms of the BF from gilts fed four different concentrations of CLA.

Table 2. Setting and melting properties of backfat of gilts from the four dietary treatments.

Treatment	Control	0.25% CLA	0.5% CLA	1% CLA	Sign. level
Setting:					
T max	13.3^{a}	18.3^{b}	19.2^{b}	23.6^{c}	$p < 0.001$
ca 20°C dH	ND	2.5^{a}	3.2^{a}	10.2^{b}	$p < 0.001$
ca 17°C dH	ND	4.41	4.42	ND	NS
ca 10°C dH	2.8^{a}	35.5^{b}	34.6^{b}	36.6^{b}	$p < 0.001$
ca 5°C dH	30.9	ND	ND	ND	NSA
ca -22°C dH	33.1^{c}	27.9^{bc}	21.8^{ab}	18.5^{a}	$p < 0.001$
T min	-38.9^{b}	-42.3^{a}	-41.6^{a}	-40.4^{ab}	$p < 0.01$
Melting:					
ca 0°C dH	-20.0^{ab}	-20.7^{a}	-13.1^{ab}	-11.6^{b}	$p < 0.05$
T min	-18.8^{a}	-17.0^{ab}	-16.2^{ab}	-14.3^{b}	$p < 0.05$
ca 30°C dH	-28.3^{b}	-42.2^{a}	-43.1^{a}	-43.1^{a}	$p < 0.001$
ca 40°C dH	ND	-7.1^{b}	-8.3^{ab}	-11.1^{a}	$p < 0.001$
T max	37.0^{a}	42.8^{b}	43.7^{bc}	45.0^{c}	$p < 0.001$

Means with different superscripts in the same row differ significantly
ND = Not Detected; NS = Not Significant; NSA = Not Statistically Analysed

Upon further cooling this was followed by a large exotherm at ca. 5°C, between 9 and 4°C, of ca. 32 W/g. The setting of the fats from pigs fed 0.25 and 0.5% CLA commenced at ca. 20°C, with a peak at ca. 17°C. A shift was therefore noted from a 10°C exotherm to a 17°C exotherm. Upon cooling, this was followed by a large exotherm between 14 and -8°C, with a peak at ca. 8°C. Setting of the fats from pigs fed 1% CLA commenced at ca. 26°C, with a peak at ca. 20°C. A shift was therefore noted, from a 17°C exotherm to a 20°C exotherm. Upon cooling, this was followed by a large exotherm between 16 and -9°C, with a peak at ca. 10°C. Again another shift was noted, from an 8°C exotherm to a 10°C exotherm.

Melting of fat from the control pigs commenced at ca. -18°C to 11°C, with an endotherm peak at ca. -2°C. The onset of melting of some fats from CLA fed pigs commenced at temperatures up to -12°C, with peaks at between 0 and -2°C. The dH of the 0°C endotherm was large for the fat from the control and 0.25% CLA fed pigs, and smaller for the fats from the 0.5 and 1% CLA fed pigs, but only significant ($p<0.001$) between the fat from the 1% CLA fed pigs and the controls, as well as the BF of the 0.25% CLA pigs. Upon heating, a large endotherm followed between 11 and 38°C, which contained small peaks between 11 and 25°C, and a large peak at ca. 30°C. Also, upon heating the fats from the pigs fed 0.25 and 0.5% CLA, similar endotherms were shown, however, with higher enthalpies between 11 and 25°C, and the large peak at ca. 30°C. Additional endotherms were seen between ca. 36 and 42°C, with a peak at ca. 38°C. The same was observed for the fat from the 1% CLA fed pigs, but the high temperature endotherm was extended to a final melting temperature of 45°, and peaked at 40°C. Because the enthalpies between ca. 11 and 25°C, and the 30° C peak were not clearly separated, they were regarded as one peak for statistical calculations. The dH of this isotherm was higher for the fat from all CLA fed pigs. In general, there was hardly any significant ($p<0.001$) difference between the melting isotherms, between the fat from the 0.25 and 0.5% CLA fed pigs, although the fatty acid composition was different. The shift to higher

melting isotherms is most probably due to the formation of higher melting β'- and β-crystals of triacylglycerols containing C-16, C18 and C18:1 fatty acids, which melt between 37 and 41°C [14]. Similar observations of harder adipose fat showing high melting peaks, compared to softer subcutaneous fat have been reported [15]. These results showed that the physical hardness, observed for the different fats (Table 1), might also be ascribed to the melting properties of fats.

IV. CONCLUSIONS

Elevated CLA levels in backfat, resulted in improved technological properties, as demonstrated by an increase in firmness, an increase in C18:0/C18:2 ratio and a decrease in DBI. Results also show that the physical hardness of backfat from pigs with dietary CLA supplementation may be ascribed to the melting properties of the fat as demonstrated by DSC.
This findings offer a solution for the dilemma of the inverse relationship between the nutritional and technological qualities of backfat [5]. This research indicate that CLA being a PUFA can be used to increase the technological properties of backfat. This findings is of importance for manufacturers of high value meat products. Firmer backfat will result in less cutting losses during bacon packaging [16], and salami of better consistency and oxidative stability [17].

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Red Meat Research and Development Trust of South Africa (RMRD-SA), the Technology and Human Resources Industry Program (THRIP) of the Department of Trade and Industry for research funding.

REFERENCES

1. Hallenstvedt, E., Kjos, N.P., Øverland, M., & Thomassen, M. (2012). Changes in texture, colour, and fatty acid composition of male and female pig shoulder fat due to different dietary fat sources. Meat Science, 90, 519-527.
2. Wood, J.D. (1984). Fat deposition and the quality of fat tissue in meat animals. In J. Wiseman (Ed.), *Fats in animal nutrition* (pp.401-435). London: Butterworths.
3. Maw, S.J., Fowler, V.R., Hamilton, M., & Petchey, A.M. (2003). Physical characteristics of pig fat and their relation to fatty acid composition. *Meat Science*, 63, 185-190.
4. Hadorn, R., Eberhard, P., Guggisberg, D., Piccinali, P., & Schlictherle-Cerny, H. (2008). Effect of fat score on the quality of various meat products. *Meat Science*, 80, 765-770.
5. Hugo, A., & Roodt, E. (2007). Significance of porcine fat quality in meat technology: a review. *Food reviews International*, 23, 175-198.
6. Dugan, M.E.R., Aalhus, J.L., Jeremiah, L.E., Kramer, J.K.G., & Schaefer, A.L. (1999). The effects of feeding conjugated linoleic acid on subsequent pork quality. *Canadian Journal of Animal Science*, 79, 45-51.
7. Folch, J., Lees, M., & Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, 226, 497-509.
8. Park, Y., Albright, K. J., Cai, Z. Y., & Pariza, M. W. (2001). Comparison of methylation rocedures for conjugated linoleic acid and artefact formation by commercial (trimethylsilyl) diazomethane. *Journal of Agricultural and Food Chemistry*, 49(3), 1158–1164.
9. Alam, S.Q., & Alam, B.S. (1986). Effect of essential fatty acid deficiency on acyl group composition, membrane fluidity and adenylate cyclase activity in plasma membranes of rat submandibular salivary glands. *Journal of Nutrition,* 116, 1620-1630.
10. NCSS. (2007). Statistical System for Windows. Number Cruncher Statistical Systems, Kaysville, Utah, USA.
11. Han, X.F., Feng, F.J., Yu, J.P., Tang, S.X., Bamikole, M.A., Tan, Z.L., *et al*. (2011). Effects of conjugated linoleic acid supplementation on growth, carcass characteristics and fatty acid profiles of muscle and fat in growing-finishing pigs. *Journal of Animal and Feed Sciences*, 20, 171-185.
12. Honkavaara, M. (1989). Influence of porcine stress and breed on the fatty acid profiles of subcutaneous and intramuscular total lipids. *Fleischwissenschaft*, 69(9), 1429-1432.
13. Prabucki, A.L. (1991). Qualitätsanforderungen an Schweinefleisch. In *Schweinefleischqualität – Qualitätsschweinefleisch.* Tagunsbericht, p 5-10, 22 May 1991, ETH-Zürich, Institut für Nutztierwissenschaften.
14. Hagemann, J.W. (1988). Thermal behavior and polymorphism of acylglycerides. In N. Garti & K. Sato (Eds.), *Crystallization and polymorphism of fats and fatty acids.* (pp 9-96). New York: Dekker.
15 Sasaki, K., Mitsumoto, M., Nishioka, T., & Irie, M. (2006). Differential scanning calorimetry of porcine adipose tissues. *Meat Science,* 72, 789–792.
16. Enser, M.B. (1983). The relationship between the composition and the consistency of pig backfat. In J.D. Wood (Ed.), *Fat Quality in Lean Pigs* (Special Report No. 2, EUR 8901 EN, CEC Workshop), (pp.53-57), Brussels, Belgium.
17. Hauser, A., & Prabucki, A.L. (1990). Ergebnisse eines "screenings"betreffend die Fettqualität bei Mastschweinen in schweizerischen Schlachthöfen. *Journal of Animal Physiology and Animal Nutrition*, 64, 36.