CONJUGATED LINOLEIC ACID AND POLY-UNSATURATED FATTY ACIDS IN INTRAMUSCULAR FAT OF BELGIAN BLUE BULLS: EFFECT OF DOUBLE-MUSCLING

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Background

With respect to human health, nutritional guidelines put an increasing emphasis on reducing the ratio n-6/n-3 poly-unsaturated fatty acids (PUFA) (Demeyer and Doreau, 1999). Recently, conjugated linoleic acids (CLA) in animal products have also gained a lot of attention. The term CLA covers a group of isomers of C18:2 fatty acids, with c9, t11 C18:2 being the most abundant one. CLA are mostly present in products of ruminants, due to the specific metabolism of the rumen. CLA have different positive effects on health, e.g. anti-carcinogenic, anti-oxydans, anti-artherosclerosis. They also influence the partitioning of energy between muscle and lipid deposition (Banni and Martin, 1998). Besides the effect of hydrogenation in the rumen, the muscle fatty acid composition is determined by several animal genetic and feeding factors. For instance, the Belgian Blue beef breed is characterised by a high frequency of double-muscling and, concomitantly, high meat-yielding carcasses (Uytterhaegen et al., 1994). Due to the low fat content, muscle samples of this breed may have a different fatty acid pattern compared with meat from other breeds, and may vary according to the genotype for the double-muscling condition.

Objectives

1. Establishing a method for CLA analysis.
2. Comparing the fatty acid composition, with emphasis on conjugated linoleic acids and poly-unsaturated fatty acids, in samples of five different muscles from Belgian Blue bulls according to the genotype for double-muscling.

Material and methods

Young bulls of the Belgian Blue breed of different double-muscling genotype (double-muscling, mh/mh; heterozygous, mh/+; normal, +/+; 10 animals per genotype) were slaughtered at the abattoir of the department. Animals originated from different farms and were fattened on high-concentrate diets. Mean (sd) age at slaughter, warm carcass weight and dressing yield were respectively 19.1 (2.2) months, 434 (57) kg and 65.9 (4.4) %. The genotype for the mutation nt821 (del11) in the myostatin gene causing the double-muscling phenotype in the Belgian Blue breed was determined following Grobet et al. (1998). Samples were taken from 5 muscles: *M. longissimus thoracis* (LT), *M. rectus femoris* (RF), *M. semitendinosus* ST), *M. triceps brachii* (TB) and *M. gluteobiceps* (GB). Frozen muscle samples of 10 g were taken for intramuscular fat extraction with chloroform/methanol (2/1; v/v) (Folch et al., 1957). Different methylation methods were first tested to determine their effect on the CLA isomers. Extracts, with C19: 0 as internal standard, were methylated as follows: a) tetramethylguanidine (TMG) in MeOH; b) H_2SO_4 in MeOH; c) NaOH in MeOH; d) NaOH in MeOH followed by HCL in MeOH. The methylated fatty acids were analysed with GC (HP 6890) using a CP-Sil88 column for FAME (100 m x 250 µm x 0.2 µm; Chrompack). The conditions were: injector: 250 °C; detector: 280 °C; p = 2 bar; oven temperature: 150 °C for 2 min, followed by an increase of 1.5 °C/min to 200 °C, then followed by 5 °C increase/min to 215 °C. Peaks were identified using retention times, by comparing them with their corresponding standards (Nu-Chek-Prep, Elysian, MN).

Results and discussion

1. Methylation procedure

The effect of the methylation procedures on the CLA isomers is presented in Figure 1. Acid methylation (H_2SO_4 in MeOH) resulted in a decrease of the major isomer c9, t11 CLA and an increase in tt isomers. Therefore, the use of base-catalysed reagents seems necessary, but these were not able to methylate free fatty acids. The best results were obtained with base-catalysed reagents (NaOH in MeOH), followed by an acid-catalysed methylation (HCl in MeOH). Hence, this method was further used. These findings are in accordance with literature (Kramer et al., 1997).

2. *Fatty acid profile*

Figure 2 shows the content of saturated (SFA; C14:0 + C16:0 + C18:0), mono-unsaturated (MUFA; C16:1 + C18:1 (c+t)) and poly-unsaturated fatty acids (C18:2 n-6 + C18:3 n-6 + C18:3 n-3 + CLA + C20:2 n-6 + C20:3 n-3 + C20:3 n-6 + C20:4 n-6 + C20:5 n-3 + C22:4 n-6 + C22:5 n-3 + C22:6 n-3) in five muscles depending on the double-muscling genotype. The amount of PUFA (mg/ 100 g meat) is almost equal for the three genotypes, hence the relative proportion of PUFA in the total fatty acid content increases with decreasing fat content. These results correspond with earlier findings at our department (De Smet et al., 2000). The higher intramuscular fat content in bulls of the mh/+ and +/+ genotype compared with the mh/mh genotype is mainly due to a 2 to 3 times higher content of SFA and MUFA (mg/100 g meat).

Table 1 shows that the relative amount of CLA (% of total fatty acids) is highest for the +/+ genotype. The amounts are in accordance with literature findings (Chin et al., 1992; Enser et al., 1999). The ratio PUFA/SFA increases significantly as the number of mh alleles increase. A further increase to the recommended value of 0.7 (Nationale Raad voor Voeding, 1996) would be advisable. The ratio n-6/n-3 PUFA is for the three genotypes already close to the recommended value of 5 (Nationale Raad voor Voeding, 1996) and is significantly higher for the +/+ genotype compared with the other genotypes.

The relative composition of the n-3 and n-6 fatty acid series is shown in Figure 3. The differences between genotypes suggest that the metabolism of the n-3 and n-6 fatty acids could vary depending on the double-muscling genotype. This needs further investigation.

Conclusions

A method for the determination of CLA is established. The relative amount of CLA (% of total fatty acids) in intramuscular fat of bulls of the Belgian Blue breed did not greatly differ between double-muscling genotypes.

The double-muscling genotype has a large effect on the intramuscular fat content, whereas minor differences between muscles were observed.

The absolute amount of PUFA in intramuscular fat (mg/100 g meat) did not greatly differ between double-muscling genotypes.

The PUFA/SFA ratio increases significantly as the number of mh alleles increase.

The n-6/n-3 ratio is somewhat higher for +/+ genotype but is close to the recommended value independent of the genotype.

The n-3 and n-6 fatty acid metabolism is possibly depending on the double-muscling genotype.

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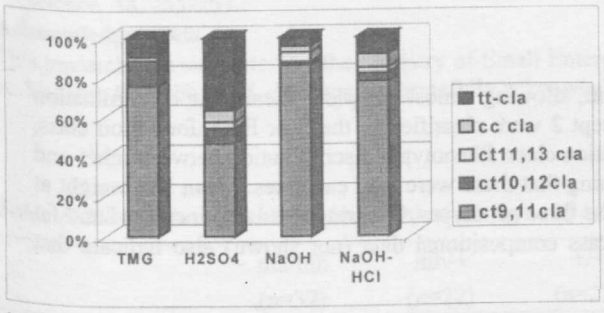


Fig. 1. Effect of methylation procedure on CLA isomers

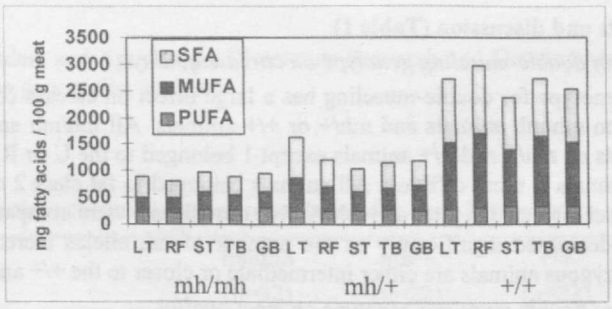
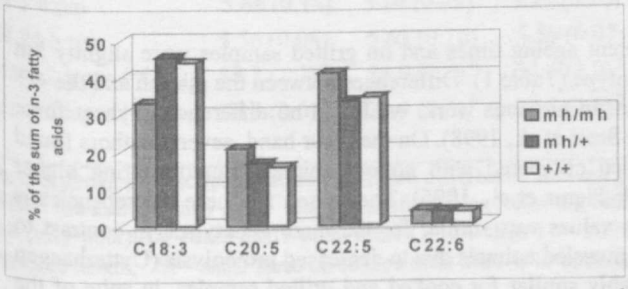


Fig. 2. Intramuscular fatty acid composition depending on the double-muscling genotype

Table 1. Amount of CLA and some fatty acid ratios in meat of Belgian Blue bulls depending on the double-muscling genotype

	mh/mh	mh/+	+/+
CLA (% of total fatty acids)	$0.45^{a,b}$	0.39^{a}	0.51^{b}
PUFA/SFA	0.56^{a}	0.40^{b}	0.19^{c}
C18:2 n-6/C18:3 n-3	12.4^{a}	8.07^{b}	10.2^{c}
n-6/n-3	5.53^{a}	5.17^{a}	6.32^{b}

^{a,b,c} Means with different superscripts are significantly different ($p < 0.05$)



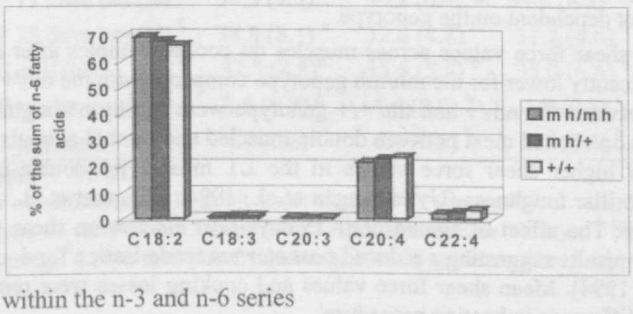


Fig. 3. Fatty acid composition within the n-3 and n-6 series