INTRODUCTION OF N-3 FATTY ACIDS AND CONJUGATED LINOLEIC ACID INTO THE INTRAMUSCULAR FAT OF BELGIAN BLUE DOUBLE-MUSCLED BULLS

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Background
Over the last years, much attention has been paid to the fatty acid composition of meat and especially beef. In relation to human health and nutrition, an increase in the intake of n-3 fatty acids is advisable. However, dietary habits are difficult to change and people in Western Europe consume too low levels of fish, which is a source rich in n-3 fatty acids, so an increase of these fatty acids in meat is recommended (Demeyer & Doreau, 1999). In Belgium, beef cattle of the Belgian Blue double-muscled breed are fattened in intensive indoor production systems on high-concentrate diets. Earlier results (Raes et al, 2001) have shown that such beef raised on commercial diets has n-6/n-3 and P/S ratios close to the advocated nutritional guidelines. Besides this, beef is also a natural source of conjugated linoleic acid (CLA), a group of isomers with potential health effects. However, commonly used polyunsaturated fat sources in concentrates are mostly rich in n-6 fatty acids, and therefore including a n-3 source could further improve the n-6/n-3 ratio of beef. In the absence of grass or grass silage in the finishing diet, the inclusion of a n-3 source in the concentrate can be a way to increase the n-3 content of beef. Although CLA is formed during the preliminar isomerisation step of hydrogenation of linoleic acid by rumen micro-organisms (Kepler & Tove, 1967), CLA seems to accumulate more if n-3 sources are fed to cows and steers (Dhiman et al, 1999; Shantha et al, 1997). Increasing both the n-3 fatty acid and the CLA content in beef seems an interesting challenge. However, polyunsaturated fatty acids in the feed are extensively hydrogenated in the rumen (Demeyer & Van Nevel, 1995; Scollan et al, 2001). Hence, attempts have been made to protect fat sources from biohydrogenation in the rumen, which may also affect the CLA content (Gulati et al, 2000). However, CLA may not only be formed as a major intermediate of the biohydrogenation of linoleic acid in the rumen (Kepler & Tove, 1967) but also through tissue desaturation of C18:1t11. Desaturase acitivity has been proposed for the mammary gland (Griinari et al, 2000), but has to our knowlegde not been identified for muscle or adipose tissue. We have compared the ratio c9,t11CLA/C18:1t11 in intestinal contents with that in subcutaneous and muscle samples to obtain some evidence for possible tissue CLA synthesis.

Objective
In this study, it was attempted to increase the n-3 content of beef by incorporating crushed linseed in the concentrates. The fatty acid profile and some important nutritional parameters were determined on three muscles. Different types of samples (muscle, subcutaneous fat, rumen contents) were analyzed to obtain more information about the main site of CLA synthesis.

Material and methods
Eight Belgian Blue young bulls were slaughtered at a live weight of 650-700 kg. During the growth stage, they were kept indoors on a maize silage/concentrate diet without added n-3 source. In the fattening stage, a maize silage/concentrate diet was given in a ratio of 20/80 for at least 45 days. The concentrate contained a premix with crushed linseed as the n-3 source, resulting in a ratio C18:2n-6/C18:3n-3 of 0.97 in the concentrate. Animals were not fasted before slaughter so rumen contents at slaughtering was still representative for the fattening stage. Subcutaneous fat and muscle samples (M. *longissimus thoracis* (LT), M. *semitendinosus* (ST) and M. *gluteus biceps* (GB)) were taken at 24 h *post mortem*. The samples were vacuum packed and frozen. Lipids were extracted with C/M (2/1; v/v) (adapted from Folch et al, 1957). Fatty acids were methylated with NaOH/MeOH followed by HCl/MeOH and analysed by GC (HP 6890) on a CP-Sil88 column for FAME (100 m x 250 µm x 0.2 µm, Chrompack) (Raes et al, 2001). The GC conditions were as followed: injector T = 250 °C; detector T = 280 °C; carrier gas = H_2, oven temperature = 150 °C for 2 min followed by an increase of 1.5 °C to 200 °C, followed by an increase of 5 °C/min to 215 °C. To determine the trans C18:1 fatty acids, a pre-separation was performed on Ag^+-TLC. After reextracting the fatty acids, they were analysed on a CP-Sil88 column for FAME (100m x 250 µm x 0.2 µm) using an oven temperature of 140 °C, followed by an increase of 0.5 °C/min to 158 °C, for 15 min on 158 °C, followed by an increase of 2 °C/min to 175 °C (Ansorena et al, in prep.). Peaks were identified by comparing the retention times with those of the corresponding standards (Nu-Chek Prep., Mn, USA). Statistical analyses were performed using SPSS version 9.0 (One-way Anova with Duncan as post-hoc test; $p < 0.05$).

Results and discussion
Results for selected fatty acids and some nutritional parameters for the different muscles are shown in Table 1. The lower PUFA content and the lower P/S ratio of the LT muscle compared with the ST and TB muscle reflects the more glycolytic nature of this muscle (Turkii & Campbell, 1967). In all muscles the n-6/n-3 ratio is below the nutritional guidelines of 5 (Richtlijnen voor de Voeding, 1996) and is lower than previously found values for Belgian Blue double-muscled bulls (Raes et al, 2001). This means that the incorporation of crushed linseed in the diet resulted in an increase of n-3 fatty acids in the muscle. The mean c9,t11 CLA content (0.43 % of total fatty acids) is comparable with other results reported for beef (Shantha et al, 1997; Enser et al, 1999; Raes et al. 2001).

Table 1. Selected fatty acids and important nutritional parameters for muscle LT, ST and TB

	LT	ST	TB	P
% of total fatty acids				
SFA	34.5 (3.24)	31.6 (2.32)	31.8 (2.45)	0.074
MUFA	29.3 (2.87)	26.2 (3.16)	27.3 (3.23)	0.163
PUFA	26.9[a] (4.47)	31.8[b] (4.23)	30.7[ab] (4.23)	0.076
mg/100 g muscle				
Sum	698 (114)	658 (109)	690 (105)	0.739
n-6	153[a] (9.54)	168[b] (11.4)	173[b] (15.0)	0.014
C18:2 n-6	116[a] (7.66)	124[ab] (10.0)	129[b] (13.2)	0.063
n-3	33.6[a] (4.78)	40.6[b] (5.61)	39.7[b] (5.57)	0.031
C18:3 n-3	17.1 (3.96)	19.8 (4.46)	20.1 (4.45)	0.329
c9,t11 CLA	3.45 (1.33)	2.89 (1.31)	3.06 (1.29)	0.683
Important nutritional parameters				
n-6/n-3	4.63 (0.59)	4.19 (0.41)	4.41 (0.59)	0.292
P/S	0.58 (0.15)	0.72 (0.15)	0.71 0.15)	0.159

Mean (standard deviation) of 8 values [a,b,c] Means with different superscripts are significantly different ($p < 0.05$)

Table 2. Selected fatty acids and c9,t11 CLA/C18:1 t11 ratio for rumen contents, subcutaneous fat and LT muscle

	Rumen contents	Subcutaneous fat	Muscle LT	P
% of total fatty acids				
SFA	54.2[a] (12.4)	45.3[b] (2.53)	34.5[c] (3.24)	0.000
MUFA	28.3[a] (6.66)	46.9[b] (2.07)	29.3[a] (2.87)	0.000
PUFA	15.2[a] (7.76)	5.27[b] (0.82)	26.9[c] (4.47)	0.000
C18:2 n-6	8.32[a] (4.42)	3.99[b] (0.69)	16.6[c] (2.81)	0.000
C18:3 n-3	5.05[a] (4.29)	0.94[b] (0.24)	2.42[b] (0.56)	0.012
c9+c11C18:1	5.52[a] (2.42)	30.0[b] (3.41)	19.9[c] (2.90)	0.000
t11C18:1	4.31[a] (1.99)	1.45[b] (0.45)	1.19[b] (0.82)	0.000
c9,t11 CLA	0. 29[a] (0.26)	0.86[b] (0.20)	0.48[a] (0.12)	0.000
c9,t11CLA/C18:1t11	0.10[a] (0.11)	0.65[b] (0.27)	0.51[b] (0.23)	0.000

Mean (standard deviation) of 8 values [a,b,c] Means with different superscripts are significantly different ($p < 0.05$)

In Table 2, the contents of some fatty acids are presented for rumen contents, subcutaneous fat and LT muscle. The higher C18:1t11 content in the rumen compared with subcutaneous fat and intramuscular fat is due to the hydrogenation of polyunsaturated fatty acids by the rumen micro-organisms. The higher c9,t11CLA/C18:1t11 ratio is of subcutaneous fat and muscle compared to rumen contents and the low CLA content in the rumen suggest that a desaturase might be active in the adipose tissue producing c9,t11 CLA from C18:1t11. However, further research (enzyme studies, *in vitro* research) will be necessary to confirm this hypothesis.

Conclusions

Using crushed linseed in the fattening diet of Belgian Blue double-muscled animals, it was possible to improve the n-6/n-3 and P/S ratios in beef. However, the CLA content was not different compared to previous research on similar animals and to literature reports. Based on the ratio c9,t11CLA/C18:1t11 in rumen, subcutaneous fat and intramuscular fat, it is suggested that endogenous synthesis of CLA by desaturase activity in adipose tissue is the main mechanism of CLA formation.

References

Demeyer, D. & Doreau, M. (1999). Proceedings of the Nutrition Society, 58, 593-607.
Demeyer, D. & Van Nevel, C. (1995). Archives of Animal Nutrition, 48, 119-134.
Enser, M., Scollan, N.D., Choi, N.J., Kurt, E., Hallett, K. & Wood, J.D. (1999). Animal Science, 69, 143-146.
Folch, J., Lee, M. & Sloane-Stanley, G.H.A. (1957). Journal of Biological Chemistry, 226, 497-509.
Gulati, S.K., Kitessa, S.M., Ashes, J.R., Fleck, E., Byers, E.B., Byers, Y.G., Scott, T.W. (2000). Animal Feed Science and Technology, 86, 139-148.
Kepler, C.R. & Tove, S.B. (1967). Journal of Biological Chemistry, 242, 5686-5692.
Nationale Raad voor Voeding (1996). Belgium, 77 p.
Raes, K., De Smet, S. & Demeyer, D. (2001). Animal Science (accepted).
Scollan, N.D., Choi, N.J., Kurt, E., Fisher, A.V., Enser, M. & Wood, J.D. (2001). British Journal of Nutrition, 85, 115-124.
Shantha, N.C., Moody, W.G. & Tabeidi, Z. (1997). Journal of Muscle Foods, 8, 105-110.
Turkii, P.R. & Campbell, A.M. (1967). Journal of Food Science, 32, 151-154.

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