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Meat Science 73 (2006) 258–268
MEAT SCIENCE
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Factors influencing proportion and composition of CLA in beef

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Received 28 August 2005; received in revised form 28 November 2005; accepted 29 November 2005

Abstract

Bovine meat is criticised for the bad nutritional image of its lipids and fatty acids. However, with dairy products, beef is the major source of conjugated linoleic acid (CLA) which could have several human health benefits. The present study compared, from data of five nutritional experiments on bovine animals performed by the laboratory, the impact of factors linked to the animals (breed, age, sex, type of muscle) and to feeding conditions (basal diet, lipid supplements) on the CLA proportion and composition in muscles. Among these factors, linseed supplementation was an efficient way to increase CLA proportion in beef (+22% to +36%) but was highly modulated by the nature of the basal diet, and by intrinsic factors (breed, age/sex, type of muscle) since these ones could modulate CLA proportion in beef from 24% to 47%. Moreover, these factors modified also the proportion of *cis,trans*-CLA, related to *cis,cis*- and *trans,trans*-isomers. Specific biological properties of these latter isomers should be determine to understand the consequences of intramuscular CLA isomer variations for the health of consumers.

Keywords: Beef; Conjugated linoleic acid isomers; Diet; Breed; Gender; Age; Muscle

1. Introduction

The fatty acid (FA) composition of ruminant meat has long been studied because of its implication in terms of meat quality and nutritional value for consumers (Wood et al., 1999). Indeed, beef is considered as a food with an excessive fat content and notably a high proportion of saturated fatty acids (SFA) which would contribute to several human diseases (Williams, 2000). Besides a moderate total fat intake, human nutritionists are claiming for higher intake of polyunsaturated fatty acids (PUFA) and especially $n-3$ PUFA at the expense of $n-6$ PUFA (Department of Health, 1994). PUFA/SFA (P/S) and $n-6$ PUFA/$n-3$ PUFA are two important FA ratios to consider for human nutrition. Generally, the recommended average values are $\leqslant 5$ for $n-6$/$n-3$ ratio (Department of Health, 1994) and 0.45–0.64 for P/S ratio (Wood et al., 1999). Consequently, there is incentive for the production of meat containing increased proportion of healthy FA such as PUFA (Scollan et al., 2005) and conjugated linoleic acid (CLA) (Mir et al., 2004).

CLA refers to a mixture of positional and geometric isomers of linoleic acid with two conjugated double bonds. The major source of CLA for humans are ruminant products (Chin, Liu, Storkson, Ha, & Pariza, 1992) with 24 isomers identified (Cruz-Hernandez et al., 2004), the *cis*9, *trans*11-CLA isomer (rumenic acid) being the

Abbreviations: CLA, conjugated linoleic acid; DM, dry matter; FA, fatty acids; LT, *longissimus thoracis*; P/S, polyunsaturated/saturated fatty acid ratio; PT, *pectoralis transversus*; PUFA, polyunsaturated fatty acids; RA, *rectus abdominis*; SFA, saturated fatty acids; ST, *semi tendinosus*; VA, vaccenic acid.

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doi:10.1016/j.meatsci.2005.11.025

major isomer (more than 80% of total CLA) (Griinari & Bauman, 1999). In recent years, CLA has received special attention because of its potential beneficial properties for human health (Belury, 2002). This explains that numerous studies aim to increase the CLA content in ruminant products (Mir et al., 2004; Parodi, 1999). Amounts of CLA in beef which ranged from 1.2 to 12.5 mg/g fat (Raes, De Smet, & Demeyer, 2004) vary mainly with feeding conditions (nature and quality of forages, proportions between forage and concentrate, oilseed supplementations) (Mir et al., 2004), but intrinsic factors such as breed (Choi, Enser, Wood, & Scollan, 2000) and sex and age of animals (Rule, Broughton, Shellito, & Maiorano, 2002) have been proposed to modulate these variations. However, no study has clearly determined the importance of such factors on the amount and the distribution of CLA isomers deposited in lipids of muscles.

In this context, the aim of this paper was to use experimental data collected in our laboratory to determine the relative importance of different factors involved in variations of CLA composition in beef such as the supplementation of diets with oleaginous seeds (linseeds), the composition of the basal diet (corn silage vs. concentrate), factors linked to animal (fatness, breed, sex, age) and the type of muscles.

2. Materials and methods

2.1. Animals and management

The data presented in this paper are taken from five experiments carried out in our laboratory in a time frame of one year and conducted in a manner compatible with the national legislation on animal care (Certificate of Authorisation to Experiment on Living Animal, No. 7740, Ministry of Agriculture and Fish Products). All the animals used in the different experimentations were raised/kept in similar pasture conditions before the beginning of the experimental period. Animals from experiments 1, 2 and 3 were slaughtered in the experimental slaughterhouse of the research centre of INRA (Saint Genès Champanelle, France) and animals from experiments 4 and 5 were slaughtered in the slaughterhouse of Socopa (Mirecourt, France).

2.1.1. Experiment 1

This experiment was performed using 8 crossbred Charolais × Salers steers (492 ± 27 day-old) having an initial live weight of 469 ± 26 kg. After an initial period of 15 days for adjustment on concentrate and hay, animals were randomly divided into two groups ($n = 4$ per group) on the basis of their live weight and daily gain. They were fed diets that differed by the level of lipid intake over the experimental period of 70 days. Ingredients and fatty acid composition of diets were given in Table 1. The control diet (diet CC) consisted of 45% of dry matter (DM) from grass hay and 55% of DM from a concentrate. The average composition of the concentrate mixture was, per kg on a DM basis, 575 g corn seed, 240 g soybean meal, 120 g dehydrated alfafa, 20 g cane molasses, 25 g urea and 20 g vitamin and mineral mixture. The experimental diet (diet CL) consisted of the same basal diet supplemented with extruded linseed (4% of lipids in diet DM) provided by Valorex (Combourtillé, France). The two diets were adjusted to be isoenergetic and isonitrogenous by decreasing the level of starch from concentrate in the CL diet. Animals were managed in pair-feeding conditions in order to reach 1.0–1.2 kg daily gain (2.95 Mcal/kg MS).

Table 1
Ingredients and fatty acid composition of treatment diets of the 5 experiments

Exp:	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5	
Diets:	CC♦	CL♦	CSC♦	CSL♦	CSC♦	CSL♦	CC♦	CL♦	CSC♦	CSL♦
Ingredients (% DM)										
Grass hay	45	39	–	–	–	–	–	–	–	–
Corn silage	–	–	70	70	66	70	–	–	63	61
Straw	–	–	–	–	–	–	30	32	–	–
Linseed	–	14	–	14	–	14	–	14	–	11
Concentrate	55	47	30	16	34	16	70	54	37	28
Fat content	3.6	7.5	4.3	8.0	4.3	8.0	2.0	5.8	2.5	5.9
FA composition (% total FA)										
14:0	1.0	0.9	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
16:0	22.8	20.3	14.9	14.1	14.7	14.1	15.2	14.0	15.5	14.4
18:0	2.8	2.8	2.1	2.3	2.1	2.3	2.1	2.1	1.6	1.7
18:1$n-9$	16.7	16.3	20.5	19.2	20.7	19.2	21.3	19.7	20.8	19.7
18:2$n-6$	32.7	29.8	46.6	40.9	47.1	40.9	49.2	45.7	51.6	48.5
18:3$n-3$	15.7	22.1	8.0	16.2	7.7	16.2	5.6	13.3	6.1	12.0
Others	8.3	7.8	7.7	7.1	7.5	7.1	6.8	5.1	4.3	3.6

♦ CC, concentrate-based diet; CL, concentrate-based diet supplemented with linseeds (4% of diet DM); CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).

2.1.2. Experiment 2

Following an initial period of 15 days for adjustment to a corn silage diet, 10 Charolais cullcows (6.2 ± 2 years old) were randomly housed in two distinct stalls ($n = 5$ per stall) on the basis of their initial live weight (752 ± 74 kg) and of their body fat score (3 ± 0.3) determined by manual palpation. Animals had ad libitum access to one of the two following diet treatments during the experimental period (84 days). The control diet (diet CSC) consisted on the kg DM basis of 700 g of corn silage and 300 g of concentrate (same composition as in experiment 1, Table 1). The experimental diet (diet CSL) consisted of the basal diet supplemented with extruded linseed (4% of lipids, Table 1). Diets were adjusted for the energy and nitrogen content by decreasing the level of starch from concentrate in the CSL diet to reach a mean daily gain of 1.3 kg/day (2.53 Mcal/kg MS). All Charolais cullcows were slaughtered at the final live weight of 840 ± 72 kg and with a body fat score of 4 ± 0.2.

2.1.3. Experiment 3

This experiment was managed in the same way than the experiment 2. Following an initial 2-week period for adjustment to a corn silage diet, 12 Holstein cullcows (6.0 ± 2 years old) were randomly housed in two distinct stalls ($n = 6$ per stall) on the basis of their initial live weight (600 ± 55 kg) and of their body fat score (2.6 ± 0.5). Animals had ad libitum access to the same diets than those used in experiment 2 (CSC and CSL, respectively, Table 1) for an experimental period of 70 days (2.54 Mcal/kg MS).

2.1.4. Experiment 4

Twenty Charolais bulls (358 ± 47 day-old, initial live weight: 530 ± 34 kg) were used in this experiment. After an adaptation period of three weeks on concentrate and straw, the animals were randomly housed in two distinct stalls on the basis of their live weight and of their age. Animals had ad libitum access to one of the two diets (Table 1): the control diet (diet CC, $n = 6$) consisting on the kg basis DM of 700 g of concentrate and of 300 g of straw, the experimental diet (diet CL, $n = 14$) consisting of the same basal diet to which was added 4% of lipids given as extruded linseeds. The concentrate feed contained, on the basis of DM, 305 g wheat seed, 300 g corn seed, 114 g soybean meal, 100 g barley seed, 93 g dehydrated alfafa, 62 g cane molasses, 10 g of vitamin and mineral mixture, 10 g bicarbonate sodium, 6 g carbonate. Diets were adjusted for the energy and the protein content by decreasing the level of starch from concentrate in the CL diet to reach a mean daily gain of 1.6 kg/day for a mean average experimental period of 97 days (3 Mcal/kg MS). Animals were slaughtered at the final live weight of 675 ± 29 kg.

2.1.5. Experiment 5

Twenty Charolais bulls (350 ± 48 day-old, initial live weight: 534 ± 27 kg) were allocated for a mean average experimental period of 97 days to two dietary treatments (Table 1): a control diet (diet CSC, $n = 6$) consisting of 63% of DM from corn silage and of 37% of DM from a concentrate, an experimental diet (diet CSL, $n = 14$) consisting of the same control diet supplemented with 4% of lipids provided from extruded linseeds (3 Mcal/kg MS). Animals were slaughtered at the final live weight of 690 ± 26 kg.

2.2. Lipid analysis

In the experiments 1, 2 and 3, four muscles [*Longissimus thoracis* (LT, 12th–13th rib), *Rectus abdominis* (RA), *Semi tendinosus* (ST) and *Pectoralis transversus* (PT)] were taken from beef carcasses just after slaughtering. In the experiments 4 and 5, only the RA muscle was taken after chilling of the carcasses at 4 °C for 24 h. In all experiments, muscles samples (approximatively 200 g), trimmed of subcutaneous adipose tissue, were diced, packaged in 10 g portions and stored at −20 °C until lipid analysis. Total lipids were extracted from muscle samples (4 g) by chloroform/methanol (2/1, v/v) according to the method of Folch, Lees, and Sloane-Stanley (1957). Extraction and transmethylation of FA into methyl esters (FAME) were realized at room temperature by using sodium methylate (1 M) and boron trifluoride in methanol (14% vol/vol) according to the method of Christie (2001). FAME composition was determined by gas liquid chromatography (Peri 2100, Perichrom, Saulx-les-Chartreux, France) using a glass capillary column (100 m lengh, 0.25 mm internal diameter, Varian, USA) coated with CP SIL 88. The oven temperature was initially at 70 °C for 30 s, and then subsequently increased to 175 °C at a rate of 20 °C/min, held at 175 °C for 25 min, then increased to 215 °C at a rate of 10 °C/min, and finally held at 215 °C for 41 min; injector and detector temperatures were set at 235 °C and 250 °C, respectively. Hydrogen, generated by Nicrocraft H_2 generator (Nitrocraft, Herbignac, France), was used as the carrier gas (at a flow rate of 1.1 ml/min). Chromatographic signals were analyzed by the Winilab II Chromatography Data System software (Perichrom). Response coefficients were calculated for each individual fatty acid by using the quantitative mix C4–C24 methyl esters provided by Supelco (USA) and total FA content was calculated by using the internal standard method (C19:0).

Values for CLA *cis*-9, *trans*-11 content include those of CLA *trans*-7, *cis*-9 and CLA *trans*-8, *cis*-10 because the complete separation of these CLA isomers was not possible by GLC under our experimental conditions (Fritsche et al., 2000). FA composition (saturated, monounsaturated and polyunsaturated) is not detailed; only the P/S and $n-6/n-3$ FA ratios are presented in tables. P/S ratio corresponds to $(18{:}2n-6+18{:}3n-3)/(12{:}0+14{:}0+16{:}0+18{:}0)$ and $n-6/n-3$ ratio was calculated as the ratio of the sum of $n-6$ PUFA ($18{:}2n-6+18{:}3n-6+20{:}2n-6+20{:}3n-6+20{:}4n-6+22{:}4n-6$) on the sum of $n-3$ PUFA ($18{:}3n-3+20{:}3n-3+20{:}5n-3+22{:}5n-3+22{:}6n-3$).

2.3. Statistical analysis

All values were expressed as the mean values with the standard error of the model (SEM). The effects of the different factors on beef CLA content such as factors linked to the animal (fatness, breed, age, sex and type of muscles) and feeding conditions (dietary lipid supplements, i.e., linseed rich in linolenic acid, composition of the basal diet, i.e., corn silage vs. concentrate) were tested by analysis of variance (ANOVA) with the GLM procedure of SAS (SAS Inst., Inc., Carry, NC). Comparisons between dietary treatments (Control diet vs. Linseed diet) for each of the five experiments were analyzed by using the Student's *t* test for unpaired data after verification of the normal distribution of data.

3. Results and discussion

3.1. Factors influencing beef CLA content

3.1.1. Influence of lipid supplements

Management strategies and, among them, feeding has been shown to have strong effects on FA composition of animal tissues (Demeyer & Doreau, 1999; Mir et al., 2004). To determine the impact of dietary lipid supplementation on intramuscular composition of CLA, the effects of linseed supplementations were investigated taking into account the five experiments (for details see Section 2). Time of feeding differed in these different experiments (from 70 to 97 days) but was probably superior to the minimal experimental feed time necessary to modulate fatty acid composition in tissues. Indeed, previous studies showed that, from 42 days of experimental diets (lipid supplementations), the fatty acid composition of muscle tissues is marked and does not vary significantly any more for longer times of experimental diet (Griswold et al., 2003; Mandell, Buchanan-Smith, Holub, & Campbell, 1997). In experiments 1–3, beef CLA contents were compared in four muscles (*Longissimus thoracis*, LT, *Rectus abdominis*, RA, *Semi tendinosus*, ST and *Pectoralis transversus*, PT), whereas in experiments 4 and 5, only the RA muscle was analyzed. Total CLA contents (0.4–1.0% of total FA, Table 2) in intramuscular beef fat were in the same range than values previously reported (Enser et al., 1999; Mir, Paterson, & Mir, 2000; Nurnberg et al., 2002; Raes et al., 2004). Dietary lipid supplementation with linseeds (expressed as "suppl." in Table 2) resulted in a significant increase of total CLA proportion in intramuscular fatty acids ($P < 0.01$, Table 2) in agreement with previous studies in bovines given diets enriched in $n-3$ PUFA (linseed supplement, grass or grass silage) (Bauchart, Gladine, Gruffat, Leloutre, & Durand, 2005; Enser et al., 1999; French et al., 2000). This increase resulted in a higher proportion of all classes of CLA isomers, more particularly of *cis,cis*-CLA isomers in Charolais cows experiment (experiment 2, $P < 0.03$). However, this effect was not observed in all

Table 2
Effects of dietary linseed supplementation compared to basal diet on CLA proportions (% of total fatty acids), on CLA composition (% of total CLA) and on some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 1[A] C × S steers		Exp. 2[A] Charolais cows		Exp. 3[A] Holstein cows		Exp. 4[B] Charolais bulls		Exp. 5[B] Charolais bulls		SEM	*P* effects (*P*=)		
Diets:	CC[♦]	CL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CC[♦]	CL[♦]	CSC[♦]	CSL[♦]		Suppl.[C]	Exp.[D]	Suppl. × exp.
	% total FA													
Total CLA	0.72[a]	0.88[b]	0.56[a]	0.72[b]	0.46	0.40	1.02	0.99	0.67[a]	0.91[b]	0.07	*0.009*	*0.001*	*0.014*
CLA *c*9,*t*11	0.45[a]	0.57[b]	0.49[a]	0.59[b]	0.38	0.32	0.77	0.71	0.53	0.68	0.06	0.070	*0.001*	0.313
∑*c*,*t* CLA	0.54[a]	0.67[b]	0.52[a]	0.65[b]	0.39	0.34	0.85	0.77	0.58	0.74	0.06	0.058	*0.001*	*0.013*
∑*c*,*c* CLA	0.10	0.11	0.02[a]	0.03[b]	0.03	0.03	0.07	0.09	0.04	0.10	0.02	*0.024*	*0.001*	0.584
∑*t*,*t* CLA	0.08	0.10	0.02	0.04	0.04	0.03	0.10	0.13	0.05	0.07	0.01	0.059	*0.001*	0.105
	% total CLA													
∑*c*,*t* CLA	75.0	76.1	92.9	90.3	84.8	85.0	83.3[a]	77.8[b]	86.6	81.3	3.51	0.212	*0.001*	0.570
∑*c*,*c* CLA	13.9	12.5	3.6	4.2	6.5	7.5	6.9	9.1	6.0	11.0	2.57	0.237	*0.001*	0.912
∑*t*,*t* CLA	11.1	11.4	3.6	5.6	8.7	7.5	9.8	13.1	7.5	7.7	2.18	0.540	*0.001*	0.245
P/S[E]	0.23	0.24	0.09[a]	0.12[b]	0.09	0.07	0.26	0.28	0.15[a]	0.24[b]	0.02	*0.006*	*0.001*	*0.007*
$n-6/n-3$[F]	3.13[a]	2.36[b]	2.53[a]	1.90[b]	2.36	1.77	4.22[a]	2.20[b]	4.19[a]	1.82[b]	0.22	*0.001*	*0.001*	*0.001*

C × S, Charolais × Salers.

[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).

[♦] CC, concentrate-based diet; CL, concentrate-based diet supplemented with linseeds (4% of diet DM); CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).

[A] Muscles LT, RA, ST and PT were analyzed.

[B] Muscle RA was analyzed.

[C] Effect of linseed supplementation compared to control diet (CC vs. CL or CSC vs. CSL).

[D] Effect of experiment including the nature of the basal diet and the factors linked to animals (fatness, breed, sex and age).

[E] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).

[F] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22: 4$n-6$)/(C18:3$n-3$ + C20:3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

experiments since linseed supplementation increased the proportion of total CLA in muscle fatty acids in experiments 1, 2 and 5 (from 22% to 36%), whereas no change was observed in experiments 3 and 4. This implied that additional experimental factors (termed "Exp." in Table 2), including the ingredient composition of basal diets and the animal intrinsic factors (age, sex and breed) were strongly involved in the modulation of CLA composition in intramuscular fat ($P < 0.001$ for the five experiments). Moreover, the significant interaction noted between linseed supplementation and experimental factors (presented as "suppl. × exp." in Table 2) on proportions of total CLA and of *cis,trans*-isomers indicated that experimental factors influenced the efficiency of the dietary supplementation to enhance the CLA deposition in fat of muscles.

3.1.2. Influence of the basal diet composition (experiments 4 and 5)

The impact of the basal diet on beef CLA composition (expressed as "diet" in Table 3) was investigated in RA muscle by comparing the CLA composition of lipids in bovines fed a diet consisting in straw/concentrate (30/70) supplemented (CL) or not (CC) with extruded linseeds with that of bovines fed a corn silage/concentrate diet (60/40) supplemented (CSL) or not (CSC) with extruded linseeds. Amounts of total CLA ($P < 0.02$) as well as those of *cis, trans* ($P < 0.06$) and *trans,trans*-CLA isomers ($P < 0.001$) were higher (on average +26%, +23% and +77%, respectively) in the group given a diet rich in concentrate (70%) than in the group given the low concentrate (40%) silage-based diet. This could be explained mainly because feeding high concentrate diets reduces the intensity of ruminal PUFA biohydrogenation (Doreau & Ferlay, 1994) favoring the production of vaccenic acid (18:1Δ11trans, VA) available for CLA synthesis and its accretion in peripheral tissues (Griinari & Bauman, 1999). Another explanation would be an increase in tissue fat of 18:1Δ10trans with the corn silage-based diet as previously observed in milk fat by Ferlay, Capitan, Ollier, and Chilliard (2003). This confirms the theory proposed by Bauman and Griinari (2001) on 18:1Δ10trans pathway which would decrease the yield of ruminal vaccenic acid and its availability for CLA synthesis in tissues. The tendency of the interaction (expressed as "suppl. × diet", $P = 0.12$) between linseed supplementation and the level of concentrate in diet observed in Charolais bulls (experiments 4 and 5), indicated that the basal diet might modulate the efficiency of the linseed supplementation to increase the proportion of CLA deposited in intramuscular fat. Indeed, linseed supplementation increased the proportion of total CLA (+33.8%, $P < 0.05$) in muscles of Charolais bulls fed a corn silage-based diet low in concentrate (experiment 5, Table 3), whereas the same supplementation did not affect the CLA proportion in intramuscular fat of Charolais bulls fed diet rich in concentrate (experiment 4, Table 3). Among possible hypothesis, dietary conditions (such as a concentrate rich diet) favoring CLA deposition in muscle and fat tissues would reduce the extent of bioconversion of vaccenic acid into CLA by a mechanism of retroinhibition of the activity of the stearoyl CoA desaturase (Δ9 desaturase)

Table 3
Effects of the basal diet composition (30% straw/70% concentrate (CC and CL) vs. 60% corn silage/40% concentrate (CS and CSL)) on CLA proportions (% of total fatty acids), CLA composition (% of total CLA) and some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 4[A] Young Charolais bulls		Exp. 5[A] Young Charolais bulls		SEM	*P* effects (*P*=)		
Diets:	CC[♦]	CL[♦]	CSC[♦]	CSL[♦]		Diet[C]	Suppl.[B]	Suppl. × diet
	% total FA							
Total CLA	1.02	0.99	0.67[a]	0.91[b]	0.08	*0.021*	0.251	0.116
CLA *c*9,*t*11	0.77	0.71	0.53	0.68	0.06	0.058	0.544	0.131
∑*c*,*t* CLA	0.85	0.77	0.58	0.74	0.07	0.052	0.604	0.113
∑*c*,*c* CLA	0.07	0.09	0.04	0.10	0.02	0.714	*0.041*	0.205
∑*t*,*t* CLA	0.10	0.13	0.05	0.07	0.01	*0.001*	*0.049*	0.528
	% total CLA							
∑*c*,*t* CLA	83.3[a]	77.8[b]	86.6	81.3	1.53	*0.045*	*0.012*	0.946
∑*c*,*c* CLA	6.9	9.1	6.0	11.0	1.50	0.946	0.166	0.386
∑*t*,*t* CLA	9.8	13.1	7.5	7.7	1.01	*0.004*	0.068	0.236
P/S[D]	0.26	0.28	0.15[a]	0.24[b]	0.02	*0.001*	*0.006*	0.136
$n-6/n-3$[E]	4.22[a]	2.20[b]	4.19[a]	1.82[b]	0.17	0.289	*0.001*	0.381

[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).

[♦] CC, concentrate-based diet; CL, concentrate-based diet supplemented with linseeds (4% of diet DM); CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).

[A] Muscle RA was analyzed.

[B] Effect of linseed supplementation compared to control diet (CC vs. CL or CSC vs. CSL).

[C] Effect of the nature of the diet (30% straw/70% concentrate, CC and CL vs. 60% corn silage/40% concentrate, CSC and CSL).

[D] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).

[E] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22:4$n-6$)/(C18:3$n-3$ + C20:3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

(Griinari & Bauman, 1999). Indeed, it was shown that high levels of CLA as well as of PUFA decrease the Δ9 desaturation activity in both hepatic and adipose tissues either directly or indirectly by decreasing its mRNA expression (Ntambi, 1999; Park et al., 2000).

3.1.3. Influence of the factors linked to animals

The other factors which could modify the CLA content in beef are intrinsic factors linked to animals (age, sex, breed and type of muscles). Such factors influence fat deposition (body fat score, BFS) which was only determined in experiments 1–3 (Table 4). CLA content in beef was not related to final body score, but was influenced more specifically by the rate of fattening of animals (difference between final and initial body fat score of animals: ΔBFS, Table 4). Variations of ΔBFS modified significantly the level of CLA in muscle FA, especially of *cis*9, *trans*11- ($P < 0.009$) and *cis,cis*- ($P < 0.05$) isomers. Intramuscular fat of steers with a low ΔBFS (+14%, experiment 1) contained a higher proportion of CLA (+25% and +86%) than that of animals with a high ΔBFS (+38% and +90% in experiments 2 and 3, respectively). In the same way, linseed supplementation increased significantly the proportion of CLA deposited in muscles only in animals with a low ΔBFS. These results, not expected, showed clearly that the rate of CLA deposition does not depend on the final body fat score of animals but is favored only in conditions favoring a low rate of fat deposition.

Differences in fattening of bovine tissues are influenced by the genotype, the sex and the age of animals (De Smet, Raes, & Demeyer, 2004; Nurnberg, Wegner, & Ender, 1998). As presented in Table 5, the effects of these factors (expressed as "type") on the level of CLA in beef FA were highly significant ($P < 0.001$) and affected all classes of CLA isomers ($P < 0.005$). Individual effects of age and of sex of animals were not tested separately in our experimental conditions. When taken together, age and sex (Table 5) influenced significantly total CLA proportion in intramuscular fat ($P < 0.004$) and *cis,cis*- and *trans,trans*-CLA isomer proportions ($P < 0.02$ and $P < 0.001$, respectively), intramuscular fat of young Charolais bulls containing higher proportions of CLA (on average +41%) than that of mature Charolais cows. Sex has a large effect on fatness of animals since, at equal slaughter weights, bulls are leaner than steers, themselves being leaner than females as reported earlier (Enser, 1991; Nurnberg et al., 1998). Moreover, the effect of breed was tested by comparing CLA proportions present in lipids of LT, RA, ST and PT muscles of Charolais and Holstein cows (Table 6). Intramuscular fat of Charolais cows contained more total CLA than that of Holstein cows (on average +47%, $P < 0.001$), and especially more *cis,trans*-isomers (on average +55%, $P < 0.001$). These effects could be explained by differences in the muscle fat deposition as discussed above. However, it has been previously reported that, even after corrections to remove the effect of fatness, significant differences between breeds

Table 4
Effects of the body fat score (BFS) and of the rate of fattening (ΔBFS) of bovines on CLA content (% of total fatty acids), CLA composition (% of total CLA) and some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 1[A] C × S steers		Exp. 2[A] Charolais cows		Exp. 3[A] Holstein cows		SEM	*P* effects of BFS (*P*=)			*P* effects of ΔBFS (*P*=)		
Diets:	CC[♦]	CL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]		BFS	Suppl.[B]	BFS × suppl.	ΔBFS	Suppl.[B]	ΔBFS × suppl.
BFS	3.125	3.125	3.88	4.20	3.35	3.50							
ΔBFS[■](%)	+9.3	+19.3	+33.8	+42.4	+76.3	+105.9							
	% total FA												
Total CLA	0.72[a]	0.88[b]	0.56[a]	0.72[b]	0.46	0.40	0.07	0.249	*0.040*	0.143	0.105	*0.037*	0.279
CLA *c*9,*t*11	0.45[a]	0.57[b]	0.49[a]	0.59[b]	0.38	0.32	0.05	0.200	*0.041*	0.312	*0.009*	0.072	*0.014*
∑*c*,*t* CLA	0.54[a]	0.67[b]	0.52[a]	0.65[b]	0.39	0.34	0.06	0.275	0.063	0.438	0.051	0.147	0.098
∑*c*,*c* CLA	0.10	0.11	0.02[a]	0.03[b]	0.03	0.03	0.02	0.996	0.503	0.259	*0.042*	*0.024*	0.830
∑*t*,*t* CLA	0.08	0.10	0.02	0.04	0.04	0.03	0.02	0.730	0.275	0.440	0.551	0.511	0.794
	% total CLA												
∑*c*,*t* CLA	75.0	76.1	92.9	90.3	84.8	85.0	4.89	0.992	0.683	0.353	0.105	0.159	0.722
∑*c*,*c* CLA	13.9	12.5	3.6	4.2	6.5	7.5	3.54	0.984	0.810	0.249	0.066	*0.049*	0.920
∑*t*,*t* CLA	11.1	11.4	3.6	5.6	8.7	7.5	3.00	0.919	0.702	0.911	0.781	0.962	0.572
P/S[C]	0.23	0.24	0.09[a]	0.12[b]	0.09	0.07	0.03	0.373	0.551	0.755	0.379	0.978	0.869
$n-6/n-3$[D]	3.13[a]	2.36[b]	2.53[a]	1.90[b]	2.36	1.77	0.27	0.564	*0.001*	0.478	0.579	*0.001*	0.361

[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).

[♦] CC, concentrate-based diet; CL, concentrate-based diet supplemented with linseeds (4% of diet DM); CSC: corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).

[■] Variation of body fat score (ΔBFS) = [(final BFS − initial BFS)/final BFS] × 100.

[A] Muscles LT, RA, ST and PT were analyzed.

[B] Effect of linseed supplementation compared to control diet (CC vs. CL or CSC vs. CSL).

[C] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).

[D] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22:4$n-6$)/(C18:3$n-3$ + C20:3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

Table 5
Effects of the type (breed, sex and age) and of the sex and age of animals on CLA proportions (% of total fatty acids), CLA composition (% of total CLA) and some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 2[A] Mature Charolais cows		Exp. 3[A] Mature Holstein cows		Exp.5[A] Young Charolais bulls		SEM	*P* effects of type of animals (*P*=)			*P* effects of sex and age of animals (*P*=)		
Diets:	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]		Type	Suppl.[B]	Type × suppl.	Sex/age	Suppl.[B]	Sex/age × suppl.
	% total FA												
Total CLA	0.48	0.65	0.48	0.42	0.67[a]	0.91[b]	0.07	*0.001*	0.071	0.125	*0.004*	*0.012*	0.633
CLA *c*9,*t*11	0.47	0.53	0.42	0.37	0.53	0.68	0.05	*0.005*	0.313	0.267	0.105	0.106	0.440
∑*c*,*t* CLA	0.48	0.59	0.44	0.38	0.58	0.74	0.06	*0.002*	0.231	0.226	0.071	0.056	0.694
∑*c*,*c* CLA	nd	0.03	0.005	0.002	0.04	0.10	0.02	*0.001*	0.088	0.280	*0.013*	*0.050*	0.517
∑*t*,*t* CLA	nd	0.03	0.03	0.04	0.05	0.07	0.01	*0.004*	0.128	0.760	*0.001*	0.069	0.593
	% total CLA												
∑*c*,*t* CLA	100.0	90.8	91.7	90.5	86.6	81.3	2.75	*0.001*	*0.026*	0.508	*0.001*	*0.010*	0.299
∑*c*,*c* CLA	–	4.6	1.0	0.5	6.0	11.0	1.84	*0.002*	0.112	0.420	*0.017*	0.054	0.790
∑*t*,*t* CLA	–	4.6	6.3	9.5	7.5	7.7	1.92	*0.015*	0.084	0.543	*0.002*	0.052	0.140
P/S[C]	0.07	0.10	0.09[a]	0.06[b]	0.15[a]	0.24[b]	0.01	*0.001*	0.410	*0.001*	*0.001*	*0.002*	0.078
$n-6/n-3$[D]	2.59	2.14	2.85	2.32	4.19[a]	1.82[b]	0.18	*0.007*	*0.001*	*0.001*	*0.003*	*0.001*	*0.001*

[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).
[♦] CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).
[A] Muscle RA was analyzed.
[B] Effect of linseed supplementation compared to control diet (CSC vs. CSL).
[C] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).
[D] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22:4$n-6$)/(C18:3$n-3$ + C20: 3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

Table 6
Effects of the animal breed on CLA proportions (% of total fatty acids), CLA composition (% of total CLA) and some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 2[A] Mature Charolais cows		Exp. 3[A] Mature Holstein cows		SEM	*P* effects (*P*=)		
Diets:	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]		Breed[C]	Suppl.[B]	Suppl. × breed
	% total FA							
Total CLA	0.56[a]	0.72[b]	0.46	0.40	0.06	*0.001*	0.391	*0.005*
CLA *c*9,*t*11	0.49[a]	0.59[b]	0.38	0.32	0.05	*0.001*	0.842	*0.030*
∑*c*,*t* CLA	0.52[a]	0.65[b]	0.39	0.34	0.05	*0.001*	0.588	*0.008*
∑*c*,*c* CLA	0.02[a]	0.03[b]	0.03	0.03	0.02	0.791	0.236	0.900
∑*t*,*t* CLA	0.02	0.04	0.04	0.03	0.02	0.950	0.934	*0.015*
	% total CLA							
∑*c*,*t* CLA	92.9	90.3	84.8	85.0	4.21	0.140	0.641	0.161
∑*c*,*c* CLA	3.6	4.2	6.5	7.5	3.09	0.408	0.373	0.850
∑*t*,*t* CLA	3.6	5.6	8.7	7.5	2.91	0.207	0.784	0.069
P/S[D]	0.09[a]	0.12[b]	0.09	0.07	0.01	*0.001*	0.597	*0.001*
$n-6/n-3$[E]	2.53[a]	1.90[b]	2.36	1.77	0.23	0.153	*0.001*	0.820

[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).
[♦] CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).
[A] Muscles LT, RA, ST and PT were analyzed.
[B] Effect of linseed supplementation compared to control diet (CSC vs. CSL).
[C] Effect of breed (mature Charolais cows, Exp. 2 vs. mature Holstein cows, Exp. 3).
[D] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).
[E] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22:4$n-6$)/(C18:3$n-3$ + C20:3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

occur for FA concentrations in intramuscular fat (Angus vs. Simmental; Wagyu vs. Waguy × Limousin vs. Limousin) (Laborde, Mandell, Tosh, Wilton, & Buchanan-Smith, 2001; Mir et al., 2002) and for CLA content in milk (Holstein-Friesian vs. Montbeliarde vs. Normande) (Lawless, Stanton, Devery, Dillon, & Murphy, 1999). In contrast, no effect of breed on CLA content in muscle was reported when two dairy breeds (Holstein vs. Montbeliarde vs. Normande) or two beef breeds (Wagyu vs. Limousin) were compared (Laborde et al., 2001; Lawless et al., 1999; Mir et al., 2002). However, stearoyl CoA desaturase and elongase activities in muscles differed between beef and dairy breeds (Jersey vs. Limousin) (Malau-Aduli, Siebert, Bottema, & Pitchford, 1997; Malau-Aduli, Siebert, Bottema,

& Pitchford, 1998) which could explain, at least in part, the differences in muscle CLA content observed in our experiments (Charolais and Holstein bulls or cullcows, Tables 5 and 6).

Differences in fat and CLA content between muscles have also to be considered (Table 7). The type of muscles strongly influenced proportions of total CLA ($P < 0.001$) and of all CLA isomer classes ($P < 0.01$) in intramuscular fatty acids. In ruminant muscles, it is know that CLA is mainly associated to the triacylglycerol fraction (Bauchart et al., 2005; Fritsche et al., 2000; Raes, De Smet, Balcaen, Clayes, & Demeyer, 2003) which is linked to the fat content of tissues (Ashes, Siebert, Gulati, Cuthbertson, & Scott, 1992). Consequently, when CLA content was expressed in quantity (mg/g tissue), RA and LT muscles contained more CLA than ST and PT muscles (data not shown) since the two first muscles were the fattiest (Table 7). However, the high proportion of triacylglycerol in muscles is also associated with higher amounts of SFA (Raes, Haak et al., 2004) as reflected by the P/S ratio presented in Table 7, leading to a dilution of the CLA proportion. Thus, when CLA content was expressed in proportion (% of total FA), ST and PT muscles contained higher proportions (on average +24%) of CLA than RA and LT muscles. Moreover, estimation of Stearoyl CoA desaturase activity by the desaturase index developed by Malau-Aduli et al. (1997, 1998) led to higher values in ST and PT muscles than in LT and RA muscles (data not shown) which could explain, at least in part, differences in proportion of CLA isomers between muscles.

In conclusion, extrinsic factors such as basal diet composition and lipid supplements and factors linked to animal such as the type of animal (including the breed, the sex and the age of animal) are of major importance for the proportion of CLA in intramuscular fat of bovines. The greatest proportion of CLA occurred in lean muscles of young males from beef breed fed a diet rich in concentrate supplemented or not with linseeds, the efficiency of such conditions depending on the initial amount of CLA and of PUFA in muscles.

3.2. Factors influencing composition of beef CLA isomers

The biological properties, especially anticancer properties, of bioformed CLA isomers in milk vary mainly with the proportion of *cis,trans*- *cis,cis*- and *trans,trans*-isomers (Miller, Stanton, & Devery, 2002). Moreover, comparison of antiproliferative activities of different CLA isomers present in beef on a set of human tumour cells demonstrates that all CLA isomers possess antiproliferative properties, the 9t,11t isomer being the most active (De La Torre et al., 2005). In the same way, CLA mixtures extracted from beef can inhibit proliferation of human cancer cells, a high content in *cis,trans* isomers leading the maximal antiproliferative effect (De La Torre et al., in press). In this context, it appears important to determine the variations of the distribution of CLA isomers in beef since these proportions could influence the biological properties of bioformed CLA.

Dietary supplementation with linseeds did not modify the distribution of CLA isomers (expressed as % of total CLA) in intramuscular FA of animals in our five experiments, whatever the type of animals and muscles (Table 2). These results confirmed recent data reported by Gillis, Duckett, and Sackmann (2004) in beef cattle given corn oil supplement. In contrast, there was an important impact of additional experimental factors linked to the composition of basal diet and to animals on the proportions of CLA isomers (Table 2, $P < 0.001$). Intramuscular fat of animals fed a concentrate-based diet (experiment 4, Table 3) presented a proportion of *trans,trans*-CLA isomers 1.4-fold higher ($P < 0.004$) than that of animals fed a corn silage-based diet (experiment 5) to the detriment of the proportion of *cis,trans* isomers (on average 80.9% vs. 83.3% of total CLA in experiments 4 and 5, respectively, $P < 0.05$). The influence of the basal diet on the CLA isomer profile in muscles was studied very little except for studies of Teter et al. (1999) and Piperova et al. (2000) on milk fat and of Dannenberger et al. (2004) and Nurnberg et al. (2002) on beef fat. However, in all these studies, authors compared pasture vs. concentrate-based diets and not concentrate- vs. corn silage-based diets as in the present experiments. However, the variations observed here could be the result of modifications of rumen FA biohydrogenations which are affected by the feed intake, the composition of the basal diet and the type and the amount of dietary FA (Chouinard et al., 2001; Latham, Sharpe, & Sutton, 1971; Piperova et al., 2000) which regulate the amount and the activity of the bacterial population in the rumen. The consequences are modifications in the rate of production of CLA (especially of different *cis* and *trans* octadecenoic acids), of their utilization by ruminal microbes and of their availability for CLA formation in peripheral tissues (Griinari & Bauman, 1999).

Among factors related to the animals, the body fat score at the end of the experimental period (BFS) did not alter the distribution of the different CLA isomers deposited in muscle fats (Table 4). Only the difference between final and initial body score (ΔBFS, Table 4) slightly modulated the proportion of *cis,cis*-CLA isomers accumulated in muscles ($P < 0.07$), the proportion of these isomers being higher in animals with a low ΔBFS (Charolais × Salers steers) than in those with a high ΔBFS (Charolais or Holstein cullcows). In contrast, the effect of breed, age and sex of animals was highly significant on the proportion of *cis,trans*-, *cis,cis*- and *trans,trans*-isomers in RA muscle ($P < 0.001$, $P < 0.002$, $P < 0.02$, respectively, Table 5), mature cullcows possessing around 1.2-fold higher proportion of *cis,trans* isomers compared with young bulls. Among these intrinsic factors, the joint effect of the sex and the age, obtained by comparison of Charolais mature cows (experiment 2) with Charolais young bulls (experiment 5), significantly influenced the proportions of *cis,trans*-, *cis,cis*- and *trans,trans*-CLA isomers in muscle lipids (Table 5). Intramuscular fat of young males presented greater proportions of *cis,cis*- (3.7×, $P < 0.02$) and

Table 7
Effects of the type of muscles (*Longissimus thoracis*, LT; *Rectus abdominis*, RA; *Semi tendinosus*, ST and *Pectoralis transversus*, PT) on CLA proportions (% of total fatty acids), CLA composition (% of total CLA) and some important nutritional ratios of intramuscular fat of animals

Animals:	Exp. 2 mature Charolais cows								Exp. 3 mature Holstein cows								SEM	*P* effects (*P*=)		
Muscles:	LT		RA		ST		PT		LT		RA		ST		PT					
Diets	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]	CSC[♦]	CSL[♦]		Tissue	Suppl.[A]	Tiss. × suppl.
	g/100 g fresh tissue																			
Total lipids	4.47	3.96	5.26	3.10	2.09	1.88	3.77	2.53	5.00	6.20	4.80	5.80	3.20	2.90	4.70	4.70				
	% total FA																			
Total CLA	0.44	0.61	0.48	0.63	0.56	0.67	0.78	0.78	0.39	0.29	0.48	0.45	0.46	0.49	0.50	0.44	0.06	*0.001*	0.557	0.793
CLA *c*9,*t*11	0.39	0.53	0.47	0.50	0.40	0.45	0.68	0.63	0.36	0.29	0.42	0.41	0.35	0.38	0.39	0.31	0.05	*0.015*	0.849	0.498
$\sum c,t$ CLA	0.41	0.55	0.48	0.56	0.49	0.55	0.71	0.71	0.36	0.29	0.44	0.41	0.35	0.40	0.42	0.32	0.05	*0.001*	0.565	0.438
$\sum c,c$ CLA	nd	0.02	nd	0.03	0.02	0.03	0.05	0.04	0.01	nd	0.01	nd	0.03	0.06	0.04	0.08	0.02	*0.010*	0.437	0.995
$\sum t,t$ CLA	0.03	0.04	nd	0.04	0.05	0.09	0.02	0.03	0.02	nd	0.03	0.04	0.08[a]	0.03[b]	0.04	0.04	0.02	*0.007*	0.991	0.205
	% total CLA																			
$\sum c,t$ CLA	93.2	90.2	100.0	88.9	87.5	82.1	91.0	91.0	92.3	100.0	91.7	91.1	76.1	81.6	84.0	72.7	4.26	*0.005*	0.383	0.616
$\sum c,c$ CLA	nd	3.3	nd	4.8	3.6	4.5	6.4	5.1	2.6	nd	2.1	nd	6.5	12.2	8.0	18.2	*3.20*	*0.017*	0.313	0.997
$\sum t,t$ CLA	6.8	6.6	nd	6.3	8.9	13.4	2.6	3.8	5.1	nd	6.3	8.9	17.4[a]	6.1[b]	8.0	9.1	2.88	*0.014*	0.749	0.657
P/S[B]	0.07	0.08	0.07	0.10	0.14	0.17	0.10[a]	0.15[b]	0.07	0.05	0.09	0.06	0.11	0.09	0.09	0.10	0.01	*0.001*	0.455	0.470
$n-6/n-3$[C]	2.88[a]	2.10[b]	2.59	2.18	2.07	1.65	2.59[a]	2.07[b]	3.15[a]	1.69[b]	2.85	2.42	1.59	1.46	1.95	1.58	0.22	*0.001*	*0.001*	0.052

nd, non detected.
[a,b] Mean values with different superscripts were significantly different tested by Student's *t* test for unpaired data ($P < 0.05$).
[♦] CSC, corn silage-based diet; CSL, corn silage-based diet supplemented with linseeds (4% of diet DM).
[A] Effect of linseed supplementation compared to control diet (CSC vs. CSL).
[B] Ratio of (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0 + C18:0).
[C] Ratio of (C18:2$n-6$ + C18:3$n-6$ + C20:2$n-6$ + C20:3$n-6$ + C20:4$n-6$ + C22:4$n-6$)/(C18:3$n-3$ + C20:3$n-3$ + C20:5$n-3$ + C22:5$n-3$ + C22:6$n-3$).

trans,trans-isomers (×3.6, $P < 0.002$) than that of mature cows which contained, in turn, higher proportions of *cis, trans*-isomers in (+14.5%, $P < 0.001$). Such differences might be explained, either, by a variability of the bacterial population between animals, or by disruptions of intestinal transit or by differences in dietary intake. In contrast, the breed did not have any impact on the proportion of CLA isomers present in lipid of muscles (Table 6). The type of muscles (Table 7) also modulated the proportions of *cis,-trans*-, *cis,cis*- and *trans,trans*-CLA isomers in beef fat ($P < 0.005$, $P < 0.02$ and $P < 0.02$, respectively), ST and PT muscles containing higher proportions of *cis,cis* (×5) and *trans,trans* (+61.2%) isomers than RA and LT muscles to the detriment of *cis,trans* isomers (−12.2%).

In conclusion, several factors could influence the proportion of CLA isomers in beef fat, the type of animals including sex/age of animals and the type of muscles and the nature of the basal ration being of major importance. The greatest proportion of *cis,trans*-isomers to the detriment of others occurred in the fattiest muscles of mature cows fed a diet rich in corn silage.

4. Conclusions

The proportion of CLA in ruminant products is dependent on the ruminal formation of both vaccenic acid and CLA and on the tissue ability to desaturate vaccenic acid into CLA. Extrinsic (lipid supplementation, nature of the basal diet) and/or intrinsic (breed, age and sex of animals and type of muscles) factors influence the amount but also the composition of CLA isomers deposited in beef fat. In our experiments, lipid supplements with oleaginous seeds (i.e., linseeds) were an effective way which could induce an increase of CLA proportion in beef from 22% to 36%. But the efficiency of this intervention was highly dependent on other factors, such as the nature of the basal diet which could influence the proportion of CLA of about 26%, and others factors such as breed, sex and age of animals, as well as the type of muscle which could modulate the CLA proportion in beef of about 47%, 41% and 24%, respectively. These results showed for the first time that the whole of these factors must be taken into account within a framework of development of strategies of breeding aimed at increasing the CLA content in beef. These results must be completed to develop a tool of modelling making it possible to anticipate the variability of the distribution of CLA in the bovine muscles, since it has been previously demonstrated that such distribution is not further modified by preserving and cooking of meat (Bauchart, Durand, Thomas and Peyron, unpublished data). Moreover, further investigations are necessary to determine with precision the biological activities (as anticancer properties, De La Torre et al. (2005), De La Torre, Debiton, Juanéda et al. (in press)) of *cis,cis*- and *trans,trans*-isomers compared to those of *cis*9, *trans*11-CLA isomer since the distribution of CLA isomers can be altered.

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