Linseed and rapeseed supplements diversely altered *trans* 18:1 isomers in total lipids of *Longissimus thoracis* muscle of finishing Normand cows

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SUMMARY

The aim of the study was to determine the impact of lipid supplements rich in unsaturated FA provided by extruded linseed (rich in 18:3n-3) alone or with rapeseed (rich in 18:1n-9*cis* and at a lower extent in 18:2 n-6 and 18:3n-3) on *trans* 18:1 isomers of *Longissimus thoracis* muscle in Normand cull cows given a concentrate/straw based diet (70/30) for a 100d finishing period. Vaccenic acid (Δ11*tr* 18:1) was known to be beneficial for the human health by its protective effect against atherosclerosis whereas Δ9*tr* 18:1 *(*elaidic acid*)* and Δ10*tr* 18:1 would be detrimental since they were known to be pro-inflammatory and pro-atherogenic in animal models and humans. Beef *trans* 18:1 were purified by preparative HPLC and the relative distribution and amount of their 11 isomers (from Δ6*tr* to Δ16*tr)* were determined by GLC-MS. In the control diet (C), *trans* 18:1 were dominated by the Δ10*tr* (33.7%) and Δ11*tr* (36.1%) isoforms, the Δ9*tr* representing only 8.5%. Addition of linseed (diet L) highly decreased the Δ9*tr* (-41.2%) and Δ10*tr* (-53.7%) isomers ($P< 0.05$) to the benefit of only Δ12*tr* up to Δ16*t*r isomers (x2.4, ($P< 0.05$). On the other hand, when compared to that in diet C, addition of the mixture rapeseed (2/3) and linseed (1/3) significantly decreased the Δ9 *tr* (-24.7%) and Δ11*tr* (-30.7%) isoforms to the benefit of the Δ10*tr* isoform (+22.0%) ($P< 0.05$). We concluded that addition of lipids rich in unsaturated FA from linseed or rapeseed to a basal diet rich in cereals, can diversely modified the health value of beef *trans* 18:1 on the basis of its Δ9*tr*, Δ10*tr* and Δ11*tr* 18:1 contents. Linseed rich in 18:3n-3 had a positive effect on beef health value by decreasing both Δ9*tr* and Δ10*tr* 18:1. Inversely, association of linseed with rapeseed rich in 18:1n-9 would altered beef health value by decreasing Δ11*tr* 18:1.

Keywords: lipid supplements, unsaturated FA, *trans* 18:1 isomers, *Longissimus thoracis,* Normand cows

INTRODUCTION

Trans fatty acids (FA), especially *trans* isomers of 18:1, are notably formed during partial hydrogenation of polyunsaturated fatty acids (PUFA) provided by forages, cereals or plant oils in the rumen and then deposited in tissues of ruminants, especially in muscle adipose tissues. In bovine given conventional diets such as fresh forage (Noci et al, 2003, Danneberger et al, 2004) or concentrate / dry forage mixture (Bauchart et al, 2005; Danneberger et al, 2004; Habeanu et al, 2008), total *trans* 18:1 represented a minor class of monounsaturated FA in beef lipids (<3.5% of total FA). In lipid-enriched diets, this level can reach 6% of total FA (Bauchart et al, 2005, Habeanu et al, 2008), but the effects of lipid supplements on the composition of *trans* 18:1 isomers were not yet determined.

Among *trans* 18:1 isomers found in ruminant products, 18:1Δ11*tr* (vaccenic acid) is generally the most abundant (Wolff, 1995; Danneberger et al, 2004) and considered to be innocuous or even protective against cardiovascular disease (CHD) for consumers whereas 18:1Δ9*tr* (elaidic acid) and 18:1Δ10*tr* are rather detrimental for the human health by favouring atherosclerosis and CHD inflammation, diabetes and by altering infant development (Bauchart et al, 2007, Dalainas and Ioannou, 2008).

The objective of the study was to determine the impact of lipid supplements rich in unsaturated FA provided by extruded oleaginous seeds (linseed rich in 18:3n-3 or rapeseed rich in 18:1n-9*cis* and at a lower extent 18:2 n-6 and 18:3n-3) on distribution and amount of the different *trans* 18:1 isomers in FA of total lipids of the *Longissimus thoracis* muscle in Normand cull cows during a 100d finishing period. An original and performant method for the specific analysis of beef trans 18:1 isomers was used in this study. It was based, first, on the selective isolation of total *trans* 18:1 from FA of total lipids by preparative HPLC, second, on the efficient separation and subsequent specific analysis of different *trans* 18:1 isomers by gas-liquid chromatography and mass spectrometry.

MATERIAL AND METHODS

Animals and diets

The experiment was performed at the Experimental Station of the Research Unit on Herbivores of the INRA Centre of Clermont-Ferrand-Theix (France) with 19 Normand cull cows [48-60 months old, mean live weight 638 kg] selected for their live weight, age and body fat score for a 100d finishing period. Animals, randomly assigned to three iso-energetic and iso-nitrogenous rations, were straw (30% diet DM) and concentrate (70%)-based. They were given the basal diet (diet C) or the same diet supplemented with only extruded linseeds (diet L) or with a mixture of extruded rapeseeds (2/3) and linseeds (1/3) (diet RL) (Table 1).

Lipid supplements amounted to 40g / kg diet DM for a mean DM intake of 10.5 kg/d. Animals were fed 90% of the requirements for growing adapted individually for each animal, function of body fat score and live weight. This strategy allowed a complete ingestion of lipid supplements. Animals were slaughtered at a mean live weight of 787 (SD 66) kg for the three diets with a mean body fat score of 3.5 and a mean body weight gain of 1.52 kg/d for the 100d finishing period. Samples (150g) of *Longissimus thoracis (*LT) muscle were collected 1d *post mortem*, cut into small pieces, mixed in N_2 liquid as a fine and homogeneous powder and finally stored at -20°C until *trans* 18:1 analysis.

Table 1: Chemical composition of experimental diets

Diets	Control (C)	Linseed (L)	Rapeseed + Linseed (RL)
Dry matter (%)	87	90.6	89
Total proteins (% diet DM)	14	15.2	15.3
Crude cellulose (% diet DM)	10.0	11.1	11.0
Starch + other sugars (% diet DM)	33	23	22
Total minerals (% diet DM)	5.3	7.2	6.8
Total fat (% diet DM)	2	6.7	6.8
C18:3 (g/kg diet DM)	2.1	27.4	12.1

Beef trans 18:1 analysis

Total beef lipids were extracted by mixing the beef powder with chloroform /methanol 2/1 (Vol/Vol) according to the method of Folch et al (1957). Their fatty acids (FA) were extracted and transmethylated as FA methyl esters (FAME) with sodium methanolate followed with BF3-methanol 14% according the method described by Morrison and Smith (1964). Total FA composition was determined by gas-liquid chromatography (GLC) in a CP Sil 88 glass capillary column (100m x 0.25mm, Varian).

Total *trans* 18:1 were isolated from FAME by preparative reversed-phase high pressure liquid chromatography (HPLC) using a series of two Kromasil KR100-5C18 inverse phase columns (5µm, 250mm x 10mm) with acetonitrile as the eluting solvent (4mL/min) (Juaneda, 2002) and detected at 206 nm. Specific distribution of *trans* 18:1 isomers, converted into dimethyl disulfide (DMDS) adducts, was achieved by GLC-MS (Figure 1) in the Agilent 7890A GC (HP5 MS, 30m x 25mm, carrier gas: He) linked to the mass spectrometer Agilent 5975E (ionizing energy 70eV), owing the structural characterization and quantification of each individual *trans* 18:1 isomer (Figure 2).

RESULTS AND DISCUSSION

GLC analysis of beef FA showed that *trans* 18:1 represented 2.5% of total FA in the control group (Diet C). Lipid supplements increased *trans* 18:1 by

33.7% in cows given the linseed diet (Diet L) and by 105.5% in cows given the mixture linseed/rapeseed diet (Diet LR) (P<0.05).

Total *trans* 18:1 were well separated from other FA (especially from *cis* 18:1 isomers) by preparative HPLC (profile not given), allowing the specific analysis of their isomers by GLC-MS (Figure 1).

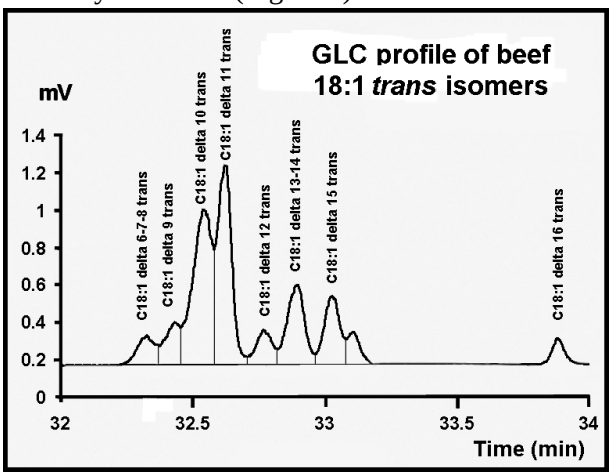

Figure 1. Gas-liquid chromatography analysis of beef *trans* 18:1 isomers previously purified by preparative HPLC

Mass spectrum analysis of each *trans* 18:1 isomer (Figure 2) determined their chemical structure and relative importance in total *trans* 18:1 isomers. It showed that each isomer was eluted as a single peak, excepted for Δ6*tr* associated to Δ7*tr* and Δ8*tr*, for Δ13*tr* associated to Δ14*tr*, and for Δ15*tr* sometimes contaminated by 18:1Δ9*cis*.

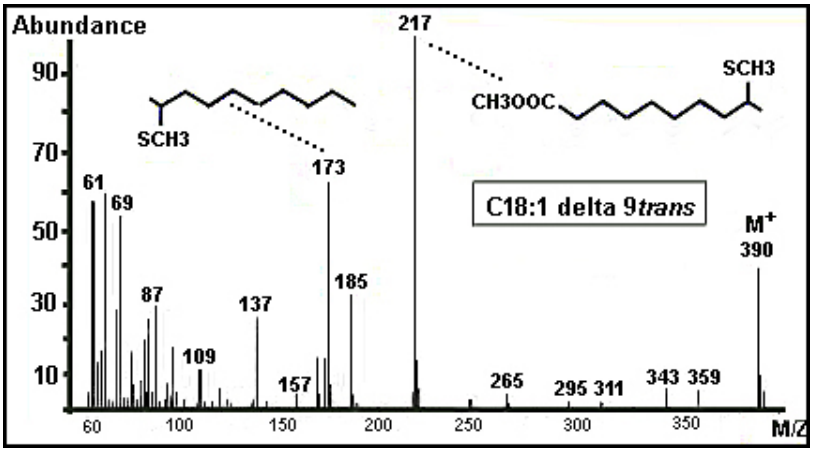

Figure 2. Example of mass spectrum of dimethyl disulfide adduct of methyl Δ9trans 18:1 analyzed by GLC-MS

In diet C (rich in cereals), beef *trans* 18:1 (Table 2) were dominated by the Δ10*tr* and Δ11*tr* forms, the Δ12*tr* up to Δ16*t*r isomers representing each less than 4.5% and the Δ6*tr* up to Δ8*tr* each less than 2.0%, as reported earlier (Wolff, 1995). Diet L, rich in 18:3n-3 (31.6% total FA, 27.4 g/kg diet DM), deeply decreased proportions of Δ9*tr* (-41.2%) and Δ10*tr* (-53.7%) isomers ($P< 0.05$) to the benefit of Δ12*tr* up to Δ16*t*r 18:1 isomers (x2.4, ($P< 0.05$) which represented 43.7% of total *trans* 18:1 isomers (Table 2).

Table 2: Effects of lipid supplements (L= linseed; R = rapeseed) on the distribution of trans 18:1 (in % total trans 18:1, mean ± SD) in beef LT muscle determined by GLC-MS (*, P< 0.05).

Trans 18:1	6*tr*	7*tr*	8*tr*	9*tr*	10*tr*	11*tr*	12*tr*	13*tr*	14*tr*	15*tr*	16*tr*
Diet C	1.3 ± 0.9	0.5 ± 0.1	1.9 ± 0.3	8.5 ± 1.5	33.7 ± 18.6	36.1 ± 14.4	4.3 ± 1.2	3.4 ± 1.0	4.0 ± 1.3	3.4 ± 1.8	2.9 ± 1.9
Diet L	0.6 ± 0.5	0.4 ± 0.1	1.6 ± 0.4	5.0* ± 0.8	15.6* ± 6.7	33.2 ± 11.8	6.1* ± 0.3	8.7* ± 0.8	9.1* ± 0.9	10.9* ± 9.0	8.9* ± 2.7
Diet RL	0.5 ± 0.4	0.6 ± 0.1	2.3 ± 0.6	6.4* ± 0.9	41.1 ± 16.4	25.0* ± 12.4	4.9 ± 9.2	5.8 ± 1.8	5.5 ± 1.6	5.0 ± 1.8	3.1 ± 1.1

Such great modulation of *trans* 18:1 isomer distribution was associated to a global increase of *trans* 18:1 isomers in total fatty acids with L diet when compared to that in the C (control) diet (4.08 vs 2.46%, ($P< 0.05$).
With the diet providing the mixture rapeseed (2/3) and linseed (1/3) (diet RL) lower in 18:3n-3 (18.8% total FA, 12.1g/kg diet DM) but higher in 18:1 Δ9*cis* (28.5% vs 18.0% total FA) when compared to the linseed diet (L diet), beef *trans* 18:1 isomers were significantly deeply lower in Δ11*tr* isoform (-30.7%) to the benefit in Δ10*tr* isoform (+22.0%) ($P< 0.05$). As for the L diet, the great modulation of *trans* 18:1 isomer distribution with RL diet was associated to a global increase of *trans* 18:1 isomers in beef total fatty acids when compared to that in the C (control) diet (5.04 vs 2.46%, ($P< 0.05$).

CONCLUSIONS

This study showed for the first time the important alteration of beef *trans* 18:1 isomers when oleaginous seeds rich in unsaturated FA were added to the basal (C) diet. The results clearly showed differential effects on *trans* 18:1 isomers with a direct impact of the health value of beef when dietary FA were dominated by 18:3 n-3 (diet L) or by 18:1 n-9 associated with lower amounts of 18:2n-6 and 18:3n-3 (diet RL).

With such a basal diet rich in cereals, lipid supplements can diversely modified the health value of beef *trans* 18:1 (on the basis of the Δ9*tr*, Δ10*tr* and Δ11*tr contents)*, with a positive effect when 18:3n-3 was mainly provided (diet

L) or, inversely, with a rather negative effect when 18:3n-3 was associated to 18:1n-9 (diet RL).

The high increase of *trans* isomers in the range of Δ12*tr* up to Δ16*t*r isomers observed in beef from our cows given the linseed supplemented diet (L diet) was never reported earlier whatever the dietary conditions of bovine animals. Additional studies on human or animal models for human are needed to determine the specific health values of Δ12*tr* up to Δ16*t*r 18:1 isomers to validate the strategy of linseed supplementation in finishing bovines for a better health value of beef FA for consumers.

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