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

D. Bauchart1*, Esperanza Bispo Villar1,2, Agnès Thomas1, B. Lyan3, Mihaela Hăbeanu4, D. Gruffat1, D. Durand1

1INRA, Herbivore Research Unit, 63122 St-Genès-Champanelle, France, 2Centro de Investigaciones Agrarias de Mabegondo, 15080 A Coruña, Spain, 3INRA, Human Nutrition Unit, 63122 St-Genès-Champanelle France, 4National Research-Development Institute for Animal Biology and Nutrition, Balotesti, Romania

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-9cis 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 (Δ11tr 18:1) was known to be beneficial for the human health by its protective effect against atherosclerosis whereas Δ9tr 18:1 (elaidic acid) and Δ10tr 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 Δ6tr to Δ16tr) were determined by GLC-MS. In the control diet (C), trans 18:1 were dominated by the Δ10tr (33.7%) and Δ11tr (36.1%) isoforms, the Δ9tr representing only 8.5%. Addition of linseed (diet L) highly decreased the Δ9tr (-41.2%) and Δ10tr (-53.7%) isomers (P< 0.05) to the benefit of only Δ12tr up to Δ16tr 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 Δ11tr (-30.7%) isoforms to the benefit of the Δ10tr isofrom (+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 Δ9tr, Δ10tr and Δ11tr 18:1 contents. Linseed rich in 18:3n-3 had a positive effect on beef health value by decreasing both Δ9tr and Δ10tr 18:1. Inversely, association of linseed with rapeseed rich in 18:1n-9 would altered beef health value by decreasing Δ11tr 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, 18:1\(\Delta 11\)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 18:1\(\Delta 9\)tr (elaidic acid) and 18:1\(\Delta 10\)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-9cis 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 N₂ 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

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

**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).

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 Δ6tr associated to Δ7tr and Δ8tr, for Δ13tr associated to Δ14tr, and for Δ15tr sometimes contaminated by 18:1Δ9cis.
In diet C (rich in cereals), beef trans 18:1 (Table 2) were dominated by the Δ10tr and Δ11tr forms, the Δ12tr up to Δ16tr each less than 4.5% and the Δ6tr to Δ8tr 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 Δ9tr (-41.2%) and Δ10tr (-53.7%) isomers (P<0.05) to the benefit of Δ12tr up to Δ16tr 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).

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

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 Δ9cis (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 Δ11tr isoform (-30.7%) to the benefit in Δ10tr 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 Δ9tr, Δ10tr and Δ11tr 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 Δ12tr up to Δ16tr 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 Δ12tr up to Δ16tr 18:1 isomers to validate the strategy of linseed supplementation in finishing bovines for a better health value of beef FA for consumers.

REFERENCES

Bauchart D., Gladine C., Gruffat D., Leloutre L., Durand D. 2005. Effects of diets supplemented with oil seeds and vitamin E on specific fatty acids of rectus abdominis muscle in charolais fattening bulls. 55th Annual Meeting of the EEAP, Bled (Slovenia), In "Indicators of milk and beef quality" EAAP Publication No 112 (editors JF Hocquette and S Gigli), pp. 431-436.


