Lipids and fatty acid composition of *Longissimus thoracis* and *Semitendinosus* muscles in finishing Normand cows

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SUMMARY

The study investigated the influence of dietary lipid (from linseed and rapeseed providing n-3 polyunsaturated fatty acids. PUFA) and of antioxidant (vitamin E plus plant extracts rich in polyphenols) supplements on lipid and FA characteristics of *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles in 25 Normand (fat breed) cull cows for a 100d finishing period. Animals were randomly assigned to 5 isoenergetic and isonitrogenous diets consisting in a concentrate/straw based diet (70/30) (C group) or the same basal diet supplemented with extruded linseed (L) alone (40g lipid/kg DM diet) (L group) or with antioxidants (vit E -250 UI /Kg + extract of plants rich in polyphénols - 1%. LEP group) or with a mixture of extruded rapeseed (66) and linseed (33) (RL) (40g lipid/kg DM diet) alone (RL group) or with antioxidants (RLEP group). Beef samples collected 24 h post-mortem were homogenized in N_2 liquid. Classes of their lipid were separated and analyzed by HPLC and the detailed FA composition of total lipids was analysed by GLC. Lipids of LT were 36% higher than that of ST ($P<0.002$) mainly due to the higher content of triglycerides. Dietary L and RL lipids did not modify beef lipid content but significantly increased in both tissues proportions of 18:3n-3, 18:1 Δ11*trans* and CLA beneficial for human health. The addition of antioxidants reinforced their positive impact on the nutritional value of beef lipids.

Keywords: cull cows, extruded linseed and rapeseed, antioxidants, fatty acids, lipid classes

INTRODUCTION

Lipids deeply control the nutritional/health value and the sensorial qualities of meats, especially in ruminant animals (Geay et al. 2001. Chilliard 1999). Nutritional properties of fat are influenced especially by their fatty acid (FA) component. Factors linked to animals (age. sex. breed) and their feeding conditions [characteristics of ingredients and their proportions in diets. FA composition and physical form of lipid supplements] can more particularly

modulate in the fattening period characteristics of beef lipids such as i) their quantity in muscles and associated adipose tissues. ii) the distribution of their major (triglycerides and. at a lower extend. phospholipids) and minor classes (free and cholesteryl ester, diglycerides and free FA). iii) their FA composition. Contrary to other fatty acids, linoleic (18:2 n-6) and alpha-linolenic (18:3n-3) acids cannot be synthesized de novo by mammalian cells; therefore they are termed ‘‘essential’’ and must be obtained in adequate amounts from diet (El-Brady. 2007). Thus, the impact of dietary n-3 PUFA sources (grass. oleaginous seeds, fish oil) given in the finishing period to improve the nutritional value of beef FA has been extensively described in young (<2 years-old) bovines (bulls. heifers. steers) of which muscles were relatively low in lipids (Bauchart et al. 2005; Scollan et al. 2005). On the other hand, little is known on the effect of dietary n-3 PUFA rich lipids on beef FA in oldest and fat bovine animals. The aim of this study was to analyze the impact, on beef lipids and FA composition, of linseed given alone or with rapeseed (rich in n-3 and n-6 PUFA) in association or not with antioxidants (vit. E + extracts of plants rich in polyphenols) given to cull Normand (fat breed) cows during the finishing period.

MATERIAL AND METHODS

Animals and diets.

The experiment was performed with 25 Normand cull cows [48-60 months old, mean live weight 642 kg] selected for their live weight. age and body fat score (BFS) for a 100d finishing period. Animals were assigned at random (n=5 for each diet) to five isoenergetic and isonitrogenous rations (UFV= 0.92, PDIN = 105, PDIE = 100) for a 100 d feeding study (table 1). All rations were straw (30%) and concentrate (70%) -based. Animals were given the basal diet without any supplements (diet C) or with extruded linseed alone (diet L) or with vitamin E (250 mg /kg diet) and a mixture of plant antioxidant extracts rich in polyphenols (P. 10g/kg diet, from rosemary, grape, citrus and marigold) (diet LEP) or with a mixture of extruded rapeseed (2/3) and linseed (1/3) alone (diet RL) or with vitamin E and PE (diet RLEP). Lipid supplements amounted to 40g lipid/kg diet DM for a mean DM intake of 10.5 kg/d. In order to equilibrate the diets and for a good digestibility of concentrate fibres was add to diet as molassed straws (300 g/kg). This formula simplifies manipulations and quantity ingested by animals. During a 100 day period (usually used for beef finish period) diet consumption was calculated to obtain daily weight gain of 1150g and to increase the body fat score (BFS) by two points. Animals were fed 90% of their requirements for growing adapted individually for each animal function of BFS and live weight. This strategy allowed a complete ingestion of lipid supplements.

Table 1. Compounds feed formulation

*Composition of concentrate	Concentrate				
% raw	C	L	LEP	RL	RLEP
Wheat	17	13.6	13.2	14.1	14
Corn	21.4				
Dehydrated corn	12.8				
Beet pulp	25.05	25	25	25	24.6
Sunflower cake		3.4	3.4	2	2
Rapeseed cake		4.4	4.2	6.1	5.9
Soybean cake	14.1				
Wheat bran		15	15	15	15
Cereals bran		10	10	10	10
Alfalfa	7.6				
Extruded linseed		25	24.95		
Extruded linseed (33%) + rapeseed (66%)				24	24
Limestone		1.55		1.75	0.25
Bicarbonate Na	1.3	1.3	1.3	1.3	1.3
Vitamins and oligoelement mixture	0.75	0.75	0.75	0.75	0.75
Vitamin E 2%			1.2		1.2
Plant extracts rich in polyphenols (P)			1.0		1.0
TOTAL	100	100	100	100	100
Chemical characteristics					
DM %	88	89	89	89	89
Total nitrogen Protein	14	15.2	15.2	15.3	15.3
Crude cellulose (%diet DM)	10.5	10.5	10.5	10.8	10.7
Starch (%diet DM)	33	23	22	22	22
UFV	0.92	0.92	0.92	0.92	0.92
PDIN g	100	105	105	105	105
PDIE g	106	100	100	99	99
Ca (g)	8	13	9	14	10
P (g)	2	3.5	3.6	3.7	3.7
Fat (% diet DM))	2	7	7	7	7
C18:3 g/kg diet DM)	0.7	27.4	27.4	12.1	12.1
Vit E UI / kg	15	15	250	15	250
AOV %			1		1
quantity delivered- kg crude	10.2	10.2	10.2	10.2	10.2
quantity delivered - kg DM	9	9	9	9	9

**Straws delivered separated supplied 85.9% DM; UFL-0.50. UFV- 0.50 and PDIN 24 g.PDIE 50 g and PDIA 10 g*

Animals were slaughtered at a mean live weight of 803 (SD 49) kg with a BFS of 3.53 on a scale varying from 1 (very lean) to 5 (very fat). Mean individual body weight gain amounted to 1.56 kg/d for the 100d finishing

period with no marked variations between diets. Samples (100g) of *Longissimus thoracic (*LT) and of *Semitendinosus* (ST) muscles were collected 1 day *post mortem,* cut into small pieces and frozen in N_2 liquid. Finally, they were mixed in N_2 liquid to produce a homogenous and fine powder stored at -20°C until lipid and FA analysis.

Lipid and fatty acid analysis

Meat dry matter was determined gravimetrically after drying at 80°C for 48h. Total lipids of LT and ST muscles were extracted by mixing 6g of meat powder with chloroform /methanol 2/1 according to the method of Folch et al (1957) and determined gravimetrically. Their different lipid classes were separated by high-performance liquid chromatographic (HPLC) (Kontron. Switzerland) on silica 5µm column (250 mm long. internal diameter 4.6 mm) with a ternary solvent gradient [isooctane/tetrahydrofurane (99/1), acetone/dichloromethane (2/1) and 2-propanol/water + acetic acid + ethanolamine (85/15) and quantified by evaporative light-scattering detection (Sedere. France) as described by Reynolds et al (1998). Fatty acids were extracted from total lipids and transmethylated first with Na methanolate and second with BF3-methanol (Morrison et al 1964). Their detailed composition was determined by gas liquid chromatography (GLC) analysis (Perichrom. France) using the CP Sil 88 glass capillary column, H_2 as the gas vector. Response coefficient of each individual FA was calculated by using the quantitative mix C4-C24 FAME (Supelco. USA).

Statistical analysis

Results were expressed as mean values.± SEM. The effects of dietary treatments and the muscle on FA composition have been analysed by ANOVA linear model (SAS 9X) and differences between treatments by the Student's test. Effects were considered significant at $P<0.005$; $P< 0.001$ or $P< 0.0001$ and a tendency at $P<0.10$.

RESULTS AND DISCUSSION

Lipid classes in LT and ST muscles

Dry matter and lipids of LT muscle were 6 and 31% higher than that of ST muscle respectively whatever the diet given to animals (Table 2). Beef total lipids were mainly constituted by triglycerides and secondly by phospholipids. They mainly varied with that of triglycerides (Figures 1 and 2) while phospholipids remained stable. Triglycerides represented 72 and 68% and phospholipids 16% and 19.5% in total lipids of LT and ST muscles respectively.

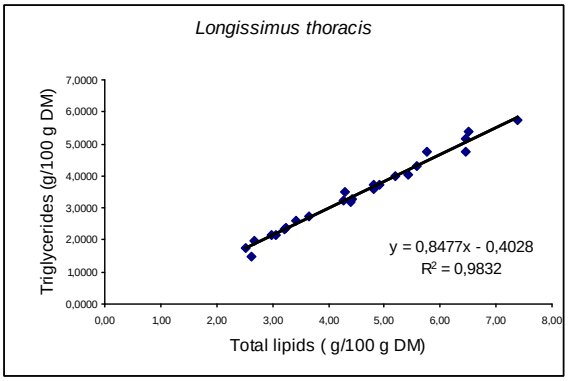

Figure 1. Relation between triglycerides and total lipids in LT muscle

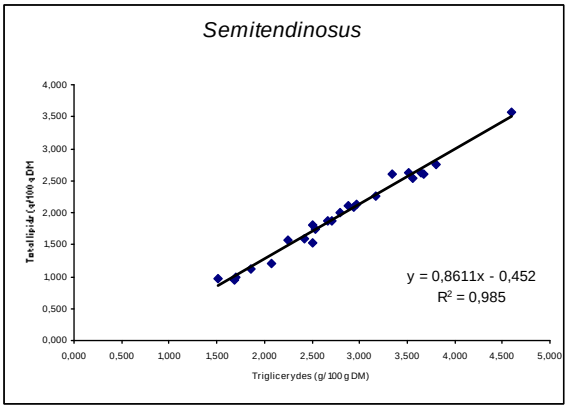

Figure 2. Relation between triglycerides and total lipids in ST muscle

Table 2. Effects of lipid and antioxidant supplements on the major classes of lipids in *Longissimus thoracic* (LT) and *Semitendinosus* (ST) muscles of cull cows.

Diets*	*Longissimus thoracis* muscle				
	C	L	LEP	RL	RLEP
Dry matter	27.27 ± 1.53	26.67 ± 0.71	27.79 ± 0.26	26.54 ± 1.11	26.39 ± 0.63
Total lipids	4.98 ± 2.04	4.09 ± 0.94	5.13 ± 0.64	3.89 ± 1.28	3.42 ± 0.64
Triglycerides	4.04 ± 1.80	3.03 ± 0.79	3.85 ± 0.60	2.87 ± 1.02	2.60 ± 0.60
Phospholipids	0.60 ± 0.15	0.67 ± 0.07	0.75 ± 0.05	0.58 ± 0.09	0.48 ± 0.03
Diets*	*Semitendinosus* muscle				
	C	L	LEP	RL	RLEP
Dry matter	25.57 ± 1.10	25.45 ± 0.41	25.73 ± 0.51	24.88 ± 0.23	25.08 ± 1.15
Total lipids	2.95 ± 1.10	3.23 ± 0.38	2.96 ± 0.62	2.97 ± 0.12	2.62 ± 1.32
Triglycerides	2.12 ± 0.98	2.33 ± 0.26	2.0 ± 0.55	2.12 ± 0.08	1.75 ± 1.12
Phospholipids	0.61 ± 0.09	0.60 ± 0.10	0.60 ± 0.03	0.52 ± 0.02	0.59 ± 0.08

Minor lipids, constituted by free cholesterol, cholesteryl esters, diglycerides and free fatty acids (Table 3) represented only 9.8 and 8.9% of total lipids in LT and ST muscles respectively.

Dietary unsaturated lipid supplements from extruded linseed alone or with rapeseed did not modify significantly total lipids and their major classes on LT or ST muscles. On the other hand, free fatty acids and diglycerides tend to be higher in the lipid supplemented groups in the two muscles due to a possible lipolysis of triglycerides (Table 3).

Table 3 Effects of lipid and antioxidant supplements on the minor classes of lipids in *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of cull cows

Diets*	*Longissimus thoracis* muscle				
	C	L	LEP	RL	RLEP
Cholesteryl esters	0.06 ± 0.03	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Free cholesterol	0.07 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Diglycerides	0.07 ± 0.03	0.07 ± 0.01	0.10 ± 0.01	0.10 ± 0.03	0.07 ± 0.00
Free fatty acids	0.13^{a} ± 0.10	0.22^{ab} ± 0.06	0.31^{b} ± 0.10	0.24^{ab} ± 0.12	0.19^{ab} ± 0.05
	Semitendinosus muscle				
	C	L	LEP	RL	RLEP
Cholesteryl esters	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
Free cholesterol	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.02
Diglycerides	0.04^{a} ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
Free fatty acids	0.10^{a} ± 0.06	0.16^{ab} ± 0.05	0.22^{b} ± 0.07	0.18^{ab} ± 0.01	0.15^{ab} ± 0.06

*$^{a.b.c}$, P≤0.10;*For details, see the materials and methods section*

Beef fatty acid composition

ANOVA (linear model) test allowed us to determine the significance of the diet effect on muscle characteristics as well the interaction diet x muscle. For all individual FA, no interaction *muscle x diet* was noted but only differences due to the diet effect on muscle (P<0.0001) (figure 2).

For LT and ST muscles dietary unsaturated lipid supplements from linseed and the mixture rapeseed + linseed increased significantly proportions of 18:3 n-3 (LT: +38 and +89%; ST: +23% and +50% respectively) of 18:1 Δ11*trans* (LT: x2.0 and x3.0; ST: x2.0 and x2.2) and of 9*cis*11*trans* 18:2 (CLA) (LT :+32% and +43%; ST : +58% and +42%) (Tables 4 and 5) which are known to be beneficial for the human health. In a unexpected way, the addition of extruded rapeseed with extruded linseed in the basal diet increased more efficiently beef content in fatty acids desirable for human health than the addition of linseed alone. Addition of antioxidants (vitamin E + PE) in lipid supplemented diets was initially proposed for limiting peroxidation in the blood and tissues during the life of animals and therefore the risk of production of free radicals affecting the health of animals and their growth performances. The results showed that the mixture of antioxidants reinforced also the stimulating effect of lipid supplements on proportions of the three considered FA in lipids of LT (Table 4) and ST (Table 5) muscles.

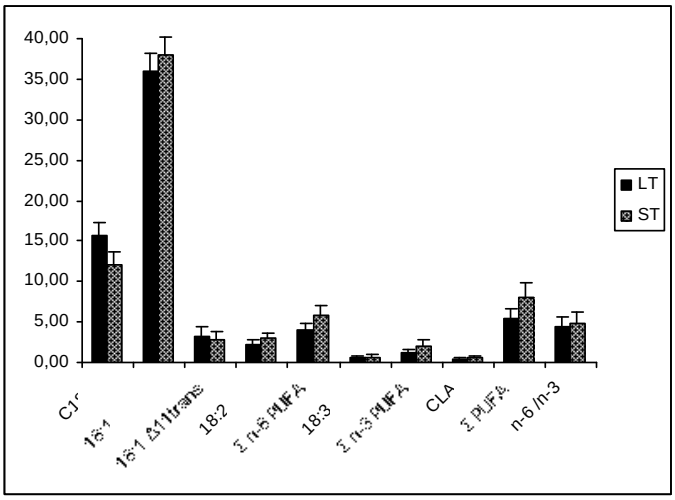

Figure 3 Most important effects in the ST muscle than LT muscle (*P<000.1).

These effects of antioxidants were already reported in young Charolais bulls given similar linseed and vit. E supplements in the finishing period (Bauchart et al. 2005) suggesting a modulation of the microbial lipid metabolism by antioxidants in the rumen. Similar significant stimulating effects concerned total *trans* MUFA and n-3 PUFA leading to a decrease of the ratio n-6 PUFA/n-3 PUFA, especially when antioxidants were added to the diets.

Table 4 Effects of lipid and antioxidant supplements on centesimal fatty acid composition of total lipids in *Longissimus thoracic* (LT) and *Semitendinosus* (ST) muscles of cull cows.

Diets*	*Longissimus thoracic* muscle				
	C	L	LEP	RL	RLEP
16:0	27.0	27.0	26.0	25.8	25.9
18:0	15.2	15.5	16.1	16.4	15.5
18:1 Δ9*cis*	38.8^{a}	36.3^{b}	35.2^{bc}	34.1^{c}	34.9^{c}
18:1 Δ11*trans*	1.36^{a}	2.71^{b}	3.11^{b}	4.07^{c}	3.90^{c}
18:2 n-6	2.35	2.13	2.29	2.29	2.66
18:3 n-3	0.37^{a}	0.51^{b}	0.69^{b}	0.70^{b}	0.67^{b}
9*cis*.11*trans* 18:2 (CLA)	0.28^{a}	0.37^{b}	0.48^{c}	0.40^{b}	0.41^{b}
Σ SFA	47.3	47.9	47.9	47.8	46.8
Σ *cis* MUFA	45.4^{a}	41.5^{b}	41.1^{b}	40.6^{b}	41.5^{b}
Σ *trans* MUFA	2.05^{a}	3.77^{b}	4.07^{b}	5.41^{c}	5.01^{c}
Σ n-6 PUFA	3.82	3.83	3.94	4.16	4.58
Σ n-3 PUFA	0.86^{a}	1.08^{ab}	1.43^{b}	1.33^{b}	1.41^{b}
Σ PUFA	4.83	5.17	5.71	5.66	6.17
PUFA/SFA	0.10^{a}	0.11^{a}	0.12^{a}	0.12^{a}	0.13^{b}
n-6 /n-3	4.58^{a}	3.78^{b}	2.96^{c}	3.27^{bc}	3.22^{bc}

$^{a.b.c}$ *P*<0.05;**For details see the materials and methods section*

These results confirmed previous observations in LT and RA muscles of bulls given similar supplements showing a paralleled increase in 9*cis*11*trans* 18:2 (CLA) and in its precursor the 18:1 Δ11*trans* (Bauchart et al. 2005). Combination of linseed and rapeseed led to a similar impact on deposition of n-3 PUFA and their *trans* derivates. On the other hand, such increases observed in our fat and relatively old bovines were lower in intensity than that observed in the young males, suggesting a decreased reactivity of muscles and its connected fat tissues towards n-3 PUFA-rich lipid supplements (Bauchart et al 2005). Thus a more important level of fatty acids should be justify in the diets for expecting an effect higher on tissues of cows during the finishing period.

Table 5 Effects of lipid and antioxidant supplements on centesimal fatty acid composition of total lipids in *Semitendinosus* (ST) muscles of cull cows.

Diets*	*Semitendinosus* muscle				
	C	L	LEP	RL	RLEP
16:0	24.8	24.9	23.9	24.7	23.9
18:0	12.3	11.4	13.1	11.7	12.0
18:1 Δ9*cis*	40.3^{a}	38.6^{ab}	36.6^{b}	37.4^{b}	36.9^{b}
18:1 Δ11*trans*	1.38^{a}	2.53^{b}	2.85^{c}	3.01^{c}	3.60^{c}
18:2 n-6	3.23^{a}	2.37^{b}	3.15^{a}	2.92^{a}	3.41^{a}
18:3 n-3	0.46^{a}	0.57^{ab}	0.93^{c}	0.69^{b}	0.78^{c}
9*cis*. 11*trans* 18:2 (CLA)	0.38^{a}	0.60^{b}	0.69^{b}	0.54^{b}	0.58^{b}
Σ SFA	41.3	42.2	41.4	42.7	40.9
Σ *cis* MUFA	48.5^{a}	46.7^{ab}	44.9^{b}	44.8^{b}	44.9^{b}
Σ *trans* MUFA	2.12^{a}	3.53^{b}	3.97^{b}	4.06^{b}	4.56^{c}
Σ n-6 PUFA	5.89^{a}	4.98^{a}	6.05^{ab}	5.64^{a}	6.41^{b}
Σ n-3 PUFA	1.53^{a}	1.58^{a}	2.56^{b}	1.90^{ab}	2.22^{b}
Σ PUFA	7.62	6.97	8.99	7.78	8.92
PUFA/SFA	0.19	0.17	0.22	0.18	0.22
n-6 /n-3	3.96^{a}	3.33^{ab}	2.59^{b}	3.14^{b}	2.94^{b}

$^{a.b.c}$ *P*<0.05;**For details see the materials and methods section*

CONCLUSIONS

Incorporation of n-3 PUFA in diets did not increase fat deposition in muscles and improved the nutritional quality of beef FA both with the combination of linseed and rapeseed or with linseed alone. However, in intensity, the beneficial effects on FA composition were less important than that reported in the young bovines suggested a low reactivity of muscle tissues in older and fat bovines. Incorporation of antioxidants, initially proposed to limit blood and muscle lipoperoxidation, reinforced the beneficial impact of dietary n-3 PUFA on the health value of FA muscle and therefore should be associated to lipid-rich diets.

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