Lipid and fatty acid composition of *Longissimus thoracis* and *Semitendinosus* muscles in finishing Normand cows given unsaturated lipids and antioxidants

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Abstract

The study investigated the influence of lipid (rich n-3 polyunsaturated fatty acids, FA) and of antioxidant (vitamin E plus plant extracts rich in polyphenols) supplements on lipid and FA characteristics of *Longissimus thoracis* and *Semitendinosus* 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 (LEP group) or with a mixture of extruded rapeseed and linseed (RL, 66/33) (40g lipid/kg DM diet) alone (RL group) or with antioxidants (RLEP group). Beef samples, collected 24 h post-mortem, were homogenized into N_2 liquid. Their lipids were analyzed by HPLC and their detailed FA composition by GLC. Lipids of LT were 36% higher than that of ST ($P<0.002$), mainly due to higher content of triglycerides. Dietary L and RL lipids did not modify beef lipid content but they significantly increased, in both tissues, proportions of 18:3n-3, 18:1 Δ11*tr* and CLA beneficial for human health. The addition of antioxidants reinforced their positive impact on the nutritional value of beef lipids.

Introduction

Lipids deeply control the nutritional/health value and the sensorial qualities of meats, especially in ruminant animals (Geay et al. 2001). Factors linked to animals (age, sex, breed) and their feeding conditions [characteristics of ingredients and their proportions in diets, fatty acid (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 esterified cholesterol. diglycerides and free FA), iii) their FA composition. 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, of linseed given alone or with rapeseed (rich in n-3 PUFA) in association or not with antioxidants given to cull Normand (fat breed) cows during the finishing period.

Materials 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 to five isoenergetic and isonitrogenous rations (n=5 for each diet) for a 100 d feeding study. 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 (155 IU/kg diet DM) and a mixture of plant extracts (P, 0.7 g/kg diet DM) from rosemary, grape, citrus and marigold rich in antioxidants (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. 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 thoracis (*LT) and of *Semitendinosus* (ST) muscles were collected 1d *post mortem,* cutted into small pieces and frozen in N_2 liquid. Finally, they were mixed in N_2 liquid to produce a homogenous and fine powder. Meat powders were stored at -20°C until lipid and FA analysis.

Lipid and fatty acid analysis. Total lipids of LT and ST muscle tissues were extracted by mixing 6g of meat powder with chloroform /methanol 2/1 (V/V) and determined gravimetrically. Their different lipid classes were separated by HPLC (Kontron, Switzerland) on silica 5µm column (150mm long, i.d. 4.6mm) with a ternary solvent gradient and quantified by evaporative light-scattering detection (Sedere, France) as

described by Reynolds et al (1998). Fatty acids (FA) were extracted from total lipids and transmethylated with BF3-methanol. Their detailed composition was determined by GLC analysis (Perichrom. France) using the CP Sil 88 glass capillary column. Response coefficient of each individual FA was calculated by using the quantitative mix C4-C24 FAME (Supelco, USA). Results were expressed as mean values. The effects of dietary treatments on lipids and FA have been analysed by the Student's test.

Results and discussion

Dry matter and lipids contents were 6 and 31% higher in LT muscle than ST muscle respectively (Table 1). Total lipid contents varied with that of triglycerides while phospholipids remained stable. Mean contents in minor lipids (constituted by free cholesterol, cholesteryl esters, diglycerides and free fatty acids) represented only 9.8 and 8.9% of total lipids in LT and ST muscles respectively (Table 1). The dietary unsaturated lipid supplements 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 for the two muscles due to a possible lipolysis of triglycerides.

Table 1. Effects of lipid and antioxidant supplements on the major and minor classes of lipids in *longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of cull cows

Diets*	*Longissimus thoracis* muscle				
	C	**L**	**LEP**	**RL**	**RLEP**
Dry matter	27.27	26.67	27.79	26.54	26.39
Total lipids	4.98	4.09	5.13	3.89	3.42
Triglycerides	4.04	3.03	3.85	2.87	2.60
Phospholipids	0.60	0.67	0.75	0.58	0.48
Cholesteryl esters	0.06	0.04	0.06	0.04	0.04
Free cholesterol	0.07	0.05	0.06	0.05	0.04
Diglycerides	0.07	0.07	0.10	0.10	0.07
Free fatty acids	0.13^{a}	0.22^{ab}	0.31^{b}	0.24^{ab}	0.19^{ab}

Diets*	*Semitendinosus* muscle				
	C	**L**	**LEP**	**RL**	**RLEP**
Dry matter	25.57	25.45	25.73	24.88	25.08
Total lipids	2.95	3.23	2.96	2.97	2.62
Triglycerides	2.12	2.33	2.0	2.12	1.75
Phospholipids	0.61	0.60	0.60	0.52	0.59
Cholesteryl esters	0.54	0.04	0.03	0.03	0.03
Free cholesterol	0.05	0.05	0.04	0.05	0.04
Diglycerides	0.04^{a}	0.06	0.06	0.06	0.06
Free fatty acids	0.10^{a}	0.16^{ab}	0.22^{b}	0.18^{ab}	0.15^{ab}

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

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%) (Table 2) which are known to be beneficial for the human health.

Addition of antioxidants (vitamin E + PE) in lipid supplemented diets reinforced the stimulating effect of lipid supplements on proportions of the three considered FA in lipids of the two muscles (Table 2). 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. 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.

Table 2. Effects of lipid and antioxidant supplements on centesimal fatty acid composition of total lipids in *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of cull cows

Diets*	*Longissimus thoracis* 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}

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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