A milk diet containing plant shortening lipids deeply altered *trans* 18:1 isomers of muscle and liver lipids in the preruminant calf: a possible negative impact on the image of veal products for consumers

E.B. Villar[1, 2], G. Gandemer[3, 4], D. Durand[1] & D. Bauchart[1]

[1]INRA, Herbivore Research Unit, Nutrients and Metabolisms Group, 63122 St-Genès-Champanelle, France, E-mail: bauchart@clermont.inra.fr.

[2]Centro de Investigacións Agrarias de Mabegondo, 15080 A Coruña, Spain, E-mail: espebazarra@yahoo.es.

[3]Centre d'Information des Viandes, 75009 Paris, France.

[4]INRA, Centre de Recherche de Lille, 2 Chaussée Brunehaut, 80203 Péronne, France, E-mail : Gilles.Gandemer@mons.inra.fr.

Abstract

In the preruminant calf, a functional monogastric given only a milk replacer, dietary fatty acids (FA) can influence directly meat FA composition. From the BSE crisis, lipids from oleaginous plants are preferentially incorporated into the milk diet, but their polyunsaturated FA content has been limited to 5% of total FA Thus, partially hydrogenated oils (shortenings) were proposed for calf milk feeding, but they contained *trans* 18:1 of which some isomers (Δ9, Δ10) have harmful effects on human's health. This study described the detailed composition of *cis* and *trans* 18:1 isomers of muscle and liver tissues in the calf given commercial shortenings. *Trans* and *cis* FA were isolated by preparative HPLC and their 18:1 isomers were analysed by GLC. Compared to that in ruminant meats, calf muscle and liver tissues were twice higher in *trans* 18:1, dominated by Δ9 and Δ10 *trans* isomers which deeply altered their health value for humans.

Introduction

Meats from veal calves are frequently consumed in Europe, especially in France and in Italy. Veal calves are given only a milk replacer from birth to slaughter at the age of 6 months. Due to its liquid feeding, calves are keeping the oesophageal groove reflex which hinders ruminal fermentation. Thus, theses animals are known as preruminants of which the digestive system functions were similar to that of monogastric animals. Due to BSE crisis and the resulting constraints for use of animal fats in animal feeding, lipids from oleaginous oils were preferentially incorporated into the milk replacer given to young calves.

The incorporation of soybean oil rich in n-6 polyunsaturated FA (PUFA) into the milk diet as the sole source of lipids for the veal calves led to lipid (as triglycerides) infiltration of the liver, leading to adverse effects on animal health (Leplaix-Charlat et al, 1996). Consequently, the excess of PUFA of plant (oleaginous) lipids justified to hydrogenate unsaturated FA, in the same way than for fats used in bread and cake industries. However, this catalytic process of partial PUFA hydrogenation generated *trans* 18:1 of which some isomers (Δ9*trans* and Δ10*trans*) were known for having harmful effects on human health by favouring cardiovascular diseases (Ascherio et al., 1999). On the other hand, vaccenic acid (18:1 Δ11*trans*), another *trans* 18:1 isomer produced by microbial biohydrogenation of PUFA in the rumen which is abundant in the ruminant products (meat, milk) would play a positive effect on human health (Belury, 2002). Indeed, when associated to its derivate, the 9*cis*,11*trans* 18:2 (CLA), 18:1 Δ11*trans* was shown to have a hypocholesterolemic effect in the rabbit, an animal model for humans (Bauchart et al, 2007). The aim of the study was to describe the selective distribution of *trans* and *cis* 18:1 isomers of total lipids in muscle and liver tissues of the preruminant calf given a shortening rich milk diet and to compare the distribution of these FA to that in an adult bovine given a conventional corn silage and concentrate diet.

Materials and methods

Animals and diets. One preruminant Friesian Holstein calf was given, from birth to slaughter at 6 months of age, a milk replacer contained, on the basis of 1000g dry matter, 650g skimmed milk powder, 150g vegetal proteins (from cereals) and 200g fat composed of 80g of lard, 40g of coconut oil and 80g of a mixture of partially hydrogenated palm (2/3) and soybean (1/3) oils. One 5 year-old cull Friesian Holstein cow was given, on the basis of 1000g dry matter intake, 650g conventional corn silage and 350g concentrate. One hundred grams of liver (from calf)and of *Longissimus thoracis* (LT) (from calf and cow) muscle tissues were collected 1d 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 and stored at -20°C until FA analysis.

Trans fatty acid analysis. Total lipids of liver and LT muscle tissues were extracted by mixing 6g of meat powder with chloroform and methanol 2/1 (V/V). Their fatty acids (FA) were extracted and transmethylated with sodium methanolate and BF3-methanol at 20ºC. Total *cis* and *trans* 18:1 were isolated as FA methyl esters (FAME) by preparative reversed-phase HPLC using a series of two Kromasil-C18 columns (5µm, 250mm length, i.d. 10mm; Thermo Electron, France) equipped with a precolumn Modulo-cart QS (10mm) as described by Juanéda (2002). FAME were analysed with an UV detector and *cis* and *trans* FAME were selectively recovered with a fraction collector. Specific distribution of different *cis* and *trans* 18:1 isomers were achieved by GLC using a chromatograph PERI 2100 (Périchrom, France) fitted with a CP Sil 88 column (100m length; i.d. 0.25mm, Varian USA). Milk replacer and its components were analysed using the same methods than those described earlier.

Results and discussion

Complete separation of *cis* and *trans* 18:1 isomers by HPLC analysis from total muscle or liver FAME (solubilized in acetone) was obtained in an isocratic system by using acetonitrile as the carrier solvent (Figure 1). As validated by external standards for *cis* (methyl *cis*-9-octadecenoate) and *trans* isomers (methyl *trans*-11-octadecenoate), one peak corresponded to *cis* 18:1 isomers (including trace of 16:0 in tissue FAME) and another peak to *trans* 18:1 isomers (including 16:0 in tissue FAME) (Figure 1).

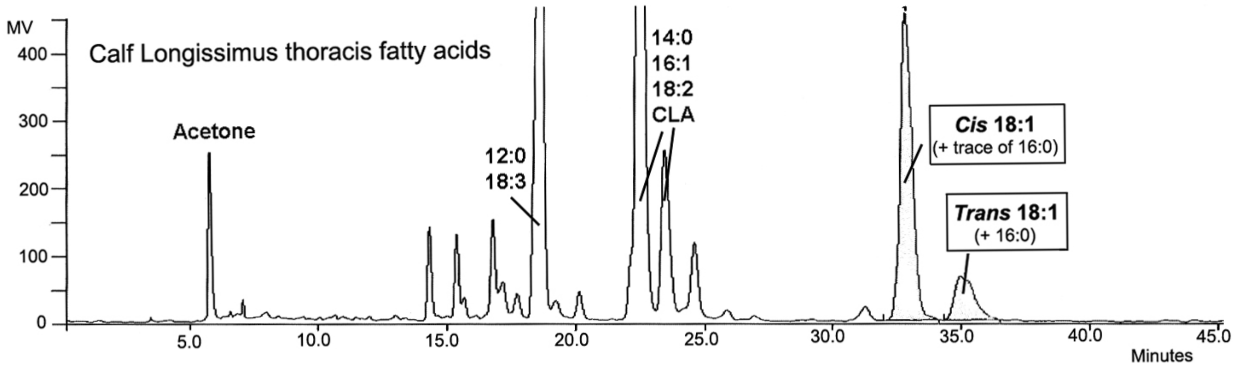


Figure 1. Separation of *cis* and *trans* 18:1 isomers of calf LT muscle FAME by HPLC analysis.

Trans 18:1 isomers represented 19.6 and 25.2% of total 18:1 isomers in calf LT muscle and liver tissues whereas they represented only 12.4% in cow LT muscle. Total *cis* and *trans* 18:1 were then collected and centesimal proportions of their isomers were separately determined by GLC analysis (Tables 1A and 1B).

Table 1. Distribution of *cis* (1A) and *trans* (1B) 18:1 isomers [% total *cis* (1A) or *trans* (1B) 18:1] determined by GLC in milk ingredients and total milk powder of calf and in calf and cow tissues

	Calf milk ingredients			Calf		Cow
1A : % *cis*	**Palm oil +soybean oil**	**Lard**	**Total milk**	**Liver**	**LT muscle**	**LT muscle**
Δ6-7-8	0.8	traces	traces	0.6	0.6	0.2
Δ9 (+10)	94.4	91.4	93.6	81.1	82.2	95.4
Δ11	1.9	7.9	5.4	12.6	7.4	3.7
Δ12	1.5	0.2	0.7	2.4	1.4	0.3
Δ13	1.0	0.4	0.3	1.1	0.9	0.4
Δ14	0.4	traces	traces	1.2	0.8	traces
Δ15	traces	traces	traces	0.6	0.4	traces
Δ16	traces	traces	traces	0.5	0.2	traces
	Calf milk ingredients			**Calf**		**Cow**
1B : % *trans*	**Palm oil +soybean oil**	**Lard**	**Total milk**	**Liver**	**LT muscle**	**LT muscle**
Δ6-7-8	8.6	ND	11.1	1.8	6.5	traces
Δ9	15.1	ND	17.0	7.5	13.8	0.7
Δ10	22.1	ND	20.2	17.2	32.6	6.0
Δ11	15.6	ND	16.0	24.3	18.6	52.0
Δ12	9.9	ND	8.9	29.0	11.6	6.5
Δ13	11.2	ND	11.8	15.7	11.4	15.1
Δ14	13.0	ND	13.1			
Δ15	1.6	ND	1.7	3.5	5.5	15.3
Δ16	2.7	ND	traces	0.8	traces	3.4

Trans vaccenic acid (Δ11*trans*18:1) was the most abundant *trans* 18:1 isomer in cow LT muscle (Table 1B, Figure 2A), which is commonly observed in beef of adult bovine, whereas Δ10*trans* 18:1 was the highest *trans* 18:1 isomer in the LT muscle of calf (Table 1B, Figure 2B) given the shortening-rich milk replacer (Table 1B). Compared to that in beef of adult bovine (cow), milk replacer and calf liver and LT muscle samples were far higher in Δ9trans (17.0, 7.5 and 13.8% vs. 0.7%), Δ10 trans (20.2, 17.2, 32.6% vs 6.0) and Δ6-8trans 18:1 (11.1%, 1.8% and 6.5% vs. 0%) (Table 1B).

Figure 2A : Trans isomers of 18:1 in *Longissimus thoracis* muscle of adult bovine

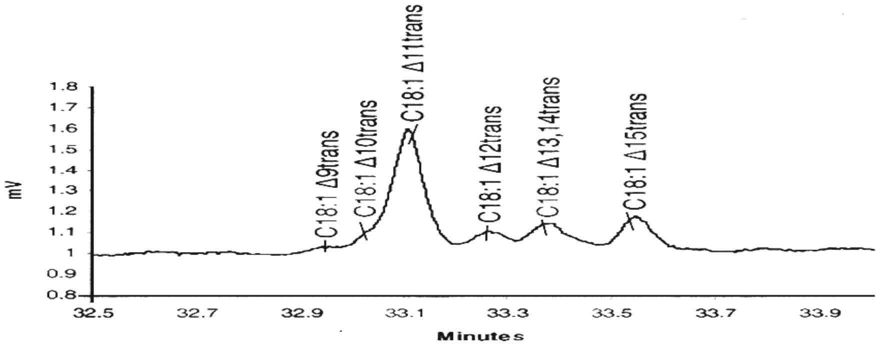
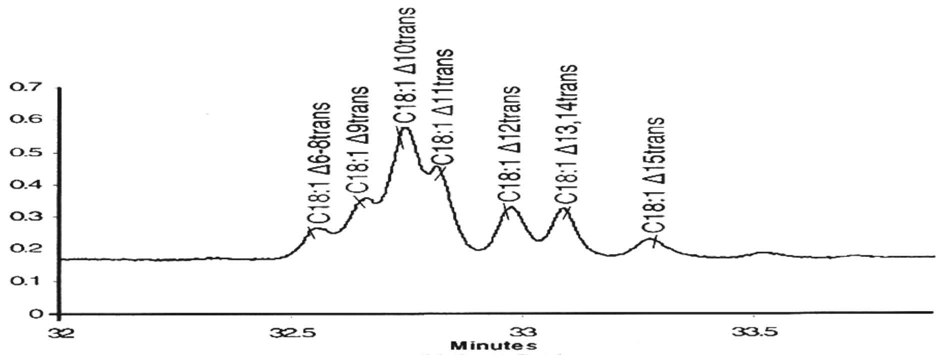


Figure 2B : Trans isomers of 18:1 in *Longissimus thoracis* muscle in the calf given a milk diet containing plant shortening lipids



Conclusion

Incorporation of partially hydrogenated fatty acids from oleaginous oils (shortenings) in the preruminant calf milk replacer directly influenced *trans* 18:1 isomer profile of meats differing to that of beef from adult bovine. Meat *trans* 18:1 from calves given shortenings were characterized by the abundance of Δ9 (13.8%) and Δ10 (32.6%) *trans* 18:1 known to have a hypercholesterolemic effect in monogastric animals and in human. Indeed, food manufacturers are presently in the way of the complete elimination of *trans* fats from margarines or shortenings (Korver and Katan, 2006) which could be readily incorporated as lipid sources in the lipid free milk powder for calves without any risks of adverse effects on the health value of meat lipids for consumers.

References

Ascherio A, Katan M. (1999). Trans fatty acids and coronary heart disease. New England Journal of Medicine, 340, 1994-1998.

Bauchart D., Roy A., Lorenz S., Chardigny J.M., Ferlay A., Gruffat D., Sébédio J., Chilliard Y., Durand D. (2007). Butters varying in trans 18:1 and cis-9,trans-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. Lipids, 42, 123-133

Belury, M.A. (2002). Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annual Review of Nutrition, 22, 505-531.

Juanéda P. (2002). Utilisation of reversed-phase high-performance liquid chromatography as an alternative to silver-ion chromatography for the separation of cis- and trans-C18:1 fatty acid isomers. Journal Chromatography A, 954, 285-289.

Korver O., Katan M.B. 2006. The elimination of trans fats from spreads: how science helped to turn an industry around. Nutrition Reviews, 64, n°6, 275-279.

Leplaix-Charlat L., Durand D., Chilliard Y., Williams P., Bauchart D. 1996. Effects of diets containing tallow and soybean oil with and without cholesterol on hepatic metabolism of lipids and lipoproteins in the preruminant calf. Journal of Dairy Science, 79, 1826-1835.