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Study of Genetic Variability of Fatty Acid Profile in Bovine Milk and Fat Using Mid-Infrared Spectrometry

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Résumé: La composition en matières grasses influence tant la qualité nutritionnelle du lait que les propriétés technologiques du beurre. L'effet de l'alimentation sur le profil en acides gras est connu mais l'effet de la génétique est peu étudié. L'objectif général de cette thèse de doctorat était d'étudier la variabilité génétique du profil en acides gras de la matière grasse laitière bovine. Une telle étude nécessite une quantité importante de données. Le dosage des acides gras dans la matière grasse est actuellement onéreux. Le premier objectif de cette thèse était de développer une méthode alternative rapide et bon marché. Des droites de calibration prédisant les concentrations en acides gras dans le lait à partir du spectre du moyen infrarouge ont été établies. Les acides gras saturés et monoinsaturés, les oméga-9, les acides gras à courte, moyenne et longue chaîne étaient les mieux prédits. Par l'implémentation de cette méthode lors du contrôle laitier wallon, plus de 20.000 échantillons de lait ont été analysés. Cette base de données a permis de modéliser les variations des taux en acides gras. Grâce à cela, l'existence de la variabilité génétique des acides gras a été montrée. Au fur et à mesure de l'augmentation des données disponibles, les modèles utilisés se sont complexifiés. Les différences raciales entre 7 races laitières ont été étudiées par l'emploi de modèles uni- et multi-caractère. Les matières grasses et l'activité delta-9 désaturase issus de vaches Jersey et Blanc Bleu de type Mixte différaient de l'Holstein. Le choix de la race modifie la composition en matières grasses. Les héritabilités des acides gras étudiés s'étalaient de 0,05 à 0,42. Les acides gras saturés étaient plus héritables que les insaturés. Des héritabilités modérées ont été observées pour l'activité de la delta-9 désaturase (±0,20) et pour la dureté du beurre (0,27). Ces deux caractères sont définis par des rapports d'acides gras. L'héritabilité estimée par le modèle multi-caractère avec régressions aléatoires pour les acides gras saturés (0,42) était similaire à celle obtenue pour le taux en matière grasse (0,37). En considérant l'effet de la sélection sur le taux en matière grasse, son effet sur la composition en acides gras devrait être non négligeable. De hautes corrélations génétiques ont été observées entre les acides gras présentant des similitudes dans leur synthèse. Les héritabilités et les corrélations variaient au cours de la lactation. Dû au nombre élevé d'acides gras, définir un index résumant le profil en acides gras pourrait être intéressant pour une sélection future. Cette thèse de doctorat fournit les données de base nécessaires aux développements d'études futures basées sur l'impact d'une sélection animale sur la composition en matières grasses.

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Summary: Changes in milk fat composition influence its nutritional quality as well as the technological properties of butter. The impact of feed on fat composition is well known; however, limited information is available on the genetic variability of fatty acids in bovine milk. The overall aim of this PhD thesis was to study the genetic variability of fatty acid profile in bovine milk and fat. This type of research needs a large amount of data. Expensive reference analysis is used to measure the fatty acid contents in fat. The first objective of this thesis was to develop an alternative method that could be faster and cheaper than traditional methods. Calibration equations predicting the contents of fatty acid from mid-infrared spectrum were established. The contents of saturated and monounsaturated fatty acids, omega-9, fatty acids with short, medium and long chain were the best predicted. Thanks to the implementation of this method in the Walloon routine milk recording, more than 20,000 milk samples were analyzed. This database permitted to model the variation of fatty acid contents in milk and fat. From these models, the genetic variability of fatty acid profile was shown. The complexity of models increased throughout this project due to the increase of new available data. Differences across 7 dairy breeds were estimated using single and multi-trait mixed models. Milk fat and delta-9 desaturase activity of Jersey and dual purpose Belgian Blue differed significantly from Holsteins. Therefore, the choice of a given breed could modify the fat composition. Heritability values obtained for studied fatty acids with multi-trait mixed model ranged from 0.05 to 0.42. Higher values were observed for saturated compared to unsaturated fatty acids. Moderate heritability estimates were observed for the activity of delta-9 desaturase (±0.20) and the hardness of butter (0.27). These two traits were estimated by specific fatty acid ratios. The heritability observed using a multi-trait random regressions test day model for the content of saturated fatty acids (0.42) was similar to the one observed for the percentage of fat (0.37). Considering the impact of selection on fat content, the selection could have a great impact on fat composition. High genetic correlations were observed between some fatty acids having similarities in their synthesis. Heritability and correlations varied through the duration of the lactation. Due to the large number of fatty acids, the estimation of an index, which includes the proper fatty acid profile, could be interesting for a future selection program. This PhD thesis provides the background required by future studies to estimate the impact of animal selection on milk fat composition.

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%FAT = percentage of fat
%MONO = percentage of MONO in fat
%PROT = percentage of protein
BSW = Brown-Swiss breed
CAP = common agricultural policy
CLA = conjugated linoleic acid
DIM = days in milk
DPB = dual purpose Belgian Blue breed
FA = fatty acid
HOL = Holstein-Friesian breed
JER = Jersey breed
MILK = milk yield
MIR = mid-infrared
MON = Montbeliarde breed
MONO = monounsaturated fatty acids
MT = multiple-trait
NOR = Normande breed
PLS = partial least squares regression
POLY = polyunsaturated fatty acids
R^2c = calibration coefficient of determination
R^2cv = cross-validation coefficient of determination
RED = non-Holstein Meuse-Rhine-Yssel (Red and White breed)
RPD = ratio of SECV to SD
RR = random regressions
SAT = saturated fatty acids
SCD = Stearoyl-CoA desaturase
SD = standard deviation
SEC = standard error of calibration
SECV = standard error of cross-validation
SNPs = single nucleotides polymorphisms
TD = test day
UNSAT = unsaturated fatty acids
WTO = World Trade Organization

Chapter I

GENERAL INTRODUCTION

With the addition of 10 new members in the European Union and the concept of fair trade supported by the World Trade Organization (WTO), improving the competitiveness of European milk producers becomes a crucial issue. In 2004, the milk price in New-Zealand, a major dairy exporting country in the world, was approximately 60% lower than the average price observed in the European Union. Moreover, the reform of the Common Agricultural Policy (CAP), decided in June 2003, forces the producers to change their conception of milk production. Indeed, the majority of agricultural subsidies established by CAP will not be linked to the volume of produced milk. Even if the current price of milk is increasing, the price of inputs (cereals, energy,…) increases also. So, the situation of European dairy producers on a world scale may retain relatively unstable. Consequently, dairy farmers should produce milk more in closer agreement with the demand of dairy industry and consummers. The dairy industry will have to increase the range of products, as, for instance, the functionnal products. In Belgium as in The Netherlands or France (especially in Normandy), for four years, some farmers have begun to produce milk with more polyunsaturated fatty acids as, namely omega-3 and conjugated linoleic acid (CLA), by giving specific feed to their cows. For instance, one of this type of specific feeding, Nutex©, was used by 180 farms in Belgium, 550 in The Netherlands, and 140 in Normandy. The market in Spain has begun with 4 producers. In total, approximately 55,000 cows in the European Union receive this specific feeding. Due to the increase of demand, 20 new producers in Belgium bought the Nutex© last year (Vanvolsem, 2007, personnal communication).

According to the World Health Organization (WHO), unhealthy nutrition increases the risk of chronic diseases as diabetes, cardiovascular diseases and cancer. One of the five proposals suggested by WHO to improve the diet is to limit the energy intake and prefered fat composition with more unsaturated fatty acids compared to saturated fatty acids. This last statement is based on the results obtained by many researches conducted during the most recent years. In comparison with unsaturated fatty acids, the consumption of saturated fatty acids, particularly C12:0, C14:0 and C16:0, seems to increase the level of cholesterol in blood and, thus, should increase the risk of cardiovascular diseases. Besides this hypocholesterolemic effect, the intake of CLA, polyunsaturated fatty acids produced only by ruminants and particularly

C18:2 *9-cis, 11-trans* and C18:2 *10-trans, 12-cis*, would have positive impacts on the prevention of cancer and obesity.

Fat composition influences the nutritional quality of milk but also modifies the technological properties of butterfat. Early researches on fatty acid profile studied these technological properties. Coulter and Hill (1934) focused on the variation of fatty acid contents among dairy breeds based on the estimation of iodine value. Recently, Bobe *et al.* (2003) showed that butter produced by cows with a more unsaturated fatty acid composition was more spreadable, softer, and less adhesive.

Consequently, increasing the proportion of unsaturated fatty acids in bovine milk presents many advantages for human health as well as for the technological properties of butter. Average milk fat contains 70% of saturated fatty acids, 25% of monounsaturated fatty acids and 5% of polyunsaturated fatty acids. The aim of recent studies which focus on the improvement of nutritional quality of milk fat was to decrease the content of saturated fatty acids in fat. The modification of fat composition is feasible using the different sources of variation as feeding or maybe genetic variability (breed differences or individual genetic effect). Although the feed impact is well known; limited information exists to characterize the genetic variability of fat composition. The overall objective of this PhD thesis was to estimate the genetic variability of fatty acid profile in bovine milk and fat.

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Chapter II

GENETIC VARIABILITY OF FATTY ACIDS IN BOVINE MILK

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2.1. Abstract

2.1.1. Genetic Variability of Fatty Acids in Bovine Milk

Fatty acids composition of bovine milk influences the technological properties of butterfat and also presents some potential benefits for human health. Impact of feeding on fat composition is well described in the literature; less information is available about the impact of genetics. Based on few studies, essentially conducted to isolate some feeding effect, the breed seemed to influence the fatty acids composition. The variation in the activity of delta-9 desaturase, key enzyme in the production of monounsaturated fatty acids and conjugated linoleic acids in milk, could explain these differences. Very few studies have been focussing on the estimation of genetic parameters of fatty acids composition. However, the moderate heritability estimates observed by these studies for the major fatty acids could suggest a potential genetic effect.

Keywords : fatty acids, milk, genetic, heritability, breed, delta-9 desaturase

2.1.2. Variabilité Génétique des Acides Gras du Lait d'Origine Bovine

La composition en acides gras du lait influence les propriétés technologiques du beurre et montre des effets potentiellement positifs sur la santé humaine. L'effet de la supplémentation de la ration animale sur le profil en acides gras est bien décrit dans la littérature mais peu d'information est disponible sur les effets génétiques. Sur base d'un nombre limité d'études, généralement menées pour mettre en évidence un effet alimentaire, l'effet de la race semble influencer la composition en acides gras du lait. Une variation de l'activité delta-9 désaturase, enzyme clé dans la production des acides gras monoinsaturés et des acides linoléiques conjugués (CLA) pourrait expliquer ces différences. Très peu d'études se sont focalisées sur l'estimation des paramètres génétiques des acides gras. Pourtant, les valeurs d'héritabilité modérées observées pour les principaux acides gras du lait pourraient suggérer un effet génétique potentiel.

Mots clé : acides gras, lait, génétique, héritabilité, race, delta-9 desaturase

2.2. Introduction

For a long time, the perception of consumers about fat from animal origin was linked to the increased risk of cardiovascular diseases, especially due to the large amounts of saturated fatty acids. The dairy products provide 15 to 25% of fat consumed by humans and 25 to 35% of saturated fat (Chillard *et al.*, 2001). This statement of cognizance, even if the potential effects of saturated fatty acids are more nuanced (Chilliard *et al.*, 2001), has forced the dairy industry to consider new options to produce and promote healthy milk. A way of doing this, is to modify the milk fat composition for improved the nutritional quality of dairy products.

Bovine milk fat, which the percentage varies between 3 and 5% (Jensen *et al.*, 1990), is emulsified in the aqueous phase of milk. Milk fat globule is protected by a membrane partially derived from the plasma membrane of the lactating cell (Danthine *et al.*, 2000; Jensen *et al.*, 1990). The centre of this droplet is essentially composed by the lipids. The latter are complex both with the respect to lipids classes and to fatty acid components (Jensen and Clark, 1988). Triglycerides compose 98% of lipids present in bovine milk. The last 2% includes small amounts of free fatty acids, mono- and diglycerides, phospholipids, sterols and hydrocarbons (Jenness, 1988). The analysis of fatty acids is difficult due to the volatile trend of short chain fatty acids and the large amounts of fatty acids present in milk (Jensen and Clark, 1988). Currently, 406 fatty acids are listed (Debry, 2001). However, few fatty acids represent the majority of fat present in bovine milk. Table 1 shows the contents of the principal fatty acids present in bovine milk based on the results obtained by Collomb and Bühler (2000). The 16 major fatty acids represented approximately 79% of milk fat content (Table 1). The fatty acids with the highest contents were C16:0 and C18:1 *9-cis* (Table 1).

Typical milk fat from dairy cows contains 70% of saturated, 25% of monounsaturated and 5% of polyunsaturated fatty acids (Grummer, 1991). Based on the previous results on the impacts of fatty acids on human health (*e.g.* Noakes *et al.*, 1996; Hu *et al.*, 1999; Parodi, 1999; Simopoulos, 2003), a composition of milk fat with potential positive effects on human health should be composed for 60% of monounsaturated (Pascal, 1996), 30% of saturated and 10% of polyunsaturated fatty acids (Hayes and Khosla, 1992). Even if obtaining this particular fat composition is an utopia, fatty acid profile could be improved using different sources of variation. The interest to study the variation of fat composition in bovine milk is not recent. For instance, Smith *et al.* in 1916 discussed the effect of cottonseed oil on fat composition. Then, many studies focussed on feed supplementation with particular feeds to modify the fatty acid profile (*e.g.* Chilliard *et al.*, 2000 and 2001), especially to increase the fraction of unsaturated fatty acids in milk fat. Even if many results confirm the feeding impact, less information relates to genetic effect including breed differences and individual variability of the fatty acids. The aim of this paper was to review the impacts of genetic factors on the composition of bovine milk fat.

Table 1. *Average contents of the main fatty acids present in bovine milk estimated from 35 milk samples by Collomb and Bühler (2000).*

Tableau 1. *Concentrations moyennes des principaux acides gras du lait d'origine bovine estimées à partir de 35 laits par Collomb et Bühler (2000).*

Fatty acid	Mean (g of fatty acids/100 g of fat)
C4:0	3.23
C6:0	2.02
C8:0	1.19
C10:0	2.66
C10:1	0.29
C12:0	2.84
C14:0	9.51
C14:1 cis	0.83
C15:0	1.00
C16:0	23.90
C16:1 *9-cis*	1.25
C18:0	8.98
C18:1 *9-cis*	17.36
C18:2 *9-cis,12-cis*	1.21
C18:2 *9-cis, 11-trans*	1.44
C18:3 *9-cis,12-cis, 15-cis*	0.86

2.3. Breed Differences

2.3.1. Fatty Acids

Variation of milk fat contents among dairy breeds has been known for a long time and is well described in the literature even if actual averages differ according to country and period. Table 2 gives an example of breed averages in the 1970s in the

USA, representing lactation average fat content in milk produced by five dairy breeds (Gaunt, 1980). These data included over 23,000 lactation records for cows milked twice a day for 305 days in 22 states in United States. Highest and lowest milk fat contents were observed for milk from Jersey and Holstein cows, respectively (Table 2). As the fatty acids compose the milk fat, logically variability of fatty acid profiles due to breed differences should also exist.

Table 2. *Lactation average fat content of milks of five breeds (Gaunt, 1980).*

Tableau 2. *Pourcentage moyen en matière grasse estimé sur une lactation à partir d'échantillons de lait collectés chez 5 races (Gaunt, 1980).*

Breed	Number of records	Fat (g/100 g)	
		Mean	SD
Ayrshire	3,362	3.99	0.33
Brown-Swiss	2,621	4.16	0.35
Guernsey	6,956	4.87	0.45
Holstein	9,102	3.70	0.39
Jersey	6,354	5.13	0.54

Generally the breed influences on fatty acid profile were studied based on phenotypic differences observed for limited numbers of cows from various breeds fed with the same diet. Consequently although the obtained results did not represent exactly the breed differences, this type of research gives some indications. The first studies linked differences in milk fat composition to manufacturing properties. One of the oldest studies linked the technological properties of milk fat to the hardness of butterfat. Coulter and Hill (1934) studied the variation of fatty acid contents among dairy breeds based on the estimation of iodine value. These authors observed that the butterfat produced by Channel Island cows was firmer than that produced by Holstein or Ayrshire cows.

Table 3 summarizes the results of breed differences obtained by several authors based on chromatographic data. All results are expressed in % of produced fatty acid content compared to that produced by Holstein breed. Majority of the studies focussed on the differences in fat composition between Jersey and other dairy breeds, especially to study the effect of a highest fat content on fatty acid profile.

Table 3. *Differences for the fatty acids profile across dairy breeds compared to Holstein breed.*

Tableau 3. *Différences de profil en acides gras entre diverses races laitières comparées à la race Holstein.*

	Differences of fatty acid contents compared to Holstein (in %)							
	Guernsey	Jersey			Brown-Swiss		Montbeliarde	Normande
	Stull[1]	Stull[1]	Beaulieu[2]	DePeters[3] [3]	DePeters [3]	Kelsey[5]	Lawless[4]	Lawless[4]
N	25	10	8	23	29	106	29	27
C4:0			-2.43	-4.90	-1.47	+12.36	-5.50	-2.75
C6:0	+20.73	+8.54	+16.67 $^{(*)}$	+3.32 $^{(*)}$	+2.21	+7.32	-2.54	+0.85
C8:0	+13.16	+15.79	+38.46 $^{(**)}$	+7.55 $^{(**)}$	+5.03 $^{(**)}$	+13.13	+1.02	+5.10
C10:0	+14.29 $^{(**)}$	+34.10 $^{(**)}$	+43.33 $^{(***)}$	+13.59 $^{(**)}$	+4.08 $^{(**)}$	+14.22	+6.98	+9.30
C10:1	+12.5	+70.83					-16.67	0.00
C12:0	+7.59 $^{(**)}$	+36.90 $^{(**)}$	+42.86 $^{(***)}$	+16.90 $^{(**)}$	+6.34 $^{(**)}$	+14.41	+6.46	+10.77
C14:0	+5.64	+9.26	+8.62 $^{(*)}$	+2.36	+2.14	+4.66	+2.61	+1.87
C14:1 *cis*	-11.31	-4.76				-1.64	-28.09	-10.11
C15:0	-6.80	-2.04				-6.76		
C16:0	+7.20 $^{(**)}$	+5.63 $^{(**)}$	-6.79 $^{(*)}$	-1.24	-1.70	+0.96	-11.49	-8.15
C16:1 *9-cis*	-7.14 $^{(**)}$	-16.67 $^{(**)}$		-9.55	-1.51	-13.08		
C18:0	+4.64	+1.12	+12.50	+6.61 $^{(**)}$	-6.83 $^{(**)}$	-3.42	+10.89	+14.93
C18:1 *9-cis*	-11.15 $^{(**)}$	-12.92 $^{(**)}$	-12.72 $^{(**)}$	-9.51	+3.91	-1.96	+5.37 *	+1.37 *
C18:2	-4.92	-4.64	0.00	+1.58	-4.74	-5.80	+5.94	+3.96
CLA						-6.82	+13.07	-5.11
C18:3	-19.79	-32.29	-16.67	+15.50 $^{(*)}$	-6.98	-2.56	+1.22	-6.10

[1] Stull and Brown, 1964; [2] Beaulieu and Palmquist, 1995; [3] DePeters *et al.*, 1995; [4] Lawless *et al.*, 1999 (compared to Dutch Holstein; P values not indicated);

[5] Kelsey *et al.*, 2003 (P values not indicated); * total of C18:1; * = P < 0,05; ** =P<0,01; ***=P<0,001

In 1964, Stull and Brown reported that the composition of milk fat produced by Holstein breed was significantly different compared to that produced by Jersey and Guernsey for five individual fatty acids (C10:0, C12:0, C16:0, C16:1 *cis* and C18:1 *cis*) (Table 3). Higher contents of fatty acid with short and medium chain and a lower content of C18:1 *9-cis* were observed by Hermansen and Lund (1990) in Jersey milk fat than in fat produced by heavier breeds. Palmquist and Beaulieu (1992) studied the differences of milk fat composition between Jersey and Holstein. These authors reported that the contents of C6:0 until C14:0 were 8 to 42% higher in Jersey compared to Holstein. Jersey fat contained a higher content of C18:0 (Palmquist and Beaulieu, 1992). Few years later, these same authors confirmed that Jersey milk fat contained a higher proportion of short and medium chain fatty acids and lower proportions of C18:1 *9-cis* than milk fat from Holstein cows (Beaulieu and Palmquist, 1995) (Table 3). They found also that the content of C16:0 was lower in Jersey than in Holstein. This observation was not in agreement with Stull and Brown (1964) but was also observed by DePeters *et al.* (1995) (Table 3). Beaulieu and Palmquist (1995) indicated that the amount of fat yield stayed unchanged in function of the quantity of supplement fed. Consequently, the observed changes in the fatty acid profile in milk fat reflected a modification in the amount of fatty acids synthesized by the mammary gland. These authors postulated that the *de novo* synthesis of fatty acids in Jersey cows was more inhibited than in Holstein breed at the first amount of added fat. The differences on fatty acids composition between Holstein, Jersey and Brown-Swiss cows were studied by DePeters *et al.* in 1995. They reported that the content of short chain fatty acids did not change a lot between Jersey and Holstein milk fat (Table 3). Stull and Brown (1964) and Beaulieu and Palmquist (1995) reported that the proportion of medium chain fatty acids was higher and the content of C18:1 *9-cis* was lower in Jersey than in Holstein milk fat. Higher proportions of C6:0 until C12:0 were produced by Jersey cows (Table 3). The total unsaturated fatty acids content was lower for Jersey than other studied cows. As for the previously reported results, White *et al.* (2001) showed that Jersey produced significantly higher concentrations of C6:0, C8:0, C12:0 and C14:0 than Holstein. This last breed produced significantly higher contents of C16:1 and C18:1. Based on these results, Jersey cows seemed to produce more fatty acids with short and medium chain compared to Holstein. Consequently, the production of a highest content of fat in bovine milk seems to be linked to a lowest content of unsaturated fatty acids. So, this fat composition involves a production of an harder butter and milk with less interest for human health. Indeed, different studies based on the impact of fatty acids on human health (*e.g.* Bonanome and Grundy, 1988; Grummer, 1991; Hu *et al.*, 1999) showed that the fatty acids with medium carbon chain, especially C14:0, increased more the risk of cardiovascular diseases compared to other fatty acids and especially unsaturated fatty acids.

Only one study was found to describe the differences between Brown-Swiss and Holstein. DePeters *et al.*, 1995 showed that Brown-Swiss milk fat contained the lowest content of C18:3.

The study of Lawless *et al.* (1999) focussed on the differences on fatty acid profile between Holstein, Montbeliarde and Normande. These authors showed that Dutch Holstein had higher C16:0 concentrations that the other studied breeds. Normande and Montbeliarde produced the milk fat with the highest C18:0 (Table 3).

Thanks to its potential positive effects on human health, a specific type of fatty acids, the conjugated linoleic acids (CLA), shows a large interest. CLA is a term representing a mixture of positional and geometric isomers of octadecadienoic acid with a conjugated double bond system (Kelsey *et al.*, 2003). Rumenic acid, C18:2 *9-cis*, *11-trans* is the major CLA isomer found in dairy products accounting for 75 to 90% of the total CLA in milk fat (Bauman *et al.*, 1999). This isomer is known for its anti-carcinogenic properties showed on animal model (Parodi, 1999). A other isomer, C18:2 *10-trans, 12-cis* seems to be also interesting for its action on the lipid status (Chilliard, 2001; Pariza *et al.*, 2001). Moreover, other effects of CLA were shown : anti-obesity (Park *et al.*, 1997), suppression of carcinogenesis (Ip *et al.*, 1994; Belury, 1995), immune modulation by increasing of IgA, IgG et IgM and by decreasing IgE (Cook *et al.*, 1993; Debry, 2001), anti-atherogen (Nicolosi *et al.*, 1997; Debry, 2001) and effect on diabetes (Houseknecht *et al.*, 1998). Due to these positive effects on human health, some studies are focussed on feed supplementation to increase CLA content in milk and more less studied the breed differences. However, Lawless *et al.* (1999) observed a variation of CLA between dairy breeds. These authors reported that breed had some influence on CLA content of milk and that a cow yielding high levels of milk fat CLA sustained this production over time. Montbeliarde milk fat contained the highest level of CLA and Normande the lowest content compared to Dutch

Holstein (Lawless *et al.*, 1999) (Table 3). Lawless *et al.* were not the only authors to study the variation of CLA among dairy breeds. White *et al.* (2001) showed that Holstein breed produced significantly higher concentrations of CLA than Jersey. Kelsey *et al.* (2003) studied the variation of fatty acids, especially of CLA, among Holstein and Brown-Swiss cows. Holstein milk fat contained a higher content of CLA in milk. However, these authors mentioned that the difference among studied dairy breeds were not significant. Breed accounted for less than 0.1% of the total variation in the CLA concentration in milk fat.

Independently of the variation of fatty acids across dairy breeds, Lawless *et al.* (1999) showed also some significant differences, especially for C16:0, on the fatty acid profiles among Holstein cows from two separate origins, Irish and Dutch Holstein. These individual variations suggest that additional factors must affect the fat composition in bovine milk (*e.g.* the rumen environment (pH, time of rumination, physiological differences (size of intestine,*etc*), the activity of mammary gland,…).

2.3.2. Delta-9 desaturase

The variation of milk fat composition observed among dairy breeds could be partially explained by the metabolic process of fatty acids synthesis. Aforementioned, Beaulieu and Palmquist (1995) have used a metabolic interpretation based on the *de novo* synthesis to explain the differences in the response of Jersey and Holstein cows to the addition of fat in the animal ration.

By the introduction of a cis-double bond between carbons 9 and 10 in the carbon chain of fatty acids (Bauman *et al.*, 1999), delta-9 desaturase is an important enzyme in the production of unsaturated fatty acids. The iron which composes the delta-9 desaturase enzyme, also named stearoyl-CoA desaturase (**SCD**), with the action of NADPH, cytochrome b5 reductase, cytochrome b5 and oxygen, catalyzes this desaturation (Ntambi, 1995; Yahyaoui *et al.*, 2002). As its activity permits the conversion of C18:1 *11-trans* into C18:2 *9-cis, 12-cis*, this enzyme is responsible to the endogenous production of the major isomer of CLA in bovine milk, the rumenic acid (Kinsella, 1972; Bauman *et al.*, 1999). The conversions of C18:0 into C18:1 *9-cis*, C16:0 into C16:1 *9-cis*, C14:0 into C14:1 *9-cis* and C10:0 into C10:1 *9-cis* in mammary cells are also regulated by this enzyme. Consequently, the variation of delta-9 desaturase activity could partially explain the fluctuation of the contents of monounsaturated fatty acids and CLA in bovine milk fat. Lock and Garnsworthy (2003) estimated the ratio product/substrate (*e.g.* C14:1 *9-cis*/C14:0) to study the seasonal variation of the delta-9 desaturase. These authors suggested that a molecule contained in the grass could induce a higher delta-9 desaturase activity, and thus, explain the seasonal variation. Based on the ratio substrate/product (*e.g.* C14:0/C14:1 *9-cis*), Chouinard *et al.* (1999) showed that the delta-9 activity was influenced by dietary supplementation. In addition to these extrinsic factors, Peterson *et al.* (2002) suggested that the variation of CLA content in milk fat among individuals was related to the rumen biohydrogenation and delta-9 desaturase activity. If the variation of the delta-9 desaturase activity permitted to explain partially some phenotypic differences observed in the CLA content, the activity of this enzyme should vary genetically. In this sense, Kelsey *et al.* (2003) showed a delta-9 desaturase activity differenced between Holstein and Brown-Swiss breeds from the estimation of the ratio substrate/(substrate + product) (*e.g.* C14:0/(C14:1 *9-cis* + C14:0). Holstein cows showed the highest delta-9 desaturase activity. The lowest contents of monounsaturated fatty acids and CLA observed by Kelsey *et al.* (2003) for Holstein breed could be explained by this enzymatic activity (Table 3). As the ratio C18:1/C18:0 could reflect the delta-9 desaturase activity, the lowest value of this ratio observed for Jersey could also explain the lowest contents of monounsaturated fatty acids and CLA observed by DePeters *et al.* (1990) for this breed.

2.4. Heritability

2.4.1. Fatty acids

Gibson (1995) mentioned that effective genetic improvement requires genetic variation, a mechanism of selection, and an economic incentive for the improvement. Economic incentives will not be discussed as evolution of milk price is yet uncertain; however several authors reported estimation of genetic parameters for the fatty acid profile in milk. Unfortunately even if already several feeding studies concluded towards a possible genetic effect only a restricted number focussed on the estimation of genetic parameters of fatty acid contents. Most feeding studies, as for Beaulieu and

Palmquist (1995), observed differentiated reactions of cows from different breeds to dietary changes, a clear indication that the genetic factor might be important.

One of the first studies about the genetic variability of fatty acid profile was conducted by Edwards *et al.* in 1973. Very high heritability estimates ranging between 0.64 and 0.98 were obtained by these authors. In their research, the environmental variance was estimated as the sum of variances within monozygotic twins and half of the genetic variance added to the environmental variance was the sum of variances within dizygotic twins. This estimation method is today considered unreliable and that these values were probably highly overestimated. Few years later, Renner and Kosmack (1974a) reported heritability values equal to 0.26, 0.06 and 0.04 for the contents of fatty acid with short and medium chain and for the C18 family in milk fat, respectively. Estimates of 0.26, 0.25 and 0.02 for the contents of fatty acids with short and medium carbon chain and for the C18 family in milk (% in milk), respectively, were also showed by these authors. From these estimates, fatty acid contents in milk seemed to be more heritable than the contents of fatty acid in milk fat (% in milk fat). Values of heritability estimated by Karijord *et al.* (1982) were different from those observed by Renner and Kosmack (1974a). They were on average 0.13, 0.14 and 0.10 for fatty acid contents with short and medium carbon chain and for the C18 family in milk fat, respectively.

2.4.2. Delta-9 desaturase

Even if no articles about the estimation of genetic parameters for the delta-9 desaturase were found, some authors which focussed on the variation of this enzymatic activity concluded towards the existence of some genetic influences. For instance, Kelsey *et al.* (2003) showed that the average content of CLA in the studied animal population was 4.3 mg/g of fatty acids but the range among individuals was approximately threefold (2.3 to 7.2 mg/g of fatty acid). Even if the stage of lactation, the milk production level, and the diet were identical among cows, a substantial variation among individuals was still observed as reported by Kelly *et al.* (1998). However one abstract written by Royal and Garnsworthy (2005) reported the variances components and heritability of delta-9 desaturase ratios estimated on 1,520 Holstein-Friesian. These authors found a heritability values equal to 0.30, 0.19 and

0.29 for C14:1 / (C14:0+C14:1), C18:1 *9-cis* / (C18:1 *9-cis* + C18:0) and C18:2 *9-cis,11-trans* / (C18:2 *9-cis,11-trans* + C18:1 *11-trans*), respectively. Only C16:1 / (C16:0 + C16:1) showed a heritability equal to 0.01. These results confirmed an individual genetic variability of the delta-9 desaturase activity.

2.5. Genetic Correlations

Another important type of genetic parameter beside heritability, are genetic correlations. In particular, the genetic correlations among fatty acids are important to investigate the potential effects of animal selection on the fatty acid profile in bovine milk. These effects depend of the relative interdependence of fatty acids. Only two studies were found.

The genetic correlations between traditional production traits (milk yield, fat and protein contents) and fatty acids with short and medium carbon chain and for the C18 family were estimated by Renner and Kosmack (1974b). These authors observed that the relationships between milk yield and the studied group of fatty acids were relatively low. Only the content of fatty acids with short chain in milk seemed to be positively correlated with milk yield (0.24). Karijord *et al.* (1982) estimated the genetic correlations between the individual fatty acids and the traditional production traits. Their results were more diverse. Genetic correlations close to 0 were observed between C16:1, C17:0, C18:1 and milk yield. Positive genetic correlations ranged between 0.11 and 0.24 were obtained between the individual saturated fatty acids and milk yield, except for C15:0 and C16:0 (-0.58 and -0.14, respectively). Negative genetic correlations ranging between -0.11 and -0.35 were found between unsaturated fatty acids and milk yield, except for C18:2 (0.35).

Renner and Kosmack (1974b) indicated that the content of saturated fatty acids with medium chain became higher when the percentage of fat increased. As the percentage of fat in Jersey milk is high (Table 2), this observation could probably explain the higher contents of C12:0, C14:0 and C16:0 in Jersey fat compared to Holstein breed as observed by various authors (*e.g.* Beaulieu and Palmquist (1995); DePeters *et al.* (1995)). The genetic correlation between the content of fatty acids of the C18 family in fat and the fat percentage estimated by Renner could negative explain partially the lowest content of unsaturated fatty acids observed for Jersey breed. The same results were obtained by Karijord *et al.* (1982).

Table 4 describes the genetic correlations estimated by Karijord *et al.* (1982) between the contents of fatty acids in milk fat. The values of genetic correlation estimated among the contents for major fatty acids were extremely variable and ranged from -0.68 to 0.97. Generally, positive genetic correlations were observed between the fatty acids belonging to the same class of fatty acids (saturated, monounsaturated or polyunsaturated fatty acids). On the other hand, the contents of monounsaturated or polyunsaturated fatty acids were negatively correlated with saturated fatty acids. High positive correlations between fatty acids with short and medium chain except C16:0 are showed in Table 4. The same observation can be made among the polyunsaturated fatty acids and among the monounsaturated fatty acids. C16:0 and C18:0 seemed to have a different behaviour. C16:0 was negatively correlated with all studied fatty acids except C14:1 and C16:1. C18:0 was negatively correlated with C12:0, C14:0 and unsaturated C18 family. The genetic correlations between the individual monounsaturated fatty acids and C18:2 were positive except for C16:1. This observation could be explained the effect of the delta-9 desaturase. However, the results have to be interpreted cautiously because some doubts may exist. As a matter of fact the values of heritability estimated for milk and fat content by Karijord *et al.* (1982) tended to be very low (0.09 for these 2 traits), indicating potential data quantity and quality problems.

2.6. Conclusion

Our literature review focussed on the genetic variability of fatty acids in bovine milk and showed a shortage of information concerning the genetic parameters of these traits. Available results showed the existence of breed effects. Especially the differences of fatty acids composition between Holstein and Jersey breeds. Jersey produced less unsaturated fatty acids in milk fat probably due in part to a lower delta-9 activity observed in this breed. Few studies reported genetic parameters showing the existence of a genetic variability of fatty acid profile in bovine milk.

Table 4. *Genetic correlations between the major fatty acids in milk estimated by Karijord et al. (1982) from 7,000 milk samples.*

Tableau 4. *Corrélations génétiques entre les principaux acides gras du lait estimées par Karijord et al., 1982 à partir de 7000 échantillons de lait.*

	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2
C8:0	0.89												
C10:0	0.70	0.93											
C12:0	0.59	0.88	0.97										
C14:0	0.48	0.71	0.80	0.84									
C14:1	-0.03	0.12	0.01	0.21	0.10								
C15:0	-0.20	-0.07	-0.05	0.01	0.06	0.11							
C16:0	-0.25	-0.38	-0.35	-0.30	-0.27	0.01	-0.22						
C16:1	-0.28	-0.29	-0.29	-0.21	-0.32	0.32	-0.49	0.46					
C17:0	0.08	0.17	0.15	0.17	0.28	0.11	0.77	-0.36	-0.60				
C18:0	0.22	0.10	0.10	-0.08	-0.01	-0.58	0.26	-0.49	-0.70	0.33			
C18:1	-0.57	-0.60	-0.68	-0.63	-0.65	0.12	0.04	-0.28	0.25	-0.08	-0.14		
C18:2	-0.02	-0.05	-0.19	-0.20	-0.38	0.11	-0.59	-0.30	-0.12	-0.13	-0.07	0.61	
C18:3	-0.38	-0.35	-0.48	-0.39	-0.52	0.39	0.25	-0.23	0.29	-0.09	-0.30	0.84	0.65

Globally the values of heritability were moderate. Heritabilities of saturated fatty acids were higher than those observed for unsaturated fatty acids. The variation in the delta-9 desaturase activity could partially explained the variation observed in the contents of monounsaturated fatty acids and CLA in milk. Negative genetic correlations were observed between unsaturated fatty acids and fat content. It could explain why, in the available studies, the Holstein breed with the lowest fat percentage showed the highest contents of unsaturated fatty acids. The positive genetic correlations showed strong relationships between some fatty acids in bovine milk fat. Based on an unique study reporting genetic correlations among individual fatty acids, an animal selection based on the fatty acids contents could target potential interesting groups of fatty acids and not exclusively a specific fatty acids. Based on these genetic parameters and in spite of the lack of accurate estimates, the contents of monounsaturated fatty acids and CLA could increase simultaneously in bovine milk.

2.7. References

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Chapter III

OBJECTIVES

Development of selection program based on the fat composition could provide opportunities to modify permanently the fatty acid profile in bovine milk. Associated with a proper feeding regime, the fat composition of bovine milk could have an unsaturated fatty acids content that is significantly higher than the current one. The overall objective of this study was to investigate the **genetic variability of fatty acid contents in bovine milk** in order to test the feasibility of a future selection program based on the improvement of fat composition.

As explained in Chapter II, the feed effect on milk fat composition is well known and detailed; however, information of genetic effect is limited. Very few studies focussed on the genetic variability of fat composition. Moreover, these authors studied a limited number of cows as well as a limited number of analyzed milk samples. This is mainly due to the cost of gas chromatography, the reference analysis used to measure the fatty acid contents in bovine milk fat. Although this method is efficient, it is time-consuming and requires well-skilled staff and expensive reagents. A study of genetic variability needs a larger data sets including many animals and also many repetitions of milk fat analysis separated in time. Except with a lot of money, a study of genetic variability based on gas chromatographic data is unfeasible. Therefore, the first investigation of the present PhD thesis was to **identify and develop an alternative tool to estimate the fatty acid profile in bovine milk**. Chapter IV describes the methodology used to create the calibration equations to estimate the fatty acid contents based on the mid-infrared (MIR) spectral data and presents the results obtained to validate the ability of this method to estimate the milk fat composition. These results were published in the Journal of Dairy Science. Mid-infrared spectrometry was used in this research for three main reasons. The first one is related to the availability of this technology. Indeed, the MIR spectrometry is used all around the world by milk laboratories to measure the contents of some major milk components as, for instance, fat, protein or lactose. Consequently, the current selection programs uses data predicted from MIR spectral data. The second reason is the low price of this infrared analysis. MilkoScanFT6000 spectrometer, used at Battice milk committee (Belgium), can analyze up to 500 samples per hour and requires no pre-treatment of samples and no expensive reagents. The third reason linked with the two first ones is the opportunity to implement in the milk recording scheme the estimation of fatty acids by MIR spectrometry. Indeed the estimation of these fat components used the same milk sample analyzed at the same time and, thus, used the same spectral data than the one used to predict, for instance, the percentage of fat used in the current selection program.

The developed calibration equations to predict the fatty acid contents of bovine milk have permitted the study of genetic variability of fat composition on a larger scale than the one used currently. The reliability of this study is a function of the size of collected data. Consequently, the second aim of this thesis was to **implement the estimation of fatty acid contents by MIR spectrometry in the current selection structure**. As the MIR spectrometry is used during the Walloon routine milk recording, we have realized the importance of archiving MIR spectral data, which are normally erased after each infrared analysis. Working with the same milk samples than the ones analyzed during the Wallon milk recording presented the advantages to facilitate the sampling, the identification of samples (animal identity, breed composition,…) and the collection of lactation information as, for instance, days in milk and lactation number.

Thanks to the calibration equations and their implementation in the Walloon selection structures, the study of genetic variability of fat composition was possible. Consequently, the third objective of this thesis was **to investigate a model to study the genetic variability of fatty acid contents**. The accumulation of data needed for this study has taken time. Thus, the models used to study the individual variability of fat composition has been improved throughout the elaboration of this thesis, as more data became available. The obtained results were reported chronologically, thus, in function of the amount of collected data. Due to the limited size of data but thanks to the milk samples collected from multiple breeds, the first models used included only one trait but repeated data and regressions on breed composition. It permitted to study the differences in fat composition within and across breeds. These results were presented in Chapter V and published in the Journal of Dairy Science. Then, the higher amount of data allowed to consider a repeatability multiple-trait animal model including also regressions on breed composition. This model accounted for the existing links between the traditionnal production traits (milk yield, percentages of fat and protein) and fat composition. The objective of this study was to estimate the heritability of the major fatty acids of bovine milk and to estimate the genetic and phenotypic correlations among the various traits. Chapter VI described these results that were published in the Journal of Dairy Science

Many different fatty acids compose the bovine milk fat. Due to this large number of traits, it could be a priority to investigate a new trait which summarizes the most important information from the various fat composition traits. Thus, the genetic variability of delta-9 desaturase activity was investigated. The rule of this enzyme is important for the biosynthesis of unsaturated fatty acids, particularly the majority of monounsaturated fatty acids and CLA in bovine milk. Some authors suggested the possibility to estimate this enzymatic activity based on the ratios of fatty acids dependent on the delta-9 desaturase. These fatty acids ratios, the monounsaturated fatty acids content and the common production traits were studied with the multiple-trait animal model than the one used to study the genetic variability of individual fatty acids. These results are presented in Chapter VII and were submitted to the Journal of Dairy Science.

For all of these previous models, the genetic parameters were supposed constant through the lactation. However, based on previous results, it appeared that the fatty acid contents varied through the lactation. So, when the amount of data was sufficient, a multi-trait random regression test day model was used to model the lactation curve. Due to the complexity of this model, only first parity Holstein cows were studied. The genetic parameters for the saturated and monounsaturated fatty acid contents were estimated, as well as the ratio of saturated to unsaturated fatty acids reflecting the hardness of butterfat. Their relationship with the common production traits as milk yield, percentage of fat, and protein content were also analyzed. Chapter VIII describes this study submitted to the Journal of Dairy Science.

The last part of this PhD thesis discussed globally the results obtained and formulate perspectives of this research.

Chapter IV

ESTIMATING FATTY ACID CONTENT IN COW MILK USING MID-INFRARED SPECTROMETRY

FROM :
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4.1. Interpretive Summary

Mid-infrared spectrometry is a tool that may be used to determine the fatty acids profile in cow milk. Because of its potential use for regular milk recording, this new method offers the possibility of assessing and improving the quality of the cow milk produced on farms by animal selection. Using this method, the fatty acid composition for each cow in a herd could be estimated and this data then used for breeding value estimation and breeding strategies.

4.2. Abstract

Interest in fatty acid composition of dairy products is increasing; however, the measurement of fatty acids requires using gas-liquid chromatography. Although this method is suitable, it involves a time-consuming procedure, expensive reagents, and qualified staff. By comparison, the mid-infrared (MIR) spectrometry method could be a good alternative for assessing the fatty acid profile of dairy products. The objective of this study was to explore the calibration of MIR spectrometry for estimating fatty acid concentrations in milk and milk fat. Estimated concentrations in milk fat were less reliable than those for the same fatty acids in milk. Results also showed that when the fatty acid concentrations in milk increased, the efficiency of the infrared analysis method in predicting these values simultaneously increased. Selected prediction equations must have a high cross-validation coefficient of determination, a high ratio of standard error of cross-validation to standard deviation and good repeatability of chromatographic data. Results from this study showed that the calibration equations predicting 12:0, 14:0, 16:0, 16:1*cis-9*, 18:1, and saturated and monounsaturated fatty acids in milk could be used. Thus, with its potential for use in regular milk recording, this infrared analysis method offers the possibility of assessing and improving the quality of milk produced. Indeed, it enables the fatty acid composition in milk to be estimated for each cow and the estimates to be used as indicator traits to determine the genetic values of underlying fatty acid concentrations. The knowledge of these genetic values would open up opportunities for animal selection aimed at improving the nutritional quality of cow milk.

(**Keywords**: milk, fatty acids, mid-infrared, quality)

4.3. Introduction

Cow milk fat typically contains 70% saturated fatty acids (**SAT**), 25% monounsaturated fatty acids (**MONO**) and 5% polyunsaturated fatty acids (**POLY**; Grummer, 1991). A milk lipid composition more favorable to human health would be about 30% SAT (Pascal, 1996), 60% MONO, and 10% POLY (Hayes and Khosla, 1992). The fatty acid profile of cow milk is therefore far from optimal. However, the observed variations in SAT, MONO and POLY suggest that milk fat composition could be modified by various means, such as through feeding and genetics (Palmquist *et al.*, 1993), and could come closer to the optimal profile. Several researchers have been focusing on ways to improve the nutritional quality of bovine milk fat by feed supplementation (*e.g.*, Demeyer and Doreau, 1999; Chilliard *et al.*, 2000). However, all the fatty acids in a specific class (SAT, MONO or POLY) do not have the same effects on human health. In the case of SAT, although myristic acid is known for its negative effects on cardiovascular diseases, stearic acid does not seem to have this effect (Hu *et al.*, 1999). Similarly, in POLY, the n-6 fatty acids appear to have negative effects on human health because of their prevalence in Western nutrition. Indeed, the current ratio n-6:n-3 is estimated to be 15:1 to 20:1 (Simopoulos, 2003). It is therefore important to check the global fatty acid profile in milk if one wants to assess the nutritional quality of bovine milk fat. As stated earlier, influencing the nutritional quality of milk fat has been the topic of several recent research papers (*e.g.*, Demeyer and Doreau, 1999; Chilliard *et al.*, 2000), some of which have concentrated on milk fat enriched in n-3, in line with the current interest in dairy products that are enriched in n-3. However, there has been less focus on assessing the fatty acid content, because this requires chromatographic analysis. Although this method is suitable (*e.g.*, Dorey *et al.*, 1988; Collomb and Bülher, 2000), it is time-consuming and requires skilled staff.

Mid-infrared (**MIR**) spectrometry is an alternative to gas chromatography, with advantages such as a very high throughput (up to 500 samples/h; FOSS, 2005), ease of use, and availability. The infrared spectrum is caused by absorptions of electromagnetic radiation at frequencies that are correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000). The spectrum therefore illustrates these absorptions at different wavenumbers (cm^{-1}) for a specific chemical composition (Smith, 1996). Mid-infrared spectrometry (400 to 4,000 cm^{-1}) is particularly interesting because it is very highly sensitive to the chemical environment, as the fundamental absorptions of molecular vibrations occur in this region (Belton, 1997). Mid-infrared spectrometry can be used to estimate various traits quantitatively based on calibration equations. The purpose of our research was to develop the predicted equations necessary for measuring the fatty acid content in milk and milk fat using MIR spectrometry.

4.4. Materials and Methods

4.4.1. Sampling and Recording Spectra Files

Milk samples were taken from cows in 7 herds selected according to the following criteria: their participation in the milk recording system in Wallonia, the observed variation in the percentage of milk fat and the number of breeds in the herds. An 80-mL sample taken during routine milk recording was divided into 2 parts (60 mL + 20 mL). The samples were collected for all the cows milked in the herds on a given test-day. Following standard procedures (International Committee for Animal Recording, 2004), the samples represented 50% morning milk and 50% evening milk. The 20 mL sample was then analyzed using MIR spectrometry (MilkoScan FT6000; FOSS, 2005) following the normal milk recording procedure (International Committee for Animal Recording, 2004). The MilkoScan FT6000 works within the MIR region from 1,000 to 5,000 cm^{-1} and uses an interferometer. From the resulting interferogram, MIR spectra are generated by means of fast Fourier transformations (FOSS, 2005). The spectra files generated were recorded in a database. The second sample (60 mL) was frozen at – 26 ± 2°C. In this procedure, 600 samples were taken between April and June 2005 from 275 cows from 6 breeds (Dual Purpose Belgian Blue, Holstein-Friesian, Jersey, Normande, Montbeliarde, and Red and White). Not all the farms were tested 3 times, and some cows were dried off or had calved during the study. The milk fat percentage of the collected samples ranged between 2.97 and 7.73 g/dL of milk. This large variation indicated that a good calibration could be made.

4.4.2. Reference Values

Using principal component analysis based on the spectral variability, 49 samples were chosen and used from the 600 samples collected. The milk fat was extracted according to the ISO Standard 14156:2001 (International Organization for Standardization, 2001). These milk fat samples were analyzed using gas chromatography, based on a method derived from Collomb and Bühler (2000). The gas chromatograph (model 6890, Agilent technologies, Inc., Palo Alto, CA) was equipped with a CPSil–57 CB capillary column (Varian, Inc., Palo Alto, CA), with a length of 50 m, an internal diameter of 0.25 mm, and a film thickness of 0.20 µm. The retention gap was a "methyl deactivated nonpolar" (Varian, Inc.), with a length of 20 cm and an internal diameter of 0.53 mm. The conditions for the chromatographic analyses were as follows: carrier gas, helium; average velocity, 35 cm/s; cold on-column injector; flame ionization detector at 265°C; and a temperature program from 40°C (2 min) to 150°C (at 30°C/min), then 150 to 250°C (at 2°C/min). The volume injected was 0.5 µL. To measure the fatty acid concentrations, the response factors used were the same as those described by Collomb and Bühler (2000) because the experimental conditions were similar.

For each sample, 2 groups of reference values were generated (g of fatty acid/100 g of fat, and g of fatty acid/dL of milk) and were recorded in the database. The repeatability and accuracy of this method were estimated from several reference butter samples produced by BIPEA (Bureau InterProfessionnel d'Etude Analytique, http://www.bipea.org).

4.4.3. Calibration Equations

From the chromatographic and spectral data, a specific program for multivariate calibration (WINISI III, http://www.winisi.com/) was used to compute the calibration equations using partial least squares regression (**PLS**). Partial least squares regression has 2 important advantages over multiple linear regression or regression on principal components. First, like principal components regression, it uses all the spectral data for the calibration (Frank *et al.*, 1984). Second, in one step, the PLS method compresses the data (Martens and Jensen, 1982) and maximizes the variability of the dependent variable (Martens and Naes, 1987). The PLS method is therefore considered more efficient for calibration than the regression on principal components or multiple linear regression (Prévot, 2004). The number of factors used in the equation was determined by cross-validation, which was also needed to estimate its robustness. Although various pretreatments were studied, none was used on the spectral data before the calibration. Finally, 41 prediction equations were elaborated to estimate the fatty acid profile in milk and milk fat. To assess the efficiency of the calibration equations, various statistical parameters were estimated and analyzed: mean, standard deviation, standard error of calibration (**SEC**), calibration coefficient of determination, standard error of cross-validation (**SECV**) and cross-validation coefficient of determination (**R^2_{CV}**). The ratio (**RPD**) of SECV to standard deviation was also calculated (Williams and Norris, 2001) to assess the efficiency of the calibration.

4.5. Results and Discussion

4.5.1. Reference Values

Figure 1 presents a typical gas chromatogram recorded under the conditions described earlier. Because of the poor resolution between 18:1 isomers, they were not studied individually. Thus, the concentration indicated for 18:1 in this study is the result of the sum of the different 18:1 isomer contents.

The accuracy of the chromatographic method was assessed by comparing the mean values obtained with the reference values set by BIPEA (Table 1). For all fatty acids, with the exception of 4:0 and 18:1*cis-9*, the mean results obtained were in the variation limits of the reference values. The divergence for 4:0 with the values set by BIPEA might be explained by the more cautious procedure for volatile fatty acids followed during the experiment: The Sovirel tubes (VWR International, Leuven, Belgium) were closed by a hermetic cap between all manipulations. The divergence for 18:1*cis-9* might be explained by the poor chromatographic resolution between this fatty acid and other 18:1 isomers.

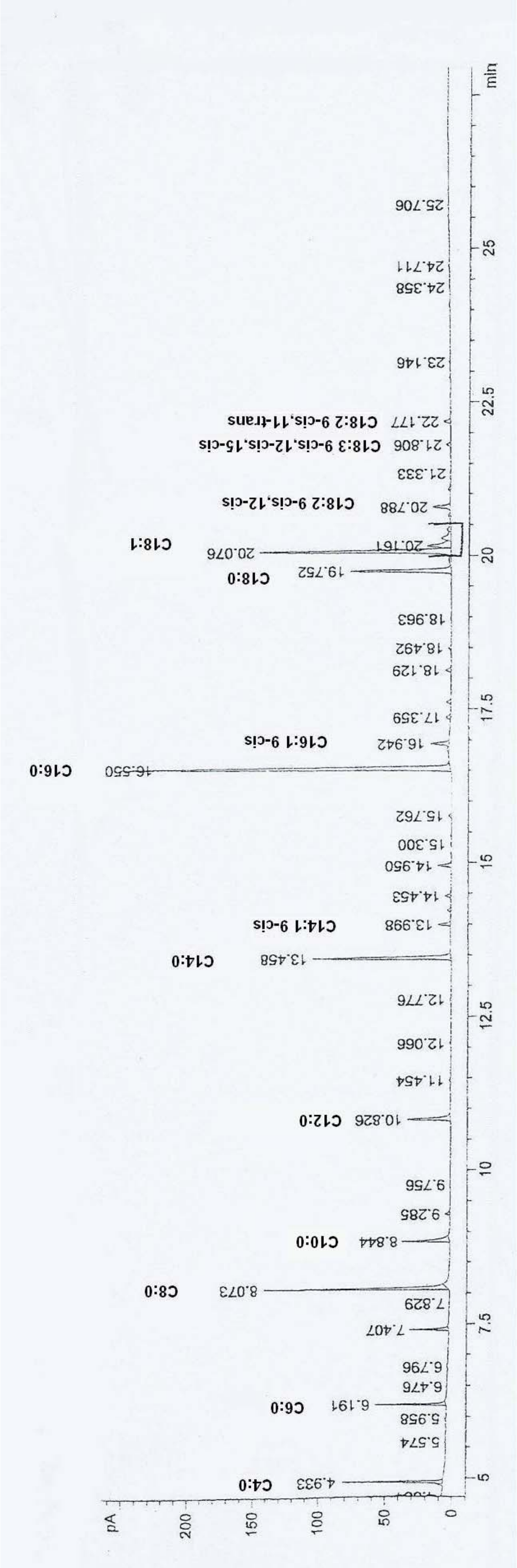

Figure 1. *Studied peaks in the chromatogram.*

Table 1 shows the coefficients of variation for the butter samples analyzed by BIPEA. In this study, repeatability for a fatty acid was considered acceptable if its coefficient of variation was less than or equal to 5 %. With good repeatability obtained for most of the fatty acids studied, 2 decimal places were used in Table 1. In this study, 6:0 and 8:0 showed poor repeatability, which was attributed to some losses owing to their relative volatility. Nevertheless, when pooled in the group of SAT, the repeatability was acceptable. Generally, good repeatability was observed for fatty acids with longer carbon chains. Overall, except for short-chain fatty acids the accuracy of the chromatographic method used was acceptable.

Table 1. *Mean concentrations of fatty acids (g/100g fat) obtained for reference butter and the reference concentrations of fatty acids in the same butter, set by BIPEA (http://www.bipea.org/)*[1].

Fatty Acids	Mean	CV	Reference value
4:0	4.43 ± 0.21	4.74	3.3 ± 0.4
6:0	2.31 ± 0.19	8.22	2.0 ± 0.4
8:0	1.72 ± 0.09	5.23	1.3 ± 0.4
10:0	3.42 ± 0.05	1.46	3.1 ± 0.4
10:1*cis-9*	0.34 ± 0.01	2.94	0.3 ± 0.4
12:0	3.83 ± 0.11	2.87	3.8 ± 0.4
14:0	12.13 ± 0.30	2.47	11.8 ± 0.9
14:1	1.08 ± 0.03	2.77	1.1 ± 0.4
15:0	1.22 ± 0.04	3.28	1.2 ± 0.4
16:0	33.35 ± 1.05	3.15	32.0 ± 2.5
16:1	1.43 ± 0.07	4.89	1.8 ± 0.4
18:0	9.97 ± 0.25	2.51	9.8 ± 0.8
18:1*cis-9*	19.41 ± 1.71	8.81	22.3 ± 1.8
18:2*cis-9,cis-12*	1.54 ± 0.05	3.25	NA
18:3*cis-9,cis-12,cis-15*	0.42 ± 0.01	2.38	0.5 ± 0.4
18:*2cis-9,trans-11*	0.64 ± 0.01	1.56	NA
SAT	69.38 ± 0.94	1.35	NA
UNSAT	30.62 ± 0.94	3.07	NA
MONO	27.73 ± 0.93	3.35	NA
POLY	2.89 ± 0.12	4.15	NA

[1] Mean = mean ± standard deviation estimated from 7 samples of the same BIPEA butter; Reference value = mean ± tolerance estimated by 26 laboratories; NA = value not set by BIPEA; SAT = saturated fatty acids; UNSAT = unsaturated fatty acids; MONO = monounsaturated fatty acids; POLY = polyunsaturated fatty acids.

4.5.2. Calibration Equations

The applied PLS analysis resulted in equations with approximately 10 factors combining more than 500 values in each equation.

The potential for estimating the fatty acid composition in milk using MIR spectrometry might be explained by the absorptions of electromagnetic radiations at frequencies that are correlated to the vibrations of specific chemical bonds within molecules (Coates, 2000). This explanation is easy with a simple matrix as a mix of 2 different components, but the milk matrix is very complex. The spectrum is therefore the result of successive interactions due to the chemical bonds from all the constituents (fatty acids, proteins, lactose, *etc*.). By comparing the milk spectrum and specific fatty acid spectrum, the principal MIR regions that were implicated in estimating the fatty acid profile were located between 1,736 and 1,805 cm^{-1} and between 2,823 and 3,016 cm^{-1}. The implication of the first region is logical because Coates (2000) indicated that 1,745 cm^{-1} is the frequency correlated to the vibration of fatty acids carbonyl group.

Table 2 shows the estimated statistical parameters for each calibration equation. These show that the correlations for predicting fatty acid concentrations in milk were better than those for predicting the same fatty acid concentrations in milk fat. This might be explained by a different dispersion of values obtained for concentrations of fatty acids in milk or milk fat. Indeed, 2 milk samples can have the same fat profile but different percentage of fat in the milk. The profile values expressed in grams per deciliter of milk were autocorrelated more closely than those expressed in grams per 100 grams of fat. The reference values used to establish the predicted equation for fat in milk came from the predicted values obtained using the Milkoscan FT6000. This explains the high result of R^2_{CV} obtained for this calibration equation.

The SEC parameter underestimates the mean square error of the model because the residual variance is not taken into account. Therefore, SEC neglects the variance of regression coefficients and, in the context of calibration model validation, SECV is preferred (Prévot, 2004). To estimate the efficiency of the calibration, the RPD was calculated (Table 2, Figure 2). For potential use, high R^2_{CV} and high RPD parameters would be required. Generally, if the value of R^2_{CV} was high, the value of RPD was high (Table 2).

Based on the results in Table 2, our study also showed that there was a second-order polynomial relationship ($y = 0.0374\ x^2 + 0.7321\ x + 1.3446$) between the fatty acid concentrations in milk and the value of RPD ($r^2 = 0.75$). Generally, therefore, if the concentration of fatty acid is high, the potential for predicting concentration using MIR spectrometry analysis is also high.

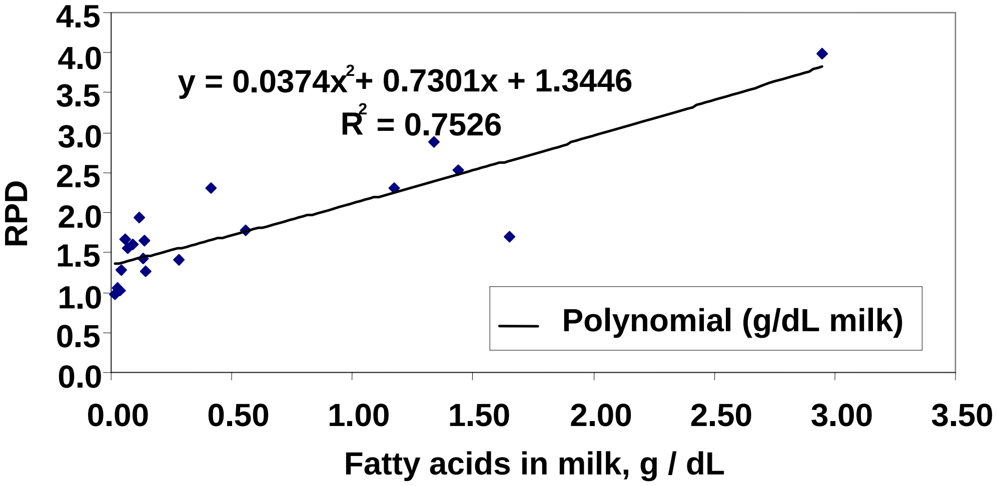

Figure 2. *Variation trend of RPD, the ratio of the standard error of cross-validation (SECV) to the standard deviation (SD) as a function of concentration of fatty acids in milk.*

To verify whether the predicted concentrations of fatty acids obtained by the calibration equations were due to real absorbance of these fatty acid or only to the correlations between total fat content and fatty acids, the correlations between total fat and the studied fatty acids were calculated (Table 3). If the calibration correlations (R_{CV}) were not due to real absorbances specific to fatty acids, these correlations would not be higher than the correlations between total fat and fatty acids. Thus, the predicted concentration for these fatty acids resulted more from a real infrared prediction than the correlation with the fat.

Table 2. *Estimated statistical parameters for each calibration equation that estimated the concentrations of fatty acid in milk (g/dL of milk) and in milk fat (g/100 g of fat).*

	g/dL of milk							g/100 g of fat						
Fatty acids	Mean	SD	SEC	R^2_C	SECV	R^2_{CV}	RPD	Mean	SD	SEC	R^2_C	SECV	R^2_{CV}	RPD
FAT	4.55	1.18	0.05	1.00	0.06	1.00	20.90	NA	NA	NA	NA	NA	NA	NA
4:0	0.28	0.11	0.07	0.59	0.08	0.51	1.41	6.26	2.02	1.42	0.50	1.60	0.39	1.27
6:0	0.13	0.06	0.04	0.69	0.04	0.52	1.43	2.90	1.26	0.97	0.41	0.98	0.41	1.28
8:0	0.07	0.03	0.02	0.75	0.02	0.59	1.55	1.54	0.68	0.43	0.60	0.50	0.46	1.35
10:0	0.14	0.06	0.03	0.77	0.04	0.64	1.65	3.06	1.31	0.69	0.72	0.90	0.53	1.45
10:1*cis-9*	0.01	0.01	0.01	0.05	0.01	0.01	0.98	0.27	0.16	0.10	0.64	0.12	0.45	1.33
12:0	0.12	0.04	0.02	0.82	0.02	0.74	1.93	2.71	0.87	0.38	0.81	0.53	0.64	1.65
14:0	0.41	0.12	0.04	0.90	0.05	0.82	2.30	9.28	1.95	0.87	0.80	1.14	0.67	1.71
14:1*cis-9*	0.03	0.01	0.01	0.12	0.01	0.07	1.02	0.71	0.32	0.26	0.34	0.28	0.23	1.13
15:0	0.04	0.01	0.01	0.58	0.01	0.40	1.28	0.98	0.29	0.18	0.61	0.20	0.53	1.44
16:0	1.17	0.39	0.11	0.91	0.17	0.82	2.30	25.67	4.89	1.63	0.89	3.50	0.50	1.40
16:1*cis-9*	0.06	0.03	0.02	0.75	0.02	0.65	1.66	1.32	0.46	0.18	0.86	0.37	0.37	1.24
18:0	0.56	0.24	0.12	0.73	0.13	0.69	1.77	11.97	2.87	2.66	0.14	2.77	0.09	1.04
18:1	1.34	0.51	0.12	0.95	0.18	0.88	2.88	29.19	5.74	3.14	0.70	3.99	0.53	1.44
18:2*cis-9,cis-12*	0.09	0.03	0.02	0.76	0.02	0.62	1.61	1.96	0.46	0.35	0.41	0.44	0.11	1.05
18:3*cis-9,cis-12,cis-15*	0.03	0.01	0.01	0.20	0.01	0.14	1.06	0.58	0.22	0.19	0.27	0.20	0.20	1.10
18:2 *9-cis,11-trans*	0.04	0.02	0.02	0.12	0.02	0.07	1.02	0.82	0.45	0.20	0.80	0.37	0.34	1.21
SAT	2.95	0.78	0.12	0.98	0.20	0.94	3.99	64.87	6.13	2.94	0.77	3.75	0.63	1.64
UNSAT	1.65	0.57	0.29	0.74	0.34	0.66	1.69	35.13	6.13	2.94	0.77	3.75	0.63	1.64
MONO	1.44	0.55	0.18	0.89	0.22	0.85	2.54	31.74	5.87	3.26	0.69	4.10	0.52	1.43
POLY	0.14	0.05	0.03	0.43	0.04	0.39	1.27	3.39	0.77	0.68	0.22	0.74	0.10	1.05

[1] SEC = standard error of calibration; R^2_C = calibration coefficient of determination; SECV = standard error of cross-validation; R^2_{CV} = Cross-validation coefficient of determination; RPD = Ratio of standard error of cross validation to standard deviation; NA = data not available; SAT = saturated fatty acids; UNSAT = unsaturated fatty acids; MONO = monounsaturated fatty acids; POLY = polyunsaturated fatty acids.

It is interesting to observe that the differences between correlation with fat and R_{CV} for short chain fatty acids (4:0, 6:0, 8:0) were higher than the others. Therefore, specific spectral information can be extracted by the PLS model independently from the correlation with total fat.

Table 3. *Correlations between the percentage of milk fat and different concentrations of studied fatty acids in milk.*

	Correlation FAT[1]	R_{CV}[2]
C4:0	0.38	0.71
C6:0	0.24	0.72
C8:0	0.21	0.77
C10:0	0.14	0.80
C10:1 *9-cis*	-0.04	0.09
C12:0	0.21	0.86
C14:0	0.36	0.90
C14:1 *9-cis*	0.08	0.26
C15:0	0.09	0.63
C16:0	0.59	0.90
C16:1 *9-cis*	0.48	0.80
C18:0	0.68	0.83
C18:1	0.62	0.94
C18 :2 9-cis,12-cis	0.51	0.79
C18 :3 9-cis,12-cis,15-cis	0.20	0.37
C18 :2 9-cis,11-trans	0.05	0.26

[1] Correlation FAT = Value for the correlation between the percentage of milk fat and different concentrations of fatty acid; [2] R_{CV} = Square root of the R^2_{CV} value.

4.6. Conclusions

The estimation of fatty acid concentrations in milk and in milk fat using MIR spectrometry seems feasible. Results from this study showed that the calibration equations that predict 10:0, 12:0, 14:0, 16:0, 16:1*cis-9*, 18:1, 18:2*cis-9,cis-12*, SAT, and MONO in milk could be used. Although some fatty acids present in low concentrations in milk could not be predicted accurately (*e.g.*, n-3 and 14:1*cis-9*), MIR spectrometry could predict most fatty acids (*e.g.*, 14:0, 16:0, 18:0, 18:1, SAT, and MONO). Indeed, most associated coefficients of determination were clearly lower than 1, but they were significantly different from zero. Therefore, and because of the speed of analysis and the present application of the methodology in routine milk testing, MIR spectrometry is an interesting alternative in the dairy sector for providing indications of the fatty acid profiles in cow milk. This option seems particularly useful for application in milk recording schemes. The composition of milk fat for each milk sample from an animal could be estimated using the calibration equations established in this study. The estimates based on MIR could be used as indicator traits for real underlying fatty acid concentrations, potentially in a multi-trait setting using the appropriate selection index theory. Knowledge of these genetic values would open up opportunities for animal selection aimed at improving the nutritional quality of cow milk.

4.7. Acknowledgments

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Chapter V

VARIATION IN FATTY ACID CONTENTS OF MILK AND MILK FAT WITHIN AND ACROSS BREEDS

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5.1. Interpretive Summary

This study showed differences on fatty acid profile in cow milk within and among breeds. These findings are useful to the farmers and for the dairy industry. By producing milk with a higher nutritional quality, the farmer could obtain a higher financial return. Cows selected on fatty acid contents could produce milk with superior quality.

5.2. Abstract

The aim of this research was to study the potential for selection of cows with a higher nutritional quality of milk fat by studying the differences in fatty acid profiles within and across the following breeds: Dual Purpose Belgian Blue, Holstein-Friesian, Jersey, Montbeliarde and non Holstein Meuse-Rhine-Yssel type Red and White. Six hundred milk samples from 275 animals were taken from 7 herds. Several types of fatty acids in milk and milk fat were quantified using mid-infrared spectrometry and previously obtained calibration equations. Statistical analyses were made using a mixed linear model with a random animal effect. The variance components were estimated by using REML. Results showed breed differences for the fatty acid profile. The repeatability estimate obtained in the present study may suggest the existence of moderate additive genetic variance for the fatty acid profile within each breed. Results also indicated variation for each analyzed milk component in the whole cow population studied. Genetic improvement of the nutritional quality of milk fat based on fatty acid profiles might be possible, and further research and development are warranted.

(**Keywords:** milk quality, breed difference, fatty acid, fat)

5.3. Introduction

Improving the nutritional quality of milk fat has been the topic of recent research. Feed supplementation, the most popular way to improve the nutritional quality of milk, presents certain disadvantages. First, this approach ignores the animal genetic effect, even through the effect of genetics on milk components such as milk fat has been demonstrated previously. Second, this improvement is not permanent. Indeed, if the supplementation is stopped, the additional nutritional quality disappears. The advantages of genetic approach are linked to these disadvantages: Genetic improvement is permanent and has the advantage of creating additional value through selection.

Average cow milk fat contains 70% saturated fatty acids (**SAT**), 25% monounsaturated fatty acids (**MONO**), and 5% polyunsaturated fatty acids (**POLY**) (Grummer, 1991). A combination of milk lipids more favorable to human health would be around 30% SAT (Pascal, 1996), 60% MONO, and 10% POLY (Hayes and Khosla, 1992). Fatty acid profile of cow's milk is therefore far from optimal. However, the observed variations in SAT, MONO, and POLY suggest that the milk fat composition can be modified by various means (*e.g.*, feeding and genetics) to be brought closer to the optimal profile (Palmquist *et al.*, 1993). Many results are available describing the influence of feeding (*e.g.*, Demeyer and Doreau, 1999; Chilliard *et al.*, 2000); however, many fewer are available on breed and individual genetic differences, even through variation in the overall milk fat among breeds is well known (*e.g.*, Interbull, 2005). Also, not all the fatty acids in a specific class (SAT, MONO, or POLY) have the same effect on human health. In the case of SAT, although myristic acid is known for its negative effect on cardiovascular diseases, stearic acid does not seem to have this effect (Hu *et al.*, 1999). Similarly, in POLY, the n-6 fatty acids appear to have negative effects on human health because of their overabundance in the Western diet. The current ratio of n-6:n-3 is estimated to be 15-20 to 1 in the human Western diet (Simopoulos, 2003). It is therefore important to check the global fatty acids profile in milk if one wants to assess the nutritional quality of bovine milk fat.

An alternative way to study the nutritional quality of milk fat is to analyze the variations of Δ^9-desaturase activity. This enzyme is important to the fatty acid composition in milk because it is responsible for the majority of MONO and the totality of conjugated fatty acids (**CLA**) in milk. The Δ^9-desaturase activity can be studied by analyzing the ratio of product to substrate (14:1 *cis-9* to 14:0, 16:1 *cis-9* to 16:0, 18:1 *cis-9* to 18:0, and 18:1 *trans-11* to CLA). Through this methodology, Lock and Garnsworthy (2003) studied the seasonal variation in the activity of this enzyme and Peterson *et al.* (2002) showed individual animal differences in Δ^9-desaturase activity.

Studying the genetics of the nutritional quality of milk requires a large amount of data. Many previous studies have used chromatographic analyses to estimate the fatty acid content in milk fat (*e.g.*, Bobe *et al.*, 1994; DePeters *et al.*, 1995). This method is efficient (*e.g.*, Dorey *et al.*, 1988; Collomb and Bühler, 2000) but requires a lengthy analysis, expensive reagents, and highly skilled staff. Therefore, these studies are generally restricted in the number of animals and samples available (*e.g.*, 234 cows for Bobe *et al.*, 1994; 90 cows for DePeters *et al.*,1995). Mid-infrared (**MIR**) spectrometry is a faster method of estimating the different components of milk (up to 500 samples/h; Foss, 2005). This technology is currently integrated into milk recording to measure different components as the percentage of fat and protein in milk. Unfortunately, it is used only for major components because adapted calibration equations are not available. A recent study by Soyeurt *et al.* (2006) provided the first calibration equations for estimating the contents of different fatty acid in milk.

Similar to its current use in routine milk recording, MIR spectrometry is an interesting alternative for providing indications of the fatty acid content in cow’s milk for the dairy sector (Soyeurt *et al.*, 2006). The predicted concentrations of fatty acid based on MIR spectrometry could be used as indicator traits for the actual underlying fatty acid concentrations. The objective of this project was to study predicted results for the fatty acid contents in dairy cattle milk and Δ^9-desaturase activity based on MIR spectrometry of milk and milk fat.

5.4. Materials and Methods

5.4.1. Animal Population and Milk Samples

Six hundred milk samples were collected from 275 cows in 7 herds. The herds were selected using several criteria: their participation in Walloon milk recording, the observed variation in the percentage of milk fat and the number of different breeds in the herds. Twenty-four-hour milk samples, representing 50% of the morning milk and

50% of the evening milk, were taken from all cows in each reference herd during the routine milk recording.

Samples were collected from April to June 2005 from 6 breeds: Dual Purpose Belgian Blue (**DPB**), Holstein-Friesian (**HOL**), Jersey (**JER**), Montbeliarde (**MON**), Normande (**NOR**) and non Holstein Meuse-Rhine-Yssel type Red and White breeds (**RED**). This time period covered no grazing feeding for the samples taken in April, partial grazing feeding for the samples taken in May, and total grazing feedings for the samples taken in June. Because of technical issues, the number of test days was not constant for all herds. Also, some cows were dried off or calved during this experiment. Breed composition was determined out of the known pedigrees of the animals. A certain percentage of genes were of unknown origin. Therefore, in the analysis, these genes were treated as though provided by another breed. Only 2 NOR cows were included in the animal population (Table 1). Also, because of technical problems with spectra files acquisition, only fatty acids data were obtained from NOR, and we decided that these data were insufficient to study the variation in fatty acid content for this breed. The presence of the HOL genes (20 to 83.33% of average breed composition of a given herd) on all farms allowed us to study them at the same time and theoretically to separate environmental, breed, and animal effects (Table 1).

Table 1. *Average breed composition of studied herds (in %)*[1].

	Herds						
Breed	1	2	3	4	5	6	7
DPB [1]		43.53	17.95				
RED [2]	30.62		25.64	14.57		5.61	12.92
HOL [3]	62.66	22.06	51.28	41.60	20.00	79.04	83.33
JER [4]					71.96		
MON [5]				41.70			
NOR [6]			5.13				
Unknown origin	6.72	34.41		2.13	8.04	15.35	3.75

[1] DPB = Dual Purpose Belgian Blue breed; RED = non-Holstein Red and White breed; HOL = Holstein-Friesian breed; JER = Jersey breed; MON = Montbeliarde breed; NOR = Normande breed.

5.4.2. Predicted Concentrations of Fatty Acid in Milk and Milk Fat

All milk samples were analyzed by an MIR spectrometer (Foss Milkoscan FT6000; Foss, Hillerød, Denmark), an instrument that also provided the standard milk recording analyses (Foss, 2006). The calibration equations used were those obtained by Soyeurt *et al.* (2006). We considered only those that were reasonably reliable, with cross-validation coefficients of determination above or equal to 0.60. The calibration equations enabled prediction of 12:0, 14:0, SAT in milk fat (g/100 g of fat), 12:0, 14:0, 16:0, 16:1 *cis-9,* 18:0, 18:1, 18:2 *cis-9,cis-12*, SAT, and MONO in milk (g/dL of milk).

5.4.3. Differences of Fatty Acid Profiles

Predicted contents for the different fatty acids in milk or milk fat were analyzed using the following single-trait mixed model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where:

$\mathbf{y}$ is the vector of observations (fat, fatty acid content in milk or milk fat); $\boldsymbol{\beta}$ is the vector of fixed effects (herd x test day, parity number, stage of lactation, regressions on breed composition because many animals were crossbred; when the fatty acid content in milk fat was analyzed, a regression for the milk fat percentage was added to prevent bias attributable to variation in the fat percentage in milk); $\mathbf{u}$ is the vector of random repetition on animal effect; $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices; $\mathbf{e}$ is the vector of random residual effects.

Animals were considered unrelated because tests using the relationship matrix and separated genetic and permanent environmental effects did not converge. Computations were done with PROC MIXED (SAS Institute, 1999), and variance components were estimated by REML. Results for regression on breed composition were reported in comparison with the reference HOL breed. To allow an optimal comparison among studied breeds and traits, results are reported in standardized values. The standard deviations used were based on the total variances given in Tables 2, 3, 4 and 5.

Animal repeatability was estimated from the ratio of animal variance to total variance. Overall individual effects were estimated from the sum of the breed and the specific animal effects. These individual effects should be considered as corrected phenotypic values for the different cows. Finally, the phenotypic correlations among traits were also estimated based on the correlations among these individual effects.

As mentioned, Δ^9-desaturase activity is important for explaining the variation in fatty acid composition because it has been implicated in the production of the majority of MONO and all CLA present in milk. With the MIR predictions of 14:1 *cis-9*, 16:1 *cis-9*, 18:1, 14:0, 16:0, and 18:0, the product-to-substrate ratios were computed and analyzed with the same mixed model as that envisaged for the study of fatty acids in milk.

5.5. Results and Discussion

5.5.1. Differences in Fatty Acid Contents in Milk and Milk Fat Among Breeds

Table 2 shows the mean and standard deviation for each milk fat component estimated for the 600 milk samples. Tables 3, 4, and 5 report differences in fatty acid content of milk, fatty acid content of fat, and Δ^9-desaturase activity among breeds, respectively. In these 3 tables, differences are presented with their associated P-values which allowed us to test the overall significance level of breed differences and the significance of comparisons between a given breed and HOL. Because the differences may not be easy to visualize, Figures 1, 2, and 3 also show graphical representations of differences among the studied breeds for the same traits.

Even though the best indicator for the Δ^9-desaturase activity is theoretically the ratio of 14:1 *cis-9* to 14:0 because 14:1 *cis-9* is derived only through desaturation (Lock and Garnsworthy, 2003), the best predictor of Δ^9 activity for this study was the ratio of 16:1 *cis-9* to 16:0. This is because of the low value for the cross validation coefficient of determination obtained from the calibration equation that predicted the 14:1 *cis-9* concentration in milk. The ratio of 16:1 *cis-9* to 16:0 would therefore be preferred (Soyeurt *et al.*, 2006). As explained before, because Δ^9-desaturase has been implicated in the production of fatty acids, its activity was studied, because it might partially explain the observed differences in the fatty acid profiles of milk fat.

Table 2. *Mean, standard deviation, individual standardized variation, repeatability and total variance estimate for milk and milk fat components (n = 600)*[1].

	Milk (g/dL of milk)						Milk fat (g/100 g of fat)					
Milk component	Mean	SD	Min	Max	Rep (%)	σ^2 (g/dL of milk)²	Mean	SD	Min	Max	Rep (%)	$\sigma^{2\ 4}$ (g/100 g of fat)²
Fat	4.30	0.90	-1.34	2.69	47.83	0.36						
SAT	2.88	0.67	-1.43	3.03	55.99	0.19	66.44	4.06	-2.38	1.68	54.64	7.25
MONO	1.27	0.32	-1.44	2.79	44.48	0.056						
12:0	0.12	0.03	-1.88	3.29	60.91	0.00058	2.84	0.47	-2.25	2.40	57.69	0.15
14:0	0.41	0.10	-1.41	2.98	55.26	0.0044	9.67	1.04	-2.18	1.75	48.24	0.69
16:0	1.13	0.31	-1.54	2.33	49.73	0.033						
16:1 *cis-9*	0.05	0.02	-1.49	2.61	41.33	0.00014						
18:0	0.52	0.15	-1.23	2.63	49.63	0.0099						
18:1	1.16	0.31	-1.45	2.76	43.91	0.046						
18:2 *cis-9,cis-12*	0.08	0.02	-1.34	3.40	51.78	0.00016						

[1] min = minimum estimated individual variation for each studied component in milk and milk fat; max = maximum estimated individual variation for each studied component in milk; Rep = repeatability; σ^2 = total variance; SAT = saturated fatty acids; MONO = monounsaturated fatty acids.

Table 3. *Standardized differences in fatty acid content in milk (g/dL of milk) between studied breeds and Holsteins, and P-values for differences among all breeds (in parentheses)*[1].

	Milk fat and fatty acids (g/dL of milk)									
	FAT	SAT	MONO	12:0	14:0	16:0	16:1	18:0	18:1	18:2
Breeds	($P < 0.001$)	($P < 0.001$)	($P = 0.027$)	($P < 0.001$)	($P <0.001$)	($P = 0.003$)	($P = 0.074$)	($P < 0.001$)	($P = 0.099$)	($P = 0.018$)
DPB	-0.48	-0.56	-0.47	-0.11	-0.30	-0.69	-0.54	-0.49	-0.21	-0.09
	($P = 0.135$)	($P = 0.087$)	($P = 0.140$)	($P = 0.731$)	($P = 0.349$)	($P = 0.033$)	($P = 0.089$)	($P = 0.128$)	($P = 0.499$)	($P = 0.786$)
RED	-0.12	-0.01	-0.40	0.32	0.13	-0.15	-0.52	-0.08	-0.31	-0.23
	($P = 0.625$)	($P = 0.982$)	($P = 0.116$)	($P =0.226$)	($P = 0.627$)	($P = 0.549$)	($P = 0.038$)	($P = 0.745$)	($P = 0.214$)	($P = 0.370$)
MON	0.56	0.73	0.08	0.97	1.12	0.50	-0.29	0.68	0.11	0.19
	($P = 0.098$)	($P = 0.037$)	($P = 0.814$)	($P =0.007$)	($P = 0.001$)	($P = 0.148$)	($P = 0.380$)	($P = 0.048$)	($P = 0.733$)	($P = 0.581$)
JER	1.46	1.60	0.95	1.37	1.40	1.16	0.43	1.45	0.88	1.25
	($P < 0.001$)	($P < 0.001$)	($P = 0.009$)	($P < 0.001$)	($P < 0.001$)	($P = 0.002$)	($P = 0.230$)	($P < 0.001$)	($P = 0.016$)	($P = 0.001$)

[1] SAT = saturated fatty acids; MONO = monounsaturated fatty acids; DPB = Dual Purpose Belgian Blue; RED = Red and White; MON = Montbeliarde; JER = Jersey.

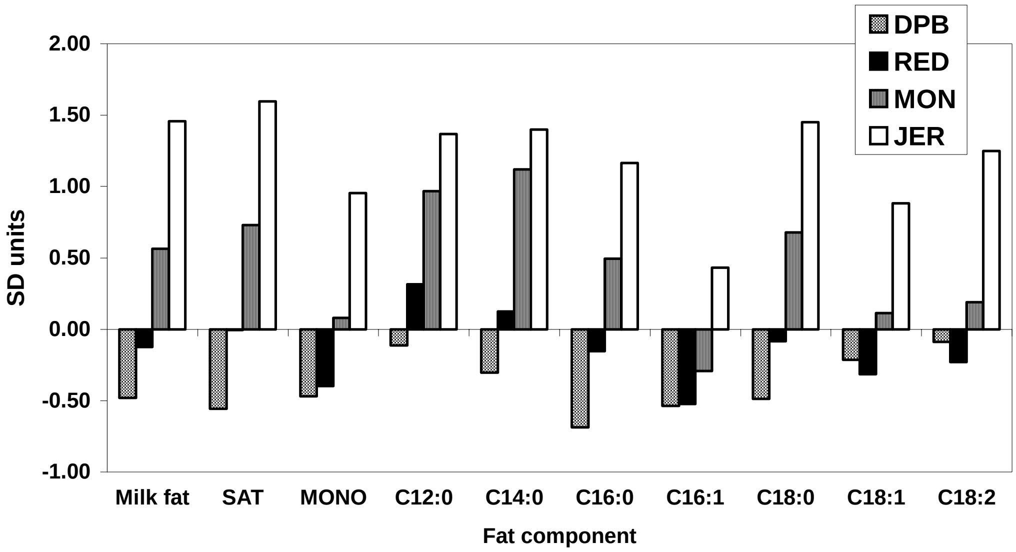

Figure 1. *Differences in fat components (18:2 = 18:2 cis-9,cis-12; MONO = monounsaturated fatty acids; SAT = saturated fatty acids) of milk [standard deviation (SD) units] for 4 breeds (DPB = Dual Purpose Belgian Blue; JER = Jersey; MON = Montbeliarde; RED = Red and White) compared with Holsteins.*

Globally, the effect of breed was often significant ($P < 0.05$). Tables 3, 4 and 5 show generally low P-values for every trait studied except for 16:1 *cis-9* ($P = 0.074$), 18:1 ($P = 0.099$) in milk, SAT in fat ($P = 0.334$), and the ratios of 18:1 to 18:0 ($P = 0.092$) and 14:1 *cis-9* to 14:0 ($P = 0.319$). In particular, Table 5 shows that breed effect had a significant impact on Δ^9-desaturase activity for the 16:1 *cis-9* to 16:0 ratio ($P = 0.044$), the best predictor used in our study. Kelsey *et al.* (2003) also showed that the Δ^9-desaturase activity differed between breeds (HOL and Brown Swiss), but this effect was minor. This minor impact might be explained by the low number of breeds studied by Kelsey *et al.* (2003).

As shown in Table 3, JER showed the greatest difference in milk fatty acid content compared with HOL. The differences between JER and HOL in fatty acid profile of milk were generally significant except for 16:1 *cis-9* ($P = 0.230$). In addition, the JER milk contained a SAT fraction of higher nutritional quality than that in HOL milk (Figure 1), a result which has previously been mentioned by Palmquist and Beaulieu (1992), DePerters *et al.* (1995), Hermansen and Lund (1990), and Beaulieu and Palmquist (1995). Although the milk of JER cows contained a higher percentage of fat than that of HOL cows (Figure 1), there were no significant differences between these 2 breeds concerning the proportion of SAT in milk fat after correcting for fat percentage differences (Table 4; P = 0.460). This observation might be explained by the similar Δ^9-desaturase activity observed for these 2 breeds (Table 5 and Figure 3). The SAT fraction in JER milk fat was slightly higher, and the unsaturated fraction was therefore a bit lower, than in HOL (Figure 2), but the difference was not significant (P = 0.460). This result was in agreement with Palmquist and Beaulieu (1992), who indicated that 18:1 was 15% lower in JER than in HOL milk fat, as well as with DePerters *et al.* (1995) and Beaulieu and Palmquist (1995).

Table 4. *Standardized differences in fatty acid content in fat (g/100 g of fat) between studied breeds and Holsteins, and P-values for differences among all breeds (in parentheses)*[1].

Breeds	Saturated milk fat and fatty acids (g/100 g of fat)		
	SAT (P = 0.334)	12:0 (P = 0.011)	14:0 (P = 0.032)
DPB	-0.22 (P = 0.495)	0.01 (P = 0.976)	0.04 (P = 0.912)
RED	0.37 (P = 0.157)	0.15 (P = 0.569)	0.19 (P = 0.452)
MON	0.39 (P = 0.257)	1.08 (P = 0.002)	0.97 (P = 0.005)
JER	0.29 (P = 0.460)	0.76 (P = 0.052)	0.57 (P = 0.129)

[1] SAT = saturated fatty acids; DPB = Dual Purpose Belgian Blue; RED = Red and White; MON = Montbeliarde; JER = Jersey.

With regard to DPB, Tables 3 and 4 show that the fatty acid profiles linked to milk or fat between this breed and HOL were slightly different; however, except for 16:0, these differences were not significant (P = 0.033). Although the DPB had the lowest concentrations in milk fat, SAT, and MONO in milk, the concentrations of 18:1 and 18:2 *cis-9,cis-12* observed for this breed were not the lowest across studied breeds (Figure 1). This confirms an unsaturated fraction in DPB milk fat that was more important than in the milk fat of the other studied breeds. This is supported by the fact that the milk fat of DPB contained the lowest proportion of SAT (Figure 2). One has to consider these results in the light of a correction for milk fat percentage; therefore, the SAT contents were comparable even though the fat percentages were different. These observations might be explained by the activity of Δ^9-desaturase for this breed (Figure 3). Indeed, for all 3 ratios, DPB had higher Δ^9-desaturase activity compared with all the other breeds. Even though these results were not significant, they may assist in understanding the findings from this study.

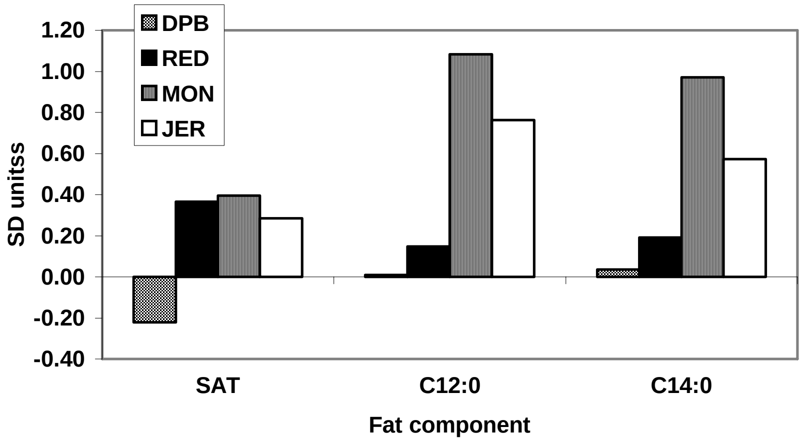

Figure 2. *Differences in fat components (SAT = saturated fatty acids) of milk fat [standard deviation (SD) units] for 4 breeds (DPB = Dual Purpose Belgian Blue; JER = Jersey; MON = Montbeliarde; RED = Red and White) compared with Holsteins.*

Table 5. *Standardized differences in Δ^9-desaturase activity between the studied breeds and Holsteins, and P-values for differences among all breeds (in parentheses)*[1].

	Δ^9-desaturase activity		
Breed	18:1 to C18:0 ratio (*P* = 0.092)	16:1 to C16:0 ratio (*P* = 0.044)	14:1 to C14:0 ratio (*P* = 0.319)
DPB	0.30 (*P* = 0.345)	0.06 (*P* = 0.853)	0.20 (*P* = 0.518)
RED	-0.38 (*P* = 0.136)	-0.60 (*P* = 0.018)	-0.21 (*P* = 0.411)
MON	-0.72 (*P* = 0.353)	-0.67 (*P* = 0.047)	-0.62 (*P* = 0.064)
JER	-0.01 (*P* = 0.989)	-0.17 (*P* = 0.643)	-0.06 (*P* = 0.866)

[1] DPB = Dual Purpose Belgian Blue; RED = Red and White; MON = Montbeliarde; JER = Jersey.

Similarly, the milk fat of RED and MON contained the highest proportion of SAT (Figure 2). In particular, the MON milk fat contained a significantly higher proportion of 12:0 and 14:0 than the milk fat from HOL cows (Table 4; respectively, P = 0.02 and P = 0.005). This might be explained by a significantly lower Δ^9-

desaturase activity for these 2 breeds compared with HOL (Figure 3). However, Lawless *et al.* (1999) indicated that the MON milk fat had a low fraction of SAT and that the concentrations of 12:0 and 14:0 were equal to those observed in HOL milk fat. This divergence might be explained by the fact that the results obtained by Lawless *et al.* (1999) were raw phenotypic values and therefore might have been much more influenced by other factors, for example, by feeding practices and stage of lactation. In the current study, breeds were compared on the same farms, and variation among individual animals was corrected for different fixed effects.

5.5.2. Differences in Fatty Acid Profiles of Milk and Milk Fat Within Breeds

For all the studied components (proportions of SAT, 12:0, and so on in milk and in milk fat), the estimated values of repeatability were high (Table 2) and ranged from to 41.33 to 60.91 %. This could suggest a reasonably moderate heritability for each studied component, similar to what is known for fat (Interbull, 2005). The first study, reported by Edwards *et al.* (1973) for estimating the heritability for each fatty acid, indicated extremely high heritabilities, between 0.64 for 12:0 and 0.98 for 12:1 and 14:1. These values can be considered as high overestimations because a biaised hypothesis was used to compute the heritability. The environmental variance was estimated from the variance component within monozygous pairs. The variance components within dizygous pairs represented the environmental variance and half the genetic variance. In spite of these overestimated values, this study was the first to show high heritability for each fatty acid in milk fat.

The repeatability of milk fat components was previously shown by Karijord *et al.* (1982). For the same contents, the values obtained by Karijord *et al.* (1982) ranged from 0.10 to 0.33. The differences in values found between these 2 studies might be explained by the data analysed and the models used. The fixed effects used by Karijord *et al.* (1982) were only the effect of stage of lactation and the effect of month. The heritabilities estimated by Bobe *et al.* (1994) were 0.18 for 18:0 and 0.39 for 14:0. A similar conclusion was drawn by Renner and Kosmack (1974a) with 0.26 for short-chain, 0.20 for medium-chain and 0.30 for long-chain fatty acids. Because the heritability is also a part of the repeatability, our results suggest that the heritability of each fatty acid could be reasonably high.

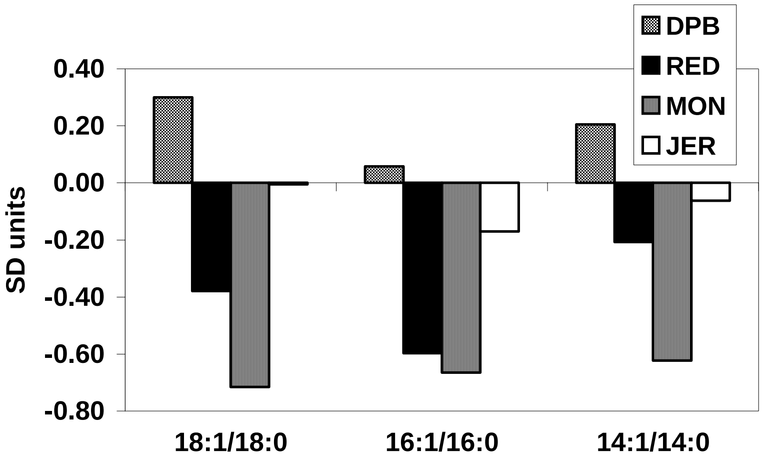

Figure 3. *Differences in activity of Δ^9-desaturase [standard deviation (SD) units] for 4 breeds (DPB = Dual Purpose Belgian Blue; JER = Jersey ; MON = Montbeliarde; RED = Red and White) compared with Holsteins.*

For Δ^9-desaturase, the estimated repeatability for the ratios of 14:1 *cis-9* to 14:0, 16:1 *cis*-*9* to 16:0, and 18:1 to 18:0 were, respectively, 43.12, 41.78, and 45.79 %. These values might also suggest also a reasonably moderate heritability. Lock and Garnsworthy (2003) previously reported the variation in Δ^9-desaturase activity within breeds. Similarly, Royal and Garnsworthy (2005) reported heritability values of 0.30 and 0.19 for the ratios of 14:1 to (14:0+14:1) and 18:1 to (18:0+18:1), respectively. Lock *et al.* (2005) and Peterson *et al.* (2002) confirmed that nutritional and individual variations in Δ^9-desaturase activity were the major factors contributing to the concentration of rumenic acid in milk.

5.5.3. Individual Variation

For the concentrations of fatty acids in milk, the mean scale of variation attributable to individual effects ranged from –1.30 to +2.80 standard deviation units, and the mean scale of variation attributable to breed effects ranged from –0.40 to +1.20 standard deviation units (Table 2). The range of individual values was higher than the variation across the selected breeds. The same conclusion applied to the concentrations of fatty acids in milk fat (Table 2).

5.5.4. Correlations

Table 6 shows the phenotypic correlations between the studied components in milk. The values shown are Pearson correlation coefficients estimated among the individual effects. In general, high positive correlations were observed, with several exceptions. The correlations between 16:1 *cis-9* and 12:0 and between 16:1 *cis-9* and 14:0 were low and negative. Low correlations between MONO and 12:0, MONO and 14:0, 14:0 and 18:1, 14:0 and 18:2 *cis-9,cis-12* were also observed. These findings are important because they suggest that animal selection based on the fatty acid profile might be possible. Bobe *et al.* (1994) obtained a phenotypic correlation of 0.69 between 14:0 and 18:0. This value is in agreement with the value obtained in this study (0.80). Also, Karijord *et al.* (1982) observed lower correlations between milk components and fat than the values obtained in this study. This might be explained by differences in the data and the models used. In addition, Renner and Kosmack (1974b) obtained high genetic correlations, with fat yield equal to 1.00 for short-chain, 0.48 for medium-chain, and –0.98 for long chain POLY. The same conclusion applies to the concentrations of fatty acids in milk fat. Indeed, high phenotypic correlations (results not shown) were found in the current study for the concentrations of fatty acids in milk fat.

Table 6. *Phenotypic correlations between studied components of milk estimated from individual effects*[1].

Component	SAT	MONO	12:0	14:0	16:0	16:1	18:0	18:1	18:2
Milk fat	0.95	0.85	0.68	0.77	0.94	0.58	0.98	0.80	0.82
SAT		0.70	0.83	0.89	0.94	0.39	0.97	0.60	0.70
MONO			0.24	0.36	0.79	0.91	0.81	0.96	0.89
12:0				0.95	0.67	-0.12	0.72	0.16	0.89
14:0					0.75	-0.01	0.80	0.31	0.39
16:0						0.54	0.95	0.68	0.72
16:1							0.52	0.87	0.75
18:0								0.75	0.79
18:1									0.87

[1] SAT = saturated fatty acids; MONO = monounsaturated fatty acids; 16:1 = 16:1 *cis-9*; 18:2 = 18:2 *cis-9,cis-12*.

5.6. Conclusions

This study revealed differences in the fatty acid content of milk across the studied breeds, which suggests the possibility of obtaining milk products with improved nutritional quality by choosing the right breed. Considerable variation within breed was also found. This might suggest a moderate heritability for each component in the fatty acid profile. The high values estimated by this study for individual variation suggest the existence of genetic variability. Finally, some low phenotypic correlations were estimated using individual effects.

Although the aforementioned results are interesting, they also suggest the need for further research, especially into the estimation of genetic parameters for milk fat components. An objective of future research should be to confirm the possibility of a differentiated quantitative selection of fatty acids to modify the nutritional quality of milk and milk fat. The final objective should be to produce healthier milk products based on genetic selection for modified global fatty acid profiles.

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Chapter VI

ESTIMATION OF HERITABILITY AND GENETIC CORRELATIONS FOR THE MAJOR FATTY ACIDS IN BOVINE MILK

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6.1. Interpretive Summary

Interest in dairy products with differentiated nutritional quality is increasing. So, the combination of feeding and genetic could give new perspectives for the dairy industry and good opportunities for farmers. The positive impact of feeding on fatty acids profile has already been shown. So, the aim of this study was to investigate the genetic variability of these milk components. The genetic parameters (moderate heritabilities and genetic correlations) observed for each major fatty acid could suggest the possibility to select animals based on the nutritional quality of their milk fat.

6.2. Abstract

The current cattle selection program for dairy cattle in the Walloon Region of Belgium does not consider the relative content of the different fatty acids (FA) in milk. However, interest by the local dairy industry in differentiated milk products is increasing. Therefore, farmers may be interested in selecting their animals based on the fat composition. The aim of this study was to evaluate the feasibility of genetic selection to improve the nutritional quality of bovine milk fat. The heritabilities and correlations among milk yield, fat, protein and major FA contents in milk were estimated. Heritabilities for FA in milk and fat ranged from 5 to 38%. The genetic correlations estimated among FA reflected the common origin of several groups of FA. Given these results, an index including FA contents with the similar metabolic process of production in the mammary gland could be used, for example, to increase the monounsaturated and conjugated fatty acids in milk. Moreover, the genetic correlations between the percentage of fat and the content of C14:0 or C12:0, C16:0 and C18:0 in fat were -0.06, 0.55, 0.60 and 0.84, respectively. This result demonstrates that an increase in fat content is not directly correlated with undesirable changes in FA profile in milk for human health. Based on the obtained genetic parameters, a future selection program to improve the FA composition of milk fat could be initiated.

(**Keywords:** heritability, genetic correlation, fatty acid, mid-infrared)

6.3. Introduction

Interest in differentiated nutritional quality of dairy products is increasing in Belgium and around the world. Due to the negative reputation of milk fat for human health, the modification of milk composition presents a real interest for the dairy industry. The fat contains mainly triglycerides (96%; Grummer, 1991; Jensen, 1995). They are composed of a glycerol linked with 3 esterified fatty acids (**FA**). Many previous studies have intensively examined the effect of FA on human health (Noakes *et al.*, 1996; Hu *et al.*, 1999; Parodi, 1999; Simopoulos, 2003). Based on these results, some studies tried to modify the FA profile by feeding to obtain a fat composition more desirable for human health (Chilliard *et al.*, 2000). Despite the large number of studies regarding the effect of nutrition on FA composition, the information about the effect of animal factors on FA profile is very poor. However, few studies (Karijord *et al.*, 1982; Palmquist *et al.*, 1993; Soyeurt *et al.*, 2006a) have suggested the possibility of genetically modifying the FA profile. Thus, it could be interesting for farmers to select cows that produce milk with a particular FA composition.

Selection for improved FA profiles would be feasible only if there is sufficient genetic variation in FA composition. Until now, very few studies have estimated genetic parameters for these traits. One of the first studies estimating heritabilities in bovine milk was by Edwards *et al.* (1973), who observed very high values that ranged from 0.64 to 0.98. However, these authors did not use an optimal model. They assumed that the environmental variance was the sum of variances within monozygotic twins and that the environmental variance added to the half of genetic variance was the sum of variances within dizygotic twins. Therefore, we can assume these heritability values were probably overestimated. Renner and Kosmack (1974a) obtained estimated heritabilities of 0.26, 0.06, and 0.04 for the content of FA with short (FA< C12:0) and medium carbon chains (C12:0 to C16:0) and for the C18 family in milk fat, respectively. They also obtained estimates of 0.26, 0.25, and 0.02 for contents of FA with short and medium carbon chain and for the C18 family in milk, respectively. From their estimates, it appeared that FA content in milk is more heritable than the content of FA in milk fat. The heritabilities estimated by Karijord *et al.* (1982) were different from those observed by Renner and Kosmack (1974a). They were on average 0.13, 0.14, and 0.10 for FA contents with short and medium carbon chains and for the C18 family in milk fat, respectively.

Renner and Kosmack (1974b) were among the first scientists to estimate the genetic correlations among different FA in milk or fat. Only the content of FA with short carbon chain in milk seemed to be positively correlated with milk yield (0.24). As expected, all studied classes of FA were positively correlated with milk fat except the correlation between the content of C18 family in fat and the content of fat (**%FAT**). Karijord *et al.* (1982) studied the genetic correlations between the content of FA in %FAT and the traditional production traits like the content of protein (**%PROT**), %FAT, and the milk yield (**MILK**). As found by Renner and Kosmack (1974b), the correlation with the C18 family and %FAT was also negative. However, the values estimated by Karijord *et al.* (1982) were greater than those obtained by Renner and Kosmack (1974b). The values of genetic correlation estimated among the contents for major FA were extremely variable and ranged from -0.68 to 0.97. Globally, the contents of FA of the same class (saturated (**SAT**), monounsaturated (**MONO**) or polyunsaturated fatty acids (**POLY**)) were positively correlated. On the other hand, MONO or POLY contents were negatively correlated with SAT. However, the results must be interpreted with caution, because the heritabilities estimated for MILK and %FAT were very low in this study (0.09 for these 2 traits), indicating potential data quantity and quality problems.

The estimation of heritability and genetic correlations requires sufficient data to obtain reliable estimates. Many studies have used the data from chromatography of FA to estimate the heritabilities of FA in milk and fat (Renner and Kosmack, 1974a; Karijord *et al.*, 1982). This method to measure FA is accurate (Dorey *et al.*, 1988; Collomb and Bühler, 2000) but requires a long time for analysis, expensive reagents and well-skilled staff. Therefore, these studies have generally been restricted in the number of animals and samples available. Mid-infrared (**MIR**) spectrometry is a faster method to estimate different milk components (up to 500 samples/h ; Foss, 2006). This technology is currently routinely used by milk recording agencies to measure different components as overall concentrations of %FAT and %PROT. A recent study (Soyeurt *et al.*, 2006b) provided the first calibration equations to estimate the major FA contents in milk.

The results available for the heritabilities or genetic correlations for FA profile in bovine milk are very variable. Consequently, the aim of this study was to estimate the heritabilities and the genetic correlations among the major FA. This study used a simple test day model and FA contents predicted by MIR spectrometry. Use of this type of data facilitates an increase in the number of records and should improve the reliability of estimates.

6.4. Materials and Methods

6.4.1. Animal Population and Milk Samples

From April 2005 to May 2006, milk samples (7,700) were collected from 25 herds that represented 7 breeds (Brown Swiss, dual-purpose Belgian Blue, Holstein-Friesian, Jersey, Montbeliarde, Normande and non Holstein Meuse-Rhine-Yssel type Red and White breeds). These herds were selected using several criteria: their participation in Walloon milk recording, which was necessary to analyze samples with MIR, and the degree of pedigree completeness. The samples taken from all cows during regular visits for milk recording and comprised equal numbers from morning and evening milkings. Due to technical issues, the number of observed test days was not constant for all herds. Also, some cows were dried off or calved during this experiment.

6.4.2. Predicted Contents of Fatty Acids in Milk and Milk Fat

All samples were analyzed by using a MIR spectrometer (Foss Milkoscan FT6000; Foss, Hillerød, Denmark). Calibration equations used to predict the contents of FA in milk (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 *cis-9,cis-12*, SAT, and MONO g/dL of milk) were those developed by Soyeurt *et al.* (2006b). Using the density of milk (1.03 g/cm^3), these FA contents were transformed in grams per 100 g of milk. Using the %FAT predicted by the MilkoScan FT6000, these FA contents in milk were then converted into content in milk fat expressed as grams per 100 g of fat. Table 1 gives the mean and SD observed for all studied traits.

***Table 1.** Mean and standard deviation for each analyzed component of milk for the studied population.*

	Milk (g/100 g of milk)		Milk fat (g/100 g of fat)	
Trait	Mean	SD	Mean	SD
Milk (kg/day)[1]	23.12	8.43		
Fat (%)[1]	4.13	0.79		
Protein (%)[1]	3.47	0.40		
C12:0[2]	0.13	0.03	3.15	0.65
C14:0[2]	0.44	0.10	11.09	1.88
C16:0[2]	1.24	0.29	30.76	4.24
C18:0[2]	0.51	0.13	12.44	1.03
C18:1[2]	0.95	0.30	23.63	6.00
C18:2 *cis-9,cis-12*[2]	0.07	0.02	1.84	0.38
Saturated fatty acids[2]	2.86	0.63	70.72	6.53
Monounsaturated fatty acids[2]	1.02	0.32	25.35	5.69

[1] n = 40,007 test-day records; [2] n = 7700 test-day records.

6.4.3. Additional Information About Milk History

To have additional data for MILK, %FAT, and %PROT, the historical records for these traits of cows and herds were added to the database. Complete historic records were added for all cows since March 2005, including those cows of which no FA were available. The final edited data set contained 40,007 records on 2,047 animals.

Breed composition was determined according to the known pedigrees of these animals. A certain proportion of genes were of unknown origin, however, and thus treated as though they were provided by another distinct breed. Table 2 describes the average breed composition for the animals with records.

6.4.4. Model

Due to the computational challenges related to the number of traits, the final data set was divided into 5 runs that contained the followed traits:

MILK, %FAT, %PROT, SAT, and MONO;

SAT, MONO, and 6 major FA (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 *cis-9,cis-12*);

MILK, %FAT, %PROT, and 3 first FA (C12:0, C14:0, and C16:0);

MILK, %FAT, %PROT, and 3 18C FA (C18:0, C18:1, C18:2 *cis-9,cis-12*); and

6 FA (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 *cis-9,cis-12*).

For these 5 runs, the same simplified multitrait mixed repeatability test-day model with a constant genetic effect was used:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Wl} + \mathbf{Zp} + \mathbf{Zu} + \mathbf{e}$$

where $\mathbf{y}$ = the vector of observations (*e.g.*, MILK, %FAT, %PROT, SAT, and MONO); $\boldsymbol{\beta}$ = the vector of fixed effects (herd x test day x class of parity number, stage of lactation x class of parity number, class of age x class of parity number, and regressions on the fractions of genes for every breed other than Holstein); $\mathbf{l}$ = the vector of permanent environment random effects within lactation; $\mathbf{p}$ = the vector of permanent environment random effects across lactations; $\mathbf{u}$ = the vector of animal effects; $\mathbf{X}, \mathbf{W}$ an $\mathbf{Z}$ = incidence matrices; and $\mathbf{e}$ = the vector of random residual effects.

Table 2. *Average breed composition of the studied animal population (%).*

Breeds	Average breed composition
Dual-purpose Belgian Blue	12.31
Meuse-Rhine-Yssel type Red and White	4.31
Holstein-Friesian	45.39
Jersey	3.92
Brown Swiss	2.90
Montbeliarde	11.21
Normande	13.12
Unknown	6.85

Fixed effects were defined as follows. Stage of lactation was divided into 24 classes of 15 d each. Records with DIM <5 and >365 were deleted. Parities were grouped as first, second and third or later lactation with 14,844, 10,132 and, 15,031 records in each of the respective groups. Age at test day was defined as number of months from birth. There were 9 classes of age (for first lactation, age less than 29, 29 to 32, 33 and older; for second lactation, age less than 42, 42 to 46, 47 and older; and for the third or later lactation, age <54, 54 to 59, 60 and older).

Pedigree completeness was good, with 18,856 animals. Due to the informative pedigree, genetic and permanent environmental effects could be separated. Variance components were estimated using expectation maximisation REML and average information REML (Misztal,2007). Standard errors of estimates were obtained using average information REML (Misztal, 2007).

The variances reported are the average values measured from the results obtained by the 5 runs. Due to the separate estimation of correlations, the correlation matrices had to be bended by applying the weighted bending procedure presented by Jorjani *et al.* (2003). The weights were the number of observations used to estimate a given correlation.

6.5. Results and Discussion

6.5.1. Heritability of MILK, %FAT, %PROT, and FA in Milk

The model used allowed the estimation of genetic, 2 permanent environmental, and residual effects. Table 3 summarizes the variance components for MILK, %FAT, %PROT, SAT, and MONO and for the major FA in bovine milk (g/100 g of milk). Heritability estimates for MILK were similar to those estimated by other authors (Veerkamp and Goddard, 1998; Lidauer and Mäntysaari, 1999; Bormann *et al.*, 2003; Gengler *et al.*, 2004). Few recent authors have reported daily %FAT and %PROT heritabilities. The results obtained in this study were lower than those mentioned by Druet *et al.* (2005). The average heritabilities obtained by those authors for the first 3 lactations were 33, 37 and 47% for MILK, %PROT and %FAT, respectively. This difference could partially be explained by the type of model (random regression) and the eigenvalue approach used by Druet *et al.* (2005).

The content of SAT in milk was more heritable than MONO. The heritability of SAT was close to the value observed for %FAT. This result could be explained by the part-whole relationship among the various measures, because SAT was a major constituent of milk fat (Table 2). The heritability difference between SAT and MONO observed in this study is in line with estimates of animal-specific relative variances obtained earlier by Soyeurt *et al.* (2006a).

Table 3. *Average estimate and average standard error of variances (% of phenotypic variance) for each studied effect (genetic, 2 permanent environments, residual) with a multitrait model including the quantity of milk, the content of milk fat, the content of protein, and the content of fatty acids in milk (g/100 g of milk).*

			Permanent environment effects					
	Genetic		Within lactation		Across lactations		Residual	
Trait	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Milk	18	1.75	31	0.73	9	1.50	42	0.24
Fat (%)	32	1.78	5	0.25	5	1.20	58	0.15
Protein (%)	28	2.20	12	0.37	9	1.72	51	0.27
Saturated	36	2.13	7	0.51	6	1.43	51	0.35
Monounsaturated	15	0.98	16	0.96	1	0.36	68	0.52
C12:0	29	2.20	13	1.20	8	1.54	49	0.60
C14:0	31	2.48	12	1.24	10	1.95	48	0.53
C16:0	38	1.97	7	0.83	2	0.89	53	0.33
C18:0	30	1.86	8	0.77	4	1.08	57	0.35
C18:1	5	0.63	18	1.03	1	0.19	75	0.47
C18:2 *cis-9,cis-12*	20	1.50	12	1.13	3	0.78	66	0.63

The greatest heritability was observed for the FA having the greatest content in milk (C16:0; Tables 2 and 3). The heritability for C18:1 was very low. One possible reason for this result could be that the simple model used is suboptimal for this trait, because it explained <25% of the variation of C18:1 in total (Table 3). Although the heritability for POLY was not studied due to the precision of the calibration equation, the principal FA of this class, C18:2 *cis-9,cis-12*, had a moderate estimated heritability (Table 3).

No relationship between the length of the carbon chain and heritability was observed in milk (Table 3). This result was in opposition to Renner and Kosmack (1974a), who reported a decreasing value of heritability as a function of FA length. Heritabilities estimated in this study [29, 31, and 38%, respectively, for C12:0, C14:0, and C16:0 (Table 3)] were moderate, as were the values found by Renner and Kosmack (1974a) for the FA with medium length chains (26%). The heritability estimated by Renner and Kosmack (1974a) for the C18 family was 2%. Although the complete family of C18 was not evaluated in this analysis, the values estimated for C18:2 *cis-9,cis-12* and C18:1 were clearly greater (Table 3) than those for other FA.

6.5.2. Relative Environmental Variances of FA in Milk

For all traits, relative permanent environmental variance across lactations was smaller than relative permanent environmental variance within lactation (Table 3). The lowest within-lactation variance was estimated for %FAT and the highest for MILK. Monounsaturated fatty acids seemed to be more variable within lactation than the content of SAT in milk, which showed the same trend as %FAT. Clear separation of both types of permanent environmental estimates would have required a larger number of repeated records within and across lactations that were available for FA in this study. The results should therefore be considered preliminary.

The estimates for the residual effects mentioned in Table 3 were important, in particular for MONO and for C18:1. This observation could be an indication that the model used missed some important source of variation in MONO content in milk.

6.5.3. Heritability of FA in Milk Fat

Estimates and SE of relative variances for each random effects for SAT, MONO, and the major FA in milk fat (g/100 g of fat) are given in Table 4. Heritability estimated for SAT in fat (Table 4) was smaller than that observed for the same component in milk (g/100 g of MILK; Table 3). This observation can be generalized for all studied saturated FA. The results obtained by Renner and Kosmack (1974a) showed the same trend. However, the heritabilities for MONO and for C18:1 in fat was greater than that in milk (Table 3 and 4).

6.5.4. Relative Environmental Variances of FA in Milk Fat

Contents of SAT and MONO in fat were highly variable within lactation (Table 4). This could be linked to seasonal effects. Saturated fatty acids in fat are lowest during the grazing period. Lock and Garnsworthy (2003) suggested that a molecule contained in the grass could activate the enzymatic activity (especially Δ^9-desaturase activity).

The residual variances in Table 4 were smaller than those shown in Table 3. This observation is an indirect indication that the model used in this study seems to be more appropriated to analyze the proportion of FA in milk fat than in milk.

6.5.5. Genetic Correlations Among MILK, %FAT, %PROT, and Different FA in Milk

Table 5 shows genetic and phenotypic correlations for SAT and MONO in milk and for traditional production traits (MILK, %FAT, and %PROT). The genetic correlations between MILK and %PROT or %FAT were negative and moderate, -0.35 and -0.48, respectively. The genetic correlation between %FAT and %PROT was positive and tended to be greater in absolute value (0.63). These results are in agreement with Roman and Wilcox (2000), who estimated that the genetic correlation expressed on a lactation basis between MILK and %FAT was -0.21 and between MILK and %PROT was -0.56. These same authors also found that the genetic correlation between %FAT and %PROT was 0.63.

Table 4. *Average estimate and average standard error of variances (% of phenotypic variance) for each studied effect (genetic, 2 permanent environments, residual) with multitrait mixed models including in particular the saturated, monounsaturated, C12:0, C14:0, C16:0, C18:0, C18:1, and C18:2 cis-9,cis-12 fatty acid contents in milk fat (g/100 g of fat).*

			Permanent environment effects					
	Genetic		Within lactation		Across lactations		Residual	
Trait	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Saturated	14	1.46	27	1.50	3	0.68	55	0.43
Monounsaturated	24	2.27	25	1.70	8	1.62	43	0.37
C12:0	9	1.17	24	1.18	5	0.97	61	0.42
C14:0	19	1.75	20	1.32	7	1.64	52	0.36
C16:0	20	2.20	8	0.94	12	1.85	60	0.47
C18:0	28	2.35	14	1.40	9	2.01	50	0.57
C18:1	15	1.57	28	1.55	4	1.00	53	0.30
C18:2 *cis-9,cis-12*	15	1.79	15	1.56	6	1.43	64	0.82

The observed genetic correlations for these traditional production traits were also similar to those estimated by others (Othmane *et al.*, 2004). Given these results, we think that this simplified model is still adapted for traditional traits; however, more research is needed to establish an optimal model for FA.

The genetic correlations obtained between MILK and FA (Table 5) were all negative. This result is probably due to the effect of dilution. When the production of MILK increased, %FAT and FA contents seemed to decrease. Other scientists (Lock and Garnsworthy, 2003) have already observed this effect between MILK and %FAT.

As expected, the genetic correlations between SAT or all of the studied saturated FA and %FAT were greater than those estimated with MONO or all of the studied unsaturated fatty acids (Table 5). In the same way, the genetic correlations estimated between MONO and unsaturated FA were greater than those that involved saturated FA (Table 5).

The genetic correlations reflect the physiological processes involved in the production of FA in milk. Consequently, the values of genetic correlations can be interpreted biologically. Bobe *et al.* (1999) have already analyzed the corrected correlations existing among the FA contents. Three groups can be isolated from Table 5. The first group contains C12:0, C14:0, C16:0, and C18:0.

The high genetic correlations observed among these FA could be explained by similarities in their origin. These FA are synthesized de novo in the mammary gland and are regulated by only 2 enzymes, acetyl-coenzyme A carboxylase and fatty acid synthase (Chilliard *et al.*, 2001). The second group is composed of C18:1, C18:2 *cis-9,cis-12*, C16:0, and C18:0. These FA are extracted from the blood. The presence of C16:0 and C18:0 in 2 groups can be explained by their double origin. These FA are partially extracted from the blood and partially synthesized de novo by the mammary gland (Chilliard *et al.*, 2001). Finally, the third group contains only C18:1 and C18:2 *cis-9,cis-12*. These FA are extracted from the blood, and the biohydrogenation acts little on them (Bobe *et al.*, 1999).

Table 5. *Genetic (above the diagonal) and phenotypic correlations (below the diagonal) among each studied trait [milk yield, content of fat, content of protein, saturated (SAT), monounsaturated (MONO), C12:0, C14:0, C16:0, C18:0, C18:1, and C18:2 cis-9,cis-12 fatty acid contents in milk].*

Trait	Milk	Fat	Protein	SAT	MONO	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
Milk (kg/d)		-0.35	-0.48	-0.26	-0.21	-0.36	-0.29	-0.25	-0.28	-0.39	-0.28
Fat (%)	-0.18		0.63	0.97	0.74	0.91	0.80	0.95	0.97	0.75	0.75
Protein (%)	-0.32	0.39		0.62	0.44	0.73	0.60	0.55	0.60	0.45	0.62
SAT (g/100 g of milk)	-0.13	0.90	0.40		0.66	0.91	0.83	0.94	0.94	0.60	0.67
MONO (g/100 g of milk)	-0.17	0.72	0.15	0.49		0.44	0.21	0.61	0.63	0.86	0.81
C12:0 (g/100 g of milk)	-0.11	0.61	0.52	0.81	0.02		0.94	0.89	0.93	0.53	0.61
C14:0 (g/100 g of milk)	-0.07	0.67	0.39	0.83	0.07	0.93		0.83	0.85	0.34	0.38
C16:0 (g/100 g of milk)	-0.16	0.88	0.34	0.91	0.60	0.67	0.72		0.97	0.67	0.72
C18:0 (g/100 g of milk)	-0.13	0.94	0.34	0.91	0.67	0.67	0.74	0.92		0.73	0.75
C18:1 (g/100 g of milk)	-0.15	0.66	0.08	0.38	0.93	-0.02	0.10	0.51	0.64		0.84
C18:2 (g/100 g of milk)	-0.22	0.66	0.46	0.48	0.80	0.21	0.18	0.57	0.67	0.77	

6.5.6. Genetic Correlations Among MILK, %FAT, %PROT, and Different FA in Milk Fat

Table 6 has the genetic and phenotypic correlations estimated for each studied traits in milk fat. Results among MILK, %FAT, and %PROT were slightly different from those reported in Table 5, because they came from different analyses, and a bending procedure was applied.

Table 6 shows low to moderate negative or positive genetic correlations between MILK and the different FA in fat. In general, SAT and saturated FA tended to be negatively correlated with MILK and MONO, and C18:1 was positively correlated. Given these results, genetic selection for MILK would be expected to increase the content of MONO in fat. For C18:2, the correlation was close to zero. The genetic correlation estimated between SAT and %FAT is also given in Table 6. Greater %FAT was genetically linked to lower MONO, higher SAT, and lower C18:2, and C18:1. Genetic selection for greater %FAT content would increase nearly all SAT and decrease MONO and C18:2. However, all saturated FA did not seem to show the same response to the increase of fat. The genetic correlation between C18:0 and %FAT was high (0.84), and the genetic correlation between C12:0 and %FAT was lower (0.55). These observations could be explained by the variation of Δ^9-desaturase activity in cows observed by Lock and Garnsworthy (2003) and Soyeurt *et al.* (2006a). In the same way, the results involving the content of myristic acid (C14:0) in milk fat are interesting, because Table 6 shows a genetic correlation between C14:0 and %FAT that is close to 0 and also a low phenotypic correlation (-0.19). Also, C14:0 is highly negatively correlated with MONO (-0.84). Given its negative effects on human health (Hu *et al.*, 1999), genetic selection to increase MONO should have a beneficial effect of reducing the C14:0 content in fat. In the same way, the greatest genetic correlations with %FAT were observed for C16:0 (0.60) and C18:0 (0.84) compared with 0.37 estimated for C14:0. Hu *et al.* (1999) found that C16:0 and C18:0 are known for their low to nonexistent effects on human health; therefore, the increase of %FAT in bovine milk does not seem to involve an undesirable milk fat composition for human health.

Table 6. *Genetic (above the diagonal) and phenotypic correlations (below the diagonal) among each studied trait [milk yield, content of fat, content of protein, saturated (SAT), monounsaturated (MONO), C12:0, C14:0, C16:0, C18:0, C18:1, and C18:2 cis-9,cis-12 fatty acid contents in milk fat].*

Trait	Milk	Fat	Protein	SAT	MONO	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
Milk (kg/d)		-0.35	-0.48	-0.09	0.22	-0.34	-0.02	0.01	-0.15	0.11	-0.01
Fat (%)	-0.18		0.63	0.76	-0.22	0.55	-0.06	0.60	0.83	-0.78	-0.37
Protein (%)	-0.32	0.38		0.51	-0.34	0.77	0.15	0.20	0.52	-0.59	-0.02
SAT (g/100 g of fat)	0.04	0.13	0.21		-0.44	0.67	0.37	0.55	0.66	-0.90	-0.66
MONO (g/100 g of fat)	-0.06	0.03	-0.18	-0.73		-0.70	-0.84	-0.34	-0.44	0.67	0.67
C12:0 (g/100 g of fat)	0.00	-0.03	0.37	0.75	-0.84		0.60	0.20	0.52	-0.78	-0.54
C14:0 (g/100 g of fat)	0.09	-0.19	0.11	0.65	-0.90	0.84		0.00	0.10	-0.46	-0.68
C16:0 (g/100 g of fat)	-0.03	0.10	0.05	0.44	-0.23	0.16	0.12		0.61	-0.62	-0.28
C18:0 (g/100 g of fat)	0.00	0.65	0.23	0.30	-0.24	0.11	0.01	0.29		-0.78	-0.38
C18:1 (g/100 g of fat)	-0.03	-0.13	-0.27	-0.93	0.83	-0.85	-0.73	-0.47	-0.33		0.70
C18:2 (g/100 g of fat)	-0.10	-0.23	0.21	-0.50	0.53	-0.34	-0.50	-0.23	-0.32	0.53	

The negative genetic correlation between SAT and MONO shows the logical opposition of these 2 types of FA (Table 6). If the content of SAT in fat increases, the content of POLY or MONO will obviously decrease.

As mentioned, the genetic correlations reflect the origin of FA. As in Table 5, the results indicated in Table 6 show the links which could exist between C12:0 and C14:0, C18:1 and C18:0 or C18:2 *cis-9,cis-12*. The genetic correlations between C16:0 or C18:0 with C12:0, C14:0, C18:1, or C18:2 *cis-9,cis-12* were lower than those mentioned in Table 5. This latter result did not confirm the previous observation about the two possibilities of production for C16:0 and C18:0.

6.6. Conclusions

The interest of consumers for the nutritional quality of dairy products is increasing. It is thus interesting to study the genetic variation of FA composition to evaluate the feasibility of selecting animals to alter the relative proportions of FA and improve the nutritional quality of the milk fat. The current study shows that the genetic variation in FA exists. The heritabilities for the major FA in milk ranged from 19 to 38% in milk with the exception of C18:1 (5.39%). Similarly, the heritabilities of FA in milk fat ranged from 15 to 28% with the exception of C12:0 (9.11%).

The genetic correlations estimated among each FA reflected the common origin of several groups of FA. Given these results, information about each distinct FA is not necessary. An index could be created to include the groups of FA with the similar metabolic origins in the mammary gland. For example, it could be interesting to use an index including the FA for which the Δ^9-desaturase is needed (*e.g.*, C14:1, C16:1, C18:1). Based on such an index, selection could be used in the future to increase MONO and conjugated FA in bovine milk.

The nearly zero genetic correlation between %FAT and the percentage of C14:0 and the greater genetic correlations between %FAT and the contents of C12:0, C16:0, and C18:0 in fat showed that the increase of %FAT is not directly associated with undesirable milk fat composition for human health.

In conclusion, genetic variability seems to exist in milk FA content. Based on the obtained estimates of genetic parameters, selection programs could be implemented in the future to improve the nutritional quality of fat in bovine milk by altering relative amounts of the various FA.

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Chapter VII

VARIATION OF Δ^9-DESATURASE ACTIVITY IN DAIRY CATTLE

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7.1. Interpretive Summary

The activity of Δ^9-desaturase influences milk fat composition. This enzyme catalyzes the endogenous production of different monounsaturated fatty acids and nearly all conjugated linoleic acids. The variability of this enzymatic activity was studied from the ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0, also named Δ^9-desaturase indices. The values of these indices increased as a function of days in milk. The observed differences between 7 dairy breeds suggested that the choice of breed modifies the fatty acid profile in milk. Based on the estimated heritability values, the selection of animals based on their Δ^9-desaturase indices is feasible. This potential selection program should increase the content of monounsaturated fatty acids and conjugated linoleic acids in bovine milk.

7.2. Abstract

The endogenous production of unsaturated fatty acids (FA), particularly some monounsaturated FA (%MONO) and nearly all conjugated linoleic acids (CLA), is regulated by the Δ^9-desaturase activity. The aims of this study were to assess the variation of this enzymatic activity within lactation, across dairy breeds and to estimate its genetic parameters. The ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0 were calculated from FA contents predicted by mid-infrared spectrometry. Variance components and standard errors were estimated using Average Information REML. The multitrait mixed model included as fixed effects, herd x test date x class of lactation number, class of days in milk x class of lactation number, class of age x class of lactation number, and regressions on breed composition. Four random effects were also included: animal genetic effect, two permanent environments (within and across lactations), and residual effect. Under the assumption that the calculated ratios are an approximate measurement of Δ^9-desaturase activity, this study showed different sources of variation for this enzymatic activity. A slight difference was observed within lactation. The ratios of C14:1 *cis-9* to C14:0 and C16:1 *cis-9* to C16:0 increased as a function of days in milk. Differences across 7 dairy breeds were observed. The values of Δ^9-desaturase indices observed for Jersey and Brown-Swiss cows were lower compared to Holstein. The opposite was observed for dual purpose Belgian Blue cows. Values of heritability for the ratios of

C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0 were 20%, 20%, and 3%, respectively. Negative genetic correlations observed between fat or protein contents and the 3 indices suggested that an increased activity of Δ^9-desaturase could inhibit the synthesis of fat and protein in bovine milk. Negative correlations were also observed between fat or protein contents and the contents of 3 studied unsaturated fatty acids in milk fat (C14:1 *cis-9*, C16:1 *cis-9*, and C18:1 *cis*). The positive genetic correlations observed between %MONO and the ratios of C14:1 *cis-9* to C14:0 (0.72), C16:1 *cis-9* to C16:0 (0.62), and C18:1 *cis* to C18:0 (0.97) showed that %MONO is linked to the Δ^9-desaturase activity.

(**Keywords:** Δ^9-desaturase, fatty acid, genetic parameter, cattle)

7.3. Introduction

Milk fat is considered hypercholesterolemic because of its high saturated fatty acids (**FA**) contents (Ulbricht and Southgate, 1991), in particular for C12:0, C14:0, and C16:0 (Fernandez and West, 2005; Hu *et al.*, 1999). By opposition, the intake of polyunsaturated and monounsaturated FA reduces the plasma cholesterol concentrations (Fernandez and West, 2005; Hu *et al.*, 2001; Noakes *et al.*, 1996; Ulbricht and Southgate, 1991). Beside its positive effect on cardiovascular diseases, the intake of conjugated linoleic acids (**CLA**), particularly C18:2 *cis-9, trans-11* and C18:2 *trans-10, cis-12*, is also beneficial for the prevention of cancer (Belury, 1995; Ip *et al.*, 1994; Parodi *et al.*, 1999) and obesity (Park *et al.*, 2001). Average milk fat contains 70% of saturated FA, 25% of monounsaturated FA (%**MONO**), and 5% of polyunsaturated FA (Grummer, 1991). Some researchers studied different possibilities to modify the FA profile, especially to increase the proportion of unsaturated FA. The sources of variation such as feed, breed, or animal genetics can be used to improve the nutritional quality of fat (Palmquist *et al.*, 1993; DePeters *et al.*, 1995). Detailed information about the feeding effect on the FA profile in bovine milk is available (*e.g.*, Chilliard *et al.*, 2000); information about the genetic effect on milk fat composition is limited (*e.g.* Renner and Kosmack, 1974; Karijord *et al.*, 1982; Soyeurt *et al.*, 2006b, 2007).

The introduction of a cis-double bond between carbon 9 and 10 of saturated FA with a chain length of 10 to 18 carbons is an important step in the synthesis of unsaturated FA (Bauman *et al.*, 1999; Ntambi, 1995; Ntambi and Miyazaki, 2004; Thomson *et al.*, 2003). The iron attached to the Δ^9-desaturase enzyme, also named stearoyl-CoA desaturase (**SCD**), catalyzes this desaturation together with NADPH, cytochrome b5 reductase, cytochrome b5, and oxygen (Ntambi, 1995; Yahyaoui *et al.*, 2002). The Δ^9-desaturase activity regulates the production of the major isomer of CLA by converting C18:1 *trans-11* into C18:2 *cis-9, trans-11* (Bauman *et al.*, 1999; Kinsella, 1972). Corl *et al.* (2001) observed that 78% of the total of C18:2 *cis-9, trans-11* secreted in milk fat was endogenously synthesized. The conversions of C10:0 into C10:1 *cis-9*, C12:0 into C12:1 *cis-9* (Thomson *et al.*, 2003), and mainly, the conversions of C14:0 into C14:1 *cis-9*, C16:0 into C16:1 *cis-9*, and C18:0 into C18:1 *cis-9* (Ntambi, 1995; Bauman *et al.*, 1999) are also regulated by this enzyme in the mammary cells. Chilliard *et al.* (2001) reported that on average 40% of C18:0 taken by the mammary gland from the circulating plasma lipids is converted into C18:1 *cis-9*. This Δ^9 desaturation contributes to more than 50% of C18:1 *cis-9* secreted in bovine milk. The impact of this enzyme on the cited FA is not equal, the favourite substrates being C18:0 and C16:0 (Ntambi and Miyazaki, 2004).

Bovine SCD gene contains 5,331 bp and is localized on chromosome 26 (Campbell *et al.*, 2001; Taniguchi *et al.*, 2004). Its opening reading frame includes 1,080 nucleotides and codes for 359 amino acids (Taniguchi *et al.*, 2004). The exact number of SCD genes in bovine is currently unknown (Campbell *et al.*, 2001). Ward *et al.* (1998) reported that the ovine SCD gene was widely expressed. Kinsella *et al.* (1972) reported that the Δ^9-desaturase activity and mRNA abundance were greater in the mammary gland for lactating ruminants. Taniguchi *et al.* (2004) studied the full length of the bovine SCD from 20 Japanese Black steers and observed 8 single nucleotide polymorphisms (**SNPs**). One of these SNPs is the site of an amino acid replacement, substitution of valine (V) for alanine (A). Mele *et al.* (2007) suggested that this locus could be a candidate gene to explain the variability of FA profile in bovine milk. They observed that the relative frequencies of bovine SCD genotypes were 27% for AA, 60% for AV, and 13% for VV. Generally the A allele was associated with higher %MONO and greater values of FA indices of C14. Moioli *et* *al.* (2007) studied the SNP on exon 5 and observed an influence of this SNP on the C14 and C16 indices.

Previous studies estimated specific FA indices to approximate the measurement of the Δ^9-desaturase activity. These indices were defined as ratios of FA dependent on the activity of this enzyme: product/substrate (Lock and Garnsworthy, 2003; Thomson *et al.*, 2003), substrate/product (Chouinard *et al.*, 1999) or product/(substrate + product) (Kelsey *et al.*, 2003; Royal and Garnsworthy, 2005). These ratios are affected by lipid supplementation (Chouinard *et al.*,1999), season (Lock and Garnsworthy, 2003), and breed (Kelsey *et al.*, 2003; Soyeurt *et al.*, 2006b). Consequently, a cow of a specific breed with a higher Δ^9-desaturase activity should produce higher contents of %MONO and CLA in milk fat. Peterson *et al.* (2002) suggested that the variation of CLA content in milk fat among individuals was related to the rumen biohydrogenation and the Δ^9-desaturase activity. Unfortunately, due to the price and the time needed by the gas chromatography to measure FA contents in milk fat, the major part of these studies were limited to a small number of animals and samples. Recently several calibration equations have been developed by our group (Soyeurt *et al.*, 2006) to predict the FA contents from mid-infrared (**MIR**) spectrum. Thanks to the low cost of this infrared analysis, the number of animals and samples analyzed can be large.

The objective of the current research was to study, in a large animal population, the variation of the Δ^9-desaturase indices within and across lactations, the differences among dairy breeds and to estimate their genetic parameters.

7.4. Materials and Methods

7.4.1. Animal Population and Milk Samples

Between April 2005 and December 2006, as part of the standard farm recording activities in the Walloon region of Belgium, 10,401 milk samples were collected once a month from 1,918 cows in 26 herds. Several criteria were used to select herds, such as their participation in Walloon milk recording and their degree of pedigree completeness. A total of 8 herds were followed since April 2005. The remaining herds were studied since November 2005. The number of lactating cows per herd ranged from 25 to 100. Herds were also chosen to have a mixed breed structure representing a diversity of breeds. There were 7 main dairy breeds with Holstein (**HOL**) being the most common (47,27%). The remaining breeds in decreasing order of abundance were: dual purpose Belgian Blue (**DPB**), 15.95%; Montbeliarde (**MON**), 12.93%; Normande (**NOR**), 8.51%; Unknown, 5.59%; Meuse-Rhine-Yssel type Red and White (**RED**), 4.11%; Jersey (**JER**), 3.43%; and Brown-Swiss (**BSW**), 2.21%. Following standard procedures (International Committee for Animal Recording, 2007), milk samples were representative of the 24h milking period (50% of morning milk and 50% of evening milk). Milk Committee of Battice (Belgium) analyzed these samples on a Foss MilkoScan FT6000 spectrometer (Foss, HillerØd, Denmark) during the regular milk analysis. All of MIR milk spectra generated by this infrared analysis were recorded. Due to different time frames and technical issues, the number of test days was not constant for all herds. Some cows were also dried off or calved during this experiment.

Due to the limited size of the spectral database, milk yields and the percentages of fat (**%FAT**) and protein (**%PROT**) measured by MIR spectrometry were added. To increase the number of contemporaries, the historic milk records were also added for all studied herds and represented records from 1995 to 2007. The edited data set contained 52,950 records from 3,217 cows.

7.4.2. Predicted Concentrations of Fatty Acid in Milk and Milk Fat

Recently, our group developed calibration equations useful to predict the FA contents in bovine milk (Soyeurt *et al.*, 2006). The reference FA concentrations were measured by gas chromatography with a capillary column of 50m length. This column showed a poor chromatographic resolution for C18:1 isomers. Therefore in this study, improved calibration equations were developed.

Between March 2005 and May 2006, 1,609 milk samples were collected in 8 herds from 475 cows representing 6 dairy breeds (DPB, HOL, JER, NOR, MON, and RED). From their spectral variability, 78 milk samples were selected using a principal components approach. The milk fat of these samples was extracted according to ISO

Standard 14156:2001 (International Organization for Standardization, 2001). Preparation of FA methyl esters was made following the ISO 15884:2002 (International Organization for Standardization, 2002). These milk fat samples were analyzed using gas chromatography from the method developed by Collomb and Bühler (2000). The gas chromatograph (model 6890N; Agilent Technologies, Inc., Palo Alto, CA) was equipped with a CPSil-88 capillary column (Varian, Inc., Palo Alto, CA) with a length of 100m, an internal diameter of 0.25mm, and a film thickness of 0.20µm. The conditions for the chromatographic analyses were as follows: carrier gas, helium; average velocity, 19cm/s; cold on-column injector; flame ionization detector at 255°C; and a temperature program from 60°C (5 min) to 165°C (at 14°C/min) during 1 min, then 165 to 225°C (at 2°C/min) during 17 min. The volume injected was 0.5µL. An anhydrous milk fat with a certified FA composition (reference material BCR-164, obtained from the Commission of the European Communities, Brussels, Belgium) was used to determine the FA methyl esters response factors, the repeatability, and the accuracy of this method.

The methodology used to develop the calibration equations was similar to the one used previously (Soyeurt *et al.*, 2006). These equations were established from chromatographic and spectral data using a specific program for multivariate calibration (WINISI III; http://www.winisi.com/) and partial least squares regressions. No treatments were applied beforehand on the spectral data. As over-fitting can occur through the use of partial least squares regressions technique, cross-validation of the developed calibration equations was used to prevent this. Cross-validation was applied to validate the number of factors used in the different equations and to estimate the validation errors of the obtained equations. These errors were calculated by partitioning the calibration set into several groups. In this study, a full cross-validation was used. Thus, a calibration was performed for each sample, until every sample had been predicted once. Validation errors were combined into a standard error of cross-validation (**SECV**; Sinnaeve *et al.*, 1994). To assess the robustness of the developed calibration equations, several statistical parameters were calculated: mean and standard deviation (**SD**) measured from reference concentrations of FA, standard error of calibration (**SEC**), calibration coefficient of determination (**R^2_C**), SECV, cross-validation coefficient of determination (R^2_{CV}), and the ratio of SECV to

SD (**RPD**). For the interested readers, Williams (2007) explains in details the calibration procedure.

The calibration equations predicting the contents of monounsaturated FA, C14:0, C14:1 *cis-9*, C16:0, C16:1 *cis-9*, C18:0 and the total C18:1 *cis* in milk (g/dL of milk) were applied to the recorded spectra. All predicted FA contents were converted into grams per 100 grams of fat using %FAT measured by the MilkoScan FT6000. From these predictions, the ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0 were calculated. Lock and Garnsworthy (2003) estimated these ratios to approximate the measurement of the Δ^9-desaturase activity. From the 78 calibration samples, canonical correlations were calculated using PROC CANCORR (SAS Institute, 1999). These correlations were used to study the similarities between the Δ^9-desaturase indices calculated from FA contents measured by gas chromatography and MIR spectrometry.

7.4.3. Statistical Model

The division of permanent environment into two distinct parts (within and across lactations) was based on the model used by Bormann *et al.* (2003). A total of 3,024 cows had repeated records within lactation and 1,838 cows had repeated records across lactations. All traits were studied with 8 separate runs using the same multitrait mixed animal model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{l} + \mathbf{Z}\mathbf{p} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where $\mathbf{y}$ was the vector of observations for each of the 8 runs (milk yield, %FAT, %PROT, and %MONO; milk yield, %FAT, %PROT, and the ratio of C14:1 *cis-9* to C14:0; milk yield, %FAT, %PROT, and the ratio of C16:1 *cis-9* to C16:0; milk yield, %FAT, %PROT, and the ratio of C18:1 *cis* to C18:0; %MONO, the ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0; milk yield, %FAT, %PROT, C14:1 *cis-9*, and C14:0; milk yield, %FAT, %PROT, C16:1 *cis-9*, and C16:0; milk yield, %FAT, %PROT, C18:1 *cis*, and C18:0); $\boldsymbol{\beta}$ was the vector of fixed effects (herd x test day x class of lactation number, stage of lactation x class of lactation number, class of age x class of lactation number, regressions on breed composition as many animals were crossbred); $\mathbf{l}$ was the vector of permanent environment random effects within lactation; $\mathbf{p}$ was the vector of permanent environment random effects across lactations; $\mathbf{u}$ was the vector of animal effects; $\mathbf{X}, \mathbf{W}$ and $\mathbf{Z}$ were incidence matrices; **e** is the vector of random residual effects.

Fixed effects were chosen from the sources of variation known for the traditional production traits as milk yield, %FAT, and %PROT. Stage of lactation was divided in 24 classes of 15 days in milk (**DIM**). Records with DIM lower than 5 and greater than 365 were deleted. Parities were grouped as first, second, and third or later lactation (18,733 records for cows in first lactation; 12,857 in second lactation, and 21,359 in third or later lactation). Age at test day was defined as the number of months from birth. A total of 9 classes of age were created: for first lactation, age below 29 mo (3.62% of test day records), 29-32 mo (6.65%), 33 mo and older (25.11%); for second lactation, age below 42 mo (2.72%), 42-46 mo (5.52%), 47 mo and older (16.04%); and for the third or later lactation, age below 54 mo (1.52%), 54-59 mo (4.28%), 60 mo and older (34.54%). Significance of the fixed effects was tested using PROC GLM (SAS Institute, 1999).

Pedigree contained 9,174 animals including 1,666 sires and 7,508 dams. Only animals born after 1980 were considered. In this context, a maximum of 6 generations per animal were covered. Breed composition was determined according to the known pedigrees of animals. A certain proportion of this breed composition was of unknown origin. To optimize the comparison between breeds, these proportions were supposed to correspond to another breed (unknown breed). Variance components were estimated using Expectation Maximisation REML and Average Information REML (Misztal, 2007). Covariance components were considered converged when the relative squared differences were equal to 10^{-11}. Variance components were supposed to be identical across breeds, lactation number, and stages of lactation. Standard errors (**SE**) of estimates were obtained using Average Information REML (Misztal, 2007). Heritability was calculated as the ratio of genetic variance ($\sigma^2_{\text{genetic}}$) to the sum of variances obtained for the genetic effect, the permanent environment within lactation (σ^2_{PEWL}) and across lactations (σ^2_{PEAL}), and the residual effect ($\sigma^2_{\text{residual}}$).

$$\text{heritability} = \frac{\sigma^2_{\text{genetic}}}{\sigma^2_{\text{genetic}} + \sigma^2_{\text{PEWL}} + \sigma^2_{\text{PEAL}} + \sigma^2_{\text{residual}}}$$

Genetic or phenotypic correlations were calculated using the ratio of genetic or phenotypic covariance between 2 traits to the product of the genetic or phenotypic standard deviations estimated for these 2 traits. Heritability values and correlations for milk yield, %FAT, and %PROT were the means of the estimates obtained from the 8 computations. The genetic and phenotypic correlations among the studied FA where estimated from the variance components using the same mixed model than the one exposed previously including milk yield, %FAT, %PROT and the 6 studied FA (C14:1 *cis-9*, C14:0, C16:1 *cis-9*, C16:0, C18:1 *cis*, and C18:0). Due to the large number of traits, covariance components for this calculation were considered converged when the relative squared differences were equal to 10^{-10}.

7.5. Results and Discussion

7.5.1. Calibration Equations

If RPD is equal or higher to 2, the MIR predicted value is considered to be a good indicator of the studied trait (Sinnaeve, 1994). The calibration equations predicting the contents of monounsaturated FA, C14:0, C16:0, C18:0, and C18:1 *cis* in milk (g/dL of milk) showed a RPD higher to 2 (Table 1).

Table 1. *Mean, standard deviation (SD), standard error of calibration (SEC), calibration coefficient of determination (R²c), standard error of cross-validation (SECV), cross-validation coefficient of determination (R²cv) and the ratio of SD to SECV (RPD) calculated for the 7 calibration equations used in this study and established from 78 milk samples*[1].

Fatty acid (g/dl of milk)	Mean	SD	SEC	R²c	SECV	R²cv	RPD
%MONO	1.43	0.45	0.06	0.98	0.12	0.93	3.76
C14:0	0.50	0.15	0.02	0.97	0.05	0.90	3.22
C14:1 *cis-9*	0.04	0.01	0.01	0.58	0.01	0.53	1.45
C16:0	1.39	0.42	0.12	0.92	0.17	0.84	2.50
C16:1 *cis-9*	0.08	0.03	0.02	0.64	0.03	0.28	1.17
C18:0	0.58	0.26	0.06	0.94	0.10	0.85	2.54
C18:1 *cis*	1.08	0.39	0.06	0.97	0.12	0.91	3.25

[1] %MONO = content of monounsaturated fatty acids in milk fat.

The lowest values of RPD observed for C14:1 *cis-9* and C16:1 *cis-9* can be explained by the lower concentrations of these FA in bovine milk. The means and SD observed for their respective Δ^9-desaturase indices calculated from FA contents measured by gas chromatography and MIR spectrometry were similar (Table 2). This suggests that predictors covered a similar range. The differences between the studied indices were relatively small (Table 2). Skewness for these traits were close to 0 except for the ratio of C18:1 *cis* to C18:0 (Table 2). This value was not extremely large (-1.405). Consequently, the differences were approximately normally distributed.

Table 2. *Mean and standard deviation for the ratios of C14:1 cis-9 to C14:0, C16:1 cis-9 to C16:0, and C18:1 cis to C18:0 estimated from the contents of fatty acid measured by gas chromatography and predicted by mid-infrared spectrometry from 78 milk samples*[1]*.*

Traits (g/dl of milk)	Gas Chromatography		Mid-Infrared Spectrometry		Difference				
	Mean	SD	Mean	SD	Mean	SD	Range	IQR	Skew.
C14:1 *9-cis*/C14:0	0.081	0.024	0.083	0.019	-0.011	0.020	0.091	0.026	0.292
C16:1 *9-cis*/C16:0	0.057	0.021	0.058	0.018	-0.001	0.014	0.88	0.017	-0.67
C18:1 *cis*/C18:0	2.004	0.572	2.011	0.597	-0.007	0.361	2.549	0.291	-1.405

[1] IQR = interquartile range; Skew. = skewness.

A canonical correlation of 0.86 was observed between the first canonical variable calculated from the Δ^9-desaturase indices estimated from the FA contents measured by gas chromatography and MIR spectrometry. This first principal component explained 78.25% of the variability. The second principal component described 13.60% of the variability and the canonical correlation was 0.58. The last principal component explained 0.08% of the variability and had a canonical correlation equal to 0.48. Figure 1 illustrates the position of the indices calculated from chromatographic and spectral data on a graph representing the first and the second principal components of the chromatographic data. The positions for each pair of indices were close. Results indicated the existence of a strong relationship between the Δ^9-desaturase indices calculated from chromatographic and spectral data. This suggests, therefore, a potential use of the ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0 predicted by MIR.

Skewness values observed in Table 3 for each studied trait were lower to 1 except for the Δ^9-desaturase indices. Consequently, the distribution of these traits was close to the normality. Skewness values for the ratios of C14:1 *cis-9* to C14:0 (2.49) and C16:1 *cis-9* and C16:0 (1.04) were not high. The same comment can be formulated for kurtosis (Table 3).

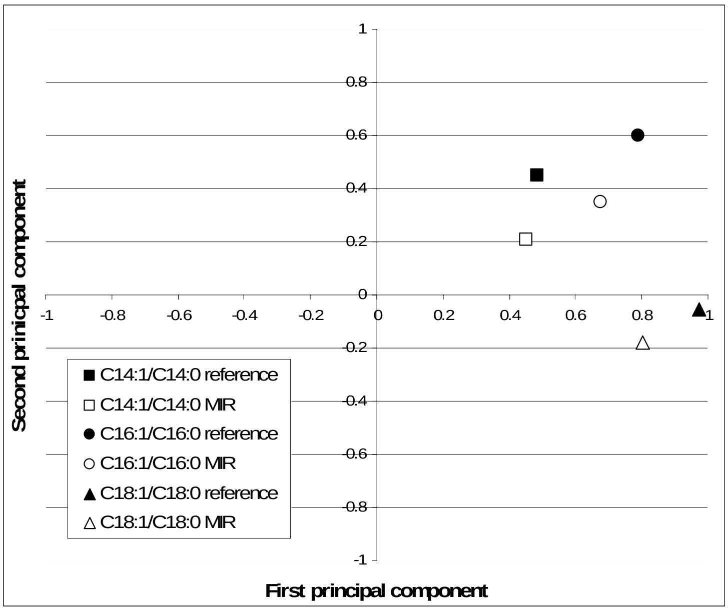


Figure 1. *Circle of correlations for the canonical variables representing C14:1 9-cis/C14:0, C16:1 9-cis/C16:0 and C18:1 cis/C18:0 estimated from the contents of fatty acid measured by gas chromatography (reference analysis) and predicted by mid-infrared (MIR) spectrometry. The axis represented the first and second principal components estimated from chromatographic data.*

Even if the distributions of the ratios of C14:1 *cis-9* to C14:0 and C16:1 *cis-9* to C16:0 can not be considered having a normal distribution, they are close enough to normality to be used in this study. The ratio of C18:1 *cis* to C18:0 had a very high value of skewness and kurtosis. The distribution of this trait was not normal. This ratio was also included in this study but the results obtained have to be interpreted cautiously.

7.5.2. Variation within and across lactations

As expected, the herd x test day x class of lactation number fixed effect explained the major part of the variability (49.6 to 97.6%; Table 4). The percentage of total variation explained by the second fixed effect, stage of lactation x class of lactation number, ranged from 2.4 to 50.4%.

Table 3. *Number of samples (N), mean, standard deviation (SD), mode, interquartile range (IQR), coefficient of variation (CV), skewness, and kurtosis of the milk components in the studied Walloon cattle populations*[1].

Trait	N	Mean	SD	Mode	IQR	CV	Skewness	Kurtosis
Milk yield (in kg)	52,950	22.54	8.56	20.00	11.6	37.95	0.47	0.24
% Fat (g/100g of milk)	52,950	4.02	0.76	3.85	0.91	18.85	0.91	2.52
% Protein (g/100 g of milk)	52,950	3.44	0.40	3.18	0.50	11.59	0.74	2.63
%MONO (g/100 g of fat)	10,401	28.42	5.05	28.32	6.80	18.16	0.84	2.22
C14:1 *cis-9*/C14:0	10,401	0.10	0.02	1.00	0.27	24.39	2.49	29.34
C16:1 *cis-9*/C16:0	10,401	0.07	0.02	0.67	0.20	26.59	1.04	4.09
C18:1 *cis*/C18:0	10,401	2.14	1.32	1.92	0.65	61.58	27.72	1,212.81
C14:1 *cis-9* (g/100 g of fat)	10,401	1.03	0.22	1.01	0.30	21.66	0.37	2.97
C14:0 (g/100 g of fat)	10,401	10.62	1.88	11.40	2.50	17.72	-0.57	0.42
C16:1 *cis-9* (g/100 g of fat)	10,401	1.88	0.40	2.02	0.51	21.18	-0.14	7.91
C16:0 (g/100 g of fat)	10,401	28.72	4.60	30.30	6.80	15.98	-0.40	1.21
C18:1 *cis* (g/100 g of fat)	10,401	22.31	4.48	20.30	5.90	20.08	0.63	0.51
C18:0 (g/100 g of fat)	10,401	11.00	2.58	10.38	3.10	23.46	-0.11	1.81

[1] %MONO = content of monounsaturated fatty acids in milk fat; Fatty acids ratios were calculated from fatty acids concentrations expressed in g/dL of milk

This effect was also significant for all traits. The class of age x class of lactation number fixed effect was significant except for %PROT, %MONO, the ratio of C16:1 cis-9 to C16:0, C14:0, C16:0, C18:1 cis, and C18:0. As this effect was significant for some studied traits, it was included in the used statistical model.

Table 4. *Percentage of variation measured on total fixed effects explained by each separate studied fixed effect after their introduction in the model and their significance*[1].

	% of total variation explained by studied effect		
Trait	Herd	DIM	Age
Milk yield (in kg)	68.81 (***)	31.11 (***)	0.08 (**)
% Fat (g/100g of milk)	84.80 (***)	14.52 (***)	0.68 (***)
% Protein (g/100 g of milk)	49.59 (***)	50.35 (***)	0.06 (NS)
%MONO (g/100 g of fat)	89.53 (***)	10.43 (***)	0.04 (NS)
C14:1 *cis-9*/C14:0	95.79 (***)	3.95 (***)	0.26 (***)
C16:1 *cis-9*/C16:0	97.55 (***)	2.38 (***)	0.07 (NS)
C18:1 *cis*/C18:0	94.45 (***)	4.68 (***)	0.87 (***)
C14:1 *cis-9* (g/100 g of fat)	87.40 (***)	12.38 (***)	0.22 (***)
C14:0 (g/100 g of fat)	91.01 (***)	8.96 (***)	0.03 (NS)
C16:1 *cis-9* (g/100 g of fat)	94.17 (***)	5.76 (***)	0.07 (*)
C16:0 (g/100 g of fat)	95.92 (***)	4.06 (***)	0.02 (NS)
C18:1 *cis* (g/100 g of fat)	83.89 (***)	16.09 (***)	0.02 (NS)
C18:0 (g/100 g of fat)	91.94 (***)	8.00 (***)	0.05 (NS)

[1] %MONO = the content of monounsaturated fatty acids in milk fat; Herd = herd x test date x lactation number; DIM = class of days in milk x class of lactation number; Age = class of age x class of lactation number; *** = P<0.001; ** = P<0.01; * = P<0.05.

The lactation curve for milk yield and %FAT were better fitted compared to the curve for %MONO (Figure 2). It explains by a larger number of observations used to trace the lactation curve of milk yield and %FAT (52,950 records). The lactation curve for %MONO represented 10,401 spectral records.

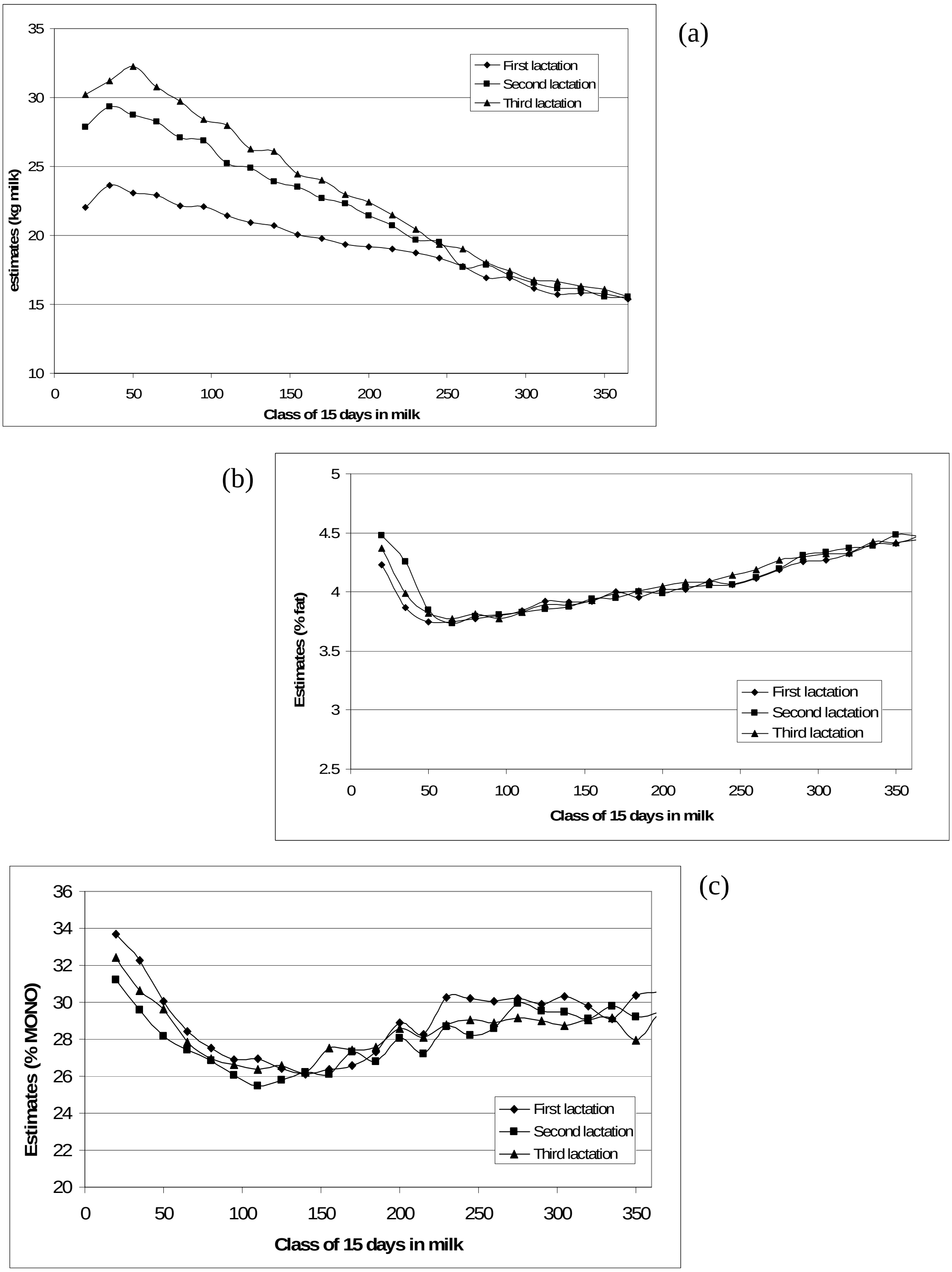


Figure 2. *Variation of unadjusted means for milk yield (a), %fat (b) and % monounsaturated fatty acids in fat (c) in function of class of 15 days in milk and lactation number. Number of samples used for estimating the value of each data point ranged from 193 to 1250.*

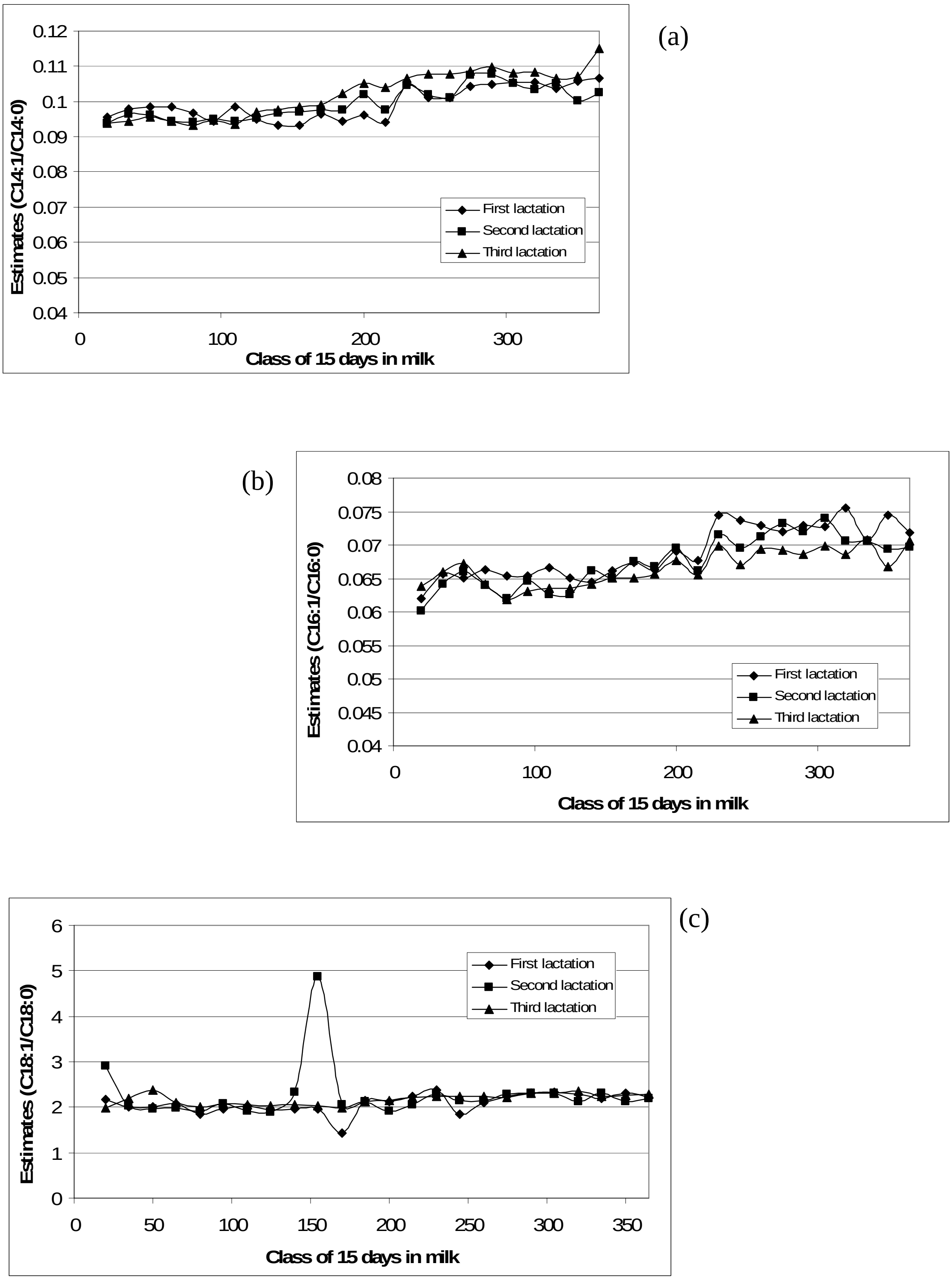
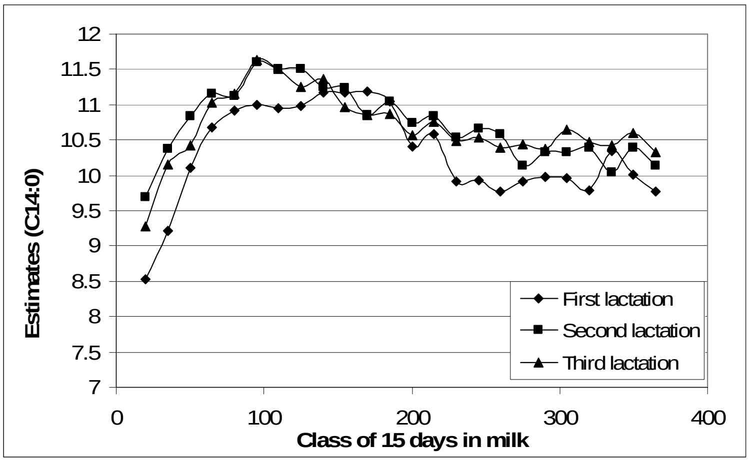


Figure 3. *Variation of unadjusted means for the ratios of C14:1 cis-9 to C14:0 (a), C16:1 cis-9 to C16:0 (b), and C18:1 cis to C18:0 (c) in function of class of 15 days in milk and lactation number. Number of samples used for estimating the value of each data point ranged from 45 to 268.*

The variation of %MONO within lactation was similar to the curve observed for %FAT. The lowest contents of %FAT and %MONO were observed at around 50 DIM for %FAT and at around 100 DIM for %MONO. As observed for %FAT, the differences between the first, second and third class of lactation number were not high. Karijord *et al.* (1982) observed a similar curve for C18:1. A slight increase of the values for the studied indices was observed within lactation, especially for the ratios of C14:1 cis-9 to C14:0 and C16:1 cis-9 to C16:0 (Figure 3). The differences across lactations were small. An outlier appeared in the Figure 3c. The contents of all FA in fat (g/100g of fat) were influenced by the days in milk (Figures 4 and 5). The impact of lactation number was slight. The curves observed for all studied FA were similar to those mentioned by Karijord *et al.* (1982). Therefore, the effect of lactation number was small. DIM affected the content of %MONO and studied FA and, more slightly, the values of Δ9-desaturase indices.

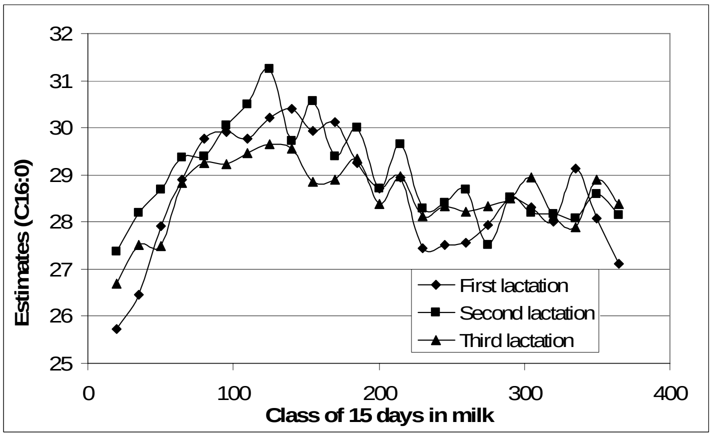
7.5.3. Heritability

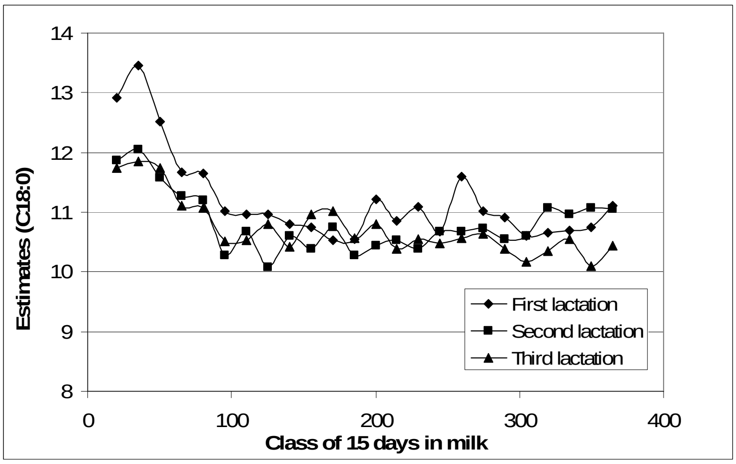
Heritability for daily milk yields was 20%. This value was rather low compared to the 305d heritability estimates used by the majority of countries in their genetic evaluations (Interbull, 2007). Average daily milk yield was modeled. Therefore the model used in this study provided an average heritability. Logically, average heritability observed for a test date is low compared to a figure representing the whole lactation, as test-day specific measurement errors are still included. Our model did also not model genetic or non-genetic variations of milk yield as a function of DIM for individual cows. Still it is similar to daily estimates reported by some other authors like Veerkamp and Goddard, 1998; Lidauer and Mäntysaari, 1999; Bormann *et al.*, 2003; and Gengler *et al.*, 2004. Heritability values were 33% for %FAT and 36% for %PROT. Ikonen *et al.* (1999) as Welper and Freeman (1992) and Miglior *et al.* (2007) estimated daily little higher values of heritability for these traits. Our model included also several breeds. The cited publications studied only Holstein cows.



(a)

(b)





(c)

Figure 4. *Variation of unadjusted means for the 3 studied saturated fatty acids in function of class of 15 days in milk and lactation number. Number of samples used for estimating the value of each data point ranged from 45 to 268.*

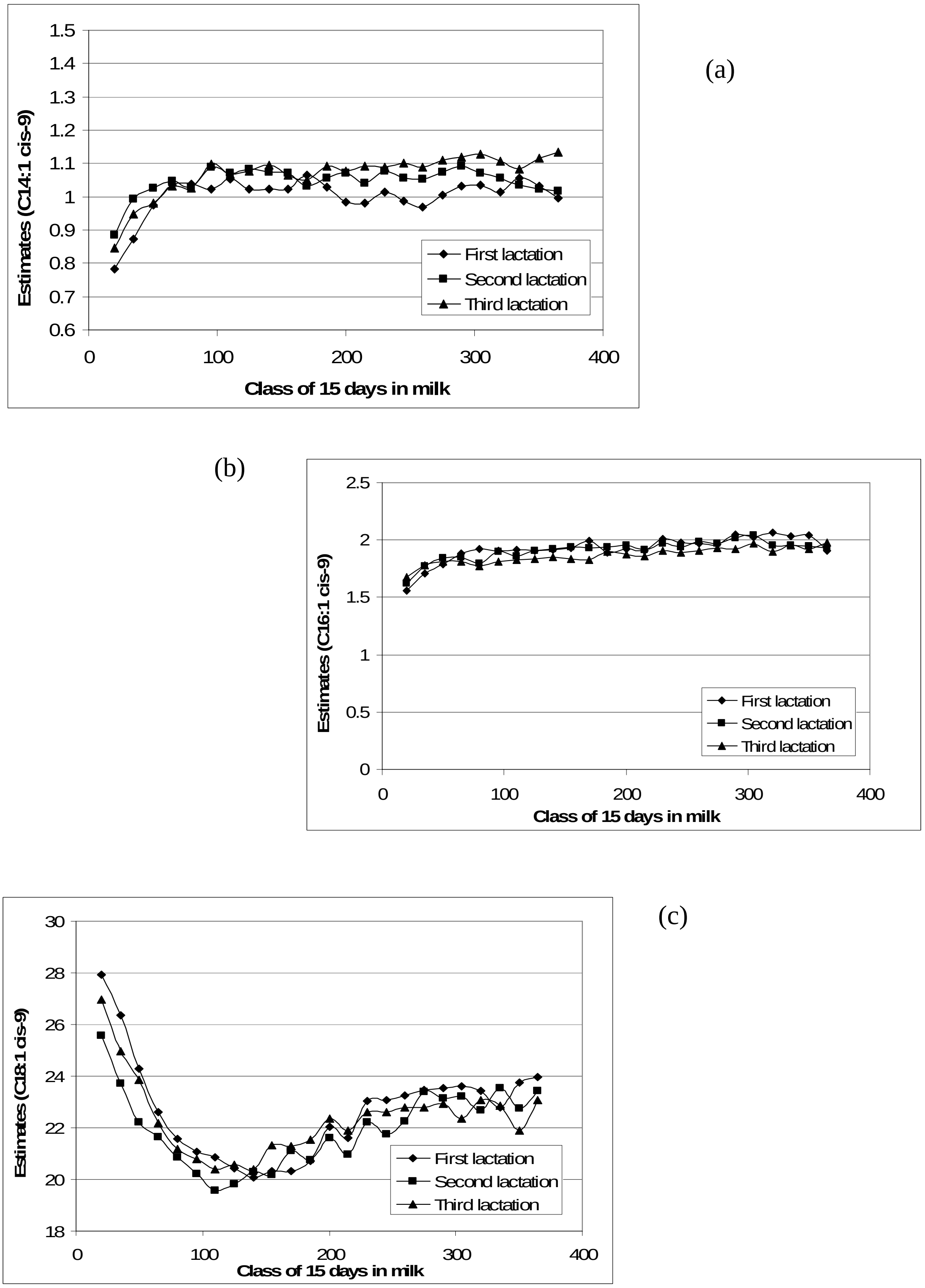


Figure 5. *Variation of unadjusted means for the 3 studied unsaturated fatty acids in function of class of 15 days in milk and lactation number. Number of samples used for estimating the value of each data point ranged from 45 to 268.*

Milk fat composition results from the production of FA by two distinct metabolic processes: *de novo* synthesis in the mammary gland (40% of FA secreted in milk; Chilliard *et al.*, 2001) and extraction from the circulating plasma lipids (60 %; Chilliard *et al.*, 2001). C4:0 to C12:0, most of C14:0, and on average 50% of C16:0 are produced by the *de novo* synthesis. All of C18 and longer chain FA are extracted from blood (Givens and Shingfield, 2004; Palmquist, 2006). Effect of dietary fat on FA profile has been extensively studied such as the effect on unsaturated FA on milk fat depression (Bauman *et al.*, 2006; Palmquist *et al.*, 2006). To circumvent the dietary impact, a fixed effect including herd x test day x class of lactation number, was included in the statistical model used. The following hypothesis was done: all cows present in the same herd received the same feeding. If this hypothesis is verified, which was largely the case in this study, the ‘herd’ fixed effect includes the main impact of feeding on milk fat composition. Consequently, the genetic variance obtained, for instance for %MONO, reflected more the endogenous production of monounsaturated FA.

Heritability for %MONO was equal to 17% (Table 5). This value is lower than the one observed for %MONO with the same model developed by Soyeurt *et al.* (2007) (24% with SE=2.3%) on a smaller data set. Two reasons can explain these differences. First, a larger spectral database was used. In this study, many cows were studied at least during one year. Secondly, the calibration equation used previously to estimate the percentage of monounsaturated FA in bovine milk (g/dL of milk) was established from FA contents measured using gas chromatography with a capillary column of 50m length (RPD=2.54). Based on the results given in Table 1, the new calibration equation used in this study can be considered more accurate (RPD=3.76). Overall, the genetic parameter estimates of both studies are similar and suggest a moderate heritability of %MONO. Heritability of unsaturated C18 obtained by Renner and Kosmack (1974) and Karijord *et al.* (1982) were close to 0. The sire model and the small number of samples used by these authors could explain this extremely low value.

Table 5. *Estimate and standard error (SE) of variances (in % of phenotypic variance) for genetic, 2 permanent environmental, residual random effects estimated for daily milk yield, fat content, protein content, percentage of monounsaturated fatty acids in fat (%MONO) and the three Δ^9-desaturase indexes (C14:1 cis-9/C14:0, C16:1 cis-9/C16:0,*

	Heritability		Permanent Environments				Residual	
			Within Lactation		Across Lactations			
Traits	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Milk yield (in kg)	20	1.8	27	0.6	15	1.5	38	0.2
% Fat	33	1.7	4	0.2	5	1.2	57	0.2
% Protein	36	1.8	10	0.3	5	1.2	49	0.4
%MONO	17	1.4	12	1.1	4	0.8	67	0.8
C14:1 *cis-9*/C14:0	20	1.8	25	1.4	4	0.9	51	0.6
C16:1 *cis-9*/C16:0	20	2.3	6	1.0	6	2.2	69	0.8
C18:1 *cis*/C18:0	3	0.2	2	0.4	0	0.0	95	1.0
C14:1 *cis-9*	20	2.1	8	0.8	10	1.8	62	0.7
C14:0	15	1.3	11	1.1	3	0.8	71	0.8
C16:1 *cis-9*	22	1.6	12	1.1	3	0.7	63	0.6
C16:0	15	1.8	10	1.1	8	1.7	66	0.8
C18:1 *cis*	17	1.3	10	1.0	5	0.7	68	0.6
C18:0	16	1.8	3	0.7	7	1.6	73	0.7

[1] Sample number = 52,950; Cow number = 3,217; Sire number = 1,666.

Heritability observed for the ratio of C14:1 *cis-9* to C14:0 was similar to the one found for %MONO (20%). This similarity between %MONO and this Δ^9-desaturase index is partially explained by their link with the Δ^9-desaturase activity. %MONO includes several FA whose production is partially catalyzed by the Δ^9-desaturase (Chilliard *et al.*, 2001). The ratio of C14:1 *cis-9* showed a similar heritability. This result was expected because of the origin of this FA. Nearly all C14:0 in milk fat is produced by *de novo* synthesis in the mammary gland. Consequently, the desaturation should be the main source of C14:1 *cis-9* (Lock and Garnswothy, 2003). Mosley and McGuire (2007) observed that 90% of C14:1 *cis-9* was the result of Δ^9 desaturation. Heritability observed for C14:0 was a little lower (15%; Table 5). Due to the endogenous production of C14:0 and C14:1, the impact of feeding on these FA contents is low. This impact could explain the low values of residue observed in Table 5 for the C14 ratio compared to the 2 other Δ^9-desaturase indices. Heritability estimated by Royal and Garnsworthy (2005), from 1,520 cows and one analyzed sample per cow, for the ratio of C14:1 *cis-9* to the sum of C14:0 and C14:1 *cis-9* was equal to 30%. Karijord *et al.* (1982) obtained heritability values equal to 7% for C14:0 and 26% for C14:1.

The ratio of C16:1 *cis-9* to C16:0 had a heritability equal to 20%. Palmitic acid is synthesized *de novo* in de mammary gland and also extracted from blood. Mosley and McGuire (2007) showed that 50% of the C16:1 *cis-9* came from the Δ^9-desaturation. The heritability for this ratio was more similar to that observed for the ratio of C14:1 *cis-9* to C14:0 even if C16:0 and C16:1 *cis-9* whose contents are influenced by feeding. Our result was different from the one obtained by Royal and Garnsworthy (2005) who found the lowest heritability for the ratio of C16:1 *cis-9* to the sum of C16:0 and C16:1 *cis-9*. Similar heritability values than those observed for C14:0 and C14:1 *cis-9* were estimated for C16:0 (15%) and C16:1 *cis-9* (22%). Heritability values obtained by Karijord *et al.* (1982) were 15% for C16:0 and 12% for C16:1 *cis-9*.

The heritability estimates for C18:1 *cis* to C18:0, C18:1 cis, and C18:0 were 3%, 16%, and 17%, respectively (Table 5) which is lower than for the ratio of C18:1 *cis-9* to the sum of C18:0 and C18:1 *cis-9* by Royal and Garnsworthy (2005). The low heritability can be explained partly by the fact that these FA are primarily extracted from blood (Bobe *et al.*, 1999; Chilliard *et al.*, 2001; Mosley and McGuire, 2007).

7.5.4. Correlations

Genetic correlations between milk yield and %FAT, %PROT were -0.36 and -0.50, respectively. Genetic correlation between %FAT and %PROT was 0.68 (Table 6). These values were in agreement with the one found in the literature (*e.g.*, Roman and Wilcox, 2000). Genetic correlation between %FAT and %MONO was -0.70. This value was higher than the one obtained by Soyeurt *et al.* (2007) (-0.22). This difference between those results has most likely similar reasons that those mentioned for heritability values. Karijord *et al.* (1982) and Renner and Kosmack found similar genetic correlation. The positive genetic correlation between milk yield and %MONO (0.23) was similar to the one found by Soyeurt *et al.* (2007) but differed from results obtained by Karijord *et al.* (1982) and Renner and Kosmack (1974b). Genetic correlations between %MONO and the C14, C16 and C18 ratios were 0.72, 0.62 and 0.97, respectively (Table 6). This observation is explained because the major part of monounsaturated FA produced endogenously is catalyzed by the Δ^9-desaturase. The higher genetic correlation between the ratio of C18:1 *cis* to C18:0 and %MONO is explained by the large content of C18:1 present in %MONO (Mosley and McGuire, 2007).

A negative genetic correlation observed between the ratio of C14:1 *cis-9* to C14:0 and %FAT was equal to -0.66. Negative genetic correlations were also observed for the 2 other studied indices (Table 6). Genetic correlations obtained for the studied indices could suggest the inhibition of milk fat synthesis when a higher Δ^9-desaturase activity is observed. This enzyme catalyzes the production of CLA (Bauman *et al.*, 1999). Some previous studies indicated that some isomers of CLA inhibit milk fat synthesis. For instance, Chouinard *et al.* (1999) and Perfield *et al.* (2007) suggested that CLA altered milk FA composition and inhibited milk fat synthesis. This observation is confirmed by the genetic correlations obtained between %FAT and each individual studied monounsaturated FA (Table 7).

Table 6. *Genetic (above the diagonal) and phenotypic correlations (below the diagonal) among milk yield, content of fat and protein, percentage of monounsaturated fatty acids in fat (%MONO) and the 3 Δ^9-desaturase indexes.*

	Milk	%FAT	%PROT	MONO	C14:1/C14:0	C16:1/C16:0	C18:1/C18:0
Milk yield (in kg)		-0.36	-0.50	0.23	0.27	0.10	0.39
% Fat	-0.19		0.68	-0.70	-0.66	-0.43	-0.80
% Protein	-0.32	0.40		-0.48	-0.56	-0.38	-0.29
% MONO	0.02	-0.17	-0.17		0.72	0.62	0.97
C14:1 9-*cis*/C14:0	0.01	-0.36	-0.20	0.55		0.51	0.82
C16:1 9-*cis*/C16:0	0.04	-0.14	-0.15	0.65	0.32		0.64
C18:1 *cis*/C18:0	0.03	-0.15	-0.01	0.21	0.22	0.14	

[1] Sample number = 52,950; Cow number = 3,217; Sire number = 1,666.

Genetic correlations among the 3 Δ^9-desaturase indices were positive. The values of correlations observed in Table 6, especially between the ratio of C14:1 *cis-9* to C14:0 and C16:1 *cis-9* to C16:0 or the ratios of C16:1 *cis-9* to C16:0 and C18:1 *cis* to C18:0, were lower than expected. Genetic correlations observed between the ratio of C14:1 *cis-9* to C14:0 and C18:1 *cis* to C18:0 was high (0.82). The ratio of C14:1 *cis-9* to C14:0 was more related to this last desaturase index than to the ratio of C16:1 *cis-9* to C16:0. Based on the results indicated by Mosley and McGuire (2007), a higher content of oleic acid compared to palmitic acid (59% vs 50%) resulted from the Δ^9 desaturation. This slight difference could be explained by the higher genetic correlation observed between the ratio of C14:1 *cis-9* to C14:0 and C18:1 *cis* to C18:0. Indeed, as 90% of myristoleic acid is converted via the action of Δ^9-desaturase (Mosley and McGuire, 2007), these FA are more influenced by the Δ^9-desaturase activity. The phenotypic correlation between these 2 indices was low (0.22).

If the genetic parameters estimated in this study reflect mainly the endogenous production of FA via the Δ^9-desaturase activity, the phenotypic correlation is also influenced by the permanent environments relative to a specific animal and other factor of variation not explained by the statistical model used (for example, some specific diet effects). Consequently, the lower phenotypic correlation observed between the ratio of C14:1 *cis-9* to C14:0 and C18:1 *cis* to C18:0 was expected because C18:0 and C18:1 *cis-9* are more influenced by feeding. In the same way, the higher phenotypic correlations between the ratio of C14:1 *cis-9* to C14:0 and C16:1 *cis-9* to C16:0 (0.32) compared to the observed genetic correlation between these two index was expected.

Phenotypic correlations between milk yield and the 3 Δ^9-desaturase indices were close to 0 (Table 6). So in contrast with %FAT and %PROT, the products secreted by the action of Δ^9-desaturase did not seem to influence milk production. Perfield *et al.* (2007) observed that the different treatments of CLA made by these authors did not influence the milk yield of studied cows.

Table 7. *Genetic (above the diagonal) and phenotypic correlations (below the diagonal) among milk yield, content of fat and protein, percentage of C14:1 cis-9, C14:0, C16:1 cis-9, C16:0, C18:1 cis, and C18:0.*

	Milk	%FAT	%PROT	C14:1	C14:0	C16:1	C16:0	C18:1	C18:0
Milk		-0.36	-0.50	0.21	-0.18	0.14	-0.04	0.32	-0.25
%Fat	-0.19		0.68	-0.48	0.40	-0.24	0.52	-0.73	0.36
%Protein	-0.32	0.40		-0.34	0.43	-0.24	0.42	-0.56	0.05
C14:1	0.08	-0.55	-0.10		-0.05	0.40	-0.16	0.40	-0.60
C14:0	0.05	-0.12	0.19	0.34		-0.26	0.36	-0.75	-0.22
C16:1	0.05	-0.09	-0.09	0.09	-0.28		-0.18	0.41	-0.13
C16:0	0.00	0.15	0.14	0.10	0.41	-0.03		-0.72	0.03
C18:1	0.02	-0.13	-0.20	-0.06	-0.78	0.39	-0.64		-0.29
C18:0	-0.09	0.30	-0.15	-0.53	-0.41	-0.11	-0.31	0.14	

[1] Sample number = 52,950; Cow number = 3,217; Sire number = 1,666

7.5.5. Differences across breeds

The difference in %MONO produced by DPB compared to HOL cows was highly significant (Table 8). DPB cows had a higher content of %MONO in milk fat. The low %FAT synthesized by these cows was in line with the negative value of genetic correlation observed between %FAT and %MONO. This breed showed a delta-9 activity similar (or slightly higher) to that estimated for HOL cows. The difference of delta-9 desaturase activity for the C18:1 *cis*/C18:0 was close to significance, as was previously observed by Soyeurt *et al.* (2006b).

In the contrast to DPB, JER produced more fat content than HOL cows. The %MONO in JER milk fat was lower than the one observed for HOL. The low activity of Δ^9-desaturase indices observed for these cows could explain the significant differences observed in fat composition. This result was in agreement with Soyeurt *et al.* (2006b). Generally the breed effects were studied using the phenotypic differences observed on a limited number of cows fed with the same diet. Consequently although the obtained results did not represent exactly the breed differences excluding individual cow differences, these publications gave some indications. Many studies observed the same difference between JER and HOL: Stull and Brown (1964) on 40 to 50 for HOL, and 10 to 15 for JER; White *et al.* (2001) on 18 JER and 19 HOL cows; Drackley *et al.* (2001) on 4 JER and 4 HOL, Beaulieu and Palmquist (1995), and DePeters *et al.* (1995), and Sol Morales *et al.* (2000) on 12 JER and 12 HOL. These last authors found that differences disappeared when cows were fed with soybean.

Similar to JER, BSW cows produced a higher content of %MONO essentially because of the low Δ^9-desaturase activity observed for this breed. The differences between BSW and HOL shown in Table 8 were significant for %MONO and the 3 Δ^9-desaturase indices. Kelsey *et al.* (2003) found a significant difference between BSW and HOL for the activity of Δ^9-desaturase indices calculated from 113 HOL and 106 BSW. The value for the ratio of C16:1 *cis-9*/(C16:1 *cis-9* + C16:0) measured by these authors for BSW was lower than that calculated for HOL.

Table 8. *Differences for milk yield, %fat , %protein, the content of monounsaturated fatty acids in fat (%MONO) and the 3 Δ^9-desaturase indexes (C14:1 cis-9/C14:0; C16:1 cis-9/C16:0, and C18:1 cis/C18:0) between studied breeds and Holstein. The level of signification was added in the brackets*[1].

	Dual Purpose Belgian Blue	Red and White	Jersey	Brown-Swiss	Montbeliarde	Normande
Number of Pure breed cows	275	35	46	61	85	162
Milk yield	-5.13 (***)	-1.77 (**)	-5.54 (***)	-1.90 (**)	-3.42 (***)	-7.05 (***)
% Fat	-0.23 (**)	0.04 (NS)	1.15 (***)	0.40 (***)	0.05 (NS)	0.10 (NS)
% Protein	0.04 (NS)	0.05 (NS)	0.40 (***)	0.28 (***)	0.20 (***)	0.20 (***)
% MONO	1.34 (**)	0.03 (NS)	-1.93 (***)	-1.56 (***)	0.08 (NS)	0.23 (NS)
C14:1 9-*cis*/C14:0	0.0021 (NS)	-0.0068 (**)	-0.0160 (***)	-0.0130 (***)	-0.0030 (NS)	-0.0031 (NS)
C16:1 9-*cis*/C16:0	0.0026 (NS)	0.0010 (NS)	-0.0044 (**)	-0.0031 (*)	0.0023 (NS)	0.0030 (**)
C18:1 *cis*/C18:0	0.03 (NS)	-0.14 (NS)	-0.38 (***)	-0.22 (*)	0.03 (NS)	-0.19 (**)

[1] Number if crossbred cows = 1,311; Number pure breed Holstein cows = 1,242; *** = P<0.001; ** = P<0.01; * = P<0.05.

The ratio of C18:1/C18:0 calculated by DePeters *et al.* (1995) from 16 HOL and 29 BSW was higher for BSW than HOL cows. The ratio of C16:1 *cis* to C16:0 calculated from the results mentioned by these authors were similar.

MON cows did not produce a different %MONO in milk fat. Consequently, Table 8 shows that this breed had Δ^9-desaturase indices similar to the ones observed for HOL cows. The ratio of C14:1 to C14:0 calculated from FA contents measured by Lawless *et al.* (1999) from 23 HOL and 29 MON was lower value for MON than HOL, whereas the C18 ratio was similar. Soyeurt *et al.* (2007) have found a significant difference between HOL and MON. This difference could be explained by a larger number of MON cows in this study. Even if the value observed for the ratio of C14:1 *cis-9* to C14:0 was lower than the one observed from HOL cows, %MONO produced by RED cows was not significantly different. The 2 other Δ^9-desaturase indices were not different than the ones observed in HOL cows. %MONO produced by NOR cows was not significantly different from the one observed for HOL cows. The values observed for the ratios of C16:1 *cis-9* to C16:0 and C18:1 *cis* to C18:0 were different between HOL and NOR (Table 8). The ratios of C14:1 to C14:0 and the total C18:1 to C18:0 calculated from the results obtained by Lawless *et al.* (1999) showed lower values for NOR than HOL cows.

7.6. Conclusions

Under the assumption that Δ^9-desaturase activity can be approximated by the ratio of product to substrate, this study showed different sources of variation for this enzyme. The effect of DIM was significant for all studied traits. A slightly increase within lactation was observed for the Δ^9-desaturase indices. The variation of these indices did not explain completely the variation of monounsaturated FA observed in this study within the lactation. Heritability values obtained for the ratios of C14:1 *cis-9* to C14:0, C16:1 *cis-9* to C16:0, and C18:1 *cis* to C18:0 were 20%, 20% and 3%, respectively. The low heritability observed for the third ratio could be explained by the influence of feeding in the production of C18:0 and C18:1 *cis* in milk. Negative genetic correlations observed between %FAT or %PROT and the studied indices suggested that an increase of Δ^9-desaturase activity by some of its products could inhibit the synthesis of milk fat or protein in the mammary gland. Some previous studies observed that some isomers of CLA altered milk fat composition. The implication of Δ^9-desaturase in the endogenous production of %MONO in milk fat was suggested by the positive genetic correlations observed between %MONO and the 3 Δ^9-desaturase indices. The values of indices differed between the studied dairy breeds. Particularly, JER cows had lower values compared to HOL and, consequently, had lower content of %MONO in milk fat. The same observation was made for the BSW cows. In contrast, DPB cows produced a higher content of % MONO in milk fat, had slightly higher values. Based on the estimated heritability values, the selection of animals based on their Δ^9-desaturase indices is feasible. This potential selection program should increase the content of monounsaturated fatty acids and conjugated linoleic acids in bovine milk.

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7.8. References

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Chapter VIII

GENETIC PARAMETERS OF SATURATED AND MONOUNSATURATED FATTY ACID CONTENTS IN BOVINE MILK AND AN INDICATOR OF BUTTER HARDNESS ESTIMATED BY TEST-DAY MODEL

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8.1. Interpretive Summary

The texture of butter is influenced notably by the changes of milk fatty acid composition. Milk fat with high unsaturated fatty acid contents produces butter more spreadable and softer. The natural sources of variation of fatty acid profile can be used. Feeding and genetics could be associated. This research studied the genetic variability as well as the effects of season and stage of lactation on fatty acid composition and the ratio of saturated to unsaturated fatty acids that is one of indicators reflecting the hardness of butter.

8.2. Abstract

Fatty acid composition influences the nutritional quality of milk as well as the technological properties of butter. Using a prediction of fatty acid (**FA**) contents by mid-infrared (**MIR**) spectrometry, a large amount of data concerning the FA profile in bovine milk was collected. The large size of this data set permitted to consider more complex models than those used in previous studies. The aim of the current study was to estimate the effects of season and stage of lactation as well as genetic parameters of saturated (**SAT**) and monounsaturated (**MONO**) fatty acid contents in bovine milk and milk fat, and the ratio of SAT to unsaturated fatty acids reflecting the hardness of butter, using 7 multiple-trait random regressions test-day models. The relationship between these FA traits with common production traits was also studied. The dataset contained 100,841 test-day records from 11,626 Holstein heifers. The seasonal effect was studied based on unadjusted means. These results confirmed the production of milk fat with more UNSAT during spring and summer compared to winter (on average 63.13% *vs.* 68.94% of SAT in fat). With the same method, the effect of stage of lactation on fatty acid profile was studied. Holstein heifers at early stage of lactation produced lower content of SAT in their milk fat. Variance components were estimated using Bayesian method via Gibbs sampling. Heritability of SAT in milk (0.42) was higher than heritability of SAT in milk fat (0.24). Estimates of heritability for MONO were also different in milk and fat (0.14 *vs.* 0.27). The hardness of butter was moderately heritable (0.27). For all of these traits, the obtained heritability estimates and the genetic and phenotypic correlations varied through the lactation. These heritability differences could be partly explained by the variation of energy balance of cows within lactation. In conclusion, this study confirms the genetic variability of FA in bovine milk and suggests a possible link between the changes of fatty acid composition and the energy balance of cows.

(**Keywords:** genetic parameters, energy balance, milk, fatty acid, fat, mid-infrared)

8.3. Introduction

The World Health Organization points out that an unhealthy nutrition contributes to some chronic diseases such as diabetes, cardiovascular diseases, and cancer. One of the 5 proposals formulated by this organization is to decrease the energy intake and prefer a fatty acid composition composed of a higher proportion of unsaturated fatty acids (**UNSAT**). This statement is related to the results obtained by several studies which mentioned the hypercholesterolemic effect of saturated fatty acids (**SAT**), especially C12:0, C14:0, and C16:0 (*e.g.*, Hu *et al.*, 1999; Fernandez and West, 2005). By comparison to SAT, the intake of UNSAT seems to decrease the level of cholesterol in blood (Ulbricht and Southgate, 1991; Noakes *et al.*, 1996; Hu *et al.*, 2001; Fernandez and West, 2005) and thus, reduces the risk of cardiovascular diseases.

Besides these effects on human health, increasing the proportion of UNSAT in bovine milk fat has also positive impacts on the technological properties of butter. Butter spreadability is improved by increasing the contents of UNSAT and short chain fatty acids (**FA**) in milk fat (Bobe *et al.*, 2007). In accordance with Bobe *et al.* (2003), the textural properties of butterfat could be modified by using the phenotypic variation of fatty acid composition observed by these authors from cows fed with the same diet. This variation could be partially genetic. First results obtained by Soyeurt *et al.* (2007b) confirmed this hypothesis.

Few authors as Renner and Kosmack (1974), Karijord *et al.* (1982), Soyeurt *et al.* (2006, 2007, and 2008), and Stoop *et al.* (2008) have studied the individual variability of FA. The results obtained by these authors suggested the presence of significant genetic variability of FA profile in bovine milk and fat. Generally, the contents of individual SAT were more heritable than individual UNSAT. This genetic variability of FA should be sufficient to implement a selection program based on the improvement of fatty acid composition. These studies suffered from different disadvantages. Renner and Kosmack (1974) as well as Karijord *et al.* (1982) and Stoop *et al.* (2008) analyzed a limited number of samples without repetitions on animals (even if Karijord *et al.* (1982) analyzed 7,000 milk samples) likely due to the expensive cost of gas chromatographic analysis needed to measure the FA contents in milk fat. Due to this relatively low number of samples and the lack of repeated measurements, Renner and Kosmack (1974) and Karijord *et al.* (1982) estimated the genetic parameters of FA contents in bovine milk fat using a sire model. Stoop *et al.* (2008) used a single trait animal model. Thanks to the development of calibration equations to predict FA contents in bovine milk by mid-infrared (**MIR**) spectrometry (Soyeurt *et al.*, 2006b), Soyeurt *et al.* (2007) estimated the genetic parameters of FA using a multiple-trait (**MT**) test-day (**TD**) animal model on a larger number of samples including repeated measurements of fatty acid composition. The use of a MT-TD model presents major advantages: 1) more efficient use of collected data, 2) a genetic model that fits better the biology of dairy cows, 3) accounting for environmental effects at each different test-day milk recording (useful, for instance, for the herd management), and 4) more accurate estimation of cow indices (Schaeffer *et al.*, 2000; Mayeres *et al.*, 2004; Muir *et al.*, 2007). Parametric curves as Ali Schaeffer curve (Ali and Schaeffer, 1987), the Wilmink curve (Wilmink, 1987), or orthogonal polynomials have been used to model the random regressions. The disadvantage of TD model is their computation cost (Druet *et al.*, 2003). The database related to FA contents is generally small. Thus, the computational cost for a TD model using a limited database stays acceptable. The TD model used by Soyeurt *et al.* (2007 and 2008) did not include random regressions (**RR**) to model the shape of lactation curve, assuming that the genetic parameters of FA were constant through the lactation. Karijord *et al.* (1982) and Soyeurt *et al.* (2008) showed a phenotypic variation of FA contents within lactation. In accordance with Karijord *et al.* (1982), the contents of each individual SAT increased until the 4th month of lactation and then decreased slowly. The opposite trend was observed by these authors for UNSAT. Soyeurt *et al.* (2008) found that the lowest content of MONO in fat was reached around 100 DIM. Soyeurt *et al.* (2007b) showed that the ratio of SAT to UNSAT, approximating the hardness of butter, varied also through the lactation. The values increased rapidly until 100 DIM where it was close to 2. Then, the values decreased slowly to be equal to 1.5 at 365 DIM. All FA traits seem to be influenced by the stage of lactation.

The aim of this study was 1) to study the effects of season and stage of lactation on the contents of SAT and MONO, and for the ratio of SAT to UNSAT, 2) to estimate their genetic parameters, 3) to study the relationship between these FA traits and production traits (milk yield, percentage of fat, and protein content).

8.4. Materials and Methods

8.4.1. Animal Population and Milk Samples

Following the standard procedures edited by the International Committee for Animal Recording (2007), 27,959 samples representing 50% of morning milk and 50% of evening milk were collected between April 2005 and July 2007 from 96 herds during the Walloon milk recording. Herds were selected from their degree of pedigree completeness, their number of dairy breeds in the farm, and their geographical position. Eight herds were followed since April 2005. Eighteen herds were studied since November 2005. The remaining herds were controlled since January 2007. Due to technical issues, the number of TD was not constant for all herds. Also, some cows were dried off or calved during this experiment. The percentages of fat (**%FAT**) and protein (**%PROT**) were measured using a Foss MilkoScan FT6000 spectrometer (Foss, HillerØd, Denmark). The spectra generated during this infrared analysis were recorded for all analyzed milk samples. For this study, only heifers containing more than 84% of Holstein genes were studied and represented 1,167 cows. To increase the number of contemporaries, the milk yield, %FAT, and %PROT known for all studied heifers and herds were added. Test-day records with DIM less than 5 and greater than 365 were deleted. The final edited data file contained 100,841 TD records from 11,626 first parity Holstein cows (Holstein genes > 84%) recorded from 1991 to 2007. Pedigree contained 18,573 animals including 1,895 sires.

8.4.2. Predicted contents of Fatty Acids in Milk and Milk Fat

Soyeurt *et al.* (2006b) developed 42 calibration equations to predict the fatty acid composition in bovine milk by MIR spectrometry. New calibration equations were built from FA concentrations of 114 samples measured using a gas chromatograph (model 6890N; Agilent Technologies, Inc., Palo Alto, CA) and a CPSIL-88 capillary column (length: 100m; internal diameter: 0.25mm; film thickness: 0.20µm; Varian, Inc., Palo Alto, CA).

A total of 78 samples were selected using principal components approach (**PCA**) on the 1,609 frozen milk samples collected between March 2005 and May 2006 from 475 cows representing 6 dairy breeds: dual purpose Belgian Blue, Holstein Friesian, Jersey, Normande, Montbeliarde, and non-Holstein Meuse-Rhine-Yssel type Red and White. In order to increase the spectral variability of calibration set, 36 milk samples were also chosen based on the results of successive PCA conducted during the routine Walloon milk spectral analysis.

The methodology used to measure the FA contents was the one described previously by Soyeurt *et al.* (2008). The milk fat of these selected samples was extracted according to ISO Standard 14156:2001 (International Organization for Standardization, 2001). Preparation of FA methyl esters was made following the ISO 15884:2002 (International Organization for Standardization, 2002). These milk fat samples were analyzed using gas chromatography as described above. The conditions for the chromatographic analyses were as follows: carrier gas, helium; average velocity, 19cm/s; cold on-column injector; flame ionization detector at 255°C; and a temperature program from 60°C (5 min) to 165°C (at 14°C/min) during 1 min, then 165 to 225°C (at 2°C/min) during 17 min. The volume injected was 0.5µL. The FA concentrations were measured according to the method developed by Collomb and Bühler (2000). An anhydrous milk fat with a certified FA composition (reference material BCR-164, obtained from the Commission of the European Communities, Brussels, Belgium) was used to determine the FA methyl esters response factors, the repeatability, and the accuracy of this method.

The methodology used to develop the calibration equations was identical to the one mentioned previously by Soyeurt *et al.* (2006b and 2008). More details about the calibration procedure are available in the course of Williams (2007). The calibration equations were built from chromatographic and spectral data using a specific program for multivariate calibration (WINISI III; http://www.winisi.com/) and partial least squares regressions. No treatment was applied beforehand on the spectral data. As over-fitting can occur through the use of partial least squares regressions technique, cross-validation of the developed calibration equations was used to prevent this. Cross-validation was applied to validate the number of factors used in the different equations and to estimate the validation errors of the obtained equations. These errors were calculated by partitioning the calibration set into several groups. In this study, a full cross-validation was used. Thus, a calibration was performed for each sample, until every sample had been predicted once. Validation errors were combined into a standard error of cross-validation (**SECV**). In order to assess the robustness of the developed calibration equations, several statistical parameters were calculated: mean and SD measured from reference concentrations of FA, standard error of calibration (**SEC**), calibration coefficient of determination ($\mathbf{R^2_C}$), SECV, cross-validation coefficient of determination ($\mathbf{R^2_{CV}}$), and the ratio of SD to SECV (**RPD**). The calibration equations predicting the contents of SAT and MONO in milk (g/dL of milk) were applied to the recorded spectra. Using the density of milk, these FA contents were converted into gram per 100 grams of milk. Using the %FAT measured by the Foss MilkoScan FT6000 (Foss, Hillerød, Denmark), these FA contents were converted into gram per 100 grams of fat. The hardness of butter was defined as the ratio of SAT to UNSAT in fat. The content of UNSAT was estimated by: 100 – the content of SAT in fat.

The ability of established calibration equations to predict the contents of SAT and MONO in bovine milk were also assessed by analyzing 14 independent samples. An additional larger validation is planned in a parallel study. These samples were taken randomly in the 1,609 frozen milk samples collected previously.

8.4.3. Statistical Model

Variance components were estimated by Bayesian method with Gibbs sampling (Misztal, 2007) using 7 MT-TD-RR models:

$$Y = \mathbf{X\beta} + \mathbf{Q(WI} + \mathbf{Zp} + \mathbf{Zu)} + \mathbf{e}$$

where Y was the vector of observations (milk yield, %FAT, %PROT, and SAT in milk and fat; milk yield, %FAT, %PROT, and MONO in milk and fat; milk yield,

%FAT, %PROT, and the ratio of SAT to UNSAT; the ratio of SAT to UNSAT, SAT, and MONO in milk and fat); $\boldsymbol{\beta}$ was the vector of fixed effects (herd x test day, stage of lactation (24 classes of 15 DIM), age (3 classes: < 29 mo, 29-32 mo, > 32 mo)); $\mathbf{Q}$ was the covariate matrix of second-order Legendre polynomials; $\mathbf{I}$ was the vector of random herd x date of calving effects; $\mathbf{p}$ was the vector of random permanent environmental effects; $\mathbf{u}$ was the vector of animal effects; $\mathbf{X}, \mathbf{W}$ and $\mathbf{Z}$ were incidence matrices; $\mathbf{e}$ corresponded to the vector of random residual effects.

Priors of variance components were estimated using Expectation Maximisation REML (Misztal**,** 2007) using 12 MT-TD models (milk yield and SAT or MONO in milk and fat; %FAT and SAT or MONO in milk and fat, %PROT and SAT or MONO in milk and fat). The fixed and random effects included in these models were the same as those mentioned previously. Priors of variance components for the ratio of SAT to UNSAT were those obtained previously by Soyeurt *et al.* (2007b). Residual variances were assumed to be independent. Posterior means of (co)variance components were calculated using 90,000 samples after a burn-in of 10,000 samples.

Average daily heritability values were defined as a ratio of average genetic variance to the average sum of genetic, herd x date of calving, permanent environment, and residual variances for each DIM from 1 to 365. The same method was used to calculate the estimates of herd x date of calving random effect, permanent environment, and residual effect expressed in percentage of phenotypic variances. These values showed in the current study for milk yield, %FAT, and %PROT were the average of values obtained from the 5 MT-TD-RR models including these traits. Daily phenotypic and genetic correlations between 2 traits (a and b) were described as:

$$r = \frac{\mathrm{cov}(a, b)}{\sqrt{\mathbf{T}\sigma_a^2\mathbf{T'}*\mathbf{T}\sigma_b^2\mathbf{T'}}}$$

where $\mathbf{T}$ was the transformation matrix corresponding to the sum of Legendre polynomials for each DIM from 1 to 365; σ_a^2 and σ_b^2 were the variances for traits a and b. Heritability values for milk yield, %FAT, and %PROT and correlations among these traits shown in the current study were the average of values obtained from the 5 MT-TD-RR models including these traits.

8.5. Results and Discussion

8.5.1. Calibration Equations

Table 1 describes the results obtained during the calibration procedure for the mean, SD, SEC, R²c, SECV, R²cv, and RPD. The values of RPD were equal to 5.78 and 3.75 for SAT and MONO, respectively. If RPD is equal or superior to 2, the infrared predicted value is considered to be a good indicator of the studied trait (Sinnaeve *et al.*, 1994). In the same way, the estimates of R²cv were 0.97 for SAT and 0.93 for MONO. If R²cv ranged between 92% and 96%, Williams *et al.* (2007) mentioned that the predicted values could be used in most applications, including quality assurance.

Table 1. *Descriptive statistics calculated for the 2 calibration equations used in this study and established from 114 milk samples.*

Fatty acid (g/dL of milk)	Mean	SD	SEC	R²c	SECV	R²cv	RPD
Saturated	3.20	0.85	0.09	0.99	0.15	0.97	5.78
Monounsaturated	1.40	0.43	0.08	0.97	0.12	0.93	3.65

[1] SD = standard deviation; SEC = standard error of calibration; R²c = calibration coefficient of determination; SECV = standard error of cross-validation; R²cv = cross-validation coefficient of determination; RPD = the ratio of SD to SECV.

In order to confirm the ability of established calibration equations to predict the contents of SAT and MONO in bovine milk, a validation with 14 independent milk samples chosen randomly was conducted. Table 2 presents descriptive statistics obtained from chromatographic and spectral analysis of these 14 selected samples. The variation of fatty acid composition for these samples was relatively large. The concentrations of SAT and MONO in milk fat ranged from 61.69% to 77.89% and from 19.14% to 33.03%, respectively (Table 2). Based on the values shown in Table 2, bias corresponding to the mean difference between MIR and chromatographic data were calculated and were equal to 0.17% for SAT and -0.11% for MONO.

Table 2. *Mean, standard deviation (SD), minimum (Min.) and maximum (Max.) values of saturated and monounsaturated fatty acid contents in milk fat (g/100 g of fat) observed from the chromatographic and spectral analysis of 14 milk samples collected randomly from the 1,060 samples collected previously.*

g/100 g of fat	Mean	SD	Min.	Max.
Chromatographic analysis				
Saturated fatty acids	67.98	4.69	61.69	77.89
Monounsaturated fatty acids	27.33	4.26	19.14	33.03
Mid-infrared spectrometry				
Saturated fatty acids	67.81	5.27	58.67	77.44
Monounsaturated fatty acids	27.44	5.10	19.54	38.44

Due to the low estimates of bias, the predicted values of SAT and MONO in milk obtained from the developed calibration equations can be considered as good indicator of these FA. Figure 1 illustrates the reference contents of SAT and MONO measured by gas chromatography on the x axis and the infrared predicted values for these same FA on the y axis. This figure confirms the linear relation between these 2 data sets.

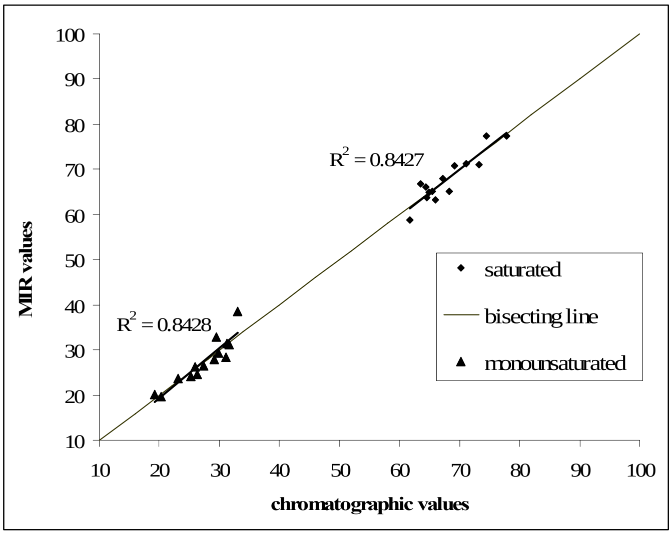


Figure 1. *Relation between the contents of saturated and monounsaturated fatty acids measured by gas chromatography and predicted by mid-infrared (MIR) spectrometry from 14 milk samples.*

The validation coefficients of determination (**R^2v**) were equal to 84.27% (R=91.80%) and 84.28% (R=91.80%) for SAT and MONO, respectively. The values of R^2v were inferior to those estimated from the cross-validation. In accordance with Williams *et al.* (2007), these values of R^2v are sufficient to be considered in some research applications. By using successive PCA during the Walloon milk recording, our group is continuing to select new samples to increase the robustness of developed calibration equations.

8.5.2. Seasonal Effect

The variation for the contents of SAT (66.26% with SD=6.15) and MONO (27.55% with SD=4.80) was large (Table 3). Based on the ratio of SAT to UNSAT, the milk fat contained twice as much SAT than UNSAT (2.06 with SD=0.55). Stoop *et al.* (2008) calculated from 1,918 samples a mean value for the ratio of SAT to UNSAT equal to 2.80. These authors mentioned that this value was overestimated because the UNSAT content included only the major UNSAT fatty acids in milk.

Table 3. *Mean, standard deviation (SD) for milk yield, the percentages of fat and protein (100,799 test-day records), the contents of saturated and monounsaturated fatty acids in milk and milk fat, and the ratio of saturated to unsaturated fatty acids approximating the hardness of butter (4,666 test day records). To assess the distribution of these data, the values of skewness and kurtosis were added.*

	Mean	SD	Skewness	Kurtosis
Milk (kg/day)	22.54	6.13	0.17	0.08
Fat (g/100g of milk)	4.05	0.68	0.59	1.70
Protein (g/100g of milk)	3.32	0.34	0.64	2.16
Saturated (g/100 g of milk)	2.63	0.54	0.57	1.89
Monounsaturated (g/100 g of milk)	1.08	0.26	1.85	7.46
Saturated (g/100 g of fat)	66.26	6.15	-0.60	0.31
Monounsaturated (g/100 g of fat)	27.55	4.80	0.65	0.47
Hardness of butter	2.06	0.55	0.39	0.26

[1] Hardness of butter = the ratio of saturated to unsaturated fatty acids.

The coefficient of variation for this traits observed in the current study (26.70%) was higher than the one observed by Stoop *et al.* (2008) likely due to the difference in the number of analyzed samples.

Figure 2 illustrates the seasonal effect on the contents of SAT and MONO in bovine milk (g/100g of milk) and milk fat (g/100g of fat), and the ratio of SAT to UNSAT. This effect was estimated from unadjusted means. These means calculated for July, August, and September were different than the estimates normally expected based on the trend of the curve drawn in Figure 2. These differences can be explained by the month of TD and the number of observations. During the summer, there is one month without infrared analysis for the routine Walloon milk recording.

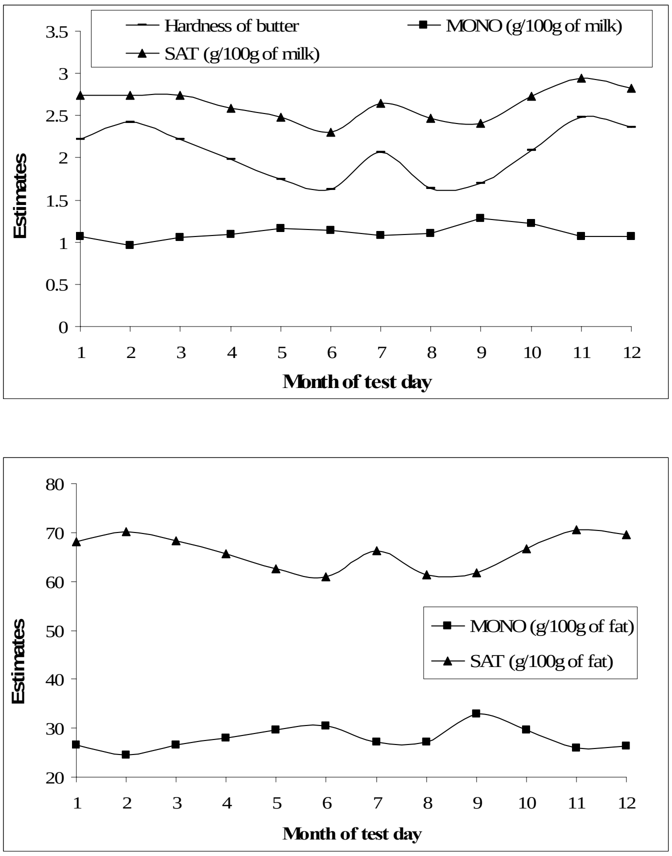


Figure 2. *Effect of season on the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk (g/100 g of milk) and milk fat (g/100g of fat) and the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter. SD ranged between 0.21 and 0.59 for SAT and MONO in bovine milk, and the ratio of saturated to unsaturated fatty acids. SD ranged from 3.72 to 5.70 for SAT and MONO in milk fat.*

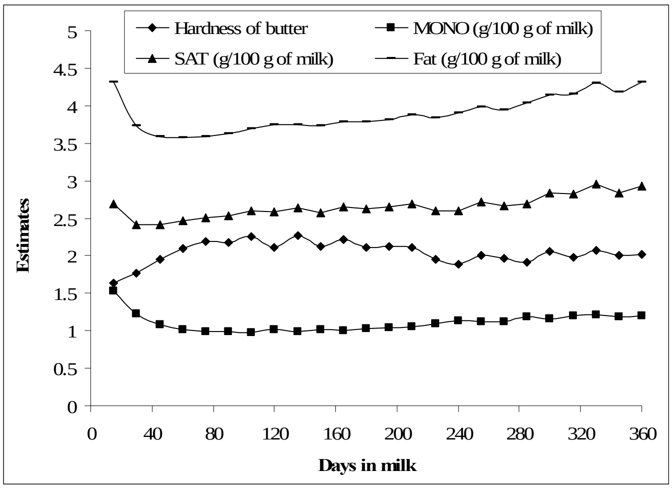
The number of observations used to calculate these average values for July, August, and September were low (52 for July, 61 for August, and 58 for September). For the other months, the minimum and maximum numbers of observations were equal to 103 and 799. Without considering the means obtained for July, August, and September, Figure 2 suggests that the content of SAT decreased until the summer and then increased (63.13% *vs.* 68.94%). The opposite relation was observed for MONO. Karijord *et al.* (1982) and Lock and Garnsworthy (2003) have observed a similar seasonal effect for these 2 traits. Gallacier *et al.* (1974) observed at the end of summer the lowest concentrations of SAT (C4 - C16) and the higher contents of C18:0 and C18:1.

The ratio of SAT to UNSAT decreased until the summer and then increased. As expected, these results confirmed that the butter is more spreadable at the end of spring and during the summer. This seasonal effect is explained mainly by the changes of feeding (Chilliard *et al.*, 2001), especially the grazing period. Taking into account the date of test in the model was important for all studied traits.

8.5.3. Lactation Effect

Figure 3 describes the effect of DIM on the contents of SAT and MONO in bovine milk and fat, and the ratio of SAT to UNSAT. This figure was built from unadjusted means. The numbers of observations for each class of 15 DIM ranged from 76 to 274. A strong decrease in %FAT in bovine milk was observed until 60 DIM where the %FAT was the lowest. De Vries and Veerkamp (2000) obtained the same curve. The highest %FAT observed at the beginning of lactation could be explained by the negative energy balance of cows in early lactation. To sustain the high milk production, the cows mobilize their lipids from adipose tissue. For example, cows, which produced average 30 kg of milk/day, secreted 1.5 kg of fat from mobilization of 1 kg of adipose tissue lipids/day (Barber *et al.*, 1997). The FA stored in the form of triacylglycerols in adipose tissue were mainly C16:0, C18:0, and C18:1 *cis-9* (Barber *et al.*, 1997; Chilliard *et al.*, 2001). This unbalanced energy status involves some changes in milk composition to sustain the milk production. The contents of SAT and MONO in milk decreased rapidly until the peak of lactation and then increased slowly (Figure 3).

The content of MONO in milk fat followed the same trend as %FAT. The content of SAT in fat increased until the peak of lactation and then decreased. The same trend was observed by Karijord *et al.* (1982) for the major saturated FA in bovine milk fat, except for C18:0. The ratio of SAT to UNSAT varied through the lactation following the same trend that the one observed for the content of SAT in fat. The peak for this trait reached around 80 DIM.



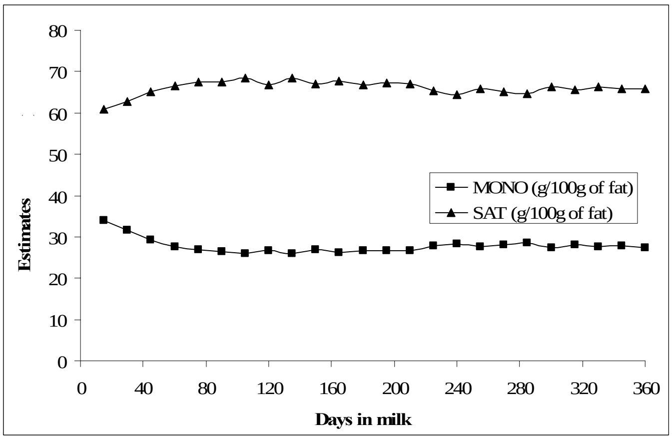


Figure 3. *Effect of days in milk on the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk (g/100g of milk) and milk fat (g/100g of fat), and the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter. SD ranged between 0.17 and 0.66 for SAT and MONO in bovine milk, and the ratio of saturated to unsaturated fatty acids. SD ranged from 3.81 to 6.56 for SAT and MONO in milk fat.*

Two conclusions can be formulated from these results. First, the observed effect of lactation on FA profile suggests that the genetic parameters for these studied traits vary through the lactation. Secondly, the effect of lactation on fatty acid composition could be used to modify the technological properties of butter.

8.5.4. Heritability

Based on the results of skewness and kurtosis shown in Table 3, the distribution of studied traits approached normality. Table 4 shows average daily estimates expressed in percentage of phenotypic variance for each studied random effect. Figure 4 illustrates the changes of heritability values through the lactation. Average daily heritability for milk yield of 0.27 (Table 4) obtained in this study was lower than that estimated for Holstein cows in Belgium (0.48) (Interbull, 2007). This value was also lower than the one observed by Miglior *et al.* (2007) (0.52). Heritability values for milk yield decreased until the lactation peak and then increased slightly (Figure 4). The values ranged from 0.25 to 0.33. Muir *et al.* (2007) observed a similar trend for Italian Holstein cows. Other studies as Veerkaamp and Goddard (1998), Gengler *et al.* (2004), Mayeres *et al.* (2004), and Druet *et al.* (2005), found the highest heritability at mid-lactation.

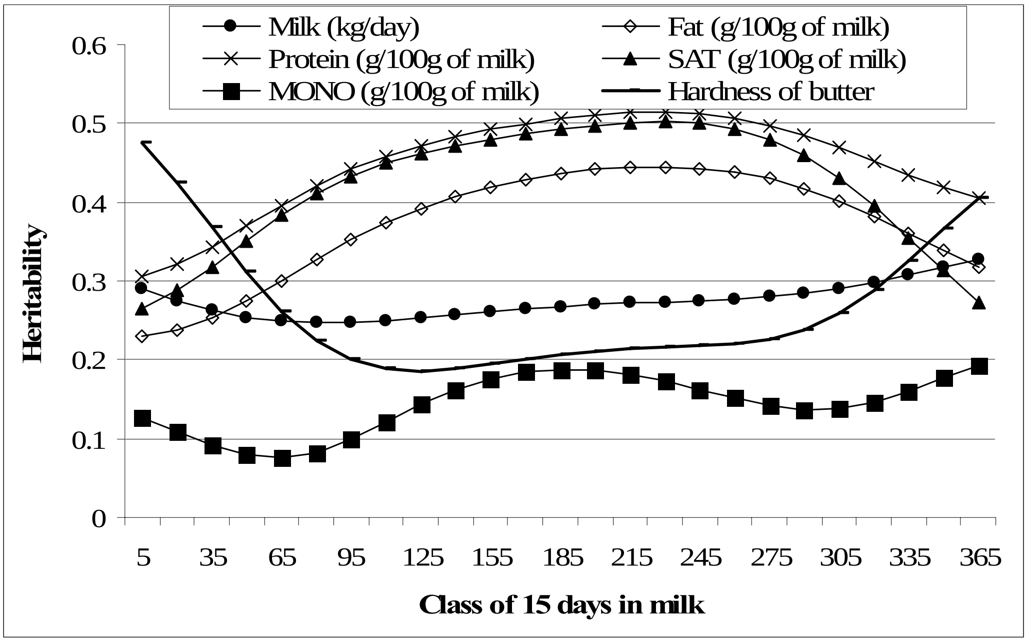
Average daily heritability estimates observed for %FAT and %PROT were equal to 0.37 and 0.46, respectively (Table 4). These values were similar to those obtained previously by Soyeurt *et al.* (2007b) with the same model but a lower number of data. Miglior *et al.* (2007) obtained higher estimates for Holstein heifers (0.55 and 0.58 for %FAT and %PROT, respectively). The changes of heritability estimates through the lactation were similar for %FAT and %PROT with higher values for %PROT (Figure 4). The heritability values ranged from 0.23 to 0.44 for %FAT, and from 0.30 to 0.51 for %PROT. The highest heritability values were reached in mid-lactation. Druet *et al.* (2005) observed similar trends for these 2 traits even if these authors found higher heritability values for %FAT compared to %PROT.

Average daily estimates of heritability were 0.42 and 0.14 for SAT and MONO in milk, respectively. Lower value (0.24) was obtained for SAT expressed in milk fat than the heritability of SAT in milk. A highest value was found for MONO in fat (0.24) compared to the heritability of MONO (Table 4). Soyeurt *et al.* (2007) observed also this difference in SAT heritability values between the contents expressed in milk and milk fat even if this difference was slightly higher in the current study. These differences in heritability values between Soyeurt *et al.* (2007) and the current one can be explained by the methodology used to estimate the heritability values. Soyeurt *et al.* (2007 and 2008) assumed that the genetic value did not vary through the lactation. By the introduction of orthogonal regressions in the model, this study takes into account the fluctuations of genetic parameters through the lactation. The heritability estimates mentioned in Table 4 represent the means of values obtained for 1 to 365 DIM. As the differences between the extremes and the middle of lactation shown in Figure 4 were high, the average values for SAT and MONO in fat (g/100g of fat) shown in the study were higher than those estimated without modelling the lactation curve.

Table 4. *Average daily estimate for each studied random effects (genetic, herd x test day, permanent environment, residual) expressed in % of phenotypic variation for the quantity of milk, the percentage of milk fat and protein, the contents of saturated and monounsaturated fatty acids in bovine milk (g/100 g of milk) and milk fat (g/100 g of fat)*[1].

	Heritability	Herd	Permanent environment	Residual
Milk (kg/day)	0.27 (0.009)	0.08 (0.012)	0.41 (0.014)	0.24 (0.001)
Fat (g/100g of milk)	0.37 (0.016)	0.05 (0.014)	0.19 (0.018)	0.39 (0.004)
Protein (g/100g of milk)	0.45 (0.008)	0.11 (0.018)	0.17 (0.019)	0.27 (0.002)
Saturated (g/100 g of milk)	0.42	0.05	0.24	0.29
Monounsaturated (g/100 g of milk)	0.14	0.04	0.33	0.49
Saturated (g/100 g of fat)	0.24	0.09	0.17	0.50
Monounsaturated (g/100 g of fat)	0.27	0.09	0.19	0.45
Hardness of butter	0.27	0.07	0.14	0.52

[1] Hardness of butter= the ratio of saturated to unsaturated fatty acids. Herd = herd x date of calving random effect; Values for milk yield, percentages of fat and protein were the average estimates calculated from the 5 runs. The standard deviations were added in the brackets.



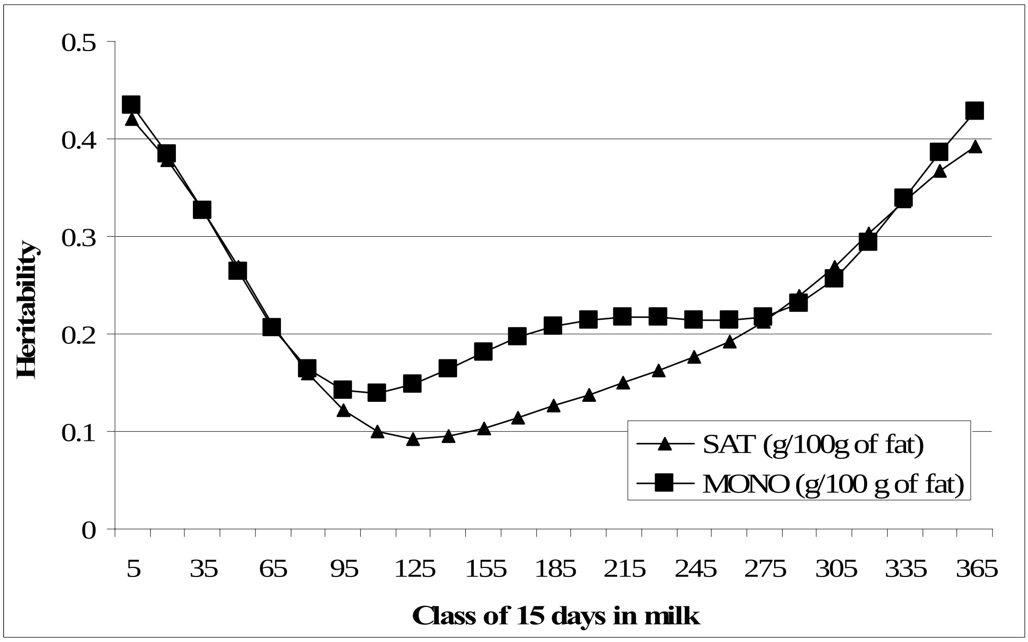
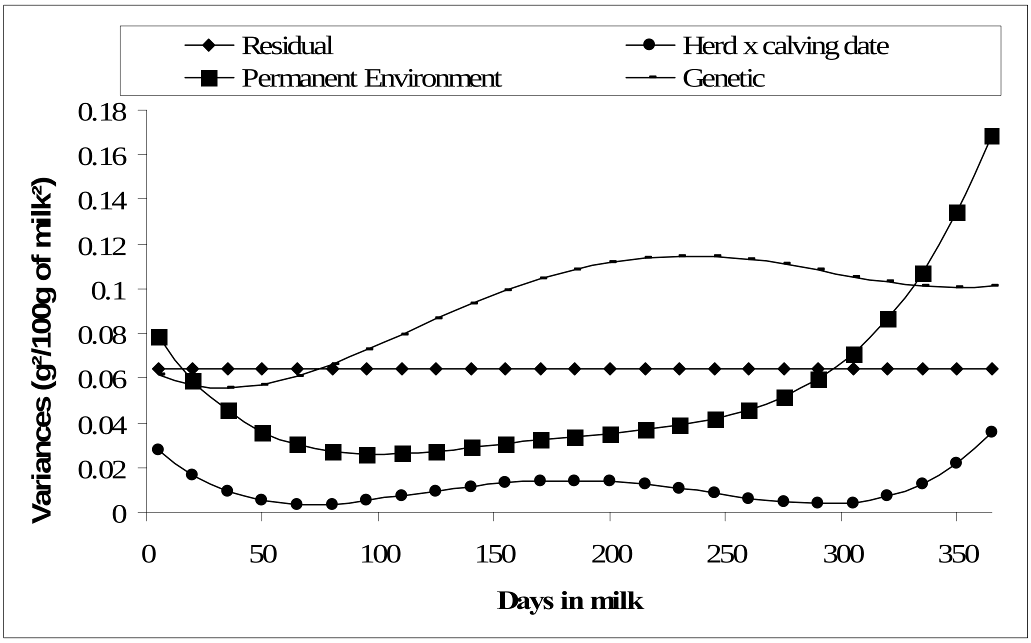


Figure 4. *Changes of heritability value through the lactation for milk yield, percentages of fat and protein, the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter and the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk (g/100g of milk) and milk fat (g/100g of fat).*

The trend of heritability values for SAT in bovine milk was similar to the one observed for %FAT and %PROT. This could be explained by the highly positive genetic correlations existing between SAT and %FAT (Karijord *et al.*, 1982; Soyeurt *et al.*, 2007) (Figure 5) and the positive genetic correlation between SAT and %PROT

(Soyeurt *et al.*, 2007). The heritability values for this trait ranged from 0.26 to 0.50 for SAT in milk.



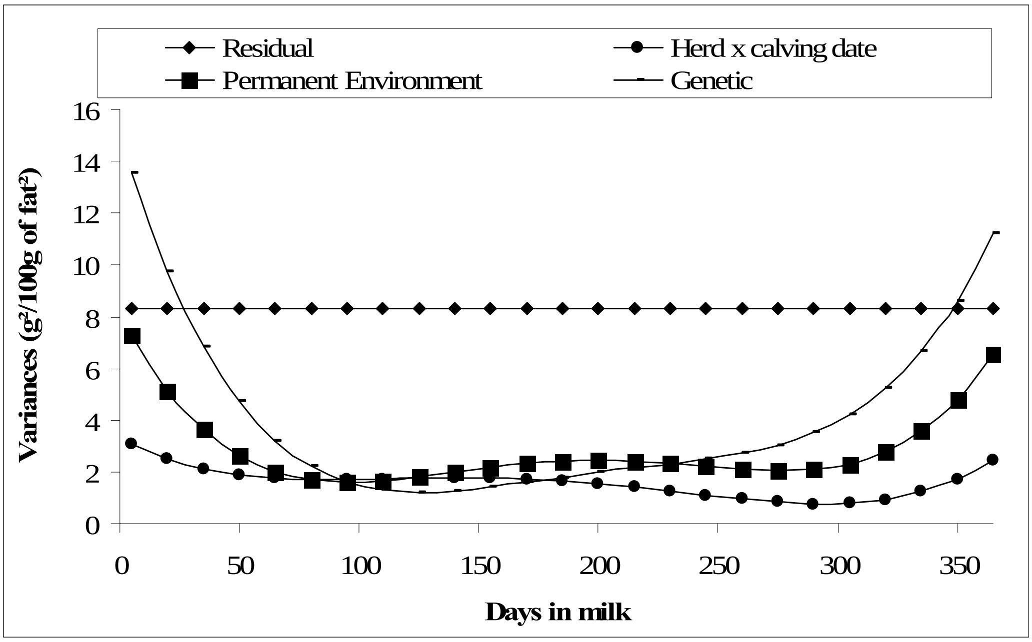


Figure 5. *Changes of additive genetic, herd x calving date, permanent environment and residual variances through the lactation for the contents of saturated (SAT) in bovine milk (g/100g of milk) and milk fat (g/100g of fat).*

The changes of heritability values for SAT and MONO in fat were extremely large through the lactation (Figure 4). Heritability values ranged from 0.09 to 0.42 for SAT, and from 0.14 to 0.43 for %MONO. The highest heritability values were observed at the beginning and the end of lactation. The highest heritability estimates observed at the early stage of lactation could be explained by the energy status of cows. As the energy balance of cows at early stage of lactation is negative, cows mobilize their lipid reserves from adipose tissue. This mobilization requires some internal mechanisms genetically regulated. The part of genetics at the early stage of lactation could be expected higher. The higher heritability estimates observed at the end of lactation could be related to the persistency of lactation. Fluctuation of heritability for SAT in milk and milk fat was similar to the one observed for the additive genetic variance of SAT in milk and milk fat (Figure 5).

Figure 4 and Table 4 present the heritability values observed for the ratio of SAT to UNSAT, reflecting the hardness of butterfat. Average daily heritability observed for this trait was 0.27 (Table 4). This value was higher compared to that found previously by Soyeurt *et al.* (2007b) (0.11) but in agreement with the value obtained by Stoop *et al.* (2008) (0.20). This value confirmed the suggestion of Bobe *et al.* (2003) concerning the possible influence of genetics on technological properties of butter. As expected, the curve of variation for heritability of this ratio followed the same trend than the one observed for %SAT in bovine milk fat (Figure 4). The heritability values ranged from 0.19 to 0.47. The highest values were observed at the beginning and at the end of lactation.

8.5.5. Correlations

Figures 6 to 8 show the trend of daily genetic and phenotypic correlations among traits through the lactation. Generally the genetic correlations between milk yield and the studied milk components (%FAT, %PROT, SAT and MONO in milk, and the hardness of butter) were negative. These negative values are explained by a dilution effect. These correlations were lower at the beginning of lactation. Changes of genetic correlation between milk yield and MONO in milk were more accentuated. The lowest correlations were observed at the beginning and end of lactation. The phenotypic correlations observed in Figure 7 between milk yield and milk components were relatively stable through the lactation, except for MONO. The phenotypic correlations between milk yield and MONO decreased through the lactation.

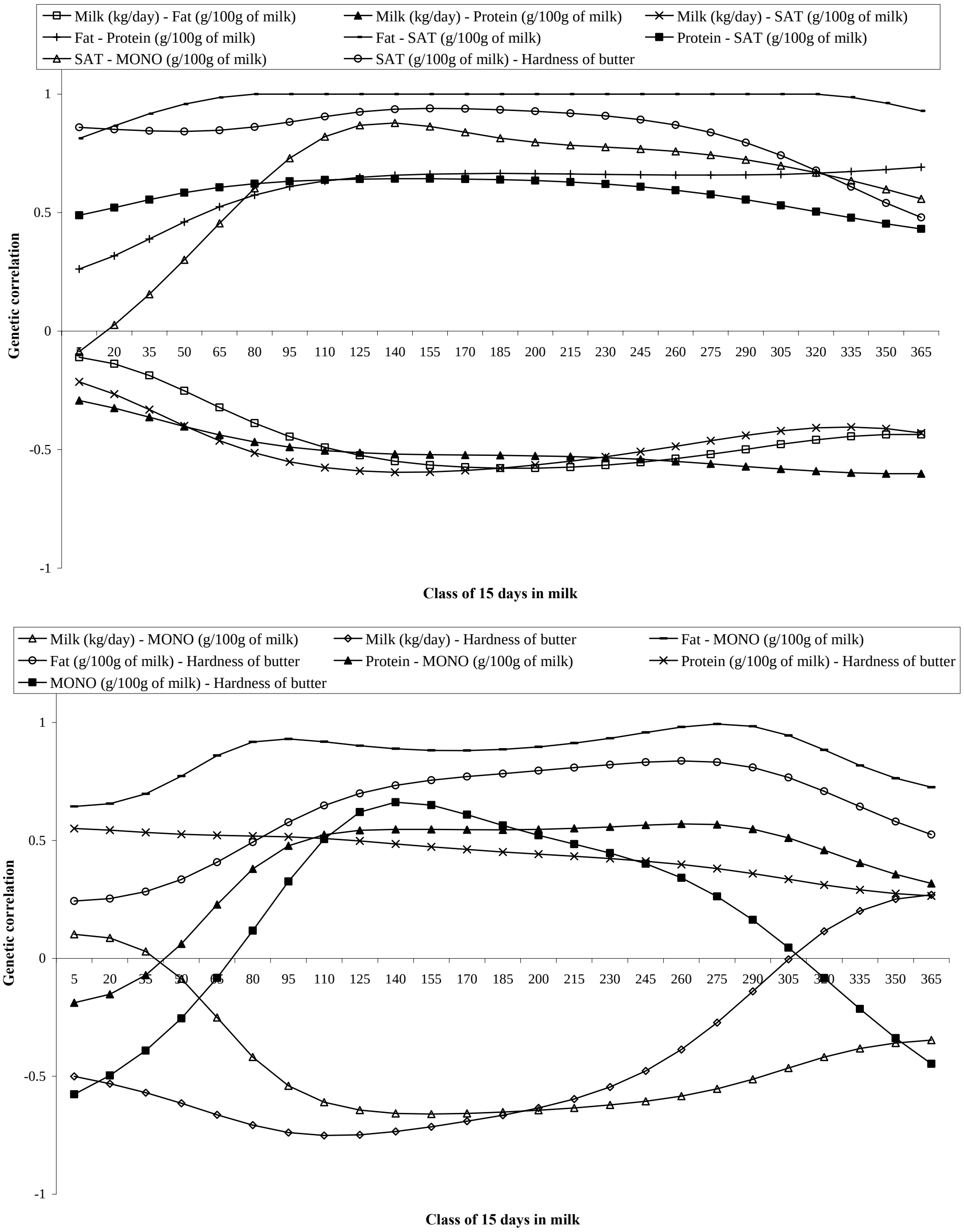


Figure 6. *Changes of genetic correlation through the lactation among milk yield, percentages of fat and protein, the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter and the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk (g/100g of milk).*

The curves of variation obtained for genetic correlations between milk yield and the fatty acid composition traits (SAT and MONO in fat) presented an opposite trend than the one observed for SAT and MONO. The highest genetic correlations were observed at the beginning of lactation and were positive for SAT and negative for MONO in fat. As the genetic correlation between SAT and MONO in fat was negative (Soyeurt *et al.*, 2007; Figure 6), it was expected that the curves observed for SAT and MONO in milk fat would be in opposite trend. The phenotypic correlations between these FA traits and milk yield stayed stable through the lactation (Figure 8).

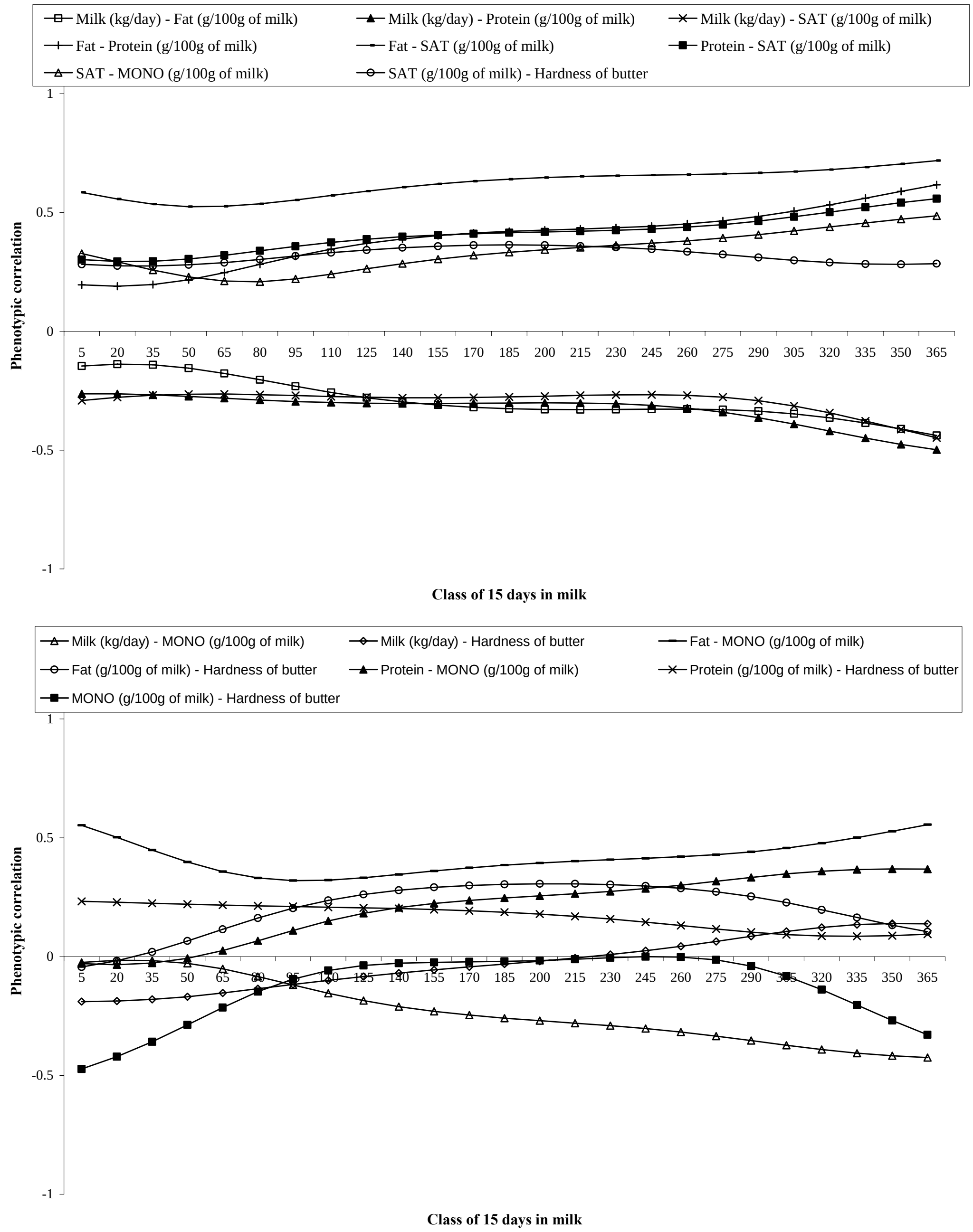


Figure 7. *Changes of phenotypic correlation through the lactation among milk yield, percentages of fat and protein, the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter and the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk (g/100g of milk).*

The genetic correlations between %FAT and SAT or MONO in milk (g/100g of milk) were positive and high through the lactation (Figure 6). The changes for genetic correlations between %FAT and the ratio of SAT to UNSAT were more accentuated. These values were lowest at the beginning of lactation. Phenotypic correlations between %FAT and SAT were stable within lactation (Figure 7). The phenotypic correlations observed between %FAT and MONO or the ratio of SAT to UNSAT changed through the lactation. As for genetic correlations, the phenotypic correlations between %FAT and the hardness of butter were lower at the beginning of lactation (Figure 7). Similar results were obtained previously (Soyeurt *et al.*, 2007b). A nadir was observed around 80 DIM for the curve of phenotypic correlations between %FAT and MONO in milk (Figure 7).

The genetic correlations between %FAT and SAT in milk fat (g/100g of fat) increased positively until the first part of the lactation, stayed relatively stable and then decreased slightly. These values ranged from 0.23 to 0.62 (Figure 8). The same trend was observed for the phenotypic correlations between these 2 traits but the values were lowest compared to the corresponding genetic correlations. The negative energy balance of cows at the beginning of lactation could partly explain the lowest correlations observed. Strong opposite trend was observed for the genetic as well as phenotypic correlations between %FAT and MONO in milk fat (Figure 8).

The changes of genetic and phenotypic correlations between %FAT and the ratio of SAT to UNSAT (Figure 6) within the lactation were the same than those observed between %FAT and %SAT (Figure 8). The genetic correlations between %FAT and SAT in milk fat (g/100g of fat) increased positively until the first one third of the lactation, stayed relatively stable and then decreased slightly. These values ranged from 0.23 to 0.62 (Figure 7). The same trend was observed for the phenotypic correlations between these 2 traits with lowest values. The negative energy balance of cows at the beginning of lactation could also explain the lowest correlations observed. Strong opposite trend was observed for the genetic and phenotypic correlations between %FAT and MONO in milk fat (Figure 7).

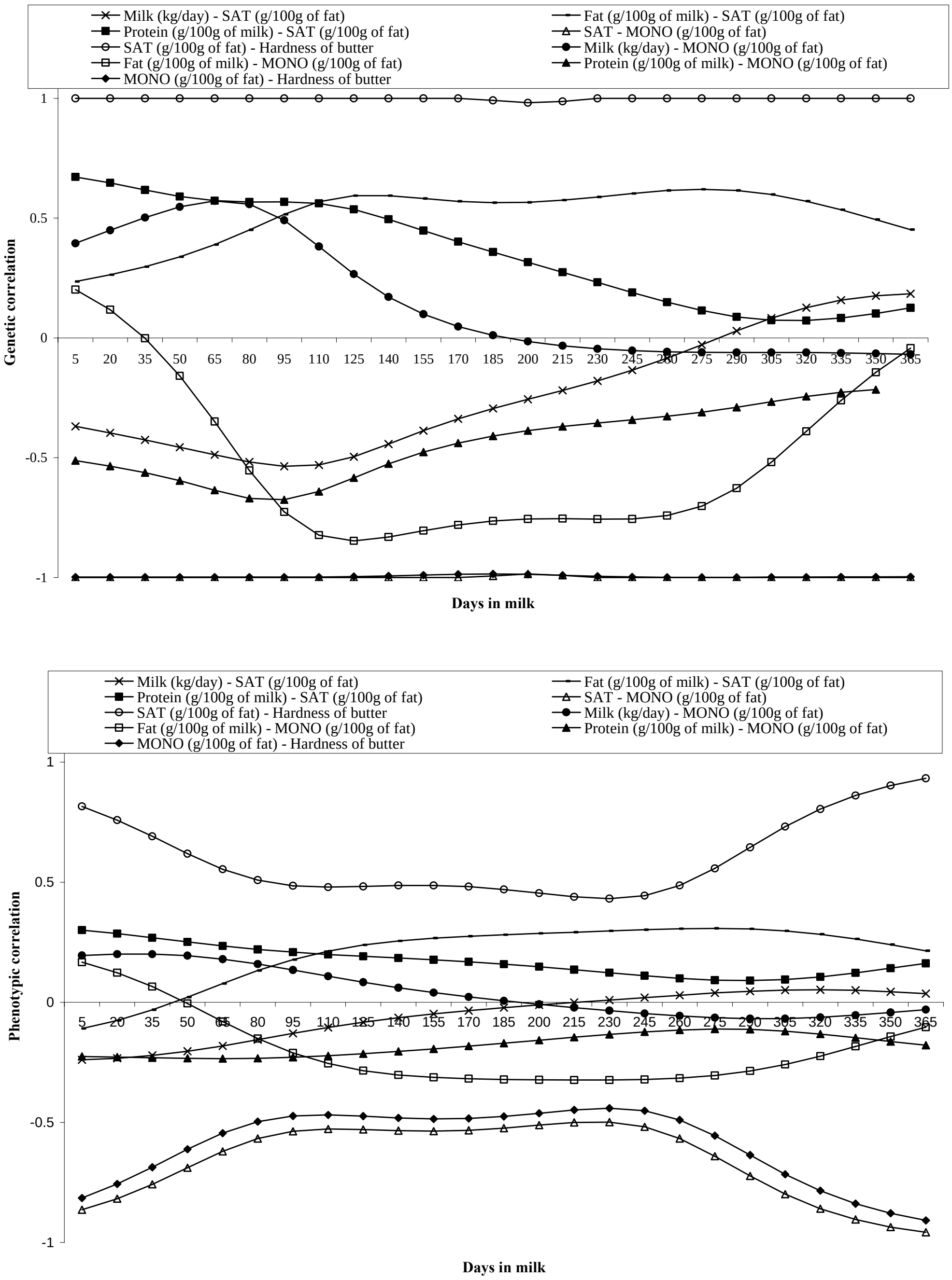


Figure 8. *Changes of genetic and phenotypic correlations through the lactation among milk yield, percentages of fat and protein, the ratio of saturated to unsaturated fatty acids reflecting the hardness of butter and the contents of saturated (SAT) and monounsaturated (MONO) fatty acids in bovine milk fat (g/100g of fat).*

The changes of genetic and phenotypic correlations between %FAT and the ratio of SAT to UNSAT (Figure 5) within the lactation were the same than those observed between %FAT and %SAT (Figure 7).

8.6. Conclusions

The seasonal effect on fatty acid composition was studied based on unadjusted means. As expected, the contents of SAT in milk fat were smaller during the spring and summer while the contents of MONO decreased. Some changes were observed for the contents of %MONO in milk fat. The highest contents of %MONO were observed during the spring and summer. The hardness of butter was influenced by stage of lactation. The milk fat produced by cows in first parity at early stage of lactation had lower contents of SAT. Therefore, butter from milk produced by cows at the beginning of lactation should be more spreadable and softer.

For all studied traits, the estimates of heritability were moderate to high and changed through the lactation. The %FAT and SAT content in milk were less heritable at the beginning of lactation. A more fluctuating curve of heritability was observed for MONO. It was interesting to observe that the contents of SAT and MONO in milk fat were strongly heritable at the beginning and at the end of lactation. One possible explanation could be that, at the early stage of lactation, cows were generally in negative energy balance. In order to sustain their milk production, cows mobilize their lipid reserves. This mobilization requires some internal mechanisms genetically regulated.

In conclusion, this study confirmed the genetic variability of FA. Based on the obtained results, it appeared that the energy balance of cows could influence the fatty acid composition in bovine milk. Thanks to the prediction of FA by MIR spectrometry during the milk recording, investigating the changes of fatty acid profile in relation to the energy balance could be interesting to help farmers to limit the period of negative energy balance.

8.7. Acknowledgments

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Chapter IX

GENERAL DISCUSSION & PERSPECTIVES

9.1. General Discussion

9.1.1. Mid-Infrared Spectrometry

A good knowledge of the fat composition of bovine milk is important to better understand the nutritional quality of milk, as well as the technological properties of butter. Some researches in the 1930’s studied the milk fat composition using the iodine value approximating the contents of unsaturated fatty acid in milk. Few years after, the gas chromatography was applied to measure the fatty acid concentrations. Currently, this method is the reference analysis to measure the fatty acid profile in bovine milk fat. However, the high cost of this analysis explains why previous studies about the genetic variability of fatty acids investigated a limited number of milk samples collected on a limited number of cows.

As the overall objective of the current PhD thesis was to study the genetic variability of fatty acid profile in bovine milk, an **alternative method** to estimate the fat composition was developed. The **MIR spectrometry** is used for the routine milk recording to predict the contents of major milk components as, for instance, fat, protein, lactose, or urea. Using the same technology than the one used routinely for the milk analysis permits to decrease the cost of analysis. As shown in Chapter IV, the first calibration equations developed to measure the contents of fatty acid in milk gave encouraging results. The cross-validation coefficients of determination were **higher for calibration equations that predicted fatty acid contents in milk than in milk fat**. This can be explained by a lower variation of fatty acid contents in fat than the contents of these same fatty acids in milk. Indeed, based on the spectral test-day records used for the last publication, the coefficients of variation for the contents of saturated fatty acids in milk and fat were equal to 22.66% and 10.04%, respectively. The same observation was made for the contents of monounsaturated fatty acids (25.89% *vs.* 17.74%).

The capillary column of 50m length used showed some problem of resolution especially for the C18:1 isomers. Consequently, new calibration equations were developed using reference contents of fatty acid measured by gas chromatography with a capillary column of 100m length. The results obtained with this longer column were better than the previous results using the shorter column. For instance, the cross-validation coefficient of determination obtained for the calibration equations predicting the contents of saturated and monounsaturated fatty acids were equal to 0.97 and 0.93 (Chapter VIII) *vs.* 0.94 and 0.85 obtained in the first study (Chapter IV). A validation procedure of these calibration equations was shown in Chapter VIII. Only 14 milk samples were chosen randomly on the 1,060 milk samples collected from March 2005 to May 2006. The validation coefficients of determination were equal to 0.84 for both of saturated and monounsaturated calibration equations. These coefficients were lower than the corresponding cross-validation coefficients of determination. Consequently, even if these results confirmed the **ability of established MIR calibration equations to predict the content of saturated and monounsaturated fatty acids,** these results suggested also the need to increase the number of samples used for the validation and for the calibration. A larger validation extending the work done with 14 samples is currently conducted. Moreover, from successive principal components approaches done during the milk infrared analysis, new milk samples are continuously searched to be introduced in the calibration set. Even if these calibration equations were not used in this PhD thesis, **the contents of omega-9 and fatty acids with short, medium and long chain can be also predicted by MIR spectrometry**.

9.1.2. Genetic Variability of Fatty Acids

Thanks to the development of fatty acid calibration equations and the record of all spectra generated during the infrared analysis executed for the Walloon routine milk recording, a very large database including the fatty acid profile was created. The quantity of information available has influenced the choice of models used to estimate the genetic parameters of fatty acids. This explained why, through this PhD thesis, the models used were chronologically “finer”.

Previous studies based on the observation of phenotypic differences suggested some breed differences of fatty acid profile in bovine milk fat (Chapter II). Based on the predicted values of fatty acids, the differences within and across 7 dairy breeds (Brown-Swiss, dual purpose Belgian Blue, Holstein Friesian, Jersey, Normande, Montbeliarde, and non-Holstein Meuse-Rhine-Yssel type Red and White breeds) were studied (Chapter V and VII) and compared to Holstein. Dual purpose Belgian Blue and Jersey differed significantly from Holstein. When a regression on fat content was included in the model, the milk fat produced by Jersey cows did not contain the highest content of saturated fat. It could be explained by the relatively high delta-9 desaturase activity observed for this breed. On the other hand, dual purpose Belgian Blue had the highest content of monounsaturated fatty acids in their milk fat because this breed showed the highest activity of delta-9 desaturase. Another investigation presented in Chapter VII measured the breed differences for the delta-9 ratios with a multiple-trait test-day animal model. This study confirmed the breed difference for the delta-9 ratios. Dual purpose Belgian Blue cows had the lowest values for the 3 studied delta-9 ratios. However, Jersey cows had the highest. The type of model used can explain the difference for Jersey between these 2 studies. Regression on the fat content was not included in the last model. All of these results suggested that **the choice of breed might allow modifying the milk fat composition**.

Based on the obtained differences within the studied dairy breed, results shown in Chapter V suggested also a genetic variability of fat composition. Thanks to the increase of spectral data, and thus, infrared predicted values of fatty acid contents, a multiple-trait test-day animal model was developed and used to estimate the genetic parameters of fatty acids. This study was presented in Chapter VI. Heritability values obtained for the contents of saturated fatty acids in milk were higher than the one obtained for these same fatty acids in milk fat. Heritability estimates for monounsaturated fatty acids were similar in milk and fat. This study **confirmed the existence of significant genetic variability of fat composition**. Heritability values for saturated fatty acids and fat were similar (0.36 and 0.32, respectively). As we know the effect of animal selection on fat content in milk, we can expect a similar impact of selection on saturated fatty acid contents. The part of residual variability, especially for unsaturated fatty acids, was important in this study and suggested the need to improve the model used. This was done in the last publication presented in Chapter VIII where random regressions to model the lactation curve were included. The residual effect was less important but the heritability estimates were relatively similar compared to those estimated in Chapter VI. Heritability values for saturated and monounsaturated fatty acids in milk were equal to 0.42 and 0.14, respectively.

Heritability and phenotypic and genetic correlations varied through the lactation. This variation could be related to the energy balance of cows.

Besides the estimation of heritability, the genetic relationship among the major fatty acids in bovine milk and the common production traits (milk yield and percentages of fat and protein) was also studied and presented in Chapter VI. **Genetic correlations among different fatty acids were high** and were explained by the similarities of metabolic production of these milk traits (de novo synthesis, blood extraction, *etc*). This result may suggest the possibility to group some fatty acids and to **develop an aggregate index**. Indeed, due to the large number of fatty acids predicted by MIR, estimating the genetic parameters for all of these traits in the same model requires a high computational cost. The estimation of genetic parameters for limited number of traits resuming the fatty acid information is easier. The question remains **which group of fatty acids has to be studied?** Currently, there is no clear answer. Indeed, before choosing a selection strategy, it is needed to know the genetic relationship existing among fatty acids in milk and also the relationship between these fatty acids and the known traits to estimate as detailed as possible the impact of this potential selection on milk. Results from Chapter VI show the genetic correlations among fatty acids as well as between the major fatty acids and the common production traits. Generally the genetic correlations between milk yield and the content of fatty acids in milk were negative and were explained by a dilution effect. As expected, the genetic correlations between fatty acids and the percentage of fat were positive and strongly positive for the correlations including saturated fatty acids. The correlations between these fatty acids and the percentage of protein were also positive but lower than those including the percentage of fat. These positive correlations can be partially explained by the requirement of some identical precursors to produce protein and fat in milk. Consequently, decreasing the content of saturated fatty acids involves a decrease of fat content, and in a lower proportion, of protein content in milk. Even if this study estimates some genetic correlations among fatty acids, the relationship between all fatty acids, especially the short chain fatty acids, was not studied. However, to improve the spreadable properties of butter and the nutritional quality of fat, increasing the unsaturated fatty acid contents in milk fat could be an option. Besides confirming the breed differences for fatty acid contents, the study in Chapter V showed also the possibility to explain the observed fatty acid differences based on the activity of delta-9 desaturase approximating by the ratios of product to substrate (C14:1 *cis-9*/C14:0, C16:1 *cis-9*/C16:0, and C18:1/C18:0). Consequently, a study on the estimation of genetic parameters of delta-9 ratios was conducted in Chapter VII. This study confirmed the moderate heritability of the monounsaturated fatty acid content in milk fat. Heritability values, on average equal to 0.20, were found for all delta-9 ratios except for C18:1 *cis*/C18:0. Under the assumption that delta-9 desaturase activity can be studied by the ratio of product to substrate, these results **confirmed the genetic variability of delta-9 desaturase activity**. Negative genetic correlations were found between these ratios and the percentage of fat. Some products generated from this desaturation, could inhibit the synthesis of milk fat in the mammary gland. This is in agreement with the results observed previously and presented in Chapter V. Dual purpose Belgian Blue cows, producing the lowest content of fat in their milk, showed the highest delta-9 desaturase activity. In the same way, the genetic correlations with the percentage of protein were also negative but lower than those including the fat content. Before choosing a proper selection objective, more studies will have to be conducted to clarify the link existing among the fatty acids and between these milk components and other production traits, especially by increasing the spectral database.

However, to evaluate the impact of a specific selection program, the economic impact of this selection has to be also known. An important question to consider is **what the dairy industry wants for the future.** It is probably too early to respond to this question. In Belgium, only one dairy firm sells dairy products with differentiated fat composition. Only an added feeding is proposed to the farmers selling their milk to this industry to modify the fat composition. Currently, the dairy industry proposed a compensation for the higher price of this type of feed. However, combining a proper feeding regime with a coherent animal selection could permit to open new possibilities for the dairy industry. With the introduction of MIR spectrometry directly in the Walloon routine milk recording and in the laboratories responsible to the milk analysis for milk payment, a milk price scheme could be based on, for instance, the content of saturated fatty acids in milk. Based on this table, an economic selection index could be developed.

Overall, this PhD thesis confirms the genetic variability of fat composition and supposed that this variability is sufficient to be considered in an animal selection program. Currently, many questions remain unanswered. The milk price scheme will stay the same that the one proposed currently or it will change to take into account the nutritional quality of the milk fat. What will be the impact of a particular selection of fatty acids group? This PhD thesis provides the background necessary to develop this type of researches in Belgium and the obtained results could be also extended to other countries.

9.2. Perspectives

9.2.1. Mid-Infrared Spectrometry

The perspectives for the application of MIR to estimate the fat composition are numerous. First, the robustness of calibration equations, which predict the fatty acid contents in milk, can be improved further by adding new samples. As explained before, thanks to successive principal components approach during the milk infrared analysis, new samples will be chosen to increase the size of the calibration set. Thanks to the international interest for these calibration equations, a large validation will be conducted during the year 2008 based on milk samples collected in Wallonia but also in Luxemburg, France, Ireland, and maybe in Austria and Scotland. Second, even if the MIR prediction of fatty acid contents will not replace the gas chromatography, the low cost of this infrared analysis (more than 75 times less expensive than traditional analysis) and the speed of this analysis (up to 500 samples per hour with the MilkoScan FT6000) permit a intensive use of these predicted values allowing a regular check of fat composition. This regular analysis of fat composition could allow fitting the animal feeding in agreement with the producer’s desires.

9.2.3. Genetic variability

This thesis confirmed the genetic variability of fatty acids and suggested that this variability is sufficient to be considered in a selection program based on the improvement of milk fat composition. Since September 2007, all spectra data generated during the Walloon milk recording are stored. Thus, breeding values for cows studied during the Walloon milk recording could be estimated in a near future using variance components estimated in this PhD thesis. As mentioned previously, before the development of this type of animal selection program, two questions have to be answered. What the dairy industry wants? Which group of fatty acids have to be selected? The first question needs the strong involvement of the dairy industry in the research. Based on the current dairy products, one hypothesis could be to increase the contents of unsaturated fatty acids. However, increasing the proportion of unsaturated fatty acids could have some negative effect on the conservation of milk. Studies on the relationship between the different fatty acid and the traditional production traits are also needed in order to better estimate the impact of selection for fatty acids. Furthermore, to prevent some disadvantages related to the health status or morphology, the impact of fat composition on other traits like longevity, fertility will have to be also estimated.

Even if this thesis was focussed first on the nutritional quality of milk fat, the development of the MIR tool to estimate the fatty acid profile, especially their introduction in the Walloon milk recording, opens new perspectives. Depending on the health status and energy balance of cows, the fatty acid profile changes. Consequently these changes could be used to develop some management tools permitting the detection of some metabolic disease (*e.g.* acidosis) or mastitis. Based on the fatty acid data, a theoretical curve of fatty acid modifications through the lactation could be estimated. So, the obtained fatty acid concentrations for a specific test-day record could be compared to the estimated theoretical concentrations. The importance of the deviation could reflect the presence of metabolic disease or even mastitis.

All of these developments will permit to select more robust cows that could produce an improved milk fat composition. This is the objective of an European Project named RobustMilk.

9.2.4. Beyond the Fatty Acid Profile

The development of calibration equations, which predict the contents of fatty acid in bovine milk, has shown the interest to use the MIR spectral data. The spectral data represents the entire composition of milk. There are two possibilities: using directly the spectral data or making new calibration equations to study other aspects of milk.

The MIR milk spectrum generated by the MilkoScan FT6000 contains 1,060 data points. Estimating simultaneously the genetic parameters of these traits is still impossible due to the high computational cost required. A study not presented in this thesis was conducted to develop a method to decrease the dimensionality of traits (Soyeurt *et al.*, 2007a). This method used a principal components approach. Only 48 principal components described more than 99% of spectral variability. The genetic parameters of these components were estimated. Results showed that not all wavenumbers contained in the milk spectrum were interesting. After deletion of these less informative MIR regions, only 7 principal components described more than 99% of remained spectral variability. This number of traits allows the use of these principal components to, for instance, detect some metabolic disease or develop a selection program based on the global improvement of milk related to the expected milk valorisation (cheese, yogurt, *etc*).

The second approach is to make calibration equations to predict the contents of new milk components. The development of calibration equation which predict the content of lactoferrin in milk was done in a conducted study (Soyeurt *et al.*, 2007b). The prediction of lactoferrin content by MIR has allowed the estimation of genetic parameters for this milk component. The moderate heritability observed for this trait is promising because the lactoferrin is involved in the immune processes of cows and humans. The estimation of some minerals contained in milk as calcium, phosphorus, *etc* is also conducted currently in the context of a master thesis.

In conclusion, besides the study of genetic variability of fat composition and thanks to the use of MIR spectrometry, the current PhD thesis opened new perspectives for the dairy research.

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List of Publications, Oral Presentations, and Posters

1. Publications

Soyeurt, H., P. Dardenne, G. Lognay, D. Veselko, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690-3695.

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