COMMUNAUTÉ FRANÇAISE DE BELGIQUE
ACADÉMIE UNIVERSITAIRE WALLONIE-EUROPE
UNIVERSITÉ DE LIÈGE -GEMBLOUX AGRO-BIO TECH

Development of innovative and practical management tools to improve sustainability of milk production and quality of dairy products

VALERIE ARNOULD

Essai présenté en vue de l'obtention du grade
de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Dr. Ir. Nicolas Gengler

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Développement d'outils de gestion innovatifs et utiles aux éleveurs soucieux d'améliorer leur système de production et la qualité de leurs produits

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Valérie Arnould. Development of innovative and practical management tools to improve sustainability of milk production and quality of dairy products. Gembloux, Belgium, Gembloux Agro-Bio Tech, University of Liège.

ABSTRACT

In the current complex economical context, novel strategies are needed to help local dairy farmers to face the European dairy sector crisis. This thesis was initiated in the framework of ManageMilk project and was globally aimed to investigate the possibility to develop some innovative and practical management tools helping dairy farmers in their daily decisions. To develop such management tools, several conditions must be fulfilled. Firstly, used data must be relevant. According to the literature, the milk composition, and in particular, the milk fatty acid (FA) profile, appears to be a suitable trait allowing useful information about the dairy cow's health status or about the management system efficiency. These data must also be easily available at low cost from milk recording organization. Recently, the MIR spectrometry offers the possibility to build routinely cheaper and more important databases. To develop management tools, milk samples have to be collected using comparable sampling methods. Unfortunately, in order to decrease the milk quality control costs, the International Committee for Animal Recording allows alternative sampling schemes including the collection of samples from morning or evening only milkings. This alternative sampling scheme can interact with phenotypic and genetic parameters. Therefore, additionally to the development of conversion equations, this thesis is establishing if morning or evening only milkings are genetically different traits. Last condition concerns a useful phenotypic and genetic variability. Milk FA profile is, among others, altered by genetics. So, one paper of this thesis concerns the setup of a useful genetic evaluation model able to estimate accurately the genetic part of milk fat composition variations. Routine genetic evaluation of production traits in dairy cattle commonly uses random regression model (RRM). Recently, "splines" have been advocated as a good alternative to Legendre polynomials (LP) for analyzing test-day yields in RRM. Therefore, several models are compared. Obtained results show the possibility to propose a practical and robust method for estimating accurate daily major FA production from single milking, useful for a further development of practical management tools helping dairy farmers in their daily decisions.

Valérie Arnould. Développement d'outils de gestion innovants et utiles aux éleveurs soucieux d'améliorer leur système de production et la qualité de leurs produits. (PhD Dissertation en Anglais). Gembloux, Belgium, Gembloux Agro-Bio Tech, Université de Liège.

RESUME

Dans un contexte économique difficile, de nouvelles stratégies doivent être proposées à nos producteurs laitiers locaux afin de leur permettre de faire face à la crise européenne du secteur laitier. Cette thèse s'inscrit dans le cadre du projet belgo-luxembourgeois ManageMilk dont l'objectif global est la contribution au développement d'outils innovatifs et utiles aux éleveurs laitiers dans leurs décisions quotidiennes. Le développement de tels outils est soumis à plusieurs conditions nécessaires. La première condition est la construction d'une base de données pertinente. La composition du lait et, plus particulièrement le profil en acides gras (AG) du lait, apparaît comme autant de sources d'information utiles reflétant la santé du bovin et l'efficacité du système de production. Les données enregistrées doivent être facilement récoltées et ce, à moindre coût. Récemment, la mise au point de la technologie de spectrométrie MIR permettait la construction, en routine, de bases de données, plus importantes et moins onéreuses que les systèmes d'analyses traditionnels. Les échantillons de lait utilisés doivent également être prélevés selon des protocoles similaires. Cependant, dans le but de réduire les coûts du contrôle laitier, le Comité International d'Enregistrement des Animaux permet la collecte d'échantillons proportionnés selon le moment de traite. Le dernier objectif de cette thèse est donc l'étude de l'effet du moment de traite sur les paramètres génétiques des caractères étudiés. Selon les résultats obtenus, il est possible de construire une méthode pratique et robuste permettant l'estimation de la production d'AG à partir des données d'une seule traite. La dernière condition concerne les variabilités phénotypique et génétique des caractères précités. Les évaluations génétiques appliquées en routine utilisent essentiellement des modèles de régression aléatoires. Selon certaines recherches, l'utilisation de « splines » permettrait de corriger certains défauts des polynômes de Legendre. La comparaison de différents modèles permettant l'étude/estimation des paramètres génétiques de la production laitière est également réalisée dans le cadre de cette étude. En conclusion, les données AG laitiers peuvent être utilisées en tant qu'outils de gestion d'une production laitière bovine.

« Soit A, un succès dans la vie.
Alors A = x+y+z.
Où x = travailler. y = s'amuser. z = se taire. »

Albert Einstein

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TABLE OF CONTENT

AIC	Akaike's information criterion
AM	Ante meridian (morning)
BIC	Bayesian information criterion
BHBA	ß-hydroxybutyrate
CLA	Conjugated linoleic acid
cDIM	Class of days in milk
DC	Daily content
DIM	Days in milk
DGAT-1	Diacylglycerol O-acyltransferase
EBV	Estimated breeding value
FA	Fatty acids
FPratio	Ratio of milk fat/protein
FTIR	Fourier transform infrared spectrometry
GHG	Greenhouse gases
ICAR	International Committee for Animal Recording
LCFA	Long chain fatty acids
LTF	Lactoferrin
LP	Legendre polynomial
MCFA	Medium chain fatty acids
MIR	Mid-infrared
MI	Milking interval
MUFA	Mono-unsaturated fatty acids
NPN	Non-protein nitrogen
PM	Post meridian (evening)

PSB	Percentage square biases
PUFA	Poly-unsaturated fatty acids
QTL	Quantitative trait loci
R	Correlation value
R^2	Coefficient of determination
R^2cv	Cross-validation coefficient of determination
RMSE	Root mean squared error
RPD	Ratio of SECV to SD
RRM	Random regression model
RRTDM	Random regression test-day model
SCC	Somatic cells count
SCD	Delta-9 desaturase
SCFA	Short chain fatty acids
SCS	Somatic cell count
SD	Standard deviation
SEC	Standard error of calibration
SECV	Standard error of cross-validation
SFA	Saturated fatty acids
SNP	Single-nucleotide polymorphisms
SP	Spline
SSE	Sum of squares error
TD	Test-day
UFA	Unsaturated fatty acids

CHAPTER I. GENERAL INTRODUCTION

1.1. Context

Over recent years Europe encounters a serious agricultural, and in particular, dairy crisis. According to the European Commission and the European Council, the crisis in the European dairy sector is a result of a combination of several factors such as the Russian embargo on Europe agri-food products, a lower than expected Chinese demand for dairy products, the increasing production of volumes of milk in Europe after the abolition of the milk quota system, as well as an overall observed increasing production of milk in New Zealand and Australia.

In this complex economical context, the policy of the European Commission Rural Development aims to improve sustainability of agriculture, as well as to improve the quality of life in rural areas and the diversity of rural economies. Therefore, milk recording organizations in Europe, have a strong role to play in the development of management tools helping to improve sustainability of dairy farms.

CONVIS s.c., as an agricultural and breeding cooperative in Luxembourg, is a provider of services such as performance testing for breeding animals, consultancy in animal husbandry related areas such as feeding and farm management. CONVIS s.c. is a service provider for the official milk recording, offers advisory services for animal breeding, performs evaluations of cost and analysis of production characteristics. Thus, CONVIS s.c. actively supports the Luxembourgish dairy farmers by making available, relevant management tools in order to improve sustainability of dairy farms.

Compared to other European countries, Luxembourg presents some particularities. Among the European Member States, Luxembourg has the smallest number of agricultural holdings (2,200) in 2010 (Eurostat, 2010). Over the 2000-2010 timeframe, 540 farms ceased their activities. In parallel, as widely observed across the EU-28, Luxembourg also encountered an important decrease in the number of people working on farm falling by 21% between 2000 and 2010 (4,960 workers).The used agricultural land experienced the opposite trend by an increase to 131,110 hectares in 2010 (+2.9 % vs. 2000). The most common farms are farms with at least 50 hectares of agricultural land representing about half (49%) of the number of agricultural holdings and occupied 86% of the country's agricultural land in 2010. Further, in

terms of the number of holdings, dairy farms are the most common category of farms: they accounted for 27% of the country's farm population and 47% of the Standard Output in 2010 (Standard Output is defined as the average monetary value of the agricultural output at farm gate price of each agricultural product in a given region). Consequently, developing management tools for farmers in Luxembourg is relevant.

From this general context, this thesis which is the result of a collaboration between CONVIS s.c. and the Animal Science Unit of Gembloux Agro-Bio Tech (University of Liège, Belgium) aims to contribute to the development of innovative and practical management tools helping dairy farmers to improve the sustainability of their farms.

In particular, the innovative aspect of this thesis is to strengthen the use of data routinely recorded to develop such tools. The use of test-day records is an interesting opportunity to develop management tools. Indeed, test-day yield records from the milk recording system provide an important source of information for both breeding and management (Caccamo et al., 2008). Historically, herd management improvement and breeding values' estimation have been separate processes but the use of dairy records should be more than a simple reporting of yield performance or inputs for the estimation of breeding values (Bastin et al., 2009). However, there are clear advantages of using the same data and statistical procedures for both management purposes and genetic evaluation (Caccamo et al., 2008).

Further, in practice, routine measurement of milk components offers the potential for early detection of systemic and/or local alteration and, consequently, provides assistance for strategic and management decisions. Mid-infrared (**MIR**) spectrometry is already used routinely by milk recording organizations and milk laboratories to quantify the contents of fat, protein and lactose in milk samples. However, this use could be easily extended to other milk components. Indeed, several papers (Soyeurt et al., 2006, 2008a, 2008b, and 2011; Rutten et al., 2009) have shown the potential of MIR spectrometry for quantifying small fractions, such as fatty acids (Soyeurt et al., 2006 and 2011).

Consequently, the working hypothesis of this thesis is that several milk components predicted by MIR spectrometry could be used as indicators of the metabolic status of dairy cows and/or the nutritional quality of milk and/or the environmental sustainability. Particularly, the knowledge of the milk fatty acid (**FA**) profile produced by dairy cows seems to be interesting with regard to this perspective. To refine the analysis and correct the noisy background related to the natural sources of variation such as dietary composition, genetics, lactation stage, energy balance and animal status (e.g. parity, days in milk, health status) (Arnould et al., 2009; Beaulieu et al., 1995; Chilliard et al., 2001;Gross et al., 2011; Grummer, 1991; Palmquist et al., 1993), computer-based systems are very useful in interpreting differences between the observed and expected values of given milk components predicted by MIR, which could be used as a guideline for health management and preventive systems. Such an approach would result in the development of easy, cheap and useful sustainable management tools assisting dairy farmers in their daily decisions.

1.2. Outline

This thesis is a compilation of published scientific papers and is structured in 8 chapters.

As mentioned in the title, the global objective of this thesis is to contribute to the development of innovative management tools for dairy farmers allowing them to improve their dairy production system and product quality. As aforementioned, a first literature review permitted to identify the contents of milk fatty acid as potential interesting traits with regard to their environmental, animal health and nutritional aspects (Chapter II).

The development of management tools takes profit of the existing genetic and phenotypic variability of the studied traits. A second literature review was conducted to summarize the state of the knowledge in genetic variability of FA (Chapter III). Unfortunately, no information was available for dairy cattle in Luxembourg. Therefore a study was conducted to define a model allowing the estimation of genetic parameters. This study compared two

approaches using more or less computational resources to model the evolution of genetic parameters throughout the lactation (Chapter V).In milk production, quality control samples are typically taken during both the AM and PM milkings. CONVIS s.c. proposes alternative procedures that restrict sampling to a single time of day as approved by the International Committee for Animal Recording. These alternative testing schemes present economic advantages in daily routine explaining the interest of farmers in Luxembourg. However, the composition of AM- and PM-collected milk samples can differ and impact the estimation of genetic and phenotypic variability. Therefore, a study conducted in the context of this thesis had two objectives: 1) the estimation of FA genetic parameters for dairy cattle in Luxembourg and 2) the estimation of the impact of an alternate milking testing scheme on the phenotypic and genetic variability (Chapter VI). As variability was observed among times of milking, one solution studied in this thesis was to develop conversion equations allowing the prediction of daily yields of production traits (milk, milk fat and FA) from readily available field data (Chapter VII).

In order to better assess the results obtained in this thesis and their potential added value for the dairy cattle sector, the final chapter of the present thesis contains a general discussion presenting perspectives and conclusions (Chapter VIII).

1.3. Framework

This thesis was initiated in the framework of ManageMilk project financed by A.F.R.-F.N.R. (AFR PHD-09-119-RE) (Fonds National de la Recherche Luxembourg). This project was jointly conducted by CONVIS s.c. (Ettelbruck, Luxembourg) and the Animal Science Unit of Gembloux Agro-Bio Tech, University of Liège (GxABT - ULg, Gembloux, Belgium).

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Soyeurt H., Arnould V.M.R., Vanderijck S., and N. Gengler. 2011. Feasibility of a Walloon genetic evaluation for milk fat composition. J. Dairy Sci. 93: 744.

CHAPTER II.

REVIEW : MILK COMPOSITION AS MANAGEMENT TOOL OF SUSTAINABILITY

From: Arnould V. M. R., Reding R., Bormann J., Gengler N., and H. Soyeurt. 2013. Review: milk composition as management tool of sustainability. *Biotechnologie, Agronomie, Société et Environnement.* 17 : 613-621

2.1. Abstract

The main objective of this paper is the use of milk composition data as a management tool. Milk composition, and in particular, milk fat content and fatty acid profiles may be significantly altered due to a variety of factors. These factors are reviewed in the literature; they include diet, animal (genetic) selection, management aspects and animal health. Changes in milk composition can be used as an indicator of the animal's metabolic status or the efficiency of the feed management system. The advantages of using this kind of data as a management tool would be to allow the early detection of metabolic or management problems. The present review suggests that milk and, especially milk fat composition may be used as a sustainability management tool and as a monitoring and prevention tool for several pathologies or health disorders in dairy cattle. Further, due to the use of mid-infrared spectrometry (MIR) technology, these tools may be easily implemented in practice and are relatively cheap. In the field, milk labs or milk recording agencies would be able to alert farmers whenever threshold values for disease were reached, allowing them to improve their dairy production from an economic, ecological and animal (welfare) point of view.

Keywords. Cow milk, composition, management techniques, sustainability, fatty acids, spectrometry, decision support systems, and livestock management.

2.2. Résumé

L'objectif principal de cette synthèse bibliographique est l'étude de l'utilisation de la composition laitière en tant qu'outil de décision et de gestion. La composition laitière est relativement variable et de nombreux facteurs de variation sont répertoriés dans la littérature tels que le régime alimentaire, la sélection animale, la conduite du troupeau ou le statut sanitaire de l'animal. La composition laitière se révèle être un véritable miroir du statut métabolique de la vache laitière et de l'efficacité du système de gestion du troupeau. Cet article

suggère donc l'utilisation pratique de la composition laitière en tant qu'outil d'aide à la décision, en vue d'améliorer la durabilité de la production laitière grâce au contrôle, au suivi et à la détection précoce de dysfonctionnements métaboliques ou de gestion du troupeau. Des valeurs limites sont disponibles en tant qu'exemple dans la littérature pour certains composés. Par ailleurs, l'utilisation de l'outil MIR facilitera (d'un aspect pratique et économique) l'application d'un tel outil sur le terrain. Enfin, les organismes chargés d'assister les éleveurs dans leurs décisions pourront alerter les producteurs laitiers en cas de risque de maladie, leur permettant de traiter tout problème préventivement et d'améliorer ainsi leur production laitière d'un point de vue économique, écologique et animal (bien-être). Mots-clés. Lait de vache, composition, technique de gestion, durabilité, acides gras, spectrométrie, système d'aide à la décision, conduite d'élevage.

2.3. Introduction

In the last two decades, the beef and dairy sectors have faced new challenges regarding sustainability issues. The current challenge is to improve the economic efficiency of dairy cows by improving productivity and lowering costs (*e.g.* feed, veterinary). Firstly, human consumption patterns of beef and dairy products have changed and, currently, besides being driven by price, consumers are basing their choices more often on health aspects of food. Thus, it is becoming increasingly important for dairy farmers to take into account these considerations and to adapt their milk production system to consumer and dairy industry needs. Fortunately, milk composition and, in particular, milk fat content and fatty acid (FA) profiles may be significantly altered through management interventions such as changes in diet (e.g. Grummer, 1991; Chilliard et al., 2000; Chilliard et al., 2001; Forsbäck et al., 2010), but also through animal (genetic) selection (Arnould et al., 2009a). The high elasticity of milk fat content offers the opportunity to respond to industry and consumer requirements.

Secondly, efficient milk production requires dairy cows to experience gestation and parturition every year. Most of the metabolic diseases of dairy cows occur within the first weeks

of lactation. Indeed, the cow's high nutrient demand due to an increased mammary gland activity cannot always be met. The most economically relevant diseases in higher yielding cows are milk fever, ketosis (or acetonemia) and mastitis. Understanding the variation in milk composition can be useful for providing information about the health status of dairy cows. Indeed, modifications in the metabolic process, and changes in milk yield, fatty acids, protein fractions or mineral content can be used as indicators for the metabolic status of the cow (Fleischer et al., 2001; Mulligan et al., 2006). In this case, the efficiency of a cow health management system is determined by the ability to diagnose changes in animal health status at an early stage and the ability to develop preventive measures (Hamann et al., 1997). The earlier health problems are identified, the higher the chance of successful health management, with positive consequences for farm management, economical, ecological and animal welfare issues. Indeed, a more effective prevention system for common dairy diseases and the improvement of the health status of dairy cows would, indirectly, help to improve dairy farming from an economical and ecological point of view. Such a system would limit labor investment, medical treatment costs, and animal suffering (social aspects), and would also increase milk yield and milk quality, including the animal's lifetime production. In addition, analysis of milk composition could provide some interesting information about the efficiency of the feed management system. Furthermore, feed management issues are highly related to the agricultural environmental footprint. Indeed, methane production, for instance, corresponds to a loss of productive energy in cows and is negatively correlated to feed conversion (Boichard et al., 2012).

In practice, routine measurement of milk components during milk recording offers the potential for early detection of systemic and/or local alteration and, consequently, provides assistance for strategic and management decisions. Limiting negative influences on dairy cows is the key issue in achieving this objective. The majority of analytical techniques (*e.g.*, gas chromatography, ELISA, or immuno-diffusion methods) used for measuring specific milk components in bovine milk are expensive and time consuming, and require skilled staff. Therefore, these methods are not feasible for making regular measurements relating to individual cows. This inconvenience can be solved by using mid-infrared (MIR) spectrometry.

This technology is already used routinely by milk recording organizations to quantify, for instance, fat, protein and lactose content in milk samples. Several papers (Soyeurt et al., 2006, 2008a, 2008b, and 2011; Rutten et al., 2009) have shown the potential of MIR spectrometry for quantifying small fractions, such as fatty acids (Soyeurt et al., 2006 and 2011). Therefore, MIR spectrometry could be used to routinely quantify various milk components. Furthermore, computer-based systems could be very useful in interpreting differences between the observed and expected values of a milk component, which could be used as a guide for health management and preventive systems.

Several milk components that can be predicted by MIR spectrometry could be used as indicators of the metabolic status of dairy cows. For instance, the milk fatty acid (FA) profile is a dynamic pattern influenced by several factors such as dietary composition, genetics, lactation stage, energy balance and animal status (*e.g*. parity, days in milk, health status) (Grummer, 1991; Palmquist et al., 1993; Beaulieu et al., 1995; Chilliard et al., 2001; Arnould et al., 2009a; Gross et al., 2011). Moreover, protein, fat, the fat:protein ratio, levels of acetone, etc. could be used as disease, feeding and environmental management indicators, and indirectly, as economic indicators, using observed deviations from normal concentrations and their trends of change (Hamann et al., 1997; Chilliard et al., 2009).

The objective of this review is to determine the practical aspects of measuring milk composition and milk fat in order to propose an easy, cheap and useful sustainable management tool to help dairy farmers in their daily decisions.

2.4. Health management

2.4.1. Acetonemia and energy balance

At the beginning of lactation, the dairy cow must cope with an important increase in energy demand by the mammary gland for milk production. This is achieved partly by increasing

feed intake and partly by fat mobilization from the cow's adipose tissue. However, excessive fat mobilization may induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies (ß-hydroxybutyrate [BHBA], acetoacetate, and acetone), a state called hyperketonemia. Hyperketonemia, in its clinical manifestation (ketosis or acetonemia), has an economical effect through decreased milk production and a greater risk of periparturient diseases such as mastitis and left displaced abomasum (Enjalbert et al., 2001; Mulligan et al., 2006). Subclinical ketosis and negative energy balance are closely linked and numerous studies available on the relationship between modifications in milk composition and the metabolic status of dairy cows focus on the energy metabolism (Hamann et al., 1997). Ketone bodies are produced as by-products when FAs are used in energy metabolism in the liver and kidney. Consequently, subclinical ketosis frequently results from an over-long negative energy balance. Because of its importance in dairy cattle (some studies conclude that approximately 50% of all lactating cows develop subclinical ketosis in early lactation), numerous authors have reviewed this kind of metabolic disorder in dairy cattle (*e.g*. Hamann et al., 1997; Van Haelst et al., 2008; Gross et al., 2011; Van Der Drift et al., 2012).

Even if there are no clinical signs of ketosis, milk composition may still be affected (Enjalbert et al., 2001). Current detection methods are based on the measurement of ketone bodies in body fluids (blood, urine or milk) (Van Haelst et al., 2008; Van Der Drift et al., 2012). As expected, both clinical and subclinical ketosis results in increased concentrations of ketone bodies in blood, tissues and milk. As blood sampling is not very convenient for farmers, analyzing milk composition would seem to be an interesting and more practical alternative. A few authors have reported studies comparing concentrations of ketone bodies in milk and blood (*e.g*. Van Haelst et al., 2008; Van Der Drift et al., 2012). In 2001, Enjalbert et al. observed high correlation coefficients between blood and milk acetone (0.96) and moderate correlation coefficients between blood and milk acetoacetate (0.74). The detection of milk acetone and BHBA could therefore be considered as a good predictor of ketosis in dairy cows. In some countries, such as The Netherlands, acetone and BHBA are already routinely analyzed by Fourier transform infrared spectrometry (FTIR) without any extra cost to the dairy farmer (Van Der Drift

et al., 2012). Unfortunately, the number of false-positive test results restricts the usability of acetone and BHBA for reliable detection of acetonemia.

The FA composition of milk could also be used to detect preclinical ketosis. Indeed, milk fat production is the main expenditure for milk production in dairy cows. Moreover, the milk fat profile has been shown to change markedly during the first weeks of production (from week 1 to week 12) and to remain unchanged thereafter (Gross et al., 2011). For all these reasons, and as mobilization of adipose tissue precedes the development of ketosis and incorporation of mobilized FAs into milk fat, changes in milk FA composition might be an early indicator of hyperketonemia (Van Haelst et al., 2008; Van Der Drift et al., 2012). To summarize, milk FAs can be derived from four major pathways: diet, the mammary gland (*de novo* synthesis), rumen (bacterial synthesis) and body fat mobilization (Stoop et al., 2009). Thus where a negative energy balance occurs, there may be several reasons underlying the changes in FA composition. Several studies (*e.g*. Van Haelst et al., 2008; Gross et al., 2011) have proposed relative increases in the proportions of omega-9 (or C18:1 cis9) and long chain fatty acids (LCFAs) as an interesting indicator of subclinical ketosis. Nutrient and energy deficiencies are compensated by mobilization of body fat reserves, predominantly of adipose tissue, associated with the release of FAs. Indeed, the major FAs released during fat mobilization are C16:0, C18:0 and C18:1 cis-9. Unfortunately, milk LCFAs may be largely influenced by diet. Correlations between energy balance and the proportion of C18:1 cis-9 have been shown to range from 0.77 (van Haelst et al., 2008) to 0.92 (Gross et al., 2011). These results confirm that a high proportion of LCFAs (especially if combined with lower medium chain fatty acid [MCFA] proportions) and, in particular, a high proportion of C18:1 cis-9 in milk fat can be considered as a good predictor of subclinical ketosis (Van Haelst et al., 2008).

According to several authors (Hamann et al., 1997; Heuer et al., 1999; Mulligan et al., 2006), the ratio of milk fat/protein (FPratio) is a useful risk predictor for numerous pathologies observed in dairy cattle, such as negative energy balance, ketosis, displaced abomasums, lameness and mastitis. In 1999, Heuer et al. proposed threshold values for diagnosing health problems in a given dairy cow using analysis of its milk composition. Using these threshold

values, Mulligan et al. (2006) established that milk containing an FPratio > than 1.4, a milk protein percentage lower than 2.9%, a milk fat percentage higher than 4.8% and a milk lactose value lower than 4.5% was an indicator for health problems in cows (**Table 1**). Toni et al. (2011) studied three large Italian dairy herds with 1,498 Holstein dairy cows, 35.8% of which were in first lactation. According to the data collected, the FPratio category with the lowest disease prevalence was between 1 and 1.5. On the other hand, cows presenting an FPratio < than 1 showed a higher risk of developing disease. However, it would seem to be important to use these threshold values carefully, as they would need to be adapted to the particular dairy cow population under study.

In addition, many of these milk production traits (fat and protein content, acetone and BHBA concentration) can vary according to the breed, parity number, season, etc. As a result, some authors, such as Van Der Drift et al. (2012), proposed improvements that could be made in order to improve the reliability of acetonemia detection by including the following components in the final diagnostic model: fat content, fatty acid composition (SCFAs, MCFAs, LCFAs and C18:1 cis-9), protein content, FPratio, acetone concentration, BHBA concentration, and other relevant factors, such as parity and season.

Table 1. Effects of metabolic diseases on milk composition. The composition of mature milk (vs colostrum) is shown for comparison — *Effet des maladies dites métaboliques sur la composition du lait. Afin de permettre les comparaisons, la composition du lait est également indiquée*.

		Mature	Energy	Ketosis	Mastitis
SCC					↑ (5)
Milk pH					↑ (5)
Mineral	Sodium	470 $mg.l^{-1}$			↑ (5)
	Potassium	1320 $mg.l^{-1}$			↓ (5)
	Chloride	1190 $mg.l^{-1}$			↑ (5)
	Calcium	1130 $mg.l^{-1}$			↓ (5)
Protein		30-35 $g.L^{-1}$	< 2.9% (1)		
	Lactoferrin	0.1 to 0.4 $g.L^{-1}$			↑ Mastitis: can reach 2.3g/L (6;7)
Fat		35-40 $g.L^{-1}$	> 4.8% (1)	> 4.8% (1)	↑ (9)
	C18:1 cis-9			↑ (2) ($g.100g^{-1}$ of FAMEs)	
	SCFA			↓ (2)	
	MCFA			↓ (2) ($g.100g^{-1}$ of FAMEs)	
	LCFA			↑ (2) ($g.100g^{-1}$ of FAMEs)	
Ratio milk fat/protein			> 1.4 (1)	>1.4 (1;4)	
BHBA				> 100mol/L (3)	↑ (5)
Lactose		45-50 gL^{-1}	< 4.5% (1)	< 4.5% (1)	↓ (5;8;9)
Urea					↓ (5)

SCFA= short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long chain fatty acids; BHBA = ß-hydroxybutyrate; FAMEs = Fatty acids methyl esters. Numbers in parentheses indicate the reference(s) corresponding to the effect (↑: increase; ↓: decrease). Values are threshold values. Les numéros indiqués entre parenthèses correspondent aux références bibliographiques utilisées afin de décrire l'effet (↑: augmentation; ↓: diminution). Les valeurs indiquées sont des valeurs seuil. References: (1) Mulligan et al., 2006; (2) Van Haelst et al., 2008; (3) Toni et al., 2011; (4) Van Der Drift et al., 2012; (5) Brandt et al., 2010; (6) Kutila et al., 2004; (7) Soyeurt et al., 2007; (8) Pyörälä, 2003; (9) Rajcevic et al., 2003

2.5. Indicators of mammary inflammation

In a well managed dairy herd, both clinical mastitis and subclinical mastitis should be efficiently detected (Pyörälä, 2003). Unfortunately, bacteriological sampling is not feasible as a routine test to identify subclinical mastitis. It is well known that mastitis affects the quality of

milk, and several authors (*e.g.* Pyörälä, 2003; Brandt et al., 2010) have reported changes in the composition of milk obtained from cows with infection. Thus, these changes in milk composition could be used as indirect mammary inflammation indicators. The most important changes are: an increasing permeability of the mammary epithelium, leading to a leak of ions, proteins and enzymes from the blood into milk (Bannerman et al., 2003), the invasion of phagocyting cells into udder compartments (Pyörälä, 2003; Komine et al., 2006), and a decreasing production capacity of the mammary gland, resulting in reduced concentrations of certain milk components (Pyörälä, 2003). Besides a high somatic cells count (SCC), the most important changes in milk composition resulting from subclinical mastitis are an increase in free FA concentration; a reduction in casein combined with an increase in whey protein; a reduction in lactose concentration; changes in the concentration of minerals such as sodium, chloride, potassium, and calcium, and an increase in milk pH (Brandt et al., 2010).

An increase in the somatic cell count (SCC) is the first indicator of inflammation. A high SCC found in the milk of healthy cows is essentially due to the presence of macrophages (66-88%), neutrophils (1-11%), epithelial cells and mononuclear cells (Pyörälä, 2003; Forsbäck et al., 2010). In the case of inflammation of the mammary gland, the proportion of neutrophils may increase by up to 90%. The SCC in milk has been commonly used as a mastitis indicator since the 1960s. In order to take into account SCC fluctuations in relation to a cow's number of days in milk (DIM), different SCC thresholds have been proposed within lactation. The proposed SCC threshold values for the Canadian Holstein population are: 500,000; 300,000; and 200,000 cells.ml^{-1}for the following DIM classes: 5 to 10, 11 to 30, and 31 to 305 DIM, respectively (Koeck et al., 2012). Bovine lactoferrin (LTF) is also moderately correlated with the SCC. Arnould et al. (2009b) estimated positive genetic and phenotypic correlations between LTF and the SCC (0.24 and 0.31, respectively; $P < 0.0001$). These values would seem to indicate that the LTF content in milk may increase proportionally to the SCC value. Bovine LTF is mainly present in milk and the protein shows important physiological and biological functions (such as antibacterial, antiviral, antifungal and antiparasitical characteristics). This multifunctional protein plays a key role in the health of the mammary gland. According to several authors (Seyfert et al., 1996; Wojdak-Maksymiec et al., 2006; Soyeurt et al., 2007; Arnould et al., 2009b), the LTF gene could be

considered as a potential candidate gene for selection of mastitis resistance. Concentration of LTF in bovine milk increases significantly during mammary infections such as mastitis, and the degree of increase is related to the severity of the disease. The LTF concentration ranges from 0.1 g.l-1 (mature milk: 0.1 to 0.4 $g.l^{-1}$) to 5 $g.l^{-1}$ (colostrum: 1.5 to 5 $g.l^{-1}$). Although LTF is present in low concentrations in the milk of healthy cows, LTF concentrations in milk may increase up to a level of 2.3 $g.l^{-1}$ during clinical mastitis (Kutila et al., 2004; Soyeurt et al., 2007) (**Table 1**).

A recent genetic study in Canadian Holsteins shows a genetic correlation of 0.69 between mastitis and the average somatic cell score (SCS) (Koeck et al., 2012). Other studies have estimated genetic correlations between mastitis and the SCC, ranging from 0.3 to 0.8, with an average of 0.6 (Heringstad et al., 2000). Thus, according to Koeck et al. (2012), even though mastitis and the SCS have a common genetic background, they may not be regarded as the same trait.

Lactose values are also capable of displaying disorders in the secretory tissues. Indeed, udder infections cause the biosynthesis of lactose to decrease (Pyörälä, 2003; Rajcevic et al., 2003; Brandt et al., 2010; Forsbäck et al., 2010). In 2003, Rajcevic et al. obtained a correlation between SCC and lactose of -0.42. According to Pyörälä (2003), lactose may be a more reliable indicator of mammary gland disorders as compared to SCC. Lactose presents an interesting advantage as an indicator as its day-to-day variation is very low (0.9%) compared to the day-to-day variations in fat content (7.7%), milk yield (7.0%) and the SCC (2.0%) (Forsbäck et al., 2010). Thus, net observed decreases in lactose content are useful predictors of inflammation risk (**Table 1**).

Mastitis also causes some changes in milk conductivity, due to damage in the mammary epithelium, thus altering the balance of specific minerals, such as potassium, sodium and chlorine ions. These minerals may thus be useful predictors for mastitis (van Hulzen et al., 2009; Brandt et al., 2010). Similar to the variation in lactose values, variations in the milk mineral concentration are obviously a response to udder inflammation, though these variations might, to some extent, also be related to other effects. By contrast, changes in milk fat and total protein content in milk are influenced by several other factors, including feed composition. Early

detection of mastitis might be related to the correlation between different indicators, such as the SCC, lactose and protein.

2.6. Feeding and environmental management

In the last few decades, numerous studies have dealt with the negative impact of dairy cattle on the environment. Nowadays, an ever increasing number of studies are focusing on environmental issues in agriculture, and more specifically, in animal production systems. In 2010, agriculture was believed to contribute about 10% to the total EU-25 emissions of greenhouse gases (GHG) (EUROSTAT, 2012; Schils et al., 2005) and about 6% to the total GHG emissions in the United States (EPA, 2013). Animals are considered to contribute about 36% to the total emission values (Weiske et al., 2006). Methane (CH_4) and nitrous oxide (N_2O) are considered to be the primary greenhouse gases emitted by agricultural activities (EPA, 2013). Among the various agricultural activities, most GHG production is, directly or indirectly, caused by animal production, particularly of ruminants. Indeed, the main sources of agriculture-related GHG emissions are enteric fermentation, rumination of cattle and sheep, handling of manure (CH_4) and agricultural soil practices (N_2O). Thus we can conclude that $CH_4$and N_2O emissions are closely related to dairy production. Another important source of GHG is linked to the importation of feedstuffs, mostly protein-rich feeds (transport).

Milk composition mainly depends on the quality of the feed, *i.e.* feed composition, energy and fiber content, etc. Milk urea content can be used as an indicator for dietary crude protein concentration (Kuterovac et al., 2005). In 2001, Godden et al. described the relationship between milk urea content and nutritional management, production, and economic variables in commercial dairy herds. There are three main sources of urea in milk: the final product of protein decomposition, the digestion of non-protein nitrogen (NPN), and the catabolism of amino acids in the mammary gland. Milk protein contains true-protein (95%) and NPN (5%). Milk urea contributes most to the NPN fraction (30-35% of the NPN: Bastin et al., 2009; Biswajit et al., 2011). According to Kuterovac et al. (2005) and Biswajit et al. (2011), average herd milk

urea concentrations are positively related to the dietary crude protein and rumen (un)degradable protein. Biswajit et al. (2011) also showed that milk urea measurements determined by infrared test methodology provide a useful tool for monitoring the efficiency of nitrogen utilization in dairy cattle. Diets those are too rich in protein lead to higher feed costs, environmental pollution and fertility problems (Biswajit et al., 2011). On the other hand, very low milk urea content could indicate protein deficiencies in the diet of dairy cattle, potentially leading to a loss of production. Target values for milk urea content used by farm advisors (Belgium, Walloon Region) are generally within a range between 200 and 400 $mg.l^{-1}$ (Bastin et al., 2009). However, it still remains important to take into account certain characteristics of the cow, such as her stage of lactation, her parity number, etc. Consequently, monitoring milk urea content presents several economic and ecological benefits. Indeed, this tool could be useful in decreasing milk urea concentrations, in reducing the excretion of excess nitrogen into the environment, and consequently in lowering feed costs, while maintaining the cow's level of milk yield (Kuterovac et al., 2005; Bastin et al., 2009; Biswajit et al., 2011).

Boichard et al. (2012) looked at the relationship between feed management practices and the environmental footprint. In that study, feed costs were decreased through the use of alternative diet compositions, all while maintaining the herd's milk yield at a constant level. This led to a decrease in the GHG emissions by the dairy cattle under study. This proves that the environmental concerns of consumers could be met by adapting herd management techniques, lowering the impact of dairy farming on GHG emission values and, indirectly, improving the economical sustainability of dairy production systems. In addition, despite the fact that GHG emissions currently have no direct value on the market, decreasing the agricultural environmental footprint becomes a major challenge for the future (Boichard et al., 2012) (*e.g.* increased importance of environmental policies, introduction of "emissions taxes" to be paid by farmers [Moraes et al., 2012]).

Unfortunately, only a few studies have analyzed the relationship between milk composition and GHG production levels. Recent studies have shown that FA profiles may be used as indicators for the "environmental quality of milk" (*e.g.* Chilliard et al., 2009; Dijkstra et al.,

2011). Fatty acid profiles thus represent a valuable tool for reducing methane emissions. The incentive for reducing methane emissions is two-fold. Firstly, a reduction in methane emissions leads to a decrease in the impact of GHG on the environment. Secondly, since methane production corresponds to a loss of productive energy by the dairy cow (Chilliard et al., 2009), there is great interest in providing indicators that will allow for a decrease in methane emission values. Various milk FAs show a moderate relationship with methane production in dairy cattle. According to the study of Chilliard et al. (2009), which was based on eight lactating multiparous Holstein cows, saturated FAs (SFAs) showed the highest positive correlation value with methane output (r = 0.87 to 0.91). Extremely high correlations were obtained for 8:0 to 16:0 (r = 0.94) and for the sum of C18-FA (r = -0.94). The relationship between milk FAs and methane output is easy to explain. As stated previously, milk FAs are derived from four major pathways: diet, the mammary gland, bacterial synthesis and body fat mobilization (Chilliard et al., 2009; Stoop et al., 2009). On the one hand, synthesis both of FAs and of methane, acetate, and butyrate presents a common biochemical pathway. On the other hand, dietary FAs, especially SCFAs, MCFAs, LCFAs, and poly-unsaturated FAs (PUFAs), present a negative impact on protozoa, cellulolytic bacteria, or archaea methanogene populations, and consequently, on methane production (Chilliard et al., 2009). In addition, in the FAs studies carried out by Chilliard et al. (2009), most FAs presented an interesting correlation with methane production levels (*trans*-16 18:1; cis-9, *trans*-13 18:2; *trans*-12 18:1; *trans*-13+14 18:1; *trans*-6+7+8 18:1; cis-15 18:1; and *trans*-11, cis-15 18:2). These FAs are known as biohydrogenation intermediates, and are indirectly linked to the dietary 18:3 content. This explains why diets showing high concentrations of PUFAs tend to decrease methane production.

2.7. Using milk components as multiple (health and environmental) indicators in dairy cattle management

The main objective for using various milk components as management tools for promoting the sustainability of dairy production systems (health, feeding and environmental aspects) is to

obtain valuable indicators for diseases and unbalanced diets, which might be helpful for preventing metabolic problems at an early stage. **Table 1** shows normal concentrations of several milk components, such as their range of changes according to different diseases or metabolic disorders. However, the evidence from the authors cited shows that expression levels of disease can vary from one study to another. Some authors prefer to present normal levels and threshold values for disease, while others display these values as a ratio (pathological value divided by physiological value).

As indicated previously, some milk components may present different day-to-day variations (*e.g.* lactose *vs* fat content) (Forsbäck et al., 2010). Fortunately, for the majority of metabolic disorders, at least two parameters may vary, thus allowing the setting up of a pre-diagnostic test. In practice, normal physiological variances would need to be determined for each parameter used (Hamann et al., 1997).

In order to improve the interpretation of milk composition patterns, a reference system needs to be set up for easy interpretation of available data. Indeed, values presented in this review may only be valid for that given dairy cow population or herd and may not be used as reference values for different dairy herds or cow populations. Threshold values for disease must be used with caution. It is important to study each dairy cow population separately and to adapt the threshold values accordingly. Values given in this review would still need to be adapted and validated in the field. In addition, due to variations between individual cows, this kind of strategy needs to be applied on a herd level, not on the basis of individual cows. Finally, the measurement of milk composition is meant as both a monitoring and a prevention tool. In other words, this tool can never replace close monitoring of a herd by the farmer and appropriate veterinary care, but may be used as an efficient alert system for preventing health disorders in cows.

2.8. Conclusion

Milk yield, milk and milk fat composition may be used for developing strategies for the prevention and monitoring of production dysfunctions in dairy cattle and for the improvement of the sustainability of dairy production systems. The threshold values for disease in cattle presented in this review were obtained from the current literature. These values are examples and should be used with caution. Nevertheless, the present review suggests that milk and milk fat composition may be used both as a sustainability management tool and as a monitoring and prevention tool for several pathologies and health disorders in dairy cattle. The FA profile of milk may also be used to predict methane production in dairy cattle; however, more data reflecting a wide range of diets will be required to confirm the usefulness of the prediction model. In addition, due to the use of MIR technology, these tools may be easily implemented in practice and are relatively cheap. Milk labs or milk recording agencies would be able to alert farmers whenever threshold values for disease were reached, representing a valuable tool for health and environmental management. Using such prevention tools could thus help to improve the sustainability of dairy farmers. Indeed, in addition to avoiding losses arising from clinical diseases (milk yield losses, veterinary costs, etc.), and unbalanced diets, the prevention of production dysfunctions would also improve the reproductive performance of dairy herds, their udder health, animal welfare aspects (consumer concerns), labor input (medical treatment of cows), environmental aspects (decrease in the use of drugs and decrease in GHG emissions), etc. In this way, the introduction of prevention tools would contribute to improvements in dairy production from an economical, ecological and animal welfare point of view.

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CHAPTER III.

GENETIC VARIABILITY OF MILK FATTY ACIDS

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3.1. Abstract

3.1.1. Genetic variability of milk fatty acids.

The milk fatty acid (FA) profile is far from the optimal fat composition in regards to human health. The natural sources of variation, such as feeding or genetics, could be used to increase the concentrations of unsaturated fatty acids. The impact of feeding is well described. However, genetic effects on the milk FA composition begin to be extensively studied. This paper summarizes the available information about the genetic variability of FAs. The greatest breed differences in FA composition are observed between Holstein and Jersey milk. Milk fat of the latter breed contains higher concentrations of saturated FAs, especially short-chain FAs. The variation of the delta-9 desaturase activity estimated from specific FA ratios could explain partly these breed differences. The choice of a specific breed seems to be a possibility to improve the nutritional quality of milk fat. Generally, the proportions of FAs in milk are more heritable than the proportions of these same FAs in fat. Heritability estimates range from 0.00 to 0.54. The presence of some single nucleotide polymorphisms could explain partly the observed individual genetic variability. The polymorphisms detected on *SCD1*and *DGAT1* genes influence the milk FA composition. The *SCD1* V allele increases the unsaturation of C16 and C18. The *DGAT1* A allele is related to the unsaturation of C18. So, a combination of the molecular and quantitative approaches should be used to develop tools helping farmers in the selection of their animals to improve the nutritional quality of the produced milk fat.

Keywords: delta-9 desaturase, diacylglycerol O-acyltransferase and genetic, milk fatty acids.

3.2. Introduction

Milk fat is a complex mix of tri- and diglycerides, complex lipids, and liposoluble substances (Debry 2001). On average, 96% of milk fat is composed of triglycerides (Jensen 1995), each made up of glycerol esterified with 3 fatty acids (FAs). These are carboxylic acids with aliphatic chains, whose length and degree of saturation vary. According to the saturation, the FAs are

divided into 3 classes: saturated FAs (SFAs), monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs).

Dairy products account approximately for 15–25% of fat intake and for 25–35% of SFA intake in human nutrition. Due to the negative effects of some SFAs on human health, milk fat has a bad reputation, because it is composed of 65–75% of SFAs (Debry 2001). Diets rich in SFAs, such as the lauric (C12:0), myristic (C14:0), and palmitic acids (C16:0), are highly related to an increased risk of atherosclerosis, obesity, and coronary heart diseases (e.g. Ulbricht and Southgate 1991; Cox et al. 1995; Hu et al. 1999; Haug et al. 2007). However, not all SFAs increase the cholesterol level in blood with the same proportion. According to Mensink et al. (2003), C12:0 markedly increases the total cholesterol content but decreases the ratio of total cholesterol to HDL cholesterol. This last property is favorable and more marked for C12:0 than for C14:0 and stearic acid (C18:0). C16:0 increases this ratio. The risk of cardiovascular diseases is not influenced by C18:0 (Hu et al. 1999). So, judging the nutritional quality of milk fat only basing on their total SFA content seems to be too generalist.

The unsaturated FAs are usually called 'healthy fats', especially for their impact on the level of cholesterol in blood (Ward et al. 1998; Haug et al. 2007). PUFAs decrease the cholesterol content more strongly than MUFAs (Williams et al. 2000). Oleic acid (C18:1 *cis-9*) and linolenic acid (C18:3 *cis-9, cis-12, cis-15*), belonging to the ω-3 family, have anticancer and anti-atherogenic properties (Williams et al. 2000; Haug et al. 2007). Besides its effect on cholesterol level, linoleic acid (C18:2 *cis-9, cis-12*), the most important in the ω-6 family, improves the sensibility to insulin and thus reduces the incidences of type 2 diabetes (Hu et al. 2001). This FA has also bactericidal impact on *Lysteria monocytogenes* (Petrone et al. 1998). Western diets are known to be deficient in ω-3 and excessive in ω-6. This disequilibrium promotes many diseases, such as cardiovascular diseases, cancer, and inflammatory or autoimmune diseases (Simopoulos 2002). So, reaching and keeping a lower ratio of ω-6 to ω-3 is important. This ratio is usually higher than 12 in industrialized societies. Current dietary recommendations propose dietary ω-6: ω-3 lower than 5 to reduce the risk of cardiovascular diseases, cancer, autoimmune disorders, allergies, obesity, some mental disorders, etc. (Sabikhi 2004). Excess of ω-6 can lead to

disruption of the biosynthesis of prostaglandins and consequently to inflammation, obesity, high blood pressure, irritation of the digestive tract, depressed immune function, and other disorders. Deficiency in ω-3 can also lead to other physiologic disorders, such as asthma and heart diseases (Sabikhi 2004). This ratio is naturally low in milk products (1.6; Haug et al. 2007). Dairy and beef products are rich sources of conjugated linoleic acid (CLA) (2.5–18.0 mg g^{-1} of fat in bovine milk), which is a mixture of positional and geometric isomers of C18:2 *cis-9, cis-12*. This structural variability explains the several functions, sometimes contradictory, attributable to CLA (Lock and Bauman 2004; Parodi 1997; Whale et al. 2004). The most important isomers are the rumenic acid, C18:2 *cis-9, trans-11,* which represents about 75–90% of the total CLA, and C18:2 *trans-10, cis-12*. According to several animal models, CLA exhibits anti-atherogenic, antiobesity, and anticarcinogenic proprieties (e.g. Corl et al. 2001, MacDonald 2000; McGuire and McGuire 2000, Parodi 1997). CLA are also able to modulate the immune response and bone growth, to promote cell growth, etc. (e.g. Keating et al. 2005; Lock and Bauman 2004; MacDonald 2000; Tanaka 2005; Whale et al. 2004). More details can be found in many reviews about the effects of FAs on human health (e.g., Hu et al. 2001; Chilliard et al. 2000).

Basing on these health aspects, it would be interesting to modulate the quality of milk fat, and then to promote the production of some FAs in relation to the others. Even if the consumption of dairy products is lower than recommended [450–600 mL of milk and 20–40 g of cheese (Devriese et al. 2006)], the improvement of nutritional quality of milk could have a significant impact only in the context of a balanced diet. Numerous investigations described the feeding effects on milk fat composition (e.g., Chilliard et al. 2000), but information about genetic effects on the FA profile of bovine milk is scarce in the literature. The aim of this paper was to review the impact of genetic factors on the FA composition of bovine milk fat.

3.3. Quantitative approach

3.3.1. Breed differences

Several authors observed breed differences in the milk FA profile. Table 1 summarizes the breed differences in FA concentrations in milk fat, observed in various studies and expressed in comparison with Holstein (Soyeurt et al. 2008a). The papers referenced in Table 1 are some examples of available studies on breed differences in FAs. Holstein and Jersey milk fats present the greatest differences. Higher concentrations of SFAs, especially of FAs with short and medium carbon chains, are observed in Jersey milk fat (e.g., Hermansen and Lund 1990; Beaulieu and Palmquist 1995; White et al. 2001; Table 1). However, DePeters et al. (1995) reported that the concentrations of FAs with short and medium chains did not differ. Moreover, the proportion of C16:0 did not differ significantly between Holstein and Jersey milk fat. According to Lawless et al. (1999), Normande and Montbeliarde produce milk fat with the highest proportions of C18:0. In contrast to Normande, however, Montbeliarde milk fat has higher CLA content, as compared to Dutch Holstein milk fat (Table 1).

Unfortunately, the studies focusing on the breed differences in FA composition analyzed generally small numbers of milk samples and cows (Table 1). This is related to the cost of the gas chromatographic analysis needed to measure FA concentrations in bovine milk. Recently, Soyeurt et al. (2006a) showed the possibility to estimate the FA concentrations by mid-infrared spectrometry. This technology is faster and cheaper than the reference chemical analysis. Thanks to these estimations of FAs by infrared, Soyeurt et al. (2006b and 2008b) studied the differences across dairy breeds on a large dataset using mixed models. The obtained results for Jersey, Montbeliarde, and Normande breeds were generally in agreement with those mentioned in Table 1. Those authors also observed that the milk fat produced by dual-purpose Belgian Blue cows had the highest concentrations of unsaturated FAs. The observed breed differences were partly explained by the values of C14:1 cis-9/C14:0, C16:1 cis-9/C16:0, and C18:1/C18:0, reflecting the activity of delta-9 desaturase.

Table 1. Breed differences of fatty acid profile on bovine milk fat obtained by different studies from a limited number of cows (N) fed with the same diet (Soyeurt et al., 2008).

	Differences of fatty acid contents compared to Holstein (in %)								
	Guernsey	Jersey				Brown-Swiss		Montbeliarde	Normande
	Stull[1]	Stull[1]	Beaulieu[2]	DePeters[3]	White	DePeters[3]	Kelsey[4]	Lawless[5]	Lawless[5]
	N=25	N=10	N=8	N=23	N=18	N=29	N=106	N=29	N=27
C4:0			-2.43	-4.90	+3.81 (**)	-1.47	+12.36(***)	-5.50	-2.75
C6:0	+20.73	+8.54	+16.67 (*)	+3.32 (*)	+16.33(**)	+2.21	+7.32(**)	-2.54	+0.85
C8:0	+13.16	+15.79	+38.46 (**)	+7.55 (**)	+27.17(**)	+5.03 (**)	+13.13(***)	+1.02	+5.10
C10:0	+14.29 (**)	+34.10 (**)	+43.33 (***)	+13.59 (**)	+34.00(**)	+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 (**)	+34.19(**)	+6.34 (**)	+14.41(***)	+6.46	+10.77
C14:0	+5.64	+9.26	+8.62 (*)	+2.36	+10.71(**)	+2.14	+4.66(*)	+2.61	+1.87
C14:1 *cis*	-11.31	-4.76			+1.69		-1.64	-28.09	-10.11
C15:0	-6.80	-2.04					-6.76(**)		
C16:0	+7.20 (**)	+5.63 (**)	-6.79 (*)	-1.24	-1.11	-1.70	+0.96	-11.49	-8.15
C16:1 *9-cis*	-7.14 (**)	-16.67 (**)		-9.55	-10.71(**)	-1.51	-13.08(***)		
C18:0	+4.64	+1.12	+12.50	+6.61 (**)	+0.72	-6.83 (**)	-3.42	+10.89	+14.93
C18:1 *9-cis*	-11.15 (**)	-12.92 (**)	-12.72 (**)	-9.51	-10.35(**)	+3.91	-1.96(***)	+5.37 *	+1.37 *
C18:2	-4.92	-4.64	0.00	+1.58	0.00	-4.74	-5.80(***)	+5.94	+3.96
CLA					-21.95(**)		-6.82(***)	+13.07	-5.11
C18:3	-19.79	-32.29	-16.67	+15.50 (*)	-2.63	-6.98	-2.56	+1.22	-6.10

[1] Stull et al. (1964); [2] Beaulieu and Palmquist (1995); [3] DePeters (1995); [4] Kelsey et al (2003); [5] Lawless et al (1999);[6] White et al. (2001) * = P<0.05; ** = P < 0.01 and *** = P < 0.001

Delta-9 desaturase (SCD), also named stearoyl coenzyme-A desaturase (E.C. 1.14.19.1), catalyses the introduction of a *cis*-double bond between carbons 9 and 10 of SFAs with a chain length of 10-18 carbons (Bauman et al. 1999; Thomson et al. 2003). So, it converts specific medium- and long-chain SFAs into the corresponding MUFAs (Reh et al. 2004). This last activity is an essential step in the synthesis of unsaturated FAs. Up to 90% of the CLA in bovine milk is formed due to the activity of this enzyme in the mammary gland (Keating et al. 2005). According to Feng et al. (2007) and Lock and Garnsworthy (2003), the C14 desaturase index is considered as the best indicator of desaturase activity. In fact, 90% of C14:1 *cis-9* is the result of SCD activity (Mosley and McGuire 2007). The total concentrations of MUFAs and CLA should increase in fat if SCD activity rises, improving in this way the nutritional quality of milk. Some studies estimated SCD activity by specific FA indices, defined as ratios of FAs dependent on this enzymatic activity: product/substrate (e.g., Lock and Garnsworthy 2003), substrate/product (e.g., Chouinard et al. 1999) or product/(substrate + product) (e.g., Kelsey et al. 2003). Kelsey et al. (2003) observed that Holstein cows showed higher FA indices compared to Brown-Swiss cows, except for CLA index. The greatest concentrations of MUFAs and CLA observed by those authors for Holstein breed could be explained by this enzymatic activity (Table 1). Soyeurt et al. (2008b) observed the greatest FA indices for the dual-purpose Belgian Blue, explaining partially the greatest concentrations of unsaturated FAs observed for this breed. In the same way, the FA indices of Jersey cows were lower, compared to Holstein cows, explaining partly the high SFA content observed in this breed.

3.3.2. Individual genetic variability

The effects of feeding on FA composition of bovine milk are well known. For a few years, some Belgian and Dutch breeders used specific feeding to increase the concentrations of unsaturated FAs in their milk, especially of ω-3 and CLA. Although this method is efficient, the effects are not durable. If the feeding supplementation stops, the improvement of FA composition disappears. So, animal selection using the genetic variability of FAs should transmit from generation to generation this nutritional improvement. For this purpose, a selection index

needs to be developed. The estimation of genetic parameters for FA concentrations in bovine milk is the first step.

The heritability values mentioned in Table 2 differ between the cited studies. The number of analysed samples and the methodology used for estimating the genetic parameters could explain these differences. This section presents the heritability values obtained in various studies, describes the particularities of each study, and discusses all the obtained results.

To our knowledge, Edwards et al. (1973) were the first authors who estimated the genetic parameters of FA concentrations in bovine milk fat. They expressed FA concentrations as molar percentage. The genetic parameters were calculated from 50 winter milk samples (2 × 10 samples from Ayrshire monozygotic twins and 2 × 15 samples from Ayrshire dizygotic twins). Heritabilities were high and ranged between 0.64 and 0.98 (Table 2). This may be partly due to the specific unit, but these values can also be considered as overestimated because of the low number of analyzed samples and the biased hypothesis used to calculate the variance components. Environmental variance was estimated from the variance component within monozygotic pairs. The variance components within dizygotic pairs represented the environmental variance and half of the genetic variance. In spite of these overestimated values, this study was the first one showing high heritability for each FA in milk fat.

One year later, Renner and Kosmack (1974a) estimated the genetic parameters of various groups of FAs based on 2082 milk samples collected from the progeny of 10 AI sires by using a sire model. They obtained some heritability estimates of 0.26, 0.06 and 0.04 for the concentrations of FA classes with short and medium carbon chains and of C18 family in milk fat, respectively. Heritability values were 0.26, 0.25 and 0.02 for the same classes of FAs in milk, respectively. From these estimates, it appears that the FA concentrations in milk seem to be more heritable than the concentrations of FAs in milk fat.

Table 2. Heritability of fatty acid composition in bovine milk and milk fat.

Fat or fatty acids	Heritability estimates of fat content and fatty acids content						
	Karijord[1] N=7000	Soyeurt[2] N=40,007		Soyeurt[3] N=52,950	Stoop[4] N=1,918	Bobe[5] N=592	
	g/100 g	g/100g	g/100g fat	g/100g fat	%wt	g/L milk	%wt
%Fat	0.09	0.32	0.32	0.33	0.47	nd	nd
C4:0	nd	nd	nd	nd	0.35	0.01	0.00
C6:0	0.11	nd	nd	nd	0.39	0.19	0.00
C8:0	0.13	nd	nd	nd	0.48	0.37	0.18
C10:0	0.16	nd	nd	nd	0.54	0.40	0.22
C12:0	0.17	0.29	0.09	nd	0.35	0.36	0.18
C14:0	0.07	0.31	0.19	0.15	0.49	0.18	0.00
C14:1	0.26	nd	nd	0.20	nd	nd	nd
C16:0	0.15	0.38	0.20	0.15	0.31	0.20	0.09
C16:1	0.12	nd	nd	0.22	nd	0.34	0.49
C18:0	0.15	0.30	0.28	0.16	0.19	nd	0.24
C18:1	0.06	0.05	0.15	0.17	0.18	0.25	0.06
C18:2	0.11	0.20	0.15	nd	0.13	0.27	0.00
CLA	nd	nd	nd	nd	0.21	nd	nd
C18:3	0.09	nd	nd	nd	0.09	nd	nd
SAT	nd	0.36	0.14	nd	nd	0.27	0.05
MONO	nd	0.15	0.24	0.17	nd	0.09	0.08
POLY	nd	nd	nd	nd	nd	0.25	0.00

[1]Edwards et al. (1973) ; [2]Karijord et al. (1982); [3] Soyeurt et al. (2007); [4]Soyeurt et al. (2008b); [5]Stoop et al. (2008); [7] Bobe et al. (2008); %wt= fatty acid (FA) weights as a proportion of Total fat weight; CLA= conjugate linoleic acid; MUFAs =monounsaturated FAs; PUFAs= polyunsaturated FAs; SFAs= saturated fatty acids; nd= no data

Renner and Kosmack (1974a), Karijord et al. (1982) used also a sire model to estimate the genetic parameters but they calculated the heritability values for the major individual FAs. A total of 7000 milk samples collected from about 30 daughters of each of the 114 selected AI test bulls between January 1979 and August 1979 were used in this study. As in the previous studies, concentrations of FAs were measured by gas chromatography. The heritability values of the FA concentrations in fat (g/100g of fat) ranged from 0.06 to 0.26 (Table 2). The comparison of the studies conducted by Renner and Kosmack (1974) and Karijord et al. (1982) with the one of Edwards et al. (1973) is impossible because the methods and units used were clearly different. Compared to the methodology used by Edwards et al. (1982), the sire model used by Renner and Kosmack (1974) and Karijord et al. (1982) gave more accurate variance components.

Using an animal model instead of a sire model permits to estimate directly the genetic effects of all relatives. Further, this model permits to take into account the performances of ancestors, descendants and collateral relatives, and thus improves the accuracy of the estimation. More recent studies, such as Soyeurt et al. (2007a and 2008b), Stoop et al. (2008) and Bobe et al. (2008), used an animal model to estimate the genetic parameters of FAs.

The previous studies used gas chromatography to measure FA concentrations in milk fat. Although this method is efficient, it requires skilled staff, expensive reagents, and takes time, so only small numbers of samples were analyzed. The estimation of the genetic parameters needs a large amount of data; hence Soyeurt et al. (2006a) proposed to use mid-infrared spectrometry to predict the FA concentrations directly in bovine milk. Thanks to the large data set including the spectral data and, thus, the FA concentrations estimated by applying the developed calibration equations on these collected spectra, Soyeurt et al. (2007a) estimated the genetic parameters of FAs by using a multi-trait test-day animal mixed model. A total of 7700 milk samples were collected in 25 herds between April 2005 and May 2006, and analyzed by mid-infrared spectrometry. The generated spectra were recorded. To increase the number of contemporaries, milk history of studied animals and herds was added. The final edited data set contained 40 007 records on 2047 cows. Heritability estimates ranged from 0.05 to 0.38 for the individual FA concentrations in milk (g $100g^{-1}$ of milk) and from 0.09 to 0.32 for FA concentrations in fat (g $100g^{-1}$ of fat) (Table 2). One year later, the same authors (Soyeurt et al.

2008b) used the same model but with a larger data set containing 52 950 records (including 10 401 spectral data collected from April 2005 to December 2006) from 3217 cows. Only FA concentrations (g $100g^{-1}$ of fat) related to the delta-9 activity were estimated (C14:0 to C18:1 and MUFAs). Heritability values ranged from 0.15 to 0.33. The results were slightly lower than those estimated previously by the same authors except for C18:1. These differences could be explained mainly by the data (the second data set contained spectral data from a larger number of winter milk samples) and partly by the improvements of the calibration equations (78 reference milk samples used to build the calibration equations instead of 49 used in the previous study).

Stoop et al. (2008) used a single-trait animal mixed model to calculate the heritability of the individual FA measured by gas chromatography and expressed as %wt (FA weight as a proportion of total fat weight), based on 1918 milk samples collected from 1918 cows between February and March 2005. The benefit of using gas chromatography instead of mid-infrared spectrometry is a more accurate measurement of FAs with low concentrations in bovine milk fat. In fact, if the concentration of an individual FA in bovine milk decreases, the accuracy of its prediction by mid-infrared spectrometry decreases (Soyeurt et al. 2006a). Stoop et al. (2008) studied a large number of various FAs, especially of several isomers of C18:1. The various studied isomers showed similar heritabilities, ranging between 0.11 and 0.18. Heritability values for the major FAs ranged from 0.09 to 0.54 (Table 2).

Bobe et al. (2008) calculated the genetic parameters of FAs measured by gas chromatography, using single-trait mixed animal models based on 592 milk samples collected between August 1993 and July 1994 from 233 cows. Heritability values ranged between 0.01 and 0.40 in milk (g L^{-1} of milk) and between 0.00 and 0.49 in fat (%wt) (Table 2).

The comparison of results among the cited studies is difficult because of the diversity of the units used to express the concentrations of FAs, the model used, and the amount of data available. However, some observations made by various authors can be compared. All of these studies confirmed the existence of the genetic variability of the FA concentrations in bovine milk and fat, suggesting a potential future animal selection. Results obtained by Soyeurt et al.

(2007a) and Bobe et al. (2008) and presented in Table 2 suggest that the concentrations of FAs in milk (expressed as g $100g^{-1}$ of milk and g L^{-1} of milk) are generally more heritable than the concentrations of FAs in milk fat (expressed as g $100g^{-1}$ of fat and %wt). Renner and Kosmack (1974a) also observed this trend. This observation was expected because the fat content of bovine milk is strongly heritable. Heritability of fat percentage ranged from 0.32 to 0.47 (Table 2). Karijord et al. (1982) found a lower value, equal to 0.09. Stoop et al. (2008) as Renner and Kosmack (1974a) suggested a relation between FA length and the heritability estimates. The other cited studies did not observe the same trend.

The improvement of models used to describe the variability of FAs is related to the facilities needed to obtain the FA data. Gas chromatography is too expensive to be used on a large scale to develop the tools needed for the implementation of animal selection based on FA concentrations. The use of mid-infrared spectrometry to predict the FA concentrations in bovine milk is a good alternative method, even if the prediction of FAs with low concentrations in milk is not accurate enough. The implementation of this methodology in the different milk labs used to collect the data for the routine milk recording is a crucial point before thinking about developing a selection program based on the FA profile. Currently, the Walloon and Luxembourg milk recordings are, to our knowledge, the only ones that record the spectral data during the routine milk infrared analysis used to measure the concentrations of fat, protein, lactose, and urea. However, recently, Foss (Hillerod, Denmark) proposed different calibration equations to predict the FA concentrations in milk. All this suggests that in the near future a larger number of labs could predict the FA concentrations needed for a selection program. Thanks to a larger data set, a test-day animal mixed model could be used to describe the variability of FAs, as done by Soyeurt et al. (2007a, 2007b, 2008b). The use of this type of model presents some advantages, such as a more efficient use of the collected data, a genetic model that accounts better for the biology of dairy cows, a better accounting for short-term environmental effects at each test-day milk recording, and finally, more accurate estimations of cow indices (Schaeffer et al. 2000; Mayeres et al. 2004; Muir et al. 2007). Also due to the availability of data, the model could be improved by the addition of some regressions, to take into account the variation of genetic parameters throughout the lactation. So, parametric

curves, such as the Ali-Schaeffer curve, the Wilmink curve, or orthogonal polynomials, could be used to model the random regressions. However, the disadvantage of the test-day model is the computation time, cost, or both (Druet et al. 2003).

The number of FAs is very large: 406 registered currently (Debry 2001). Consequently, it could be interesting to find an indicator that reflects the most important information contained in the FA variability, especially to decrease the computation cost and time. By its implication in the production of MUFAs and CLA, the FA indices could reflect the nutritional quality of bovine milk fat. Royal and Garnsworthy (2005) reported heritability values of 0.30, 0.19, and 0.29 for C14:1/(C14:0+C14:1), C18:1 cis-9/(C18:1 cis-9+C18:0), and C18:2 cis-9, trans-11/(C18:2 cis-9, trans-11+C18:1 trans-11), respectively. Only C16:1/(C16:0+C16:1) showed a heritability equal to 0.01. Heritability for C14:1 cis-9/C14:0, C16:1 cis-9/C16:0, and C18:1 cis/C18:0 obtained by Soyeurt et al. (2008b) were equal to 0.20, 0.20, and 0.03, respectively. These results showed the individual genetic variability of the FA indices.

The FA composition influences the nutritional quality of milk fat but also the technological properties of butter (Soyeurt et al. 2007b). Increasing the concentrations of unsaturated FAs and short-chain FA improves butter spreadability (Bobe et al. 2007). Bobe et al. (2003) suggested that the phenotypic variation of FA composition was sufficient to modify the textural properties of butterfat. One of the indicators used to determine the hardness of butterfat is the ratio of SFAs to unsaturated FAs. Heritability of this ratio estimated by Soyeurt et al. (2007b) was equal to 0.22. Stoop et al. (2008) found a heritability of 0.20. As expected, the genetic variability of the ratio of SFAs to unsaturated FAs exists.

3.3.3. Molecular approach

Few authors pointed out the *SCD* gene level expression as one of the possible origins of FA variation in milk (e.g., Baumgard et al. 2002; Keating et al. 2005). The bovine *SCD* mRNA, completely cloned and sequenced, spans 5.1 kb and codes for a 355-amino-acid enzyme. The

SCD gene is identified in various species (e.g., Tabor et al. 1998, Kuchel et al. 2004). Currently, two *SCD* genes are identified on BTA6 and BTA26 (Campbell et al. 2001; Lengi and Corl 2007). The first SCD gene is expressed in several tissues and organs, principally in mammary glands and adipose tissue, but also in the liver, muscle, lung, brain, heart, etc. The second one is principally expressed in the brain (Ward et al. 1998; Yahyaoui et al. 2001). Medrano et al. (1999) identified 8 single-nucleotide polymorphisms (**SNP**) in various bovine breeds (Holstein, Jersey, and Brown-Swiss): 3 SNPs were detected on exon 5 and the others in the 3' UTR of the SCD gene. Keating et al. (2005) have characterized the bovine SCD gene promoter and studied its regulation on 9 Holstein cows having high and low concentrations of CLA and on 10 cows of various dairy breeds. According to their results, no polymorphic sites between the bovine SCD promoters of these 19 cows were shown by the sequence comparison. Keating et al. (2005) concluded that the variations in the levels of CLA in milk could not be explained by polymorphisms of the SCD promoter regions. However, these variations could be explained by other hypotheses, such as differences in ruminant synthesis of CLA (or CLA precursors), differences in the regulatory proteins themselves, or by polymorphisms in the coding sequences of the bovine SCD gene (Keating et al. 2005). Moioli et al. (2007) and Mele et al. (2006) studied the effect of the SNP (C/T) located on exon 5 of the SCD gene from 79 cows belonging to 3 breeds (27 Piedmontese, 27 Valdostana, and 25 Jersey) and from 297 Holstein Italian Friesian cows, respectively. They concluded to a higher enzymatic activity of SCD polymorphism essentially on C14:0 and caproleic acid (C10:1). Recently, Schennink et al. (2008) observed that the SCD1 V allele was related to higher concentrations of C10:0, C12:0, C14:0, C16:1 cis-9 and CLA in milk fat.

The diacylglycerol O-acyltransferase (**DGAT-1**) is also implied in the FA composition of bovine milk (Schennink et al. 2007). DGAT-1 is considered as a microsomal enzyme (E.C. 2.3.1.20) able to catalyze the only committed step in triacylglycerol synthesis by using diacylglycerol and fatty acyl CoA as substrates (Cases et al. 1998). Situated on BTA14, the DGAT1 gene encodes 489 amino acids and comprises 17 exons. By sequencing the bovine DGAT1 gene, a non-conservative lysine to alanine substitution was observed at position 232 (K232A) and seems to influence the major milk production traits, such as milk yield and milk composition (Grisart et al. 2004; Thaller et al. 2003; Winter et al. 2002). Winter et al. (2002) observed that

the lysine variant is associated with greater milk fat content than the alanine variant. According to results of Schennink et al. (2007), K232A led to a larger fraction of C16:0 in milk fat but less C14:0, less unsaturated C18 and less CLA. Further, K232A had a positive effect on the ratio of SFAs to unsaturated FA. This could be explained by the fact that the presence of alanine residue at position 232 could inhibit the acyl-CoA-binding capacity of this enzyme, and this leads to a greater activity or an alteration of specificity of DGAT-1 (Schennink et al. 2007; Winter et al. 2002)

3.3.4. Impact on animal selection

As mentioned previously, thanks to the development of FA calibration equations (Soyeurt et al. 2006a) and the possibility to record all spectra generated during the infrared analysis executed during the milk recording, the creation of a large database including the FA profile is now possible. This data set should permit the development of selection indexes to improve the nutritional quality of milk fat. Which FA should be included in this selection index? The answer to this question is not easy. The genetic correlations among some FAs are high (Soyeurt et al. 2007a; Stoop et al. 2008). This relationship is explained by the similarities in their metabolic production processes. For instance, it will be impossible to increase the concentrations of C18:2 *cis*-9, *cis*-12 without increasing the concentrations of C18:3 *cis*-9, *cis*-12, *cis*-15. Besides the relationships among FAs, these milk components are also related to the traditional production traits, such as milk yields, fat or protein contents, and fat or protein yields. The FA composition of milk fat is influenced by fat and protein contents. Negative genetic correlations were observed between the unsaturated FAs and the fat and protein contents (Karijord et al. 1982; Soyeurt et al. 2007a; Stoop et al. 2008). Consequently, as the fat and protein contents influence positively the milk payment, increasing the concentrations of unsaturated FAs should have negative economic impacts for farmers. A new procedure of milk payment needs to be developed, basing on, e.g. the concentrations of some FAs. Thanks to that, many farmers should be interested in improving the nutritional quality of their milk fat, and thus

a large selection program could be developed, basing on the genetic variability of FA concentrations in dairy cattle.

During the last few decades, quantitative genetics permitted important genetic progress without knowing the genes responsible for livestock performance. Even if molecular approach is expensive, it permits to identify these genes, so it complements the quantitative approach. For instance, molecular approach permits the quality control of selection, the major gene identification, and development of new methods enabling better estimates of animal performance. Currently, several genetic marker maps are available for many species, and various QTL regions have been identified. Marker-assisted selection is useful in many situations, especially when the accuracy of conventional selection is low, e.g. when studied traits have low heritability or are measured late in life. Some SNPs have been identified on *DGAT1* and *SCD* genes, permitting early selection of animals. Molecular analyses are interesting for the testing animals. For global animal selection on FA composition, the molecular and quantitative approaches should be associated.

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CHAPTER IV.

OBJECTIVES

This thesis aimed to investigate the opportunity of using milk (fat) composition as an innovative, robust and practical management tools. Development of such tools could help dairy farmers in their daily decisions.

As explained in Chapter II, the milk, and more especially, the milk fat composition could be used in routine as a practical tool allowing the early detection of metabolic or management problem.

In order to develop such a tool based on milk fat composition, a second literature review, presented in Chapter III, was proposed. It summarized the current available information about the genetic variability of milk FA. Further, this review described also the impact of genetic factors on the FA composition of bovine milk.

Therefore, the first objective of the present thesis was to investigate the development of a genetic evaluation for milk FA. The current genetic evaluation model for production traits in the Walloon region of Belgium is a multiple-lactation, multiple-trait random regression test-day model using second-order Legendre polynomials. However, according to the available literature, this kind of model using Legendre polynomials present undesirable properties, as an overestimation of variances at the edges of lactation. On the other hand, some previous researches have reported that splines might be less sensitive to the data that Legendre polynomials. Consequently, splines could be considered as a good alternative to polynomials. The objective of this study was to compare different models used for estimating genetic parameters of milk saturated fatty acids production. This comparison was based on the goodness of models fit and concerned 3 functions: 1) Legendre polynomials; 2) linear splines with 10 knots and 3) linear splines with the same 10 knots reduced to 3 variables. The obtained results were presented in Chapter V, and published in the Journal of Dairy Science.

Next Chapter, Chapter VI, was also concerned by the development of models. In Luxembourg, CONVIS s.c. is the current milk recording organization. The standard milk recording

scheme consists in a physical visit to each participating dairy herd every 4 weeks, and milk samples are collected from all milked cows during consecutive PM and AM milkings. This standard scheme is called “S”. Some alternative recording schemes are proposed to interest dairy farmers. So, milk samples could be also collected every 2, 4 or 6 weeks, during only PM or only AM milkings (scheme “M”) or during on alternate monthly PM and AM milkings (scheme “T”). These alternative schemes present several advantages for dairy farmers. Indeed, they are less disruptive to the daily routine and present an interesting reduce of costs.

However, the use of different sampling schemes could also influence the development of management tools. Indeed, it is well known that phenotypic differences exist in milk composition between AM and PM milkings. Further, very few literatures exist about the impact of milking moment on genetics parameters. So, the Chapter VI, concerns the study of effect of the milking recording time (AM or PM) on genetic parameters of milk yield and milk fat composition (fat and fatty acids groups). These results were presented at ADSA 2012 and recently submitted.

The use of different sampling schemes represents another obstacle to the development of above mentioned tools. Indeed, to develop robust management tools, it is very important that the used phenotypic data were homogenous. However, the use of different sampling methods can bring heterogeneity, and, consequently prevents the comparison of all productions on the different dairy farms.

One possibility is to develop methods allowing the standardization of the milk composition. Chapter VII investigates estimations of milk and milk FA groups productions on 24 hours. The originality of this study was to develop some equations permitting the estimation of FA daily production based only on accurate and available data.

In 2000, ICAR approved a model able to estimate daily milk, fat and protein yields based on AM or PM milking. At our knowledge, nothing is done currently about FA. So, the second

innovative aim of this study was, therefore, to develop equations to estimate the daily yields of the major FA present in milk. Results and conclusions of this study were published in Animal.

The last part of the thesis (Chapter VIII) discussed globally the possibility to use obtained results in order to develop the previous mentioned management tools. This last chapter also formulates perspectives of this research.

CHAPTER V.

SHORT COMMUNICATION: GENETIC VARIATION OF SATURATED FATTY ACIDS IN HOLSTEINS IN WALLOON REGION OF BELGIUM

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5.1. Abstract

Random regression test-day models using Legendre polynomials are commonly used for the estimation of genetic parameters and genetic evaluation for test-day milk production traits. However, some researchers have reported that these models present some undesirable properties such as the overestimation of variances at the edges of lactation. Describing genetic variation of saturated fatty acids expressed in milk fat might require the testing of different models. Therefore, 3 different functions were used and compared to take into account the lactation curve: (1) Legendre polynomials with the same order as currently applied for genetic model for production traits; 2) linear splines with 10 knots; and 3) linear splines with the same 10 knots reduced to 3 parameters. The criteria used were Akaike's information and Bayesian information criteria, percentage square biases, and log-likelihood function. These criteria identified Legendre polynomials and linear splines with 10 knots reduced to 3 parameters models as the most useful. Reducing more complex models using eigenvalues seemed appealing because the resulting models are less time demanding and can reduce convergence difficulties, because convergence properties also seemed to be improved. Finally, the results showed that the reduced spline model was very similar to the Legendre polynomials model.

Keywords: spline, Legendre polynomials, random regression test-day model

Random regression test-day models (**RRTDM**) using Legendre polynomials (**LP**) remain a commonly used methodology for the estimation of genetic parameters and genetic evaluation for daily milk production traits (Misztal, 2006; Bohmanova et al., 2008). The current genetic evaluation model for production traits in the Walloon region of Belgium is a multiple-lactation, multiple-trait RRTDM using second-order LP (constant, linear, quadratic) for additive genetic and environmental effects (herd × year of calving and permanent environmental). Jamrozik and Schaeffer (2002) showed that RRTDM with orthogonal polynomials outperform models using

lactation curves based on the Wilmink function (Wilmink, 1987) and the Ali and Schaeffer function (Ali and Schaeffer, 1987), even using the same number of parameters for additive genetic and environmental effects. Nevertheless, Bohmanova et al. (2008) reported that RRTDM using LP have undesirable properties, mainly the overestimation of variances at the edges of lactation, which could be explained by lack of asymptotes of LP. López-Romero et al. (2004) reported also that LP models resulted in poor performance of fitting data at the extremes of lactations.

Mathematically speaking, splines are piecewise polynomial functions. They are defined as curves that consist of individual segments themselves connected in "knots." The simplest case of a spline function is the linear spline where the segments are fitted by linear polynomials. Some previous research established that splines might be less sensitive to the data than LP and have been considered as a good alternative to polynomials (Druet et al., 2003; Meyer, 2005; Bohmanova et al., 2008).

For the new fatty acid traits expressed in milk fat (g/100 g of fat), models required are still under scrutiny. Recently, Soyeurt et al. (2008) estimated genetic parameters for content of saturated and unsaturated fatty acids using a RRTDM with the similar order of LP for genetic and environmental effects. The current study aimed to compare different models to study genetic parameters of the milk saturated fatty acids production. Three functions were tested and compared to take into account the lactation curve: 1) Legendre polynomials with the same order as currently applied for genetic model for production traits, 2) linear splines with 10 knots, and 3) linear splines with the same 10 knots reduced to 3 variables. The comparison will be based on the goodness of models fit.

A total of 57,953 milk samples were collected between March 2005 and December 2007 from 3,140 primiparous Holstein (>84% Holstein blood) cows in 98 herds. Samples were collected during the official Walloon milk recording managed by the Walloon Breeders Association (Ciney, Belgium). The samples were analyzed by mid-infrared spectrometry using a

Foss Milkoscan FT600 (Foss, Hillerød, Denmark) by the milk committee (Battice, Belgium). Records collected before 5 or after 365 DIM were discarded. Only test-day records from cows with age at first calving between 640 and 1,500 d were kept.

Test-day saturated fatty acid (SFA) content in fat (g/100 g of milk fat) was estimated from collected mid-infrared spectra using the calibration equation developed by Gembloux Agro-Bio Tech (Animal Science Unit, University of Liege, Gembloux, Belgium) and Walloon Research Centre (Quality Department, Gembloux, Belgium).

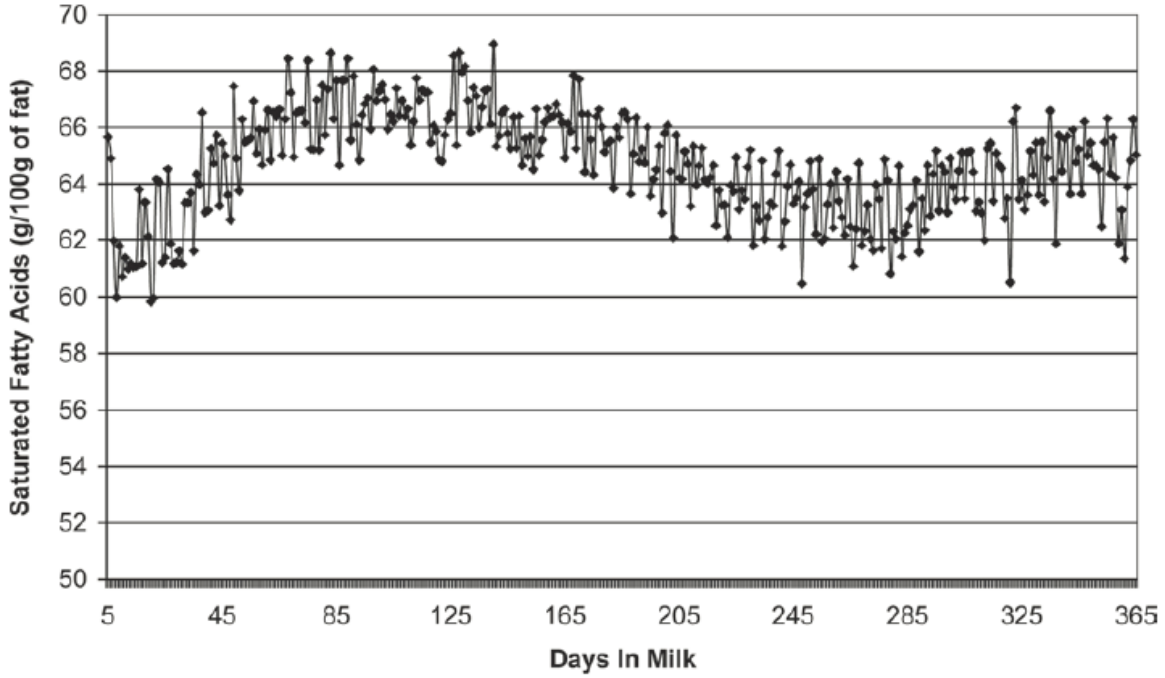

Figure 1. Saturated fatty acids content in fat (g/100 g of milk fat) from 5 to 365 DIM. The number of observations ranged from 10 to 83 for each DIM and from 301 to 930 for each class of 15 DIM.

Figure 1 displays the variation of SFA throughout the first lactation. The content of SFA in fat increased until DIM 85 and then decreased with a slight increase at the end of the lactation. A similar trend was observed in reports in the literature (e.g., Soyeurt et al., 2008) for most SFA expressed in bovine milk fat. The mean value of test-day SFA was 64.99 (SD = 5.98; g/100 g of fat). The minimum and maximum values were 33.70 and 81.35 g/100 g of fat, respectively.

The data were analyzed with 3 RRTDM. The general matrix notation for these models was

$$\mathbf{y= X\beta+ Q(Za+Zp+Wh) + e,}$$

where **y** was the vector of observations (SFA content in milk, g/100 g of fat), **β** was the vector of fixed effects (herd × test day, stage of lactation: 20 classes of DIM, age at calving: 20 classes); **a** was the vector of additive genetic animal effect; **p** was the vector of permanent environment random effect; **h** was the vector of herd-year of calving. The **Q** matrix, which was the matrix of regressors, was different for the 3 models studied containing LP of order 2 (model LP), linear splines with 10 knots (model SP10) or linear splines reduced to 3 transformed variables (model SP3); **X**, **Z**, and **W** were incidence matrices; and **e** was the vector of random residual effects.

In the SP10 model, 10 knots were equally spaced on the lactation curve (interval of 40 DIM). The chosen knots [$\mathbf{T}_{(i)}$] were 5, 45, 85, 125, 165, 205, 285, 325, and 365 DIM. Coefficients of linear splines were calculated as the interpolation coefficient between 2 adjacent knots as (Misztal, 2006):

$$\text{if DIM} = T_{(i)}, \text{then } \phi_{(i)} = 1,$$

$$\text{if DIM is between } T_{(i)} \text{and } T_{(i+1)}, \text{ then } \phi_{(i)} = \left(\frac{T_{(i+1)} - DIM}{T_{(i+1)} - T_{(i)}}\right) = \alpha, \text{ and } \phi_{(i+1)} = 1 - \alpha,$$

where $\phi_{(i)}$was the i th covariate at DIM t, and $T_{(i)}$was the i th knot.

With linear spline coefficients, all $\phi_{(i)}$ are equal to zero except when DIM is between $T_{(i)}$ and $T_{(i+1)}$. Therefore, $\phi_{(i)}$ vector had, at most, 2 nonzero elements, and the sum of all elements was equal to 1.

Computational requirements for the SP10 model were obviously very high. To reduce the complexity of that model, it was reduced toward the SP3 model based on the reduction of

the 10 knots applied to only 3 variables. To reduce the number of parameters, the eigenvectors of the obtained covariance matrices were calculated. In preliminary studies, it was shown that the first 3 eigenvectors of the genetic, permanent environment, and herd × year of calving (co)variance components were the 3 most important. This fact is in accordance with results reported by Druet et al. (2003) and Torres and Quaas (2001). The method applied to do the rank reduction was based on the elimination of dimensions with very small eigenvalues. In this study, the 3 retained eigenvalues explained 99.3% of the genetic variability. The different steps were as follows:

Step 1. Let **G**, **H**, and **P** be the 10 × 10 matrix of (co)variance components between the 10 genetic, herd × year of calving, and permanent environmental linear splines, respectively. These matrices were summed to a matrix of phenotypic (co)variance components (**S**) representing the (co)variances among the regressors.

Step 2. Create a 10 × 10 matrix **R** containing $\phi_{(i)}$ values for DIM included between DIM 5 and DIM 365. The dimensions of this matrix were (361, 10).

Step 3. Compute the (co)variance matrix **V** among the 361 test-days:

$$\mathbf{V= RSR'.}$$

Step 4. Compute the eigenvalues and eigenvectors of **V** matrix. Let the matrix of eigenvectors be $\mathbf{V_{sp}}$ and that of the eigenvalues $\mathbf{D_{sp}}$; $\mathbf{V_{sp}}$ is a 10-rank matrix.

Step 5. Create $\mathbf{V_{sp\_red}}$ by choosing the 3 dimensions with highest eigenvalues.

Step 6. Re-estimate new matrices $\mathbf{G_{red}}$, $\mathbf{H_{red}}$, and $\mathbf{P_{red}}$ for these new regressions based on $\mathbf{V_{sp\_red}}$. These matrices are 3-rank matrices.

For the 3 models, the genetic parameters for SFA were estimated by REML (Misztal, 2007). Average heritability values as the ratio of genetic variance (σ_G^2) to the sum of variances obtained for the genetic effect (σ_G^2), the herd × year of calving (σ_H^2), the permanent environment (σ_P^2), and the residual effect ($\sigma_{residuals}^2$) for DIM for SFA were defined as follows:

$$\text{Heritability} = \frac{(\sigma_G^2)}{(\sigma_G^2 + (\sigma_H^2) + (\sigma_P^2) + (\sigma_{residuals}^2)}$$

The choice of optimal RRTDM was based on statistical criteria. Akaike's information criterion (**AIC**), developed by Akaike (1973), is a measure of the goodness of fit of an estimated statistical model. This criterion is widely used in statistics for comparing models (e.g., Druet et al. 2003). Akaike proposed a simple and useful criterion for selecting the best-fit model among alternative model. In the general case, the AIC presents the following form:

$$AIC = 2k - 2\ln(L),$$

Where k is the number of parameters in the statistical model, and L is the maximized value of the likelihood function for the estimated model. The model with the lower AIC, and thus with the highest value [ln(L)] for the number of parameters corrected log-likelihood, is considered as the best. The models were also compared by Bayesian information criterion (**BIC**) values. Several competing models may be ranked according to their BIC values, with the one having the lowest BIC being the best. In statistics, BIC is very closely related to AIC. However, the penalty for additional parameters is stronger than that of the AIC. The BIC is a criterion for model selection among a class of parametric models with different numbers of parameters. It was calculated as

$$BIC = -2\ln(L) + k\ln(\lambda),$$

Where *L* is the maximized value of the likelihood function for the estimated model, *k* is the number of variance components estimated, and λ is the number of samples. The interpretation of BIC is analogous to AIC. Both correct for the number of parameters, but BIC also corrects for samples. Despite this correction, the basis of minus twice the logarithm of the likelihood (**−2logL**) is another useful measure to evaluate the fitness of models. In statistics, the

likelihood function is a function of the parameters of a statistical model that plays a key role in statistical inference:

Log-likelihood function = −2log (L),

where *L* is the maximized value of the likelihood function for the estimated model. Models with the lowest Log-likelihood function and therefore the highest log (*L*) are the best.

The last criterion is for the analysis of residuals. The percentage square biases (**PSB**; Ali and Schaeffer, 1987) was computed as

$$PSB = \frac{\sum_{r=1}^{n}(yr-xr)^2}{\sum_{r=1}^{n}(yr)^2} \text{x } 100,$$

where y_r was the *r*th observed record, x_r was the *r*th predicted record, and *n* was the number of records. The model with the lowest PSB is the best one. The models were also compared according to EBV. Sires with more than 10 daughters were ranked according to their EBV. Spearman rank correlation coefficients were computed for all models to assess the similarity (or lack thereof) between sire rankings obtained with the different applied models.

Table 1 reports model selection criteria. The AIC of the SP10 model was 23 and 28% higher than that for the SP3 and the LP models, respectively. The BIC of that model was also 28% higher than the BIC of LP model. In general, the AIC and the BIC values of SP3 model were closer to the values of LP model (AIC and BIC of LP were only 7 and 3% lower, respectively, than their corresponding values for the SP3 model). This was expected because the SP3 model had the same number of parameters as the LP model. The third criterion was the basis of −2logL. Similar observations were realized for the log-likelihood function. The obtained value for the SP10 model was approximately 28% higher than those obtained for the SP3 and LP models. These last 2 models were very close (less than 1% of difference). The PSB criterion evaluated the 3 models differently. Indeed, the lowest value of PSB was obtained for the SP10 model. This was

expected because fitting 10 knots created a better fit, but at the expense of many more parameters. In general, the comparison of models based on AIC, BIC, and log-likelihood function favored the LP model.

Trends of estimated genetic (co)variances among the first lactation for SP3, SP10, and LP models are shown in Figure 2. The overall obtained shape of the variance functions was similar among models with higher variances at the beginning. The model SP10 and, to a lesser extent, model SP3 showed very high variances at the beginning of the lactation. All models showed a tendency to increase at the end of the lactation, with LP reaching levels comparable to those at the beginning. As expected from the way linear splines are defined for the SP10 model, some parabolic shapes were observed in the genetic variance function. According to Bohmanova et al. (2008), this parabolic shape is specific to linear splines and does not translate to a biological mechanism. Results for SP10 were most likely influenced by the way knots were distributed throughout the lactation. In this study, knots were equally spaced on the lactation curve (intervals of 40 DIM) and no efforts were made to optimize their location. As shown in the literature, the general shape of a spline variance function is usually influenced by the number of knots (e.g., Bohmanova et al., 2008). It can be also noticed that the SP3 shape is smoother than the SP10 shape. This seems to be logical because the SP3 model was obtained by reducing 10 knots (SP10 model) to 3 regressions. As reported earlier, the genetic variance for LP model showed a U shape, despite the fact that a herd × year of calving effect was introduced in the model. For production traits, Gengler and Wiggans (2001) had shown that the inclusion of this effect could better catch variance at the beginning and at the end of the lactation. Unreasonably high variances at the beginning of the lactation were also reported in several studies, such as by Bohmanova et al. (2008) for production traits and by Soyeurt et al. (2008) for milk quality traits.

Figure 3 depicts the change of heritability values throughout the lactation. Large changes of heritability values for SFA in fat were observed throughout the first lactation. The SP10 model

on one hand and the LP and SP3 models on the other hand showed different patterns throughout the first lactation. The shape followed by the SP10 model was very different from the shape obtained for the 2 remaining models. The trend of genetic variance obtained for the SP10 model was much smoother than the obtained trend of heritability. The irregularity of the trend of heritability could be explained by the permanent environmental component (not shown).

Table 1. Estimates of Akaike's information criterion (AIC), Bayesian information criterion (BIC), −2 log-likelihood, and percentage of squared bias (PSB)

Item	SP10	SP3	LP
AIC	87.415	67.463	62.916
BIC	88.339	80.228	63.139
-2Log(L)	87.083	62.878	62.836
PSB	432.76	642.139	619.83

[1]SP10 = splines with 10 knots; SP3 = linear splines with the same 10 knots reduced to 3 parameters; LP = Legendre polynomials.

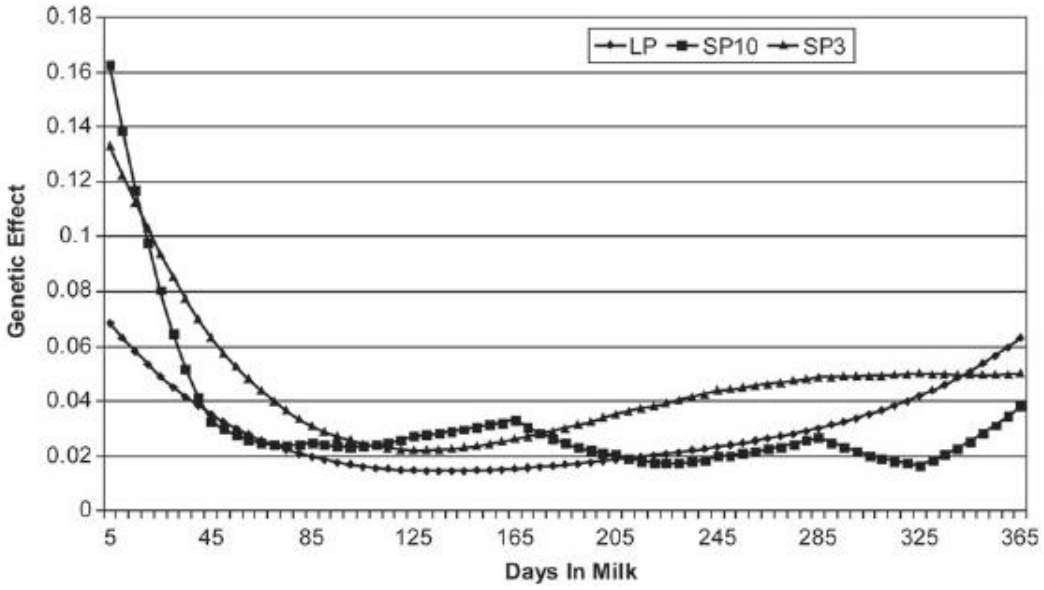

Figure 2. Variation of estimated genetic (co)variance over the first lactation. LP = Legendre polynomials; SP10 = splines with 10 knots; and SP3 = linear splines with the same 10 knots reduced to 3 parameters.

For LP and SP3 models, the highest value of heritability was observed at the beginning of the lactation (0.33). The minimum was close to 0.12 and was found around 140 DIM for the LP model. For the SP3 model, the lowest value was observed at the end and around 140 DIM of lactation and was close to 0.11. This observation could be due to the changes of energy status of the cow throughout the lactation. Energy balance is known to be negative at the beginning of the lactation, causing mobilization of adipose fatty acids. This could explain why the genetic part is higher in the early stage of lactation (Stoop et al., 2009). At the end of lactation, the LP and SP3 models present different shapes. Heritability estimates by the LP model increased at the end of the first lactation compared with estimates by SP3 model, which decreased.

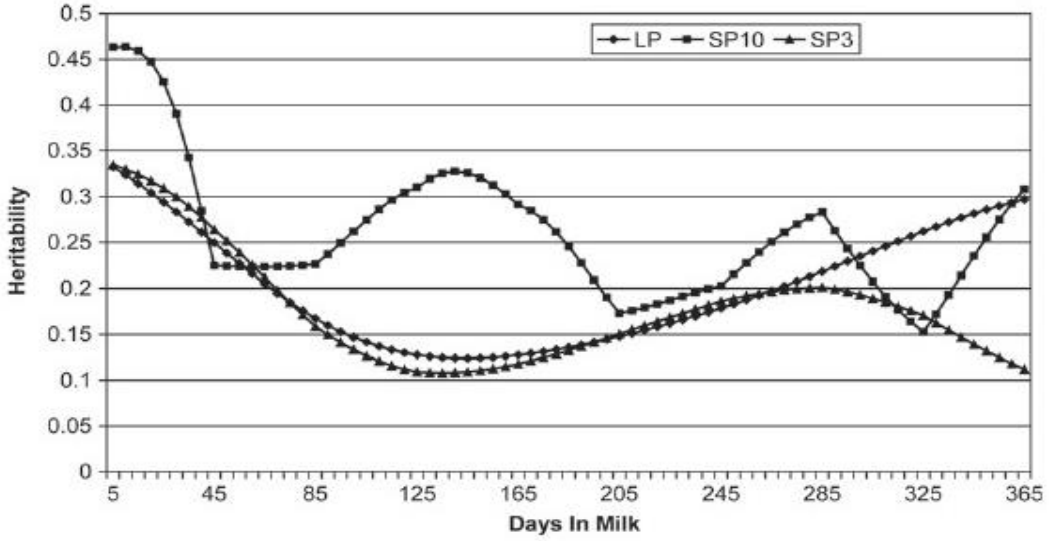

Figure 3. Variation of heritability values over the first lactation. LP = Legendre polynomials; SP10 = splines with 10 knots; and SP3 =linear splines with the same 10 knots reduced to 3 parameters.

Average heritability estimates throughout the lactation were 0.12, 0.16, and 0.20 for LP, SP3, and SP10 models respectively. Heritability for SFA obtained in this study was similar to the value of 0.24 reported by Soyeurt et al. (2008), who used LP with the same polynomials as in the current study. According to a literature review done by Arnould and Soyeurt (2009), the estimates of heritability for milk quality traits differ very much among the studies. Also, few authors have reported heritability values of the milk quality trait defined here (SFA expressed in

fat, g/100 g of fat). The ranking of animals for EBV using SP10, SP3, and LP did not change very much, which supports the use of simpler models such as SP3 and LP with reduced number of parameters.

This study aimed to compare different models to study genetic parameters of milk SFA production. The presented models gave similar overall shape of the genetic variance function. However, the trend of heritability was very different between models. The AIC, BIC, and log-likelihood function identified the LP and SP3 models as the most useful models. Model SP10 was the worst model for each function. Indeed, all function estimates were less favorable for this model. Using 10 splines (regressions) was also very computationally demanding. Indeed, LP and SP3 models needed the least time to converge (2 h 50 min and 17 h 10 min, respectively) and had the lowest number of rounds (1,153 and 978). Convergence rate of SP10 model was much slower (more than 1 month and more than 5,000 rounds).

Finally, SP10, SP3, and LP did not differ in the ranking of sires with respect to Spearman rank correlation. Hence, the 3 methods showed the same ability to rank sires based on their EBV.

Based on results from this study, the reduced SP3 model was very similar to the LP model. Except for the PSB value that was lowest for SP10 model (indicating that this model had the best fit), the AIC, BIC and, −2log(L) ranked the models in the same way. However, SP3 was not found to be superior to LP. These 2 models require the same limited number of parameters. Results of this study indicated, therefore, that LP was the best among the compared models. Therefore, it can be expected that LP-based models could be used to model production of SFA in fat. However, our results for SP10 were most likely influenced by the way knots were distributed throughout the lactation. Recently, Jamrozik et al. (2010) studied the selection of locations of knots for linear splines in RRTDM. They concluded that optimal locations of knots (for linear splines) could vary according to the studied population, lactation, and trait and

according to the random effects. As work with new traits is in progress, additional research on this topic is required.

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CHAPTER VI.

SHORT COMMUNICATION: EFFECTS OF ALTERNATIVE MILK RECORDING SCHEMES ON THE GENETIC PARAMETERS OF MILK FATTY ACIDS.

From: Arnould V. M.R., Reding R., Bormann J., Gengler N., and H. Soyeurt. 20XX. Short communication: Effects of alternative milk recording schemes on the genetic parameters of milk fatty acids. (In Review)

6.1. Abstract

For quality control and monitoring purposes in milk production, proportionate samples are typically collected from morning (AM) and evening (PM) milkings. Although the International Committee for Animal Recording (ICAR) guidelines allow a single sample to be collected from either the morning or evening milkings, the composition of AM- and PM-collected milk can differ. Thus, the records generated by such sampling schemes could affect differentially genetic traits, potentially impacting animal rankings. Here, we tested whether milking time (AM or PM) interacts with genetic parameters and whether correlations exist among AM and PM milk yields and fatty traits (fat content and saturated, monounsaturated, unsaturated, short chain, medium chain, and long chain fatty acids (FA)). Using an AM/PM alternating sampling scheme, a total of 58,540 test-day records were collected from Holstein cows. Using a bivariate modeling approach, we identified relatively larger phenotypic differences for unsaturated fatty acids (FAs) than for saturated FAs (7% vs. 2%, respectively). Daily h^2 estimated from AM records for unsaturated FAs were always higher (0.24 on average) than those estimated from PM records (~0.01). Greatest AM/PM differences were observed for saturated FAs (0.04). Daily estimates of the genetic correlations between the AM and PM records ranged from 0.93 to 0.94. Small relative genetic variance differences were detected between AM and PM unsaturated FAs (on average 2%: lower AM genetic variance). The relative genetic variance differences were 3-fold larger for saturated than unsaturated FA traits (mean 8%, lower PM genetic variance). However, these FA differences were much smaller than those detected for milk yield (28%). Taken together, our results suggest that milking time has a limited impact on the estimation of genetic parameters of FA contents. Therefore, we propose that either AM or PM samples could be used reliably in evaluation models for genetic purposes.

Keywords: milk, fatty acid, genetic parameter

Fatty acid (FA) composition affects the nutritional quality of milk and the technological properties of butter (Couvreur et al., 2006; Soyeurt et al. 2007a). Moreover, knowledge of any

changes in FA content can be useful for farm management purposes (Arnould et al., 2013). Previous studies have investigated the genetic variability of milk FA composition (Arnould and Soyeurt 2009; Karijord et al. 1982; Soyeurt et al. 2007a, 2007b and 2008; Stoop et al. 2008; Bastin et al. 2011). The daily heritability (h^2) values of saturated and monounsaturated FAs estimated by Soyeurt et al. (2008) (0.14 and 0.42 g/dL of milk respectively) suggest that considerable genetic progress is possible for these traits. Precise trait definitions are required during breeding program development. Therefore, milk samples intended for such applications should be collected using comparable sampling methods. However, there has been a recent global trend for milk recording organizations to increase the flexibility of their allowed sampling procedures. For instance, CONVIS s.c. (Ettelbruck, Luxembourg) allows three alternative milk sampling schemes: 1) the conventional approach, where in milk samples are collected from all milked cows during consecutive evening (PM) and morning (AM) milkings (Scheme S); 2) consistent collections being made during only PM or AM milking (Scheme M); or 3) collections being made in PM and AM milkings in alternating months (Scheme T). Such alternative milking schemes can be less disruptive and more cost-effective (Everett and Wadell, 1970) than the conventional scheme. However, Liu et al. (2000) have reported that the use of alternative sampling schemes introduces heterogeneity into studied traits (e.g., milk yield, fat and protein content). AM and PM milks differ in their composition (Forsbäck et al., 2010; Liu et al. 2000). However, the influence of AM/PM milk FA composition on the estimation of genetic parameters of FA traits has yet to be investigated. Moreover, it is unclear whether the observed differences between AM and PM milks also have genetic components, which could confound current approaches. Here, we aimed to compare the AM and PM genetic parameters estimated for milk yield, fat content, and the main FA groups in bovine milk (g/dL of milk) from primiparous Holstein cows and to estimate correlations among these traits.

Data. Milk samples were collected in Luxembourg from 13,854 first parity Holstein cows belonging to 492 herds during routine milk recording between October 2007 and February 2011 (Scheme T). These samples, composed of AM- and PM-collected milk, were analyzed by mid-

infrared spectrometry using a Foss MilkoScanFT6000 (Hillerod, Denmark) at CONVIS s.c. (Ettelbruck, Luxembourg). Milk fat content was predicted by the MIR equation provided by the manufacturer. The milk saturated FA (SFA), monounsaturated (MUFA), unsaturated (UFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA) FA contents (g/dL of milk) were predicted by applying the updated equations of Soyeurt et al. (2011) to the recorded spectra (Grelet et al., 2014). Given the current lack of International Committee for Animal Recording (ICAR) norms for FA traits, records were considered to be outliers and discarded if their values were lower or higher than the mean ± 3 times the observed standard deviation. The final dataset contained up to 29,936 AM test-day records and up to 28,604 PM test-day records.

Model. Inspired by similar models (e.g., Croquet et al., 2006), we applied a bivariate random regression test-day model (i.e., AM and PM records were considered different traits) as follows:

$$\mathbf{y=X\beta+Q(Z_1p+Z_2a)+e}$$

where **y** is the vector of observations for one of the studied traits [AM and PM milk (kg/milking), FAT, SFA, MUFA, UFA, SCFA, MCFA, and LCFA(g/dL of milk)]; **β** is the vector of the following fixed effects: herd × test-date, age at calving (3 classes: <29, 29–32 and >32 months-old), and lactation stage [24 classes of 15 days in milk (DIM)]; **p** is the vector of permanent environmental random effects; **a** is the vector of genetic animal effects; **e** is the vector of residuals; **X**, $\mathbf{Z_1}$, and $\mathbf{Z_2}$ are incidence matrices assigning observations to effects; and **Q** is the covariate matrix for second-order Legendre polynomials. The estimation of variance components was first performed using the Expectation Maximization-Restricted Maximum Likelihood algorithm (EM-REML), as implemented in the REML software by Misztal (2012). The Average Information-REML (AI-RELM) algorithm, as implemented by Misztal (2012), was used to obtain the standard errors of variance components, taking the results obtained by EM-REML as priors. This strategy combined stable estimations from EM-REML with the availability of the Hessian matrix from AI-REML, allowing the derivation of standard errors of variance components. Daily h^2, defined as

the ratio of the genetic variance to the sum of genetic, permanent environment and residual variances, was estimated for each DIM comprised between n 5 and 365. The average daily h^2 used in this study was computed as the average of all daily h^2 values. Genetic and permanent environmental correlations were estimated using the obtained co-variance components. The standard error of h^2 (se_{h^2}) and correlation (se_{rr}) were estimated as proposed by Klei and Tsuruta (2008).

Descriptive statistics. The skewness and kurtosis values were close to zero for all studied traits (Table 1), therefore the data can be assumed to be normally distributed. We detected differences in the means of the measured values between AM and PM records. AM milk yield was higher than PM milk yield (Table 1), which is in line with the findings of Everett and Wadell (1970), Gilbert et al. (1972), and Quist et al. (2008). Fat content was marginally higher in PM than in AM milk samples, which was also described by Quist et al. (2008) and Gilbert et al. (1972). Milk FA content was also lower in AM than in PM milk samples, as noted by Arnould et al. (2015). We detected greater AM/PM variation in UFA, MUFA, and LCFA than unsaturated FAs (Table 1). FAs in bovine milk are thought to be either produced *de novo* in the mammary gland or derived from the diet and body fat mobilization. Generally, 4:0 to 14:0 and some 16:0 FAs are produced *de novo* (Grummer, 1991). These *de novo* synthesized FAs (e.g., SFA, SCFA, and MCFA) had the lowest relative differences (2.1, 2.6, and 1.4% respectively), whereas UFA (7.7%), MUFA (7.3%) and LCFA (7.0%) had the highest AM/PM differences. These observations suggest that *de novo* synthesized FAs are under stronger genetic control than those produced from plasma lipids (Bastin et al., 2011; Grummer, 1991) and that they, therefore, have lower AM/PM variability.

The FA content heritabilities calculated here for Luxembourg dairy cattle (Table 2) were similar to those reported previously for Walloon cattle (Bastin et al., 2011). The average daily heritabilities for milk were 0.23 (AM) and 0.20 (PM) and those for fat were 0.30 (AM) and 0.32 (PM). Our estimates indicated that the FA groups (SFA, SCFA, and MCFA) were more heritable than LCFA and UFA, with heritabilities of 0.31 (AM) and 0.35 (PM) for SFA, 0.31 (AM) and 0.35

(PM) for SCFA, 0.32 (AM) and 0.36 (PM) for MCFA, 0.23 (AM) and 0.22 (PM) for LCFA, and 0.24 (AM) and 0.23 (PM) for UFA. Heritability estimates decreased with FA chain length, as described by Bastin et al. (2011). Indeed, heritability values reflect the physiological processes involved in the production of milk FAs. Thus, heritability values can be interpreted biologically. The h^2 values observed here could be explained by the similarities in their origin of production. FAs with high h^2 (SFA and SCFA) are synthesized *de novo* in the mammary gland, whereas the FAs with lower h^2 values (essentially long carbon chains) are synthesized from the blood, indirectly from feeding. MCFAs are partially extracted from the blood and partially synthesized *de novo* by the mammary gland (Chilliard et al., 2001). A further distinction can be made between saturated and unsaturated FA contents. Compared to saturated FA contents (SFA, SCFA, and MCFA), h^2 estimated for unsaturated FAs from AM milk samples were slightly higher than those estimated from PM milks. Although by taking into account the estimated standard errors, no statistically significant differences were observed between the daily AM and PM h^2 values for saturated and unsaturated fats, mean differences between the AM and PM h^2 values were higher for saturated FAs (0.04) than for unsaturated FAs (0.01–0.02).

Table 1. Descriptive statistics of data during morning (AM) milking and evening (PM) milking for milk yield and the content in milk (g/dL of milk) of fat, saturated (SFA), monounsaturated (MUFA), unsaturated (UFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA) fatty acids milk contents.

		N	Mean	SD	Min.	Max.	Skewness	Kurtosis
Milk	AM	30,092	11.5	3.14	2.0	26.2	0.11	-0.002
(kg/milking)	PM	28,768	10.2	2.95	2.0	34.0	0.23	0.24
	Relative absolute differences	4.5%	12.0%	6.2%	0.0%	25.9%		
FAT	AM	29,936	4.23	0.76	1.65	7.19	0.41	0.57
(g/dL milk)	PM	28,604	4.4	0.77	1.64	7.17	0.38	0.46
	Relative absolute differences	4.6%	3.9%	1.3%	0.6%	0.3%		
SFA	AM	29,955	2.82	0.59	0.85	4.93	0.36	0.3
(g/dL milk)	PM	28,621	2.88	0.60	0.86	4.93	0.33	0.2
	Relative absolute differences	4.6%	2.1%	1.7%	1.2%	0.0%		
UFA	AM	29,958	1.38	0.27	0.44	2.59	0.76	1.54
(g/dL milk)	PM	28,548	1.49	0.28	0.93	2.60	0.67	0.92
	Relative absolute differences	4.8%	7.7%	3.6%	71.5%	0.4%		
MUFA (g/dL milk)	AM	29,956	1.19	0.24	0.30	2.28	0.8	1.65
	PM	28,542	1.28	0.25	0.31	2.28	0.7	0.98
	Relative absolute differences	4.8%	7.3%	4.1%	3.3%	0.0%		
SCFA	AM	29,965	0.38	0.08	0.11	0.67	0.33	0.37
(g/dL milk)	PM	28,630	0.39	0.08	0.11	0.67	0.31	0.25
	Relative absolute differences	4.6%	2.6%	0.0%	0.0%	0.0%		
MCFA	AM	29,994	2.20	0.50	0.58	3.95	0.31	0.16
(g/dL milk)	PM	28,662	2.23	0.50	0.59	3.95	0.29	0.06
	Relative absolute differences	4.5%	1.4%	0.0%	1.7%	0.0%		
LCFA	AM	29,948	1.65	0.35	0.39	3.18	0.72	1.26
(g/dL milk)	PM	28,529	1.77	0.37	0.36	3.18	0.65	0.76
	Relative absolute differences	4.9%	7.0%	5.6%	8.0%	0.0%		

Daily genetic parameters. The average daily genetic correlations estimated between the AM and PM studied traits were strong (Table 2), ranging from 0.90 to 0.96. The highest genetic correlation was observed for milk yield. The genetic correlation observed between AM and PM FA traits were globally similar and always ≥ 0.93. Compared to h^2, no difference in terms of genetic correlations was observed between saturated and unsaturated FA contents in milk.

The average daily correlations between AM and PM FA traits for permanent environmental effect were significantly lower than their corresponding genetic correlations (Table 2), suggesting a moderate difference in the non-genetic factor between AM and PM FA traits. This difference could be accounted for by the biological processes involved in the production of milk FAs. Indeed, milk FA composition is influenced by a range of conditions, including feeding regime (Larsen et al., 2012). Lower values of average daily correlation between AM and PM samples for permanent environmental effects could be explained by a feeding effect. Indeed, according to Sahana et al. (2008), cows consuming the major part of their feed between the AM and PM milking and not being fed for the last 8 hours before morning milking, present a higher fat content and a higher content of C18 unsaturated FA in the afternoon milk compared with the morning milk, whereas the content of *de novo* synthesized FA C6:0 to C16:0 was reported to be lower. Based on our results, our hypothesis is that the underlying genetic effect does not vary strongly throughout the day. Conversely, the non-genetic (permanent environment) effect did vary between AM and PM FA traits, though the magnitude of this variation was only moderate.

Table 2. Average daily heritability and genetic and permanent environmental correlations (and their corresponding standard error) for each studied trait (milk (g/dL of milk) of fat, saturated (SFA), monounsaturated (MUFA), unsaturated (UFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA) fatty acids milk contents).

		Average daily estimate		Average daily correlation between AM and PM	
Trait		**h^2 AM**	**h^2 PM**	**Genetic**	**Permanent environment**
Milk (kg/milking)	values	0.23	0.20	0.96	0.97
	SE	*0.02*	*0.02*	*0.08*	*0.04*
Fat (g/dL milk)	values	0.30	0.32	0.90	0.78
	SE	*0.04*	*0.04*	*0.11*	*0.11*
SFA (g/dL milk)	values	0.31	0.35	0.93	0.84
	SE	*0.04*	*0.04*	*0.11*	*0.11*
UFA (g/dL milk)	values	0.24	0.23	0.94	0.75
	SE	*0.02*	*0.01*	*0.06*	*0.06*
MUFA(g/dL milk)	values	0.24	0.22	0.94	0.75
	SE	*0.02*	*0.01*	*0.07*	*0.06*
SCFA (g/dL milk)	values	0.31	0.35	0.94	0.84
	SE	*0.02*	*0.02*	*0.06*	*0.06*
MCFA (g/dL milk)	values	0.32	0.36	0.94	0.86
	SE	*0.02*	*0.02*	*0.05*	*0.06*
LCFA (g/dL milk)	values	0.23	0.22	0.94	0.77
	SE	*0.02*	*0.01*	*0.06*	*0.06*

Table 3 shows the average estimates and average standard errors of variance for permanent environmental and genetic effects for each studied trait, as well as the minimal and maximal AM/PM variance estimates. Notable value ranges were observed for each studied trait, regardless of the sampling time. Table 3 also shows the variance values estimated for

permanent environmental effects. The mean daily estimates were higher for AM estimates than for PM estimates: milk yield [244.7 (AM) vs.191.8 (PM)*10 kg²/milking²]; FAT [1225.8 (AM) vs. 1156.4 (PM)*100 g²/dL milk²]; SFA [683.7 (AM) vs. 595.8 (PM)*100 g²/dL milk²]; SCFA [13.9 (AM)vs. 12.2 (PM)*100 g²/dL milk²];and MCFA [415.4(AM) vs. 370.9 (PM)*100 g²/dL milk²]. The opposite trend was observed for the unsaturated FA contents, which also showed the highest relative differences between the AM and PM daily estimates (+19% vs. -12% on average for unsaturated and saturated FAs, respectively). The range of variation was also greater for unsaturated FA contents. By comparison, the observed relative difference of the daily permanent environmental variances for milk yield was 22%.

Compared to permanent environmental variances (Table 3), lower differences were observed between the AM and PM genetic estimated variances (Table 3, +2% and -8% for unsaturated and saturated FA contents respectively). At the genetic level, the daily variance estimates for unsaturated FA traits were greater for AM milk than for PM milk. Excepted for milk yield, this pattern was opposite to the situation observed for permanent environmental variance estimates. This inverse tendency was also observed for all studied saturated FA traits.

Table 3. Average estimate and average standard error of variance for genetic and permanent environmental effect for each studied trait (milk (g/dL of milk) of fat, saturated (SFA), monounsaturated (MUFA), unsaturated (UFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA) fatty acids milk contents).

		Genetic						Permanent environment					
		AM			PM			AM			PM		
Trait		**Value**	**Min**	**Max**	**Value**	***Min***	***Max***	**Value**	***Min***	***Max***	**Value**	***Min***	***Max***
Milk (*10 kg²/milking²)	Value	111.5	88.0	166.2	80.4	61.3	125.2	244.7	*189.8*	538.0	191.8	141.9	421.2
	SE	*10.0*			*7.4*			*10.6*			*8.3*		
FAT(*100 g²/dL milk²)	Value	1274.9	736.3	1815.8	1364.4	721.4	1976.2	1225.8	821.7	3723.3	1156.4	716.6	4055.2
	SE	183.7			183.9			*168.9*			*169.6*		
SFA (*100 g²/dL milk²)	Value	763.6	402.2	1103.8	821.8	479.9	1084.7	683.7	544.5	1475.4	595.8	464.9	1244.4
	SE	102.7			103.1			*93.7*			*93.4*		
UFA (*100 g²/dL milk²)	Value	122.3	75.8	313.1	119.4	77.1	224.5	148.0	46.20	810.5	178.2	48.7	1049.6
	SE	9.2			8.5			*9.7*			*9.9*		
MUFA (*100 g²/dL milk²)	Value	95.5	57.3	264.4	93.2	58.8	194.8	123.1	37.7	681.0	145.3	40.29	854.5
	SE	7.7			7.2			*8.2*			*8.3*		
SCFA (*100 g²/dL milk²)	Value	14.5	6.7	23.6	16.0	9.9	22.4	13.9	10.8	30.8	12.2	9.1	28.7
	SE	0.9			1.0			*0.9*			*0.9*		
MCFA (*100 g²/dL milk²)	Value	492.1	271.6	679.6	527.2	315.9	683.8	415.4	329.4	917.1	370.9	285.7	764.7
	SE	28.9			29.9			*28.0*			*26.4*		
LCFA (*100 g²/dL milk²)	Value	188.8	122.4	457.2	185.0	121.9	325.1	236.5	86.0	1195.0	280.6	90.4	1554.2
	SE	14.4			13.6			*15.7*			*16.1*		

6.2. Conclusions

From the obtained results, different conclusions and consequences for genetic evaluations can be drawn. First, unsaturated FAs displayed 1) the largest phenotypic differences between values for AM and PM FA records; 2) a range of differences between AM and PM daily permanent environmental variances similar to the range observed for milk yield and greater than the range observed for saturated FA traits; and coupled with a low permanent environmental correlation, and 3) slight genetic variance differences coupled with a high genetic correlation. Saturated FA traits showed 1) a limited phenotypic differences between AM and PM FA records (>2-fold lower than for unsaturated FAs), 2) a small permanent environmental differences (12%) which was almost 2-fold lower than the one observed for milk yield, and 3) a small genetic variance differences (2-fold higher than the one observed for unsaturated FAs but 3-fold lower than the differences observed for milk yield). Currently, when records come from different sampling time points, milk yield records are corrected before genetic evaluations (ICAR, 2014). However, the range of differences observed for all studied fat and FA traits were largely lower than that observed for milk yield. Moreover, by taking into account the standard error, the differences in terms of heritability, correlations and variances were often not statistically significant. Both mean and variance differences can be considered with appropriate modeling. Sampling type in the evaluation model can be included in the applied genetic model with the use of appropriate fixed effects which allow the correction of mean differences. This correction can be done indirectly because the milk sampling at a given herd x test-day is always done AM or PM for all milking cows, or directly by including a sampling moment x sampling type fixed effect in the model. FA trait heteroscedasticity can also be taken into account. Various adjustment strategies for milk yield, as summarized by Gengler et al. (2005), can also be applied to FA traits. However our results showed that heterogeneity of variances of AM/PM records was very different among random effects, therefore adapted corrections should be considered. Gengler et al. (2005) proposed a transformation of random regressions to take into account this problem.

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CHAPTER VII.

PREDICTIONS OF DAILY MILK AND FAT YIELDS, MAJOR GROUPS OF FATTY ACIDS, AND C18:1 CIS-9 FROM SINGLE MILKING DATA WITHOUT A MILKING INTERVAL

From: Arnould V. M.R., Reding R., Bormann J., Gengler N., Bastin C. and H. Soyeurt. 2015. Predictions of daily milk and fat yields, major groups of fatty acids, and C18:1 cis-9 from single milking data without a milking interval. *Animals*, 5: 643-661

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7.1. Simple summary

Reducing the frequency of milk recording decreases the costs of official milk recording. However, this approach can negatively affect the accuracy of predicting daily yields. Equations to predict daily yield from morning or evening data were developed in this study for fatty milk components from traits recorded easily by milk recording organizations. The correlation values ranged from 96.4% to 97.6% (96.9% to 98.3%) when the daily yields were estimated from the morning (evening) milking. The simplicity of the proposed models which do not include the milking interval should facilitate their use by breeding and milk recording organizations.

7.2. Abstract

Reducing the frequency of milk recording would help reduce the costs of official milk recording. However, this approach could also negatively affect the accuracy of predicting daily yields. This problem has been investigated in numerous studies. In addition, published equations take into account milking intervals (MI), and these are often not available and/or are unreliable in practice. The first objective of this study was to propose models in which the MI was replaced by a combination of data easily recorded by dairy farmers. The second objective was to further investigate the fatty acids (FA) present in milk. Equations to predict daily yield from AM or PM data were based on a calibration database containing 79,971 records related to 51 traits [milk yield (expected AM, expected PM, and expected daily); fat content (expected AM, expected PM, and expected daily); fat yield (expected AM, expected PM, and expected daily; g/day); levels of seven different FAs or FA groups (expected AM, expected PM, and expected daily; g/dL milk), and the corresponding FA yields for these seven FA types/groups (expected AM, expected PM, and expected daily; g/day)]. These equations were validated using two distinct external datasets. The results obtained from the proposed models were compared to previously published results for models which included a MI effect. The corresponding correlation values ranged from 96.4% to 97.6% when the daily yields were estimated from the AM milkings

and ranged from 96.9% to 98.3% when the daily yields were estimated from the PM milkings. The simplicity of these proposed models should facilitate their use by breeding and milk recording organizations.

Keywords: milk recording; fatty acid groups; prediction model; single milking

7.3. Introduction

According to Arnould *et al.* [1], milk yield, and, particularly, milk fat composition, may facilitate the development of strategies to prevent and monitor milk production dysfunction in dairy cattle, and may improve the sustainability of dairy production systems. Correspondingly, various milk fatty acids (FA) have shown a relationship with methane production in dairy cattle. For example, positive correlations between saturated FA (SFA) and methane output has been observed (r = 0.87–0.91) [2]. Another example involves ketosis detection. In reports by van Healst *et al.* and Gross *et al.* [3, 4]), a high proportion of long chain FA (LCFA; especially if combined with a lower proportion of medium chain FA (MCFA)), and especially a high proportion of C18:1 cis-9, in milk fat were found to be good predictors of subclinical ketosis. Therefore, a regular quantification of FA in milk is relevant.

Recent studies have demonstrated that mid-infrared spectrometry (MIR) has the potential to quantify the FA content of milk [5-7]). Therefore, the creation of spectral databases represents valuable resources for determining the FA profile of test-day samples collected from lactating cows that are routinely monitored using specific MIR calibration equations. For instance, this is currently realized by the Belgian (Walloon Breeding Association, Ciney, Belgium) and Luxembourg (CONVIS s.c., Ettelbruck, Luxembourg) milk recording organizations. Thanks to the easy acquisition of spectral data, other countries will realize the same work in a near future.

To develop robust management tools, the used phenotypic data should be homogenous. However, the use of different sampling methods can bring heterogeneity. Milk recording

organizations in many countries use more and more often an alternate morning (AM) and evening (PM) testing scheme since it is less expensive than analyzing one milk sample per cow that includes 50% of a representative AM milking fraction and 50% of a representative PM milking fraction. Since the 1970s, numerous equations have been evaluated for their capacity to estimate total daily yields for traditional production traits (*i.e*., milk, fat, and protein) from alternate protocols. For example, Lee and Wardrop [8] studied the effects of milking interval (MI; the duration between two consecutive milkings, expressed in h or min; AM or PM) and stage of lactation on daily milk, fat, and protein yields, and fat and protein content. In 1986, adjustment factors for daily milk, fat, and protein yields were reported [9], and these remain the most widely used factors based on their ability to take into account heterogeneous means and variances between MIs and classes of days in milk (cDIM). In 2000, this model was modified [10], and the changes were approved by the International Committee for Animal Recording [11]. At our knowledge, nothing is done currently about FA. The general aim of this paper is therefore to develop equations to estimate the daily yields of the major FAs present in milk, including SFA, unsaturated FA (UFA), mono-unsaturated FA (MUFA), short-chain FA (SCFA), MCFA, and LCFA from a single milking. In addition, C18:1 *cis*-9 was also studied because this FA is interesting for management purposes [1].

Most of the studies mentioned in the above paragraph included an MI parameter in their predictive models. However, such information might be difficult to collect on a farm since the time and duration of milking is often inconsistent. In a previous report [12], it is mentioned that changes in milk composition can occur according to the MI primarily due to a dilution effect. Thus, a high volume of milk produced during one milking would be predicted to contain less fat and protein compared to a smaller volume of milk. Based on this concept, MIs could affect the levels of detected milk components. Therefore, an additional aim of the present study was to compare the results obtained using the models of Liu [10] and Berry [13] that include an MI effect for milk, fat, and protein yields with the results obtained from models that include only factors related to milk composition and production. Potentially, such models could provide a

straight forward prediction of daily yields for production traits from more readily available information (*i.e.*, fat and protein content and other MIR predicted traits).

7.4. Materials and methods

7.4.1. Available Data

7.4.1.1. Overall strategy

To develop equations which permit the estimation of FA daily yields from one milking, measurements of milk yield and milk composition at each milking are needed, as well as milk composition data from 50% AM and 50% PM milk samples. Unfortunately, separate AM and PM milk samples at the same test day were never collected by the Luxembourg milk recording (CONVIS s.c., Ettelbruck, Luxembourg). Therefore, the innovative part of this study was to create a calibration set including AM and PM expected values estimated using selection index theory from available mixed samples. Then, the equations developed using these expected phenotypes were validated using real data. Indeed, a sampling including AM, PM and mixed milk samples was performed on a limited number of cows and herds in order to create a validation set. More details are given in the following sections.

7.4.1.2. Calibration Data

The calibration dataset included milk samples collected in Luxembourg between October 2007 and April 2013 during routine conventional milk testing (data S). These milk samples were composed of 50% morning milk and 50% evening milk and were collected from 21,582 Holstein cows in 163 herds. All of the milk samples were analyzed by MIR spectrometry using a Foss MilkoScan FT6000 (Hillerod, Denmark) at CONVIS s.c. (Ettelbruck, Luxembourg). MIR analysis of

the milk samples provided spectral data and the quantities of major milk components, including fat and protein content. By applying the updated equations of Soyeurt *et al.* [7], SFA, MUFA, UFA, SCFA, MCFA, LCFA, and C18:1 *cis*-9 content in each milk sample (g/L) were determined. As a result, the ratio of the standard error of cross-validation to the standard deviation (SD) of gas chromatography FA values used in the calibration set (referred to as a RPD parameter) greater than five was observed. Table 1 shows the statistical parameters of the calibration equations used. The data used to build the mid-infrared FA equations were not related to the data used in this study.

Table 1. Estimated statistical parameters for each calibration equation that estimated the concentration of fatty acids (FAs) in milk (g/dL of milk).

FA	N	Mean	SD	SECV	R^2cv	RPD
SFA	1176	2.69	0.79	0.051	0.9958	15.34
MUFA	1180	1.04	0.34	0.047	0.9805	7.18
UFA	1179	1.20	0.39	0.051	0.9828	7.62
SCFA	1185	0.35	0.10	0.020	0.9613	5.10
MCFA	1187	2.06	0.65	0.086	0.9824	7.53
LCFA	1188	1.50	0.52	0.087	0.9718	5.96
C18:1 *cis*-9	1194	0.71	0.26	0.051	0.9610	5.06

FA = fatty acid; SD = standard deviation; SECV = standard error of cross-validation; R^2cv = cross-validation coefficient of determination; RPD = ratio of standard error of cross-validation to standard deviation; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SCFA= short chain fatty acids, MCFA=medium chain fatty acids; LCFA=long chain fatty acids.

Records were discarded from the dataset if test-day records were lower or higher than mean ± three times the observed SD. Furthermore, only spectral data with known production factors such as DIM, and parity were kept. After these edits, the final calibration dataset contained 79,971 records.

Data from the S milk recording scheme included observed $FAT_{50/50}$, $SFA_{50/50}$, $MUFA_{50/50}$, $UFA_{50/50}$, $SCFA_{50/50}$, $MCFA_{50/50}$, $LCFA_{50/50}$, and C18:1 *cis-9* $_{50/50}$. This dataset also contained AM, PM, and 24h milk yields. However, this dataset did not contain records for milk composition related to AM or PM milking. Therefore, a method similar to that of the selection index theory was used to calculate expected values for: $SFA_{AM,}$ SFA_{PM}, $MUFA_{AM,}$ $MUFA_{PM}$, UFA_{AM}, UFA_{PM}, $SCFA_{AM}$, $SCFA_{PM}$, $MCFA_{AM}$, $MCFA_{PM}$, $LCFA_{AM}$, $LCFA_{PM}$, and C18:1 *cis-9*$_{AM}$, C181 *cis-9*$_{PM}$. This method was based on a linear combination of phenotypic data and the following two equations:

$$observed\_trait_{50/50} = 0.5 \times value_{AM} + 0.5 \times value_{PM} \quad (1)$$

$$\exp ected\_trait_{\text{AM or PM}} = f(milk\_yield_{\text{AM or PM}}, fat\_yield_{\text{AM or PM}}) \quad (2)$$

Equation (1) assumes that the milk samples contained 50% AM milk and 50% PM milk. In addition, a non-zero correlation between milk fat composition during the AM or PM milking and the milk and fat yields during the same milking were also assumed (Equation (2)).

Equations (1) and (2) can then be combined to generate Equation (3):

$$\begin{bmatrix} observed\_trait_{50/50} \\ \exp ected\_trait_{AM} \\ \exp ected\_trait_{PM} \end{bmatrix} = \begin{bmatrix} 0.5 & 0.5 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} value_{AM} \\ value_{PM} \end{bmatrix}$$

$$\begin{bmatrix} 0.5 & 1 & 0 \\ 0.5 & 0 & 1 \end{bmatrix} \begin{bmatrix} 0.5 & 0.5 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} value_{AM} \\ value_{PM} \end{bmatrix} = \begin{bmatrix} 0.5 & 1 & 0 \\ 0.5 & 0 & 1 \end{bmatrix} \begin{bmatrix} observed\_trait_{50/50} \\ \exp ected\_trait_{AM} \\ \exp ected\_trait_{PM} \end{bmatrix}$$

$$\begin{bmatrix} value_{AM} \\ value_{PM} \end{bmatrix} = \begin{bmatrix} 1.25 & 0.25 \\ 0.25 & 1.25 \end{bmatrix}^{-1} \begin{bmatrix} 0.5 & 1 & 0 \\ 0.5 & 0 & 1 \end{bmatrix} \begin{bmatrix} observed\_trait_{50/50} \\ \exp ected\_trait_{AM} \\ \exp ected\_trait_{PM} \end{bmatrix} \quad (3)$$

$$\begin{bmatrix} value_{AM} \\ value_{PM} \end{bmatrix} = \frac{1}{3} \begin{bmatrix} 1 & 2.5 & -0.5 \\ 1 & -0.5 & 2.5 \end{bmatrix} \begin{bmatrix} observed\_trait_{50/50} \\ \exp ected\_trait_{AM} \\ \exp ected\_trait_{PM} \end{bmatrix}$$

$$i = \mathbf{A} \begin{bmatrix} observed\_trait_{50/50} \\ \exp ected\_trait_{AM} \\ \exp ected\_trait_{PM} \end{bmatrix} \text{ with } \mathbf{A} = \frac{1}{3} \begin{bmatrix} 1 & 2.5 & -0.5 \\ 1 & -0.5 & 2.5 \end{bmatrix}$$

where **i** is the vector that contains the AM and PM values that will be used to build the equations to predict daily yield from AM or PM data for the trait considered and **A** is the matrix containing the coefficients used to combine observed studied_trait$_{50/50}$ with the expected_trait$_{AMorPM}$, these values being equal to b × milk_yield$_{AMorPM}$. The b coefficients for each studied traits were calculated based on regression analyses performed using Statistical Analysis System (SAS) software where the yield of the studied trait (calculated as content × milk yield) for the AM (PM) milking is the dependent variable and the milk yield observed after the AM (PM) milking is the independent variable.

The b coefficients were obtained from a second dataset that included 225,890 milk samples collected between October 2007 and February 2013 during the Luxembourg routine alternative milk recording (type T) from 31,510 cows (Holstein) in 491 herds (data T). During this milk testing, only one milk sample was collected per cow at one milking (AM or PM). Therefore, FAT_{AM} (FAT_{PM}), SFA_{AM} (SFA_{PM}), $MUFA_{AM}$ ($MUFA_{PM}$), UFA_{AM} (UFA_{PM}), $SCFA_{AM}$ ($SCFA_{PM}$), $MCFA_{AM}$ ($MCFA_{PM}$), $LCFA_{AM}$ ($LCFA_{PM}$), and C18:1 *cis-9*$_{AM}$ (C181 *cis-9*$_{PM}$) were available for dataset T. This dataset also contained the AM or PM milk yield.

Based on this approach, expected AM and PM records were obtained for the dataset S. The daily average quantities (g/day) for all of the studied traits were estimated as the sum of yields after both milkings (AM and PM). Therefore, the final calibration dataset contained 79,971 records related to 51 traits [milk yield (expected AM, expected PM, and expected daily); fat content (expected AM, expected PM, and expected daily); fat yield (expected AM, expected PM, and expected daily; g/day); levels of seven different FAs or FA groups (expected AM, expected PM, and expected daily; g/dL milk), and the corresponding FA yields for these seven FA types/groups (expected AM, expected PM, and expected daily; g/day)].

7.4.1.3. Validation Data

The equations to predict daily yields were validated using two distinct external validation datasets that included data for representative milk samples collected during two successive milkings in Luxembourg (between February and April 2013) and in the Walloon Region of Belgium (from October 2007 to June 2012).

The first validation dataset included representative milk samples (50 mL) collected from two consecutive milkings from 687 dairy cows (Holstein) belonging to 43 herds between February 2013 and April 2013 by CONVIS s.c. (Ettelbruck, Luxembourg; LUX data). This dataset contained observed yields from consecutive AM and PM milkings. Daily yields were also calculated. These samples were analyzed by MIR spectrometry using a FOSS Milkoscan FT6000 (Foss, HillerØd, Denmark). FA content (g/dL of milk) was estimated by applying the MIR calibration equations described in Table 1.

The second validation dataset included milk samples composed of 50% morning milk and 50% evening milk. These samples were collected from 138,141 Holstein cows belonging to 1291 herds that participated in the Walloon milk recording system from October 2007 to June 2012. Samples were collected from all of the cows milked in the herds on a given test day, and these samples were analyzed using MIR spectrometry (MilkoScan FT6000; FOSS, 2005) according to the normal milk recording procedure [11]. The final Walloon validation dataset contained 1,079,318 records (WAL data). AM and PM values were estimated by the same methodology used to create the calibration set.

7.4.2. Development of Statistical Models for Estimating Daily Yields from AM or PM milking

Models were developed to investigate whether daily yields can be estimated by replacing the MI effect [10] with different traits that are easily recorded and that are related to

changes in milk composition. Several variation factors were tested in order to build a robust model that uses information easily collected by milk recording organizations, including: stage of lactation (DIM), parity, yield traits (g/milking) during AM and PM milking, and the month of recording [14]. Stage of lactation is known to be one of the most influential factors affecting milk composition [10, 15, 16], and a month of recording was included in order to consider the season effect, and, indirectly, the feeding effect which affects the FA composition of milk [17, 18]. Considering that not all of these influential factors may have statistically significant effects on all of the traits examined, an appropriate subset of variables for each model was determined using the stepwise GLMSELECT procedure in the SAS/STAT software package [19]. The data used to develop the models came from the calibration set. The TEST dataset required by the GLMSELECT procedure was the LUX validation dataset collected in Luxembourg and including real observed AM/PM data. The VAL dataset, required by the GLMSELECT procedure, corresponded to the WAL validation dataset which was collected in the Walloon Region of Belgium. This procedure allowed a model to be selected from the framework of general linear models. All of the models that were developed were compared for all of the studied traits: milk yield, FAT, SFA, MUFA, UFA, SCFA, MCFA, LCFA, and C18:1 *cis*-9.

The accuracy of the AM-PM predictions was evaluated using two criteria. First, root mean squared error (RMSE) was calculated (Equation (4)), which represents the SD of the difference between observed and estimated daily yields. The model with the smallest RMSE and the highest coefficient of determination (or correlation) was considered to provide the best fit.

$$RMSE = \sqrt{SSE / n - p} \quad (4)$$

where n is the number of observations in the statistical model, p is the number of parameters (including the intercept), and SSE is the error sum of squares (*i.e.*, the sum of the squared differences between each observation and its predicted value) for the estimated model.

The second criterion was R^2, defined as the coefficient of determination. The square root of this value is the correlation ($Ry,\hat{y}$) which represents the relationship between the observed and predicted values. Statistical parameters were calculated using the GLMSELECT procedure in the SAS/STAT software package [19].

A validation was applied on the best fitted model using the two available validation sets. The estimated statistical parameters were RMSE, the standard deviation of prediction ($\sigma\hat{y}$) and $Ry,\hat{y}$.

7.5. Results and discussion

7.5.1. Available data

Tables 2–4 present descriptive statistics of the traits studied. Daily average values showed the same direction for the three datasets except for milk production. The origins of each dataset could explain these differences. For example, the calibration dataset (Table 2) and the first validation set (Table 3) were obtained from Luxembourg, with the latter including milk samples that were collected over a short period of time (between February 2013 and April 2013). In contrast, the second validation dataset (Table 4) was generated from cows recorded in the Walloon Region of Belgium from October 2007 to June 2012.

Table 2. Descriptive statistics of the calibration dataset (N = 79,971).

Variable	Collection of milk sample	Mean	SD	Min	Max.	Mean	SD	Min	Max
Milk (kg/day)	Expected AM	12.79	4.27	1.20	37.30				
	Expected PM	13.57	4.41	1.20	39.20				
	Expected Daily	26.36	8.33	2.40	72.80				
		g/dL milk				g/day			
Fat	Expected AM	4.11	0.71	1.01	7.23	515.31	164.29	22.13	1629.96
	Expected PM	4.55	0.78	1.12	8.00	605.64	191.21	24.48	1822.35
	Expected Daily	4.34	0.75	1.07	7.67	1120.94	340.33	46.61	3345.67
SFA	Expected AM	2.76	0.56	0.51	6.95	344.33	111.28	14.49	1272.59
	Expected PM	2.95	0.60	0.54	7.44	392.11	127.91	15.51	1268.89
	Expected Daily	2.86	0.58	0.53	7.21	736.41	229.39	30.00	2482.20
MUFA	Expected AM	1.15	0.24	0.29	3.84	144.14	53.26	6.08	678.89
	Expected PM	1.36	0.29	0.35	4.56	181.38	64.96	7.22	963.86
	Expected Daily	1.26	0.27	0.32	4.22	325.55	114.28	13.31	1562.22
UFA	Expected AM	1.34	0.27	0.37	4.25	168.57	61.34	7.14	765.02
	Expected PM	1.59	0.32	0.44	5.02	210.87	73.76	8.45	1047.49
	Expected Daily	1.47	0.29	0.40	4.66	379.44	130.42	15.59	1700.99
SCFA	Expected AM	0.38	0.08	0.10	1.02	47.77	16.50	1.63	200.32
	Expected PM	0.40	0.08	0.10	1.08	53.83	18.69	1.73	188.64
	Expected Daily	0.39	0.08	0.10	1.06	101.57	33.93	3.36	388.96
MCFA	Expected AM	2.16	0.47	0.06	5.77	268.89	87.51	6.98	975.09
	Expected PM	2.29	0.50	0.06	6.11	302.83	99.40	9.45	937.77
	Expected Daily	2.22	0.49	0.06	5.96	571.72	179.28	16.43	1891.53
LCFA	Expected AM	1.58	0.35	0.31	5.47	198.89	75.77	6.65	1028.49
	Expected PM	1.87	0.42	0.37	6.48	249.47	92.22	7.88	1352.77
	Expected Daily	1.73	0.39	0.34	6.01	448.39	162.72	14.53	2195.18
C18:1 *cis-9*	Expected AM	0.75	0.19	0.07	2.97	94.13	38.48	4.51	501.97
	Expected PM	0.91	0.23	0.09	3.59	120.85	48.53	5.46	777.79
	Expected Daily	0.83	0.21	0.08	3.30	214.98	84.65	9.96	1251.72

Min: minimum; Max: maximum; AM = morning milking; PM = evening milking, Daily = daily content; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

In the calibration set, the average milk production between October 2007 and April 2013 (Table 2) was 26.36 kg/day, with 4.34 g fat/dL milk having a saturated part equal to 65.9%. Based on the WAL dataset, the average production was 24.11 kg milk/day, with 4.25 g fat/dL milk composed of 68.2% SFAs (Table 4). These values for fat and SFA content were slightly higher than those observed for the calibration set (Table 2). Overall, the quantities and content of individual FAs present in the milk samples and fat were consistent with those previously reported for the Walloon data [20-22]. The milk and fat yields had similar descriptive statistics compared to the results mentioned by Liu *et al.* [10] from their calibration set.

Table 3. Descriptive statistics of the Luxembourg (LUX) validation dataset (N = 687).

Variable	Collection of milk sample	Mean	SD	Min	Max	Mean	SD	Min	Max
Milk (kg/day)	AM	12.83	4.46	2.40	30.10				
	PM	15.13	5.18	2.80	33.00				
	Daily	27.96	9.41	5.20	57.00				
		g/dL milk				g/day			
Fat	AM	4.27	0.80	1.05	7.51	537.47	187.33	115.64	1257.44
	PM	4.68	0.80	1.59	7.51	695.33	239.95	165.56	1550.10
	Daily	4.49	0.71	2.33	7.33	1232.80	404.26	321.01	2656.58
SFA	AM	2.91	0.59	0.74	5.03	364.69	126.28	80.44	801.65
	PM	3.14	0.59	1.13	5.90	465.74	159.42	96.15	1075.73
	Daily	3.03	0.53	1.50	5.22	830.43	268.59	181.14	1698.26
MUFA	AM	1.18	0.29	0.27	3.67	148.79	61.37	29.71	448.47
	PM	1.33	0.30	0.39	3.37	198.17	85.18	52.20	763.26
	Daily	1.26	0.27	0.62	3.02	346.97	139.58	111.48	1094.06
UFA	AM	1.40	0.32	0.33	4.08	176.17	70.56	36.82	506.42
	PM	1.56	0.34	0.49	3.75	233.58	97.09	61.22	853.54
	Daily	1.49	0.30	0.75	3.37	409.75	159.88	128.22	1229.88
SCFA	AM	0.40	0.08	0.10	0.71	51.00	18.47	7.36	112.88
	PM	0.44	0.08	0.17	0.79	65.16	23.18	8.80	145.92
	Daily	0.42	0.07	0.24	0.71	116.16	39.48	16.16	240.74
MCFA	AM	2.30	0.47	0.56	3.95	288.14	98.17	53.54	622.87
	PM	2.47	0.47	0.94	4.11	365.12	121.71	67.72	799.04
	Daily	2.39	0.42	1.15	3.78	653.33	206.66	121.27	1314.99
LCFA	AM	1.66	0.41	0.46	5.29	209.24	87.77	48.90	669.326
	PM	1.86	0.44	0.55	5.09	278.76	122.40	71.67	1123.44
	Daily	1.77	0.39	0.80	4.49	488.00	200.03	147.86	1582.02
C18:1 *cis*-9	AM	0.81	0.23	0.21	2.85	102.13	46.85	23.20	361.59
	PM	0.91	0.24	0.27	2.54	136.21	65.34	35.44	593.22
	Daily	0.86	0.22	0.43	2.27	238.35	107.11	75.06	852.25

Min: minimum; Max: maximum; AM = morning milking; PM = evening milking, Daily = daily content; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

Table 4. Descriptive statistics of the Walloon (WAL) validation dataset (N = 1,079,318).

Variable	Collection of milk sample	Mean	SD	Min	Max	Mean	SD	Min	Max
Milk (kg/day)	AM	11.41	4.27	0.20	49.00				
	PM	12.70	4.63	0.40	49.00				
	Daily	24.11	8.64	3.00	75.40				
		g/dL milk				g/day			
Fat	Expected AM	4.00	0.70	0.09	6.61	448.07	166.73	7.21	2730.67
	Expected PM	4.47	0.79	0.11	7.40	558.72	204.05	12.86	3418.45
	Expected Daily	4.25	0.75	0.10	7.22	1006.79	360.00	30.89	4640.37
SFA	Expected AM	2.78	0.56	0.00	5.35	311.57	119.42	0.62	1590.42
	Expected PM	3.00	0.60	0.01	5.79	375.79	142.26	0.42	1854.17
	Expected Daily	2.90	0.58	0.00	5.56	687.55	254.50	1.05	2792.34
MUFA	Expected AM	1.06	0.24	0.05	3.79	117.76	48.00	1.80	1022.04
	Expected PM	1.27	0.29	0.06	4.56	157.73	62.68	3.98	1458.89
	Expected Daily	1.17	0.27	0.06	4.18	275.49	107.92	8.62	1943.96
UFA	Expected AM	1.20	0.26	0.03	3.72	134.10	54.00	2.10	1127.60
	Expected PM	1.44	0.32	0.04	4.47	179.10	70.40	2.80	1588.10
	Expected Daily	1.33	0.29	0.03	4.10	313.10	121.30	5.50	2117.50
SCFA	Expected AM	0.36	0.08	0.01	0.93	40.79	17.00	0.67	191.11
	Expected PM	0.38	0.08	0.01	0.99	48.52	19.90	0.57	221.20
	Expected Daily	0.37	0.08	0.01	0.96	89.31	36.03	1.25	383.54
MCFA	Expected AM	2.18	0.49	0.00	4.49	243.85	94.77	0.63	1095.67
	Expected PM	2.36	0.53	0.01	4.86	294.22	113.42	0.43	1257.14
	Expected Daily	2.27	0.51	0.00	4.71	538.08	202.61	1.06	2125.97
LCFA	Expected AM	1.46	0.35	0.04	4.41	163.50	68.96	1.50	1503.15
	Expected PM	1.73	0.41	0.04	5.23	215.80	88.64	0.71	2066.22
	Expected Daily	1.60	0.38	0.04	4.83	379.30	153.91	2.21	2762.95
C18:1 *cis*-9	Expected AM	0.75	0.20	0.01	2.67	83.99	36.72	0.31	820.82
	Expected PM	0.89	0.23	0.02	3.17	110.90	47.33	0.29	1185.75
	Expected Daily	0.83	0.21	0.01	2.97	194.89	82.20	0.60	1585.61

Min: minimum; Max: maximum; AM = morning milking; PM = evening milking, Daily = daily content; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

7.5.2. Phenotypic Correlation

Table 5 shows the correlations identified between AM and PM collection times, and for daily contents and yields, for all of the studied traits. Correlations between AM and PM values varied according to trait and were lower than one, suggesting that AM and PM records represent two distinct types of traits, and, therefore, need to have individual equations developed for estimating daily yield and content. Correlation values between yield traits were higher than those observed between content traits. For both units of expression, the correlations were lower for the fatty traits than milk yield. Moreover, for both content and yield traits, the PM milking records showed higher or similar correlation values with daily traits compared with the AM milking records. The same observation was done also by Berry *et al.* [13] from fat content and yield. However, Liu *et al.* [10] observed globally similar correlations between AM and PM values with a very slight tendency to have higher correlations for AM values.

Correlations between milking and daily yield traits varied from 92.4% (SFA; AM-DY) to 97.9% (milk yield; PM-DY). The strong positive correlations between daily and AM or PM yields observed in Table 5 suggest that it may be possible to estimate daily FA yields from AM or PM FA yields.

The FAT content and FAT yield correlation values were similar than those observed by Liu *et al.* [10]. These authors found correlation values equal to 59.0%, 86.4%, and 85.8% for AM/PM, AM/daily content (DC) and PM/DC correlations related to the fat content, respectively. The correlation values for the fat yield observed by these authors for the AM/PM, AM/daily yield (DY) and PM/DY were 83.9%, 92.7%, and 92.2%, respectively. Similar results were also obtained for milk yield. Liu *et al.* [10] found 90.8%, 97.9%, and 97.5% for AM/PM, AM/DY, and PM/DY correlations, respectively. The correlation values obtained by Berry *et al.* [13] were often lower than the ones found in this study. For fat content (yield), these authors calculated AM-

PM, AM-DC, and PM-DC correlations equal to 36% (54%), 80% (84%), and 84% (90%), respectively. As observed in this study, the correlations related to fat yield were higher compared to the one observed for fat content. The same observation was done also by Liu *et al.* [10]. For milk yield, Berry *et al.* [13] found 85%, 97%, 95% for AM-PM, AM-DY, and PM-DY correlation values, respectively. The milk correlations between AM and PM values and between AM and PM values were slightly lower than the ones observed in this study but can be both considered as strong positive correlations. The differences in term of correlation values between Berry *et al*. [13] and Liu *et al*. [10] or our study can be probably explained by differences of herd management (feeding system), milking interval and milk production.

Table 5. Correlation values among morning (AM), evening (PM), and daily records for each studied trait expressed in g/dL of milk and kg/day. The values were obtained from LUX data (*i.e*., real observations, N = 687).

	g/dL of milk			kg/day		
Studied Trait	AM-PM	AM-DC	PM-DC	AM-PM	AM-DY	PM-DY
Milk				90.4	97.2	97.9
Fat	55.9	86.6	89.3	78.7	93.0	95.8
SFA	58.1	87.5	89.8	76.4	92.4	95.3
MUFA	63.7	88.3	92.1	80.9	93.3	96.6
UFA	63.3	88.3	92.0	81.4	93.6	96.6
SCFA	58.0	87.0	90.2	79.4	93.4	95.9
MCFA	60.8	88.3	90.6	76.4	92.5	95.2
LCFA	63.4	88.2	92.1	80.6	93.2	96.6
C18:1 cis-9	65.9	89.0	92.7	81.8	93.6	96.8

AM = morning milking; PM = evening milking; DC = daily content; DY=daily yield; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long chain fatty acids.

As also shown by Liu *et al*. [10] and Berry *et al*. [13] for milk fat, all correlations considered in Table 5 were lower for fatty traits compared to milk yield. This suggests that the prediction of daily yield or content from AM-PM records will be less accurate for fatty traits than milk yield.

7.5.3. Models Selected Using PROC GLMSELECT

Table 6 describes the equations that were selected using the GLMSELECT procedure for all of the studied traits. In other words, the models provided the best fit of data are described in Table 6. These models showed the smallest RMSE and the highest correlation between observed and estimated values.

Based on these results, it appears that there were similarities between the effects included in the equations that used AM records and the ones included in the equations that used PM records for each studied trait. This observation suggests that PM and AM values had a similar evolution pattern but the differences came only from a question of scale. Indeed, PM values were always higher than AM values (Tables 2–4).

The PROC GLMSELECT procedure selected always combined effects. There were not individual effects such as only DIM or only lactation number in the selected equations. Such complexity of equations was not mentioned in previous studies [9, 10, 13]. However, Berry *et al*. [13] mentioned heterogeneous means and variances for 24-h yield over different parities, season of calving and DIM. Therefore, they realized 54 subclasses taken into account the parity, DIM and the season of calving. For all of these subclasses, they estimated the coefficients of regression. The same methodology was previously used by Liu *et al*. [10]. Based on the composition of selected equations mentioned in Table 6, this study confirmed this heterogeneity because separate regression coefficients were estimated following DIM, parity and month of test.

Table 6. Models selected by PROC GLMSELECT procedure.

Studied trait	Milking moment	Selected models
Milk	AM	a+b* DIM+c*month of test+d*(milk_AM*DIM* parity*month of test)
	PM	a+b* DIM+c*month of test+d*(milk_PM* DIM* parity*month of test)
Fat	AM	a+b*(qFAT_AM*milk_AM* DIM)+c*(parity)+d*(milk_AM* parity)+e*(qFAT_AM*milk_AM* parity)
	PM	a+b*(milk_PM* DIM)+c*(qFAT_PM*milk_PM* DIM)+d* parity+e*(qFAT_PM*milk_PM*month of test)
SFA	AM	a+b*(qSFA_AM*milk_AM* DIM)+c* parity+d*(milk_AM* parity)+e*(milk_AM*month of test)
	PM	a+b*(milk_PM* DIM)+c*(qSFA_PM*milk_PM* DIM)+d*(parity)+e*month of test
MUFA	AM	a+b*(milk_AM* DIM)+c*(milk_AM* parity)+d*(qMUFA_AM*milk_AM* DIM* parity)+e*(qMUFA_AM*milk_AM*month of test)
	PM	a+b*(milk_PM* DIM)+c* parity+d*(qMUFA_PM*milk_PM* DIM* parity)+e*(milk_PM*month of test)
UFA	AM	a+b*(milk_AM* DIM)+c*(qUFA_AM*milk_AM* DIM)+d* parity+e*(milk_AM* parity)
	PM	a+b*(milk_PM* DIM)+c*(qUFA_PM*milk_PM* DIM* parity)+d*(milk_PM*month of test)+e*(qUFA_PM* DIM* parity*month of test)
SCFA	AM	a+b*(qSCFA_AM*milk_AM* DIM)+c* parity+d*(milk_AM* parity)+e*month of test
	PM	a+b*(qSCFA_PM*milk_PM* DIM)+c*(milk_PM* parity)+d*(milk_PM*month of test)+e*(qSCFA_PM* DIM* parity*month of test)
MCFA	AM	a+b*(qMCFA_AM*milk_AM* DIM)+c* parity+d*(qMCFA_AM*milk_AM*month of test)+e*(qMCFA_AM* DIM* parity*month of test)
	PM	a+b*(milk_PM* DIM)+c*(qMCFA_PM*milk_PM* DIM)+d*(milk_PM* parity)+e*month of test+f*(qMCFA_PM* DIM* parity*month of test)
LCFA	AM	a+b*(milk_AM* DIM* parity)+c*(qLCFA_AM*milk_AM* DIM* parity)+d*(milk_AM*month of test)+e*(qLCFA_AM*milk_AM*month of test) + f* (qLCFA_AM* DIM* parity*month of test)
	PM	a+b*(milk_PM* DIM)+c*(qLCFA_PM*milk_PM* DIM* parity)+d*(milk_PM* parity*month of test)+e*(qLCFA_PM* DIM* parity*month of test)
C18:1 cis-9	AM	a+b*(milk_AM* DIM)+c*(milk_AM* parity)+d*(qC18:1 cis9_AM*milk_AM* DIM* parity)+e*(qC18:1 cis9_AM*milk_AM* parity*month of test)+f*(qC18:1 cis9_AM* DIM* parity*month of test)
	PM	a+b*(milk_PM* DIM)+c*(qC18:1 cis9_PM*milk_PM* DIM* parity)+d*month of test+e*(qC18:1 cis9_PM*milk_PM*month of test)+ f*(qC18:1 cis9_PM* DIM* parity*month of test)

7.5.4. Goodness of Fit

Table 7 shows the correlation values calculated between the observed and estimated daily yields (Ry,ŷ), RMSE, and SDs of the daily yield predictions (σŷ) for each studied trait estimated from the milk samples collected during the AM or PM milking using the calibration set and the two available validation sets. The tested models were the models selected by PROC GLMSELECT and described in Table 6.

In order to appreciate the good fitting of a model, Liu *et al*. [10] indicated that σŷ should be close to the SD of the observed daily yield but must not be greater. In the present study, all of the estimates had smaller σŷ values than the observed SD values (Tables 2–4).

Except for milk yield, the observed correlations suggested that the estimations of daily yield were better when PM milking data were used. Indeed, the calibration correlation values were found to range from 96.4% to 97.6%, and from 96.9% and 98.3%, when estimations were realized from AM or PM milkings, respectively (Table 7). Except for milk yield, this is not in agreement with the observations made by Liu *et al*. [10] and Berry *et al*. [13]. However, the differences between AM and PM Ry,ŷ values were lower than 0.8%.

Table 7. Calibration and validation statistics (correlation values between true and estimate daily yield (Ry,ŷ), root mean square errors (RMSE) and standard deviation for each studied predicted trait (σŷ)) for the best model selected by PROC GLMSELECT. Ry,ŷ were expressed in % and RMSE and σŷ were expressed in kg/day for milk and g/day for the remaining studied traits.

Milking moment	Studied trait	σŷ			RMSE			Ry,ŷ		
		Calib	LUX	WAL	Calib	LUX	WAL	Calib	LUX	WAL
AM	MILK	8.08	8.81	8.48	2.03	2.67	2.25	97.0	96.8	96.5
	FAT	328.00	385.88	350.93	90.52	160.62	98.47	96.4	92.7	96.2
	SFA	221.70	255.19	248.40	58.93	113.88	66.51	96.6	92.1	96.6
	MUFA	110.95	133.37	105.22	27.42	51.30	28.22	97.1	93.2	96.5
	UFA	127.50	152.31	118.81	32.40	58.14	32.36	96.9	93.4	96.4
	SCFA	32.95	37.34	35.37	8.10	15.90	8.58	97.1	93.1	97.1
	MCFA	173.37	198.66	195.92	45.66	89.12	52.81	96.7	92.0	92.7
	LCFA	158.29	188.74	151.19	37.72	73.34	38.71	97.3	93.3	96.8
	C18:1	82.63	105.13	82.50	18.53	38.66	20.11	97.6	97.0	93.4
PM	MILK	8.00	9.36	8.22	2.26	2.57	2.41	96.5	97.5	97.0
	FAT	331.70	405.91	352.63	85.49	124.65	91.27	96.8	95.6	97.1
	SFA	222.38	272.71	247.44	56.30	90.49	61.22	96.9	95.0	97.4
	MUFA	111.80	144.42	106.43	23.82	38.28	24.33	97.8	96.4	97.8
	UFA	127.39	165.01	119.82	27.91	43.56	27.98	97.7	96.5	97.7
	SCFA	33.01	40.71	35.09	7.85	13.01	8.06	97.3	95.5	97.7
	MCFA	173.77	207.82	198.22	44.13	70.49	49.00	96.9	94.8	97.4
	LCFA	159.35	208.85	151.48	32.95	55.78	33.06	97.9	96.4	97.9
	C18:1	83.21	109.27	79.94	15.54	27.81	16.41	98.3	96.7	98.1

Calib= calibration set including expected Luxembourg records (N=79,971); LUX = Validation set including real collected Luxembourg records (N=687); WAL= Validation set including expected Walloon records (N=1,079,318); AM= morning milking; PM = evening milking; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; SFCA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long chain fatty acids.

Regarding the estimations of daily milk yield, the calibration correlation values were slightly lower than those obtained by Liu *et al*. [10] (e.g., 97.0% *vs.* 97.7% and 96.5% *vs.* 97.4% for the AM and PM milking data, respectively) (Table 7). The σŷ and RMSE values were also slightly higher in our study (for the AM and PM milking data: 8.08 and 8.00 kg/day *vs.* 7.85 and 7.83 kg/day for the σŷ values, respectively; and 2.03 and 2.26 kg/day *vs.* 1.72 and 1.84 kg/day for the RMSE values, respectively) (Table 7).

For the estimates of daily fat yield, obtained values for Ry,ŷ, and RMSE corresponded with a better fit of the model compared with Liu *et al*. [10] (for the AM and PM milking data: 96.4% *vs.* 94.3% and 96.8% *vs.* 94.0% for Ry,ŷ, respectively; and 90.52 *vs.* 106.0 g/day and 85.49*vs.* 109.0 g/day for RMSE, respectively). The σŷ values were slightly higher in the present study (328.0 *vs.* 301.6 g/day and 331.7*vs.* 300.6 g/day, respectively) (Table 7).

Better AM/PM predictions were observed for milk yield compared to fat content and yield. It was also observed by Liu *et al*. [10] and Berry *et al*. [13]. These last authors suggested that factors were missing in their equations permitting to predict AM/PM values for fat traits. However, in this study, the differences in terms of Ry**,**ŷ between milk and fat were lower. This is explained by a better fitting of fat traits in the current study.

Observed AM/PM calibration Ry,ŷ values for fatty acid traits were all within the same range and were higher than 96% suggesting a good prediction.

7.5.5. Model Validation

As expected, validation Ry**,**ŷ values obtained from the two validation sets were lower than calibration Ry,ŷ values. Validation RMSE values were higher than the observed calibration RMSE values (Table 7). However, RMSE observed for the LUX validation set (*i.e*., real observed data) were bigger than the WAL validation set (*i.e*., expected daily records). One hypothesis is that these differences were due to the initial step used to predict AM/PM values for the

calibration set. A potential confirmation of this hypothesis comes from the fact that small differences were observed between the RMSE or Ry,ŷ observed from the first and second validation data sets for the equations predicting daily milk yield whose AM and PM milk records were always observed. However, the predictability stayed good with Ry,ŷ never lower than 92.0%.

Small differences observed between calibration and WAL validation results (*i.e.*, these results were predicted using the same methodology as the one used for the calibration set) suggest a good robustness of the developed equations which was the main interest of the proposed methodology to build the calibration dataset. Indeed, as the first validation set which was composed of real records, was not large enough to cover the entire lactation, many parities, herds or cows, the theory of selection index was used to predict AM–PM records from 50% AM/50% PM collected records. Better results could be obtained by using only real observations but a large sampling procedure (larger than the one conducted for the LUX data) should be conducted to present a sufficient variability for DIM, parity, month of test as well as studied traits. The advantage of the selection index theory applied in this study is to use data routinely available at large scale to build the predictive models and, therefore, to require a smaller dataset containing real observations to validate the obtained models.

7.5.6. Milking Interval

The models proposed in the present study demonstrated that it is possible to estimate milk, fat, and FA yields without the use of MI recorded on site. To explain this observation, different regressions including the effects and covariates related to changes in milk were tested in order to estimate MI values (Table 8). An R^2 value of 0.86% was observed between MI and milk daily yield. Additional covariates and fixed classification effects can be included in the regression model (such as milk and fat yields obtained during one milking record) if we assume

that the milk composition is also influenced by the MI due to the dilution effect. To predict daily yields for milk, fat, and protein, Berry *et al*. [13] introduced milk yield and fat yields of one milking a day. By using this approach, the obtained R^2 increased to 17.6% (Table 8). When the stage of lactation was added, the R^2 obtained was 18.2% (Table 8), while inclusion of the parity effect resulted in an R^2 value of 18.4%. All of the effects proposed to describe variations in MI were significant. Therefore, nearly 20% of the MI variability observed can be explained by a combination of effects related to milk composition and production. Consequently, we can assume that the MI effect can be partially replaced by a combination of data that are generally available and are easily recorded by milk recording organizations. In addition, the accuracy of reported MI can be problematic because, with increasing herd sizes and milking times, the actual MI for a given cow can be very different from the reported herd MI. Indirect predictors as used in this study have the advantage that they will be always known very precisely on an individual level.

Table 8. Regression coefficients (in %) for the regressions explaining the milking interval (MI) in function of milk production, fat (g/milking or /day), dim, and parity (N = 79,971).

MI	R^2
Milk daily yield	0.86
Milk daily yield + Milk (AM or PM) yield	17.22
Milk daily yield + Milk (AM or PM) yield +FAT (AM or PM) (g/dL of milk)	17.64
Milk daily yield + Milk (AM or PM) yield +FAT (AM or PM) (g/dL of milk) + DIM	18.23
Milk daily yield + Milk (AM or PM) yield +FAT (AM or PM) (g/dL of milk) + DIM + parity	18.40

MI: milking interval; AM= morning milking; PM = evening milking; DIM= Days in milk.

7.6. Conclusions

The main objective of this study was to propose a practical, simple, and robust method for accurately estimating daily FA yields from a single milking (*i.e*., AM or PM milking). The obtained results show the interest to use the theory of the selection index to construct the calibration set in order to have more robust equations thanks to a large calibration set. With validation Ry,ŷ

higher than 92% obtained from observed records for all studied traits, the results are promising, although further studies are needed to confirm these results by using a larger database. Moreover, the results obtained also shows that it is possible to replace the MI parameter with a combination of more reliable parameters such as: milk production and fat content, stage of lactation classes, the test month, and calving month. The application of the models developed in this study has the potential to reduce the number of collected samples per test-day (*i.e*., only one AM or PM sample is necessary instead of the two samples needed for the 50/50 sample), thereby reducing the costs associated with official milk recording (*i.e*., only one visit of the milk recorder in the farm), while still maintaining a high accuracy of predicted daily yields.

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Author Contributions

The study was conceived and managed by Arnould and Soyeurt. Both authors contributed to the presentation and interpretation of findings. Data collections were undertaken by CONVIS s.c. All authors contributed to writing of the manuscript. The authors thank the anonymous referees for helpful comments improving the quality of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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CHAPTER VIII.

GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES

8.1. General Discussion

The main objective of this thesis is to contribute to the development of innovative management tools for dairy farmers helping them to improve the sustainability of their dairy production systems and the quality of milk and dairy products. Indeed, development of such tools could help dairy farmers in their daily decisions.

To achieve this objective, several necessary conditions must be fulfilled:

Condition 1: The selected traits (or combination of traits) must be **relevant** in order to develop management tools allowing an improvement of **dairy production system** and **product quality**;

Condition 2: The selected traits must be **easily available** for the milk recording organization, and therefore the dairy farmers, at **low (free) cost**, on a **large scale** and at **individual level**;

Condition 3: The used records must be **comparable** to allow a comparison between practices of farmers, whatever the adopted milk recording scheme.

Condition 4: The selected traits must be **phenotypically and genetically variable**. Indeed, the development of management tools takes profit of the genetic and phenotypic variability of these selected traits.

8.1.1. Condition 1: Relevant traits for management purposes

Dairy farmers need management tools for helping them in their daily decisions (culling, feeding or preventing metabolic diseases) and to improve their product quality and reducing environmental impact of dairy production systems.

Throughout the first review (Chapter II), we demonstrated that milk composition could be interpreted as a mirror of the dairy cow's health or of the efficiency of the management system. This review aimed to determine the practical aspect of using milk composition and milk FA profile in order to build an interesting (i.e., easy, useful, cheap) management tool to help dairy farmers in their daily decisions.

The high plasticity of milk fat composition is very well known in literature. Indeed, milk FA profiles may be significantly altered through numerous interventions, such as changes in diet (e.g. Chilliard et al., 2000 and 2001; Grummer, 1991) but also through animal health (e.g. Gross et al., 2011; Van Haelst et al., 2008) or through animal genetic selection as discussed in Chapter III.

Among others, two practical cases should be again outlined. Firstly, milk composition can be used in order to avoid or to prevent metabolic diseases. For example, according to the available literature (e.g. Fleischer et al., 2001; Gross et al., 2011; Mulligan et al., 2006; Van Haelst et al., 2008), some FAs, such as LCFA, MCFA, and C18:1 *cis*-9 can be used as relevant predictors/indicators of subclinical ketosis. Knowing and detecting abnormal increases of LCFA, and C18:1 *cis*-9 (especially if it is combined with a decrease of MCFA content) could be helpful to dairy farmers and veterinarians to develop preventive measures. An effective prevention system for common dairy disease would limit labor investment, medical treatment costs and animal suffering. Indeed, the earlier health problems are identified, the higher the chance of successful health management, with numerous positive consequences for farm management, economical, ecological, and animal welfare issues.

Secondly, recent studies have shown that FA profiles may be also used as indicators for the "environmental quality of milk" (e.g. Chilliard et al., 2009; Dehareng et al., 2012; Dijkstra et al., 2011). These papers present FA profile as a valuable tool for reducing methane emissions of dairy cattle. In fact, dairy cattle are considered to contribute to about one third to the total emissions of GHG. Unfortunately, few papers focus on this subject matter. In Chapter II, we

showed that some recent studies such as Chilliard et al. (2009) or Dijkstra et al. (2011) presented FA profile as a valuable tool for reducing GHG emissions.

According to this review, milk fat composition could be interpreted as a relevant mirror of the dairy cow's health or of the management system efficiency. Knowing that, understanding the variation in milk fat composition can be useful for providing information about the dairy production system. Our observations indicated that FA could be used separately as indicators of metabolic or management malfunctions. In order to build more relevant and reliable tools, a solution could be to combine several selected FAs or other milk components. For instance, as mentioned before, several studies (e.g. Gross et al., 2011; Van Haelst et al., 2008) showed that abnormal increases of LCFA and C18:1 *cis*-9 content could be interpreted as an interesting indicator of subclinical ketosis, especially if it is combined with decreasing MCFA content.

Finally, some threshold values are already available in literature. However, it would seem to be important to use these thresholds carefully as they would need to be adapted to the particular dairy cow population under study. This aspect will be developed in the perspective section.

8.1.1. Condition 2 : Easy and cheap acquisition of large-scale individual traits

The second advantage to use milk composition as an indicator of management system efficiency is the availability of data. Indeed, according to the milk recording scheme selected by Luxembourg dairy farmers, information about the milk composition and FA profile are available by MIR every 2, 4 or 6 weeks without any extra-cost.

Several papers (e.g. Rutten et al., 2009; Soyeurt et al., 2006; 2008a; 2008b and 2011) have presented the potential value of MIR spectrometry for quantifying FAs. MIR spectrometry

is an interesting alternative to the reference gas chromatography, with advantages such as a very high throughput (up to 500 samples/h), the convenience of use, and availability. Moreover, this versatile technology can be used to estimate various traits quantitatively based on calibration equations (Soyeurt et al., 2006). Consequently, thanks to the use of this technology, these management tools may be easily implemented in practice and are relatively cheap. In the field, milk recording agencies or dairy advisors (as CONVIS s.c.) would be able to alert farmers whenever threshold values for diseases or malfunction were reached. This kind of alert allows dairy producers to check their management system and therefore to improve their dairy production on numerous (economical, ecological and animal (welfare)) points of view.

The availability of data has allowed the development of a first practical tool for CONVIS s.c. Milk recording data are compiled once a month into a table for each registered farm, and for each milk recording scheme. Monthly, descriptive statistics are obtained for milk production and traditional and novel milk composition traits (especially fat, protein, urea, lactose, cells and major groups of FAs). These statistics are available for each dairy farm independent of the milk recording scheme and allow the CONVIS s.c. farm advisors to study the evolution of milk composition within a specific herd, or to compare a given herd's production to the national mean.

8.1.2. Condition 3: Easy comparison between used data independent of the recording system

ICAR guidelines allow the use of different sampling procedures (ICAR, 2014). Consequently, milk recording organizations around the world are able to increase flexibility in their recordings. In Luxembourg, two principal schemes are applied. The first one: the scheme "T" consists in one milk sample of only one milking (AM or PM milking). The second one is the scheme "S", consisting in one proportionate sample of all daily milking (50% morning and 50%

evening milking). Two other schemes are proposed to dairy farmers: milk samples could be also collected every 2, 4 or 6 weeks, during only AM or only PM milking (scheme "M"). The last scheme relates to milking robots.

For a practical point of view, the adoption of an alternative testing scheme could be an interesting response to increasing pressure to reduce breeding costs. Indeed, implementing such an alternate scheme presents several practical advantages: it is less disruptive to the daily routine, and presents reduced economical costs compared to the conventional milk testing scheme ("S") (Everett and Wadell, 1970).

To develop robust management tools, the used phenotypic data should be homogenous. However, the use of different sampling schemes impacts significantly on the milk fat composition (Liu et al., 2000) and, therefore, brings heterogeneity.

Data used in Chapter VI were recorded in Luxembourg from October 2007 to February 2011 from 13,854 first parity Holstein belonging to 492 herds. These samples were composed of AM or PM collected milk ("T" scheme). According to our observations, we detected phenotypic differences in the means of the obtained values between AM and PM records independent of the studied trait. For instance, AM milk yield (11.5 kg) was higher than PM milk yield (10.2 kg). This first observation was confirmed by the findings of other authors as Gilbert et al. (1972) and Quist et al. (2008). We observed several other differences for the studied traits. Unlike milk yield, milk fat content appeared lower in AM than in PM milk samples (4.3 *vs.* 4.4 g/dL of milk, respectively for AM and PM milking; 3.9% of relative difference). Further, greater AM/PM variations were detected in UFA (1.38 *vs.* 1.49; 7.7% of relative difference), MUFA (1.19 *vs.* 1.28; 7.3% of relative difference), and LCFA (1.65 *vs.* 1.77; 7.0% of relative difference) than unsaturated FAs such as SCFA (0.38 *vs.* 0.39; 2.6% of relative difference). These observations were confirmed by Liu et al. (2000). These differences could be explained by the different origins of these FAs. The first one is *the novo* synthesis. These FAs are synthesized in the mammary gland and consist in short chain FA (<15 carbon), while about half of the total FA (a portion of C16 and ≥ C17) are synthesized from dietary lipids and adipose tissues reserves (e.g.

Bauman et al., 1999; Samkova et al., 2012). These *de novo* synthesized FAs (e.g., SFA, SCFA, and MCFA) had the lowest relative differences (2.1, 2.6, and 1.4% respectively), whereas UFA (7.7%), MUFA (7.3%) and LCFA (7.0%) had the highest AM/PM differences. As mentioned in Chapter VI, these observations suggest that *de novo* synthesized FAs are under stronger genetic control than those produced from plasma lipids (Bastin et al., 2011; Grummer, 1991) and that they, therefore, have lower AM/PM variability.

Consequently, to develop robust management tools, the used phenotypic data should be collected by using comparable sampling methods. A suggested solution is to propose some equations allowing the estimation of daily milk, fat and FA yields from single milking data. The advantage of this daily estimation is the possible comparison between dairy breeding using different sampling scheme, and further, the comparison throughout the lactation in dairy breeding using alternate sampling scheme.

The development of such equations is presented in Chapter VII. These new developed equations present several particularities. Numerous papers studying this issue are available in scientific literature since 1970's (e.g. Berry et al., 2006; Delorenzo and Wiggans, 1986; Liu et al., 2000). Anyhow, during the conception of the CONVIS s.c. research database, some breeding data appeared as missing, hard to collect or unreliable. Milking interval was one of these cited data. Nevertheless, the majority of authors working on the issue of conversion equation used the milking interval in their predictive models. Based on the results of Ouweltjes (1998), the milking interval considers as an influent factor on the milk composition, due to the dilution effect. Therefore, a high volume of milk produced during one milking should contain lower contents of fat compared to the milk composition obtained from a lower amount of milk (dairy cows milked twice a day). Our first difficulty, considering the poor reliability of milking interval parameter, was to find how to replace this parameter by another one or combination of reliable parameters to build practical conversion equations. So, based on the conclusions of Ouweltjes (1998), the milking interval could be reflected by the content of specific milk components. In order to propose such a combination of parameters, several regressions including effects

related to the change of milk composition were tested to estimate the milking interval parameter. The obtained results show the possibility to replace this problematic parameter by a combination of more reliable parameters. Among these parameters, we can find the milk yield, fat and protein contents, classes of stage in lactation, month of test and month of calving. These parameters present the advantage to be more reliable and available than milking interval.

Despite the large number of papers considering the 24h-conversion equations of milk composition, none of them propose this kind of equation for other traits than milk yield, fat or protein production. So, our second objective was to enlarge the scope by developing 24h-conversion equations on records from alternate milk recording schemes to predict the daily production of the major groups of FAs.

The presented results in this paper (Chapter VII) were very promising. Daily estimations based on PM records were slightly higher (R comprised between 97.9 and 98.4) than daily estimation based on AM records (R ranged from 97.0 to 97.3). Further, some comparisons were realized between our proposed equations and the equations of Liu et al. (2000). Obtained results for accuracy of prediction criteria (such as R, RMSE, AIC, BIC, and ASE) were similar for all the tested equations (Liu's ones). Thus, on the basis of our observations, it is possible to estimate daily milk, fat and FA yields without the use of milking interval.

A first perspective of this study will be to validate proposed models on larger databases in order to confirm obtained results and to refine suggested equations. Indeed, the validation of such models requires more important, complete, and reliable databases than the one used in this thesis. Unfortunately, collecting enough samples from both, AM and PM milkings and recording all these data from a sufficient number of dairy cows could represent a very important, expensive, and potentially long term work. Currently, the used validation databases were not sufficient to confirm the proposed equations in routine.

Even if the development of conversion equations could allow the phenotypic comparison between used data independent of the recording system, the use of different sampling schemes

could also influence the estimation of genetic parameters of milk and milk fat composition and, therefore, have a potential impact on the genetic parameters and the ranking of animals. Unfortunately, unlike the phenotypic differences between the AM and PM milk composition none or very few information about the influence of the time of milking on the genetic parameters of milk yield, milk and milk fat composition is reported in literature.

Chapter VI was dedicated to this topic, and was focused on the effect of the time of milk recording (AM or PM) on the genetic parameters of milk yield and milk fat composition. In this study, a total of 58,540 test-day records were collected from primiparous Holstein cows in Luxembourg.

Firstly, heritabilities of FA content were calculated. Heritability values obtained from the Luxembourg database were similar to those reported previously by Bastin et al. (2011) carrying out work on Walloon cattle. Our results indicate that the average daily heritabilities for milk were higher in the morning than in the evening (0.23 vs. 0.20, respectively for AM and PM). Unlike milk yield, fat content presented higher heritability values during PM (0.32) than for AM (0.30) milking. Although few or no statistically significant differences were observed between heritability values for FA content between AM and PM, mean differences between the AM and PM heritabilities were higher for saturated FAs (0.04) than for unsaturated FAs (0.02). According to obtained results, estimated heritabilities for saturated FAs from PM milk samples are slightly higher than those estimated from AM milk samples.

Further, we observed that the average daily genetic correlations estimated between AM and PM studied FAs traits were globally ≥ 0.93. These values were higher than those obtained for the average daily correlations between AM and PM FAs traits for permanent environmental effects. This difference could be explained by the numerous biological processes involved in the production of milk FAs. As mentioned previously, milk FAs profile is influenced by a large range of conditions such as diet (Larsen et al., 2012). Lower values of average daily correlation between AM and PM samples for permanent environmental effects could be explained by a feeding effect. Indeed, according to available literature (e.g. Sahana et al. (2008)), cows

consuming the major part of their feed between the AM and PM milking present a higher fat content and a higher content of unsaturated FAs in the afternoon milk compared with the morning milk, whereas the content of *de novo* synthesized FAs is reported to be lower. Consequently, based on our results, our hypothesis is that the underlying genetic effect does not vary strongly throughout the day.

From the obtained results, by taking into account the standard error, the differences in terms of heritability and correlations were often not statistically significant. Consequently, both mean and variance differences can be corrected with appropriate modeling. Sampling type in the evaluation model can be included in the applied genetic model with the use of appropriate fixed effects which allow the correction of mean differences. Our first suggestion of correction is to include a sampling moment x sampling type fixed effect in the model. Secondly, FAs trait heteroscedasticity can also be taken into account as it is done for milk yield (Gengler et al., 2005). However, the observed differences were largely lower than the ones observed for milk yield, for example. So, these results suggest a very limited impact of the time of milking on the genetic evaluation of FA traits.

8.1.3. Condition 4: Genetic and phenotypic variability of selected traits

FAs present the most important part of milk fat, and constitute about 90% of its weight (Samkova et al., 2012). They differ in carbon chain length, on degree of unsaturation, position and number of double bonds. A particularity of milk fat is its high plasticity, compared to the other milk constituents. The milk FA profile can be easily altered using factors changing milk fat composition. These factors could be classified in three main groups: feeding, animal and environment (Samkova et al., 2012). It is well known that milk fat composition is also significantly altered through herd management intervention such as changes in feeding (e.g. Chilliard et al., 2000 and 2001; Grummer et al., 1991). While the effect of cow nutrition on FA

composition is well documented in literature, information about the effect of animal factors is more limited. Breed, stage of lactation and cow individuality are the most frequently studied animal factors affecting milk fat profile (e.g. Kelsey et al., 2003; Soyeurt et al., 2006). Several papers have reported that breed is an important factor affecting milk fat composition. Further, according to Kelsey et al. (2003) and Soyeurt et al. (2006), milk FA profile varies also within a breed.

Data used in Chapter VII were recorded from Luxembourgish dairy herds between October 2007 and April 2013 during conventional milk testing ("S"). These milk samples are composed of 50% AM milk and 50% PM milk and are collected from 21,582 Holstein cows in 163 herds. The observed average milk production was 26.36 kg/day. The fat content was 4.34 g/dL milk having a saturated part equal to 65.9%. Overall, the quantities and content of individual FAs present in the milk samples and fat were consistent with those previously reported for Walloon data (Bastin et al., 2011). The milk and fat yields had similar descriptive statistics compared to the results mentioned by Liu et al. (2008).

We reported previously that milk composition and milk yield could vary with the time of milking (AM or PM). Indeed, milk FA profile from AM milk or PM milk were different. This could be partly explained by the effect of diet. Indeed, the feeding effect is well known and largely described in available literature (e.g. Chilliard et al., 2009; Sahana et al., 2008). A second important factor is the individual animal in terms of breed, lactation number, DIM, etc (e.g. Kelsey et al., 2003; Liu et al., 2000; Mayeres et al., 2004; Soyeurt et al., 2006; Stoop et al., 2008). In Chapter III, the effect of breed on the milk composition is described using a non-exhaustive comparison of differences in FA concentrations in milk fat according to the breed. Holstein and Jersey milk fats present the greatest differences. Higher concentrations of SFAs, especially of FAs with short and medium carbon chains, are observed in Jersey milk fat (e.g., Beaulieu and Palmquist 1995; White et al., 2001). The proportion of C16:0 do not differ significantly between Holstein and Jersey milk fat. According to Lawless et al. (1999), Normande and Montbeliarde cows produce milk fat with the highest proportions of C18:0.

DIM is considered as another important factor affecting milk yield and composition. The stage of lactation or DIM are known as one of the largest factors affecting milk composition (Kelsey et al., 2003; Liu et al., 2000; Stoop et al., 2009). The inclusion of month of test is aimed to catch the effect of season and, indirectly, the feeding effect whose impact on the milk FA composition is largely described in the literature (e.g. Arnould and Soyeurt, 2009; Chilliard et al., 2000). As reported by Arnould et al. (2012), milk yield varies through lactation. Similar observations were found for FA contents. Indeed, according to Figure 1 of Chapter V, SFA content in fat (expressed in g/100g of milk fat) increased until DIM 85 with a decrease at the end of the lactation. According to additional non-published results, we observed a strong decrease in fat content until 45 DIM when fat is at its lowest level. Mean fat and SFA tended to follow the typical curves obtained for milk components decreasing in early lactation and rising again as DIM increased, in contrast to the curve obtained for milk yield. De Vries and Veerkamp (2000), Quist et al (2008), and Soyeurt et al. (2008) obtained similar curves.

Another important effect is the effect of parity. Although the literature data on the effect of parity on the milk FA profile are limited, it is unquestionable that this factor also affects milk fat composition (e.g. Craninx et al., 2008; Kelsey et al., 2003; Samková et al., 2012; Soyeurt et al., 2008). Most available papers categorise cows into two groups, primiparous and multiparous cows. According to the available data, first-calves produce milk fat with a higher proportion of UFA and lower proportion of SFA than cows in second and further lactations (Craninx et al., 2008; Samková et al., 2012; Thomson et al., 2003). Wathes et al. (2007) suggest that there are differences between primiparous and multiparous cows in the control of tissue mobilisation which may promote nutrient partitioning between growth and milk production during the first lactation.

Consequently, information like the individual animal, breed, herd, parity and stage of lactation effects must be taken into account during the development of models. So, in order to limit the effect of animal on the milk FA profile and to optimize the evaluation of genetic parameters, we only kept data from Holstein primiparous cows.

Random regression test-day models (**RRTDM**) remain a commonly used methodology for the estimation of genetic parameters and genetic evaluation for daily milk production traits (Bohmanova et al., 2008; Misztal, 2006). The basic idea of RRM consists of fitting average lactation curves, while random animal specific curves describe deviations from these average curves (Bohmanova et al., 2008). For that, several functions can be used to fit fixed and random regressions. Early applications used parametric functions and lactational shape functions such as the Ali and Schaeffer (1987)and Wilmink (1987) functions. However, those functions, especially for random effects, were subsequently replaced by LP. Models with LP as regressions are orthogonal and, therefore, have better convergence properties than models with parametric or lactational shape functions (Bohmanova et al., 2008). However, to fit the shape of lactation appropriately, higher order polynomials are required. Nevertheless, Bohmanova et al. (2008) reported that RRTDM using LP present undesirable properties, mainly the overestimation of variances at the edges of lactation, which could be explained by lack of asymptotes of LP. Recently, splines have been advocated as a good alternative to LP for analyzing test-day yields in RRM (Bohmanova et al., 2008; Druet et al., 2003; Meyer, 2005). Indeed, some previous research established that splines might be less sensitive to the data than LP and higher flexibility of fitting lactation curves (Misztal, 2006). Mathematically speaking, splines are piecewise polynomial functions. They are defined as curves that consist of individual segments themselves connected in “knots”. The simplest case of a spline function is the linear spline where the segments are fitted by linear polynomials. Consequently, coefficients of linear splines are simple interpolation coefficients between the 2 knots adjacent to the record and 0 between all other knots. Because a maximum of 2 coefficients may be nonzero for a given record, the system of equations with splines is sparser than with LP (Bohmanova et al., 2008; Misztal, 2006).

The study, presented in Chapter V, aimed to compare different models to study genetic parameters of milk SFA production. Throughout this study, three functions were tested and compared to take into account the lactation curve: 1) LP with the same order as currently applied for genetic evaluations of production traits (**LP**), 2) linear splines with 10 knots (**SP10**), and 3) linear splines with the same 10 knots reduced to 3 variables (**SP3**).

The choice of optimal test-day RRM was based on statistical criteria such as Akaike's information criterion (**AIC**) which is widely used in statistics for comparing models (e.g., Druet et al. 2003); Bayesian information criterion (**BIC**) values which was very close to AIC (the BIC is a criterion for model selection among a class of parametric models with different numbers of parameters); Log-likelihood function, and the analysis of residuals (percentage square biases or PSB). Another criterion was the overall shape of the genetic variance function. Results are available in Chapter V.

As expected, the AIC, BIC, and log-likelihood function identified the LP and SP3 models as the most useful models. Model SP10 was the worst model for each parameter. Further, LP and SP3 models needed the least time to converge (2 h 50 min and 17 h 10 min, respectively) and need the lowest number of rounds (1,153 and 978). Convergence rate of SP10 model was much slower (more than 1 month and more than 5,000 rounds). Based on results from this study, the reduced SP3 model was very similar to the LP model. Therefore, LP was the best among the compared models. It can be expected that LP-based models could be used to model production of FA in milk fat for the next researches of this thesis.

The FA content heritability values presented in Chapter VI were similar to those reported previously for Walloon cattle (Bastin et al., 2011). Firstly, according to results, heritability estimates seemed to decrease with FA chain length. Indeed, FA groups (SFA, SCFA, and MCFA) were more heritable than LCFA and UFA, with heritabilities of 0.31 (AM) and 0.35 (PM) for SFA, 0.31 (AM) and 0.35 (PM) for SCFA, 0.32 (AM) and 0.36 (PM) for MCFA, 0.23 (AM) and 0.22 (PM) for LCFA, and 0.24 (AM) and 0.23 (PM) for UFA. These findings were already reported by Bastin et al. (2011). This is easily explained by physiological processes involved in the production of milk FAs. It seems logical that FAs synthesized *de novo* in the mammary gland (SFA and SCFA; 0.31 for both FAs) presented higher heritabilities than those synthesized from blood, so, indirectly from feeding (LCFA; 0.23 and UFA; 0.24). This hypothesis was also confirmed by Chilliard et al. (2001) and Bobe et al. (2008). However, some differences can be noticed with literature values and explained by the origin or the quality of used databases, by the

improvements of the calibrations equations and by the applied genetic model. Indeed, using an animal model instead of a sire model permits to estimate directly the genetic effects of all relatives. Further, this model permits to take into account the performances of ancestors, descendants and collateral relatives, and thus improves the accuracy of the estimation. More recent studies, such as Bobe et al. (2008), Soyeurt et al. (2008b) and, Stoop et al. (2008), used an animal model to estimate the genetic parameters of FAs. These papers reported similar heritability values than those presented in the current thesis.

8.2. Conclusion

Milk FAs, which can be altered by genetics and management practices, are useful indicators of the metabolic status of dairy cows, the nutritional quality of their milk and their environmental impact. The bovine milk FA profile presents interesting properties which could be taken into account in the development of management tools. Indeed, the use of information collected within milk recording can be more than the reporting of performances or the estimation of breeding values. By using milk records differently, value is added to the available data. This thesis proposes methodologies to acquire and to model these traits in view of their use as management tools. This thesis showed the complexity and the heterogeneity of data acquisition due to alternative sampling scheme and proposed two levels of correction: phenotypically by using conversion equations and genetically by modeling separately morning and evening data. For this last correction, the use of Legendre polynomials based models were the most efficient compared to splines based models. The results of this thesis are available for further use in the development of management tools by CONVIS sc.

8.3. Perspectives

A first practical tool was proposed to CONVIS s.c. within this thesis. Monthly descriptive statistics were obtained for milk production and traditional and novel milk composition traits (especially fat, protein, urea, lactose, cells and major groups of FAs). These statistics were available for each dairy farm regardless the milk recording scheme. A first prototype is under scrutiny at CONVIS s.c. The next step will be to apply the 24h-conversion equations obtained in this thesis. As explained previously, this will allow a comparison between dairy farms using different sampling schemes. This thesis contributes also to a better understanding of the phenotypic and genetic variation of FA and to the interpretation of milk fat profile as indicators of dairy cattle system efficiency.

In order to develop practical, useful and innovative tools, some conditions have been presented previously. These four conditions are necessary but not sufficient. Indeed, a fifth condition must be fulfilled. In order to develop a useful tool and to improve the interpretation of milk composition patterns, a reference system must be set up allowing an easy interpretation of available data. Indeed, values obtained and presented in the current studies are only be valid for a given dairy cow population/herd and may not be used as reference values for different dairy herds or cow populations. Some threshold values (e.g. for diseases (Van Haelst et al., 2008)) are already available in literature. However, it would seem to be important to use these thresholds with caution. Indeed, they need to be adapted to the particular dairy cow population under study and validated in the field. Consequently, it is very important to study each dairy cow population separately and to adapt the threshold values accordingly. Finally, the measurement of milk composition is presented as both a monitoring and a prevention tool. So, it can never replace close monitoring of a herd by the farmer and appropriate veterinary care, but may be used as an efficient alert system for preventing health disorders in cows.

As explained previously, CONVIS s.c. proposes several sampling procedures to increase flexibility in their recordings. We already discussed the topic of the milking moment. Another

issue could be the frequency of sampling. Indeed, milk samples could be collected every 2, 4 or 6 weeks. However, 6 weeks interval between two successive milk samplings could be considered as a too long interval, and consequently, decrease the effectiveness of proposed management tools. Further, some diseases such as ketosis could occur, in dairy cattle, in the first few weeks of lactation. Therefore, it could be interesting to apply such management tool more frequently during the first period of lactation (until the week 9).

The development of management tools based on the methodologies proposed in this thesis would open new perspectives for dairy management. This should allow the detection of some metabolic diseases (*e.g.* hyperketonemia) due to management system deficiencies. Indeed, in 2004, Mayeres et al. showed the possibility to predict, with reasonable accuracy, the production of milk, fat and protein production for the next test day by using test-day model (Gillon et al., 2010; Mayeres et al, 2004). This prediction model permits to develop useful management tools. Indeed, the prediction of such values enables a direct comparison between the actual and the expected performances of a cow on each test day specifically for the considered farm. In practice, routine management of milk components during milk recording provides assistance for strategic and management decision. Cows with suspicious production records might be looked after or treated immediately after milking, meeting their individual needs more precisely, thus saving on medical treatment costs and expenses caused by lost milk increasing productivity in the long term. On the herd level, this comparison between actual and estimated production values might be an indicator for malfunctioning of milking equipment, for the existence of general health problem, or for a global management error in the milking herd (Gillon et al., 2010; Mayeres et al., 2004).

There is a real interest to extend such study to the bovine milk FA. Indeed, milk FA profile could be used as sustainability, monitoring, and prevention tools for several pathologies or health disorders in dairy cattle (Arnould et al., 2013). For example, the FA composition of milk could be used to detect ketosis, a metabolic disorder that affects high-producing cows and causes a loss of production and infertility (De Marchi et al., 2014). At the beginning of lactation,

the dairy cow must cope with an important increase in energy demand by the mammary gland for milk production. This is achieved partly by increasing feed intake and partly by fat mobilization from the cow's adipose tissue. However, excessive fat mobilization may induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies (ß-hydroxybutyrate, acetoacetate, and acetone), a state called hyperketonemia.

Subclinical ketosis and negative energy balance are closely linked. Numerous studies are focusing on the energy metabolism (Arnould et al., 2013). The milk fat profile has been shown to substantially change during the first weeks of production (from week 1 to week 12) and to remain unchanged thereafter (Gross et al., 2011). For all these reasons, and as mobilization of adipose tissue precedes the development of ketosis and incorporation of mobilized FAs into milk fat, changes in milk FA composition might be an early indicator of hyperketonemia (Van Der Drift et al., 2012; Van Haelst et al., 2008). Several studies (*e.g.* Gross et al., 2011; Van Haelst et al., 2008) have proposed relative increases in the proportions of LCFA, and especially, C18:1 *cis*9 (g/100g fat) as an interesting indicator of subclinical ketosis (Arnould et al. 2013).

In conclusion, thanks to the study of variability of milk FA profile and to the use of MIR spectrometry, the current PhD thesis opened new promising perspectives for the dairy herd management.

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LIST OF TABLES

CHAPTER II. GENETIC VARIABILITY OF MILK FATTY ACIDS

CHAPTER III. REVIEW : MILK COMPOSITION AS MANAGEMENT TOOL OF SUSTAINABILITY

CHAPTER V. SHORT COMMUNICATION: GENETIC VARIATION OF SATURATED FATTY ACIDS IN HOLSTEINS IN WALLOON REGION OF BELGIUM

CHAPTER VI. SHORT COMMUNICATION: EFFECTS OF ALTERNATIVE MILK RECORDING SCHEMES ON THE GENETIC PARAMETERS OF MILK FATTY ACIDS.

CHAPTER VI. PREDICTIONS OF DAILY MILK AND FAT YIELDS, MAJOR GROUPS OF FATTY ACIDS, AND C18:1 CIS-9 FROM SINGLE MILKING DATA WITHOUT A MILKING INTERVAL

LIST OF FIGURES

CHAPTER V. SHORT COMMUNICATION: GENETIC VARIATION OF SATURATED FATTY ACIDS IN HOLSTEINS IN WALLOON REGION OF BELGIUM

LIST OF PUBLICATIONS, ORAL PRESENTATIONS, AND POSTERS

1. Publications

Arnould V.M.-R., Soyeurt H., Gengler N., Colinet F., Georges M., Bertozzi C., Portetelle D., and R. Renaville. 2009. Genetic analysis of lactoferrin content in bovine milk. J. Dairy Sci. 92: 2151-2158.

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2. Oral Presentations

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3. Posters

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