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PHENOTYPIC AND GENETIC VARIATION OF MILK FATTY ACID COMPOSITION PREDICTED BY MID-INFRARED SPECTROSCOPY IN DAIRY CATTLE

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RIASSUNTO

Il potenziale della spettroscopia nel medio infrarosso (MIRS) è ben noto in letteratura. Numerosi autori hanno testato la capacità del MIRS nella predizione degli acidi grassi (AG) del latte con il desiderio di applicare questa innovativa tecnologia a livello di popolazione, nel tentativo di ottenere grandi quantità di fenotipi da usare in campo genetico. L'accuratezza dei modelli predittivi differisce largamente all'interno dei vari gruppi acidici e in maniera ancor maggiore tra i principali AG individuali; inoltre, risultano difficili le comparazioni tra diversi studi a causa di numerosi fattori come, ad esempio, l'unità di misura utilizzata per esprimere il contenuto di AG, gli algoritmi impiegati per costruire i modelli di predizione, i pre-trattamenti degli spettri in fase di editing e modellizzazione, il numero di campioni e la variabilità biologica dei caratteri nella popolazione usata per mettere a punto le equazioni di predizione. In generale, i modelli costruiti per i gruppi di AG (in particolare SFA e UFA) risultano sufficientemente accurati mentre quelli relativi agli AG individuali sono meno accurati. Lo scopo generale di questo lavoro di tesi è stato quello di studiare gli aspetti fenotipici e genetici del profilo acidico del latte bovino predetto tramite l'utilizzo della tecnologia MIRS.

Il capitolo 1 ha dimostrato l'effettiva possibilità di incremento di accuratezza dei modelli di calibrazione per i gruppi di AG (SFA, UFA, MUFA e PUFA) e per i singoli AG (C14:0, C16:0, C18:0 e C18:1) attraverso l'uso di un metodo di selezione di variabili (UVE, *uninformative variable elimination*) ed uno euristico di ottimizzazione (GA, *genetic algorithm*). Entrambi questi metodi si sono dimostrati capaci nel discriminare le aree di assorbimento dello spettro correlate agli AG nel latte, migliorando le statistiche di calibrazione dei modelli per tutti i caratteri analizzati, soprattutto nel caso di utilizzo di GA. Metodi come questi hanno dimostrato di possedere un grande potenziale di impiego sia come "strumenti di screening" per analisi di tipo qualitativo, sia come "metodi di selezione" per la costruzione di modelli di calibrazione robusti e conservativi. Sono tuttavia

necessari ulteriori approfondimenti per capire l'effettiva efficacia di questi algoritmi, aumentando nel contempo la numerosità campionaria iniziale sia in fase di calibrazione che di validazione.

Il capitolo 2 ha indagato le fonti di variazione fenotipiche che caratterizzano il profilo acidico del latte predetto in routine utilizzando la tecnica MIRS, in quattro razze di vacche da latte allevate in un'area montana: Frisona, Bruna, Pezzata Rossa e Grigio Alpina. I risultati hanno evidenziato differenze statisticamente significative tra le razze in termini di composizione acidica del latte. Tra gli effetti studiati, razza e allevamento si sono rivelati le fonti di variazione più importanti nello spiegare la variabilità del profilo acidico nel latte, seguiti dallo stadio di lattazione e dall'ordine di parto della bovina. Il latte della razza Grigio Alpina ha presentato una composizione acidica definibile più "salutistica", con un minore contenuto di SFA e un più alto contenuto di UFA, MUFA, PUFA e C18:1. Si è inoltre osservato come la fraziona satura del grasso aumentasse dal parto fino a circa 120 giorni di lattazione. Infine, le vacche primipare hanno evdienziato una concentrazione minore di C14:0 e C16:0 rispetto alle pluripare.

Lo scopo del capitolo 3 è stato quello di esplorare l'esistenza di una varianza genetico-additiva per i gruppi di AG (g/100mL di latte) predetti con tecnologia MIRS in un dataset di vacche di razza Frisona. Le ereditabilità stimate per SFA, UFA, MUFA e PUFA sono risultate, rispettivamente, 0.246, 0.069, 0.082 e 0.078, e le correlazioni genetiche tra questi caratteri hanno evidenziato che un incremento del contenuto di grasso può portare ad un aumento di SFA, con effetti indesiderati sulla qualità salutistica finale del prodotto. Inoltre, il contenuto di UFA nel latte è risultato molto correlato (0.95) con la sua parte monoinsatura; pertanto, agire con gli strumenti della selezione sull'incremento degli UFA, determinerà un incremento di MUFA.

Infine, nel capitolo 4 sono stati stimati i parametri genetici dei maggiori AG individuali (espressi come g/100 g di AG totali identificati) predetti con il MIRS nel latte di quattro razze bovine da latte: Frisona, Bruna, Pezzata Rossa e Grigio Alpina. Nel complesso tutti i parametri genetici stimati sono risultati simili nelle quattro razze. Le ereditabilità sono risultate medio-basse, con valori compresi tra 0.18 e 0.30 per i gruppi e tra 0.16 e 0.30 per gli AG individuali. Le correlazioni

genetiche positive tra grasso e SFA, C14:0 e C16:0, e quelle negative tra questi ultimi AG e il C18:0 hanno confermato in parte i risultati ottenuti nel capitolo 3, suggerendo che è possibile utilizzare gli strumenti della selezione per migliorare, dal punto di vista salutistico, il profilo acidico del latte vaccino.

ABSTRACT

The potential of mid-infrared spectroscopy (MIRS) has been well assessed in the literature. Several authors tested the capability of MIRS to predict milk fatty acid (FA) composition with the aim of applying this technology at population level to collect phenotypes for genetic purposes. The accuracy of prediction models largely differs across groups and individual FA, and comparisons among studies are difficult due to several factors such as the unit of measure to express the FA content (milk or fat basis), the computational algorithm used to construct the model, the spectra pretreatments, the number of samples and the biological variability of the trait in the population used to build the prediction equation. In general, prediction models for FA groups (in particular SFA and UFA) are quite reliable whereas those for individual FA are less accurate (especially for FA that are present in low concentration). The overall aim of the present thesis was to study the phenotypic and genetic aspects of FA composition of bovine milk predicted by MIRS.

Chapter 1 demonstrated the feasibility of improving calibration models for both FA groups (SFA, UFA, MUFA and PUFA) and individual FA (C14:0, C16:0, C18:0 and C18:1) through the use of a variable selection method (UVE, uninformative variable elimination) and a heuristic optimization algorithm (GA, genetic algorithm). Both methods were able to discriminate the spectrum areas closely related with FA in milk enhancing the fitting statistics of all the analyzed traits, with GA providing more accurate models than UVE. Methods like UVE and GA have great potential to be used both as "screening tools" for a qualitative analysis and as "selection methods" for the construction of robust and conservative calibration models. Further research is needed to understand how well these algorithms perform in larger dataset in both calibration and validation processes.

Chapter 2 investigated the sources of variation of milk FA profile routinely predicted by MIRS in four Italian cattle breeds reared in mountain area: Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey. Results underlined that a phenotypic variation is present and the four breeds differed in terms of milk FA composition. Among the studied effects, breed and herd were the most

important to explain the variation of FA profile followed by days in milk and parity. In particular, Alpine Grey presented the "healthier" milk FA profile with lower content of SFA and greater content of UFA, MUFA, PUFA and C18:1. The saturated fraction of FA increased from calving until approximately 120 days in milk. Furthermore, milk of first parity cows presented lower concentration of C14:0 and C16:0 than milk of multiparous cows.

Chapter 3 reported the existence of an exploitable additive genetic variation for predicted milk FA groups (g/100 mL milk) in Italian Holstein-Friesian cows. Heritability estimates for SFA, UFA, MUFA and PUFA were moderate to low (0.246, 0.069, 0.082 and 0.078, respectively) and the genetic correlations between these traits suggested that an increase of fat content leads to an increase of SFA with undesirable effects on the healthy quality of milk. Also, if the content of UFA is predisposed to be higher, it will be mostly its MUFA component to increase due to the strong positive genetic correlation between these 2 traits (0.95).

Finally, Chapter 4 estimated genetic parameters of the major FA (expressed as g/100 g of total identified FA) predicted by MIRS in milk of four cattle breeds: Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey. Overall, all the estimated parameters were comparable across breeds and heritability estimates were moderate to low ranging from 0.18 to 0.30 and from 0.16 to 0.30 for groups and individual FA, respectively. The positive genetic correlations between milk fat, SFA, C14:0 and C16:0, and the negative correlations between these FA and C18:0 confirmed findings of Chapter 3 suggesting that genetic selection to address milk FA to a better healthy profile is possible.

MILK FATTY ACIDS AND THEIR SYNTHESIS IN THE MAMMARY GLAND

Bovine milk and dairy products are fundamental part of human diet providing nutrients such as high quality protein, calcium, phosphorus, potassium, magnesium, fat-soluble vitamins and essential fatty acids (FA) (Depeters et al., 1995; Parodi et al., 2004). Milk fat is the most complex of dietary fats existing in microscopic globules surrounded by a membrane called Milk Fat Globule Membrane (MFGM), in an oil-in-water emulsion and distributed to the 95-98% in the nucleus of the globules, to 0.5% in the MFGM and to 1.5-4% in the serum. Milk lipids are mainly triglycerides (98%) with phospholipids, cholesterol, diacylglycerols, monoacylglycerols, free FA and fat-soluble vitamins (A, D, E, K) merging the remaining 2% (Parodi et al., 2004).

The main reason behind milk fat complexity is that it contains over 400 different FA, varying in chain length and number, position and geometry of double bonds (Jensen, 2002). Fatty acids are molecules formed by an aliphatic chain of different length with a single carboxyl group (-COOH) at the end. The aliphatic chain is usually linear and his length, in association with the number of bonds it contains, determines the physical and chemical properties of the specific FA. Although there are several ways to classify FA, the one based on length and degree of unsaturation of the carbon chain seems to be the most useful to summarize their biological functions. Following this criterion, FA can be divided into 3 categories: “short chain” if they contain 6 or less carbon atoms in the aliphatic chain, “medium chain” if the number of carbon atoms is between 7 and 12, and “long chain” if the carbon atoms are beyond 12. Moreover, FA with no double bond in their aliphatic chain are named saturated FA (SFA) and those with one or more double bonds are named unsaturated FA (UFA). Unsaturated FA can be further divided into 2 groups; those with a single double bond are called monounsaturated FA (MUFA) and those with more than one double bond are named

polyunsaturated FA (PUFA). It is also possible to use Greek letters to identify the carbon atoms starting from the carbon next to the carboxyl group (α) and remembering the last one is always called omega (ω) or n allowing the grouping of unsaturated FA into three important families: omega-9 (n-9), omega-6 (n-6) and omega-3 (n-3). In addition, the stereochemistry of the double bond may be in the cis or trans form: a cis double bond inserts a twist in the hydrocarbon chain, which does not happen in the case of the trans conformation.

Up to 70% of the total FA in milk are SFA, whereas the remaining 25% includes MUFA (20%) and PUFA (5%) (Grummer, 1991: Barlowska et al., 2011; Keszycka et al., 2013). Palmitic (C16:0, 30%) is the major individual FA, followed by stearic (C18:0, 12%), myristic (C14:0, 11%) and oleic (C18:1 n-9, 23%) (Malacarne et al., 2002).

The biosynthesis of FA in the secreting cells of mammary gland takes place in the cytosol, following 2 different pathways. Short and medium chain FA (C4:0 to C14:0) and about half of palmitic acid (C16:0) are synthesized de novo from acetate and β-hydroxybutyrate produced inside the rumen. The acetyl-CoA-carboxylase leads to the formation of malonil-CoA from acetic acid synthase and condenses it with molecules of acetate and β-hydroxybutyrate. Subsequently, it is possible to extend the FA chain up to maximum of 14 or 16 carbon atoms (Barber et al., 1997). The remaining 50% of C16:0 and all C18:0 are derived from blood steam absorption of not esterified FA (NEFA), chylomicrons, very low-density lipoproteins (VLDL) and mobilization of adipose tissues through the use of the lipoproteinlipase (LPL) enzyme (Schennink et al., 2008). Oleic FA (C18:1), which is the most important FA in bovine milk, is synthesized starting from stearic acid (C18:0) by the action of Δ-9 desaturase enzyme and, for a minimum percentage, it is produced also by biohydrogenation in rumen (Bauman et al., 2003). Other unsaturated FA, C14:1, cis-9 and C16:1, cis-9 are also produced by unsaturation activity of Δ-9 desaturase in the mammary gland starting from C14:0 and C16:0, respectively . Fatty acids both synthetized *de novo* as well as derived from diet may be used by the mammary gland and adipose tissue for the production of triglycerides and phospholipids.

FATTY ACIDS AND HUMAN HEALTH

Milk and dairy products provide only 15 to 25% of the total fat in human diet but it is considered as the main source of saturated fat, bringing as much as 25% of the total saturated fat in the diet (Palmquist et al., 2006). For this reason, it is important to understand the relationships between milk fat and human health.

First studies on this topic suggested a strong relationship between SFA and an increased risk of atherosclerosis and coronary heart diseases (CHD) (Zyriax and Windler, 2000; Vessby et al., 2001; Sacks and Katan, 2002; Mensink et al., 2003; Rasmussen et al., 2006) leading to a "demonization" of milk and dairy products among the consumers. However, recent studies demonstrated that generalizations about milk FA groups are of little weight and only specific SFA could have negative effects on human health while others may have even positive responses. For instance, short chain FA (SCFA) have no effect on human health, and C4:0 has been recognized as protector of the colonic mucosa reducing the risk of colon cancer by regulating host gene expression involved in intestinal homeostasis (Parodi et al., 1999; Hu et al., 2002; German and Dillard, 2004). Myristic (C14:0) and palmitic (C16:0) FA have been reported to increase low-density lipoprotein (LDL) cholesterol in blood, that is well known for its strong correlation with different coronary heart diseases (Mensink et al., 2003); the same authors also argued that stearic acid (C18:0) does not influence the level of serum cholesterol in human blood, probably due to its rapid conversion into oleic acid (C18:1) in the body.

The unsaturated part of milk fat (MUFA and PUFA) is related to positive effects on human health and they is considered "functional food" (Kris-Etherton et al., 1999; Mensink et al., 2003). Different studies have underlined that MUFA were able to reduce the level of LDL but, unlike PUFA, they mantained constant the level of HDL cholesterol (Mensink et al., 1998). This part is foundamental because HDL exert a protective role against CHD, being able to remove cholesterol

from peripheral cells and reducing the entry of LDL in the vessel walls at the same time. In particular, among MUFA, oleic acid (C18:1) contributes to the maintenance of the structural integrity of cell membranes, increasing the level of unsaturation and cellular metabolism (Chilliard et al., 2000). The most important part of the unsaturated fraction, however, is represented by PUFA. Linoleic acid (C18:2) is part of lipid complexes which lead to the formation of permeability barriers of the epidermis (Downing, 1992) and its deficiency leads to a significant increase in risk of CHD and neurological disorders (Horrobin et al., 1999). All the FA belonging to n-3 nd n-6 series are related to two important functions: they mantain cell structures, in particular in the brain, retina and reproductive system, and they act as catalysts of the enzymes responsible for the contraction of smooth muscle, for the control of chemotaxis and for the modulation of cytokine release (Nielsen et al., 1992; Kremer, 2000); n-3 in particular have preventive properties for hypertension, neurological disorders, depressive stages and they play an important role in brain and retina development (Connor, 2000; Lauritzen et al., 2001). Experimental studies conducted on patients at high risk of cardiovascular diseases have demonstrated that the oral administration of two metabolic derivatives of n-3, the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA), significantly decrease the LDL cholesterol levels on blood.

It is also worth mentioning that there is a kind of "competition" between the n-3 and n-6. High levels of linoleic acid (n-6) inhibit the formation of EPA and DHA (n-3) at the level of their precursor, the linolenic acid. This suggests the enormous importance of the n-6 / n-3 ratio in the diet (Williams, 2000; Dal Bosco et al., 2002).

SOURCES OF VARIATION OF MILK FATTY ACID COMPOSITION

There are several factors that affect the amount and composition of FA in cow milk and they can be divided into two main groups: (i) factors related to the environment ("exogenous" factors) and (ii) factors related to the animal ("endogenous" factors).

Exogenous factors include diet and seasonal effects whereas endogenous factors include physiological effects such as parity, stage of lactation, and breed and animal genetic. The diet is considered as far as the most important source of variation for milk FA and several authors reported that it is possible to change milk fat composition by controlling animals diet (Palmquist et al.,1993; Ferlay et al., 2008). According to these authors, a diet with a fermentable starch intake greater than 50% causes milk fat depression, reducing the total amount of SCFA and increasing the C18:0 fraction. Jensen (2002) reviewed several studies demonstrating that dietary supplements often follow changes in FA profile. Feeding animals with soybean and cottonseeds produced milk with an increasing amount of conjugated linoleic acid (CLA) (>50%), C18:0, C18:2 and C18:3, and a reduction of C16:0 and C18:1. Rations with an increase content of rapeseed and sunflowers increased C18:0 and C18:1 (>55 and >80%, respectively), and decreased SCFA and medium chain FA (MCFA) (< 30%) (Grummer, 1991; Jensen, 2002). Carroll et al. (2006) studied the effect of increasing the level of dietary fat on the composition of milk from primiparous Holstein, Jersey and Brown Swiss cows reporting a modification of the composition of milk fat. The same authors reported that milk fat content of C18:0 and C18:1 increased linearly with increasing supplemental fat whereas C18:2 and C18:3 decreased. It is also possible to change FA composition by alteration of the rumen flora through diet (Heck et al., 2009). The use of not-protected lipid sources leads to an increasing biohydrogenation of C18:2 and C18:3 by the rumen flora eventually resulting in an increment of C18:1 trans 11. Consequently, a diet rich in C18:0 and C18:1 will produce milk with a greater amount of C18:1 cis isomers whereas a diet rich in PUFA will lead to a larger amount of

C18:1 trans isomers. Dietary supplements are the most common and immediate methods to improve the nutritional quality of milk. This approach presents however some important disadvantages: it is expensive, not permanent and it does not take into account the individual genetic of the animal that has been shown to exist (Soyeurt et al., 2006).

The seasonal effect is difficult to study because it is often a confounder of the diet effect. Heck et al. (2009) noticed that FA derived from *de novo* synthesis present a minimum concentration in summer while preformed FA have an opposite trend with a minimum in winter. However this conclusion is really hard to confirm; during the end of spring and the start of summer concentrates are usually replaced by fresh grass and the variation of milk FA occurred just in proximity of this transition period with an increasing of C18:0, C18:1 cis-9, C18:1 trans-11 CLA and C18:3 cis-9, 12, 15. This suggests that seasonal effects on milk FA composition are probably due to dietary origins (Palmquist et al., 1993; Heck eta l., 2009).

Another source of variation of milk FA is the stage of lactation. Recent findings suggest that the composition of milk fat significantly change with the stage of lactation, influencing all the FA with an even number of carbon atoms. Short and MCFA (C6:0 to C14:0) are more concentrated during the 3rd month of lactation; C16:0 reaches the maximum between the 11th and the 21st week of lactation, while the C18:0 present the opposite trend during the same period (Soyeurt et al., 2008; Mele et al., 2009; Stoop et al., 2009). Milk FA can originate from four major pathways: the diet, *de novo* synthesis in the mammary gland, the rumen and body fat mobilization, and milk FA composition changes in relation to the changes in these pathways (Stoop et al., 2009). For this reason, the stage of lactation is often related to the energy status of the animal. The low concentrations of C14:0 and the greater level of C18:0 at the very beginning of the lactation could be explained with the negative energy status of the cows during the first 100 days; this condition leads to lipids mobilization from body reserves (mainly C18:0) and consequently an inhibition of d*e novo* synthesis in the mammary gland (Chilliard et al., 2001). The contribution of *de novo* FA

increases as lactation progresses (Bauchart et al., 1993; Palmquist et al., 1993; De Peters et al., 1995; Bastin et al., 2011).

The effect of parity on FA composition has been widely reported in the literature even if with controversial results. Secchiari et al. (2003) and Kgwatala et al. (2009) reported no parity effect on milk FA composition of Italian and Canadian HF cows, whereas Kelsey et al. (2003) observed significant parity effect on most of the milk FA studied in milk from US Holstein cows. Also, Bilal et al. (2014) reported a significant parity effect analyzing milk of Canadian HF cows reporting a relatively greater proportion of desirable FA on primiparous than multiparous cows. Miller et al. (2006) demonstrated that the primiparous bovine mammary gland was metabolically less active than that of later parity cows, probably leading to a lower expression of FA synthase in the mammary gland. This could be the reason that leads to the lower content of C14:0 and C16:0 in first parity cows, due to the fact that these FA are synthesized *de novo* in the mammary gland (Schennink et al., 2008). This is also a characteristic of diet-induced milk fat depression (MFD), although the reduction in SCFA and MCFA tends to be proportionally greater when the decrease in milk fat yield is more pronounced (Peterson, 2003). Moreover, primiparous cows have lower rumen capacity and consequently lower dry matter intake (DMI) along the first 2/3 of the lactation. This lower capacity to adapt to the lactation diets (i.e. capacity to absorb volatile FA produced by diet fermentation) and this could affect groups and individual FA levels across parity categories. Primiparous cows are more susceptible to developing acidosis after parturition than multiparous cows (Penner, 2007; Krause and Oetzel, 2006). Moreover, after experiencing acidosis, cows become more susceptible to subsequent bouts of acidosis and can have long-term consequences on health, productivity and FA composition (Penner, 2007; Dohme et al., 2009).

Several studies have investigated the differences between FA among dairy breeds asserting the importance of this factor in explaining the variation of FA in milk. De Peters et al. (1995) in their study observed that, under the same feeding and management conditions, Jersey cows presented milk with the highest proportion of FA with chain length from C6:0 to C14:0 and the lowest

proportion of UFA. In contrast, milk fat of Holsteins contained the lowest proportion of FA from C6:0 to C14:0. The ratio of C18:1 to C18:0 was highest for Brown Swiss cows and lowest for Jerseys. Soyeurt et al. (2006) compared the FA profile of Holstein Friesian cows with other 6 different breeds (Belgian Blue, Jersey, Montbeliarde, Normande and Meuse-Rhine-Yssel) finding significant differences ($P < 0.05$) among breeds for all the FA with the exception of C16:1 cis9. The same authors in a later work (Soyeurt et al., 2008) confirmed that Jersey cows produced a milk higher in fat and lower in MUFA percentage than Holstein Friesian animals. They also highlighted the significance of breed effect on Δ9-desaturase activity important for C16:1 cis9/C16:0 ratio. Gottardo et al. (2013) investigated sources of variation on milk FA groups predicted by mid-infrared spectroscopy (MIRS) in mixed dairy herds (Holstein Friesian, Brown Swiss and Simmental) reared under the same herd conditions. The Holstein Friesian breed showed the highest milk production and better FA composition than Simmental and Brown Swiss cows. The Simmental breed was intermediate between Holstein Friesian and Brown Swiss for FA groups. Finally, no significant differences were found among breeds for PUFA. All these studies suggest the possibility to enhance the nutritional quality of milk choosing the breed with the most desirable characteristics (Soyeurt et al., 2008).

GENETIC VARIATION OF MILK FATTY ACIDS

The overall goal of genetic selection is to permanently change the FA composition of milk fat in dairy cows population in order to increase the proportion of UFA and PUFA, and decrease those SFA who are negatively related to human health, in particular C14:0 and C16:0. This tool however is effective only if sufficient additive genetic variation exists (Soyeurt et al., 2007). Stoop et al. (2008) estimated genetic parameters of milk FA using 1,918 first lactation Dutch Holstein cows from 398 herds and reported heritabilities between 0.10 (C18:3) and 0.54 (C10:0). Bobe et al. (2008) reported heritability estimates of 0.01 (C4:0, C6:0, C14:0 and C18:2), 0.18 (C12:0), 0.49

(C16:1), 0.24 (C18:0) and 0.06 (C18:1), using data from a small number of cows (233) from a single herd. Studying 990 Italian Holstein cows from 34 herds, Mele et al. (2009) reported values of heritability for 8 individual FA ranging from 0.03 to 0.19. Moreover, the authors claimed that a selection to increase the fat content of milk could lead to increase C16:0 and to a decrease of UFA. Garnsworthy et al. (2010) observed a similar range of heritability estimates (0.04 to 0.28) for 17 individual FA in 2,048 UK cows coming from 325 herds and parity from 1 to 3. The authors used a sire model and most of their estimates were not statistically different from zero.

Genetic associations between FA in milk are limited to few studies. Renner and Kosmack (1974) and Karijor et al. (1982) were the first scientists that estimated genetic correlations among different FA in milk or fat. They reported positive phenotypic and genetic correlations between SCFA and strong negative correlations between SFA and MUFA. Mele et al. (2009) reported weak positive genetic correlations between C14:0, C16:0 and C18:0, and a strong negative correlation between C16:0 and C18:0 (-0.84). Soyeurt et al. (2007) presented positive phenotypic and genetic correlations between C12:0, C14:0 and C16:0, and negative correlations between these FA and those composing MUFA and PUFA. Penasa et al. (2015) reported similar results with strong and positive genetic correlations between UFA and MUFA, and between SFA and PUFA. The overall results for genetic correlations on this paper indicated that cows are genetically predisposed to produce more SFA when content in milk increases and more UFA when MUFA content increases.

Most studies used data from chromatographic analysis to estimate heritabilities and genetic correlations of FA in milk and fat. This method is accurate and precise (Collomb and Bütikofer, 2002) but is also costly and time-demanding making impossible the collection and the analysis of a large number of phenotypes. As recently reviewed by De Marchi et al. (2014), MIRS seems to be the right answer to collect phenotypes at population level and many studies have investigated the use of predicted phenotypes for genetic purposes: Tiezzi et al. (2013) and Visentin et al. (2015) for milk coagulation properties, Toffanin et al. (2015) for milk major minerals and acidity, McParland et al. (2014) for residual feed intake, and Soyeurt et al. (2007), Bastin et al. (2013), Lopez-

Villalobos et al. (2013) and Tullo et al. (2014) for FA composition. Mid-infrared spectroscopy presents some advantages over other analytical techniques. This technology is fast (one sample scanned in less than 1 minute), cheap (no reagents are needed and a single person can analyze a large number of samples) and easily applicable in different environments (industries, laboratories, universities/research centers etc.).

FOURIER-TRANSFORM MID INFRARED SPECTROSCOPY

Mid-infrared technology is an indirect method of analysis based on the study of the interaction between matter and electromagnetic radiation employing photon energy through the Planck-Einstein relation $E=hv$ where h is the Planck constant and v is photon's frequency, in the mid region of infrared spectrum, between 2,500 and 25,000 nm (Figure 1).

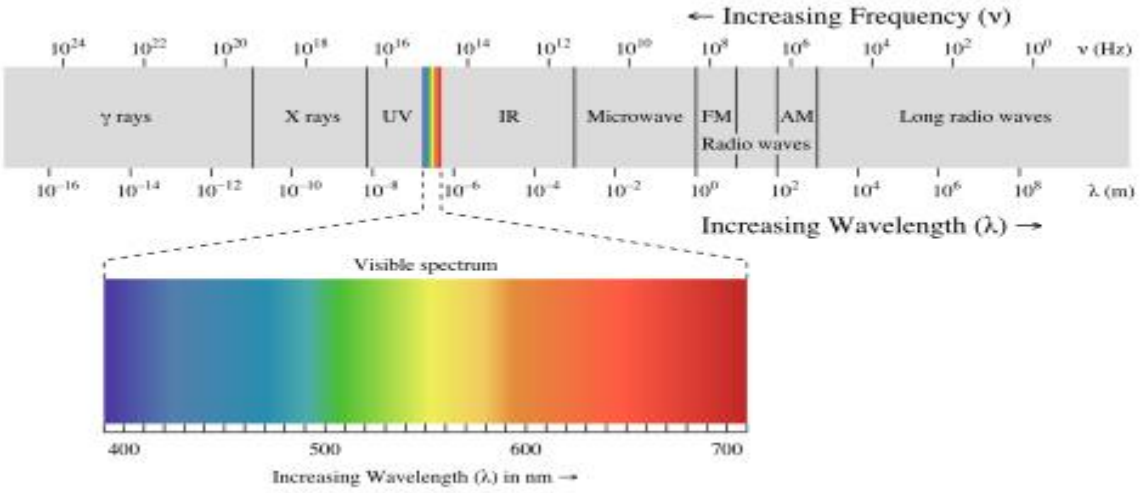

Figure 1: The electromagnetic spectrum

The basic principle of MIRS technique relies on the ability of each chemical compound to absorb, reflect or transmit energy (i.e. light radiation) producing vibrational motions defined as stretching (symmetric or asymmetric) and bending. In the MIR wavelength range some frequencies will be completely absorbed, some other will not be absorbed and some will be partially absorbed

creating a complex figure of absorption intensities called absorption spectra of a substance. Specifically the absorption can only occurs when the vibrational motions generate a change in the dipole moment of the specific molecule or in the local group of vibrating atoms. The magnitude of the absorption band is associated with the magnitude of the dipole change and with its degree of anhamonicity (Riovanto, 2011).

Mid infrared spectroscopy instruments for milk analysis usually work in transmittance mode (T), being able to measure the radiation that the sample does not absorb or reflect, through the relation: $\%T = \frac{P}{P_0}$, where P_0 is the intensity of the incident radiation and P is the intensity of the energy going out to the sample. The absorbance (A) can be calculated solving the equation: $A = Log_{10}\frac{P_0}{P}$. This equation follows the Beer-Lambert law principle establishing a linear relationship between the absorbance (or the transmittance) and the concentration of an absorber of electromagnetic radiation. This linear relationship can be written as: $A = a\lambda * b * c$ where $a\lambda$ is a wavelength dependent absorptivity coefficient of the material, b is the path length trough the sample and c is the analyte concentration. The linear relationship between A and c guarantees the validity of the Lambert-Beer law and it ceases to exist at elevated constituent concentrations in the sample or when a dispersion of energy (light) is present.

The very first infrared instruments where called “dispersive” or “grating” instruments. The key component of these instruments was the monochromator, also known as “grating”. The source of energy was directed along both sample and reference path and then in the monochromator where the light was dispersed. The dispersed light was then directed, by moving the grating trough a narrow slit, into the detector. Each wavelength was measured one at time with the slit monitoring the spectral bandwidth and the monochromator to select the wavelength being measured. The main difficulty with these instruments was the slow scanning process and Fourier Transform Infrared spectrometry (FTIR) was developed to overcome this limitation. This technology replaced the monochromator with a new optical device called interferometer, which produces a unique signal

with all the infrared frequencies encoded into it (Figure 2). Most interferometers employ a beamsplitter that divides the light beam into two different beams: one is reflected off a flat fixed mirror and the other reflects off a flat mobile mirror which moves away from the beamsplitter. The two beams are reflected from the mirrors and recombined at the beamsplitter, generating an interference due to the different travel distances. This interference is "constructive" if the two beams arrive in the same phase and "destructive" if they arrive out of phase. The recombined beam is then directed to the sampling compartment where it will interact with the sample and with the detector (Van de Voort, 1994). The resulting signal is called "interferogram" where every data point is the intensity of energy measured versus the position of the moving mirror in the time domain. This means that as the interferogram is measured, all the frequencies are being measured simultaneously.

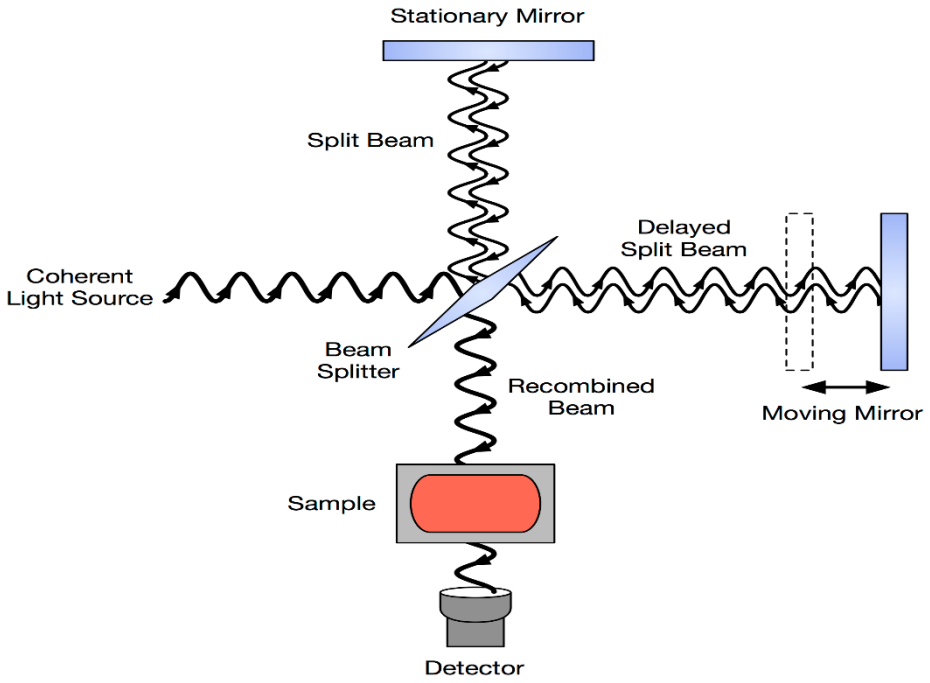

Figure 2: Interferometer scheme (Michelson interferometer)

Because the analyst requires a frequency spectrum, the interferogram (time domain) is converted into a conventional infrared spectrum (frequency domain) through the use of the Fourier Transform, that is basically an integral applied to every point of the interferogram:

$$A(r) = \sum X(k) \exp\left(-2\pi \frac{irk}{N}\right)$$

where A(r) and X(k) are the frequency domain and time domain points, respectively, for a spectrum of N points (Figure 3).

The spectra acquired using MIRS technology contain information related to the samples they come from. In order to extract valuable information about the chemical properties of the samples, mathematical and statistical procedures become fundamental; this is where chemometrics comes into play giving to spectroscopists many different ways to analyze spectral data. Some methods are very simple to understand, while other require a strong background in linear algebra.

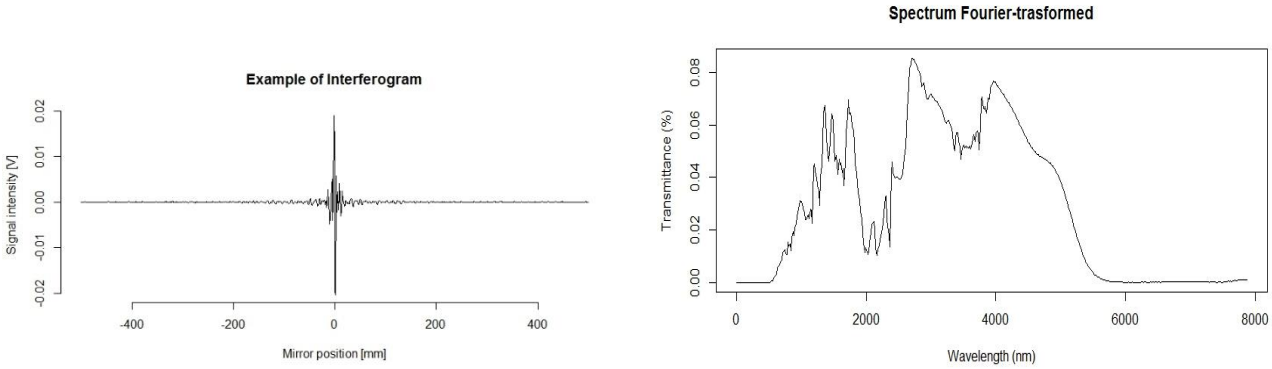

Figure 3: From interferogram to spectrum (Fourier Transform)

The main goal of chemometric tools on MIRS field is to create a calibration equation that relates spectral data matrix to its specific vector of concentrations under the assumption of Lambert-Beer's Law, which when applied to data of unknown samples measured in the same way, will accurately predict the quantities of the constituents of interest. Although different regression models could be used to build a linkage between the independent variables (spectral data) and the dependent variable/s (concentrations of constituents), the most favored and reliable regression technique is partial least squares (PLS) regression. It is based on linear transformation from a large number of

original independent variables (wavelengths or wavenumbers) to a new variable space based on a small number latent factors (or PC, principal components). These latent factors are mutually independent linear combinations of the original descriptors (i.e. orthogonal) and ordered according to the explained variance (i.e. the amount of information they contain). The particularity of PLS regression is that it chooses the latent variables in order to provide maximum covariance with dependent variable and generates two sets of vectors and two sets of corresponding scores (scores are generated multiplying the latent factors and the original independent variables); one set for the spectral data and one set for the constituent concentrations. The two scores matrices are uncorrelated (i.e. orthogonal), allowing the use of a classical linear model to obtain the calibration equation. A key point to perform a "good" and valid PLS regression is to decide the number of latent factors to be retained (i.e. to understand when the information finishes and the noise begins). In fact, the inclusion of excessive factors in the model may decrease the predictivity power of the model as model starts to represent not only the true relations between descriptor but also the random noise of the training dataset (overfitting). On the other hand selecting a lower number of factors would remove useful information decreasing the accuracy of the model (underfitting). Unfortunately there is no clear indication of how many factors are required to avoid overfitting or underfitting but there are some parameters that can help to determine this value monitoring the validity and the feasibility of the calibration equations. These methods are known as "fitting statistics" parameters.

FITTING STATISTICS

Fitting statistics are fundamental to understand the goodness of PLS models in both calibration and validation; these parameters allow us to understand the fitness of a calibration curve and give us some indication about how well the model will predict concentrations in unknown samples (prediction ability). The most common and useful fitting statistics are coefficient of determination (R^2), standard error of calibration (SEC), standard error of prediction (SEP), standard error of cross validation (SECV or 1-VR), predicted residual error sum of squares (PRESS) and the ratio of performance deviation (RPD).

Coefficient of determination (R^2)

The R^2 is defined by the following formula:

$$R^2 = \frac{\sum_{i=1}^{N}(\hat{y}_i - \overline{y})^2}{\sum_{i=1}^{N}(y_i - \overline{y})^2}$$

where $\hat{y}_i$ is the predicted value of y and $\bar{y}$ is the mean of y. This value tells us the amount of variation in the data explained by our model and it goes from 0 to 1. A theoretical value of 1 would indicate that the calibration model explains 100% of the variation within the data, meaning that no differences exists between measured and predicted values (i.e. $\hat{y}_i - y = 0$). Values approaching to 1 are attempted when building a calibration model. This statistic is usually performed in both calibration (R^2_C) and validation (R^2_V) models.

Standard error of calibration (SEC)

The SEC is calculated as:

$$SEC = \left(\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N - K - 1} \right)^{1/2}$$

where N is the number of samples and K is the number of wavelengths used in developing the equation. The SEC will decrease with the number of wavelengths used in the equation because increasing the number of term will allow more variation within the data to be fitted. This value is very useful to compare the differences between values obtained from the reference method and those predicted by MIRS. It is also very helpful to estimate a theoretical accuracy using a specific set of wavelengths during the development of the equation (Riovanto et al., 2011). The SEC takes the name of standard error of prediction (SEP) when is performed for samples outside the calibration set using a specific calibration equation (i.e. is performed as validation statistic) and is usually greater than SEC, giving indications of overall calibration performance.

Standard error of cross-validation (SECV)

The SECV is obtained as:

$$SECV = \left(\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N - 1} \right)^{1/2}$$

This statistics is useful to understand the optimal number of PC to be used for building the model and it must be as small as possible. The SECV is considered as an estimation of SEP with N-1 degrees of freedom. It measures the ability of the calibration equation to predict unknown values through the use of an iterative algorithm which selects samples from a sample set population to

build the prediction equation (train set) and tests it on the remaining unselected set of samples (test set).

Predicted residual error sum of squares (PRESS)

The PRESS is the best method to decide the optimal number of PC to be included in the model. This algorithm fits the model using just one-factor and N-1 samples. After the calibration is developed, the algorithm uses it to predict the sample left out and records the residual value $(y_i - \hat{y}_i)$ for this sample. This procedure iterates for the entire sample set and the sum of squares for the residuals is reported. The PRESS algorithm then starts again with two-factors and will stop when a pre-decided number of factors is reached. The best model is the one with the lowest number of factors and the smallest sum of squares of the residuals.

Ratio of performance deviation (RPD)

The RPD is calculated as follows:

$$RPD = \frac{SD}{SEP}$$

where SD is the standard deviation of the analyzed trait. This simple statistic enables the relative evaluation of a SEP in term of the SD of the reference data. If the SEP value is equal to the SD of the reference data it means that the MIR instrument is not able to predict accurately any value and it should be much lower than the SD. The ideal RPD ratio should be 5 or higher; however a value larger than 2 is considered adequate for analytical purposes (Karoui et al., 2006).

In recent years, some authors have attempted to predict milk FA composition using MIRS and these studies were conducted using different number of samples, different spectra pretreatments, different reference methods and different units of measure. For all these reasons, it is often difficult to compare studies in the literature. The unit of measure of FA, in particular, is a crucial point in the development of accurate MIRS prediction models (De Marchi et al., 2014). Soyeurt et al. (2006) and Rutten et al. (2009) predicted FA composition expressed in two different units of measure, i.e., per unit of milk (g/dL of milk) and on total fat (g/100 g of fat). Both authors reported that the accuracy of MIRS predictions were better when FA were expressed on a milk basis.

Prediction models achieved better results for SFA than UFA (Soyeurt et al., 2006; Ferrand et al., 2011; Maurice-Van Eijndhoven et al., 2013), and individual FA that performed better were those with a medium number of carbon atoms in their aliphatic chain, from C10:0 to C16:0 (De Marchi et al., 2014). Polyunsaturated FA, especially those usually present in low concentrations, were predicted with moderate to low accuracy. The relationship between FA content in milk and the accuracy of prediction was discussed by Rutten et al. (2009) and De Marchi et al. (2011) who reported a strong relationship between FA concentration and coefficient of determination in cross validation (R^2_{CV}) and RPD. Only Soyeurt et al. (2011) obtained high R^2_{CV} for PUFA (0.81), probably due to the huge variability of calibration data (different breeds, different countries and different production systems) used in their study. It is necessary to emphasize the importance of the dataset structure in the development of good calibration models. Breed of cows, stage of lactation, season and area of sampling are the main aspects to take into account during sampling collection (Rutten et al., 2009; De Marchi et al., 2014). The development of MIRS equations using milk from different breeds, countries and seasons, coupled with the use of the same reference method and unit of measure seems the best way to improve the accuracy and the robustness of MIRS models.

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AIMS OF THE THESIS

The general aim of the present thesis was to investigate the phenotypic and genetic aspects of milk fatty acid (FA) composition of bovine milk predicted by mid-infrared spectroscopy (MIRS). Specific aims were to:

1. compare two variable selection methods, uninformative variable elimination and genetic algorithm, in the development of MIRS calibration models for milk FA profile;
2. describe the phenotypic variation of individual FA and FA groups on four cattle breeds (Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey), predicted by MIRS;
3. estimate the genetic parameters of milk FA groups predicted by MIRS in Italian Holstein dairy cattle;
4. estimate the genetic parameters of FA profile predicted by MIRS in four dairy (Holstein Friesian and Brown Swiss) and dual-purpose (Simmental and Alpine Grey) cattle breeds.

CHAPTER 1

MILK FATTY ACID COMPOSITION PREDICTED BY MIRS

Variable selection procedures before partial least squares regression enhance the accuracy of milk fatty acid composition predicted by mid-infrared spectroscopy

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ABSTRACT

Mid-infrared spectroscopy is a high-throughput technique that allows the prediction of milk quality traits on a large-scale. The prediction is usually robust for constituents that are more abundant in milk, whereas fine milk composition, including fatty acid (FA) profile, is difficult to determine through the use of partial least squares (PLS) regression only. Therefore two variable selection methods, uninformative variable elimination and a genetic algorithm combined with PLS analysis were used to investigate their impact on the accuracy of prediction equations for milk FA profile. Both algorithms before PLS analysis improved the accuracy of prediction for FA composition of milk, especially for FA that are usually difficult to predict. These results might favor the use of prediction equations in the dairy industry for genetic purposes and payment system.

Key words: genetic algorithm, milk fatty acid, mid-infrared spectroscopy, variable selection

INTRODUCTION

Infrared technologies are fast, cheap and largely used to determine the concentration of a large number of milk components, and the amount of data they produce is outstanding (De Marchi et al., 2014) and not always easy to handle. Methods such as partial least squares (**PLS**) and principal component regression are useful to extract relevant information and potentially build reliable and robust models using the whole spectrum (Geladi and Kowalski, 1986; Thomas and Haaland, 1990). These methods are considered almost insensitive to noise and therefore it is a common belief that a variable selection is not necessary for models construction (Haaland and Thomas, 1988); however in the last decade some researchers have suggested that an efficient variable selection is useful and sometimes necessary to obtain suitable models for both routine and research purposes (Martens and Naes, 1989; Helland, 2001; Chun and Keleş, 2010). In fact, the PLS regression is a projection-based method because it ignores the directions in the variable space where noisy and irrelevant variables are present; however when the number of variables is much greater than the number of observations this property of the PLS estimator ceases to exist. Faber et al. (1995) studied the error propagation

in principal component analysis and reported that the bias of the model is greatly influenced by the number of variables and by the measurement error; if there are several uninformative variables (i.e., a model with a lot of noise) the final PLS estimators will be biased.

Prediction of concentration of individual FA in cow's milk using mid-infrared spectroscopy (**MIRS**) applying PLS is robust and precise for the major FA groups and for some individual FA, but the quality of the prediction decreases for FA that are present in low concentrations (Soyeurt et al., 2006, 2011; De Marchi et al., 2011). The application of a variable selection procedure before PLS regression could lead to a better and less complex prediction model (Mehmood et al., 2012). The present study compared two different methods of variables selection, namely the uninformative variable elimination (**UVE**) procedure proposed by Centner et al. (1996) and a genetic algorithm (**GA**). The UVE procedure has been already tested with satisfactory results on titratable acidity and calcium content of bovine milk (Gottardo et al., 2015), and GA has been mainly used on traits predicted by near infrared spectroscopy, but also reviewed and developed on MIRS by Leardi and González (1998), and tested on milk FA composition by Ferrand et al. (2011). The main goal of the present study was to investigate which of the two methods is the best to select variables and thus to be practically implemented on field conditions.

MATERIALS AND METHODS

Sample Collection and Mid-Infrared Spectra Acquisition

Individual milk samples of 63 Holstein-Friesian, 24 Brown Swiss and 25 Jersey cows from parity 1 to 7 and from 7 to 408 DIM were collected in 4 herds between February and March 2015, during the morning milking. Milks were immediately added with preservative (Bronopol, 2-bromo-2-nitropropan-1,3-diol), transferred at 4°C to the laboratory of the South Tirol Dairy Association (Bolzano, Italy) and analysed for milk chemical composition using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). For each sample, the absorbance spectrum contained 1,060 infrared data points over the spectral range from 900 to 5,000 cm^{-1}. An aliquot of each sample was

transferred to the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy) for FA analysis.

Milk Fatty Acid Analysis

Milk lipids were determined with accelerated solvent extraction method using Dionex ASE 350 system (Thermo Scientific, Dreieich, Germany) with petroleum ether in isopropanol (2:1) as solvent. Methyl esterification of FA was carried out according to Palmquist and Jenkins (2003) with a basic/acid reaction. Fatty acid separation and quantification were performed by a gas chromatography Agilent 7820A GC System equipped with an automatic sampler G4567A (Agilent Technologies, Santa Clara, CA) and flame ionization detector. The column used was a Supelco Omegawax capillary column (30 m of length, 0.25 mm of inner diameter and a film thickness of 0.25 μm). Temperatures of injector and flame ionisation detector were set at 250°C. Oven temperature was initially 50°C for 2 min and then increased at 4°C/min to 220°C and held for 18 min. Hydrogen was the carried gas and its flow was set at 1 mL/min with average speed of 21 cm/s. Fatty acid standard Supelco FAME mixC4–C24 #18919-1AMP (Sigma-Aldrich, Castle Hill, Australia) was analysed before gas chromatographic analysis for FA identification. Determination of FA values were obtained using GC ChemStation software (Agilent Technologies, Santa Clara, CA) and were expressed both on total identified FA and on a milk basis. Individual FA were C4:0, C6:0, C8:0, C12:0, C14:0, C16:0, C16:1n7, C18:0 and C18:1n9, and groups of FA were SFA, unsaturated FA (**UFA**), MUFA and PUFA.

Statistical Analysis

Spectral Information. Spectral data were transformed to absorbance using the $\log_{10}$ of the reciprocal of the transmittance and the 1,060 wavenumbers were reduced to 480 through the elimination of two spectra regions (1,601 to 1,717 and 3,052 to 5,011 cm^{-1}) which are known to be related to water absorption and thus characterized by high noise (Hewavitharana and van Brakel,

1997). Prediction equations were derived based on PLS regression using the ChemometricsWithR package (Wehrens, 2011) and the possible presence of outliers was checked using the robust Mahalanobis distance procedure. Due to the relatively low number of observations we decided to perform a leave-one-out cross-validation instead of an external validation. Wavenumbers to be included in as dependent variables of the PLS regression were selected based on two methods, UVE and a GA. Both UVE and GA were run using R (64 bit) statistical software (R Core Team, 2015). The UVE procedure was performed using a homemade script whereas GA using the "rgba.bin" function implemented in the "genalg" package of R.

Uninformative Variable Elimination Procedure. The UVE procedure was proposed by Centner et al. (1996) and involves the addition of artificial noises variables to the original wavelength matrix of predictors in order to create a filter. All the original variables having lower stability than the noisy artificial variables are eliminated. The procedure is repeated until a stop criterion is reached. The optimal number of principal components (**A**) must be determined for each trait before starting the procedure and using the lowest root mean square error of cross-validation (**RMSE**$_{CV}$) as decision criterion, defined as:

$$RMSEcv = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{N - k - 1}}$$

where y_i is the actual concentration of a FA, $\hat{y}_i$ is the predicted concentration of the FA, N is the number of samples and k is the number of principal components retained in the model. The UVE procedure consists of the following steps:

1. Generation of a random noise matrix with dimensions N*p, where N is the number of observations and p is the number of predictors (wavenumbers) in the dataset. Random entries must be generated from an uniform distribution in the interval 0.0 to 1.0 and multiplied by a very small constant (10^{-10} in our case).
2. Combination of the original wavelength matrix with the pseudo-random numbers matrix, which leads to a new matrix called Z, with dimension N*2p.

3. Application of a PLS regression on the Z matrix using a leave-one-out cross-validation with the optimal number of principal components determined as above.
4. Use of the leave-one-out coefficients obtained through jack-knifing to compute a stability criterion (**c_j**) for each variable as: $c_j = \frac{mean(\hat{\beta}_j)}{sd(\hat{\beta}_j)}, for\ j = 1, 2, 2p,$ where $mean(\hat{\beta}_j)$ and $sd(\hat{\beta}_j)$ are the average and standard deviation of the vector of n coefficients obtained by leave-one-out jackknifing validation.
5. Setting the filter using the absolute highest stability criterion among random noisy variables as threshold value. If the absolute highest stability criterion of a random variable is greater than the one of an original variable, then the original variable is uninformative and discarded from the model.
6. Performing a new PLS with the remaining variables and check the model performances using the $RMSE_{CV}$. If the new $RMSE_{CV}$ is larger than $RMSE_{CV}$ obtained from point 3, the algorithm stops, otherwise the procedure restarts from point 1, but with A-1 principal components.

Genetic Algorithm. Genetic algorithm is a heuristic approach that combines Mendelian genetics and Darwinian evolution to find a nearby optimal solution through the simulation of these two principles. There is not an "ideal" GA as the success of the algorithm is strictly depending on how well it has been adapted to the specific problem to be solved (Leardi, 2003). The basic idea of GA is quite simple; variables that produce models with better fitting statistics have higher probability to survive and to be reused on another generation of solutions. Variables (wavenumbers in our case) take the name of "genes" and the whole spectra "chromosome". After a fixed number of generations or when the fitting statistics remain constant for a pre-decided consecutive number of generations the algorithm will stop. The main risks of GA are over fitting and random correlation among variables. Some criteria have been proposed to avoid these problems. According to Leardi (2003), the original number of variables must not exceed 200 and the ratio observations to variables

must not be greater than 5. To meet these requirements we reduced the data to 160 dependent variables (wavenumbers) by using the mean of three contiguous wavenumbers and GA was performed on this new dataset. The GA was carried out according to the following steps:

1. Generation of an initial population of genes expressed by bits where "1" represents selected variable and "0" not selected variable. The selection of the initial population was performed using a pseudo-random cycle that selected an average of 5 genes per chromosome. A maximum of 30 genes per chromosome were selected.
2. Generation of 30 chromosomes as solutions through a pseudo-random step.
3. Fitting a PLS model to each solution and running a full leave-one-out cross-validation. The fitting statistic used was the $RMSE_{CV}$, defined as above.
4. Retention of the best solutions, i.e. solutions with the lowest $RMSE_{CV}$. We retained the 20% best solutions. These solutions will survive until the "next" generation.
5. Mutation was applied with a probability of 1% per gene (one unselected variable becomes selected and vice versa).
6. Creation of a "new generation"; a new pool of solutions including the best 20% of the previous generation.
7. This new set of variables serves as input for point 3.
8. Steps 3 to 5 were repeated 100 times (100 generations).

In order to avoid a sub-optimal variable selection, the procedure was repeated 30 times. The variables were selected according to the percentage of their selection during each of the 30 runs and a threshold of 80% was chosen for this task.

Fitting Statistics. The goodness of fit of the equations in the validation dataset was checked according to $RMSE_{CV}$, as described above, and the coefficient of determination in leave-one-out cross validation ($\mathbf{R^2_{CV}}$), calculated as:

$$R^2_{CV} = \frac{\sum_{i=1}^{N}(\hat{y}_i - \overline{y})^2}{\sum_{i=1}^{N}(y_i - \overline{y})^2}$$

where $\bar{y}$ is the mean of the actual values. In addition, the practical utility of prediction models was investigated through the ratio performance deviation (**RPD**):

$$RPD = \frac{SD}{RMSE_{CV}}$$

where SD is the standard deviation of reference values of the trait. Values of RPD greater than 2 are considered adequate for analytical purposes (Sinnaeve et al., 1994).

RESULTS AND DISCUSSION

Fatty Acid Composition

Means and SD of FA expressed as g/100 g of total identified FA and g/100 mL of milk are presented in Tables 1 and 2, respectively. The composition of both FA groups and individual FA was consistent with findings reported by several authors (De Marchi et al., 2011; Ferrand et al., 2011; Soyeurt et al., 2011). Saturated FA were the most abundant followed by MUFA and PUFA. Among individual FA, C16:0 and C18:1n9 were the most represented and averaged 29.78 and 18.78 g/100 g of total identified FA, and 0.99 and 0.60 g/100 mL of milk, respectively. The coefficient of variation for FA groups and individual FA expressed on total identified FA ranged from 0.05 (SFA) to 0.23 (C16:1n7), and for FA groups and individual FA expressed on a milk basis it ranged from 0.25 (C8:0) to 0.40 (C12:0 and C16:1n7). Overall, the traits exhibited an appreciable variation, which facilitated the development of prediction models.

Selected Variables

Both UVE and GA procedures selected wavenumbers that corresponded to the absorption areas closely associated to FA: the first one between 2,823-3,016 cm^{-1} and the second one between 1,736-1,805 cm^{-1}. The absorption area between 1,736-1,805 cm^{-1} is of particular importance because it corresponds to the zone of absorption of carbonyl groups (Coates, 2000). On average, UVE algorithm selected more wavenumbers (160) than GA (114). Also, UVE retained more

wavenumbers on FA expressed on a milk basis (172) than as g/100 g of total identified FA (140). The average number of wavenumbers selected by GA was almost independent from the unit of measure of FA (Tables 1 and 2).

Fitting Statistics

Calibration equations of FA composition expressed as g/100 g of total identified FA (Table 1) were less accurate than equations of FA composition expressed as g/100 mL of milk (Table 2). This result is mainly related to a different dispersion of values for FA concentration in milk and milk fat, i.e., two milk samples could have the same fat profile but different fat percentage in milk (Soyeurt et al., 2006; De Marchi et al., 2014).

Prediction models for FA composition obtained by UVE + PLS and GA + PLS regressions exhibited better fitting statistics than models developed using PLS regression only, and GA + PLS led to better results than UVE + PLS (Tables 1 and 2). For traits expressed on total identified FA, the R^2_{CV} from PLS, UVE + PLS, and GA + PLS regression ranged from 0.43 (PUFA) to 0.78 (SFA), 0.52 (C16:n17) to 0.83 (SFA and C12:0), and 0.54 (PUFA and C16:1n7) to 0.85 (SFA), respectively (Table 1). When expressed on a milk basis, the R^2_{CV} ranged from 0.60 (PUFA) to 0.93 (C8:0), 0.72 (PUFA) to 0.94 (SFA, UFA and MUFA), and 0.74 (PUFA) to 0.98 (SFA), respectively (Table 2).

Compared to PLS regression, the UVE + PLS approach improved the accuracy of prediction models of SFA, UFA, MUFA and PUFA expressed as g/100 g of total identified FA by 7%, 6%, 15% and 22%, respectively (Figure 1). These percentages increased to 9%, 11%, 24%, and 25%, respectively, when GA + PLS approach was used. Similar findings were reported for FA expressed on a milk basis, with the exception of MUFA, whose accuracy improved marginally compared to PLS regression only (Figure 2). Compared to UVE + PLS, GA + PLS regression improved the accuracy of prediction of FA groups between 2% (SFA) and 7% (MUFA) when expressed as g/100 g of total identified FA, and between 1% (UFA and MUFA) and 4% (SFA) when expressed on a

milk basis. The accuracy of prediction models for individual FA expressed as g/100 g of total identified FA increased between 5% (C12:0 and C18:0) and 13% (C6:0) when UVE + PLS instead of PLS regression only was used. These values ranged from 6% (C12:0) to 16% (C8:0) when UVE + PLS was applied (Figure 1). The accuracy of FA expressed on a milk basis was less influenced by the statistical approach compared with FA on total identified FA, particularly for C4:0, C6:0, C8:0 and C16.0 (Figure 2). Overall, it appeared that the two methods performed better on traits that were poorly predicted by PLS regression only.

Results for FA expressed on a milk basis were comparable to those reported by Ferrand et al. (2011), after a GA optimization, except for PUFA which were much better predicted in the present study (R^2_{CV} of 0.74) than in Ferrand et al. (2011) (R^2_{CV} of 0.38). To our knowledge, no other studies dealt with selection algorithms for variable selection before PLS analysis for assessing milk FA composition. However, several authors (e.g., Soyeurt et al., 2006, 2011; De Marchi et al., 2011; Lopez-Villalobos et al., 2014) used PLS regression on MIRS spectra collected to predict FA profile of bovine milk expressed on a fat or milk basis, and results from those studies were worse than those obtained in the present work using UVE + PLS or GA + PLS.

The practical utility of prediction models was ascertained using the RPD. When UVE + PLS regression was applied on traits expressed as g/100 g of total identified FA, two FA groups (SFA and UFA) and four individual FA (C4:0, C6:0, C12:0 and C14:0) exhibited RPD values equal or greater than 2, suggesting that prediction models for these features are useful for analytical purposes (Sinnaeve et al., 1994). In the case of GA + PLS regression, the practical utility of prediction models was reached also by MUFA and C18:1 n9. When the two statistical algorithms were applied to traits expressed on a milk basis, RPD values were much higher than those calculated for FA expressed as g/100 g of total identified FA and were always greater than 2, with the only exception of PUFA for UVE + PLS.

Computational Time

All the analyses were performed using a quad-core (2.40 GHz) laptop with 8 Gb of RAM and a 64-bit operating system. The UVE + PLS procedure required approximately 8 min to complete the analyses, whereas GA + PLS required an average of more than 1 h per trait. As reported by Ferrand et al. (2011), this limit can be justified if counterbalanced by a significant improvement of the accuracy. In the present study, the use of variable selection procedures before PLS regression improved the accuracy of prediction models for milk FA composition, and thus both UVE and GA are advisable to build more robust equations compared with PLS regression only. Also, the improvement concerned mainly FA that are usually difficult to predict (e.g. PUFA). The comparison between UVE + PLS and GA + PLS showed that the second led to overall slightly better accuracies than the first approach, especially when FA were expressed on a milk basis, and thus UVE + PLS could be preferred in routine conditions. However, further research is needed to better compare the two variable selection procedures.

One feature that should be emphasized is the objectivity of the two selection algorithms. The construction of the UVE selection filter is absolutely not influenced by any external decision (Centner et al., 1996; Mehmood et al., 2012) as well as the choice of the various parameters in the GA procedure (Leardi and González, 1998; Leardi, 2003; Ferrand et al., 2011).

CONCLUSIONS

There is a growing interest for the determination of the fine composition of milk and MIRS might be a fast and cheap technique to collect new phenotypes at both herd bulk and individual cow level. However, the routine application of prediction models requires the equations to be accurate. The present study highlighted that variable selection approaches (UVE and GA) before PLS analysis are useful to improve the accuracy of prediction for FA composition of milk. This would

facilitate the use of prediction equations in the dairy industry for genetic purposes and for payment system.

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Table 1. Fitting statistics[1] of validation set obtained by partial least squares (PLS) regression only, uninformative variable elimination (UVE) + PLS regression, or genetic algorithm (GA) + PLS regression for milk fatty acid (FA) composition expressed in g/100 g of total identified FA

		PLS			UVE + PLS				GA + PLS			
Trait	Mean (SD)	#PC	R^2_{CV}	$RMSE_{CV}$	W	R^2_{CV}	$RMSE_{CV}$	RPD	w	R^2_{CV}	$RMSE_{CV}$	RPD
Group of FA												
SFA	67.53 (3.46)	16	0.78	1.63	105	0.83	1.40	2.46	105	0.85	1.35	2.56
UFA	32.47 (3.42)	17	0.74	1.74	184	0.78	1.58	2.16	132	0.82	1.45	2.36
MUFA	27.12 (3.42)	17	0.61	2.14	150	0.70	1.87	1.83	123	0.75	1.70	2.02
PUFA	5.35 (1.08)	18	0.43	0.77	82	0.53	0.70	1.46	96	0.54	0.69	1.48
Individual FA												
C4:0	1.84 (0.33)	15	0.73	0.17	189	0.82	0.14	2.35	147	0.84	0.13	2.49
C6:0	1.76 (0.24)	13	0.72	0.13	160	0.82	0.10	2.35	114	0.83	0.10	2.46
C8:0	1.20 (0.18)	18	0.64	0.11	135	0.71	0.10	1.85	138	0.74	0.09	1.95
C12:0	3.14 (0.58)	13	0.79	0.27	95	0.83	0.24	2.41	105	0.84	0.24	2.47
C14:0	10.04 (1.18)	17	0.69	0.65	174	0.75	0.59	2.00	135	0.78	0.55	2.13
C16:0	29.78 (2.72)	16	0.66	1.58	145	0.72	1.43	1.89	126	0.73	1.40	1.94
C18:0	10.99 (2.20)	20	0.60	1.39	150	0.63	1.34	1.64	144	0.64	1.32	1.67
C16:1n7	1.43 (0.33)	18	0.47	0.24	144	0.52	0.23	1.45	105	0.54	0.22	1.47
C18:1n9	18.78 (3.19)	20	0.68	1.80	119	0.73	1.64	1.94	132	0.77	1.51	2.11

[1]PC = optimal number of principal components; R^2_{CV} = coefficient of determination in leave-one-out cross validation; $RMSE_{CV}$ = root mean square error in cross validation; w = number of variables (wavenumbers) selected after UVE and GA procedures; RPD = ratio performance deviation, calculated by dividing the SD of reference values to the $RMSE_{CV}$ of the trait.

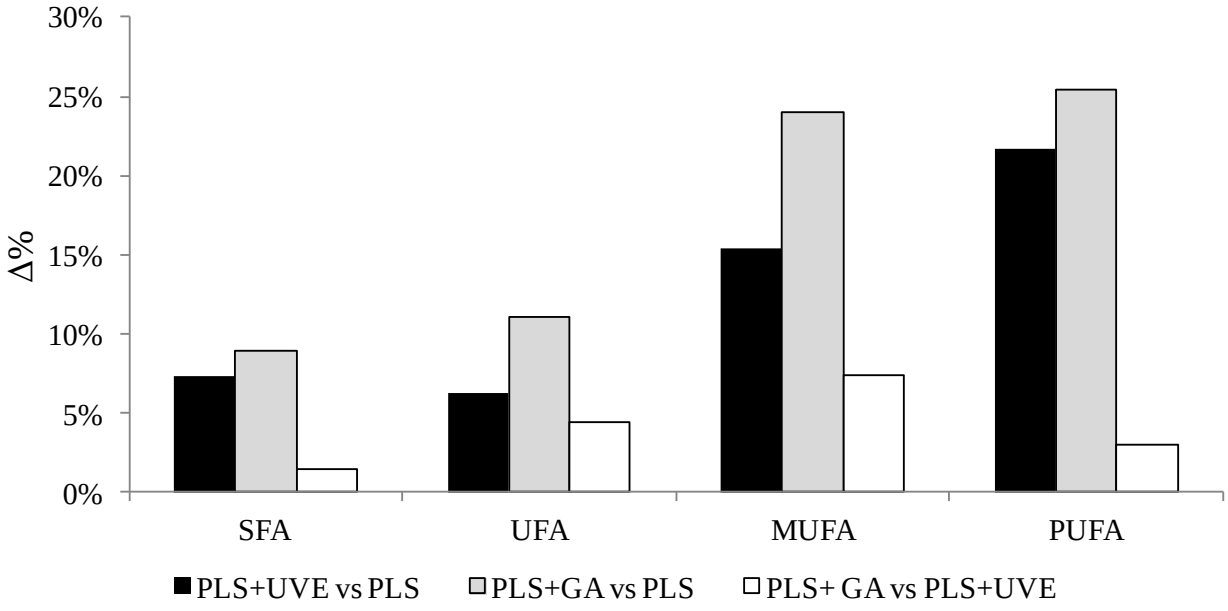
Table 2. Fitting statistics[1] of validation set obtained by partial least squares (PLS) regression only, uninformative variable elimination (UVE) + PLS regression, or genetic algorithm (GA) + PLS regression for milk fatty acid (FA) composition expressed in g/100 mL of milk

		PLS			UVE + PLS				GA + PLS			
Trait	Mean (SD)	#PC	R^2_{CV}	$RMSE_{CV}$	W	R^2_{CV}	$RMSE_{CV}$	RPD	w	R^2_{CV}	$RMSE_{CV}$	RPD
Group of FA												
SFA	2.24 (0.61)	5	0.90	0.16	350	0.94	0.13	4.68	150	0.98	0.10	6.08
UFA	1.05 (0.30)	19	0.87	0.11	138	0.94	0.08	4.05	144	0.95	0.07	4.42
MUFA	0.90 (0.27)	20	0.90	0.09	190	0.94	0.07	4.07	123	0.95	0.06	4.33
PUFA	0.15 (0.05)	15	0.60	0.03	90	0.72	0.02	1.95	90	0.74	0.02	2.07
Individual FA												
C4:0	0.06 (0.02)	15	0.91	0.01	259	0.92	0.01	3.56	102	0.92	0.01	3.61
C6:0	0.06 (0.02)	12	0.92	0.00	149	0.93	0.00	3.79	129	0.94	0.00	4.02
C8:0	0.04 (0.01)	12	0.93	0.00	125	0.93	0.00	3.91	117	0.94	0.00	4.18
C12:0	0.10 (0.04)	14	0.85	0.01	68	0.91	0.01	3.30	87	0.93	0.01	3.89
C14:0	0.33 (0.09)	11	0.89	0.03	40	0.92	0.03	3.61	75	0.93	0.02	3.89
C16:0	0.99 (0.30)	6	0.90	0.09	300	0.91	0.09	3.36	135	0.92	0.08	3.64
C18:0	0.36 (0.13)	15	0.73	0.07	252	0.79	0.06	2.17	111	0.80	0.06	2.26
C16:1n7	0.05 (0.02)	15	0.76	0.01	100	0.81	0.01	2.30	120	0.82	0.01	2.37
C18:1n9	0.60 (0.21)	20	0.83	0.08	167	0.91	0.06	3.28	147	0.93	0.05	3.80

[1]PC = optimal number of principal components; R^2_{CV} = coefficient of determination in leave-one-out cross validation; $RMSE_{CV}$ = root mean square error in cross validation; w = number of variables (wavenumbers) selected after UVE and GA procedures; RPD = ratio performance deviation, calculated by dividing the SD of reference values to the $RMSE_{CV}$ of the trait.

Figure 1. Percentage variation (Δ%) of coefficient of determination in leave-one-out cross validation (R^2_{CV}) of prediction models for A) milk fatty acids (FA) groups and B) individual milk FA (expressed in g/100 g of total identified FA) obtained by comparing uninformative variable elimination (UVE) + partial least squares (PLS) regression vs. PLS regression, genetic algorithm (GA) + PLS regression vs. PLS regression, and GA + PLS regression vs. UVE + PLS regression.

A)



B)

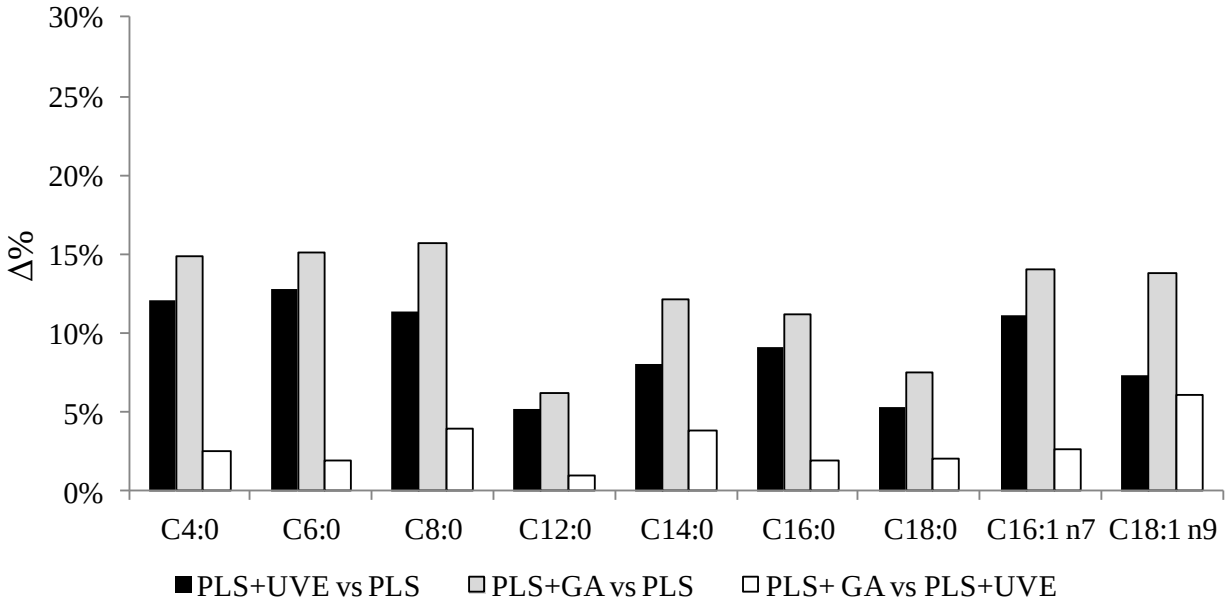
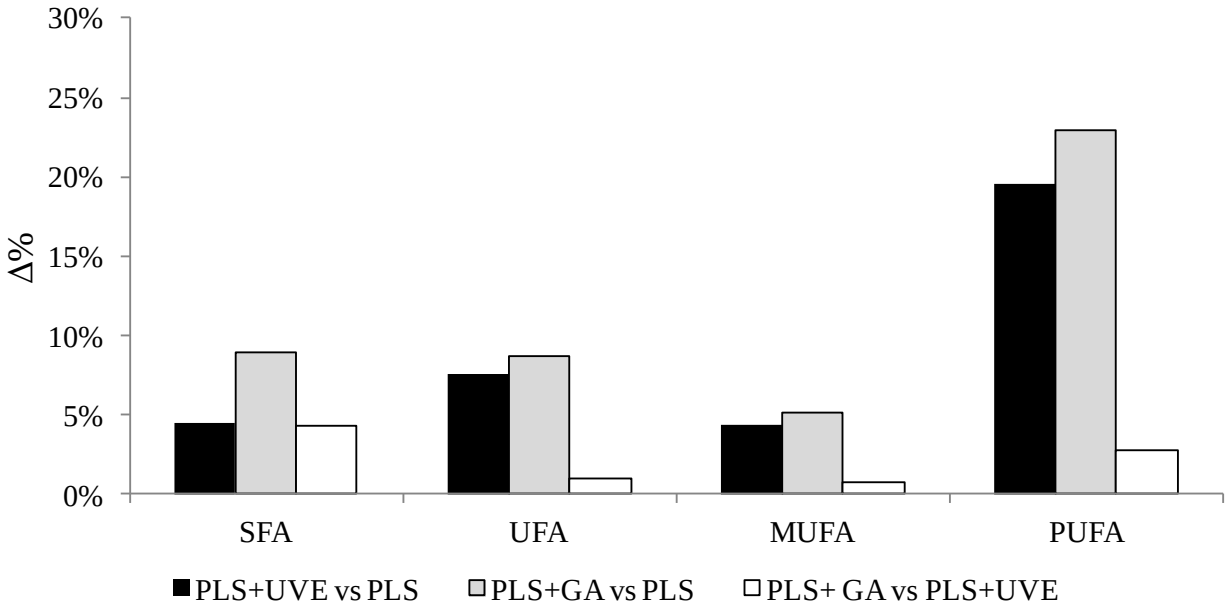
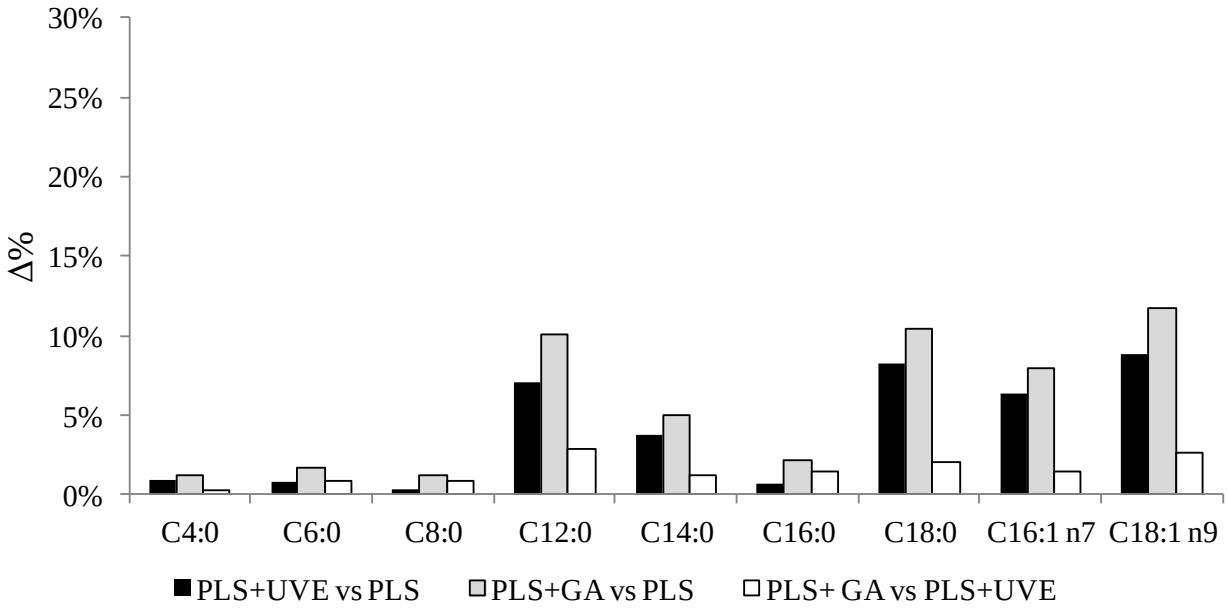


Figure 2. Percentage variation (Δ%) of coefficient of determination in leave-one-out cross validation (R^2_{CV}) of prediction models for A) milk fatty acids (FA) groups and B) individual milk FA (expressed in g/100 mL of milk) obtained by comparing uninformative variable elimination (UVE) + partial least squares (PLS) regression vs. PLS regression, genetic algorithm (GA) + PLS regression vs. PLS regression, and GA + PLS regression vs. UVE + PLS regression.

A)



B)



CHAPTER 2

FATTY ACID PROFILE OF BOVINE MILK

Fatty acid composition of milk from Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey cows predicted by mid-infrared spectroscopy

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ABSTRACT

The aim of this study was to investigate the sources of variation of groups and major individual fatty acids (FA) routinely predicted by mid-infrared spectroscopy (MIRS) in four cattle breeds reared in an alpine area of northeast Italy. Individual milk samples (n = 153 801) of 14 301 Holstein-Friesian (HF), Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG) cows from 1000 single breed herds were analysed for traditional milk quality traits and groups and major individual FA through MIRS. Sources of variation were investigated using a linear mixed model which included fixed effects of breed, month of sampling, year or sampling, stage of lactation (DIM), parity and first order interactions between them, and the random effects of herd nested within breed, cow nested within breed and the residual. Breed and herd effects were the most important to explain the variation of FA composition of milk, followed by DIM and parity. Saturated FA, C14:0 and C16:0 increased from calving until 120 DIM while unsaturated FA and C18:1 decreased. Furthermore, first parity cows presented lower concentration of *de novo* FA (C14:0 and C16:0) than multiparous cows. The greatest content of unsaturated FA and C18:1 was detected during summer, whereas the content of saturated FA, C14:0, C16:0 and C18:0 decreased in summer and increased in winter. Milk from AG cows presented the lowest content of saturated FA and the highest content of unsaturated FA, whereas milk from BS presented the opposite behaviour. Holstein-Friesian and SI were intermediate between AG and BS breeds for all the FA except for C18:1, which was greater in HF.

Keywords: FTIR, cow milk, mountainous areas, days in milk, season

INTRODUCTION

Milk and dairy products contribute to less than 15% of the total fat in human diet and about 25% of total saturated fat. Average bovine milk fat contains 70% saturated fatty acids (SFA), 25% monounsaturated FA (MUFA) and 5% polyunsaturated FA (PUFA) (Palmquist, 2006). Several authors reported an association between SFA and the risk of atherosclerosis, coronary heart diseases, elevated blood pressure, insulin resistance and hyperlipidemia (Zyriax and Windler, 2000; Vessby *et al.*, 2001; Sacks and Katan, 2002; Mensink *et al.*, 2003; Rasmussen *et al.*, 2006). In particular, C12:0 may increase serum high density lipoprotein (HDL) cholesterol, which has cardioprotective effects (Astrup *et al.*, 2011; Kromhout *et al.*, 2011), whereas C14:0 and C16:0 are associated with high serum low density lipoprotein (LDL) cholesterol, which has negative effects on human health (Hillbrick and Augustin, 2002).

Milk FA composition is influenced by several factors, the main being breed, stage of lactation, metabolic status of the animal and feeding strategies. The negative energy balance that may occur during early stages of lactation is responsible for a rapid decrease of milk SFA until the peak of lactation and an increase thereafter, whereas unsaturated FA (UFA) and MUFA exhibit an opposite trend (Arnould and Soyeurt, 2009; Gross *et al.*, 2011; Samková *et al.*, 2012; Arnould *et al.*, 2015). Feeding practice is another important factor that influences milk FA composition. Several studies reported an increase of UFA and a decrease of SFA content during grazing season; in particular, *de novo* FA had a minimum in summer whereas blood-derived FA had an opposite trend and a minimum during winter (Heck *et al.*, 2009). The most notable differences in the composition of FA are between Holstein-Friesian (HF) and Jersey breeds. Milk from Jersey cows tends to have greater concentration of some short- and medium-chain saturated FA, such as C6:0, C8:0, C10:0, C12:0 and C14:0 but lower concentration of some UFA, such as C14:1, C16:1 and C18:1 relative to milk from HF cows (Arnould and Soyeurt, 2009). This variation could be exploited in a crossbreeding program to

achieve a preferred FA profile. The majority of studies reported in the literature have dealt with FA composition of milk from HF cows, and only few papers investigated milk FA profile of other cattle breeds (e.g. Soyeurt *et al.*, 2007; Maurice-Van Eijndhoven *et al.*, 2013). Concentration of FA in milk is usually determined by gas chromatographic technique, which requires a highly skilled staff and is time expensive; this prevents the collection of phenotypes on a large scale. As recently reviewed by De Marchi *et al*. (2014), the use of mid-infrared spectroscopy (MIRS) has great potentiality to predict milk FA concentration (Soyeurt *et al*., 2008; Penasa *et al*., 2015) as applied to a large date set in New Zealand dairy cattle with thousands of MIRS spectra from milk samples used for normal herd-testing to determine fat and protein concentration (Lopez-Villalobos *et al*., 2014). The present work aimed at describing the phenotypic variation of the amount of major milk FA groups and individual FA predicted by MIRS using a large dataset of four Italian cattle breeds.

MATERIALS AND METHODS

Data and editing

Information on individual milk samples from HF, Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG) cows spanning a 2-year period (January 2012 to December 2013) were retrieved from the database of the South Tirol Dairy Association (Bolzano, Italy) and the Breeders Association of Bolzano province (Bolzano, Italy). Samples were collected during routine cow milk testing, combined with preservative immediately after collection and processed according to International Committee for Animal Recording (ICAR) procedures at the milk laboratory of the South Tirol Dairy Association (Bolzano, Italy).

Milk fat, protein, casein, lactose, urea (MU) and FA composition was determined by Milko-Scan FT6000+ using MIRS prediction models developed by FOSS (FOSS Electric A/S, Hillerød, Denmark). Fatty acid composition (g/100 g of total FA) included 7 groups, namely SFA, UFA, MUFA, PUFA, short chain FA (SCFA), medium chain FA (MCFA) and long

chain FA (LCFA), and 4 individual FA, namely C14:0, C16:0, C18:0 and C18:1. Mid-infrared spectroscopy models to predict FA composition (g/100 of total FA) were developed and commercialized by FOSS (Foss Electric A/S, Hillerød, Denmark) and fitting statistics are summarized in Table 1. Somatic cell count (SCC) was assessed by Cell Fossomatic 250 (Foss Electric A/S, Hillerød, Denmark) and was transformed to SCS according to the formula SCS = $3 + \log_2(SCC/100\ 000)$. Information on daily milk yield was also available.

Editing procedure aimed at retaining records from cows between 6 and 450 days in milk (DIM) and from parity 1 to 15. Moreover, animals with less than 3 observations within lactation were discarded from the database. The normality assumption was checked for all the traits and records were deleted if they deviated more than 3.5 standard deviations from the respective mean of each trait. After editing, the dataset consisted of 310 365 observations belonging to 30 317 cows from 2096 herds. Due to computational issues a subset of 1000 herds was randomly extracted, leading to a final dataset of 153 801 records from 14 301 cows.

Data were analysed through a generalized linear model using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The model was as follows:

$$Y_{ijklmnop} = \mu + B_i + M_j + Year_k + DIM_l + Parity_m + (B \times M)_{ij} + (B \times Year)_{ik} + (B \times DIM)_{il} + (B \times Parity)_{im} + (M \times Y)_{jk} + (DIM \times Parity)_{lm} + H_n(B_i) + Cow_o(B_i) + e_{ijklmnop},$$

where $Y_{ijklmnop}$ is the dependent variable; μ is the overall intercept of the model; B_i is the fixed effect of the i^{th} breed (i = HF, BS, SI, AG); M_j is the fixed effect of the j^{th} month of sampling (j = 1 to 12); $Year_k$ is the fixed effect of the k^{th} year of sampling (k = 2012, 2013); DIM_l is the fixed effect of the l^{th} class of stage of lactation (l = 6 to 30, 31 to 60, 61 to 90, 91 to 120, 121 to 150, 151 to 180, 181 to 210, 211 to 240, 241 to 270, 271 to 300, 301 to 330, 331 to 360, 361 to 390, 391 to 450 days); $Parity_m$ is the fixed effect of the m^{th} class of parity of the cow (m = 1 to 5 with the last including parities 5 to 15); $(B \times M)_{ij}$ is the fixed interaction effect between breed and month of sampling; $(B \times Year)_{ik}$ is the fixed interaction between breed and year of sampling; $(B \times DIM)_{il}$ is the fixed interaction effect between breed and stage of

lactation; (B x Parity)$_{im}$ is the fixed interaction effect between breed and parity; (M x Year)$_{jk}$ is the fixed interaction effect between month and year of sampling; (DIM x Parity)$_{lm}$ is the fixed interaction effect between stage of lactation and parity; $H_n(B_i)$ is the random effect of the n^{th} herd nested within the i^{th} breed ~$N(0,\sigma^2_{herd(B)})$; $Cow_o(B_i)$ is the random effect of the o^{th} cow nested within the i^{th} breed ~$N(0,\sigma^2_{cow(B)})$; and $e_{ijklmnop}$ is the random residual ~$N(0,\sigma^2_e)$. Significance of breed effect was tested on the mean squares for herd within breed. A multiple comparison of means was performed for the main effect of breed, using Bonferroni's test ($P < 0.05$).

RESULTS AND DISCUSSION

Descriptive statistics and significance of fixed effects

Descriptive statistics of milk and FA composition are reported in Table 2. The coefficient of variation for milk quality traits ranged from 4% (lactose) to 76% (SCS). The overall mean for FA groups was 69.90 (SFA), 29.81 (UFA), 25.16 (MUFA), 2.99 (PUFA), 10.79 (SCFA), 43.86 (MCFA) and 31.40 (LCFA) g/100 g of total FA, with a coefficient of variation between 5% (SFA) and 20% (PUFA). Regarding individual FA, C14:0, C16:0, C18:0 and C18:1 averaged 11.97, 31.85, 10.03 and 21.59 g/100 g of total FA, respectively, with a coefficient of variation between 10% (C16:0) and 17% (C18:0 and C18:1).

All fixed effects included in the model were highly significant ($P < 0.001$) in explaining the variation of milk FA composition, with the exception of year of sampling for SCS ($P = 0.96$). The first order interaction effects between month and year, DIM and parity, breed and parity, and breed and month were highly significant ($P < 0.001$) for all the studied traits (data not shown).

Breed effect

Least squares means of milk quality traits and FA composition across breeds are reported in Table 3. The HF produced about 5 kg/day more milk than BS and SI, and approximately 11 kg/day more milk than AG breed ($P < 0.05$). Brown Swiss cows yielded milk with greater percentages of fat, protein and casein compared with other breeds ($P < 0.05$), and milk from SI cows was characterized by the lowest SCS ($P < 0.05$). Penasa *et al*. (2015) reported a smaller superiority (about 3 kg/day) of HF over BS cows in higher input systems and De Marchi *et al*. (2007) carried out a field study to compare the milk composition of bulk milk samples from Alpine cattle breeds; the authors demonstrated the superiority of BS for protein and fat contents compared with other cattle breeds.

Milk urea averaged 21.06 mg/100 mL (Table 2) which corresponds to a MU nitrogen of about 9.8 mg/100 mL; this value is classified as slightly low by Ishler (2008) for HF cows producing less than 32 kg milk/day. Urea was greater in milk of BS and AG than HF and SI breeds ($P < 0.05$; Table 3). The level of MU in BS cows (21.03 mg/100 mL) was lower than the value (25.9 mg/100 mL) reported by Samorè *et al*. (2007) using a large dataset from 26 provinces of Italy, and the average value of urea (20.95 mg/100 mL) in milk of AG breed was lower than that (28.95 mg/100 mL) reported by Bobbo *et al*. (2014), who conducted a study in a mountain area close to Bolzano province (Italy). The management of the herd influences MU and Bobbo *et al.* (2014) reported values of MU that were lower in modern than in traditional dairy systems of North of Italy. Nutrition, regarded both as feeding techniques and diet composition, is probably one of the main factors differentiating farm systems in the present study and in general the key to reduce MU is to provide adequate rumen available carbohydrates in the diet, which provide the energy for the rumen microbes to convert ammonia into microbial protein (Ishler, 2008). According to Ishler (2008) it is possible that the different levels of MU among breeds reported in the present study are related to

differences in rumen population metabolism and to a different capacity of the various breeds to recycle urea.

The AG cows produced milk with the lowest fat percentage (3.95%) and SFA content (68.67 g/100 g of total FA), and with the greatest content of UFA (31.34 g/100 g of total FA) and PUFA (3.19 g/100 g of total FA) ($P < 0.05$). On the contrary, BS cows yielded milk with the greatest fat percentage (4.28%) and SFA content (70.18 g/100 g of total FA), and the lowest content of UFA (29.66 g/100 g of total FA) and MUFA (24.59 g/100 g of total FA) ($P < 0.05$). Also, the BS breed showed the greatest content of SCFA (11.07 g/100 g of total FA) and C14:0 (12.10 g/100 g of total FA), and the lowest content of LCFA (30.53 g/100 g of total FA) and C18:1 (20.94 g/100 g of total FA) ($P < 0.05$). Holstein-Friesian breed produced milk with the lowest content of SCFA (10.04 g/100 g of total FA) and C18:0, and the greatest content of C18:1 (22.89 g/100 g of total FA) ($P < 0.05$). Finally, milk from SI breed exhibited greater content of MCFA (46.19 g/100 g of total FA) compared with other breeds ($P < 0.05$) (Table 3).

The comparison among studies is difficult because of differences in the methodology used for the determination of milk FA composition and in the expression of milk FA. Also, most papers have been carried out on limited number of milk samples of HF breed and using gas-cromatography as a reference method for the determination of FA composition; the present study and some others (e.g., Rutten *et al.*, 2010; Bastin *et al.*, 2013; Lopez-Villalobos *et al.*, 2014; Penasa *et al.*, 2015) have used large data of FA predicted using MIRS at the moment of normal herd-testing for fat, protein and lactose or at *a posteriori* prediction. De Marchi *et al.* (2011) investigated the potential of MIRS technology to predict FA profile of milk from BS cows; the comparison with our study however is not possible because group and individual FA were expressed on a milk basis in the study of De Marchi *et al.* (2011) and on g/100 g of total FA in the present research. Mele *et al.* (2009) analysed milk of 990 Italian HF cows reared in 34 commercial herds and reported lower content of MUFA (19.97%), C14:0

(9.18%), C16:0 (24.75%) and C18:0 (9.04%) compared with the present study. Soyeurt *et al.* (2007) obtained values of SFA (70.72%), MUFA (25.35%), C14:0 (11.09%), C16:0 (30.76%), C18:0 (12.44%) and C18:1 (23.63%) in milk of HF breed that were comparable with findings of our study.

Effect of parity number

Least squares means of FA profile across parity for specialized dairy (HF and BS) and dual-purpose (SI and AG) breeds are reported in Tables 4 and 5, respectively. Regardless of the breed, the contents of C14:0 and C16:0 were lower, and those of C18:0 and C18:1 were higher in primiparous than multiparous cows. The effect of parity on FA composition has been widely investigated in the literature, with controversial results. Kgwatala *et al.* (2009) and Secchiari *et al.* (2003) reported no parity effect on milk FA composition of Canadian and Italian HF cows, respectively, whereas Kelsey *et al.* (2003) observed significant effect of parity on most of the milk FA studied in US Holstein cows. Also Bilal *et al.* (2014) reported a relatively greater proportion of desirable FA in milk of primiparous than multiparous Canadian HF cows, but no parity effect on C16:0 and C18:0. Miller *et al.* (2006) demonstrated that the mammary gland of a primiparous cow is metabolically less active than that of later parity animals, leading to a lower expression of FA synthase in the mammary gland. This could explain the lower content of C14:0 and C16:0 in first lactation cows, as these FA are synthesized *de novo* in the mammary gland (Schennink *et al.*, 2007). This is also a characteristic of diet-induced milk fat depression, although the reduction in SCFA (4 to 8 carbons) and MCFA (10 to 14 carbons) tends to be proportionally greater when the decrease in milk fat yield is more pronounced (Peterson et al., 2003).

Moreover, primiparous cows have on average lower rumen capacity and consequently lower dry matter intake along the first two thirds of the lactation (NRC, 2001), and lower capacity to adapt to the lactation diet (i.e. capacity to absorb volatile FA produced by diet fermentation);

this could have affected levels of groups of FA and individual FA across parity. Primiparous cows are more susceptible to developing acidosis after parturition than multiparous cows (Krause and Oetzel, 2006; Penner *et al.*, 2007). Moreover, after experiencing acidosis, cows become more susceptible to subsequent bouts of acidosis and can have long-term consequences on cow health and productivity (Penner *et al.*, 2007; Dohme *et al.*, 2008).

Effect of stage of lactation

Trends of FA profiles through the lactation were similar across breeds (data not shown), and thus only least squares means adjusted by breed effect are presented in Table 6. The content of SFA in milk increased until 120 DIM and decreased thereafter, whereas UFA, MUFA and PUFA exhibited an opposite trend with minimum values at around 120 to 150 DIM. The content of SCFA was almost constant across lactation, whereas MCFA and LCFA showed specific patterns. In particular, MCFA content had a minimum at 60 DIM and increased thereafter until reaching a plateau between 180 and 450 DIM. Regarding LCFA, their content in milk decreased until 150 DIM and then increased slightly until the end of lactation.

The variation of C14:0 across DIM resembled that of SFA, and C16:0 exhibited increasing values between calving and 90 DIM and reached a plateau thereafter. The contents of C18:0 and C18:1 showed an opposite trend compared with C14:0, with decreasing contents from calving to 210 and 150 DIM, respectively, and increasing values thereafter.

The variation in the FA composition across DIM has been largely discussed in the literature. Results from the present study confirmed those of Palmquist *et al.* (1993), Soyeurt *et al.* (2008) and Mele *et al.* (2009). During the first period of lactation (within 100 DIM) cows are more likely in a negative energy balance using preformed LCFA for milk production, whereas there is a larger contribution to milk from *de novo* FA as lactation progresses (Palmquist *et al.*, 1993). The greater C18:0 and C18:1 content at the beginning of lactation is responsible for the inhibition of the mammary gland lipogenic enzymes that synthesize *de novo* FA,

especially C14:0 and C16:0, positively correlated with high serum LDL cholesterol levels (Grummer, 1991; Palmquist *et al.*, 1993; Mele *et al.*, 2009; Bastin *et al.*, 2011).

Assuming a general specific feeding management of fresh cows in the studied farms, the characteristics of early lactation diet can partially explain the variations in milk FA. Highly concentrated diets, often containing plant oils which directly affect milk PUFA content, show in fact a depressing effect on fat secretion of the mammary gland. This is related to a coordinated downregulation of mammary lipogenic gene expression and is accompanied by a decrease in the proportion of milk FA synthesized *de novo* (Shingfield *et al.*, 2013). In particular, according to Bauman and Griinari (2003) and Enjalbert *et al.* (2008), a reduction in ruminal pH, induced by highly concentrated diets, can affect the activity of ruminal bacteria that are responsible for dietary PUFA biohydrogenation inducing an increase in the C18:2n-6 and 18:1*trans*-10, escaping the rumen and transported to the mammary gland where they act as strong inhibitors of fat synthesis, consistently to our data. This reduction in the biohydrogenation process tends to disappear in mid and late lactation when cows are generally fed more fibrous diets.

Season effect

Least squares means of FA composition across month of sampling are depicted in Figure 1. Saturated FA, SCFA, MCFA, C14:0 and C16:0 content decreased during the summer season (June to September) and increased during fall, whereas an opposite trend was generally detected for UFA, MUFA, PUFA, LCFA and C18:1. No specific pattern was observed for C18:0 even if season effect was highly significant ($P < 0.001$) and this is probably related to the high number of observations. With a very large sample in fact, the standard errors calculated become extremely small, so that even very small distances between the estimate and the null hypothesis become statistically significant (Tukey, 1991).

The effect of sampling month may be explained by specific management practices throughout the year. Herds included in the present study were located in a mountainous area where cows are often given access to grazing areas from June to September. As summarized by Dewhurst *et al.* (2003), plants have the unique capacity to synthesise *de novo* C18:3 n3 which represents the building unit of the n-3 series of essential FA whose content has been demonstrated in various studies to be higher in fresh herbage than in conserved forages (Kalač and Samková, 2010). The consumption of fresh forage therefore, increases the contents of C18:3 n3 and conjugated linoleic acids, especially in presence of concentrated feeds reducing biohydrogenations (Bargo *et al.*, 2006) but also in cattle grazing alfalfa, with conjugated linoleic acids and trans 18:1 positively correlated with the proportion of alfalfa pasture in the spring diets (Castillo *et al.*, 2006).

CONCLUSIONS

Results of the present study revealed that the four breeds reared under similar herd conditions largely differed in terms of milk FA composition. Alpine Grey breed presented the best FA composition with lower content of SFA and greater content of UFA, especially MUFA, PUFA and C18:1. The variation of FA during lactation was very different between SFA and other groups of FA as well between C14:0 and C16:0 and the other individual FA; the pattern of milk saturated fraction of FA followed the lactation curve, suggesting a close relation with body energy status of the cow and probably with diet characteristics (i.e. proportion of concentrates). Further research will focus on the estimation of genetic parameters of the major FA predicted by MIRS in milk of dairy and dual-purpose cattle breeds**.**

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Table 1 *Fitting statistics of mid-infrared prediction models for groups and individual fatty acids*

Trait[1] (g/100 g of total FA)	n[2]	Range	SD[3]	1-VR[4]
Groups of FA				
SFA	1690	45.92-86.39	5.04	0.80
MUFA	1671	13.66-48.50	4.01	0.81
PUFA	1664	1.90-8.60	1.11	0.72
SCFA	332	7.97-23.77	3.94	0.98
MCFA	336	13.66-48.50	4.01	0.79
LCFA	452	20.10-63.21	6.71	0.80
Individual FA				
C14:0	480	4.23-16.80	1.80	0.69
C16:0	830	18.83-48.06	4.14	0.55
C18:0	840	3.89-21.10	2.19	0.67
C18:1	843	12.09-40.89	4.33	0.81

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

[2]Number of samples.

[3]Standard deviation.

[4]Coefficient of determination of validation.

Table 2 *Descriptive statistics of milk yield, quality and fatty acid (FA) composition*

Trait[1]	N	Mean	SD	Minimum	Maximum
Milk yield (kg/day)	153 801	22.37	7.64	1.90	71.00
Milk quality (%)					
Fat	153 801	4.07	0.68	1.89	7.31
Protein	153 801	3.54	0.42	2.06	5.45
Casein	153 801	2.77	0.31	1.65	4.00
Lactose	153 801	4.77	0.20	3.80	5.58
Urea (mg/100 mL)	153 657	21.06	7.39	0.10	65.60
SCS (units)	153 801	2.44	1.86	-3.64	6.32
Groups of FA (g/100 g of total FA)					
SFA	153 801	69.90	3.71	50.24	84.85
UFA	153 801	29.97	4.18	7.30	53.48
MUFA	153 800	26. 65	3.70	4.13	49.56
PUFA	153 792	3.32	0.59	0.06	11.53
SCFA	153 792	10.79	1.21	0.15	16.07
MCFA	153 787	43.86	7.30	0.48	98.67
LCFA	153 799	31.40	4.94	2.21	58.92
Individual FA (g/100 g of total FA)					
C14:0	153 801	11.97	1.36	2.75	37.00
C16:0	153 801	31.85	3.20	15.71	65.37
C18:0	153 796	10.03	1.74	0.43	20.42
C18:1	153 799	21.59	3.71	4.43	46.20

[1]SCS = somatic cell score; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

Table 3 *Least squares means of milk yield, quality and fatty acid (FA) composition for different breeds (HF = Holstein-Friesian; BS = Brown Swiss; SI = Simmental; AG = Alpine Grey)*

Trait[1]	HF	BS	SI	AG
Milk yield (kg/day)	26.10[a]	20.83[b]	20.77[b]	14.78[b]
Milk quality (%)				
Fat	4.07[a]	4.28[b]	4.15[c]	3.95[d]
Protein	3.40[a]	3.70[b]	3.59[c]	3.56[c]
Casein	2.66[a]	2.89[b]	2.81[c]	2.78[c]
Lactose	4.72[a]	4.72[a]	4.71[a]	4.77[b]
Urea (mg/100 mL)	19.09[a]	21.03[b]	19.81[a]	20.95[b]
SCS (units)	2.57[a]	2.76[b]	2.49[a]	2.59[a]
Groups of FA (g/100 g of total FA)				
SFA	69.68[a]	70.18[b]	69.84[a]	68.67[c]
UFA	30.08[a]	29.66[b]	30.02[a]	31.34[c]
MUFA	25.72[ac]	24.59[b]	25.58[a]	26.12[c]
PUFA	2.92[ab]	2.95[a]	2.88[b]	3.19[c]
SCFA	10.04[a]	11.07[b]	10.36[c]	10.56[d]
MCFA	42.03[a]	44.72[b]	46.19[c]	41.57[a]
LCFA	32.15[ac]	30.53[b]	31.92[a]	32.35[c]
Individual FA (g/100 g of total FA)				
C14:0	11.90[a]	12.10[b]	11.77[a]	11.84[a]
C16:0	32.23[ab]	31.93[b]	32.26[a]	31.46[c]
C18:0	9.58[a]	9.79[b]	10.16[c]	10.16[c]
C18:1	22.89[a]	20.94[b]	22.02[c]	22.16[c]

[a-d]Least squares means with different superscripts within the same row are significantly different ($P < 0.05$).

[1]SCS = somatic cell score; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

Table 4 *Least squares means of* milk *fatty acid (FA) composition of Holstein-Friesian (HF) and Brown Swiss (BS) cattle breeds at different parities*

	HF					BS				
Trait[1] (g/100 g of total FA)	First	Second	Third	Fourth	Fifth and later	First	Second	Third	Fourth	Fifth and later
Groups of FA										
SFA	68.64	69.79	69.94	69.94	70.08	69.24	70.16	70.42	70.47	70.63
UFA	30.90	29.81	29.86	29.95	29.86	30.31	29.54	29.49	29.54	29.41
MUFA	27.18	25.56	25.38	25.32	25.18	25.83	24.50	24.30	24.27	24.05
PUFA	3.15	2.91	2.85	2.87	2.85	3.16	2.94	2.90	2.89	2.87
SCFA	10.27	10.12	9.99	9.92	9.90	11.29	11.12	11.06	10.98	10.88
MCFA	41.71	42.30	42.31	41.91	41.92	44.43	44.94	44.66	44.76	44.81
LCFA	33.42	31.85	31.87	31.88	31.73	31.74	30.44	30.28	30.24	29.98
Individual FA										
C14:0	11.49	11.96	11.97	12.00	12.02	11.71	12.10	12.19	12.21	12.30
C16:0	31.55	32.34	32.34	32.42	32.49	31.42	31.98	32.05	32.06	32.16
C18:0	9.67	9.45	9.56	9.63	9.62	9.97	9.75	9.78	9.75	9.69
C18:1	24.00	22.66	22.62	22.61	22.57	21.96	20.78	20.67	20.71	20.57

[1]SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

Table 5 *Least squares means of* milk *fatty acid (FA) composition of Simmental (SI) and Alpine Gray (AG) cattle breeds at different parities*

	SI					AG				
Trait[1] (g/100 g of total FA)	First	Second	Third	Fourth	Fifth and later	First	Second	Third	Fourth	Fifth and later
Groups of FA										
SFA	68.99	69.93	70.05	70.09	70.14	67.53	68.78	68.95	68.99	69.08
UFA	30.65	29.85	29.85	29.89	29.87	32.26	31.11	31.10	31.12	31.09
MUFA	26.99	25.55	25.25	25.14	24.96	27.72	26.04	25.75	25.61	25.47
PUFA	3.07	2.86	2.83	2.82	2.82	3.41	3.18	3.12	3.12	3.11
SCFA	10.55	10.45	10.34	10.28	10.21	10.79	10.68	10.53	10.42	10.36
MCFA	45.74	46.20	46.16	46.33	46.54	40.79	41.52	41.59	41.76	42.16
LCFA	33.26	31.77	31.63	31.57	31.38	34.04	32.17	32.05	31.88	31.62
Individual FA										
C14:0	11.36	11.79	11.88	11.89	11.95	11.36	11.87	11.94	12.01	12.02
C16:0	31.89	32.40	32.34	32.35	32.32	30.79	31.56	31.66	31.64	31.67
C18:0	10.30	10.03	10.14	10.18	10.17	10.43	10.10	10.14	10.10	10.05
C18:1	23.31	21.93	21.71	21.65	21.51	23.57	22.03	21.85	21.71	21.66

[1]SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

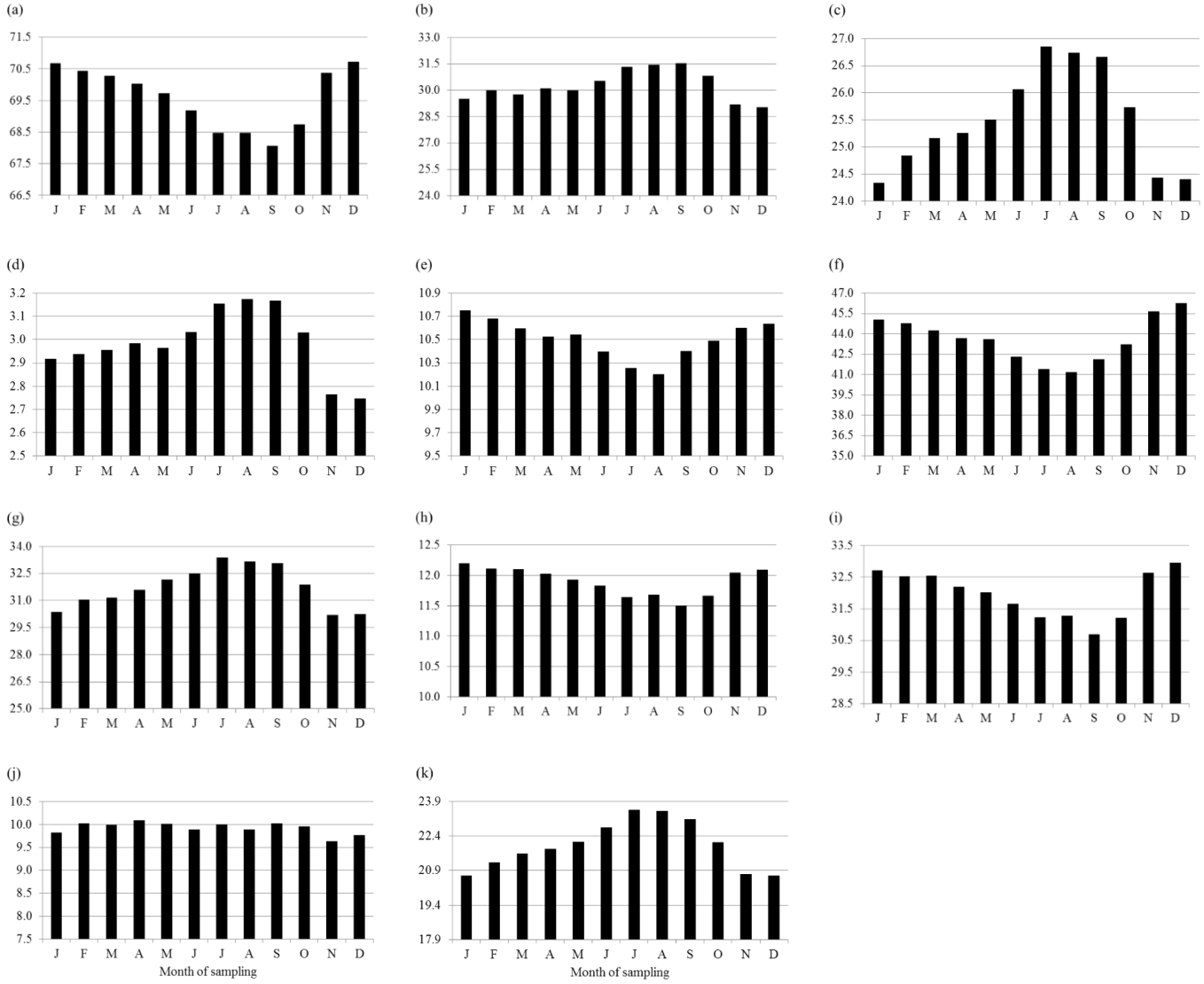
Table 6 *Least squares means of milk fatty acid (FA) composition at different stages[1] of lactation*

Trait[2] (g/100 g of total FA)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Groups of FA														
SFA	65.88	68.67	70.41	70.93	70.89	70.75	70.55	70.25	69.82	69.38	69.21	69.20	69.21	69.14
UFA	34.85	31.59	29.31	28.52	28.48	28.60	28.87	29.36	29.97	30.55	30.79	30.86	30.96	31.12
MUFA	28.83	26.42	24.75	24.13	24.15	24.27	24.49	24.86	25.31	25.73	25.89	25.97	26.06	26.17
PUFA	3.23	3.12	2.96	2.89	2.88	2.87	2.87	2.91	2.97	3.02	3.03	3.03	3.01	3.02
SCFA	10.80	10.41	10.44	10.50	10.55	10.54	10.52	10.53	10.50	10.45	10.43	10.47	10.49	10.50
MCFA	40.37	39.35	41.39	42.89	43.78	44.56	45.14	44.82	44.37	44.14	44.35	44.77	45.22	45.62
LCFA	39.17	34.53	31.45	30.21	29.97	29.99	30.19	30.49	30.89	31.32	31.45	31.48	31.56	31.66
Individual FA														
C14:0	10.16	11.58	12.28	12.50	12.47	12.40	12.27	12.18	12.02	11.86	11.79	11.76	11.70	11.66
C16:0	27.87	30.42	32.14	32.80	32.87	32.88	32.79	32.61	32.40	32.22	32.17	32.14	32.22	32.09
C18:0	13.25	11.37	10.26	9.71	9.45	9.35	9.33	9.35	9.39	9.48	9.51	9.51	9.49	9.51
C18:1	25.92	23.52	21.55	20.77	20.68	20.76	20.97	21.28	21.66	22.05	22.15	22.17	22.24	22.30

[1]Stages of lactation were: 1 = 6 to 30, 2 = 31 to 60, 3 = 61 to 90, 4 = 91 to 120, 5 = 121 to 150, 6 = 151 to 180, 7 = 181 to 210, 8 = 211 to 240, 9 = 241 to 270, 10 = 271 to 300, 11 = 301 to 330, 12 = 331 to 360, 13 = 361 to 390, and 14 = 391 to 450 days.

[2]SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.

Figure 1 *Least squares means of fatty acid (FA) composition for month of sampling effect: (a) = saturated FA (SFA); (b) = unsaturated FA (UFA); (c) = monounsaturated FA (MUFA); (d) = polyunsaturated FA (PUFA); (e) = short chain FA (SCFA); (f) = medium chain FA (MCFA); (g) = long chain FA (LCFA); (h) = C14:0; (i) = C16:0; (j) = C18:0; (k) = C18:1.*



CHAPTER 3

Genetics of milk fatty acid groups predicted during routine data recording in Holstein dairy cattle

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ABSTRACT

The aim of this paper was to estimate genetic parameters for groups of milk fatty acids (FA), namely saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA), in Holstein cows. Mid-infrared spectroscopy (MIRS) was used to predict FA groups (g/100 g of milk) of 72,848 samples recorded on 17,873 cows between September 2011 and November 2012. Univariate and multivariate models were implemented in a Bayesian framework to estimate (co)variance components for SFA, UFA, MUFA, PUFA, daily milk yield, milk fat and milk protein. Statistical models included fixed effect of parity by stage of lactation, and random effects of herd-test-date, cow permanent environmental, animal additive genetic and residual. Posterior means of heritability estimates for SFA, UFA, MUFA and PUFA were 0.246, 0.069, 0.082 and 0.078, respectively. Estimates of genetic correlations between FA groups ranged from 0.405 (SFA and PUFA) to 0.952 (MUFA and UFA). The increase of fat content led to an increase of all groups of FA, in particular SFA, with undesirable effects on the healthy quality of the product. The study highlighted the existence of exploitable additive genetic variation for groups of FA routinely predicted by MIRS and thus there is potential to address the selection to healthy milk FA composition.

Keywords: milk fatty acid, Holstein, heritability, genetic correlation

INTRODUCTION

The saturated fatty acids (SFA) increase blood cholesterol, which in turn is associated with increased blood pressure, risk of cardiovascular diseases, obesity, and insulin resistance (Mensink et al., 2003; Rasmussen et al., 2006; Sacks and Katan, 2002; Vessby et al., 2001). This has often led to the "demonisation" of bovine milk fat, as it typically contains 70% SFA, 25% monounsaturated FA (MUFA), and 5% polyunsaturated FA (PUFA).

Fat composition of cow milk is influenced by metabolic status and stage of lactation of the animal, as negative energy balance directly impacts the presence of unsaturated FA (UFA). The

mobilization of fat reserves has been found to increase the content of UFA in milk (Gross et al., 2011; Samková et al., 2012), whereas contents of SFA decrease rapidly till the peak of lactation and then increase weakly (Soyeurt et al., 2008). Stoop et al. (2009) found similar results, showing that proportions of SFA, in particular C6:0 to C14:0, peaked around the third month of lactation. Seasonality is another factor which determines milk FA composition, with a decrease of SFA content and an increase of UFA and trans-FA in grazing season (Heck et al., 2009; Jahreis et al., 1996). Heck et al. (2009) reported that FA synthesized *de novo* had a minimum in summer, whereas blood-derived FA had a minimum in winter. Moreover, the effect of animal nutrition on FA composition has been widely studied because feeding supplementation is an efficient way to modify FA composition of milk (Palmquist et al., 1993). The interest on FA profile of animal products, including milk, is also relevant for the new labeling procedures required by European Union with regulation No. 1169/2011 which will be mandatory starting December 2014 (http://ec.europa.eu/food/food/labellingnutrition/foodlabelling/proposed_legislation_en.htm). The regulation establishes that energy level and amount of fat (particularly SFA) of products destined to human consumption must be reported on their label.

Differences in FA profile of milk among dairy cattle breeds have been highlighted either in the Netherlands (Maurice-Van Eijndhoven et al., 2013) and in the Walloon region of Belgium (Soyeurt et al., 2006). Moreover, several studies have estimated genetic parameters for major FA groups in Holstein populations (e.g., Gion et al., 2011; Mele et al., 2009; Soyeurt et al., 2007; Tullo et al., 2014). Recently, genetic evaluation for FA composition has been developed in the Walloon region of Belgium, and it was suggested to evaluate animals on the basis of a Nutritional Quality Index (Gengler et al., 2012). The major constraint for the implementation of genetic evaluations for milk FA profile is the reference analysis (gas-chromatography), which is costly and time-consuming. The use of mid-infrared spectroscopy (MIRS) has revealed great potentiality for the genetic analysis of FA at population level, as recently reviewed by De Marchi et al. (2014). The aim of this work was

to estimate genetic parameters for groups of FA predicted by MIRS in milk of Italian Holstein-Friesian cows collected during routine test-day milk recording.

MATERIALS AND METHODS

Data

Starting September 2011, the assessment of FA groups has been routinely implemented in milk recording system of Veneto region (northeast Italy), along with milk coagulation properties (De Marchi et al., 2012; Tiezzi et al., 2013). Models for the prediction of FA content (g/100 g of milk) have been developed and commercialized by FOSS (Hillerød, Denmark), and installed on Milko-Scan FT6000 (FOSS) in the laboratory of the Breeders Association of Veneto region (Padova, Italy).

A total of 91,218 morning milk samples from 25,317 cows were collected between September 2011 and November 2012. Somatic cell count (SCC) was assessed by Cell Fossomatic 250 (FOSS), and MF, milk protein (MP), SFA, MUFA and PUFA by Milko-Scan FT6000. Unsaturated FA were not directly predicted by MIRS and they were calculated from milk fat (MF) and SFA as: (% MF * 0.95) - % SFA. Records on studied traits were retained if they deviated less than 3.5 standard deviations from the respective mean. Moreover, records from cows with known sire and dam, in parity 1 to 5, between 5 and 365 days in milk and with at least 2 observations in a given lactation were retained in the dataset. Sires of cows were considered if they had at least 3 daughters in 3 herds. Cows were required to have recorded performance in a single lactation and at least 3 animals were required to be controlled on each herd-test-date (HTD). After editing of the data as above, 72,848 records from 17,873 cows were available for statistical analysis. Animals were sampled in 347 herds and were daughters of 1235 sires. Sires were born between 1988 and 2008, with the majority (n = 728) born between 2004 and 2007.

Statistical analyses

The following linear animal model was used to analyze the data:

$$y = \mathbf{X}b + \mathbf{Z_h}h + \mathbf{Z_p}p + \mathbf{Z_a}a + e,$$

where *y* is the vector of phenotypic values for the analyzed trait, *b* is the vector of fixed effect of parity by stage of lactation (three classes of parity, with the last including parities 3 to 5, and twelve monthly classes of days in milk, 6 to 35 days, 36 to 65 days, 66 to 95 days, 96 to 125 days, 126 to 155 days, 156 to 185 days, 186 to 215 days, 216 to 245 days, 246 to 275 days, 276 to 305 days, 306 to 335 days, and 336 to 365 days), *h* is the vector of solutions for HTD random effect, *p* is the vector of solutions for cow permanent environmental effect, *a* is the vector of solutions for cow additive genetic effect and *e* is the vector of random residuals. Vectors *h*, *p*, *a* and *e* were assumed normally distributed with mean 0 and variance estimated from the data (σ^2_h, σ^2_p, σ^2_a, and σ^2_e, respectively). **X**, $\mathbf{Z_h}$, $\mathbf{Z_p}$ and $\mathbf{Z_a}$ are the respective incidence matrices of appropriate order.

Univariate analyses were performed to estimate variance components and sets of 4-trait analyses including MY, MF, MP and one of the four groups of FA were performed to estimate genetic correlations. A 4-trait analysis was used to estimate the correlations between groups of FA.

Models were implemented in a Bayesian framework using the software GIBBS3F90 (Misztal, available at: http://nce.ads.uga.edu/%7Eignacy/programs.html). For univariate models, 150,000 iterations were run discarding the first 50,000 samples as burn-in and storing samples every 10 iterations, and for the 4-trait multivariate analyses, chains included 600,000 iterations with the first 100,000 samples discarded as burn-in and a thinning interval of 50 iterations.

Heritability (h^2), intra-herd heritability (h^2_{IH}), repeatability (rep), and genetic correlation (r_{gen}) were defined as:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

$$h^2_{IH} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_e}$$

$$rep = \frac{\sigma^2_a + \sigma^2_{pe}}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

$$r_{gen} = \frac{cov_a}{\sqrt{\sigma^2_{x,a} * \sigma^2_{y,a}}}$$

where σ^2_a is the additive genetic variance, σ^2_{pe} is the cow permanent environmental variance, σ^2_{htd} is the HTD variance, σ^2_e is the residual variance and cov_a is the additive genetic covariance. The posterior means of the marginal posterior distributions were considered as estimates of (co)variance components and related parameters, and the 95% highest probability density intervals (HPD95) were calculated as dispersion measure of the posterior distribution.

RESULTS AND DISCUSSION

Descriptive statistics

Saturated FA and UFA accounted for 2.58% and 1.11% of milk, respectively (Table 1). Among UFA, the most represented were MUFA (0.91%), whereas PUFA represented only 0.09%. Milk yield, MF and MP averaged 31.6 kg/day, 3.70% and 3.40%, respectively. Compared to our findings (Table 1), Bastin et al. (2011) reported higher mean values for SFA (2.79), UFA (1.31), MUFA (1.13) and PUFA (0.17), probably due to the higher MF than in the present study (3.96 vs 3.70). Finally, Soyeurt et al. (2007) found higher values for SFA (2.86) and MUFA (1.02), due to higher MF than in the present research (4.13 vs 3.70).

Heritability and repeatability

Saturated FA showed the highest estimates of heritability (0.246) and intra-herd heritability (0.291), whereas UFA exhibited the lowest values (0.069 and 0.086, respectively; Table 1). Repeatability estimates ranged from 0.176 (PUFA) to 0.416 (SFA). Daily MY exhibited low heritability (0.104), intra-herd heritability (0.138) and moderate repeatability (0.412). Milk fat and

MP showed heritability and intra-herd heritability of 0.201 and 0.267, and 0.239 and 0.313, respectively (Table 1).

Estimates of heritability for groups of FA were moderate to low, suggesting that an improvement through selective breeding is possible but slow. The impact of additive genetic component decreased with the increase of unsaturation level of FA, and PUFA showed the lowest heritabilities. The difference between heritabilities and intra-herd heritabilities reflected the magnitude of HTD effect (i.e., feeding strategies), and was strong for the UFA groups. Repeatabilities of groups of FA resembled the trend of heritabilities, but they were higher in magnitude (Table 1), suggesting that the contribution of the permanent environmental effects to the phenotypic variation is relevant. However, repeatabilities are moderate, and thus several measurements per cow and lactation are needed to obtain reliable results.

Bobe et al. (2008) reported heritability and repeatability of 0.27 and 0.44, respectively, for SFA in US Holsteins; these estimates are very similar to findings of the present study. Bastin et al. (2011) found heritabilities of 0.426 for SFA, 0.223 for UFA, 0.212 for MUFA and 0.298 for PUFA, which do not reflect the pattern found in the present study, and Soyeurt et al. (2007) estimated heritabilies and repeatabilities of 0.36 and 0.70 for SFA, and 0.24 and 0.25 for MUFA, respectively. Gion et al. (2011) reported a different pattern in Holsteins, with much higher heritabilities for PUFA (0.28, 0.14, 0.15 and 0.22 for SFA, UFA, MUFA, and PUFA, respectively). However, measures of phenotypic variation were also different in the cited studies, and it is not surprising that groups of FA may have such different heritabilities across countries, farming systems and populations, as these are traits highly influenced by environmental conditions: the same genotype might show different pattern for FA composition if fed different diets.

Genetic correlations

The strongest genetic correlation was between UFA and MUFA (0.952) and the lowest between SFA and PUFA (0.405; Table 2). Milk yield was moderately and negatively correlated to all groups of FA (-0.470 with SFA to -0.346 with PUFA). Milk fat was highly correlated with SFA (0.991), UFA (0.838) and MUFA (0.812), and moderately with PUFA (0.473), and MP was moderately related to groups of FA (0.441 with PUFA to 0.634 with UFA; Table 3).

In the present study, SFA is linked to variation in MF much more that any unsaturated component is, supporting that an increase in MF can lead to a decrease of the unsaturation level of fat, which is undesirable in terms of nutritional quality. Variation in the unsaturated component seems to depend on MUFA, due to their strong link (0.952). The polyunsaturated component can be depicted as quite independent of variations of MF and other groups, as an increase in PUFA will be reflected on moderate increase in MF. Genetic correlations between all groups of FA and MY were specular although much weaker than those with MF, probably mitigated by the genetic correlation between MF and MY (-0.490). Similarly, MP follows the pattern found for MF, with less magnitude, probably due to the genetic correlation between MF and MP (0.636).

Soyeurt et al. (2007) reported genetic correlation of 0.66 between SFA and MUFA, similar to that estimated in the present work. Also, genetic relationships of SFA and MUFA with MY and MP were moderate (-0.26 and -0.21, and 0.62 and 0.44, respectively), but strong with MF (0.97 and 0.74, respectively). On the contrary, Bastin et al. (2013), working across parities 1 to 3, found similar relationships of MY, MF and MP with SFA (-0.36, 0.91, 0.50), UFA (-0.36, 0.73, 0.48), MUFA (-0.35, 0.72, 0.45) and PUFA (-0.37, 0.69, 0.56). Genetic correlations of SFA with UFA, MUFA and PUFA were 0.62, 0.61 and 0.62, respectively, of UFA with MUFA and PUFA were 0.99 and 0.77, respectively, and between MUFA and PUFA was 0.72. Using random regression models, Bastin et al. (2011) found similar values for the correlations with MY (-0.40 with SFA and UFA, and -0.39 with MUFA and PUFA), MP (0.49, 0.52, 0.48 and 0.60 with SFA, UFA, MUFA

and PUFA, respectively), and MF (0.97, 0.76, 0.72 and 0.77 with SFA, UFA, MUFA and PUFA, respectively). Moreover, they found very similar genetic correlations between the groups of FA: medium to high between SFA and the unsaturated components (0.61, 0.60 and 0.61 with UFA, MUFA and PUFA, respectively), full genetic correlation between UFA and MUFA (1.00), and high between UFA and PUFA (0.79), and MUFA and PUFA (0.73).

Overall, results for genetic correlations indicated that cows are genetically predisposed to produce more saturated fat when fat content in milk increases. Also, if the content of UFA is predisposed to be higher, it will be mostly its monounsaturated component to increase, as the polyunsaturated component will be less linked to any other group or milk quality parameter.

CONCLUSION

The present study provided evidence that additive genetic variation for groups of FA of bovine milk predicted by mid-infrared spectroscopy during test-day milk recording exists. An increase of fat content in milk is related to an undesirable increase of its saturation index, in agreement with findings from other studies. However, as phenotypic information is now routinely available thanks to the use of MIRS prediction models, and additive genetic variation of FA profile exists, there is room to improve the healthy profile of milk for human consumption without impairing fat content if appropriate weights are given to these traits in the selection index of Italian Holstein-Friesian dairy population.

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Table 1.Descriptive statistics and posterior means and 95% highest probability density intervals (HPD95) for heritability (h^2), intra-herd heritability (h^2_{IH}) and repeatability of groups of fatty acids, milk yield and quality (n = 72,848).

Trait[a]	Mean	CV[b] (%)	h^2		h^2_{IH}		Repeatability	
			Mean	HPD95	Mean	HPD95	Mean	HPD95
Fatty acids (g/100 g of milk)								
SFA	2.58	20.5	0.246	0.217; 0.278	0.291	0.254; 0.326	0.416	0.405; 0.427
UFA	1.11	21.6	0.069	0.054; 0.084	0.086	0.068; 0.106	0.188	0.180; 0.196
MUFA	0.91	22.0	0.082	0.065; 0.099	0.102	0.080; 0.123	0.200	0.192; 0.208
PUFA	0.09	33.3	0.078	0.064; 0.092	0.148	0.123; 0.175	0.176	0.168; 0.184
Milk production and composition								
Milk yield (kg/day)	31.6	28.5	0.104	0.082; 0.124	0.138	0.110; 0.165	0.412	0.400; 0.424
Milk fat (%)	3.70	18.9	0.201	0.172; 0.229	0.239	0.204; 0.271	0.361	0.351; 0.371
Milk protein (%)	3.40	11.8	0.267	0.234; 0.297	0.313	0.275; 0.348	0.485	0.474; 0.495

[a] SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

[b] Coefficient of variation.

Table 2. Posterior means and 95% highest probability density intervals (HPD95) for genetic correlations between groups of fatty acids.

Trait[a]	UFA		MUFA		PUFA	
	Mean	HPD95	Mean	HPD95	Mean	HPD95
SFA	0.767	0.714; 0.824	0.753	0.696; 0.809	0.405	0.310; 0.496
UFA			0.952	0.938; 0.965	0.617	0.535; 0.693
MUFA					0.623	0.547; 0.698

[a] SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Table 3. Posterior means and 95% highest probability density intervals (HPD95) for genetic correlations of groups of fatty acids with milk yield and quality traits.

Trait[a]	Milk yield (kg/day)		Milk fat (%)		Milk protein (%)	
	Mean	HPD95	Mean	HPD95	Mean	HPD95
Fatty acids (g/100 g of milk)						
SFA	-0.470	-0.574; -0.359	0.991	0.989; 0.993	0.601	0.539; 0.660
UFA	-0.422	-0.547; -0.280	0.838	0.798; 0.876	0.634	0.546; 0.717
MUFA	-0.398	-0.528; -0.256	0.812	0.765; 0.853	0.566	0.473; 0.657
PUFA	-0.346	-0.482; -0.212	0.473	0.390; 0.563	0.441	0.348; 0.532

[a] SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

CHAPTER 4

Genetic parameters of predicted milk fatty acid composition in dairy and dual-purpose cattle breeds

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ABSTRACT

One strategy to enhance nutritional quality of bovine milk is genetic selection. The aim of this study was to estimate genetic parameters of milk fat composition in four Italian cattle breeds: Holstein Friesian (HF), Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG). Mid-infrared spectroscopy (MIRS) was used to predict FA groups; saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) and major individuals; myristic (C14:0), palmitic (C16:0), stearic (C18:0) and oleic (C18:1), expressed as g/100 g FA of 541 028 samples recorded on 38 337 cows from 2201 herds between January 2011 to December 2013. Breed composition was determined according to known pedigrees of animals and divided as follow 4 095 HF cows, 14 407 BS cows, 12 070 SI cows and 7 765 AG cows. Variances and covariances were estimated using univariate and bivariate models through REML procedure using the software ASREML. Statistical models included fixed effects of herd-test-date, parity, stage of lactation and random effects of cow permanent environmental, animal additive genetic and residuals. Heritability estimates were moderate to low ranging from 0.18 to 0.30 and from 0.16 to 0.30 for groups and individual fatty acids respectively, confirming the findings of other authors. The positive correlations between milk fat, SFA, C14:0 and C16:0 could aid in the improvement of health quality if selection against total milk fat quantity is performed. This study highlighted the existence of exploitable additive genetic variation for both FA groups and individuals predicted by MIRS and, based on this results, a future selection program to improve milk FA composition could be initiated.

Keywords: FTIR, milk fatty acids, genetic parameters, Italian breeds

INTRODUCTION

Milk and dairy products are important sources of nutrients such as high quality protein, essential macro and micro minerals, vitamins and fat (Boland et al., 2001). Milk fatty acids (FA) are aggregated into globules and this structure is one of the most complex in nature. Up to 70% of total FA are saturated (SFA), whereas the remaining 25% includes monounsaturated FA (MUFA, 20%)

and polyunsaturated FA (PUFA, 5%) (Grummer, 1991; Barlowska et al., 2011; Markiewicz-Kęszycka et al., 2013).

Milk and dairy products contribute to approximately 25% of total saturated fat in human diet (Grummer, 1991), and this leads to an overall negative consumer perception of milk-derived products. Several authors reported an association between SFA and some important diseases such as atherosclerosis, coronary heart disease, hypertension, insulin resistance, and hyperlipidemia (Samuelson et al., 2001; Sacks and Katan, 2002; Mensink et al., 2003; Rivellese et al., 2003; Rasmussen et al., 2006). Among the major individual saturated FA, C14:0 and C16:0 are associated with high serum total and low-density lipoprotein cholesterol levels, and C12:0 showed a strong correlation with high-density lipoprotein cholesterol levels, famous for cardio-protective effects (De Roos et al., 2001; Kromhout et al., 2010; Astrup et al., 2011;).

Besides human health aspects, FA are important also in the dairy industry for their technological role. Butter produced with greater content of unsaturated FA (UFA) has softer texture (Stegeman et al., 1992; Bobe et al., 2003), and a greater susceptibility to oxidation during storage is observed when linoleic acid is greater than 20% (Chen et al., 2004; Palmquist, 2006). Milk fat plays an important role also in ice cream production, influencing sensory properties like flavor, texture and melting point (Walstra and Jonkman, 1998). In particular, ice cream manufactured with modified milk fat containing high levels of linoleic (C18:1) and linolenic (C18:2) FA presented lesser viscosity and lower melting point (Gonzalez et al., 2003).

For all these reasons, the modification of FA profile in milk is strongly advisable in order to obtain a healthier milk and to enhance dairy sector efficiency, especially in those countries where milk is predominantly destined to processing. Some studies suggested the possibility to use genetic selection as a tool to achieve this goal affirming that there is enough genetic variation in FA composition among animals (Renner and Kosmack, 1974; Karijord et al., 1982; Bobe et al., 2008; Stoop et al., 2008; Mele et al., 2009). The reference method for the determination of milk FA is gas chromatography. This analysis is costly and time-demanding, and thus not applicable on a large

scale. Recently, De Marchi et al. (2014) reviewed the potential of mid-infrared spectroscopy (MIRS) to collect phenotypes at population level, which is useful for genetic purposes as reported by several authors (Soyeurt et al., 2007; Bastin et al., 2013; Tiezzi et al., 2013; Lopez-Villalobos et al., 2014; McParland et al., 2014; Tullo et al., 2014; Toffanin et al., 2015). There is a paucity of knowledge regarding differences of FA composition in milk of Italian dairy cattle breeds (Gottardo et al., 2013) and there is a lack of estimates of genetic parameters for FA profile in Italian breeds them (Tullo et al., 2014; Penasa et al., 2015). Therefore, the aim of this work was to estimate heritability of and genetic correlations between major milk FA predicted by MIRS during official milk-testing in four Italian breeds.

MATERIAL AND METHODS

Data

Information on individual milk samples from Holstein-Friesian (HF), Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG) cows of a 3-year period (January 2011 to December 2013) were retrieved from the database of the South Tirol Dairy Association (Bolzano, Italy) and the Breeders Association of Bolzano province (Bolzano, Italy). Samples were collected during routine cow milk testing, combined with preservative immediately after collection and processed according to International Committee for Animal Recording (ICAR) procedures at the milk laboratory of the South Tirol Dairy Association. Milk fat, protein and FA composition was determined by Milko-Scan FT6000+ using MIRS prediction models developed by FOSS (FOSS Electric A/S, Hillerød, Denmark). Fitting statistics of MIRS prediction models for FA composition can be retrieved from Penasa et al. (2015). Fatty acid composition (g/100 g of total FA) included 4 groups, namely SFA, UFA, MUFA and PUFA, and 4 individual FA, namely C14:0, C16:0 C18:0 and C18:1. Somatic cell count (SCC) was assessed by Cell Fossomatic 250 (Foss Electric A/S, Hillerød, Denmark) and was transformed to SCS according to the formula $SCS = 3 + \log_2(SCC/100\ 000)$. Information on daily milk yield was also available.

The present study analysed records from cows between 6 and 450 days in milk (DIM) and from parity 1 to 15. Animals with less than 3 observations within lactation were discarded from the database. The normality assumption was checked for all the traits and records were removed from the dataset if they deviated more than 3.5 standard deviations from the respective mean of each trait. After editing, the dataset consisted of 541 028 observations belonging to 38 337 cows from 2201 herds for milk quality traits and 283 608 observation belonging to 30 663 cows from 2100 herds for milk FA profile. The number of records for each breed are reported in Table 1.

Statistical analysis

Data were analysed using the following linear animal model:

$$y = Xb + Z_p p + Z_a a + e$$

where y is the vector of phenotypic records (milk yeld, fat percentage, protein percentage, SCS, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0, C18:1); b is the vector of fixed effects of herd-test-date (HTD with 5 193 levels for HF, 28 120 for BS, 19 564 for Si and 15 891 for AG), parity (5 classes with the last one containing parities from 5 to 15) and stage of lactation (14 monthly classes, 6 to 30, 31 to 60, 61 to 90, 91 to 120, 121 to 150, 151 to 180, 181 to 210, 211 to 240, 241 to 270, 271 to 300, 301 to 330, 331 to 360, 361 to 390, 391 to 450 days); p is the vector of solutions for cow random permanent environmental effect; a is the vector of solutions for animal genetic effect; and e is the vector of random residuals. X, Z_p and Z_a are the respective incidence matrices of the appropriate order. Variances and covariance components for the random effects were estimated using univariate and bivariate models through REML procedures in ASREML (Gilmour et al, 2009).

Heritability (h^2), repeatability (rep) and cow permanent environmental effect (h_{pe}) were defined as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

$$rep = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

$$h_{pe} = \frac{\sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

where σ^2_a is the additive genetic variance, σ^2_{pe} is the cow permanent environmental variance and σ^2_e is the residual variance.

RESULTS AND DISCUSSION

Descriptive statistics

Descriptive statistics of milk yield, milk quality traits and FA composition of HF, BS, SI and AG breeds are reported in Table 1. Holstein-Friesian produced more milk than other breeds and BS yielded milk with the greatest content of fat and protein. Earlier studies demonstrated similar patterns of milk yield, and milk fat and protein content across breeds (Kelsey et al., 2003; De Marchi et al., 2007; Cecchinato et al., 2011).

Saturated SFA were more abundant in milk of BS and SI (70.32 and 70.21g/100 g of total FA, respectively) and lower in milk of AG (68.94 g/100 g of total FA) cows. The latter breed exhibited slightly higher amount of UFA (30.75 g/100 g of total FA), MUFA (25.71 g/100 g of total FA), and PUFA (3.22 g/100 g of total FA) compared with the other 3 breeds. Also, the milk of AG breed presented lower C16:0 and greater C18:0 than milk of the other breeds. Oleic acid (C18:1) was higher in milk of HF (22.64 g/100 g of total FA) and lower in BS (20.78 g/100 g of total FA) cows.

Genetic parameters

The overall coefficients of genetic variation were relatively small, ranging from about 2% (SFA) to 9% (PUFA) for FA groups and from 3% (C16:0) to 6% (C18:1) for individual FA. This variation was similar to that estimated for protein (5.5%) and fat (10%) contents (Table 2), suggesting that it

might be feasible to alter the milk FA profile by genetic selection even if the genetic progress would be slow due to moderate to low heritabilities. Estimates of heritability of FA composition were comparable among breeds. The greatest and lowest values were observed for PUFA (0.28 to 0.33) and UFA (0.18 to 0.23) among FA groups, and C18:0 (0.23 to 0.30) and C14:0 (0.16 to 0.20) among individual FA, respectively. To our knowledge, this is the first paper that has estimated heritability and repeatability of milk FA composition in AG cows. This local breed is mainly reared in the Alpine areas of northeast Italy and it is quite popular for its robustness and the close linkage to local culture. In fact, AG cows contribute to the preservation of ancient local traditions and food products (De Marchi et al., 2007). The comparison of results from the present study and those reported in the literature is not easy because of several aspects such as the unit of measure used to express FA composition (milk basis vs. fat basis), the method of analysis (e.g. gascromatography vs MIRS), the model used and the breed of the cow. Estimates of heritability for milk FA were in general higher than those reported by Soyeurt et al. (2007) in mixed-breed population of the Walloon region of Belgium and lower than those reported by Lopez-Villalobos et al. (2014) in New Zealand dairy cattle.

Estimates of repeatability were comparable with those reported by Bobe et al. (2008) in US Holsteins, Penasa et al. (2015) in Italian HF cows, and slighter lower than those reported by Lopez-Villalobos et al. (2014). The moderate values of repeatability underlines the importance of collecting repeated measures of FA profile in order to reduce the contribution from the general environmental variance to the total variance of each measurement.

In order to understand if a breeding program could be applied the genetic relationships between FA in milk must be investigated. Genetic correlations were comparable across breeds. Low to near-zero genetic correlations were found between milk yield and the different FAs in fat ranging from -0.05 (C16:0, C18:0) to about 0.30 (MUFA, UFA ,PUFA and C18:1). In addition a weak negative genetic correlation (-0.25) was found between milk yield and the content of SFA in milk fat.

In general, all the individual saturated FAs tended to be not correlated with milk yield whereas MUFA, PUFA and C18:1 was positively correlated with this trait. These results are comparable with those reported by Soyeurt et al. (2007) confirming that a selection for milk yield would lead to an increase of MUFA and PUFA in milk fat (data not show).

Strong and negative genetic correlations existed between milk fat and UFA, MUFA, PUFA and C18:1 whereas a strong positive correlation were reported between this trait and the saturated part of milk fat (data not show).

Selecting for milk fat will probably decrease the content of unsaturated fatty acids and increase the unsaturated part of fat in milk. However, it is worth to mention that the SFAs did not seem to present the same response to the increase of fat. We noticed a positive correlation between Fat and C16:0 (0.48) whereas no correlation was underlined between fat and C14:0 (0.07) or C18:0 (0.05). We also noticed a positive genetic correlation between C16:0 and C14:0 (0.49) and a negative one between these fatty acids and C18:0 (-0.53 and -0.34 respectively). Finally MUFA were strongly negatively correlated with C14:0 (-0.56) and C16:0 (-0.63), both well known for their negative effects on human health (i.e. cardiovascular diseases); genetic selection to increase MUFA should reduce C14:0 and C16:0 content in milk fat (data not show)

These correlations are in agreement with those reported by the authors mentioned before and completely not comparable with those found by other authors (Bobe et al., 2008; Stoop et al., 2008; Mele et al., 2009; Tullo et al., 2014; Penasa et al., 2015) and this is probably due to the different units of measure used in these different studies. The different units of measure in fact has a big effect on correlations. When expressed as g/100f g of total fat for example genetic correlations are inflated by the fact that SFA and UFA must sum to 100 and for this reason, when expressed in other volume measures (i.e. percentage of milk) they become much lower or even opposite.

CONCLUSIONS

The aim of this paper was to estimate the genetic parameters of the major FAs in cow's milk using MIRS as phenotyping tool in four different Italian breeds: Holstein Friesian (HF), Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG). Mid-Infrared spectroscopy can be successfully applied to large spectral data to predict rapidly milk fatty acids composition at the population level with irrelevant marginal cost. Results from the present study suggest that additive genetic variability for groups and individual fatty acids existed, and this genetic variability may be exploitable in a breeding program to enhance healthy aspects of cow milk. The genetic correlations suggested that selecting for milk fat would increase the content of SFA fatty acids, in particular C16:0 and decrease the content of UFA, MUFA PUFA and C18:1 so a selection against milk fat content is advisable.

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Table 1 *Descriptive statistics of milk yield, quality, and fatty acid (FA) composition for dairy (HF = Holstein-Friesian and BS = Brown Swiss) and dual-purpose (SI = Simmental and GA = Alpine Grey) breeds*

Trait[1]	HF			BS			SI			AG		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Milk yield, kg/day	58 294	28.04	8.14	205 817	22.81	6.95	169 994	23.42	7.18	106 923	17.25	5.86
Milk quality												
Fat, %	58 294	4.04	0.71	205 817	4.24	0.67	169 994	4.06	0.68	106 923	3.82	0.62
Protein, %	58 294	3.38	0.41	205 817	3.67	0.42	169 994	3.50	0.39	106 923	3.46	0.41
SCS, units	58 294	2.50	1.91	205 817	2.60	1.89	169 994	2.26	1.85	106 923	2.37	1.85
Groups of FA, g/100 g of total FA												
SFA	27 603	69.95	3.62	106 169	70.32	3.59	92 403	70.21	3.53	57 383	68.94	3.95
UFA	27 603	29.66	4.16	106 169	29.28	4.07	92 403	29.43	3.94	57 383	30.75	4.43
MUFA	27 603	25.47	3.56	106 169	24.39	3.48	92 403	25.23	3.47	57 383	25.71	3.90
PUFA	27 603	2.94	0.55	106 169	3.00	0.57	92 403	2.90	0.56	57 383	3.22	0.64
Individual FA, g/100 g of total FA												
C14:0	27 603	12.04	1.44	106 169	12.16	1.33	92 403	11.93	1.34	57 383	11.99	1.48
C16:0	27 603	32.35	3.11	106 169	31.93	3.13	92 403	32.45	3.08	57 383	31.34	3.40
C18:0	27 603	9.52	1.75	106 169	9.87	1.68	92 403	10.19	1.69	57 383	10.32	1.78
C18:1	27 603	22.64	3.65	106 169	20.78	3.57	92 403	21.73	3.58	57 383	21.87	3.97

[1]SCS = somatic cell score calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 2 *Coefficient of additive genetic of variation (σ_a), heritability (h^2) and repeatability (rep) of milk yield, quality, and fatty acid (FA) composition for dairy (HF = Holstein-Friesian and BS = Brown Swiss) and dual-purpose (SI = Simmental and GA = Alpine Grey) breeds*

Trait[1]	HF			BS			SI			GA		
	σ_a	rep	$h^2 \pm SE$	σ_a	rep	$h^2 \pm SE$	σ_a	rep	$h^2 \pm SE$	σ_a	rep	$h^2 \pm SE$
Milk yield, kg/day	1.89	0.40	0.13 ± 0.01	1.44	0.42	0.12 ± 0.01	1.54	0.40	0.12 ± 0.01	1.3	0.42	0.14 ± 0.01
Milk quality												
Fat, %	0.35	0.42	0.31 ± 0.02	0.26	0.28	0.20 ± 0.008	0.3	0.33	0.26 ± 0.01	0.22	0.25	0.17 ± 0.01
Protein, %	0.18	0.50	0.35 ± 0.02	0.16	0.48	0.32 ± 0.01	0.16	0.50	0.35 ± 0.01	0.15	0.47	0.30 ± 0.02
SCS, units	0.36	0.17	0.04 ± 0.008	0.32	0.20	0.03 ± 0.004	0.4	0.26	0.06 ± 0.007	0.37	0.26	0.04 ± 0.008
Groups of FA, g/100 g of total FA												
SFA	1.38	0.39	0.22 ± 0.02	1.13	0.33	0.20 ± 0.01	1.26	0.37	0.22 ± 0.01	1.14	0.32	0.19 ± 0.01
UFA	1.49	0.36	0.23 ± 0.02	1.26	0.32	0.18 ± 0.01	1.4	0.37	0.23 ± 0.01	1.34	0.31	0.18 ± 0.01
MUFA	1.33	0.37	0.24 ± 0.03	1.19	0.34	0.21 ± 0.01	1.3	0.38	0.25 ± 0.03	1.28	0.34	0.21 ± 0.01
PUFA	0.25	0.47	0.33 ± 0.02	0.22	0.42	0.29 ± 0.02	0.23	0.46	0.33 ± 0.02	0.24	0.43	0.28 ± 0.02
Individual FA, g/100 g of total FA												
C14:0	0.42	0.25	0.17 ± 0.01	0.39	0.28	0.16 ± 0.01	0.44	0.30	0.20 ± 0.01	0.42	0.26	0.16 ± 0.01
C16:0	1.16	0.40	0.27 ± 0.02	1.06	0.40	0.25 ± 0.02	1.1	0.38	0.24 ± 0.01	1.18	0.36	0.26 ± 0.02
C18:0	0.55	0.38	0.23 ± 0.02	0.57	0.41	0.28 ± 0.02	0.63	0.44	0.30 ± 0.01	0.66	0.43	0.30 ± 0.02
C18:1	1.43	0.39	0.22 ± 0.03	1.2	0.35	0.18 ± 0.02	0.64	0.38	0.26 ± 0.01	1.37	0.36	0.22 ± 0.01

[1]SCS = somatic cell score calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

GENERAL CONCLUSIONS

The main conclusions of this thesis are:

- Mid-infrared spectroscopy is a reliable method to obtain mass phenotypes for milk FA composition. Variable selection method (UVE) and heuristic algorithm (GA) before PLS, help to build more conservative models (i.e. less number of wavenumbers) and better accuracies for both FA groups and individuals, enhancing the efficiency of MIRS models to predict phenotypes at population level for genetic parameters estimation.
- Phenotypic variation of milk FA profile is present, and breed and herd are the most important sources of variation, followed by days in milk and parity. Breeds reared under the same herd conditions largely differ in terms of milk FA composition. Milk of Alpine Grey cows exhibited the best FA composition with lower content of SFA and higher content of UFA, especially MUFA, PUFA and C18:1. The variation of FA groups and individual FA during lactation suggests a close relation between milk FA profile and body energy status of the cow.
- Additive genetic variation of milk FA groups in Italian Holstein-Friesian cows exists. Estimates of genetic correlations suggest that an increase of milk fat content could lead to an undesirable increase of its saturated component and a decrease of its unsaturated part. In addition, if the content of UFA is predisposed to be higher, this will affect mostly its MUFA part, due to the high genetic correlation between these two traits.
- Overall, the content of the major milk individual FA and groups of four Italian breeds (Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey) is moderately heritable and there is room to improve the healthy profile of milk for human consumption. The positive genetic correlations between milk fat, SFA, C14:0 and C16:0 could aid in improving the healthy quality of milk.

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