On the role of mid-infrared predicted phenotypes in fertility and health dairy breeding programs¹

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ABSTRACT

Fertility and health traits are of prime importance in dairy breeding programs. However, these traits are generally complex, difficult to record, and lowly heritable (<0.10), thereby hampering genetic improvement in disease resistance and fertility. Hence, indicators are useful in the prediction of genetic merit for fertility and health traits as long as they are easier to measure than direct fitness traits, heritable, and genetically correlated. Considering that changes in (fine) milk composition over a lactation reflect the physiological status of the cow, mid-infrared (MIR) analysis of milk opens the door to a wide range of potential indicator traits of fertility and health. Previous studies investigated the phenotypic and genetic relationships between fertility and MIR-predicted phenotypes, most being related to negative postpartum energy balance and body fat mobilization (e.g., fat:protein ratio, urea, fatty acids profile). Results showed that a combination of various fatty acid traits (e.g., C18:1 *cis*-9 and C10:0) could be used to improve fertility. Furthermore, occurrence of (sub)clinical ketosis has been related to milk-based phenotypes such as fat:protein ratio, fatty acids, and ketone bodies. Hence, MIR-predicted acetone and β -hydroxybutyrate contents in milk could be useful for breeding cows less susceptible to ketosis. Although studies investigating the genetic association among mastitis and MIR-predicted phenotypes are scarce, a wide range of traits, potentially predicted by MIR spectrometry, are worthy of consideration. These include traits related to the disease response of the cow (e.g., lactoferrin), reduced secretory activity (e.g., casein), and the alteration of the blood-milk barrier (e.g., minerals). Moreover, direct MIR prediction of fertility and health traits should be

further considered. To conclude, MIR-predicted phenotypes have a role to play in the improvement of dairy cow fertility and health. However, further studies are warranted to (1) grasp underlying associations among MIR-predicted indicator and fitness traits, (2) estimate the genetic parameters, and (3) include these traits in broader breeding strategies.

Key words: health, fertility, mid-infrared spectrometry

INTRODUCTION

For several decades, in many countries, breeding goals in dairy cattle have focused mainly on increased milk production, leading to the deterioration of most functional traits, some reaching a critical point and needing to be restored. This was particularly true for female fertility, mastitis resistance, longevity, and metabolic disorders (Boichard and Brochard, 2012). Improved management practices and the inclusion of functional traits in broader breeding goals have restricted, to some extent, the deterioration in these traits or even to reverse the trends (Miglior et al., 2012). However, genetic selection for disease resistance and fertility is still hampered by the low heritability of these traits and the difficulty in routinely collecting, at low cost, a sufficient amount of relevant direct phenotypes. The collection of high-quality direct phenotypes can still be a major bottleneck, especially for disease traits (Egger-Danner et al., 2015). Therefore, indicator traits could be very useful as indirect traits (i.e., traits included in the selection index) to improve dairy cow fertility and health, which are the breeding goal traits. Indicator traits have to be readily measurable at a low cost and highly correlated with direct fertility and health phenotypes (Shook, 1989). For instance, SCC has been widely used as an indicator trait to breed for reduced incidence of both clinical and subclinical mastitis (Heringstad et al., 2000; Rupp and Boichard, 2003). Also, several traits have been investigated as potential indicators traits for fertility, most being related to negative postpartum energy balance (**EB**) and body fat mobilization such as BCS, BW (Berry et al., 2003), and milk composition

Received July 8, 2015.

Accepted November 2, 2015.

¹Presented as part of the Joint Interbull JAM Symposium: Milk Spectral Data—Cost-Effective Information to Improve Expensive and Limited Traits in Dairy Cattle Breeding at the ADSA-ASAS Joint Annual Meeting, Orlando, Florida, July 2015.

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traits (Bastin et al., 2014). Because changes in the biochemical profile of milk can be used to mirror a cow's physiological status (Hamann and Krömker, 1997), the variation in milk composition could be related to fertility and health of dairy cows.

Over the range of potential indicator traits of fertility and health, milk-based traits that could be obtained through regular milk recording schemes are of particular interest because collection is noninvasive, and a new data collection channel would not need to be implemented. Mid-infrared (**MIR**) spectrometry is the method used worldwide in regular milk recording schemes and milk payment systems to quantify the major milk components (i.e., fat, protein, casein, lactose, and urea). Over the last decade, the usefulness of MIR spectrometry to obtain new milk phenotypes such as detailed milk composition, technological properties of milk, or cow physiological status has been demonstrated (De Marchi et al., 2014). Hence, MIR spectrometry is a rapid and cost-effective tool to obtain new milk phenotypes that could be used as indicator traits in dairy breeding programs for enhanced fertility and health.

Therefore, this paper will discuss the opportunity of using MIR-predicted phenotypes as indicators of health and fertility in dairy breeding programs. First, to understand the underlying relationships between milk composition and fitness traits, we will review the phenotypic association of fertility and health with milk components that can be predicted by MIR spectrometry. The focus will be on MIR-predicted phenotypes that are related to (1) negative postpartum EB, and (2) the inflammatory response of the cow to an udder infection. Second, we will examine the genetic associations of MIR-predicted phenotypes with fertility, mastitis, and other disease traits based on the recent literature and also on a study investigating the genetic correlations between clinical mastitis and several MIR-predicted traits by using data from 7,033 Walloon Holstein cows. Finally, we will discuss the inclusion of MIR-predicted indicator traits in broader breeding programs.

CHANGES IN THE BIOCHEMICAL PROFILE OF MILK IN RELATION TO FERTILITY AND HEALTH

Postpartum Changes in Milk Composition

Typically, high-yielding cows experience a period of negative EB in early lactation when nutrient requirements for growth, activity, maintenance, and lactation exceed the energy intake of the animal. In response to the energy deficit, the cow mobilizes tissue reserves. The duration and the magnitude of the postpartum negative EB are critical for productivity, health status, and fertility. Severe and long negative EB is related to

lower fertility in dairy cows through effects exerted both early in lactation and later during the breeding period (Butler, 2003). Additionally, any physiological imbalance of the cow might reduce tolerance or resistance of the cow to the infection. Hence, cows that experience severe postpartum negative EB are at higher risk of health disorders (Collard et al., 2000). In particular, severe negative EB has been related to an impairment of udder defense mechanisms (Suriyasathaporn et al., 2000).

Previous studies reported that the biochemical profile of milk in early lactation is affected by the postpartum EB status of the cow (e.g., de Vries and Veerkamp, 2000; Reist et al., 2002). Hence, changes in milk composition due to negative EB could be associated with reproductive performance (i.e., the ability of the cow to conceive and to maintain pregnancy) and health. Only milk components that are (potentially) predicted by MIR spectrometry are examined in this paper.

Fat, Protein, and Fat:Protein Ratio. During energy balance in early lactation, mobilization of body fat reserves results in an elevated fat percentage in milk (de Vries and Veerkamp, 2000; Reist et al., 2002). Moreover, a decrease in fat percentage over the first 15 wk of lactation was significantly correlated with the extent and the duration of the postpartum negative EB (de Vries and Veerkamp, 2000). In addition, a lower protein percentage is expected in early lactation because of inadequate energy intake (Negussie et al., 2013). Consequently, a high fat:protein ratio (**FPR**) in early lactation has been related to more severe energy balance (de Vries and Veerkamp, 2000; Reist et al., 2002; Butchereit et al., 2011). These patterns were confirmed over a range of studies (Table 1) indicating that poor fertility performances were associated with elevated fat content and low protein content in early lactation, and therefore to high FPR. Moreover, Heuer et al. (1999) indicated that cows with an FPR >1.5 at the first test-day were at higher risk for mastitis, ketosis, and other postpartum disorders. Finally, it should be noted that other studies reported associations of limited magnitude between fertility and fat content (Reksen et al., 2002; Podpecan et al., 2008; Madouasse et al., 2010; Martin et al., 2015), protein content (de Vries and Veerkamp, 2000; Reksen et al., 2002; Demeter et al., 2010; Martin et al., 2015), or FPR (Reksen et al., 2002; Madouasse et al., 2010; Martin et al., 2015).

Fatty Acids. Altered activities in the 2 major pathways for the production of milk fatty acids are expected when cows experience negative EB (Palmquist et al., 1993; Gross et al., 2011). The 2 major pathways for production of milk fatty acids are (1) *de novo* synthesis of fatty acids in the mammary gland, and (2) excretion from the blood to the udder of preformed lipids that

are derived either from the digestion and absorption of dietary fat or from mobilization of adipose tissue (Palmquist et al., 1993; Grummer, 1991). The C4:0 to C14:0 and almost half of C16:0 fatty acids are synthesized de novo in the mammary gland, and the remaining C16:0 and almost all of the longer-chain fatty acids are preformed fatty acids (Grummer, 1991). A higher content of preformed fatty acids is expected in early lactation due to the release in the mammary gland of long-chain fatty acids from the mobilization of body fat reserves (Palmquist et al., 1993; Gross et al., 2011). Concomitantly, the content of de novo synthesized fatty acids tends to be lower in early lactation because the high uptake of long-chain fatty acids in the udder inhibits the de novo synthesis of FA by mammary gland tissue (Palmquist et al., 1993; Gross et al., 2011). This inhibition intensifies with increasing chain lengths (Palmquist et al., 1993). Then, along with increasing DIM and declining adipose tissue mobilization, the proportion of fatty acids derived from blood decreases, whereas the proportion of de novo synthesized fatty acids increases (van Knegsel et al., 2007; Stoop et al., 2009; Gross et al., 2011). These patterns are consistent with the relationship between fertility and milk fatty acids profile presented in Table 1, indicating that a higher content of preformed fatty acids (e.g., C18:1 *cis*-9) and a lower content of de novo synthesized fatty acids (e.g., C14:0) in early lactation tend to be related to poor fertility. In addition, van Haelst et al. (2008) reported that subclinically ketotic cows showed an elevated proportion of C18:1 *cis*-9 in milk fat.

Urea, Lactose, and Citrate. Milk urea reflects protein metabolism in the dairy cow and it is related to the surplus of nitrogen that is available in the rumen for microbiological growth compared with the avail-

able energy (Schepers and Meijer, 1998). Guo et al. (2004) indicated that high milk urea may be caused by many factors, including excessive protein intake or any illness increasing protein catabolism. Milk urea was unfavorably related to fertility in several studies (Table 1), although some of them reported a nonsignificant association (Melendez et al., 2000; Guo et al., 2004). It has been suggested that this association could be due to an unfavorable association between high milk urea and uterine pH, which could make the environment within the uterus unsuitable for early embryo development (Guo et al., 2004). Moreover, a higher lactose content in early lactation was favorably associated with EB (Reist et al., 2002) and fertility (Table 1). Finally, citrate has been presented as a promising indicator of negative EB (Bjerre-Harpøth et al., 2012).

Ketone Bodies. As a result of negative energy balance, the cow mobilizes body fat as a source of energy. However, there is a limit to the amount of fatty acid that can be oxidized by the liver. Goff and Horst (1997) indicated that when this limit is reached, triglycerides accumulate within the hepatocytes, impairing their function, and the acetyl-CoA that is not incorporated into the tricarboxylic acid cycle is converted to acetoacetate and BHB. The appearance of these ketone bodies in the blood, milk, and urine is diagnostic of ketosis. An unfavorable association was observed between acetone and fertility, indicating that higher acetone content in milk during the first week of lactation was related to poor fertility (Table 1). Moreover, the potential of MIR-predicted acetone and BHB contents in milk has been investigated by van Knegsel et al. (2010) and van der Drift et al. (2012) for the detection of (subclinical) ketosis with the definition of various detection thresholds. However, these studies concluded that MIR predictions

Table 1. Overview from various studies that reported significant association between milk components measured in early lactation and fertility performance of the subsequent lactation

Milk component	Association with fertility ¹	Reference
Fat content	Unfavorable	Kristula et al. (1995); Madouasse et al. (2010); de Vries and Veerkamp (2000)
Protein content	Favorable	Madouasse et al. (2010); Podpecan et al. (2008)
Fat:protein ratio	Unfavorable	Heuer et al. (1999); Podpecan et al. (2008)
Lactose content	Favorable	Reksen et al. (2002); Madouasse et al. (2010)
Urea content	Unfavorable	Rajala-Schultz et al. (2001); Butler et al. (1996); König et al. (2008)
Acetone content	Unfavorable	Reksen et al. (2002)
Proportion of fatty acid in milk fat		
SFA	Favorable	Stádník et al. (2015)
MUFA	Unfavorable	Stádník et al. (2015)
UFA	Unfavorable	Hostens et al. (2013)
C14:0	Favorable	Martin et al. (2015)
C16:0	Favorable	Martin et al. (2015)
C18:1 <i>cis</i> -9	Unfavorable	Martin et al. (2015)

¹Unfavorable = high level of this milk component in early lactation was related to poor fertility; favorable = high level of this milk component in early lactation was related to good fertility.

of acetone and BHB contents in milk might be not suitable for the detection of ketosis at the individual level but could be useful at herd level.

Changes in Milk Composition Related to the Inflammatory Response of the Cow to an Udder Infection

Mastitis is an inflammatory reaction of the mammary gland to an infection. Diagnostic methods have been developed to check the quality of the milk through detection of mammary gland inflammation and diagnosis of the infection and its causative pathogens (Viguier et al., 2009). In particular, the increase in the number of somatic cells in the milk of cows suffering from mastitis has been widely documented (Schukken et al., 2003). Somatic cells in milk are mostly cells from the immune system and therefore reflect inflammatory responses to an intramammary infection (Schukken et al., 2003). Somatic cell count in milk is generally determined by flow cytometry and not by MIR spectrometry and it is predominantly used to select for udder health in dairy herd improvement programs. Nevertheless, several other milk-based traits have been reported as useful in the diagnosis of mastitis, thereby providing a very effective pool of traits that could be used as indicator traits to select dairy cows for improved udder health.

Extended literature is available on milk parameters associated with mastitis and that could be useful to monitor udder health (Hamann and Krömker, 1997; Pyörälä, 2003; Lainé et al., 2014b). Changes in milk composition caused by mastitis include (1) the leaking of ions, proteins, and enzymes from the blood into the milk due to increased permeability; (2) the invasion of phagocytizing cells into the milk compartment; and (3) a decrease of the synthetic capacity of the gland, resulting in decreased concentrations of certain milk constituents (Pyörälä, 2003). Among the traits that can be predicted by MIR spectrometry (De Marchi et al., 2014; Bastin et al., 2015) and according to the existing literature on the relationship between milk-based traits and mastitis, the following MIR-predicted traits are potential candidates as indicators of udder health: lactose content, citrate content, titratable acidity, minerals (especially Na and K), and protein components (especially total casein, casein fractions, lactoferrin, α -LA, and β -LG). Expected changes in these traits might differ in direction and magnitude due to the causative agent or to the severity of the infection (Le Maréchal et al., 2011). However, the following modifications in milk composition are generally expected when cows are suffering from mastitis.

Overall, the level of proteins and proteinaceous compounds linked to the inflammatory and immune re-

sponse increases, whereas the level of endogenous milk proteins such as caseins decreases (Le Maréchal et al., 2011). The level of lactoferrin, which has a bacteriostatic function, increases, especially when mastitis is caused by major pathogens (Auldist and Hubble, 1998). A decrease of total casein content is expected, together with a decrease in α - and β -CN and an increase in γ -CN content (Le Maréchal et al., 2011). Moreover, the contents of milk α -LA and β -LG are also expected to decrease in case of mastitis (Auldist and Hubble, 1998). These changes are mainly linked to the reduced secretory activity of the mammary cells and to the increased permeability of the mammary epithelium (Auldist and Hubble, 1998).

Changes in mineral composition of milk are also expected in cases of mastitis. Potassium, the most abundant mineral in milk, leaks out of milk through the ruptured mammary epithelia during mastitis. Conversely, sodium, found in blood in high quantities, leaks into milk and its concentration in milk increases (Auldist and Hubble, 1998). According to Pyörälä (2003), calcium content in milk is expected to decrease. However, Auldist and Hubble (1998) indicated that there is no firm conclusion about the effect of mastitis on calcium concentration. Moreover, lactose content in milk clearly decreases during inflammation (Le Maréchal et al., 2011). Also, with increasing SCC, the pH is expected to increase and therefore the titratable acidity to decrease (Brandt et al., 2010). Finally, citrate content in milk has been pointed out as a potential indicator of mastitis (Hamann and Krömker, 1997).

GENETIC ASSOCIATION OF MIR-PREDICTED TRAITS WITH HEALTH AND FERTILITY

According to phenotypic associations with fertility and health, a number of traits, potentially predicted by MIR spectrometry, are good candidates as indicator traits in dairy breeding programs for improved robustness. The correlated response in fertility and health to selection for these indicators allows us to evaluate the benefit of using such indicators. The correlated response depends on the selection intensity in the indicator trait, the genetic correlation of fertility or health trait with the indicator trait, the heritability of both traits, and the SD of the health or fertility trait (Banos and Coffey, 2010). To further evaluate the correlated response in fertility and health to selection for MIR-predicted traits, the genetic parameters of these traits were examined based on (1) a review from the existing literature for fertility and health disorders other than mastitis, and (2) a study investigating the genetic correlations between clinical mastitis and several MIR-predicted traits for Walloon Holstein cows.

Fertility

Table 2 provides an overview from various studies of the genetic parameters of infrared-predicted traits that could be useful as indicators traits for fertility. In addition to milk components, Table 2 provides the genetic parameters for MIR predictions of body traits as defined by McParland et al. (2012). Those authors evaluated the potential of MIR analysis of milk to directly predict body energy status traits and provided

predictive equations for direct EB (MJ/d), body energy content (MJ), and the effective energy intake per day (MJ/d). Only traits measured during the first 2 mo of lactation, when milk composition is likely to reflect the postpartum negative EB status of the cow, are listed in Table 2. It also demonstrates the interest of using information that is readily available after calving (i.e., at first test-day).

Overall, the genetic correlations provided in Table 2 are consistent with the expected phenotypic associa-

Table 2. Overview from various studies of the heritability estimates (h^2) of infrared-predicted traits and their genetic correlation with fertility traits (r_g). When available, the SE or the level of significance of the correlation is provided

Infrared-predicted trait	h^2	Fertility trait	r_g	Reference
Average MUN concentration from the 2 first test-day (mg/kg)	0.13	Interval from calving to first service	0.29 (0.01)	König et al., 2008
		56-d nonreturn rate	-0.13 (0.11)	
		90-d nonreturn rate	-0.12 (0.12)	
Milk urea concentration at 5 DIM (mg/L)	0.12	Days open	0.03	Bastin et al., 2014
Fat:protein ratio at 30 DIM	0.16	Calving to first insemination	0.28 (≤ 0.01)	Negussie et al., 2013
		Days open	0.24 (≤ 0.01)	
		Number of inseminations	0.03 (≤ 0.01)	
		Nonreturn rate to 56 d	0.01 (≤ 0.01)	
Fat:protein ratio at 60 DIM	0.19	Calving to first insemination	0.14 (≤ 0.01)	Negussie et al., 2013
		Days open	0.19 (≤ 0.01)	
		Number of inseminations	0.02 (≤ 0.01)	
		Nonreturn rate to 56 d	0.01 (≤ 0.01)	
Fat:protein ratio at 5 DIM	0.17	Days open	0.23	Bastin et al., 2014
Fatty acids (g/dL of milk) at 5 DIM				Bastin et al., 2012
SFA	0.25	Days open	-0.23 (0.10)	
MUFA	0.13	Days open	0.37 (0.13)	
PUFA	0.20	Days open	0.00 (0.12)	
UFA	0.13	Days open	0.34 (0.12)	
Short-chain FA	0.30	Days open	-0.27 (0.09)	
Medium-chain FA	0.26	Days open	-0.21 (0.10)	
Long-chain FA	0.12	Days open	0.33 (0.12)	
C4:0	0.22	Days open	-0.02 (0.12)	
C6:0	0.28	Days open	-0.23 (0.10)	
C8:0	0.30	Days open	-0.30 (0.09)	
C10:0	0.28	Days open	-0.37 (0.09)	
C12:0	0.28	Days open	-0.35 (0.09)	
C14:0	0.27	Days open	-0.29 (0.09)	
C16:0	0.24	Days open	-0.17 (0.11)	
C17:0	0.25	Days open	-0.10 (0.11)	
C18:0	0.14	Days open	0.08 (0.13)	
C18:1 <i>cis</i> -9	0.13	Days open	0.39 (0.12)	
Direct energy balance (MJ) at 5 DIM	0.22	Days open	-0.20 (0.09)	Bastin et al., 2013
Body energy content (MJ/d) at 5 DIM	0.11	Days open	-0.08 (0.10)	Bastin et al., 2013
Effective energy intake (MJ) at 5 DIM	0.10	Days open	-0.28 (0.11)	Bastin et al., 2013
Log _e -transformed BHB content (mmol/L) from 5 to 20 DIM	0.14	Calving to first service	0.21***	Koeck et al., 2014
		Nonreturn rate to 56 d	-0.07	
		Number of service	0.12*	
		First service to conception	0.17***	
		Days open	0.20***	
Log _e -transformed BHB content (mmol/L) from 21 to 40 DIM	0.15	Calving to first service	0.11*	Koeck et al., 2014
		Nonreturn rate to 56 d	-0.06	
		Number of service	0.08	
		First service to conception	0.10*	
		Days open	0.11*	
BHB content (mmol/L) at 5 DIM	0.26	Days open	0.22	C. Bastin (unpublished data)
Acetone content (mmol/L) at 5 DIM	0.37	Days open	0.11	C. Bastin (unpublished data)
Citrate content (mmol/L) at 5 DIM	0.37	Days open	0.02	C. Bastin (unpublished data)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tions between milk-based traits in early lactation and fertility. Poor fertility was genetically associated with decreasing de novo synthesized FA contents in milk, with lower body energy traits, with increasing FPR and increasing contents in milk of urea, acetone, BHB, and long-chain MUFA. It should be noted that estimates provided in Table 2 are limited to the early stages of lactation. Nevertheless, based on the evolution of milk composition over DIM, it could be expected that genetic parameters change throughout the lactation. Bastin et al. (2012) estimated the genetic correlations among milk fatty acids and days open by using random regression models and indicated that genetic correlations changed considerably over the lactation. Therefore, genetic correlations between milk-based traits and fertility should be interpreted with regard to the stage of lactation when the milk sample was collected.

According to the genetic correlations with fertility shown in Table 2, milk fatty acid traits, especially contents in milk of C8:0 to C14:0 and C18:1 *cis*-9, were the best predictors of fertility, followed by urea content and FPR. However, König et al. (2008) demonstrated that genetic progress toward improvement in 56-d nonreturn rate could not be achieved via selection strategies on milk urea. A similar conclusion was provided by Mitchell et al. (2005) and Miglior et al. (2007), who indicated that the genetic correlation between milk urea and fertility was too weak to justify the use of milk urea as an indicator trait for fertility. To further investigate the usefulness of fatty acid traits in fertility breeding programs, Bastin et al. (2014) calculated the accuracy of a fertility index, including days open as direct trait, the content in milk of C10:0 at 5 DIM, the content in milk of C18:1 *cis*-9 at 5 DIM, or a combination of these. The authors concluded that (1) although direct selection for days open provided the best accuracy for the fertility index, using fatty acids traits in a fertility index would allow faster, indirect selection on reproduction performance; (2) an index including the 2 fatty acid traits had higher accuracy than an index including only 1 trait, thereby substantiating the opportunity to combine indicators related to various aspects of EB status and body fat mobilization. Finally, it is worth noting that several direct fertility traits (e.g., days open, calving interval) are only available when a cow has calved again, whereas indirect traits are available even for these cows with the worst fertility.

Udder Health

Udder health indices often include only traits based on SCC or its logarithmic transformation SCS (Interbull, 2015), especially as several countries do not have routine recording of clinical mastitis and cannot perform

genetic evaluations on direct health traits. Although SCS has the great advantage of providing information on subclinical variations in mastitis, the genetic correlation between clinical mastitis and SCS is <1 , with an average between 0.60 and 0.70 (Heringstad et al., 2000), thereby bringing into question the use of SCS solely to select for decreased susceptibility to mastitis. Therefore, other milk-based traits could be useful to supplement SCS in the prediction of genetic merit for udder health. For instance, Norberg (2005) proposed electrical conductivity, often measured by sensors installed in automatic milking systems, in addition to SCS to select against mastitis susceptibility. Norberg (2005) reported heritability estimates of electrical conductivity ranging between 0.12 and 0.36 and genetic correlations with clinical mastitis ranging from 0.65 to 0.80. Given that the concentration of anions and cations, with Na^+ , K^+ , and Cl^- being the most important, determines the electrical conductivity of milk (Norberg, 2005), MIR predictions of minerals are of great interest as additional traits in genetic selection for udder health. Nevertheless, few genetic correlation estimates between clinical mastitis and MIR predicted traits are reported in the literature. Therefore, to provide a first insight on the opportunity of using MIR-predicted traits as udder health indicators, a preliminary study investigating the genetic correlations between MIR-predicted traits and clinical mastitis was performed for Walloon Holstein cows.

Materials and Methods. Based on a data set including observations collected in 77 herds between 2007 and 2015 from 7,033 Walloon Holstein cows in parity 1 to 5, genetic parameters were estimated for clinical mastitis and traits derived from the SCS and the MIR predictions of FPR, urea, lactose, minerals (Na, Ca, K, P, and Mg), acetone, BHB, citrate, lactoferrin, and titratable acidity. Calibration equations used for lactoferrin, minerals, acetone, BHB, citrate, and titratable acidity were obtained in previous work (Colinet et al., 2010; Soyeurt et al., 2009, 2011, 2012; Bastin et al., 2015). Clinical mastitis data used in this study were voluntarily recorded by dairy farmers through a web-based system. To ensure good quality of the training data set, several edits were applied, as explained by Lainé et al. (2014b). Clinical mastitis (CMAS) was defined as a binary trait (0 = no mastitis, 1 = mastitis) based on whether the cow suffered from at least one episode of mastitis in the period from 10 d before calving to 10 d before the next calving. For SCS and each MIR prediction, 3 alternative traits were defined: (1) the average of the trait from 5 to 305 DIM, (2) the SD of the trait from 5 to 305 DIM, and (3) the average of the traits from 5 to 65 DIM. First, variance components were estimated using a bivariate linear model that combined

CMAS with one of the indicator traits. The model included year of calving, month of calving, class of age at calving within lactation as fixed effects, and herd $\times$ year of calving, permanent environment, and genetic additive as random effects. Analyses were performed by Gibbs sampling as implemented in the GIBBSF90 program (Misztal, 2015). A single chain of 200,000 cycles with a burn-in of the first 20,000 iterations was run for the analyses. Every 20th sample was retained to compute mean and SD of the posterior distribution. Results of the bivariate analysis are provided in Table 3. Considering that the selection intensity as well as the heritability and the SD of CMAS remained constant, the product of the square root of the heritability of the indicator trait with its absolute genetic correlation with CMAS was added in Table 3 as a proxy for the relative correlated response in udder health to selection for this indicator (Banos and Coffey, 2010). In a second step, to assess the usefulness of MIR-predicted indicator traits to supplement SCS in the prediction of genetic merit for CMAS, a 5-trait model was developed including CMAS, the averaged SCS over the lactation, the SD of Na and citrate over the lactation, and the content in milk of acetone averaged from 5 to 65 DIM. Then, the accuracy of an index including either CMAS, the indicator traits, or a combination of them was estimated, as described by Bastin et al. (2014) for a bull having a varying number of daughters with records ($D = 20, 50, 100$) under various scenarios listed in Table 4. The trait included in the breeding objective was CMAS. Parameters used in these calculations are presented in Table 5.

Results and Discussion. On average, over the 42 bivariate models, the heritability of CMAS was 0.05. Detailed heritability estimates of indicator traits and their genetic correlations with CMAS are provided in Table 3 and ranged from 0.01 to 0.62. Genetic correlations ranged from -0.68 to 0.89 but some were subject to large standard errors. According to the relative correlated response to selection, SCS remains the best indicator trait to select against clinical mastitis susceptibility. However, other traits might be of interest according to their strong genetic association with CMAS. Overall, indicator traits defined as the average of a MIR prediction over the lactation were of limited interest because of their low genetic correlation with CMAS compared with traits reporting the variation over the lactation of some MIR-predicted milk components. However, traits defined as the SD of MIR prediction from 5 to 305 DIM had the lowest heritability. Increasing risk of clinical mastitis was strongly genetically associated with greater variation over the lactation of the contents in milk of Na, Ca, and citrate, 3 milk components that can be related to the inflammatory response of the cow to the infection (Hamann and Krömker, 1997; Pyörälä, 2003). The occurrence of clinical mastitis was positively associated with the SD from 5 to 305 DIM and the average from 5 to 65 DIM of acetone content in milk (and to a lower extent of BHB). Such traits might reflect a negative postpartum EB state of the cow that could affect udder health over the lactation. This is in line with findings of Koeck et al. (2014), who reported a significant correlation of 0.20 between EBV for milk BHB measured between 5 and

Table 3. Genetic parameters of alternative indicator traits based on SCS and mid-infrared predictions: heritability, genetic correlation (r_g) with clinical mastitis, and the product of the square root of the heritability of the indicator trait with its absolute genetic correlation with the clinical mastitis ($h \times r_g$) as a proxy of the relative correlated response in udder health to selection for this indicator

Indicator trait	Average from 5 to 305 DIM			SD from 5 to 305 DIM			Average from 5 to 65 DIM		
	h^2	r_g	$h \times r_g$	h^2	r_g	$h \times r_g$	h^2	r_g	$h \times r_g$
SCS	0.13	0.79 ^a	0.29	0.03	0.87 ^a	0.14	0.08	0.83 ^a	0.24
Fat:protein ratio	0.43	-0.38^a	0.25	0.04	0.09 ^c	0.02	0.18	-0.25^b	0.11
Urea (mg/L)	0.33	-0.03^a	0.02	0.04	0.22 ^b	0.04	0.14	-0.31^b	0.11
Lactose (g/L)	0.53	-0.10^a	0.07	0.06	0.17 ^b	0.04	0.37	-0.24^a	0.14
Na (mg/kg)	0.44	0.17 ^a	0.11	0.01	0.83 ^a	0.10	0.23	0.19 ^b	0.09
Ca (mg/kg)	0.59	-0.13^a	0.10	0.01	0.89 ^a	0.10	0.35	-0.07^a	0.04
P (mg/kg)	0.54	0.00 ^a	0.00	0.01	0.10 ^c	0.01	0.32	-0.02^a	0.01
Mg (mg/kg)	0.54	0.07 ^a	0.05	0.09	0.07 ^b	0.02	0.29	0.19 ^a	0.10
K (mg/kg)	0.59	0.21 ^a	0.16	0.02	-0.68^c	0.08	0.41	0.16 ^a	0.11
Acetone (mmol/L)	0.38	0.06 ^a	0.04	0.05	0.82 ^a	0.18	0.11	0.60 ^a	0.20
BHB (mmol/L)	0.52	0.12 ^a	0.09	0.08	0.44 ^b	0.12	0.18	0.32 ^b	0.14
Citrate (mmol/L)	0.62	0.02 ^a	0.01	0.04	0.77 ^a	0.15	0.33	0.12 ^a	0.07
Titrate acidity (Dornic degrees)	0.53	-0.11^a	0.08	0.02	-0.11^c	0.01	0.37	-0.07^a	0.05
Lactoferrin (mg/L)	0.40	-0.01^a	0.01	0.08	0.07 ^b	0.02	0.17	0.17 ^b	0.07

^{a-c}Superscript letters provide information on the SE of the estimates: ^a when $SE < 0.15$, ^b when $0.15 \leq SE < 0.25$, and ^c when $SE \geq 0.25$. SE for heritability estimates were all ≤ 0.04 .

Table 4. Accuracy of an index for clinical mastitis including a various number of traits—clinical mastitis (CMAS), average SCS from 5 to 305 DIM (SCS_{m305}), SD of Na content in milk from 5 to 305 DIM (Na_{sd305} ; mg/kg), SD of citrate content in milk from 5 to 305 DIM ($Citrate_{sd305}$; mmol/L), and average acetone content in milk from 5 to 65 DIM ($Acetone_{m65}$; mmol/L), estimated for a bull having a varying number of daughters with records ($D = 20, 50, 100$)

Trait(s) in the index	Accuracy of the index		
	D = 20	D = 50	D = 100
CMAS	0.41	0.58	0.71
SCS_{m305}	0.49	0.61	0.67
Na_{sd305}	0.28	0.40	0.51
$Citrate_{sd305}$	0.23	0.33	0.42
$Acetone_{m65}$	0.32	0.43	0.50
$SCS_{m305} + Na_{sd305}$	0.52	0.63	0.69
$SCS_{m305} + Citrate_{sd305}$	0.53	0.65	0.73
$SCS_{m305} + Acetone_{m65}$	0.56	0.68	0.75
$SCS_{m305} + Na_{sd305} + Citrate_{sd305} + Acetone_{m65}$	0.59	0.71	0.78
CMAS + SCS_{m305}	0.56	0.70	0.78
CMAS + $SCS_{m305} + Na_{sd305} + Citrate_{sd305} + Acetone_{m65}$	0.63	0.76	0.84

20 DIM and EBV for SCS. However, a negative correlation was observed between CMAS and the FPR averaged from 5 to 305 DIM and from 5 to 65 DIM (Table 3). This is in opposition to Negussie et al. (2013), who reported significant genetic correlations ranging from 0.19 to 0.21 between clinical mastitis and FPR at DIM 30, 60, and 110. Hence, Windig et al. (2005) indicated that the relationship between FPR and mastitis might be either positive or negative according to the causative pathogens. Results presented in Table 4 confirmed that traits derived from SCS are the indicator of choice to improve CMAS, given that the average SCS from 5 to 305 DIM (SCS_{m305}) alone provided the best accuracy of the index when based on one indicator trait only. Nevertheless, MIR-predicted traits could supplement SCS in an index aimed at decreasing clinical mastitis susceptibility. Hence, an indicator combining the 4 indicator traits provided an accuracy greater than an index including either CMAS only or CMAS and SCS only. Finally, an index combining both direct (CMAS) and indirect traits is the best option to achieve genetic

improvement toward improved udder health, especially as indirect traits provide information on subclinical mastitis.

Ketosis and Other Disease Traits

Because hyperketonemia and its clinical manifestation (ketosis) are characterized by elevated concentration of ketone bodies in body fluids, MIR prediction of acetone and BHB are good candidates as indicators of (sub)clinical ketosis. Hence, van der Drift et al. (2012) reported moderate genetic correlations (0.52) between plasma BHB, used as the reference test to diagnose hyperketonemia, and MIR predictions of milk BHB and acetone. They explained the moderate genetic correlations by the experimental design of the study (i.e., timing of sampling), the differences among cows in the metabolism of ketone bodies, and by the relatively low accuracy of MIR predictions of ketone bodies. Nevertheless, given that MIR-predicted acetone and BHB contents in milk can be obtained routinely at test-day,

Table 5. Genetic SD (σ_a), heritability (on the diagonal), genetic correlation (above the diagonal), and phenotypic correlation (below the diagonal) among clinical mastitis (CMAS), average SCS from 5 to 305 DIM (SCS_{m305}), SD of Na content in milk from 5 to 305 DIM (Na_{sd305} ; mg/kg), SD of citrate content in milk from 5 to 305 DIM ($Citrate_{sd305}$; mmol/L), and average acetone content in milk from 5 to 65 DIM ($Acetone_{m65}$; mmol/L)

Trait	σ_a	Trait				
		CMAS	SCS_{m305}	Na_{sd305}	$Citrate_{sd305}$	$Acetone_{m65}$
CMAS	6.69×10^{-3}	0.04	0.75 ^a	0.80 ^a	0.63 ^c	0.62 ^b
SCS_{m305}	2.28×10^{-1}	0.20	0.15	0.90 ^a	0.35 ^b	0.36 ^b
Na_{sd305}	5.78	0.02	0.05	0.03	0.43 ^c	0.23 ^b
$Citrate_{sd305}$	3.92×10^{-3}	0.02	0.00	0.13	0.03	0.70 ^b
$Acetone_{m65}$	7.75×10^{-5}	0.03	0.01	0.10	0.17	0.07

^{a-c}Superscript letters provide information on the SE of the genetic correlation estimates: ^a when $SE < 0.10$, ^b when $0.10 \leq SE < 0.15$, and ^c when $0.15 \leq SE < 0.25$. SE of phenotypic correlation estimates were not available; SE of heritability estimates were ≤ 0.02 .

van der Drift et al. (2012) concluded that these indicators might be a practical alternative for breeding programs aimed at reducing hyperketonemia in early lactation.

Other studies related directly MIR-predicted traits with diseases records. Such studies mostly reported genetic correlations estimated between clinical diseases and indicator traits that are measured in early lactation. Table 6 provides an overview from various studies reporting genetic parameters of MIR-predicted traits that could be useful as indicators for clinical disease. First, it should be highlighted that SE of genetic correlation estimates were generally high and should therefore be considered with caution. Fat:protein ratio, especially at the very beginning of the lactation, seems to be one of the most interesting traits to select against the susceptibility to clinical ketosis or displaced abomasum. Other ratios might also of interest, such as the fat:lactose ratio, which has been related to severe negative EB (Reist et al., 2002). Furthermore, Koeck et al. (2014) reported a genetic correlation of 0.48 between clinical ketosis and MIR-predicted BHB at first test-day. Although the estimate was not significant, it supported the opportunity to use MIR-predicted ketone bodies to select against clinical ketosis.

ON THE INCLUSION OF MIR-PREDICTED TRAITS IN HEALTH AND FERTILITY BREEDING PROGRAMS

On the Choice of Indicator Traits and Their Inclusion in the Selection Index

Over the last 2 decades, growing emphasis has been placed on functional traits in breeding programs to breed healthy and long-lived cows that are also highly productive (Egger-Danner et al., 2015). Direct selection on health and fertility traits, by their inclusion in both breeding goal and selection index, remains the main option for the improvement of dairy cow robustness. However, the collection of relevant phenotypes for fertility and disease is still hampered, notably, by the implementation of effective recording systems, the quality of data, the long period required to validate some phenotypes (e.g., calving interval), or the censoring for some traits (e.g., no calving interval records for unfertile animals). Moreover, these traits are generally of low heritability (Shook, 1989; Veerkamp and Beerda, 2007). Consequently, indicator traits might be included in the selection index toward improvement in fertility and health if they have a high genetic correlation with an economically important trait and if they have a

Table 6. Overview from various studies of the heritability estimates (h^2) of infrared-predicted traits and their genetic correlation with disease traits (r_g ; when available, the SE of correlation estimates is provided)

Disease trait	Mid-infrared predicted trait	h^2	r_g	Reference
Clinical ketosis	Log _e -transformed BHB content (mmol/L) at first test-day (5–40 DIM)	0.12	0.48 (0.35)	Koeck et al. (2014)
	Fat:protein ratio at first test-day (5–30 DIM)	0.15	0.30 (0.14)	Koeck et al. (2013)
	>1.5 at first test-day (5–30 DIM)	0.07	0.35 (0.16)	
	at second test-day (31–60 DIM)	0.14	0.05 (0.16)	
	>1.5 second test-day (31–60 DIM)	0.03	−0.06 (0.21)	
	Fat:protein ratio at first test-day (5–40 DIM)	0.12	0.56 (0.32)	Koeck et al. (2014)
	Fat:protein ratio at first test-day	0.16	0.38 (0.10)	Ederer et al. (2014)
	at second test-day	0.14	0.25 (0.09)	
	Fat:lactose ratio at first test-day	0.19	−0.25 (0.10)	
	Protein:lactose ratio at first test-day	0.29	−0.21 (0.09)	
	Urea content (mg/100 mL) at first test-day	0.10	−0.11 (0.11)	Ederer et al. (2014)
				Koeck et al. (2013)
Displaced abomasum	Fat:protein ratio at first test-day (5–30 DIM)	0.16	0.26 (0.08)	
	>1.5 at first test-day (5–30 DIM)	0.08	0.25 (0.09)	
	at second test-day (31–60 DIM)	0.17	0.11 (0.09)	
	>1.5 second test-day (31–60 DIM)	0.04	0.09 (0.12)	
	Fat:protein ratio at first test-day (5–40 DIM)	0.12	0.25 (0.20)	Koeck et al. (2014)
	Log _e -transformed BHB content (mmol/L) at first test-day (5–40 DIM)	0.12	0.07 (0.21)	Koeck et al. (2014)
Milk fever	Fat:protein ratio at first test-day	0.16	0.18 (0.05)	Ederer et al. (2014)
	at second test-day	0.14	0.01 (0.06)	
	Fat:lactose ratio at first test-day	0.19	−0.07 (0.05)	Ederer et al. (2014)
	Protein:lactose ratio at first test-day	0.29	−0.13 (0.05)	Ederer et al. (2014)
	Urea content (mg/100 mL) at first test-day	0.10	−0.21 (0.06)	Ederer et al. (2014)
Metabolic disorders	Fat:protein ratio from 11 to 180 DIM	0.30	0.69 (0.91)	Buttchereit et al. (2012)
Claw and leg diseases	Fat:protein ratio from 11 to 180 DIM	0.30	0.14 (0.16)	Buttchereit et al. (2012)

lower recording cost, higher heritability, or can be measured earlier in life than the economically important traits they represent (Shook, 1989). Considerations provided throughout this paper indicated that several MIR-predicted traits fulfill these criteria.

First, MIR spectrometry is a rapid and cost-effective tool to obtain new indicator traits for fertility and health if (1) the trait can be predicted by MIR spectrometry with a sufficient accuracy (depending on its use), and (2) the initial capital investment for developing the prediction equations based on a sufficient data set representative of the population is overcome (De Marchi et al., 2014). It is worth noting that the accuracy of the MIR predictions is variable among traits. For instance, the SFA and MUFA contents in milk are predicted with very good accuracy (Soyeurt et al., 2011), whereas some traits have lower accuracy, such as ketone bodies (de Roos et al., 2007; Bastin et al., 2015), Na (Soyeurt et al., 2009), or lactoferrin (Soyeurt et al., 2012). However, when searching for indicator traits, the genetic correlation of the indicator with the fertility or health trait of interest has to be considered, rather than the accuracy of the MIR prediction equation. Finally, because of its use in regular milk recording, MIR spectrometry provides phenotypes that are readily available (i.e., at first test-day) for all cows recorded in the population.

Second, MIR-predicted indicator traits can be identified and characterized through study of their phenotypic and (more importantly) genetic correlations with fertility and health. Most of the traits that are potential indicators of udder health are directly related to the inflammatory response of the cow to infection. In addition, Shook (1989) reported that an indicator trait for disease will be most useful if it indicates subclinical variations in the disease. For fertility and metabolic diseases, potential indicators are traits associated with the extent and duration of postpartum negative EB. However, further research is warranted to comprehend the underlying associations among MIR-predicted indicator and fitness traits. Genetic parameters are then required to properly identify MIR-predicted traits that could be useful in dairy health and fertility breeding programs. However, studies reporting genetic correlations estimated between MIR-predicted indicators and fitness traits are scarce, mainly due to the lack of comprehensive data sets that include both groups of traits, especially health data. Hence, phenotypic studies linking indicators to direct traits can be used as proxies to assess interest of the indicators. Moreover, changes in correlations between MIR-predicted indicators and fitness traits across the lactation have to be

investigated. For instance, Bastin et al. (2012) reported that the genetic correlation between days open and the content in milk of C18:1 *cis*-9 was 0.39 at 5 DIM and -0.30 at 305 DIM. Hence, the stage of lactation when the MIR trait is recorded should be considered. Furthermore, some MIR-predicted traits might have intermediate optimums, thereby complicating their use for breeding purposes. For instance, higher lactoferrin content has been related to the occurrence of mastitis, whereas Hagiwara et al. (2003) indicated that a certain level of lactoferrin in milk was necessary to prevent certain infections.

Before including MIR-predicted indicator traits into selection indexes, some additional aspects have to be considered. First, the benefit of using MIR-predicted indicators in addition to other already used indicators or direct traits has to be demonstrated. Results from Table 4 demonstrate that MIR-predicted traits are useful to supplement SCS in an udder health index, and Bastin et al. (2014) reported that milk fatty acids could substitute for BCS in a fertility index. In both cases, the advantage of using indicator traits in addition to direct traits was demonstrated. Second, the relationships of MIR-predicted traits with other economically important traits (e.g., milk production) should be verified. The inclusion of MIR-predicted traits in breeding programs has to be considered with respect to their potential own direct economic value (e.g., fatty acid composition might have a direct impact on milk payment). Possible drawbacks of indicator traits have to be examined. For instance, breeding for a low FPR may be beneficial in early lactation, but not over a 305-d lactation period, especially in payment systems where protein is the most valuable component of the milk. Similarly, a higher content of C18:1 *cis*-9 at early postcalving was associated with poor fertility performance, whereas consumption of C18:1 *cis*-9 is considered favorable for human health (Haug et al., 2007). Thus, a higher content of C18:1 *cis*-9 in milk would be desirable for the nutritional properties of milk fat, whereas a lower content of C18:1 *cis*-9 in early lactation is desirable for improved fertility. Nevertheless, results inferred from Bastin et al. (2011) indicated that the genetic correlation between content in milk of C18:1 *cis*-9 in early lactation and its contents at 50, 100, 200, and 300 d postcalving were 0.96, 0.75, 0.27, and 0.31, respectively. It may therefore be possible to lower the content in milk of C18:1 *cis*-9 in early lactation without affecting the average MUFA content in milk over the entire lactation. Finally, weights of indicator traits in the index should be estimated with appropriate procedures (Groen et al., 1997).

On the Inclusion of MIR-Predicted Indicators in Genomic Selection

Modern breeding programs are changing through the practical introduction of genomic selection (Meuwissen et al., 2001). The objective of genomic selection is to obtain as early as possible—and therefore for animals without phenotypes—sufficiently reliable genomically enhanced EBV (GEBV). In genomic selection, a key element is the concept of a reference population, a group of animals associating reliable phenotypes and genotypes to assess the relationship between both. The obtained prediction equation can then be used to predict GEBV for animals without phenotypes. Hence, genomic selection seems appealing to achieve genetic progress in functional traits.

In genomic selection, indicator traits are particularly useful for traits with low direct genomic value accuracy, which might result from the fact that the heritability of the trait of interest is low or because the cow reference population for the trait is small (Calus et al., 2013a). Such additional gain in accuracy can be achieved when the indicator traits are recorded for both the evaluated animals and the reference population (Calus et al., 2013a; Pszczola et al., 2013). Given that MIR-predicted traits can be obtained throughout regular milk recording, they can be easily collected on both populations. The inclusion of cows in the reference population is also useful in genomic selection programs for indicator traits (Calus et al., 2013a). Furthermore, studies showed that the reliable estimation of GEBV depends also on the pedigree distance between the reference population and evaluated animals (e.g., Pszczola et al., 2012). Reliabilities of GEBV, especially for animals without records, will also decay over generations if the reference population is not updated constantly (e.g., Calus, 2010; Wolc et al., 2011). Updating the reference population may be difficult in practice for economic and practical reasons, especially for traits that involve expensive precise phenotyping of genotyped cows. Therefore, MIR-predicted traits would allow for the generation of updated phenotypic data for daughters of new generations of genotyped sires. Hence, required adapted genomic evaluation methodology allowing the optimal use of these difference sources of phenotypic information is currently under development (Calus et al., 2013b; Vandenplas et al., 2014).

On Implementation in the Field

Considerations provided throughout this paper indicate that some MIR-predicted phenotypes fulfill the criteria for indicator traits in fertility and health breeding programs. In practice, few MIR-predicted traits are

currently considered in genetic evaluations for fertility and udder health. For “traditional” MIR-predicted traits (i.e., FPR, urea) that are already available routinely, that they are not considered in genetic evaluations for fertility and udder health might be due to a limited advantage of using them as indicators, as has been demonstrated for urea (Mitchell et al., 2005; Miglior et al., 2007; König et al., 2008). For “novel” MIR-predicted traits, the opportunity of using such phenotypes might be hampered by (1) access to spectral data, and (2) access to a prediction equation that was created on a data set encompassing the variability of the population. Additionally, challenges related to the harmonization of spectral data over time and among spectrometers (Grelet et al., 2015) should be considered.

Currently, indicator traits related to ketosis have the most potential to be implemented in the field, either to supplement direct phenotypes of clinical ketosis in genetic evaluation, as has been suggested in Canada (Koeck et al., 2015), or to substitute for disease records when they are not available, as in the Netherlands (Vosman et al., 2015). In the frame of a project whose overall objective is to develop genetic evaluation for metabolic disease traits for Canadian dairy cattle, Koeck et al. (2015) proposed a multivariate model including 2 disease traits (i.e., displaced abomasum and clinical ketosis) and 3 indicator traits (i.e., BCS, MIR-predicted BHB at first test-day, and FPR at first test-day). Results indicated that the use of a multivariate model significantly increased reliability (from 28 to 64%) of sire EBV for clinical ketosis compared with a univariate model including only clinical ketosis. These results will be used as a basis to implement a routine genetic evaluation for metabolic disease in Canada in the near future. In the Netherlands, EBV for ketosis have been released since December 2014 (Vosman et al., 2015). The ketosis phenotype is established based on a combination of MIR-predicted traits: FPR, acetone, and BHB, and gave heritability estimates ranging from 0.13 to 0.18 according to lactation (Vosman et al., 2015). Furthermore, genetic trends between 2001 and 2010 were favorable, implying a decline of the incidence of ketosis, probably because of the positive correlation with udder health and fertility (Vosman et al., 2015).

New Potential MIR-Predicted Indicator Traits

As mentioned by De Marchi et al. (2014), there is growing interest in a broader application of MIR spectrometry to milk. Hence, MIR analysis of milk could be used to predict several other milk traits of interest in fertility and health breeding programs. Novel traits potentially useful to supplement the prediction of genetic merit for reproductive performance include MIR pre-

diction of body energy status or energy intake (McParland et al., 2015), direct MIR prediction of conception success (Hempstalk et al., 2015), or pregnancy status (Lainé et al., 2014a). A wide range of traits have been identified as potential udder health indicators (Pyörälä, 2003; Viguier et al., 2009). Assuming a limit of detection of 500 to 1,000 ppm (Dardenne, 2010), some are potentially predictable by MIR spectrometry such as haptoglobin (Viguier et al., 2009). Finally, this paper covered indicator traits mainly for fertility, clinical mastitis, and ketosis. However, MIR-predicted indicators would be of great interest for other novel health traits such as foot and legs diseases, which rank among the 3 most frequent reasons for culling after reproduction and udder health (Egger-Danner et al., 2015). Given that the relationship between lameness and BCS was recently highlighted (Bicalho and Oikonomou, 2013), MIR-predicted traits related to the negative EB status of the cow could be of interest as indicators to improve foot and legs health.

CONCLUSIONS

Functional traits are of prime importance in dairy breeding programs and MIR-predicted phenotypes have a role to play in genetic and genomic selection for these traits. Because changes in the biochemical profile of milk can be used to mirror the cow's physiological status (e.g., postpartum negative EB or inflammatory response of the cow to an infection), MIR-predicted phenotypes can be related to fertility and health of dairy cows. If such MIR-predicted traits are heritable and genetically correlated with fertility and health, they could either supplement direct phenotypes of fertility and health in selection indices or substitute for them when they are not (readily) available or available on only a limited number of animals. However, further studies are warranted to (1) understand the underlying associations between MIR-predicted phenotypes and health and fertility phenotypes, (2) estimate genetic parameters among these traits, and (3) include these traits in broader breeding strategies.

ACKNOWLEDGMENTS

The authors gratefully thank the Walloon partners of the FUTUROSPECTRE agreement for access to data and fruitful collaborations: the Walloon Breeding Association (Ciney, Belgium), the milk laboratory 'Comité du Lait' (Battice, Belgium) and the Walloon Agricultural Research Centre (Gembloux, Belgium). The partners of the OSAM group (Observatoire de la Santé Mammaire; www.mammitebovine.ulg.ac.be/) are also gratefully acknowledged, especially the partners of

the LAECEA project for access to mastitis data. The authors thank the INTERREG IVB NWE program and the Ministry of Agriculture of Walloon Region of Belgium (Service Public de Wallonie, Direction générale opérationnelle "Agriculture, Ressources naturelles et Environnement" – DGARNE) for their financial support to the OptiMIR project (www.optimir.eu) and NovaUdderHealth project (D31-1273). The National Fund for Scientific Research (FNRS, Brussels, Belgium) is also acknowledged for grant agreement project 2.4604.11 (COMPOMILK project). Nicolas Gengler, as a former senior research associate, also acknowledges the support of the FNRS. The authors acknowledge the support of the European Commission under the Seventh Framework Program for the GplusE project (www.gpluse.eu), grant agreement FP7-KBBE-613689. The content of the paper reflects only the view of the authors; the Community is not liable for any use that may be made of the information contained in this publication. Finally, the authors are grateful to the University of Liege (SEGI facility, Liege, Belgium) for computing facilities.

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