

Improving Dairy Cow Fertility Using Milk Based Indicator Traits

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ABSTRACT: Improvement of dairy cow fertility by means of genetic selection has become increasingly important over the last decades. Because fertility traits are difficult to measure and have low heritabilities, indicator traits are of interest to supplement the prediction of genetic merit for female fertility. This paper examines milk-based traits that could be potential predictor of fertility: changes in protein and fat composition, fat to protein ratio, urea, lactose, ketone bodies, and mid-infrared prediction of body energy traits. The pattern of genetic correlations between these traits and fertility over days in milk is likely related to the cow's energy balance state. Furthermore, changes in milk fatty acid profile were demonstrated as good potential predictors of fertility. Finally, additional research is warranted to investigate the association over the lactation between fertility and changes in milk biomarkers, potentially predicted by mid-infrared analysis of milk.

Keywords: dairy cattle; fertility; genetic correlation

Background

Optimal fertility is vital for profitable dairy production. However, dairy production systems that use cows selected, managed and fed for high milk production levels have suffered decline in cow fertility over the past decades (Lucy (2001); Norman et al. (2009)). To overcome such decline, improved management strategies and inclusion of fertility in broader breeding goals have been established and have allowed, to some extent, to stop the deterioration of reproductive performance (Norman et al. (2009)). Presently, most dairy cattle populations have routine genetic evaluations for female fertility (Interbull (2014)) and such fertility traits have been nearly unanimously included in overall breeding objective of dairy cattle (Miglior et al. (2005)).

However, direct selection for female fertility might be complicated by the following factors: 1) the difficulty in collecting large amounts of relevant direct fertility records, especially for unfertile animals (e.g. no calving interval records for unfertile animals); 2) the long time period required to validate some phenotypes (e.g. calving interval); and 3) the generally low heritability of most traditional phenotypes (from 0.01 to 0.05; Veerkamp and Beerda (2007)). These factors contribute to low accuracy of estimated breeding values, especially for cows and young bulls. Therefore, indicator traits could be very useful to supplement the prediction of genetic merit for female fertility as long as these traits are easier to measure, are recorded earlier in the cow's lactation, are heritable, and are genetically correlated with fertility (Shook (1989)).

Because energy balance (EB) has been presented as one of the most important factors influencing fertility (Butler and Smith (1989); de Vries and Veerkamp (2000)), traits related to the extent and the duration of the postpartum negative EB are of great interest as indicator traits to enhance indirect genetic improvement of reproductive performance. Negative EB occurs in early lactation when nutrient requirements for growth, activity, maintenance, and lactation exceed the ability of the cow to consume energy in the feed. In response to the energy deficit, cows mobilize tissues reserves and may be more likely to use this energy to sustain lactation at the expense of reproduction. Therefore, severe and long negative EB is unfavorably associated with fertility performance. Several traits have been associated with EB state and reproduction: body condition score, body weight, linear type traits, various metabolic and endocrine blood traits, and milk composition traits (de Vries and Veerkamp (2000); Pryce et al. (2000); Coffey et al. (2001); Berry et al. (2003); Friggens et al. (2007)). In contrast to blood metabolites, which are invasive, difficult, and expensive to measure, milk composition data are easily available.

Recent research has demonstrated the potential of mid-infrared (MIR) analysis of milk to predict new phenotypes of interest such as fine milk composition, technological properties of milk and cow status (De Marchi et al. (2014)). Because of its use by regular milk recording and milk payment systems to quantify the major milk components, MIR spectrometry is a rapid and cost-effective tool for recording phenotypes at the population level (De Marchi et al. (2014)). Hence, MIR analysis of milk appears as a method of choice to provide indicators of fertility and EB that are collected routinely and that are readily available (i.e. at the first test-day).

This paper aims 1) to review milk-based indicator traits that have been associated with reproductive performance and EB status and 2) to further investigate the opportunity of using urea content in milk, fat to protein ratio, MIR predictions of milk fatty acids (FA) and MIR predictions of body energy traits as indicators of dairy cow fertility.

Association between milk-based indicator traits, energy balance, and fertility

Changes in milk profile in early lactation. Changes in the biochemical profile of milk can be used as a mirror of the cow's physiological status (Hamann and Krömker (1997)). At the early stage of lactation, when the cow goes to negative EB, postpartum lipolysis of body fat reserves results in an elevated fat percentage (de Vries and Veerkamp (2000)). Also, altered activities in the 2 major

pathways for the production of milk FA (i.e. *de novo* synthesis of FA and excretion from the blood to the udder of pre-formed lipids) are expected (Palmquist et al. (1993); Gross et al. (2011)). This is coupled with a lower protein percentage because of inadequate intake of fermentable energy-spending carbohydrates, resulting in insufficient protein synthesis by ruminal bacteria (Negussie et al. (2013)). Given that milk urea reflects the protein metabolism in the dairy cow and is related to the surplus of nitrogen that is available in the rumen for microbiological growth compared with the available energy (Schepers and Meijer (1998)), higher milk urea content is expected when the cow is in negative EB. Furthermore, excessive fat mobilization may induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies (Van Haelst et al. (2008)). Then along with increasing lactation and the declining negative EB state, milk composition still evolves. Therefore, changes in milk profile turn out to be relevant indicators of EB state and fertility. Several studies investigated the phenotypic and genetic associations of milk-based traits with EB and reproductive performance.

Protein composition. Demeter et al. (2010) found a favorable phenotypic relationship between concentration of α S1-casein within total milk protein and non-return rates and calving rate after first insemination. Nonetheless, most of the studies indicated that protein percentage and milk protein composition were not phenotypically strongly associated with dairy cow fertility and EB state (de Vries and Veerkamp (2000); Reksen et al. (2002); Demeter et al. (2010)). Likewise, Roman and Cox (2000) presented a genetic correlation of 0.01 between protein percentage and parturition to first breeding period for Jersey first-parity cows from an experimental herd. However, Bastin et al. (2012a) reported moderate negative genetic correlation between protein content and the number of days from calving to conception (or days open; DO) for first-parity Holstein cows.

Fat composition. Milk fat is the main component determining energy expenditure for milk production in dairy cows (Gross et al. (2011)). Hence, de Vries and Veerkamp (2000) reported that a relatively strong decrease in fat percentage during early lactation was significantly correlated with lower nadir of EB, larger energy deficit, and later return to positive EB. Also, Buttchereit et al. (2011) reported a genetic correlation of -0.51 between EB and fat content at 15 days in milk (DIM). Furthermore, milk FA composition has been suggested as a relevant predictor of negative EB in early lactation (Gross et al. (2011)). Milk FAs originate from 2 major pathways: 1) C4:0 to C14:0 and almost half of C16:0 are synthesized *de novo* in the mammary gland and 2) the remaining C16:0 and almost all of the longer chain FA are excreted from the blood to the udder (Grummer et al. (1991); Palmquist et al. (1993)). Blood lipids may be derived from the digestion and absorption of dietary fat or from mobilization of adipose tissue (Grummer et al. (1991)). Changes in milk FA composition during negative EB in early lactation originate

from altered activities in these pathways (Gross et al. (2011)).

Higher content in milk and in milk fat of pre-formed FA (and especially C18:1 *cis*-9) has been reported in early lactation (Bastin et al. (2011); Gross et al. (2011)) indicating the release of long-chain FA from the mobilization of body fat reserves. Concomitantly, the content of *de novo* synthesized FA tend to be lower in early lactation (Bastin et al. (2011); Gross et al. (2011)) because the high uptake of long-chain FA in the udder inhibits *de novo* synthesis of FA by mammary gland tissue. This inhibition intensifies with increasing chain lengths (Palmquist et al. (1993)). Then, along with increasing DIM and the declining adipose tissue mobilization, the proportion of FA derived from blood decreases while the proportion of *de novo* synthesized FA increases (Kay et al. (2005); Stoop et al. (2009); Ducháček et al. (2011); Gross et al. (2011)). Furthermore, Van Haelst et al. (2008) indicated that high proportions of C18:1 *cis*-9 in milk fat were associated with subclinical ketosis resulting from excessive fat mobilization. Therefore, change in C18:1 *cis*-9 in early lactation has been proposed as suitable indicator of the EB of dairy cows.

Although they considered the level of unsaturated FA as a proxy for the dietary profile in the herd, Hostens et al. (2011) found that higher bulk tank unsaturated FA level was associated with decreased conception rate to first insemination, greater days post-calving to first insemination, and greater days to conception. Except for the concentration in fat of *trans* FA, Demeter et al. (2009) did not find a significant effect of milk fat composition on female fertility. Nevertheless, this study included cows that were likely in positive EB since all the cows were sampled after 66 DIM. Bastin et al. (2012a) reported genetic correlations between the contents in milk of major FA predicted by MIR spectrometry and the DO for first-parity Holstein cows. Genetic correlations between DO and content in milk of 17 groups and individual FA ranged from -0.39 to 0.39 according to the FA and the stage of lactation considered. Estimates varied greatly according to the trait and the stage of lactation in which the milk sample was taken. Genetic correlations with DO for the content in milk of unsaturated FA, monounsaturated FA, long-chain FA, C18:0, and C18:1 *cis*-9 were positive in early lactation but negative after 100 DIM. For the other FA, genetic correlations with DO were negative across the entire lactation. Furthermore, Bastin et al. (2012a) reported a genetic correlation between milk fat content and DO ranging from -0.17 to 0.07 indicating that detailed milk fat composition would be better indicator of fertility than fat content only.

Fat to protein ratio. Changes in fat to protein ratio were associated with the extent and the duration of EB (Loeffler et al. (1999); de Vries and Veerkamp (2000); Reist et al., (2002)). Heuer et al. (1999) indicated that cows with a fat to protein ratio > 1.5 at the first test-day showed poor reproductive performances. However, de Vries and

Veerkamp (2000) stated that changes observed in this ratio were likely derived from changes in fat percentage. Buttchereit et al. (2011) reported that the genetic correlation between EB and fat content was the highest at 15 DIM (-0.62) and then decreased towards 0. Also, Negussie et al. (2013) found genetic correlations between fat to protein ratio and interval fertility traits (i.e. calving to first insemination and DO) ranging from 0.14 to 0.28 in the first 60 days of lactation for first-parity Nordic Red cattle indicating that cows with relatively higher fat to protein ratio tend to take longer to first insemination and to conception.

Urea. Milk urea reflects the protein metabolism in the dairy cow (i.e. protein feeding efficiency and dietary protein to energy ratio; Rajala-Schultz et al. (2001)). Hence, it has been proposed as an indicator of metabolic stress in early lactation. Over a range of studies, increased milk urea nitrogen, especially in early lactation, was associated with poor reproductive performance of the dairy cow (Butler et al. (1996); Rajala-Schultz et al. (2001); Guo et al. (2004); König et al. (2008)). Likewise, an unfavorable genetic correlation between milk urea nitrogen in early lactation and fertility were reported. Mitchell et al. (2005) found a genetic correlation of 0.31 between milk urea nitrogen measured within ± 30 d of first service and DO. König et al. (2008) indicated that the genetic correlation between average milk urea nitrogen concentration from the 2 first test-days and fertility was 0.29 for calving to first service, -0.13 for 56-d non return rate, and -0.12 for 90-d non return rate. Nevertheless, both authors as well as Miglior et al. (2007) concluded that the genetic correlation was too weak to justify the use of milk urea nitrogen as an indicator trait for fertility.

Lactose. Higher lactose content in early lactation was favorably associated with EB and fertility. Gross et al. (2011) reported lower lactose percentage in the first week postpartum in comparison to later stage of lactation. Reist et al. (2002) found a positive significant phenotypic correlation of 0.36 between lactose content and EB in the first 10 weeks postpartum. Furthermore, greater milk lactose content during the first weeks postpartum of second-parity cows has been related to early luteal response (Reksen et al. (2002)). Nonetheless, Miglior et al. (2007) reported correlation between EBVs for lactose percentage and fertility close to 0, thus diminishing the potential of using lactose content as possible indicator of fertility.

Ketone bodies. Excessive body fat mobilization due to severe EB results in elevated concentration of ketone bodies (i.e. β -hydroxybutyrate, acetoacetate, and acetone) in body fluids, a state called hyperketonemia (Duffield (2000); Van Heelst et al. (2008)). Circulating serum β -hydroxybutyrate concentration has been negatively associated with reproductive performance (Walsh et al. (2007)). Furthermore, Enjalbert et al. (2001) reported that ketone body concentrations in milk and blood correlated well (correlations ranging from 0.66 to 0.96). However, studies that associated ketone bodies in milk with EB and fertility

are scarce. Reist et al. (2002) found a negative significant correlation between acetone content and EB in the first 10 weeks postpartum. Reksen et al. (2002) found that higher acetone levels from weeks 2 to 4 postpartum were associated with late luteal response in second-parity cows. Furthermore, previous studies demonstrated the potential of MIR analysis of milk for the determination of ketone bodies in milk and the detection of (subclinical) ketosis (Heuer et al. (2001); de Roos et al. (2007); van Knegsel et al. (2010); van der Drift et al. (2012)). Hence, MIR prediction of ketone bodies could be used to further investigate the association between ketones bodies and reproductive performance.

Mid-infrared predictions of body traits. Most of the milk composition traits identified as predictors of EB are predicted by MIR spectrometry. Therefore, McParland et al. (2011 and 2012) evaluated the potential of MIR analysis of milk to directly predict body energy status traits. These authors provided predictive equations for direct EB (MJ/d), body energy content (MJ) predicting body lipid and protein weight, and the effective energy intake per day (MJ/d). These traits were derived and are described in full by Banos and Coffey (2010). The accuracy of predicting these traits from MIR was moderate. Correlation coefficients of split-sample cross-validation and of external validation were respectively 0.68 and 0.65 for direct EB, 0.57 and 0.53 for body energy content and 0.80 and 0.78 for effective energy intake (McParland et al. (2012)). McParland et al. (2012) further indicated that the variation in the energy status in the population where the prediction equations are going to be exploited needs to be represented in the data set used to calibrate the equations. Bastin et al. (2013) reported genetic correlations for these MIR predicted body energy traits with DO ranging from -0.22 to -0.17 for direct EB, from -0.30 in early lactation to 0.00 in mid-lactation for effective energy intake, and from -0.05 at 5 DIM to -0.30 at 305 DIM for body energy content in first-parity Holstein cows. These estimates were weaker than those presented by Banos and Coffey (2010) for the direct measures of body energy. Those authors presented genetic correlations ranging from -0.28 to -0.64 between interval from calving to conception and direct EB and from -0.37 to -0.60 between interval from calving to conception and body energy content.

On the genetic correlations between milk-based traits and fertility. Genetic correlation estimates between milk composition traits and fertility are scarce in the literature. Moreover, some of these studies did not consider the change of the correlations between milk profile and fertility across the lactation. However, based on the evolution of milk composition over the lactation, it could be expected that correlations between milk components and fertility depend on the stage of lactation when the milk sample is collected. Hence, models like random regression models allow the estimation of genetic correlations between a longitudinal trait such as a milk component and a trait that is measured as a single lactation record such as fertility (Veerkamp et al. (2001); Bastin et al. (2012a)).

Selection response in fertility

The benefit of using various milk-based traits as potential genetic predictors of reproductive performance can be evaluated through the correlated response in fertility. According to the selection index theory (Van Vleck (1993)), the correlated response in fertility to selection for milk-based traits depends on the heritability of these indicators and their genetic correlation with fertility. Therefore, Banos and Coffey (2010) proposed the product of the square root of the heritability of the indicator trait with its absolute genetic correlation with fertility as a proxy of the relative correlated response in fertility to selection for this indicator.

Table 1 shows the genetic parameters (heritability and genetic correlation with DO) of various milk-based indicator traits available for Walloon Holstein first-parity cows: urea content, fat to protein ratio, the content in milk of 17 groups and individual FA, and the MIR prediction of direct EB, effective energy intake and body energy content. These traits were defined in early lactation (at 5 DIM) in order to investigate the interest of using information that is readily available, i.e. at first test-day. Genetic parameters for FA and body energy traits were obtained from Bastin et al. (2012a and 2013). In addition, genetic parameters for fat

Table 1. Overview of heritability (h^2) of milk-based indicator traits at 5 days in milk (DIM), their genetic correlations (r_g with days open (DO at 5 DIM and the product of the square root of the heritability of the indicator trait with its absolute genetic correlation with DO ($h \times r_g$) at 5 DIM as a proxy of the relative correlated response in fertility to selection for this indicator.

Milk-based indicator trait at 5 DIM	h^2	r_g	$h \times r_g$
Fat to protein ratio	0.17	0.23	0.10
Milk urea (mg/L)	0.12	0.03	0.01
Fatty acids (g/dL of milk)			
Saturated FA	0.25	-0.23	0.12
Monounsaturated FA	0.13	0.37	0.13
Polyunsaturated FA	0.20	0.00	0.00
Unsaturated FA	0.13	0.34	0.12
Short-chain FA	0.30	-0.27	0.15
Medium-chain FA	0.26	-0.21	0.11
Long-Chain FA	0.12	0.33	0.12
C4:0	0.22	-0.02	0.01
C6:0	0.28	-0.23	0.12
C8:0	0.30	-0.30	0.16
C10:0	0.28	-0.37	0.19
C12:0	0.28	-0.35	0.19
C14:0	0.27	-0.29	0.15
C16:0	0.24	-0.17	0.08
C17:0	0.25	-0.10	0.05
C18:0	0.14	0.08	0.03
C18:1 <i>cis</i> -9	0.13	0.39	0.14
Direct energy balance (MJ)	0.22	-0.20	0.09
Body energy content (MJ/d)	0.11	-0.08	0.03
Effective energy intake (MJ)	0.10	-0.28	0.09

to protein ratio and urea content were estimated for Walloon first-parity cows using random regression models on the same dataset than Bastin et al. (2012a). Table 1 provides the product of the square root of the heritability of the indicator trait with its absolute genetic correlation with DO ($h \times r_g$). These results confirmed previous findings indicating that milk urea is a poor indicator of fertility (Miglior et al. (2007); König et al. (2008)). Milk FA traits, especially the content in milk of C8:0 to C14:0 and C18:1 *cis*-9 showed the highest $h \times r_g$ and appear as better predictors of DO than fat to protein ratio and body energy traits predicted by MIR. The opportunity of using the content in milk at 5 DIM of C10:0 and C18:1 *cis*-9 was further investigated given that these 2 FA correlated well with DO and were poorly genetically correlated with each other (Bastin et al. (2012b)), indicating that they could be complementary indicator traits.

The accuracy of a fertility index including either DO, the 2 FA traits or a combination of them was then estimated for a bull having a varying number of daughters with records under the following scenarios: 1) selection on DO only; 2) selection on one of the 2 FA traits; 3) selection on DO and one of the 2 FA traits; 4) selection on the 2 FA traits; 5) selection on DO and the 2 FA traits. Following the selection index theory (Van Vleck (1993)), the accuracy of the index was estimated as $r_{TI} = \sigma_I / \sigma_T$ where the variance of the breeding objective was $\sigma_T^2 = w'cw$ and the variance of the index was $\sigma_I^2 = b'gw$. The economic weight on DO was $w = 1$ and c was the genetic variance of DO. The b -values were obtained as $b = P^{-1}gw$. The diagonal elements in matrix P were calculated as $\left(\frac{1+(n-1)r_i+(p-1)kh_i^2}{n} \right) \sigma_{pi}^2$

where n was the number of records per animal (set to 2 in order to consider the use of this information readily); r_i , h_i^2 and σ_{pi}^2 were respectively the repeatability, the heritability, and the phenotypic variance of trait i ; p was the number of daughters in progeny group ($p=20, 50, 100$); and k was the relationship among animals in progeny groups (0.25). The off-diagonal elements in matrix P were calculated as $\frac{\sigma_{p_{ij}}+(p-1)k\sigma_{g_{ij}}}{p}$ where $\sigma_{p_{ij}}$ and $\sigma_{g_{ij}}$ were respectively the phenotypic and genetic covariance between traits i and j . Finally, the elements in g were calculated as $a\sigma_{g_{ij}}$ where a was the relationship among animals in progeny group and the bull ($a=0.5$). Parameters used in these calculations are presented in Table 2. Additionally, the phenotypic and genetic correlations between the 2 FA traits were estimated using a bivariate random regression model on the dataset of Bastin et al. (2012b). The genetic and phenotypic correlations between the content in milk at 5 DIM of C10:0 and C18:1 *cis*-9 were -0.09 and -0.19, respectively.

As expected, direct selection on DO seemed to provide the best accuracy of the fertility index in all the scenarios with only one trait (Table 3). However, in many countries, calving interval is the only available fertility record for

routine evaluations (Veerkamp and Beerda (2007)). As a consequence, a certain period is required to obtain and validate this phenotype. Also, records for animals with the worst fertility (no DO) cannot be easily integrated. Hence, using FA traits in a fertility index would allow a more rapid indirect selection on reproduction performance. Moreover, an index including the 2 FA traits showed higher accuracy than an index including only one trait (Table 2), thereby substantiating the opportunity to combine indicators related to various aspects of EB state and body fat mobilization. Finally, the combination of DO and indicator trait(s) in a fertility index tended to provide better accuracy than an index including DO only, especially when the number of progeny was low ($p=20$).

Table 2. Genetic standard deviations (σ_a , heritability (h^2), repeatability (r , and phenotypic (r_p and genetic correlations (r_g) with days open (DO) (from Bastin et al., 2012a).

Trait	σ_a	h^2	r	r_g	r_p
DO	18.432	0.05	-	-	-
C10:0 at 5 DIM (g/dl)	0.0124	0.28	0.65	-0.37	-0.08
C18:1 <i>cis</i> -9 at 5 DIM (g/dl)	0.0763	0.13	0.64	0.39	0.04

Table 3. Accuracy of an index for fertility including a various number of traits, estimated for a bull having a varying number of daughters with records ($p=20$, 50, 100). The traits considered are days open (DO), the content in milk of C10:0 at 5 days in milk (DIM), and the content in milk of C18:1 *cis*-9 at 5 DIM.

Trait(s) in the index	Accuracy of the index		
	$p=20$	$p=50$	$p=100$
DO	0.45	0.62	0.75
C10:0	0.30	0.34	0.35
C18:1 <i>cis</i> -9	0.26	0.32	0.35
C10:0 + C18:1 <i>cis</i> -9	0.37	0.44	0.47
DO + C10:0	0.50	0.65	0.76
DO + C18:1 <i>cis</i> -9	0.49	0.65	0.76
DO + C10:0 + C18:1 <i>cis</i> -9	0.53	0.68	0.78

Contents in milk of C10:0 and C18:1 *cis*-9 at 5 DIM have been demonstrated as potential genetic predictors of fertility. Nonetheless, the inclusion of such traits in breeding schemes has to be considered with respect to the overall breeding goal, their respective economic values, their relationships with all economically important traits as well as their desirable direction of change. For instance, higher content of C18:1 *cis*-9 at early post-calving was associated with poor fertility performance while consumption of C18:1 *cis*-9 is considered to be favorable for human health (Haug et al. (2007); Ebringer et al. (2008)). Thus, higher content in milk of C18:1 *cis*-9 would be desirable for the nutritional properties of milk fat while lower content of C18:1 *cis*-9 in early lactation is desirable for improved fertility. Nevertheless, results inferred from the study of Bastin et al. (2011) indicated that the genetic correlation between content in milk of C18:1 *cis*-9 in early

lactation and its content at 50, 100, 200, and 300 post-calving 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 at 5 days in milk without impacting considerably on the average monounsaturated FA content in milk across the entire lactation. Hence, a breeding program that seeks to alter the milk FA profile should balance improvement of the nutritional properties of milk and fertility of dairy cows, potentially exploiting differences in FA profiles and their association to animal robustness across the lactation.

Conclusion and perspectives

Changes in milk composition over the lactation are closely related to the EB state of dairy cows and consequently to their reproductive performance. Among other traits, change in milk FA profile is a good indicator of fertility. Although the advantage of simultaneously including fertility and indicator traits in the selection index might be limited when fertility data are already available, the opportunity of using milk-based indicator traits as early predictors of fertility has been demonstrated when fertility information was scarce or not available yet. However, studies investigating the utilization of milk-based traits as genetic predictors of fertility are scarce. Association between other potential milk biomarkers and reproduction could be further investigated (e.g., milk citrate, Bjerre-Harpøth et al. (2012)). In such studies, the evolution of the EB-fertility-milk composition association with the stage of lactation has to be considered. Although phenotypes based on MIR analysis of milk might be less accurate than direct measures of traits related to EB, this technology offers the opportunity to record large amounts of phenotypes at the population level. Furthermore, the potential of MIR analysis of milk to assess the pregnancy status of dairy cows from 20 to 120 days after service has been reported (Lainé et al. (2014)), this status at a given moment during lactation being a potential novel trait. Finally, results presented in this study considered only DO as fertility trait. Therefore, research on other fertility traits, rather related to conception success after insemination or to the start of luteal activity, is warranted.

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