Potential of fine milk composition for cow udder health management

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Abstract

The objective of this research was to investigate the potential of mid-infrared (MIR) analysis of milk to provide indicators of the udder health status of dairy cows. Mastitis data collected in 49 Walloon herds were merged with test-day data including milk composition and MIR spectra. A total of 1987 healthy lactation and 3204 lactations with at least one mastitis, clinical or sub-clinical, chronic or new infection, were identified. First, using paired t tests, significant differences between milk composition of the test-day from 10 to 45 days before the mastitis and milk composition of the test-day occurring during the mastitis (from 10 days before to 10 days after the event) were observed for fat, protein, casein, lactoferrin, saturated FA, short chain FA, medium chain FA, Na, Mg and K. Moreover, milk composition of test-days from healthy lactations was compared with milk composition of test-days occurring from 45 to 15 days before a mastitis using t tests. Lactoferrin, Na, titrable acidity, and urea were identified as potential early indicators of mastitis. The results emphasized the potential of MIR analysis of milk to provide udder health indicators.

Keywords: mid-infrared, mastitis, indicator, fine milk composition

Introduction

Bovine mastitis is one of the most frequent and most costly diseases of the dairy industry. Mastitis affects the milk production of the cow as well as the milk composition and quality (Pyörälä, 2003; Segers *et al.*, 2003). Hence, indicators present in milk could be used for udder health management at individual and herd levels (e.g., early detection of mastitis, identification of cows suffering from chronic infections) especially if these indicators can be determined using quick, inexpensive, and easy routine techniques (Hamann & Krömker, 1997). Among these indicators, somatic cells count (**SCC**; i.e. a reflection of the inflammatory response to an intramammary infection) has been primarily used as a diagnosis tool to monitor udder health (e.g., Schukken *et al.*, 2003). Nevertheless, other traits have been identified as potential udder health indicators such as electrical conductivity (Norberg *et al.*, 2004), lactose content (Pyörälä, 2003), lactoferrin content (Soyeurt *et al.*, 2012) or lactate-deshydrogenase (Chagunda *et al.*, 2006). Some of these traits can be obtained from the mid-infrared (**MIR**) analysis of milk (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 new traits of interest at the population level (De Marchi *et al.*, 2014).

Therefore, the first objective of this research was to describe changes in milk composition which occur during clinical mastitis. The second objective was to further

investigate the potential of milk composition to provide (1) early indicators of clinical and subclinical mastitis (i.e. indicators that change earlier than SCC) and (2) early indicators of recovery from a clinical mastitis.

Material and methods

Mastitis and milk composition data

Data used in this study were obtained in commercial farms from the Walloon Region of Belgium that are involved in the milk recording program and that record clinical mastitis. Clinical mastitis data were voluntary recorded by dairy farmers through a web-based system. To ensure a good quality of the training dataset, the following edits were applied: (1) "recording period" of the herd (i.e. no. of days from the first to the last insemination recorded within the herd) ≥ 180 days; (2) no. of cows with mastitis / total no. of cows within the herd over the period > 5%; (3) only lactation that started after the beginning of the recording period; (4) only test-day data obtained during the recording period and collected during the first 305 days of lactation. The edited dataset included 44,644 test-day data from 7,878 lactations of 4,960 cows in 49 herds. From these 7,878 lactations, 23% experienced at least one mastitis event.

Milk composition data included the MIR prediction of the content in milk of fat, protein, urea, lactoferrin, total casein, minerals (Na, Ca, Mg, K, P), and 7 major groups of fatty acids (**FA**; i.e. saturated, unsaturated, monounsaturated, polyunsaturated, short chain, medium chain, and long chain). Furthermore, titrable acidity defined as Dornic degree was also considered. Calibration equations used for lactoferrin, total casein, minerals, FA and titrable acidity were obtained in previous work (Colinet *et al.*, 2010; Grelet *et al.*, 2013; Soyeurt *et al.*, 2009; Soyeurt *et al.*, 2011, Soyeurt *et al.*, 2012). To provide an indication of the accuracy of the MIR predictions of these traits, the R^2 of cross-validation was also provided for each trait in Table 1.

Furthermore, based on the primary assumption that the "raw" spectrum provided by the MIR analysis of milk is a fingerprint of the whole milk composition, MIR spectrum associated to the test-days included to the database was also investigated. First derivative was calculated on the raw spectra as the difference between a spectral value at data point X and the spectral value at data point X+5. In order to avoid introducing noises, only informative area from the spectra were kept. These ranges of informative wavelengths are the same than those used routinely to predict fatty acid contents in cow milk (Soyeurt et al., 2006).

Definition of udder health status groups

Each lactation of the edited dataset was assigned to one of the following groups: healthy lactation (**healthy**), lactation with at least one non-chronic clinical mastitis (**cl-new**), lactation with at least one chronic clinical mastitis (**cl-chron**), lactation with at least one non-chronic subclinical mastitis (**subcl-new**) and lactation with at least one chronic subclinical mastitis (**subcl-chron**). In the definition of these traits, the following SCC thresholds were considered to assign subclinical mastitis: 150,000 for first-parity cow and 250,000 for cows in parity greater than 1. Lactations were assigned to the healthy group if no clinical mastitis was recorded during the lactation and if SCC from all test-days of the lactation were below the thresholds. If at least one clinical mastitis was recorded during the lactation, the lactation was assigned either to the cl-new group if the cow presented a SCC < threshold from 10 to 45 days before the mastitis event or to the cl-chron if the cow presented

SCC ≥ threshold from 10 to 45 days before the mastitis event. If no clinical mastitis was recorded during the lactation and if the lactation was not assigned to the healthy group, the lactation was assigned either to the subcl-new group if the cow presented only one test-day over the lactation with SCC ≥ threshold or to the subcl-chron group if the cow presented at least 2 consecutive test-days with SCC ≥ threshold. A total of 5,191 lactations were assigned to one of these 5 groups.

Additionally, a cow that suffered from a clinical mastitis was considered either as cure if she presented a SCC < threshold from 15 to 45 days after the mastitis event or as not cure if she presented a SCC ≥ threshold from 15 to 45 days after the mastitis event. This leaded to the definition of 4 sub-groups: **cl-new-cure**, **cl-new-nocure**, **cl-chron-cure**, and **cl-chron-nocure**.

Identification of udder health indicators

In order to describe changes in MIR analysis of milk which occur during a clinical mastitis, 162 cl-new and cl-chron mastitis dates were associated with MIR records from a test-day occurring before the mastitis (from 10 to 45 days before the event) and with MIR records from a test-day occurring during the mastitis (from 10 days before to 10 days after the event). Paired t tests were performed on the dataset to compare MIR analysis of milk (i.e. milk components and MIR spectra) before and during the mastitis using the t-test procedure in SAS. MIR spectra were reduced into 5 latent variables using the PLS procedure in SAS. Latent variables are linear combination of the original MIR spectral points taken account relationship between variables. Paired t-tests were also performed on these latent variables to compare the response of the spectra before and during the infection.

In order to investigate the potential of MIR analysis of milk to provide early indicators of mastitis and early indicators of recovery from a clinical mastitis, the following analyses were performed.

(1) To identify indicators of clinical and subclinical mastitis that change earlier than SCC, the milk composition of the test-day that occurred from 10 to 45 days before a cl-new mastitis event or a subcl-new was compared with the milk composition of the healthy group using the t-test procedure in SAS. A sub-set of test-days attributed to the healthy group was randomly selected in order to cover a similar range of days in milk.
(2) To identify early indicators of recovery from a clinical mastitis, the milk composition of the test-day that occurred from 10 to 45 days before a cl-new was compared between the cl-new-cure and cl-new-nocure groups using the t-test procedure in SAS.

In all analysis, only the first mastitis or subclinical mastitis event of the lactation was considered.

Results and Discussion

Table 1 showed the distribution of lactations according to the mastitis traits. Results showed that less than 40% of the lactations were considered as healthy and 14% of the lactations experienced at least one mastitis event. About 30% of the lactations were assigned to the subcl-new group indicating that these cows have suffered from a subclinical mastitis but they have directly recovered from the inflammation. However, it should be noted that the edits might have changed the distribution of lactations among the various groups. Among the cl-new group, 229 lactations could be assigned to the cl-new-cure group and 89 lactations cl-new-nocure. Among the cl-chron group, 44 lactations could be assigned to the cl- chron -cure group and 100 lactations cl- chron -nocure. These results indicated that more

than 70% of cows suffering from a non-chronic clinical mastitis recovered within 15 to 45 days after the event while only 30% of cows suffering from a chronic clinical mastitis recover within 15 to 45 days after the event.

Significant differences before and during mastitis were observed for the content in milk of fat, protein, casein, lactoferrin, saturated FA, short chain FA, medium chain FA, Na, Mg, and K (Table 2). Except K, all milk components increased during the mastitis. Previous studies also reported that a decrease in K as well as an increase in lactoferrin, Na, and short chain FA contents in milk were caused by mastitis (Hamann & Krömker, 1997; Pyörälä, 2003). In opposition to our findings, these studies reported that mastitis causes decrease in casein and Mg contents. Changes in milk composition could be related to disease-combating response of the cow, reduced secretory activity and alteration of blood-milk barrier (Hamann & Krömker, 1997).

The 5 first latent variables explained more than 80% of the whole variability expressed in our MIR spectral dataset. As expected, MIR spectrum (was also significantly different before and during the mastitis. Such results emphasized the potential of using directly the MIR spectrum to provide udder health indicators.

Table 3 showed the relevant results of the t-test performed between milk composition data from the healthy group and milk composition data of the test-day recorded from 10 to 45 days before a new clinical mastitis or a new sub-clinical mastitis. Table 4 showed the same results than in Table 3 except that milk composition data of the test-day recorded from 10 to 45 days before chronic clinical and sub-clinical mastitis were added in the analysis. Same trends were observed in Table 3 and 4. The results showed that Na, lactoferrin, titrable acidity, and urea are significantly different in the milk of cows that will experience mastitis in comparison to the milk from healthy cows. No significant differences were observed for the other traits. Our results highlighted that these traits might be early indicators of to detect cows that will experience clinical or subclinical mastitis during their lactation.

Finally, no significant differences were observed between milk composition data of cows that recovered from clinical mastitis and milk composition data of cows that were not cure from 15 to 45 days after the inflammation. Interestingly, Na is not much related to chronic cases, which could be a risk for monitoring chronic cases with conductivity. Such situation is observed in automated milking system where currently no SCC is available, replaced by conductivity. Chronic cows may not be pointed by the algorithm of automated milking system software due to absence of conductivity variation.

Table 1. Distributions of lactations according to the udder health status group.

Udder health status group	N	%
Healthy	1987	38.4
cl-new	243	4.6
cl-chron	480	9.2
subcl-new	1499	28.9
subcl-chron	982	18.9

Table 2. Results of the paired t-test (average difference and p-value associated) when comparing MIR data from a test-day occurring before the mastitis (from 10 to 45 days before the event) with MIR data from a test-day occurring during the mastitis (from 10 days before to 10 days after the event).

Trait	RSQCV [(1)]	Average difference (before – during)	p-value
Milk component			
Fat (%)		-1.187	0.0128
Protein (%)		-0.806	0.0002
SCC[(2)]		-818.3	< 0.0001
SSC[(2)]		-1.620	< 0.0001
Urea (mg/L)		4.2099	0.4863
Lactoferrine (mg/L)	0.71	-39.57	< 0.0001
Titrable acidity (dornic degree)	0.80	-0.0502	0.5696
Casein (g/100g)	0.94	-0.0425	0.0131
Monounsaturated FA (g/dL)	0.97	0.0168	0.3837
Polyunsaturated FA (g/dL)	0.76	-0.0014	0.5762
Saturated FA (g/dL)	0.99	-0.1480	< 0.0001
Unsaturated FA (g/dL)	0.97	0.0143	0.4987
Short chain FA (g/dL)	0.94	-0.0118	0.0094
Medium chain FA (g/dL)	0.97	-0.1395	<0 .0001
Long chain FA (g/dL)	0.95	0.0249	0.3693
Na (mg/kg)	0.49	-10.59	0.0045
Ca (mg/kg)	0.82	-3.749	0.6685
P (mg/kg)	0.75	-3.390	0.5728
Mg (mg/kg)	0.68	-1.381	0.0212
K (mg/kg)	0.54	22.75	0.0039
Latent variables of MIR spectra			
1st latent variable		-3.9033	<0 .0001
2nd latent variable		-2.2619	<0 .0001
3rd latent variable		-1.9697	<0 .0001
4th latent variable		-1.8195	0 .0006
5th latent variable		-1.0170	0 .0006

[(1)] RSQCV: R squared of cross-validation for milk components predicted by MIR
[(2)] SCC and SCS were not predicted by MIR analysis of milk.

Table 3. Results of the t-test when comparing milk composition data from a test-day occurring before (from 10 to 45 days) a non-chronic clinical or subclinical mastitis with milk composition data from healthy lactations.

	healthy			cl-new + subcl-new			
Trait	N	Mean	Std	N	Mean	Std	p-value
Na (mg/kg)	1,834	347.0	49.4	1,148	359.8	57.3	<0.0001
Lactoferrin (mg/l)	1,589	159.5	74.0	988	170.0	73.5	0.0005
Titrable acidity (dornic degree)	1,789	16.6	1.5	1,115	16.5	1.5	0.0853
Urea (mg/l)	1,846	248.8	83.9	1,154	240.7	80.7	0.0095

Table 4. Results of the t-test when comparing milk composition data from a test-day occurring before (from 10 to 45 days) a mastitis event (clinical and sub-clinical, new and chronic) with milk composition data from healthy lactations.

	healthy			cl-new + subcl-new			
Trait	N	Mean	Std	N	Mean	Std	p-value
Na (mg/kg)	1,834	347.0	49.4	1,729	364.1	58.6	<0.0001
Lactoferrin (mg/l)	1,589	159.5	74.0	1,486	173.7	76.8	<0.0001
Titrable acidity (dornic degree)	1,789	16.6	1.5	1,682	16.3	1.6	0.0003
Urea (mg/l)	1,846	248.8	83.9	1,737	236.1	80.6	<0.0001

Conclusion and Perspectives

In this study, a comprehensive definition of the different mastitis cases was performed and provided a good overview of the distribution of these various cases in the Walloon Region of Belgium. Such definition will allow performing further detailed phenotypic and genetic analyses.

Milk composition described either by MIR-predicted milk components or by MIR spectrum was significantly different before and during a mastitis event. Such information could be used in addition to SCC data for udder health management. Furthermore, MIR predicted traits such as urea, Na, lactoferrin, and titrable acidity were identified as potential early indicators of udder health since they were significantly different in the milk of cows that will experience mastitis during their lactation in comparison to the milk from healthy cows. These indicators could be very helpful to identify dairy cows that are susceptible to develop mastitis, clinical or sub-clinical case, in the next month. Such tools can considerably reduce costs by using more accurate preventive actions. Moreover, if a combination of different information such as the SCC and MIR-predicted milk components can inform on the chronicity of the infection, helpful making decision tool could be developed.

Future studies will investigate the combination of all MIR-predicted traits into useful udder health indicators. The specificity and sensibility of such indicators will be also explored. Moreover, changes in the milk composition and especially in the MIR spectra will be further investigated to identify the pathogens involved in the udder infection.

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