Defining and using novel milk composition based heat stress resilience traits in the context of genomic selection for more robust dairy cows in Wallonia

A. Mineur[1], R. Reis Mota[1, 2], N. Gengler[1], S. Vanderick[1], H. Hammami[1]

[1] *ULiège-GxABT, Passage des Déportés 2, 5030 Gembloux, Belgium*
[2] *presenting (rrmota@uliege.be)*

Summary

Recent research showed the usefulness of using estimated breeding values (EBV) for mid-infrared (MIR) based biomarkers in genetic improvement. A novel class of biomarkers was defined based on modelling responses of milk composition (e.g., mid-infrared (MIR) based) to stress expressed on continuous scales using reaction norm models. Heat stress is an important aspect of dairy production even in temperate climates as shown in recent studies. Implementation of genomic selection for tolerance to heat stress is therefore not only an issue for Australian dairy cattle, a country that introduced recently such an evaluation. The question remains open if using milk composition based heat stress resilience genomically enhanced EBV (GEBV) is not a viable option. Genetic parameters were estimated for production and milk composition traits. Data included 155,977 test-day records for milk, fat, and protein yields, fat and protein percentages, 9 individual milk fatty acids (FA), 7 FA groups, 5 minerals, and 3 ketone bodies) predicted by mid-infrared spectrometry., and 7 FA groups. Data were from 21,375 first-lactation Holstein cows in 473 herds in the Walloon region of Belgium and were collected between 2008 and 2014. Test-day records were merged with daily temperature-humidity index (THI) values based on meteorological records from public weather stations. The maximum distance between each farm and its corresponding weather station was 13 km. Linear reaction norm models were used to estimate the intercept and slope responses of 23 traits to increasing THI values. Most yield and FA traits had phenotypic and genetic declines as THI increased, whereas C18:0, C18:1 cis-9, and 4 FA groups (unsaturated FA, monounsaturated FA, polyunsaturated FA, and long-chain FA) increased with THI. Moreover, the latter traits had the largest slope-to-intercept genetic variance ratios, which indicate that they are more affected by heat stress at high THI levels and therefore good candidate traits. Among all traits, C18:1 cis-9 was the most sensitive to heat stress. As this trait is known to reflect body reserve mobilization, using its response to THI could be a very affordable milk biomarker of heat stress for dairy cattle expressing the equilibrium between intake and mobilization, and therefore adaptation, under warm conditions. By including novel milk based composition traits to traditional production traits, correlations between EBVs and also GEBVs of those milk based traits and udder health, fertility and longevity increased considerably. This study demonstrated that milk composition resilience heat stress traits could be used as early indicators of robustness traits. Our results also suggest that marker information tend to lead to higher accuracies of prediction. Therefore, new options to improve robustness through genomic selection in Walloon Holsteins are now presented.

Keywords: milk MIR spectra, resilience, genetic improvement, genomic selection

Introduction

Robustness is a key element towards increased farm profitability by improved cattle health and welfare and is closely linked to increased adaptive capacity to climate disturbances. Animal breeding is used to improve animal features as needed for tomorrow. Genomic prediction is the method of choice to provide genetic merit estimates to breeders, especially for novel traits. It allows the identification of young animals quickly, especially for traits difficult to record such as resilience (e.g. resilience to thermal stress). Although few recent genomics studies have identified regions of the genome that appear to be involved with resilience, they were focused on limited populations and mostly by using temperature humidity index (THI) with milk yield traits decays as proxy traits of resilience (Ben Hayes et al., 2009). Thermoregulatory and production responses to heat shock are only marginally related, and the biological basis of physiological changes still is elusive (Baumgard et al., 2016). Phenotypes based on physiology, metabolism and health, when available, may be more accurate to identify valid genomic regions and genes associated with resilience to heat stress (Nguyen et al., 2016).

Current advances in the use of mid-infrared (MIR) spectroscopy had allowed the prediction of accurate large-scale of milk-based biomarkers. Many of those milk MIR predicted biomarkers were found indicator of fertility and health traits reflecting the physiological and health status of the cow such as for the negative energy balance (e.g., fatty acids profile), for body fat mobilisation (e.g., C18:1 cis-9) and for (sub) clinical ketosis (e.g., acetone contents in milk) (Bastin et al., 2016). The main objective of this study was to assess the potential of the integration of milk predicted phenotypes, meteorological, and genomic data for the selection of robust dairy cows in the Walloon Region of Belgium. For that we firstly evaluated the reaction of traditional milk production and novel milk phenotypes predicted from mid-infrared spectra to THI towards the identification of the potential novel milk composition based heat stress resilience traits. Conventional (EBV) and genomic (GEBV) slope breeding values for the high influenced predictors were estimated and investigated for their potential contribution to genetic improvement of robustness traits.

Materials and methods

Data

A total of 155,977 test-day records from 21,375 primiparous Holsteins routinely registered between 2005 and 2012 by the Walloon Breeding Association (AWE, Ciney, Belgium) were available. Each record included traditional milk records (milk yield, fat, and protein ratios) and Milk MIR spectra expressed in absorbance with 1,060 spectra points. Prediction equations were used for the prediction at each test-day of i) 9 individual fatty acids (FA): C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C18:1 cis9 and 7 groups of FA: saturated (SFA), unsaturated (UFA), monounsaturated (MUFA), polyunsaturated (PUFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA), ii) 5 minerals: calcium, phosphor, potassium, sodium, magnesium, and iv) 3 metabolites: β-HydroxyButyric acid, acetone, citrates. The predictive ability of used calibration equations was described in Soyeurt et al. (2011) for FA, Soyeurt et al. (2009) for minerals, and Grelet et al. (2016) for metabolites.

Daily meteorological records were supplied by the Belgian Crop Growth Monitoring System consortium (Brussels, Belgium). Data was collected from meteorological stations located at 5.1 and 13.3 Km in average and in maximum distances from the herds used in this study. Average 3 day lag of temperature humidity index (THI) value was assigned to each test day record from the nearest meteorological station to the herd. THI was calculated following NRC (1971):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)], \tag{1}$$

where T and RH are daily average temperature (°C) and relative humidity (%), respectively. Aiming to obtain greater data consistency, retained dataset contained only data from cows with known parents, having at least 5 test days per lactation with known traditional production, MIR spectra and THI values for each test-day record. A minimum of 3 test days been recorded under heat stress conditions (THI > 62), a threshold point for Holstein population in temperate region (Hammami et al., 2013) was required to retain the lactation record.

Genotypes based on 54,609 SNP markers (50K chip) were acquired on 2,380 animals including 1,883 bulls and 497 cows. Genotype quality control edits were implemented and 28,796 (52.7%) SNP markers remained to estimate genomic relationship coefficients between animals. Pedigree information was recovered from historical breeding records from AWE, and was comprised of 67,907 individuals. A total of 63,037 animals remained after pruning.

Statistical analysis

To evaluate the impact of thermal conditions on traditional and novel milk MIR-based traits at genetic level, individual reaction norm coefficients were estimated using random regression of the phenotypic values of the cows on THI. The magnitude of this impact was quantified by the genetic variation for the slopes of the linear reaction norms. Univariate linear reaction norm models were applied separately to each studied trait. The model can be written in matrix notation as follows:

$$\mathbf{y} = \mathbf{T_t} + \mathbf{L_l} + \mathbf{C_c} + \mathbf{M_m} + \mathbf{Q_{hs}W_w} + \mathbf{Q_{hs}(Z_1a + Z_2p)} + \mathbf{e}, \tag{2}$$

where $\mathbf{y}$ is was the vector of observation; $\mathbf{t}$, $\mathbf{l}$, $\mathbf{c}$, $\mathbf{m}$ were vectors for fixed effects: herd x test-date, lactation stage (classes of 15 DIM), classes of age at calving, classes of year x season of calving; $\mathbf{T}$, $\mathbf{L}$, $\mathbf{C}$, $\mathbf{M}$ were respective incidence matrices linking fixed effects to y; w was the vector of the fixed second-order Legendre polynomial coefficients effects to model the population mean for standardized THI (range 28-78); $\mathbf{Q_{hs}}$ was the covariate matrix for first-order Legendre polynomials for standardized THI; $\mathbf{a}$ and $\mathbf{p}$ were vectors of random regression coefficients for additive genetic and environment permanent respectively; $\mathbf{Z_1}$ and $\mathbf{Z_2}$ being their respective incidence matrices linking them to random regressions for each records; and $\mathbf{e}$ was the vector of residuals.

We applied these analyses into three (SC[1], SC[2] and SC[3]) different scenarios by including (GEBV) or not (EBV) genotype information. The EBVs and GEBVs were computed for each animal to further investigate GEBVs prediction efficiency across THI levels. Partial least square (**PLS**) analyses were performed for both scenarios in order to specify the existence of relationship between novel milk composition heat stress resilience traits (predictor variables) and udder health, longevity and fertility traits (response variables). In SC1, we used the same predictor traits (milk, fat and protein yields) as in Nguyen et al. (2016). Fat and protein percentages were added to SC1 traits to outline SC2. Finally, SC3 was composed by SC2 traits plus biomarkers traits (acetone, C4:0, C18:0, C18:1 cis-9, LFCA,

MUFA and UFA). A study was performed by using 177 genotyped bulls with 20 or more daughters with records as reference population, whereas 1,601 young bulls with no progeny as validation population.

Results and discussion

Genetic and phenotypic variation of traditional milk production and novel MIR milk predictor traits under heat stress

Many studies focused mainly on the genetics analysis of production traits (milk, fat and protein) as associated to the meteorological information in the test date from the milk recording data (Ravagnolo and Miztal, 2000; Ben Hayes et al., 2009). All authors confirmed that there is: i) genetic and phenotypic variability in the response to THI; ii) an antagonistic relationship between the level (intercept) and the tolerance to stress (slope). The slope solution was identified as heat tolerance pseudo-phenotype. Baumgard et al. (2016) reported that reaction of production traits to heat loads do not fully capture the whole effect of heat stress. The ratio of slope to intercept variances of a trait measures the magnitude of the individual response to THI. In our study, ratios of specific milk predictors (Table1) were higher by 1.5 fold (for C18:1 cis-9, acetone), 1.1 for (MUFA, LCFA and UFA) compared with milk ratio. Those traits are indicators of the energy balance and body reserve mobilization. Based on experimental protocol and using metabolomics and lipidomics data, Tin et al. (2016) find also that FA (e.g. C18:1 cis-9, UFA), ketone bodies (acetone, β-hydroxybutyrate) are ones of the 10 biomarkers in milk that can reflect heat induced metabolomic alterations.

Association between milk compositions based on heat stress resilience and robustness traits

Correlations between traditional milk production traits EBVs (milk, fat, protein yields and fat and protein percentage) with fertility, longevity, and udder health ranged from 0.14 to 0.32 (Table 2). On the other hand, correlations between GEBVs were higher (range 0.16-0.33). By adding the fat and protein percentages in this study, our values are slightly higher than correlations reported by Nguyen et al. (2016).

Our study also demonstrated that by including novel milk based composition traits to traditional production traits, correlations between EBVs and also GEBVs of those milk based traits and udder health, fertility and longevity increased considerably (Table 2). In general, it was noticeable that adding genomic information enables similar or even better predictions of the robustness traits (e.g., 0.46 vs. 0.44 for fertility; Table 2).

Conclusion

This study demonstrated that milk composition resilience heat stress traits could be used as early indicators of robustness traits. Our results also suggest that marker information tend to lead to higher accuracies of prediction. Therefore, new options to improve robustness through genomic selection in Walloon Holsteins are now presented.

List of References

Bastin, C., Theron, L., Laine, A., & Gengler, N. 2016. On the role of mid-infrared predicted phenotypes in fertility and health dairy breeding programs. J. Dairy Sci., 99(5), 4080-4094.

Baumgard, L.H., Seibert, J.T., Kvidera, S.K., Keating, A.F., Ross, J.W., & Rhoads, R.P. 2016. 0401 Production, biological, and genetic responses to heat stress in ruminants and pigs. J. Anim. Sci., 94(5), 194-195.

Grelet, C., Bastin, C., Gelé, M., Davière, J.B., Johan, M., Werner, A., Reding, R., Fernandez Pierna, J.A., Colinet, F.G., Dardenne, P., Gengler, N., Soyeurt, H., & Dehareng, F. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, β-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6), 4816-4825.

Hammami, H., Bormann, J., M'hamdi, N., Montaldo, H.H., & Gengler, N. 2013. Evaluation of heat stress effects on production traits and somatic cell score of Holsteins in a temperate environment. J. Dairy Sci., 96(3), 1844-1855.

Hayes, B.J., Bowman, P.J., Chamberlain, A.J., Savin, K., Van Tassell, C.P., Sonstegard, T.S., & Goddard, M.E. 2009. A validated genome wide association study to breed cattle adapted to an environment altered by climate change. PloS one, 4(8), e6676.

Nguyen, T.T., Bowman, P.J., Haile-Mariam, M., Pryce, J.E., & Hayes, B.J. 2016. Genomic selection for tolerance to heat stress in Australian dairy cattle. J. Dairy Sci., 99(4), 2849-2862.

Soyeurt, H., Bruwier, D., Romnee, J. M., Gengler, N., Bertozzi, C., Veselko, D., & Dardenne, P. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J. Dairy Sci., 92(6), 2444-2454.

Soyeurt, H., Dehareng, F., Gengler, N., McParland, S., Wall, E.P.B.D., Berry, D.P., Coffey, M., & Dardenne, P. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci., 94(4), 1657-1667.

Tian, H., Zheng, N., Wang, W., Cheng, J., Li, S., Zhang, Y. & Wang, J. 2016. Integrated metabolomics study of the milk of heat-stressed lactating dairy Cows. Scientific Reports volume 6, Article number: 24208.

Table 1. Slope-intercept ratios for additive genetic (*G*) and permanent environmental (*P*) effects

Traits	G variances ratios ($\sigma^2_{a\,hs} / \sigma^2_{a\,0}$) (%)	P variances ratios ($\sigma^2_{p\,hs} / \sigma^2_{p\,0}$) (%)
Production traits		
Milk (Kg)	**0,073**	0,237
Fat%	0,077	0,294
Prot%	0,065	0,285
Prot yield (Kg)	0,037	0,553
Fat (Kg)	0,028	0,704
Individual & groups of FA		
C18:1 cis9	**0,105**	**1,341**
C4:0	0,055	0,622
C18:0	0,051	0,684
C8:0	0,043	0,562
C6:0	0,041	0,588
C10:0	0,037	0,528
C12:0	0,035	0,533
C17:0	0,032	0,697
C14:0	0,027	0,558
C16:0	0,027	0,593
MUFA	**0,079**	**1,212**
LCFA	**0,073**	**1,076**
UFA	**0,072**	**1,180**
PUFA	0,038	0,669
SCFA	0,036	0,583
SFA	0,025	0,591
MCFA	0,024	0,592
Ketone bodies		
Acetone	**0,108**	**1,132**
BHB	0,071	0,630
Citrates	0,027	0,407
Minerals		
Ca	0,042	0,627
Mg	0,031	0,493
Na	0,026	0,477
P	0,023	0,462
K	0,021	0,526

Table 2. Correlation between milk composition based heat stress resilience and robustness traits (RT).

RT	Scenarios	EBV	GEBV
Fertility	S^1	0.28	0.31
	S^2	0.30	0.33
	S^3	0.44	0.46
Longevity	S^1	0.31	0.31
	S^2	0.32	0.33
	S^3	0.39	0.39
Udder health	S^1	0.13	0.16
	S^2	0.14	0.16
	S^3	0.40	0.40

S^1: Milk, fat, and protein yields (Nguyen et al., 2016); S^2: S^1 + fat and protein percentage; S^3: S^2 + acetone, C4:0, C18:0, C18:1 cis-9, LFCA, MUFA and UFA.