Comparison of Genetic Parameters Estimation of Fatty Acids from Gas Chromatography and FT-IR in Holsteins

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ABSTRACT: Fourier transform infrared (FT-IR) is routinely used in the milk recording system and IR-based solutions are therefore attractive to ensure the full potential of genomic selection data in future breeding programs. Today, IR-based models can be used to predict a wide range of milk traits, and their genetic parameters. However, IR-predicted phenotypes for detailed milk composition are often based on their correlation to other traits in a given data set rather than on direct predictions. Here, genetic parameters for individual milk fatty acids were estimated based on either IR-predicted phenotypes or on fatty acids data measured from gas chromatography in 371 Danish Holstein cows. Results showed similar heritability estimates and strong genomic correlations for most of the fatty acids. However, for some fatty acids, the choice of data affected the genetic parameter estimation, which may be due to indirect calibration models.

Keywords: dairy cattle, Milk fatty acids, genetics

Introduction

Today, the economic value of milk is primarily based on protein and fat content, and more detailed milk composition data are only achieved through costly and time-consuming analyses. Development of new high-throughput infrared (IR) based solutions for detailed milk quality are therefore welcomed and provide economic and technological benefit for the dairy industry as well as for the milk farmers. Thus, various phenotypic milk data have been successfully correlated with IR spectra, and prediction models for fatty acids (Soyeurt et al. (2006)), protein composition (Rutten et al. (2011)) and coagulation properties (Cecchinato et al. (2009)) have been generated.

In order to genetically modify the nutritional value of bovine milk by increasing the level of specific fatty acids (FA) with beneficial health effect, robust estimates for genetic parameters must be obtained. Use of prediction models for IR based FA phenotypes are excellent for this purpose, and calibration models for specific FAs have been very promising, as major FAs, such as C14:0, C16:0, C18:0 and C18:1 cis-9 are well predicted (Soyeurt et al. (2006); Rutten et al. (2009)). However, recent results have demonstrated that the correlation between individual FAs and the total fat content may be the overall enabler of successful IR based predictions of FAs (Eskildsen et al. (2014)). This means, that IR based models for predicting milk FAs are indirect, which might have implications for using IR-based phenotypes for genetic parameter estimation. The objective of this research was to examine the effect of using IR-based FA phenotypes for estimation of genetic parameters in Danish Holstein cows compared with estimation based on reference values measured by gas chromatography (GC).

Materials and Methods

Animals. Morning milk samples were collected from 371 Danish Holstein cows from 19 herds during the indoor winter period. All cows were housed in loose housing system, fed according to standard practice, and milked twice a day. The cows sampled were all in mid-lactation (day 129 – day 227 of lactation) and within the first-third parity.

Fatty acid phenotypes. FA composition was analyzed using GC (Poulsen et al. (2012)). Peak areas for individual FAs were calculated after GC separation, and FAs were identified and quantified through the use of external standards and expressed as the weight proportion of total FAs. Fourier transform infrared (FT-IR) full spectra obtained from fresh whole milk and total fat content was determined using MilkoScan™ FT2 (FOSS, Hillerød, Denmark). Samples were measured twice, and the average spectrum was used in the further analysis. The individual fatty acids were predicted by PLS1 (univariate **y**). PLS models were cross validated by random subsets, and only FAs having good coefficient of determination (R^2>0.80) were included. IR predicted FAs were converted from g FA/100 g milk to g FA/100 g FA using the fat content predicted by the MilkoScan and a conversion factor of 0.95 according to FOSS (FOSS, 2013).

SNP markers and genotyping. The animals were genotyped using the bovine HD Beadchip. Genomic DNA was extracted from ear tissue. From these markers 777,962 SNP markers were assayed, with a median interval of 2.68 kb between SNPs. The quality parameter used for selection of SNPs had minimum call rates of 80% for individuals and 95% for loci.

Calculation of the G-matrix. The genomic relationship matrix was calculated for each chromosome separately as described by the first method presented in VanRaden et al. (2008). In total 588,528 SNP markers were included to calculate the G-matrix.

Statistical analysis. A REML approach in DMU (Madsen and Jensen (2007)) was used to estimate the genomic parameters and variance components for the specific FAs measured using GC or predicted using FT-IR. The following model was used in the analysis:

$$Y_{ijk} = \mu + herd_i + parity_j + animal_k + e_{ijk}$$

where Y_{ijk} is the phenotype of individual k in herd i and lactation j, μ is the fixed mean effect, herd is a fixed effect (i = 1, 2, … , 19), parity is a fixed effect (j = 1,2,3), and animal is the random additive genetic effect based on **G** of animal k.

Univariate analyses were performed to estimate the heritability, which was defined as:

$$h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_e)$$

where σ^2_a was the genetic variation and σ^2_e was the residual variation. Genomic and phenotypic correlations were studied by fitting a series of bivariate analyses with a REML approach in DMU (Madsen and Jensen (2007)). Phenotypic correlations were studied using the cor.test command in R version 3.0.2 (R Core Team (2013)).

Results and Discussion

Heritability estimates for FAs measured using GC or predicted by IR are shown in Table 1. Heritability estimates for FAs measured using GC were low to moderate from 0 for C16:0 and C18:1 *cis*-9 to 0.32 for C8:0. Generally, these estimates were similar to those obtained using a Bayesian mixed model and pedigree information (Krag et al. (2013)), and in line with other studies showing higher heritability estimates for *de novo* synthesized saturated FAs compared with unsaturated FAs (Stoop et al. (2008); Garnsworthy et al. (2010)). IR-based heritability estimates were overall similar to the GC based heritabilities for the *de novo* synthesized saturated FAs, whereas the difference in heritability estimates was pronounced for especially C15:0 and C16:0, showing much higher heritabilites for IR predicted FAs, which suggest good opportunities for increasing these FAs through selective breeding, which is not supported by the GC derived estimates here.

The observed relationship is further supported by the phenotypic and genomic correlations. The phenotypic correlations between individual FAs measured by GC or predicted by IR (Table 2) ranged from -0.06 (C16:1 cis-9) to 0.63 (C:10), whereas the genomic correlation varied from -0.86 (C18:0) to almost 1 (C6:0, C8:0, C10:0, C12:0). The results show that except from C14:0, the *de novo* derived saturated FAs generally had relatively high phenotypic and genomic correlations suggesting that IR-based phenotypes are the same as GC phenotypes, and that the variation is controlled by the same set of genes.

Other FAs had extremely low phenotypic and genomic correlations, which may indicate that these traits are not the same and therefore not under the same genomic influence. The large heritabilities for C15:0, C16:0 and C18:0 in the IR predicted phenotypes could reflect that most of the variation in these traits was explained by the relationship to total fat, which also lead to relatively low phenotypic and genomic correlations. Previous results on the same data set have shown that IR-based FAs were mainly predicted indirectly, due to their correlation to total fat and not by absorption bands specific to each FA, as would be the case for direct models (Eskildsen et al. (2014)). The drawback for indirect models is that the models might not be valid for new samples representing other relationships between total fat and the individual FAs. The indirect correlations with total fat content were most pronounced for the even-branched saturated FAs. The unsaturated FAs have lower heritabilities in both GC and IR predicted phenotypes, but the relatively high phenotypic and genomic correlations for C18:1 *cis*-9 and C18:2 n-6 suggest that the same traits are measured. The data indicate that most of the FAs predicted by IR have strong genomic correlation to GC phenotypes and heritabilities, which are in line with those measured by GC. IR-based phenotypes obtained through the milk recording system, can thus be optimal for reliable parameter estimation and improve selective breeding for detailed milk composition. However, for some saturated FAs, the GC and IR predicted phenotypes show contrasting results in spite of good prediction models, and thus IR is not an optimal choice for estimation of genetic parameters. Rutten et al. (2010) also estimated heritabilities and calculated genetic correlations for GC and IR based FA phenotypes and showed strong genetic correlation among all FA phenotypes. However, a similar tendency towards C16:0 and C18:0 having lower genetic correlations, and C18:0 showing deviating heritability was also observed, confirming our results.

Table 1. Genomic heritability estimates (h^2) and their standard error (SE h^2) for individual fatty acids (g/100 g FA) measured by GC or predicted by FT-IR.

	GC		FT-IR	
Trait	h^2	SE h^2	h^2	SE h^2
C6:0	0.21	0.15	0.21	0.14
C8:0	0.32	0.17	0.24	0.15
C10:0	0.31	0.18	0.22	0.15
C12:0	0.23	0.17	0.29	0.16
C14:0	0.16	0.17	0.17	0.12
C15:0	0.07	0.13	0.52	0.19
C16:0	0.00	0.12	0.36	0.17
C18:0	0.14	0.15	0.33	0.18
C16:1 *cis*-9	0.06	0.13	0.14	0.14
C18:1 *cis*-9	0.00	0.13	0.07	0.14
C18:2 n-6	0.18	0.15	0.16	0.13

Conclusion

The results presented show that genetic parameter estimation based on IR predicted phenotypes are reliable for most FAs and provide estimates comparable to those based on GC phenotypes. However, for a smaller number of FAs, the results indicate that the phenotypes are not comparable. The moderate IR based heritability estimates for these FAs

may therefore reflect dependence of FA calibration models on the total fat content, which can be the drawback of using indirect IR models.

Literature Cited

Cecchinato, A., De Marchi, M., Gallo, L. et al. (2009). J. Dairy Sci. 92:5304-5313.

Eskildensen, C.E., Poulsen, N. A, Larsen, L. B. et al (2014). In preparation.

Garnsworthy, P. C., Feng, S., Lock, A. L. et al. (2010). J. Dairy Sci., 93:1743-1748.

FOSS. 2013. Application note 64, MilkoScan FT+ fatty acid prediction models, p. 1-35, FOSS Analytical, Hillerød, Denmark.

Madsen, P., and Jensen, J. (2007). An user's guide to DMU. A package for analysing multivariate mixed models. Version 6, release 4.7.

Krag, K., Poulsen, N. A., Larsen, M. K. et al. (2013). BMC Genetics, 14:79.

Poulsen, N. A., Gustavsson, F., Glantz, M. et al. (2012). J. Dairy Sci. 95:6362-6371.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rutten, M. J. M., Bovenhuis, H., Hettinga, K. A. et al. (2009). J. Dairy Sci. 92:6202-6209.

Rutten, M. J. M., Bovenhuis, H., van Arendonk, J. A. M. (2010). J. Dairy Sci. 93:4872-4882.

Rutten, M. J. M., Bovenhuis, H., Heck, J. M. L. et al. (2011). J. Dairy Sci. 94:5683-5690.

Soyeurt, H., Dardenne, P., Dehareng, F. et al. (2006). J. Dairy Sci. 89:3690-3695.

Stoop, W. M., van Arendonk, J. A., Heck, J. M. et al. (2008). J. Dairy Sci., 91:385-394.

VanRaden, P. M. (2008). J. Dairy Sci. 91:4414-4423.

Table 2. Phenotypic (Pcor) and genomic (Gcor) correlations and their standard error (SE) between fatty acids measured by GC or predicted by FT-IR.

Trait	Pcor	SE Pcor	Gcor	SE Gcor
C6:0	0.56	0.04	0.94	0.22
C8:0	0.58	0.04	0.99	0.19
C10:0	0.63	0.04	0.96	0.20
C12:0	0.61	0.04	1.00	0.28
C14:0	0.46	0.04	0.45	3.12
C15:0	0.19	0.05	0.52	0.47
C16:0	0.13	0.05	-0.15	0.63
C18:0	0.33	0.04	-0.86	1.76
C16:1 *cis*-9	-0.06	0.05	-[1]	-
C18:1 *cis*-9	0.58	0.04	-	-
C18:2 n-6	0.38	0.04	1.00	0.47

[1]Genomic correlations not estimated due to very low heritability estimates for both GC and FT-IR predicted FA phenotypes