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Genetic Analysis of Milk Fatty Acid Compositions based on Infrared Data

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ABSTRACT

Each milk fatty acid (FA) has different effect on human health and on property of dairy product. Therefore, improving nutritional quality of bovine milk is interesting. Predicted data from Fourier transform infrared spectroscopy (IR) provide more observations of detail milk FA compositions. The aims of this study were to estimate genetic parameters for fat percentage, C14:0, C16:0, C18u and ratio between saturated FA and unsaturated FA from IR data. The effect of sampling season and lactation stage were also estimated. A repeatability animal model was used for quantify variance components and genetic parameters. Phenotypic and genetic correlations among these traits as well as among sampling seasons were calculated. Data set contained 5,581 test-day records of 1,954 first lactation Dutch Holstein-Friesian cows. Result confirmed that IR data provided genetic parameters of milk FA compositions which is in line with Gas Chromatography profile. Intraherd heritability of C14:0 was greater than C16:0 and C18u. Estimates of intraherd heritability for all studied traits were lowest in spring. Phenotypic and genetic correlation of these traits were varies. The genetic correlations of the fat percentage with the content of C14:0, C16:0, and C18u were -0.09, 0.30 and -0.33, respectively. Phenotypic correlations between sampling seasons were moderate, while genetic correlations were high. Herd-test day and permanent environmental explain some part of milk FA variation. C14:0 and C16:0 have same changing pattern with advancing of lactation stage. This research showed that future selection program to improve the milk FA compositions using IR data could be possible.
Key words: milk fatty acid, mid-infrared, genetic parameters

INTRODUCTION

Nowadays, consumers are increasingly aware of health. Commercial trend has changed to improve the health aspect of dairy products. Milk fatty acid (FA) compositions have many effects on human health (Parodi, 1997; German et al., 2009) and each FA has different effects (Palmquis et al., 1993; Mensink et al., 2003; German and Dillard, 2006). Triglyceride affects the flavor of cheese and the melting point of butter (Jensen, 2002). The ratio of saturated to unsaturated FA reflects butterfat hardness. Conjugated linoleic acid posses several health benefits. Zock et al. (1994) found that myristic acid (C14:0) and palmitic acid (C16:0) caused high LDL cholesterol. Double bond FA in trans form raise LDL and lower HDL cholesterol (Brouwer et al., 2010). Hence improvement of milk FA is interesting.

To modify milk FA by genetic improvement, traditional selective breeding requires extensive recording of phenotype to quantify the variation in the population. The FA in bovine milk fat can be measured by Gas Chromatography (GC), the reference method, which is an expensive, time consuming method and need well-

skilled staff. The prediction method is an alternative method to generate milk FA profiles. Soyeurt et al. (2008) have shown the potential of using data from Mid-infrared (MIR) spectrometry for the prediction of detailed milk FA. Rutten et al. (2009) suggest that Fourier transform infrared spectrometry may be used to accurately predict proportions of several FA in milk for Dutch dairy cattle population. Results from this study showed that the calibration equations for predicting major FA, groups of FA, and ratio between saturated and unsaturated fatty acids in bovine milk can be used. The predicted FA of bovine milk can be used as indicator traits and allows the study of genetic variability of milk FA on a large scale. Infrared spectroscopy (IR) is used routinely in milk recording to quantify milk production traits like fat percentage and protein percentage. Using milk FA data from IR data lowers the cost of implementation compare to GC.

Half of milk FA is synthesized de novo in the mammary gland, around 40% from diet, and less than 10% from adipose tissue (Palmquist and Jenkins, 1980). The two main synthesis pathways imply that milk FA could be changed by changing the feed of dairy cows and by introducing genetic changes (Palmquist, 2006). The study of genetic parameters of FA in bovine milk will inform us about the possibilities for future selection program to improve milk FA and thus further improve bovine milk quality. For instance, genetic selection together with a proper feeding management, the fat composition of bovine milk could have more nutritional value than the present. Relationship of milk FA with the common milk production traits as fat and protein percentage will explain the direction of these production traits when we select for each milk FA.

Seasonal effects are mainly caused by differences in herd management, especially dietary change (Chilliard et al., 2001). Previous studies have reported the variation of milk FA over the year (Palmquist et al.,1993; Heck et al., 2009) The lactation stages also contribute to variation in milk FA (Stoop et al., 2009). Energy balance causes changing of milk FA. Short-chain FA is low in early lactation but C18:1 shows opposite pattern (Palmquist et al., 1993; Jensen, 2002). Study of the effect of seasons and lactation stages will answer the question whether the variation of milk FA would be a genetic effect or due to other factors.

The main objective of this study is to estimate genetic parameters for major milk FA from infrared data. It is also of interest to estimate variance due to the effect of seasons and lactation stages.

MATERIALS AND METHODS

Data

Data derived from of 1,954 first-lactation cows from 398 commercial herds throughout the Netherlands. Three morning milk samples were taken from each cow; they were collected from February to June 2005 (winter, spring and summer). Cows were over 87.5% Holstein-Friesian, and were between 41 and 335 days in milk (DIM). They were sired by 1 of the 5 proven bulls, 1 of 50 young bulls, or other proven bulls.

All samples were analyzed using a Fourier transform interferogram (MilkoScan FT 6000, Foss Electric, Hillerod, Denmark) for production traits (fat percentage and protein percentage). Infrared spectroscopy data were stored and used for prediction of milk FA composition for all 3 sampling seasons based on GC data from winter and summer. Prediction process was performed using calibration equations developed by Rutten et al. (2009).

A total of 5,737 samples were collected. If there were less than 2 samples per cow or less than 3 animals per herd-test day the observation was discarded. The final edited data file, used for analyzing production traits (fat percentage, protein percentage), and contained 5,581 test-day records. For the milk FA composition traits (C14:0, C16:0, C18u and ratio between saturated and unsaturated FA), 5,389 records were analyzed because records of milk FA compositions from predicted profile were missing. The number of data for each sampling time period was 1,777 for winter, 1,851 for spring, and 1,761 for summer. Pedigree was supplied by CRV (Arnhem, the Netherlands) and contained 26,300 animals.

C14:0, C16:0 and C18u data were analyzed as weight proportion of total milk fat weight. C18u contained all unsaturated C18 of the dataset. Saturated FA contained even chain FA C4:0 to C18:0 and unsaturated FA contained C10:1, C12:1, C14:1, C16:1, C18u and conjugated linoleic acid.

Statistical model and analysis

Analysis for combined dataset including three sampling seasons

The mean and standard deviation for fat percentage, C14:0, C16:0, C18u and ratio between saturated and unsaturated FA (ratio) were calculated.

Variance components and genetic parameters were estimated using a repeatability animal model in AS-Reml (Gilmour et al., 2006):

$$y_{ijklmno} = \mu + b_1 * dim_i + b_2 * e^{-0.05*dim} + b_3 * afc_j + b_4 * afc_j^2 + season_k + scode_l + htd_{m+} A_n + pe_o + e_{ijklmno}$$

where $y_{ijklmno}$ is the dependent variable (e.g., %FAT, C14:0, C16:0, C18u, and ratio between saturated and unsaturated FA (ratio)); μ is the general mean; dim_i is days in milk *i* (time between calving and date of sample), modeled with Wilmink curve (Wilmink, 1987); afc_j is a covariate describing the effect of age at first calving *j*; $season_k$ has 3 classes for season of calving: summer (June-August 2004), autumn (September-November 2004), and winter (December 2004-February 2005); $scode_l$ is a fixed effect accounting for differences between groups of proven bull daughters and young bull daughters; htd_m is a random effect defining groups of animals sampled in the same herd on the same day; A_n is the random additive genetic effect; pe_o is the random permanent environmental effect *o*; and $e_{ijklmno}$ is the random residual effect.

Intraherd heritability and repeatability were estimated using univariate analysis. Intraherd heritability (h^2_{IH}) is the parameter required to predict selection responses of breeding programs (Heringstad et al., 2006), and was calculated as:

$$h^2_{IH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{pe} + \sigma^2_e}$$

where σ^2_A is additive genetic variation, σ^2_{pe} is permanent environment variation, and σ^2_e is residual variation.

The repeatability (r) estimates the correlation between consecutive samples of the same cow in time. Repeatability was calculated as:

$$r = \frac{\sigma_A^2 + \sigma_{pe}^2}{\sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2}$$

The proportion of variance attributable to herd-test day effects and additive genetic effect ($\sigma_A^2 / \sigma_{htd}^2$) were calculated to compare their importance.

The proportion of variance due to htd (h_{htd}) was calculated as:

$$h_{htd} = \frac{\sigma_{htd}^2}{\sigma_A^2 + \sigma_{htd}^2 + \sigma_{pe}^2 + \sigma_e^2}$$

where σ_{htd}^2 is herd-test day variation.

Analysis for each sampling season separately

The mean and standard deviation for fat percentage, C14:0, C16:0, C18u and ratio were calculated. Variance components and genetic parameters were estimated using an animal model in AS-Reml (Gilmour et al., 2006):

$y_{ijklmn} = \mu + b_1 * dim_i + b_2 * e^{-0.05*dim} + b_3 * afc_j + b_4 * afc_j^2 + season_k + scode_l + htd_m + A_n + e_{ijklmn}$

Intraherd heritability (h_{IH}^2) was calculated as:

$$h_{IH}^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2}$$

where σ_A^2 is additive genetic variation, and σ_e^2 is residual variation.

The proportion of variance due to htd (h_{htd}) was calculated as:

$$h_{htd} = \frac{\sigma_{htd}^2}{\sigma_A^2 + \sigma_{htd}^2 + \sigma_e^2}$$

where σ_{htd}^2 is herd-test day variation.

To compare the relative importance of genetic and herd-test day effects, the ratio $\sigma_A^2 / \sigma_{htd}^2$ was calculated too.

Correlations

Phenotypic (r_P) and genotypic correlations (r_A) among traits of overall dataset and between time periods were estimated using bivariate analysis.

$$r_P = \frac{\sigma_{P1,P2}^2}{\sqrt{\sigma_{P1}^2 * \sigma_{P2}^2)}}$$

$$r_A = \frac{\sigma^2_{A1,A2}}{\sqrt{\sigma^2_{A1} * \sigma^2_{A2})}}$$

where $\sigma^2_{P1,P2}$ is the phenotypic covariance between trait 1 and trait2 or between sampling season 1 and 2, and σ^2_{P1} and σ^2_{P2} are the phenotypic variance of trait 1 and 2 respectively or sampling season 1 and 2, $\sigma^2_{A1,A2}$ is the additive genetic covariance between trait 1 and trait2 or between sampling season 1 and 2, and σ^2_{A1} and σ^2_{A2} are the additive genetic variance of trait 1 and 2 or sampling season 1 and 2.

Effect of lactation stage

To study the effect of lactation stage on milk FA compositions, 10 classes of lactation stage were modeled: 41 to 71 DIM (n=19); 72-102 (n=122); 103 to 133 (n=411); 134 to 164 (n=765); 165 to 195 (n=1057); 196 to 226 (n=1,203); 227 to 257 (n=999), 258 to 288 (n=677); 289 to 319 (n=287); and 320 to 335 DIM (n=41). Each class was 30 days except the last class.

Data analysis was performed using an animal model in AS-Reml (Gilmour et al., 2006). The model was:

$$y_{ijklmn} = \mu + lact_i + b_1*afc_j + b_2*afc_j^2 + season_k + scode_l + htd_m + A_n + e_{ijklmn}$$

where y_{ijklmn} is the dependent variable (e.g., %FAT, C14:0, C16:0, C18u, and ratio between saturated and unsaturated FA (ratio); μ is the general mean; $lact_i$ is a lactation stage *i*, which is a fixed effect for 10 classes of lactation stage; afc_j is a covariate describing the effect of age at first calving *j*; $season_k$ has 3 classes for season of calving: summer (June-August 2004), autumn (September-November 2004), and winter (December 2004-February 2005); $scode_l$ is a fixed effect accounting for differences between groups of proven bull daughters and young bull daughters; htd_m is a random effect defining groups of animals sampled in the same herd on the same day; A_n is the random additive genetic effect of animal *n*; and e_{ijklmn} is the random residual effect.

RESULTS

Mean and coefficient of variation

Means, standard deviation, and coefficients of variation for fat percentage, C14:0, C16:0, C18u and ratio, based on combined dataset, data from winter (1), spring (2) and summer (3) are shown in Table 1. For combined dataset, the mean fat percentage was 4.31. The major milk FA was C16:0, which accounted for 31.11% of total milk fat. The content of unsaturated C18 fatty acids (C18u) on average was 22.20 % of total milk fat. The ratio of saturated to unsaturated fatty acid (Ratio) averaged 2.65. When compare each sampling season, the fat percentage, C14:0, C16:0 and ratio were decreasing from winter to summer. Whereas, C18u was increasing. There was 0.44% less C14:0 in summer compared to winter. Similarly, C16:0 averaged 3.42% less in summer compared to winter. On the other hand, C18u was highest in summer (23.68%). Ratio was lowest in summer (2.43).

For combined dataset, the coefficient of variation for fat percentage and ratio were high (17%) compared with C14:0 (8%). Coefficient of variation for C16:0 and C18u were moderate (10% and 13%, respectively). Among data from each sampling season separately, ratio in summer had the highest CV (19%) and C14:0 in winter the lowest (6%).

Heritability and repeatability

Intraherd heritability for fat percentage, C14:0, C16:0, C18u and ratio, based on combined dataset (all), data from each sampling season: winter (1), spring (2) and summer (3) are in Table 2. For combined dataset, high intraherd heritability was found for fat percentage and C14:0. Moderate intraherd heritabilities were found C16:0 (0.27), C18u (0.26), and ratio (0.30). Repeatability of C14:0 was lower than the others (0.43) (Table 2). Imply that the correlation of C14:0 between consecutive test-day was lower than the other traits. Repeatability for C16:0, C18u and ratio were moderate (0.51 to 0.53). Fat percentage gave highest repeatability (0.69). The comparative importance of genetic with herd effects were explained by ratio of genetic variance to variance attributable to herd ($\sigma_A^2/\sigma_{htd}^2$; table 2). For fat percentage and C14:0, genetic effects were larger than herd effects, whereas for C16:0, C18u, and ratio, herd effects were larger than genetic effects. Results for the proportion of variance explained by herd-test day are also shown in table 2. Variance attributable to herd-test day for fat percentage (7%) was lower than for C14:0, C16:0, C18u, and ratio. For C18u and C16:0, the herd-test day effect moderately explained 40 and 42% of variation, while for C14:0 and ratio, herd-test day explained 28% and 38%, respectively.

Table 2 also shows intraherd heritability, the ratio $\sigma_A^2/\sigma_{htd}^2$ and the proportion of variance due to herd-test day (h_{htd}) for milk FA in winter (1), spring (2) and summer data (3). Intraherd heritabilities varied between seasons. Intraherd heritabilities for all traits were lowest at spring, except for C16:0. Intraherd heritability was lowest in summer for C16:0. There was a decrease in intraherd heritability for fat percentage from 0.57 to 0.33 from winter to spring and increase to 0.64 in summer. For C16:0, intraherd heritabilities were decreasing according to season from winter to summer. The ratio of genetic variance to variance attributable to herd ($\sigma_A^2/\sigma_{htd}^2$) also had the same trend as the intraherd heritabilities. For fat percentage and C14:0 in winter, genetic effects were generally larger than herd effects. On the other hand, for C14:0 in spring and summer, C16:0, C18u, and ratio in all 3 seasons, herd effects were larger than genetic effects. When compared among sampling seasons, proportion explains by herd-test day of winter data was smallest for all traits.

Correlation

Genetic correlations between milk FA with milk FA and with production traits

Phenotypic and genetic correlations between the studied traits are shown in table 3. The phenotypic correlation between traits ranged from -0.98 to 0.83. Genetic correlation ranged from -0.97 to 0.73.

Phenotypic correlation of C14:0 with fat percentage and protein percentage were low, ranging from -0.09 to 0.04. Phenotypic correlation between C18u and C16:0 with ratio were high, ranging from -0.98 to 0.83.

C14:0 had a weak negative genetic correlation with c18u, protein percentage and ratio, and a moderate negative genetic correlation with C16:0 (-0.54) and fat percentage (-0.24). C16:0 had a negative genetic correlation (-0.71) with C18u, while it was positively genetic correlated with production traits and ratio. C18u had a negative genetic correlation with fat percentage (-0.74), protein percentage (-0.43) and ratio (-0.97). Fat percentage showed a strong positive genetic correlation with protein percentage (0.73) and showed a weak positive genetic correlation with ratio (0.07). Ratio showed a moderate positive genetic correlation with protein percentage and high negative genetic correlation with C18u.

Genetic correlations between sampling time periods

Table 4. shows phenotypic and genetic correlation between sampling time period. Phenotypic correlation between sampling time periods were moderate, ranging from 0.36 to 0.66. Genotypic correlation between sampling time period showed a strong positive correlation, ranging from 0.84 to 0.99. Phenotypic correlations between winter and summer were lowest. Herd and residual correlation between sampling time period are shown in Table 5. Herd correlations were lower than 0.41. The residual correlation ranged from 0.14 to 0.39. Phenotypic, genetic, herd and residual correlation of C14:0 (Between winter and summer), C16:0 (Between winter and spring), and fat percentage (between winter and summer) were missing regarding to converged problem.

The effect of lactation stage

The changes in mean of milk FA during lactation are shown in figures 1. Lactation stage significantly affected all tested milk FA ($P<0.001$), except for fat percentage. Fat percentage was slightly increased from 41-71 DIM (3.84) to 289-319 DIM (4.37) and then decreased to final stage (4.26). The pattern for C14:0, C16:0 and ratio were similar to each other. They peaked at 134-164 DIM and then decreased afterward. For C14:0, C16:0 and ratio, the lowest was at 320-349 DIM. C14:0 varied from 10.52 to 11.66 w/w%. C16:0 showed large variation from 27.23 to 32.30 w/w% between134-164 DIM and 320-349 DIM. For C18u, showed a minimum at mid lactation stage (134-164 DIM) at 21.11 w/w% and maximized at last stage of lactation (25.93 w/w%). Ratio showed small change, ranging from 2.06 to 2.82.

DISCUSSION

Results of our study showed similarities in fat percentage observed previously by Stoop et al. (2009) in the same population. Fat percentage in winter was higher than in spring and summer. In winter, the main feed for Dutch dairy cows are maize silage and hay which cause an increase in fat percentage in milk (Heck et al., 2009). Our result concurred with previous observations (Soyeurt et al., 2008; Heck et al., 2009; Stoop et al., 2009). Main saturated and unsaturated FA in our study, studied from IR data, was C16:0 and C18u, which is in line with Stoop et al. (2009) and Heck et al. (2009) studied from GC profiles. There was a difference in milk FA among sampling seasons, indicating seasonal effects on these traits. In winter, there was more C14:0 and C16:0 than in summer. Earlier studies demonstrated similar pattern (Palmquist and Beaulieu, 1993; Soyeurt et al., 2008; Heck et al., 2009; Stoop et al., 2009). The studied population, Dutch Holstein Friesian cows, was kept inside during

winter and about half of these cows were grazing outside during spring and summer. Palmquist and Beaulieu (1993) also suggested that seasonal variation of milk FA are most likely because of dietary source and herd management. Elgersma et al. (2006) observed milk from cows grazing fresh grass had more unsaturated fatty acid proportion than cow fed with silage. Variation of studied traits in winter was less than in spring and summer mainly due to less herd variation in winter.

Intraherd heritability of fat percentage, C18u and ratio from combined dataset was in the same range as Stoop et al. (2009). De Jager and Kennedy (1987) estimated heritability for fat percentage in first lactation Holstein cows of 0.61. Schutz et al. (1990) estimated a lower heritability for fat percentage of 0.46, using sire model whereas Jamrozik et al. (1996) estimated at 0.28 with random regressions model. For C14:0 and C16:0, intraherd heritability estimates obtained in this study were lower than previous study (Soyeurt et al. 2008; Stoop et al., 2009). In Soyeurt et al. (2008), they used permanent environment random effects both within and across lactations. The reason for differences from Stoop et al. (2009) is due to the different number of observations and dataset. In study of Stoop et al.(2009), they studied from 1,783 GC records collected between February and March 2005. Milk fat percentage and C14:0 were more heritable than C16:0 and C18u. This pattern was similar to those obtained previously by Karijord et al. (1982).

Intraherd heritabilities estimated for milk FA differed among sampling seasons. The changes of intraherd heritability estimates over the seasons were similar for fat percentage, C14:0, C18u and ratio. For all traits except C16:0 the lowest intraherd heritability was observed in spring. This is due to an increase in residual variance during spring, indicating that other factors beyond animal and systematic environmental factors included in model were involved. Somatotropin, growth hormone, has been reported to have an effect on the whole cow body and milk production process (Etherton and Bauman., 1988; Bauman, 1992; Tyrrell et al., 1998; Jensen, 2002). Lee at al. (1976) has been reported lower corticoid in cow in spring than in winter. Glucocorticoids are inhibitor of growth hormone. Furthermore, genotype by environment may play an important role (Hammami et al., 2008). These factors can affect milk FA leading to an increase in residual variance. Intraherd heritability for C14:0 in all sampling seasons was higher than for unsaturated C18u, which is compatible with finding of Stoop et al. (2009). Fat percentage, C14:0 and C18u showed an increase in intraherd heritabilities in summer. This might be related to food changes which probably alter fat metabolism pathways, consequently increasing genetic variation (Stoop et al., 2009). Garnsworthy, (2003) suggested that a molecule contained in the grass could stimulate the enzyme activities (especially delta 9 desaturase activity). The intra-herd heritability difference between C14:0 and C16:0 and also C18u could be explained by the synthesis pathway for those traits. C14:0 and about half of C16:0 are synthesized *de novo* in mammary gland with multi-enzymes involved, but the other half of C16:0 and C18u are derived directly from blood. It was expected that genetics has a bigger effect on *de novo* synthesis of milk FA than on blood derived milk FA. The blood derived milk FA mainly depend on the composition of fat in the diet. Polyunsaturated FA are the predominantly FA in dairy cattle diets. However, the diet might contain specific FA that inhibits the *de novo* FA synthesis (Baumgard et al., 2005).

Repeatabilities of C14:0 was relatively low (0.43) so the correlation of C14:0 between the consecutive testing day was lower than the other traits. For C16:0 and C18u, the difference between repeatability and heritability was moderate (0.25). Some

part of variation in C16:0 and C18u can be explained by permanent environmental effects.

The proportion of variance attributed to herd-test day varied among sampling seasons. Our study showed that C14:0 had a smaller proportion of variance attributed to herd-test day than C16:0 and C18u. Jensen, 2002 suggested that the reason for variance attribute to herd is due to differences in feed among herds and also due to herd management. In our study, herd-test day had small effect on fat percentage (7%) and C14:0 and moderate effect on C16:0, C18u and ratio. This suggested a small effect of feed on fat percentage and C14:0, and moderate effect of feed on the others. The other study showed an effect of diet on fat (Keady et al., 2001). Herd-test day included not only effects of feed and herd management but also possible effects of sampler, measurement technique, and season of sampling. Season and region have been reported to affect milk FA (Palmquist and Beaulieu, 1993; Jensen, 2002).

Phenotypic correlations of C14:0, C16:0 and C18u with production traits were low to moderate. Soyeurt et al. (2008) observed comparable phenotypic correlation at -0.19 and 0.10 of C14:0 and C16:0 with fat percentage. Karijord et al. (1982) obtained a negative correlation between short chain FA and C18u. This result is in agreement with the result obtained in this study. A strong positive phenotypic correlation of C16:0 with ratio and strong negative phenotypic correlation of C18u with ratio were observed in our study. The reason for this is because C16:0 is the major saturated FA and C18u is a major group of unsaturated FA as described before.

Genetic correlation between fat percentage and protein percentage was positive and high (0.73), suggesting that these traits are related. This genotypic correlation was in line with other studies (Soyeurt et al., 2008; Stoop et al., 2009). Genetic correlation between C16:0 and ratio were highly positive whereas, highly negative genetic correlation between C18u and ratio. This result is in agreement with phenotypic correlation. Genotypic correlation between C16:0 and C14:0 was moderately negative. However, genotypic correlation between C16:0 and C18u was highly negative. The observed genetic correlations for these traits were similar to those estimated by Stoop et al. (2009). However, Soyeurt et al. (2008) observed genetic correlation between C14:0 and C16:0 at 0.00 using model with different fixed effects from our study. The present study shows a moderate positive genetic correlation between C16:0 and fat percentage and a small positive genetic correlation between C16:0 and protein percentage. C18u tended to be negatively correlated with fat percentage and protein percentage. Genetic selection for fat percentage and protein percentage will therefore increase the content of C16:0 and decrease C18u. This pattern was also found in study of Stoop et al. (2009). In addition, the genetic correlations reflect the synthesis pathway involved in the production of FA in milk (Chilliard et al., 2001).

We found a strong genetic correlation between sampling time periods indicating that these are genetically similar traits. Phenotypic correlations between seasons were moderate with low standard error. Stoop et al. (2009) reported high genetic correlations and moderate phenotypic correlation between winter and summer studied from GC profile. From the similar genetic parameters studied from GC and IR data, it is suggested that animal selection based on the IR profile might be possible. In addition, Soyeurt et al. (2010) showed genetic variation of milk composition studied from IR data. Samoré et al. (2007) suggested that genetic improvement for milk urea could be possible using IR data.

Fat percentage, C14:0, C16:0, C18u and ratio significantly changed with lactation stage. Stoop et al. (2009) reported significant effects of day in milk on milk FA. In our study, C16:0 had a maximum change of 5.07 w/w% with lactation stage.

This change was large compared to differences between sampling time periods. For C16:0, the difference in estimated herd-test day effect was 3.42 w/w% between winter and summer time. The current study found similar result with previous studies (Palmquist and Beaulieu, 1993; Kay et al., 2005; Stoop et al., 2009). C14:0, C16:0 and ratio slightly increased with lactation stage and then decrease after mid of lactation. The content of C18u decreased with lactation stage and then increased after mid of lactation. The reason for this is that in early lactation, the physiological inability of cows to consume enough feed to meet energy requirements (Jensen et al., 2002). Kay et al. (2005) and Jensen et al. (2002) reported that stage of lactation affects milk FA profile. The mean of day in milk for winter sample was 167 days, 204 days in spring and 247 days in summer (data not shown). This showed that cows have moved up in lactation stage in the next sampling time period. This might cause the increase or decrease in mean value between seasons. The unbalanced energy status in early lactation causes a change in milk composition (Stoop et al., 2009). Fat percentage slightly changed with advance of lactation stage. This suggests that changes in milk FA composition throughout lactation are not explained by changes in fat percentage. Coefficient of variation for all studied traits in combination with a intraherd heritability suggests that there are possibilities to change milk FA by means of selection. The fraction of the variance due to herd-test day (especially for C16 and C18u) indicates that breeding is not the only way to change these milk FA. Management method especially feeding strategy can be used to modify milk FA as well.

The main objective of this paper was to study genetic parameters from IR data. The results of this paper indicated that the IR data gives results which are in agreement with studies from GC profiles. It is clearly shown that the individual milk FA can be changed and depended on the origin of each milk FA. In summary, we can study genetic parameters from the IR data. Herd-test day, season of sampling and lactation explained some part of variation of the studied milk FA. Due to different heritability among seasons, there are other factors beside genetic and systematic environmental effects in the model affecting these traits. This might be because of changing in season affect cow metabolism. Further research; however, is need to identify the biological pathways of cows that are affecting milk FA compositions particularly during shifting of season. Regular monitoring milk FA compositions and feed management would help to identify causes of changing in milk FA compositions among seasons. In selection for improving milk FA based on IR data, the breeder should use all year round data instead of only one or two season.

CONCLUSIONS

Season of sampling had an effect on intraherd heritability for all studied traits. Phenotypic and genetic correlation of these traits were varies. Genetic correlations between sampling time period were high. C14:0 and C16:0 have a same changing pattern with advancing of lactation stage. Result confirmed that IR data provided genetic parameters of milk FA compositions which are in line with GC profile.

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Table1. Means (with standard deviation in subscript) and coefficients of variation (%) for fat percentage, C14:0, C16:0, C18u, and ratio measured on combined dataset (all), data from winter (1), spring (2) and summer (3) of 1,954 Dutch Holstein-Friesian cows.

Traits	Time period	n	Mean	CV (%)
Fat (%)	all	5,581	$4.31_{0.72}$	17
	1	1,882	$4.36_{0.70}$	16
	2	1,922	$4.30_{0.73}$	17
	3	1,777	$4.26_{0.73}$	17
C14 : 0 (w/w%)[1]	all	5,389	$11.39_{0.88}$	8
	1	1,777	$11.61_{0.72}$	6
	2	1,851	$11.40_{0.92}$	8
	3	1,761	$11.17_{0.92}$	8
C16 : 0 (w/w%)[1]	all	5,389	$31.11_{3.26}$	10
	1	1,777	$32.66_{2.44}$	7
	2	1,851	$31.41_{3.14}$	10
	3	1,761	$29.24_{3.17}$	11
C18u (w/w%)[1]	all	5,389	$22.20_{2.90}$	13
	1	1,777	$21.06_{2.12}$	10
	2	1,851	$21.90_{2.87}$	13
	3	1,761	$23.68_{2.97}$	13
Ratio	all	5,389	$2.65_{0.45}$	17
	1	1,777	$2.83_{0.34}$	12
	2	1,851	$2.69_{0.45}$	17
	3	1,761	$2.43_{0.47}$	19

[1] For each milk FA: mean is mean milk FA as w/w proportion of the total fat fraction of 100%

Table2. Intraherd heritability (h^2_{IH}), repeatability (r), the ratio $\sigma^2_A / \sigma^2_{htd}$ and the proportion of variance due to herd-test day (h_{htd}) for fat percentage, C14:0, C16:0, C18u, ratio estimated from combined dataset (all), data from winter (1), spring (2) and summer (3) of 1,954 Dutch Holstein-Friesian cows. Standard errors are given in subscript.

Trait	Time period	h^2_{IH} [1]	r^2	$\sigma^2_A / \sigma^2_{htd}$	h_{htd} [3]
Fat (%)	all	$0.52_{0.09}$	$0.69_{0.01}$	$7.38_{1.75}$	$0.07_{0.01}$
	1	$0.57_{0.11}$		$6.15_{2.01}$	$0.09_{0.02}$
	2	$0.33_{0.09}$		$3.11_{1.13}$	$0.10_{0.02}$
	3	$0.64_{0.13}$		$4.82_{1.50}$	$0.12_{0.02}$
C14 : 0	all	$0.40_{0.08}$	$0.43_{0.02}$	$1.04_{0.24}$	$0.28_{0.02}$
	1	$0.45_{0.11}$		$1.19_{0.35}$	$0.28_{0.03}$
	2	$0.27_{0.08}$		$0.60_{0.21}$	$0.31_{0.03}$
	3	$0.48_{0.12}$		$0.86_{0.27}$	$0.35_{0.03}$
C16 : 0	all	$0.27_{0.07}$	$0.52_{0.02}$	$0.37_{0.11}$	$0.42_{0.02}$
	1	$0.40_{0.11}$		$0.84_{0.27}$	$0.32_{0.03}$
	2	$0.24_{0.09}$		$0.23_{0.09}$	$0.51_{0.03}$
	3	$0.14_{0.07}$		$0.14_{0.07}$	$0.50_{0.03}$
C18u	all	$0.26_{0.07}$	$0.51_{0.02}$	$0.39_{0.11}$	$0.40_{0.02}$
	1	$0.32_{0.10}$		$0.63_{0.22}$	$0.34_{0.03}$
	2	$0.14_{0.07}$		$0.17_{0.09}$	$0.46_{0.03}$
	3	$0.21_{0.09}$		$0.25_{0.11}$	$0.46_{0.03}$
Ratio	all	$0.30_{0.07}$	$0.53_{0.02}$	$0.49_{0.14}$	$0.38_{0.02}$
	1	$0.37_{0.10}$		$0.79_{0.26}$	$0.32_{0.03}$
	2	$0.19_{0.08}$		$0.23_{0.10}$	$0.45_{0.03}$
	3	$0.24_{0.09}$		$0.30_{0.12}$	$0.44_{0.03}$

[1]for all; $h^2_{IH} = \sigma^2_A/(\sigma^2_A + \sigma^2_{pe} + \sigma^2_e)$; for 1, 2, 3 $h^2_{IH} = \sigma^2_A/(\sigma^2_A + \sigma^2_e)$

[2]for all; $r = (\sigma^2_A + \sigma^2_{pe})/(\sigma^2_A + \sigma^2_{pe} + \sigma^2_e)$

[3]for all; $h_{htd} = \sigma^2_{htd}/(\sigma^2_A + \sigma^2_{htd} + \sigma^2_{pe} + \sigma^2_e)$; for 1, 2, 3 $h_{htd} = \sigma^2_{htd}/(\sigma^2_A + \sigma^2_{htd} + \sigma^2_e)$

Table3. Phenotypic (above diagonal) and genetic correlations (below diagonal) between C14:0, C16:0, C18u, ratio, fat percentage, and protein percentage, estimated from combined dataset of 1,954 cows in first lactation. Standard errors are given in subscript.

Trait	C14 : 0	C16 : 0	C18u	Ratio	Fat (%)	Protein (%)
C14 : 0		$0.17_{0.02}$	$-0.51_{0.02}$	$0.48_{0.02}$	$-0.09_{0.02}$	$0.04_{0.02}$
C16 : 0	$-0.54_{0.14}$		$-0.85_{0.01}$	$0.83_{0.01}$	$0.30_{0.02}$	$0.06_{0.02}$
C18u	$-0.05_{0.18}$	$-0.71_{0.09}$		$-0.98_{0.00}$	$-0.33_{0.02}$	$-0.14_{0.02}$
Ratio	$-0.02_{0.18}$	$0.69_{0.10}$	$-0.97_{0.01}$		$0.07_{0.09}$	$0.31_{0.15}$
Fat (%)	$-0.24_{0.15}$	$0.67_{0.11}$	$-0.74_{0.09}$	$0.35_{0.02}$		$0.48_{0.02}$
Protein(%)	$-0.03_{0.15}$	$0.39_{0.15}$	$-0.43_{0.15}$	$0.09_{0.02}$	$0.73_{0.08}$	

Table4. Phenotypic (r_P)[1] and genetic correlation(r_A)[2] between winter, spring and summer samples, based on 5,581 test-day records of 1,954 cows. Standard errors are given in subscript.

Trait	r_P [a]	r_P [b]	r_P [c]	r_A [a]	r_A [b]	r_A [c]
C14 : 0	$0.44_{0.02}$	NA[3]	$0.40_{0.02}$	$0.96_{0.05}$	NA[3]	$0.92_{0.00}$
C16 : 0	NA[3]	$0.38_{0.03}$	$0.45_{0.03}$	NA[3]	$0.94_{0.08}$	$0.92_{0.10}$
C18u	$0.40_{0.03}$	$0.36_{0.03}$	$0.45_{0.03}$	$0.99_{0.00}$	$0.84_{0.12}$	$0.99_{0.09}$
Fat (%)	$0.66_{0.01}$	NA[3]	$0.66_{0.02}$	NA[3]	$0.99_{0.00}$	$0.99_{0.01}$
Ratio	$0.45_{0.02}$	$0.39_{0.03}$	$0.47_{0.03}$	$0.97_{0.00}$	$0.79_{0.12}$	$0.94_{0.09}$

[1] $r_P = \frac{\sigma^2_{P1,P2}}{\sqrt{\sigma^2_{P1} * \sigma^2_{P2})}}$

[2] $r_A = \frac{\sigma^2_{A1,A2}}{\sqrt{\sigma^2_{A1} * \sigma^2_{A2})}}$

[3]NA is not available due to converged problem.
[a] is correlation between winter and spring
[b] is correlation between winter and summer
[c] is correlation between spring and summer

Table5. Herd-test day (r_{htd})[1] and residual correlation(r_E)[2] between winter, spring and summer samples, based on 5,581 test-day records of 1,954 cows. Standard errors are given in subscripts.

Trait	r_{htd} [a]	r_{htd} [b]	r_{htd} [c]	r_E [a]	r_E [b]	r_E [c]
C14 : 0	$0.40_{0.07}$	NA[3]	$0.39_{0.06}$	$0.14_{0.1}$	NA[3]	$0.09_{0.04}$
C16 : 0	NA[3]	$0.31_{0.06}$	$0.40_{0.05}$	NA[3]	$0.22_{0.08}$	$0.39_{0.05}$
C18u	$0.34_{0.06}$	$0.23_{0.07}$	$0.39_{0.06}$	$0.38_{0.03}$	$0.30_{0.07}$	$0.39_{0.05}$
Fat (%)	$0.42_{0.12}$	NA[3]	$0.40_{0.11}$	$0.39_{0.03}$	NA[3]	$0.33_{0.13}$
Ratio	$0.34_{0.06}$	$0.24_{0.07}$	$0.41_{0.05}$	$0.35_{0.03}$	$0.33_{0.08}$	$0.39_{0.05}$

[1] $r_{htd} = \frac{\sigma^2_{htd1,htd2}}{\sqrt{\sigma^2_{htd1} * \sigma^2_{htd2})}}$

[2] $r_E = \frac{\sigma^2_{E1,E2}}{\sqrt{\sigma^2_{E1} * \sigma^2_{E2})}}$

[3]NA is not available due to converged problem.
[a] is correlation between winter and spring
[b] is correlation between winter and summer
[c] is correlation between spring and summer

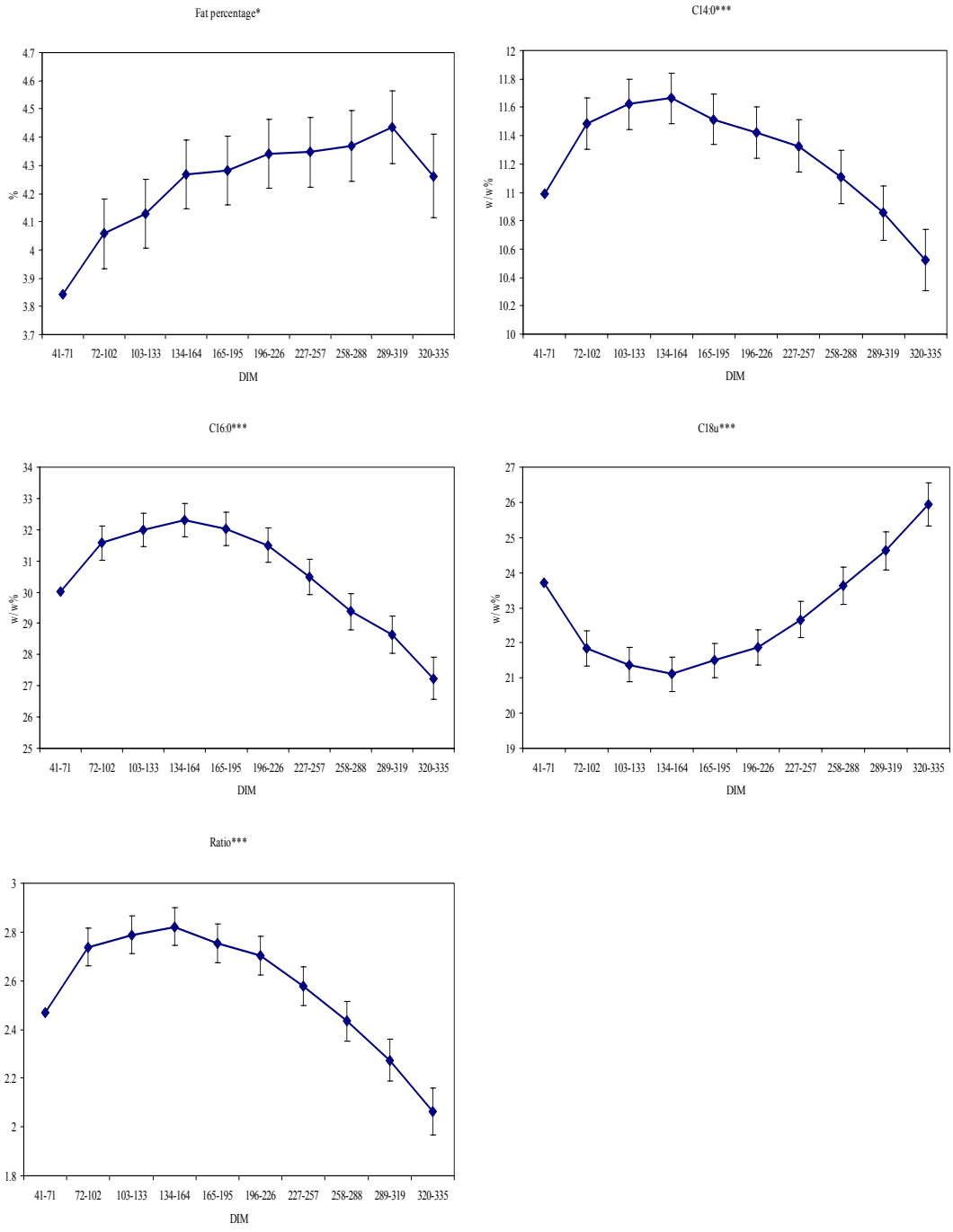

Figure1. Change in milk FA during lactation. X-axis shows class of days in milk (DIM). Y-axis shows change in fatty acid w/w%. * = P < 0.1, ** = P < 0.01, and *** = P < 0.001

APPENDIX

Table1. Herd-test day, additive genetic, permanent environment and residual variance for fat percentage, C14:0, C16:0, C18u and ratio measured on combined dataset (all), data from winter (1), spring (2) and summer (3) of 1,954 Dutch Holstein-Friesian cows.

Trait	Time period	σ^2_{htd}	σ^2_A	σ^2_{pe}	σ^2_e
Fat (%)	all	0.04	0.27	0.08	0.16
	1	0.04	0.03		0.05
	2	0.05	0.16		0.33
	3	0.07	0.33		0.18
C14 : 0	all	0.20	0.21	0.02	0.3
	1	0.15	0.18		0.21
	2	0.26	0.16		0.42
	3	0.30	0.25		0.30
C16 : 0	all	3.71	1.36	1.29	2.44
	1	1.96	1.65		2.49
	2	5.06	1.17		3.63
	3	4.93	0.71		4.20
C18u	all	2.83	1.10	1.05	2.10
	1	1.51	0.95		2.04
	2	3.67	0.61		3.69
	3	3.87	0.96		3.55
Ratio	all	0.07	0.03	0.03	0.05
	1	0.04	0.26		0.19
	2	0.09	0.02		0.09
	3	0.09	0.03		0.09

Table2. Phenotypic variance and heritability for fat percentage, C14:0, C16:0, C18u, ratio estimated from combined dataset (all), data from winter (1), spring (2) and summer (3) of 1,954 Dutch Holstein-Friesian cows. Standard errors are given in subscript.

Trait	Time period	σ_p^2	h^2
Fat (%)	all	$0.55_{0.02}$	$0.49_{0.09}$
	1	$0.50_{0.02}$	$0.52_{0.10}$
	2	$0.54_{0.02}$	$0.30_{0.08}$
	3	$0.58_{0.03}$	$0.56_{0.12}$
C14 : 0	all	$0.73_{0.02}$	$0.29_{0.06}$
	1	$0.53_{0.02}$	$0.33_{0.08}$
	2	$0.83_{0.03}$	$0.19_{0.06}$
	3	$0.85_{0.04}$	$0.30_{0.08}$
C16 : 0	all	$8.80_{0.25}$	$0.15_{0.04}$
	1	$6.11_{0.27}$	$0.27_{0.08}$
	2	$9.86_{0.47}$	$0.12_{0.05}$
	3	$9.83_{0.47}$	$0.07_{0.04}$
C18u	all	$7.09_{0.20}$	$0.16_{0.04}$
	1	$4.50_{0.19}$	$0.20_{0.07}$
	2	$7.98_{0.36}$	$0.08_{0.04}$
	3	$8.37_{0.38}$	$0.11_{0.05}$
Ratio	all	$0.18_{0.00}$	$0.19_{0.05}$
	1	$0.11_{0.00}$	$0.25_{0.07}$
	2	$0.20_{0.00}$	$0.10_{0.04}$
	3	$0.21_{0.01}$	$0.13_{0.05}$

[1]for all; $\sigma_p^2 = \sigma_A^2 + \sigma_{htd}^2 + \sigma_{pe}^2 + \sigma_e^2$; for 1, 2, 3 $\sigma_p^2 = \sigma_A^2 + \sigma_{htd}^2 + \sigma_e^2$

[2]for all; $h^2 = \sigma_A^2/(\sigma_A^2 + \sigma_{htd}^2 + \sigma_{pe}^2 + \sigma_e^2)$; for 1, 2, 3 $h^2 = \sigma_A^2/(\sigma_A^2 + \sigma_{htd}^2 + \sigma_e^2)$

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