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MODELLING VARIATION IN BOVINE MILK FAT COMPOSITION PREDICTED USING MID-INFRARED SPECTROMETRY

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SUMMARY

Applying calibration equations to mid-infrared (MIR) spectrometry is an efficient method to predict fatty acid composition in bovine milk. This investigation applied calibration equations to weekly MIR data of approximately 240 cows of mixed age and mixed calving date over a seven month period. Cows belonged to either a high yielding line selected for maximum milk fat and protein production or a control line representing the moving UK average for milk production. Random regression models with Legendre polynomials were fitted for the major fatty acids and fatty acid classes. Correlations were assessed between fatty acids and related milk production traits using bivariate regression. Results showed significant effects of genetic selection line on the fatty acid profile, whereby there was a greater proportion of saturated fatty acids in the milk fat of the high yielding line. Diet, parity number, week of lactation, date of lactation and several of their interactions also had significant affects on the fatty acid profile. Correlations were observed between fatty acids and production traits. The research demonstrates the practical application of this technique in the British dairy industry and highlights the effect of current selection practices on saturated fatty acids as an area of interest for future research.

INTRODUCTION

Research into the fatty acid profile of bovine milk has been limited in the past due to the expensive and time consuming nature of gas chromatography analysis. Mid-infrared (MIR) spectrometry is the method of choice for quantification of milk fat, protein, and lactose content of milk samples. Applying the Soyeurt *et al.* (2011) calibration equations to the MIR spectrum enables efficient analysis of fatty acid profiles for large datasets, not previously possible.

Fat content and composition of food is of growing consumer concern as related health problems continue to be a heavy burden on society. Cardiovascular disease, which is linked to high intake of saturated fatty acids (SFA), is the most common cause of death in the UK (Townsend *et al.* 2012). Milk and milk products are nutrient rich foods but suffer significant criticism as the main source of saturated fat in the diet, contributing approximately 31% and 22% of SFA in the British diet for 4-10 year olds and adults (19-64), respectively (Bates *et al.* 2011). Modifying the fatty acid profile of milk could be an effective way of reducing the SFA intake of the British population without requiring a change in dietary patterns. Four studies have reported a reduction in total and LDL-cholesterol, both risk factors for cardiovascular disease, following consumption of milk and dairy products with modified fatty acid profile (Livingstone *et al.* 2012).

Notably, the fatty acid profile of milk is highly variable, with large potential to make changes through animal nutrition (Ashes *et al,*1997). There is also evidence supporting the potential to change fatty acid profiles through genetic selection, with heritabilities reportedly as high as 0.43 for SFA (Bastin *et al.* 2011). Fatty acid profiles are also influenced by animal health, physiology and energy balance. Therefore, fatty acid profiling could potentially serve as an early indicator of these and related traits for use in cow and herd management.

This paper reports on novel methodology to model variation in the fatty acid profile. Development of appropriate models is important for more extensive genetic and phenotypic analysis.

MATERIALS AND METHOD

Animal data. Data recording, including spectral analysis, was performed weekly on morning, noon and evening milkings of approximately 240 cows of mixed age and calving date. Cows were based at the Dairy Cattle Research Centre at Crichton Royal Farm, Dumfries, Scotland. They belong to either the Langhill high-yielding line selected for maximum milk fat and protein production (select) or a control line representing the moving UK average for milk production (control).

The cows were fed on either a home-grown forage diet (home-grown) or on a bought-in by-product feed (by-product). Over summer, the animals on the home-grown forage diet were at grass during the day and overnight they were being offered a feed of appropriate home-grown ingredients to balance the high protein and relatively low neutral detergent fibre of the grass. The winter ration consisted of grass silage, maize silage, lucerne, red clover silage, field beans, crimped wheat and vitamins and minerals fed as a complete total mixed ratio (TMR).

The by-product diet was based on ingredients available following a primary production process and not normally used for human food. The ration consisted of straw, Vitagold, sugar beet pulp, biscuit meal, feed-grade breakfast cereal meal, soya bean meal, wheat distiller's dark grains, molasses, megalac (calcium soap-bound palm fatty acid distillate) plus vitamins and minerals.

Milk samples were analysed using a calibrated mid-infrared FOSS MilkoScan FT6000 spectrometer. Soyeurt *et al.* (2011) multivariate calibration equations were run on this data to quantify the fat and fatty acid composition of the milk. Notably, 4 milk samples from the Crichton dairy herd were used to add variability to the validation set of samples, and 102 milk samples from this herd were used in the cross-validation dataset to evaluate the efficacy of the calibration equations. The cross-validation demonstrated high predictive accuracy (cross-validation coefficient of determination, $R^2cv > 0.95$) for most of the SFAs, the main monounsaturated fatty acids (MUFA) and most of the main fatty acid groups, including SFA, MUFA, unsaturated, short chain, medium chain and long chain fatty acids.

From the fatty acid predictions (in g/dL of milk) the amount of the individual fatty acids and fatty acid groups as a percentage of total fatty acids was calculated. Fatty acid percentages were averaged across the morning, midday and evening milkings for each cow on each test day.

Cow information, daily milk yield (kg), fat yield (kg) and protein yield (kg), average weekly live-weight (kg) and body condition score were extracted from the routinely collected RobustMilk Langhill database for the test cattle.

Editing of data. Before statistical analysis, records were removed where all 3 milkings were not performed on a test day, where records were taken after the 45th week (310 days) of milking, where total daily milk-yield was less than 4L and where total fat content was less than 1.5g/dL of milk or greater than 9g/dL of milk. Records were eliminated for production traits and fatty acids if the value given was negative or outside 3 standard deviations of the mean.

Data analysis. The fatty acids and classes were analysed as repeated measures per cow by week of lactation using random regression models with legendre polynomials using the ASReml programme (Gilmour *et al.* 2006). Fixed effects included week of lactation, parity number, genetic group, feed group and all combinations of their interactions. Additionally, date-of-milking and date-of-milking-by-feed, animal age at milking and calving month were fitted in the model as (co)variance components. The best order polynomial was selected for the lactation cycle based on the log-likelihood ratio tests and significance of the curves at node points fitted for each trait, with the majority either fitting to order 4 or 5, plus intercept. The final models also contained the random effect of week of lactation (to the same order of polynomial, less the intercept) by individual animal.

Correlations were assessed between fatty acids and between fatty acids and related milk production traits using bivariate random regression, based on the models derived. The polynomial order of the random effect was limited to quadratic due to calculation constraints.

RESULTS AND DISCUSSION

Random regression models. Week of lactation, feed type, genetic line and date of milking had a significant effect on all of the fatty acids and fatty acid classes. Parity number had a significant effect on all fatty acids except the PUFA and trans fatty acids.

The effect of genetic line suggests that selective breeding for increased fat and protein yield could be altering the fatty acid profile of milk in an unfavourable direction. The select line cows had, on average, a 1.8% (95% C.I. 0.6-2.9) higher percentage of SFA in total fatty acids.

However, as expected, feed had a greater effect on FA profile than genetic line. There is, on average, 10.6% (95% C.I. 9.2-12.1) less SFAs as a percentage of total fatty acids in the cows fed the by-product diet. This feed is supplemented with calcium bound palm fatty acid distillate (Megalac®) which is high in both C16:0 and C18:1 fatty acids. Furthermore, feeding of supplemental fat inhibits *de novo* synthesis of the short to medium chain saturated fatty acids in the mammary glands (C4:0 to C16:0) so the percentage of C18:1 in the milk increases and the percentage of saturated fatty acids will, on balance, be lower. This is consistent with results reported previously for Megalac® (Fearon *et al*. 1994).

While there was no significant feed-by-line interaction for saturated fatty acids as a group, this affect was significant for most of the short and medium chain saturated fatty acids when analysed individually. This gene-by-environment interaction suggests the effect of diet on mammary gland synthesis differs between the control and high yield genetic lines.

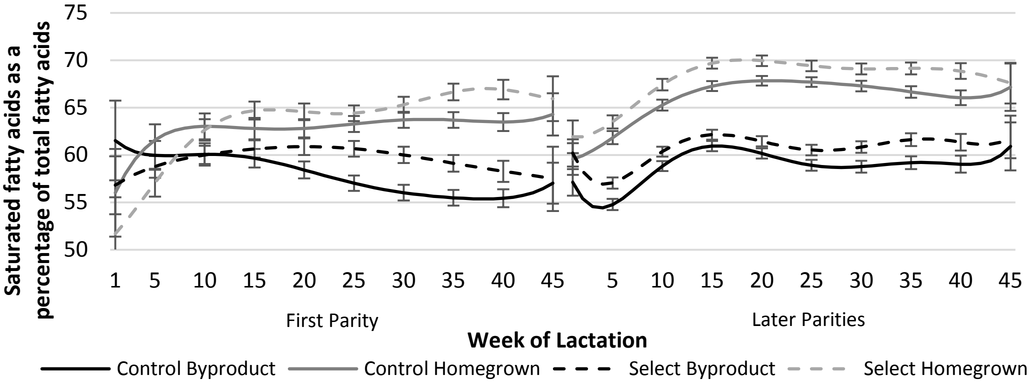

Figure 1: Predicted saturated fatty acid content as a percentage of total fatty acids, for first and later lactations across 45 weeks in milking.

Significant and nutritionally interesting variation is also seen for the main *trans* fatty acid in milk, where by the select line cows express significantly more C18:1 *trans* fat than the control line cows. *Trans* fatty acids, like SFA, have an adverse effect on cholesterol levels and cardiovascular disease. However, there was also a significantly greater proportion of beneficial omega-3 polyunsaturated fatty acids (PUFA) in the milk of the select line cows. Note that the accuracy for the omega-3 PUFA callibration equations falls below the statistical standard required for predictions (RPD 2.0, R^2cv 0.75). This may nevertheless warrant further investigation because, given that omega-3 fatty acids cannot be synthesised *de novo* by mammals, this suggests that the select line cows have greater uptake of the omega-3 fatty acids from the feed into their milk.

There was significant variation in profile over the course of lactation, especially evident in later parities where patterns are more settled (Figure 1). The low SFA percentages in early lactation corresponded to when cows were producing peak milk yield (Coffey *et al.* 2004) and therefore were typically in negative energy balance. To compensate for this imbalance, fat stores in adipose tissue, which are rich in C18:1*cis9* MUFA, are mobilised and released into the udder and make up a greater proportion of the milk fat (Stoop *et al*. 2009). Notably, the by-product control line gets as low as 54.4% (s.e. 0.60) which could have a significant effect on the functional properties of the milk e.g. in butter or cheese (Ashes *et al.* 1997).

Correlations. There were some significant correlations between fatty acids and milk yield (kg/day), fat yield (kg/day), fat content (g/dL of milk) and condition score across lactation cycle. Standard errors were typically high in early and late lactation, corresponding to low data counts.

Fat content and percentage of saturated fatty acids were generally positively correlated - between week 11 and 40 of lactation there was a significant positive correlation of between 0.45 and 0.56. This corresponds with the higher saturated fat content in the cows selected for increased fat yield.

CONCLUSIONS

Best-fit models have been developed for fatty acids and related traits based on results of calibration equations applied to MIR spectral data. The models highlight that selection for fat and protein yield had a detrimental effect on saturated fat content. Given the relationship between saturated fat intake and human health, further research into this area is warranted.

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