Association of milk MIR-derived body energy traits with fertility parameters in cows

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Introduction

The milk produced by dairy cows is a complex emulsion of proteins, carbohydrates, lipids, vitamins, enzymes, inorganic elements and, amongst others, water. Further to its composition, it is increasingly apparent that certain physiological processes may leave molecular signatures in the milk, some of which could be identified and used to inform cow management. The use of mid-infrared (MIR) spectroscopy to detect such molecular signatures has escalated over the last decade due to advantages of time, cost and labour over reference methods (de Marchi *et al.* 2014).

Building on the predictions of McParland *et al.* (2011), SRUC have developed and deployed prediction tools for energy balance and energy intake used, to date, to generate predictions for over 13.6 million animal test-date records from over 4,500 UK dairy herds as part of collaboration with National Milk Records (NMR). The literature shows us that the severity of negative energy balance in early lactation can strongly influence fertility in dairy cattle; Butler 1981 describes the delay in the commencement of normal luteal activity and ovulation when negative energy balance is severe; Berry *et al.* (2003) and Pryce *et al.* (2001) exemplify negative genetic correlations between body condition score (BCS, a proxy for energy balance) and interval to first service and positive relationships between BSC and pregnancy rate to first service. As such the objective of this study was to explore how MIR-derived energy predictions were associated with various fertility parameters and assess their use as early warning indicators of potential fertility issues.

Materials and methods

MIR-derived energy trait predictions for energy balance and energy intake were estimated as part of a routine pipeline using national data using prediction equations developed previously (Smith *et al.*, in draft) and now in use by NMR. For this analysis, we focussed on Holstein only dairy cows. Fertility records (obtained from those used in national evaluations, Wall et al, 2003) were then aligned to MIR predictions based on the same day in milk and for animals with complete milk records for lactations 1, 2 and 3. Fertility traits available were calving interval (CI), days in milk at first insemination (DFI), number of inseminations that resulted in a subsequent calving (NI) and correlated trait used in the evaluation of fertility - test milk near 110 days (TM110). This gave a dataset of predicted energy, milk and fertility traits on over 63,000 test-dates on 20,735 animals.

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Bivariate models were performed between each of energy balance and energy intake and three fertility evaluation traits (CI, DFI, NI) and TM110, then trivariate models between energy traits and a subset of fertility traits. Models were fitted with a pedigree with 4 generations which totalled 86,803 animals and consisted of:

$$y_{ijklm} = \mu + L_i + M_j + C_k + C_l^2 + a_m + p_m + e_{ijklm}$$

Where $y_{\_ijklm}$ is the observations for trait 1 and 2 (and 3 in the trivariate); μ is the trait mean, L_i is the fixed effect of the i^{th} lactation number, M_j is the fixed effect of the j^{th} month of calving, C_l, the fixed effect of the l^{th} age at current lactation, C_l^2 is the fixed effect of the l^{th} age at current lactation squared, a_o is the random additive genetic effect and p_o the random permanent environmental effect of the m^{th} individual cow and e_{ijklm} accounts for the error term. ASREML V3 (Gilmour *et al.* 2009) was used to undertake these analyses.

Results and discussion

The heritabilities for the two MIR-derived energy traits and the four fertility traits are given in Table 1, alongside their summary statistics from the dataset analysed. All heritabilities were significant and largely in line with other studies; Bastin *et al.* (2016) reported heritability for effective energy intake of 0.10, Wall *et al.* (2003) that for calving interval, days at first insemination and number of inseminations at 0.033, 0.035 and 0.02 respectively. Estimated heritability for MIR-derived energy balance range from 0.22 (Bastin *et al.* 2016), 0.10 (McParland *et al.* 2015); of which our estimated heritability is slightly below this at 0.06.

Despite being largely non-significant, the bivariate analyses revealed some correlations which are biologically meaningful and in line with previous estimates (Table 2). The negative genetic correlation (-0.19) between energy balance and calving interval is similar to that by Bastin *et al.* (2016) of -0.20 between energy balance and days open, Wall *et al.* (2013) of -0.14 between body condition score (BCS) and calving interval, but slightly lower than estimates of Pryce *et al.* (2001) of -0.36 between BCS and calving interval. In the trivariate analyses (with TM110) the negative phenotypic correlation between energy balance and calving interval was significant.

In *early* lactation the genetic correlation between energy balance and milk yield tends to be negative due to the energetic demands of milk production (Butler *et al.* 1989; Veerkamp *et al.* 1997). In this analyses, however, the genetic and phenotypic correlation between energy balance and milk yield taken at 110 days in milk (TM110) was positive in the bivariate model (0.11, 0.03) and significantly positive (0.35, 0.08) in the trivariate model with calving interval. This suggests that at this stage, following recovery from NEB, resumption of ovulation and increased feed intake may cover the energetic demands of milk production which otherwise were obtained from body reserves. Berry *et al.* (2003) suggests that selection for milk yield in later lactation is likely less detrimental to fertility due to reduce energy demands on the animals and hence greater energy balance.

Despite being small, the negative genetic correlation between energy balance and days to first insemination in the bivariate model and a significantly negative genetic correlation between them when in a trivariate analysis with TM110 is consistent with a negative relationship between BCS and interval to first service by Berry *et al.* (2003) (-0.47 to -0.30), BCS an days to first service by Pryce *et al.* (2001) (-0.54) and Wall *et al.* (2003) (-0.08). The negative genetic correlation between energy balance and number

Table 1. Summary statistics and estimated heritabilities.

Trait	Mean	Max	Min	h^2 *	S.E. *
Energy balance (EB)	5.95	86.04	-120.03	0.057	0.008
Energy intake (EI)	153.82	255.79	32.02	0.078	0.009
Calving interval (CI)	374.32	599.00	301.00	0.029	0.006
Test milk near 110 (TM110)	34.51	84.40	3.00	0.144	0.015
Days at first insemination (DFI)	65.96	466.00	20.00	0.029	0.006
Number Inseminations till conception (NI)	2.02	13.00	1.00	0.024	0.006

*Average from all bivariate analyses. All significant at $P < 0.05$

Table 2. Results of bivariate analyses between energy balance and fertility traits (upper table) and energy intake and fertility traits (lower table). 'r_p' is the phenotypic correlation and 'r_g' the genetic correlation. Significant results highlighted in bold.

Bivariate	r_p	S.E.	r_g	S.E.
EB and CI	0.006	0.005	-0.197	0.125
EB and TM110	**0.033**	0.005	0.114	0.087
EB and DFI	0.004	0.005	-0.01	0.121
EB and NI	0.007	0.005	-0.156	0.134
Bivariate	**r_p**	**S.E.**	**r_g**	**S.E.**
EI and CI	**0.038**	0.005	0.072	0.118
EI and TM110	**0.085**	0.005	0.137	0.080
EI and DFI	**0.022**	0.005	0.151	0.109
EI and NI	**0.022**	0.005	0.070	0.124

Table 3. Results of trivariate analyses between energy balance and a selection of fertility traits. Traits listed as tr1, tr2 and tr3 in order they appear in the first column. rp is the phenotypic correlation and ra the genetic correlation. Significant results highlighted in bold.

Trivariate	r_p_tr1tr2	r_p_tr1tr3	r_p_tr2tr3	r_a_tr1tr2	r_a_tr1tr3	r_a_tr2tr3
EB, CI, TM110	**-0.018**	**0.082**	**0.062**	-0.135	**0.351**	**0.164**
EB, CI, DFI	0.005	0.003	**0.417**	-0.184	-0.009	**0.709**
EB,TM110, DFI	**0.033**	**0.004**	**0.046**	**0.121**	**-0.020**	**0.403**
EI, CI, TM110	**0.0376**	**0.0852**	**0.0587**	0.0714	0.141	**0.2995**
EI, CI, DFI	**0.0363**	**0.0216**	**0.4171**	0.0791	0.1597	**0.719**

of inseminations till subsequent conception (-0.16 in bivariate) was in line with estimated correlations of 0.34 to -0.17 between BCS and number of services by Berry *et al.* (2003).

The phenotypic correlation between energy intake and each of the four fertility parameters was significant and positive (as in trivariate for EI with CI, TM110 and DFS) the largest of which was between energy intake and weight of milk, consistent with Veerkamp *et al.* (1997 and 1999). The positive phenotypic correlations between energy intake and CI, DFI and NI respectively were all extremely small (< 0.04). Bastin *et al.* (2016) estimated a genetic correlation between energy intake (from 5 days in milk) and days open at -0.28 whereas results here were obtained from around 110 days in milk and are therefore more consistent with the results of Bastin *et al.*, (2013) whereby the genetic correlation between effective energy intake and days open in mid-lactation was 0. It may be that as the demand for feed intake declines throughout lactation and that excessive feeding and over-conditioning is also detrimental to calving interval length.

The ability of the MIR spectra to relay information on energy balance is likely due to the changing molecular composition of key fats in milk as a result of mobilisation of body tissue in times of energy deficit (Bastin *et al.* 2016); a state which is well reported to affect fertility. Exploration of such correlations has been hindered in the past due to the cost and labour required to generation energy balance estimates; however the large resource of MIR-derived energy traits routinely obtained has made such exploration very feasible and initial studies such as these help indicate where to run larger models with more power. Such initial multivariate analyses have also highlighted the conclusion of others (Berry *et al.* 2003, Veerkamp *et al.* 1999) that the stage of lactation is also very important when understanding the direction of correlations between traits and as such should be considered when determining the appropriate combination of traits to select on. In this study using estimates of energy and fertility traits at approximately 110 days in milk, multivariate analyses showed positive correlations of energy traits with TM110 but antagonistic genetic correlations between energy balance and CI, DFS and NI, highlighting the potential use of MIR-derived traits as indicators of traits collected as part of fertility records. We aim to further explore correlations between the energy traits across lactation and fertility and wider functional traits as data size and structure allows. As the MIR-derived energy traits tend to have higher heritabilities than the fertility traits selection on these as indicator may be more effective as long as it is performed at a time which is least detrimental to fertility.

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