Short communication: Effect of preadjusting test-day yields for stage of pregnancy on variance component estimation in Canadian Ayrshires

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ABSTRACT

Preadjustment of phenotypic records is an alternative to accounting for the effect of pregnancy within the genetic evaluation model. Variance components used in the Canadian Test-Day Model may need to be re-estimated after preadjusting for pregnancy. The objective of this study was to assess the effect of preadjusting test-day yields on variance components and estimated breeding values using a random regression test-day model in a random sample of Ayrshire cows. A random sample of 981 Canadian Ayrshire cows from 18 complete herds (average of 54.5 cows/herd) was analyzed. Two data sets were created using the same animals, one with unadjusted milk, fat, and protein yields, and one data set with test-day records adjusted for pregnancy effects. Pregnancy effect estimates from a previous study were used for additive preadjustment of records. Variance components were estimated using both data sets. Results were very similar between the 2 data sets for all estimated genetic parameters (heritabilities, genetic, and permanent environmental correlations). The relative squared differences were very small: 0.05% for heritabilities, 0.20% for genetic correlations, and 0.18% for permanent environmental correlations. Furthermore, paired Student's *t*-tests showed that the differences between the genetic parameters of data sets adjusted and unadjusted for pregnancy effect were not significantly different from 0. Results from this study show that preadjusting data for pregnancy did not yield changes in covariance component estimates, thus suggesting that preadjusting test-day records could be a feasible solution to account for pregnancy in the Canadian Test-Day Model without changing the current model. Estimated breeding values (EBV) were calculated for both data sets to observe the impact of preadjusting for pregnancy. Overall, the largest changes in EBV seen when preadjusting for pregnancy (compared with unadjusted records) occurred for nonpregnant elite

cows, whose EBV declined. Preadjusting for pregnancy before genetic evaluations improves the estimation of breeding values by adding the negative impact of pregnancy back onto pregnant cow test-day records, causing an increase in their production EBV.

Key words: test-day model, pregnancy status, stage of pregnancy, preadjustment

Many countries preadjust phenotypes for environmental effects before using the data in genetic evaluation of dairy cattle (Interbull, 2008). Generally, all effects should be accounted for in the genetic evaluation model, but if preadjustment methods are chosen, they should be well justified (Interbull, 2001). One advantage of preadjustment is the reduced computation time for running genetic evaluations (Muir et al., 2007; Leclerc et al., 2008). Also, less memory is required to run genetic evaluations because there are fewer unknowns (Leclerc et al., 2008). Muir et al. (2007) noted that shorter computing time and “difficulties in applying methodology and explaining results” justify preadjustment. However, preadjustment factors should be updated regularly (Wilmink, 1987). The Interbull guidelines (Interbull, 2001) suggest updating preadjustment factors at least once per generation. Leclerc et al. (2008) suggest updating preadjustment factors every year. Furthermore, Mark (2004) states that a genetic evaluation is no longer a best linear unbiased prediction when phenotypes are preadjusted, so preadjustment should be used in moderation. If the preadjustment factors are not independent of genotype, then some genetic effects will be subtracted from phenotypic observations (Taylor et al., 1993), which would result in inaccurate genetic evaluations.

Determination of pregnancy is not always accurate (Bohmanova et al., 2008). For cows with lactation still in progress at the time of genetic evaluation, assumptions may have to be made about their pregnancy status if pregnancy effect is included in the model itself. This means that estimation of pregnancy effects has the potential to be very error-prone, especially if pregnancy status assumptions need to be made on many animals. However, if pregnancy effects can be estimated

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before genetic evaluation using data with limited assumptions, then more accurate pregnancy effects can be determined, making preadjustment for pregnancy effect potentially more accurate than including pregnancy in the genetic evaluation model.

Some countries use preadjustment to adjust for heterogeneous variances (Interbull, 2008). An example of heterogeneous variance is when phenotypic variances differ across time, herd, region, or parity (Wiggans and VanRaden, 1991). Canada and the United States preadjust phenotypic data for heterogeneity of variances (Muir et al., 2007). Italy has preadjusted for heterogeneous variance in their genetic evaluation model since 1993, and chose preadjustment because it was less time consuming (Muir et al., 2007). A survey by Mark (2004) found that 20 of the 31 countries questioned preadjusted for heterogeneous variances before genetic evaluations.

Interbull (2008) indicated that several countries use preadjustment for various fixed-effects factors. The Walloon region of Belgium performs additive preadjustment for age within lactation-stage-breed effects on production traits. France performs multiplicative preadjustments for the effect of parity on production traits. Italy and the United States also preadjust production trait records for various environmental effects (including age and month of calving).

Pregnancy is an environmental effect that negatively influences milk production in dairy cattle. The effect of pregnancy results from an increased regression of the mammary gland (Bachman et al., 1988; Coulon et al., 1995; Brotherstone et al., 2004; Akers, 2006) and the partitioning of nutrients toward fetal growth (Bell et al., 1995). As the fetus grows, the effect of pregnancy increases (Auran, 1974; Wiggans, 1980; Olori et al., 1997; Brotherstone et al., 2004). Recently, the assessment of the impact of pregnancy on milk production has been shown in Canada by Bohmanova et al. (2008) in Holsteins and by Loker et al. (2009) in other Canadian dairy breeds. Milk and fat yields began to decline because of pregnancy after about 4 mo of gestation, and protein began to decline after about 2 mo. Loker et al. (2009) proposed 4 different models to account for pregnancy that included: a) days open; b) days pregnant; c) days open and stage of pregnancy interaction; and d) stage of pregnancy classes. The most feasible model was the stage-of-pregnancy model. Once the proper model is chosen, pregnancy effect can be accounted for in the Canadian Test-Day Model (CTDM), either by preadjusting test-day records for milk, fat, and protein before breeding value estimation, or by including the effect of pregnancy in the genetic evaluation model. The objective of this study was to use adjustment factors from the stage-of-pregnancy model to assess the effect

of preadjusting test-day yields on variance components and EBV using a random regression test-day model applied to a random sample of Ayrshire cows.

Data were provided by the Canadian Dairy Network and were from test-day record extraction for the August 2007 genetic evaluation. Adjustment factors were estimated by Loker et al. (2009) for milk, fat, and protein yields using stage of pregnancy (classes of days pregnant with 30 d for each class, the last class including all test-day records after 210 d of pregnancy). Specifically, the model used was a fixed-effects model that included stage of pregnancy, herd test-day effect as a classification variable, and the lactation curve was modeled with a fourth-order Legendre polynomial on DIM within age-parity-season of calving class. Separate models were fitted to parities 1, 2, and 3. For this study, additive adjustment factors were assumed to be zero before the fourth stage of pregnancy for milk and fat yield. Adjustment factors were assumed to be zero for the first stage of pregnancy for protein yield. The additive adjustment factors are listed in Table 1. A fourth order polynomial regression was then fitted for each parity and trait to smooth the preadjustments at each test-day record. All test-day records for milk, fat, and protein yields were preadjusted using the estimated polynomial functions, based on the day of pregnancy of each test-day record.

After preadjusting the full data set, records from DIM <5 and >305 d were eliminated. The SCC were log-transformed to SCS. Only records from the first 3 parities that had data for all production traits on a given test-day were kept. Within cow, if parity 3 was present, parities 1 and 2 were also present, and if parity 2 was present, parity 1 was also present. Herds were required to have a minimum of 50 cows in the data set to be included in the analysis. To estimate variance components, a random sample of complete herds was extracted from the edited data set. A total of 981 cows from 18 herds (average of 54.5 cows/herd) with 14,738 test-day records were randomly selected. The total number of animals (cows with own records and pedigree) included 3,528 animals. Covariance components were estimated by Bayesian methods with Gibbs sampling using the 4-trait multiple-lactation random regression test-day model described by Miglior et al. (2007):

$$y = Hh + Xb + Za + Wp + e,$$

where y is a vector of milk, fat, protein yield, and SCS observations for the first 3 lactations, either additively adjusted (yield traits) or left unadjusted for pregnancy effect (depending on the data set, which is explained below); h is a vector of fixed herd-test-day effects;

Table 1. Adjustment factors¹ by parity and trait for each stage of gestation

Classes of days pregnant (d)	Milk yield (kg)			Fat yield (kg)			Protein yield (kg)		
	Parity 1	Parity 2	Parity 3	Parity 1	Parity 2	Parity 3	Parity 1	Parity 2	Parity 3
0	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
1–30	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
31–60	0.00	0.00	0.00	0.000	0.000	0.000	0.009	0.011	0.014
61–90	0.00	0.00	0.00	0.000	0.000	0.000	0.016	0.019	0.022
91–120	0.32	0.16	0.16	0.012	0.011	0.008	0.027	0.030	0.030
121–150	0.65	0.71	0.69	0.020	0.025	0.024	0.036	0.041	0.042
151–180	1.43	1.85	1.86	0.041	0.059	0.057	0.055	0.067	0.068
181–210	2.64	3.28	3.35	0.080	0.104	0.104	0.091	0.108	0.110
>210	3.68	4.00	3.87	0.114	0.128	0.119	0.127	0.135	0.130

¹Raw additive adjustment factors by stage of pregnancy, provided by Loker et al. (2009).

$\mathbf{b}$ is a vector of fixed regression coefficients for age-parity-season classes; $\mathbf{a}$ is a vector of random regression coefficients for genetic effects; $\mathbf{p}$ is a vector of random regression coefficients for permanent environmental (PE) effects; and $\mathbf{e}$ is a vector of residual effects; $\mathbf{H}$, $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ are incidence matrices relating observations to their respective effects. The distribution of random effects is assumed to be

$$\begin{pmatrix} \mathbf{a} \\ \mathbf{p} \\ \mathbf{e} \end{pmatrix} \sim N(0, \mathbf{V}),$$

with variances:

$$\mathbf{V} = \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & 0 & 0 \\ 0 & \mathbf{I} \otimes \mathbf{P} & 0 \\ 0 & 0 & \sum^+ \mathbf{R}^{p,s} \end{bmatrix},$$

where $\mathbf{G}$ and $\mathbf{P}$ are covariance matrices for genetic and PE regression coefficients, respectively, $\mathbf{A}$ is the additive relationship matrix and $\mathbf{R}^{p,s}$ is the matrix of residual covariances among traits with elements depending on parity (p) and the interval of DIM (s).

Custom-written software that is routinely used at the Canadian Dairy Network was used for the analysis. Two data sets were created with the same animals and records, one with unadjusted milk, fat, and protein yields, and one data set with adjusted test-day records. Somatic cell score was left unadjusted in both data sets. Variance components were estimated on both data sets. Posterior means and standard deviation of (co)variance components were estimated using 40,000 samples after a burn-in of 10,000 samples. Daily heritability was defined as a ratio of genetic variance to the sum of genetic, PE, and residual variances for each day in milk from 5 to 305 d, and averaged across the entire lacta-

tion for each of the first 3 lactations. Genetic and PE correlations between production traits were calculated using (co)variances of the first regression coefficients as described by Wood et al. (2003). A paired Student's t -test, with the null hypothesis that the mean difference between genetic parameters was 0, was performed between the genetic parameter estimates of the adjusted and unadjusted data sets.

The convergence of Gibbs samples was monitored by visual inspection of the plot of realizations for selected covariance components. The effective sample size ranged from 13 to 89 (genetic variances), from 29 to 217 (PE variances), and from 278 to 2,069 (residual variances).

Using the same random regression test-day model, breeding values were estimated for bulls and cows using the 2 complete data sets (one data set unadjusted and one pregnancy preadjusted). This was done to monitor the change in animals' EBV when preadjusting for pregnancy effect versus not accounting for pregnancy.

Genetic and PE correlations and daily heritabilities are shown in Tables 2 and 3 for the unadjusted data set and preadjusted data set, respectively. Posterior standard deviations of all estimates (heritability, genetic, and PE correlations) ranged from 0.001 to 0.013, both for adjusted and nonadjusted analyses. Results were very similar between the 2 data sets for all estimated parameters (heritabilities, genetic, and PE correlations). Table 4 shows the differences between the preadjusted and unadjusted estimates. The relative squared differences between the parameters estimated with unadjusted and adjusted were very small: 0.05% for heritabilities, 0.20% for genetic correlations, and 0.18% for PE correlations. The paired Student's t -tests between the adjusted data set and unadjusted data set genetic parameter estimates resulted in small t -values of -0.21 ($P = 0.83$), -0.11 ($P = 0.92$), and -0.51 ($P = 0.61$) for the differences in genetic correlations, heritabilities, and PE correlations, respectively. This indicates that

Table 2. Average daily heritabilities on the diagonal, genetic correlations below the diagonal, and permanent environment correlations above the diagonal (unadjusted data set)

Item	First parity				Second parity				Third parity			
	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS
First parity												
Milk (kg)	0.41	0.91	0.96	-0.27	0.59	0.50	0.57	-0.08	0.46	0.34	0.42	0.05
Fat (kg)	0.84	0.35	0.92	-0.29	0.51	0.54	0.53	-0.12	0.43	0.42	0.43	-0.01
Protein (kg)	0.93	0.86	0.39	-0.24	0.60	0.55	0.63	-0.09	0.48	0.40	0.49	0.02
SCS	-0.04	-0.06	-0.04	0.31	-0.17	-0.17	-0.16	0.44	-0.19	-0.19	-0.18	0.38
Second parity												
Milk (kg)	0.73	0.60	0.66	-0.09	0.45	0.92	0.96	-0.33	0.57	0.50	0.55	-0.11
Fat (kg)	0.58	0.70	0.58	-0.07	0.87	0.40	0.94	-0.38	0.55	0.59	0.57	-0.16
Protein (kg)	0.66	0.63	0.70	-0.06	0.93	0.89	0.43	-0.33	0.57	0.53	0.59	-0.13
SCS	-0.10	-0.09	-0.06	0.49	-0.34	-0.33	-0.32	0.40	-0.20	-0.20	-0.18	0.54
Third parity												
Milk (kg)	0.56	0.46	0.51	-0.02	0.70	0.60	0.65	-0.18	0.43	0.92	0.96	-0.38
Fat (kg)	0.39	0.53	0.41	-0.04	0.57	0.70	0.60	-0.18	0.86	0.38	0.93	-0.43
Protein (kg)	0.49	0.47	0.55	-0.03	0.62	0.59	0.68	-0.17	0.93	0.87	0.42	-0.35
SCS	-0.03	-0.08	-0.03	0.28	-0.26	-0.30	-0.27	0.55	-0.28	-0.37	-0.27	0.39

the differences between the genetic parameters of data sets adjusted and unadjusted for pregnancy effect were not significantly different from 0. Assuming similar results for the preadjustment of phenotypes for other Canadian breeds, variance components may not need to be re-estimated before using records preadjusted for pregnancy in the CTDM.

Preadjusting for pregnancy had little effect on bull EBV: the EBV of 99% of bulls did not change by more than 50 kg for milk yield (EBV SD = 630 kg), and 2 kg for fat (EBV SD = 24 kg) and protein yield (EBV SD = 20 kg). However, larger changes occurred for lactation persistency EBV, for which the relative EBV of 3.5% of bulls changed by 2 points (EBV SD = 5 points). When an adjustment is not made for pregnancy, pregnant

cows would appear to be genetically less persistent. It is therefore logical to see a change in lactation persistency EBV after preadjusting records for pregnancy effect. Overall, larger changes were observed for cow EBV; in particular, a significant decrease of cow indexes for top elite cows with large days open that have been flushed extensively for embryo transfer. As expected, cows that did not have large days open showed an increase in EBV for production traits.

Leclerc et al. (2008) compared a 2-step procedure (preadjustment, then genetic evaluation) with a 1-step genetic evaluation including all factors in the model. Preadjustment was carried out for effects related to the shape of the lactation curve, including gestation effect using 4-knot regression splines with knots at 100, 150,

Table 3. Average daily heritabilities on the diagonal, genetic correlations below the diagonal, and permanent environment correlations above the diagonal (preadjusted data set)

Item	First parity				Second parity				Third parity			
	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS
First parity												
Milk (kg)	0.42	0.91	0.95	-0.29	0.54	0.46	0.53	-0.09	0.45	0.35	0.43	0.03
Fat (kg)	0.83	0.36	0.92	-0.31	0.45	0.49	0.48	-0.12	0.42	0.43	0.42	-0.02
Protein (kg)	0.92	0.87	0.39	-0.27	0.54	0.51	0.59	-0.10	0.47	0.42	0.49	-0.01
SCS	-0.03	-0.05	-0.04	0.30	-0.16	-0.16	-0.15	0.43	-0.18	-0.19	-0.18	0.36
Second parity												
Milk (kg)	0.74	0.59	0.66	-0.09	0.44	0.92	0.96	-0.37	0.55	0.49	0.53	-0.14
Fat (kg)	0.59	0.69	0.58	-0.08	0.86	0.39	0.94	-0.40	0.53	0.57	0.55	-0.16
Protein (kg)	0.67	0.63	0.70	-0.08	0.93	0.89	0.41	-0.36	0.55	0.52	0.58	-0.14
SCS	-0.10	-0.10	-0.06	0.50	-0.29	-0.31	-0.29	0.40	-0.18	-0.18	-0.16	0.52
Third parity												
Milk (kg)	0.56	0.46	0.51	-0.06	0.68	0.59	0.63	-0.20	0.43	0.92	0.96	-0.37
Fat (kg)	0.38	0.53	0.40	-0.09	0.53	0.68	0.56	-0.23	0.84	0.38	0.93	-0.41
Protein (kg)	0.47	0.46	0.53	-0.08	0.60	0.59	0.66	-0.21	0.92	0.86	0.42	-0.33
SCS	-0.02	-0.10	-0.02	0.31	-0.20	-0.28	-0.23	0.56	-0.30	-0.40	-0.30	0.40

Table 4. Differences between estimated parameters from preadjusted data set with parameters estimated from unadjusted data set (average daily heritabilities on the diagonal, genetic correlations below the diagonal and permanent environment correlations above the diagonal)

Item	First parity				Second parity				Third parity			
	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS	Milk (kg)	Fat (kg)	Protein (kg)	SCS
First parity												
Milk (kg)	0.011	-0.004	-0.007	-0.022	-0.051	-0.041	-0.042	-0.011	-0.001	0.014	0.006	-0.018
Fat (kg)	-0.004	0.006	-0.004	-0.022	-0.057	-0.049	-0.050	0.005	-0.009	0.006	-0.005	-0.009
Protein (kg)	-0.001	0.005	0.003	-0.028	-0.057	-0.046	-0.043	-0.010	-0.002	0.017	0.007	-0.031
SCS	0.011	0.010	0.006	-0.007	0.005	0.006	0.009	-0.007	0.011	0.007	0.001	-0.016
Second parity												
Milk (kg)	0.013	-0.008	-0.005	0.003	-0.010	0.004	-0.003	-0.039	-0.020	-0.010	-0.014	-0.023
Fat (kg)	0.011	-0.001	0.002	-0.004	-0.005	-0.007	-0.001	-0.015	-0.020	-0.015	-0.015	-0.001
Protein (kg)	0.015	0.001	-0.002	-0.013	-0.003	0.002	-0.014	-0.024	-0.021	-0.010	-0.007	-0.011
SCS	0.006	-0.012	0.001	0.007	0.053	0.021	0.033	0.008	0.022	0.029	0.018	-0.019
Third parity												
Milk (kg)	0.004	0.006	-0.001	-0.045	-0.020	-0.008	-0.019	-0.024	-0.006	0.000	-0.007	0.016
Fat (kg)	-0.012	0.000	-0.015	-0.050	-0.043	-0.015	-0.036	-0.044	-0.012	-0.002	-0.002	0.024
Protein (kg)	-0.014	-0.004	-0.019	-0.056	-0.024	-0.006	-0.020	-0.038	-0.010	-0.007	-0.007	0.021
SCS	0.004	-0.012	0.009	0.031	0.057	0.018	0.045	0.009	-0.019	-0.032	-0.026	0.014

200, and 265 d pregnant. Solutions for random effects were similar between 1- and 2-step procedures, with correlations >0.99. The Interbull guidelines (Interbull, 2001) suggest that using preadjusted records for genetic evaluations should be well justified. Determination of pregnancy by Loker et al. (2009) was not always accurate. For cows with lactation still in progress, conception was assumed using date of last insemination, which was not necessarily correct. Sometimes, insemination records were not available, which would lead to further error when assuming dates of conception and pregnancy status. Bohmanova et al. (2008) separated cows into classes depending on the availability of insemination data. Because insemination date and calving date were not always known, pregnancy status was more accurate for some classes than for others. In the future, preadjustment for pregnancy should be performed using estimates for stage of pregnancy obtained after eliminating records that have erroneous pregnancy information. Conversely, if pregnancy effect was accounted for in the genetic evaluation model, there would be a large number of records with erroneous pregnancy data that would result in inaccurate corrections. Readers should note that there would still be animals for which pregnancy status is uncertain when performing genetic evaluation of records preadjusted for pregnancy. However, because adjustment factors would be accurately estimated based on data with more certain pregnancy status information, the effects of errors in determining pregnancy status for genetic evaluation will be limited compared with using all data to adjust for pregnancy.

Results from this study show that preadjusting data for pregnancy did not yield relevant changes in variance component estimates, thus suggesting that preadjusting test-day records could be a feasible solution to account

for pregnancy in the CTDM without changing the current model and programs. Re-estimation of pregnancy effects is required after elimination of records for cows whose pregnancy status is not certain. Accounting for pregnancy effect removed some bias from breeding value estimations. Evidence of this is seen in the results of this study, which showed that elite cows used for embryo transfer (cows that had longer days open) experienced a decline in EBV and other cows with shorter days open experienced an increase in EBV when the data set was adjusted for pregnancy. This is expected, because these elite cows lactate without experiencing the negative environmental effect of pregnancy within the first 305 d of lactation. Meanwhile, the negative effect of pregnancy is added back onto records of other, nonelite cows. Therefore, elite cows flushed for embryo transfer do not appear as genetically superior to other cows compared with when pregnancy is not accounted for. Aside from this difference, preadjusting for pregnancy did not have much of an effect on Ayrshire EBV. Therefore, preadjustment of test-day records for the effect of pregnancy before genetic evaluations are performed may improve the estimation of breeding values for dairy cattle, and may be applicable for use in the CTDM without having to change the current model and programs. Similar studies should be performed on other breeds, using preadjustment factors calculated after the removal of data for animals with uncertain pregnancy status.

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