GENETICS AND BREEDING

Genetic Analysis of Fertility in Dairy Cattle Using Negative Binomial Mixed Models

ROBERT J. TEMPELMAN* and DANIEL GIANOLA†
*Department of Animal Science, Michigan State University, East Lansing 48824-1225
†Department of Animal Sciences, University of Wisconsin, Madison 53706-1284

ABSTRACT

Two negative binomial mixed models with different dispersion specifications were compared for analysis of dairy reproduction count data. The first model was developed previously and had heterogeneous overdispersion in an associated logarithmic scale, assigning greater uncertainty to observations with smaller conditional expectations. The second model postulated homogeneous overdispersion across all data. A simulation study was used to compare marginal modal estimates of additive genetic variance, based on these two negative binomial models, with analogous estimates computed by an overdispersed Poisson mixed model. Estimators from the second negative binomial and overdispersed Poisson models had better frequentist properties than did those from the first negative binomial model. Nevertheless, application to a data set of number of artificial inseminations until conception in Holstein heifers suggested a slightly better fit of the first negative binomial model. A marginal likelihood ratio test indicated that the additive genetic variance was significant. Cross-validation analyses suggested that the two negative binomial mixed models had slightly better predictive ability than a linear mixed model.

(Key words: negative binomial, reproduction, generalized linear mixed model, variance component estimation)

INTRODUCTION

Genetic aspects of reproductive performance of dairy cattle have been receiving increased research attention, which may, in part, be because up to 28% of all involuntary dairy cow disposals may involve reproductive failure (11). In Sweden and in Finland, total net merit indices include number of AI until conception (NSC) (4, 15, 21). The NSC may be a better measure of fertility than calving interval, as the latter may also reflect milk producing ability (15). That is, estimates of genetic variances for calving interval may be affected by the tendency for high producing animals to be bred later postpartum (4, 11).

Heifer fertility has not been studied extensively, although it can be assessed early in the lifetime of the animal. Analyses of NSC in dairy heifers found heritability estimates of less than 0.06 (10, 21, 26). In these studies, effects of service sire and of technician were ignored, despite evidence suggesting that they were potentially important sources of variation (11). Furthermore, all parameters were estimated under a linear mixed model. In general, analysis of counts with models based on the Poisson distribution tends to be more appropriate.

Foulley et al. (6) introduced a Poisson mixed model for the analysis of count data in animal breeding. The Poisson distribution, however, constrains the conditional variance to be equal to the conditional mean. Tempelman and Gianola (32) developed a negative binomial mixed model to allow for overdispersion (i.e., for the variance being greater than the mean). This model (NB1) resulted from assuming that the Poisson parameters across all observations were independently distributed gamma variables in a hierarchical model. A simulation study (32) found that the NB1 animal model led to estimates of genetic variance having less empirical bias and mean squared error than did those from a Poisson animal model. However, this model was less accurate than an overdispersed Poisson animal model (PAM) in which the overdispersion (log normally distributed residuals) was fit as an additional random effect.
The gamma distributions on the Poisson parameters can be specified such that the resulting negative binomial mixed model (NB2) has a different dispersion structure from that of NB1. Our objectives were to develop this alternative model in a genetic context, to compare frequentist properties of variance component estimates under NB1 and NB2 in an animal breeding simulation study, and to analyze NSC in Holstein heifers with the two models, drawing comparisons with inferences from a linear mixed model analysis.

METHODOLOGY

Model Development

The NB2 model is similar to that of Albert (1) but developed here in an animal breeding context. As done by Tempelman and Gianola (32), we built a multi-stage hierarchical model. The first stage or sampling distribution of the count response \(Y_i\) for animal \(i\) is assumed to be Poisson with parameter \(\lambda_i\), so that

\[
Pr(Y = y_i | \lambda_i) = \frac{e^{-\lambda_i} \lambda_i^{y_i}}{y_i!}, \quad 0 < \lambda_i < \infty, \quad i = 1,2,...,n. \quad [1]
\]

Observations for all \(n\) animals are assumed to be conditionally independent. In the second stage, the Poisson parameters \(\lambda = \{\lambda_i\}\) are taken to be identically and independently distributed gamma variables with densities given by

\[
\pi(\lambda_i | \xi_i, \alpha) = \frac{\alpha^\xi_i}{\Gamma(\alpha)} \lambda_i^{\xi_i-1} \exp\left(-\frac{\alpha}{\xi_i} \lambda_i\right), \quad i = 1,2,...,n. \quad [2]
\]

That is, the conditional distribution of \(\lambda_i\) is gamma \(\left(\alpha, \frac{\alpha}{\xi_i}\right)\) with mean \(= E(\lambda_i | \xi_i, \alpha) = \xi_i\) and variance \(\text{var}(\lambda_i | \xi_i, \alpha) = \frac{\xi_i^2}{\alpha}\). Note that this conditional variance differs from that of NB1 in Equation [2b] of Tempelman and Gianola (32). Nevertheless, as with NB1, the limit \(\alpha \rightarrow \infty\) in Equation [2] defines a pure Poisson model with no overdispersion.

In Equation [2], let \(\lambda_i = \xi_i \epsilon_i\). Hence, \(\epsilon_i \sim \text{gamma}(\alpha, \alpha), \quad i = 1,2,...,n\), are independently and identically distributed with mean \(= E(\epsilon_i) = 1\) and variance \(= \text{var}(\epsilon_i) = \frac{1}{\alpha}\). Note that \(\log(\lambda_i) = \log(\xi_i) + \log(\epsilon_i)\) where \(\epsilon_i = \log(\epsilon_i) - \text{log-gamma}(\alpha, \alpha)\) is an extra-Poisson residual on a logarithmic scale. From the properties of a log-gamma distribution, the expectation and variance of residual \(i\) \((i = 1,2,...,n)\) are

\[
E(\epsilon_i) = \log(\alpha) - \psi(\alpha) \quad [3a]
\]

and

\[
\text{var}(\epsilon_i) = \psi^{(1)}(\alpha), \quad [3b]
\]

respectively [see also (5)]. Here, \(\psi(\alpha) = \frac{\partial \Gamma(\alpha)}{\partial \alpha}\), and \(\psi^{(1)}(\alpha) = \frac{\partial^2 \Gamma(\alpha)}{\partial \alpha^2}\) where \(\Gamma(.)\) denotes the gamma function. As \(\alpha \rightarrow \infty\), Equation [3a] \(\rightarrow 0\), and Equation [3b] \(\rightarrow \frac{1}{\alpha}\). Note that the extra-Poisson residual variance is homogeneous across all observations on the logarithmic scale, which differs from NB1 where this variance is dependent on the conditional mean \(\xi_i\) for animal \(i\) (32). That is, with NB1, the residual variance \(\psi^{(1)}(\alpha \xi_i)\) increased with decreasing \(\xi_i\) such that greater uncertainty was assigned to data with lower conditional expectations.

We assign

\[
\log(\xi_i) = \mathbf{x}_i \beta + \mathbf{z}_i \mathbf{u}. \quad [4]
\]

Here, \(\beta\) represents the \(p \times 1\) vector of fixed effects and \(\mathbf{u}\) the \(q \times 1\) vector of random effects. The row incidence vectors \(\mathbf{x}_i\) and \(\mathbf{z}_i\) connect the levels of \(\beta\) and \(\mathbf{u}\), respectively, to \(\eta_i, \xi_i, \lambda_i\), and, eventually, \(y_i\). This linked relationship between the conditional mean \(\xi_i\) of \(y_i\) and the linear structure \(\mathbf{x}_i \beta + \mathbf{z}_i \mathbf{u}\) of the model is typical of generalized linear mixed models (29).

The third stage of the hierarchical model defines the prior distributions of the two classes of location parameters:

\[
\pi(\mathbf{u} | \sigma_u^2) \propto \exp\left(-\frac{\mathbf{u} \mathbf{G}^{-1} \mathbf{u}}{2}\right) \quad [5a]
\]

and

\[
\pi(\beta) \propto 1. \quad [5b]
\]

If several, say \(c\), random factors are specified in \(\mathbf{u}\), then \(\mathbf{u} = [\mathbf{u}_1, \mathbf{u}_2, ..., \mathbf{u}_c]\) with \(\mathbf{u}_j\) being random factor \(j\). If these \(c\) factors are independent, \(\mathbf{G} = \text{var} (\mathbf{u})\) is a
function of c variance components in a vector $s = \{s_{uj}\}$ and of c known correlation (relationship) matrices. As an example, if $u_j$ constitutes the random additive genetic effects, then $\text{var}(u_j) = A \sigma^2_a$, where $A$ = known matrix of additive genetic relationships and $\sigma^2_a = \sigma^2_s$ is additive genetic variance. Covariances between effects of different random factors can be further modeled such as the covariance between additive and maternal genetic effects.

Finally, the fourth stage of NB2 specifies the prior distributions of the dispersion parameters:

$$a^2_{uj} \sim \pi(a^2_{uj}), \quad j = 1,2,\ldots,c$$ \hspace{1cm} [6a]

and

$$\alpha \sim \pi(\alpha).$$ \hspace{1cm} [6b]

One proper, but relatively vague, prior for $\alpha$ was suggested by Albert (1):

$$\pi(\alpha) \propto \frac{z_o}{(z_o + \alpha)^2}$$ \hspace{1cm} [7]

where $z_o =$ prior median of $\alpha$. This prior in Equation [7] is a generalization of a noninformative, yet proper, prior considered for the hierarchical Poisson-gamma model by Leonard and Novick (14), who used $z_o = 1$.

As indicated in Equation [3b], the variance of the extra-Poisson residuals in the logarithmic scale is homogeneous in NB2. This specification implies that heritability on the logarithmic scale would be

$$h^2 = \frac{c \sigma^2_a}{\sum_{j=1}^{c} \sigma^2_{uj} + \sigma^2_e}$$ \hspace{1cm} [8]

where $\sigma^2_e = \psi(1)(\alpha)$. This situation contrasts with the heritability parameter in NB1 (32), which is heterogeneous across all observations; that is,

$$h_i^2 = \frac{c \sigma^2_a}{\sum_{j=1}^{c} \sigma^2_{uj} + \sigma^2_{e_i}}$$ \hspace{1cm} [9]

where $\sigma^2_{e_i} = \psi(1)(\alpha \xi_i)$. Hence, $h_i^2$ is the heritability specific to record $i$ and cannot be construed as a population parameter. The heritabilities reported by Matos et al. (16) were based on NB1 yet were computed erroneously using the homogeneous specification for NB2 in Equation [8].

Yet, both Equations [8] and [9] do not give the amount of genetic variation relative to the total phenotypic variation because the residual variation from the Poisson sampling assumed in Equation [1] is ignored. Foulley et al. (6) suggested a Taylor series approximation that involves augmenting the denominator in both Equations [8] and [9] by the inverse of the Poisson parameter. As the value of this parameter is heterogeneous across observations, the use of an average Poisson parameter $\lambda = \frac{1}{n} \sum_{i=1}^{n} \lambda_i$ was suggested by Foulley et al. (6) as a further approximation. For example, to reflect both Poisson and extra-Poisson sources of residual variation, the heritability in NB2 would become

$$h^2 = \frac{c \sigma^2_a}{\sum_{j=1}^{c} \sigma^2_{uj} + \psi(1)(\alpha) + \frac{1}{\hat{\lambda}}}$$ \hspace{1cm} [10]

It should be noted that $\hat{\lambda}$ has meaning as a population parameter only when $\lambda_i$, $i = 1,2,\ldots,n$, are drawn from the same population, which is seldom the case as indicated by Equation [4].

The joint posterior density of all parameters can be written as being proportional to the product of [1], [2], [5a], [5b], [6a], and [6b]; that is:

$$\pi(\lambda,\beta,u,s,\alpha | y) \propto \left( \prod_{i=1}^{n} \frac{\lambda_i^{y_i} \exp(-\lambda_i)}{\Gamma(\lambda_i)} \right) \lambda_i^{\alpha-1} \exp\left(-\frac{\alpha}{\xi_i} \lambda_i\right)$$

$$\times |G|^{-\frac{n}{2}} \exp\left(-\frac{u'G^{-1}u}{2} \sum_{i=1}^{c} \sigma^2_{uj}\right) \pi(\alpha).$$ \hspace{1cm} [11]

As for NB1 (32), the conditional posterior density of $\lambda_i$ given $\xi_i$ and $\alpha$ in NB2 is instructive. From Equation [11], it follows that

$$\pi(\lambda_i | \alpha,\beta,u,y) \propto \prod_{i=1}^{n} \lambda_i^{y_i+\alpha-1} \exp\left(-\lambda_i \left(1 + \frac{\alpha}{\xi_i}\right)\right).$$ \hspace{1cm} [12]

Here, the Poisson parameters $\lambda_i$, $i = 1,2,\ldots,n$, follow conditionally independent gamma posterior distribu-
tions with parameters $y_i + \alpha$ and $1 + \frac{\alpha}{\xi_i}$, such that

$$E(\lambda_i | \alpha, \beta, u, y) = \frac{y_i + \alpha}{1 + \frac{\alpha}{\xi_i}} = \xi_i + \zeta_i(y_i - \xi_i)$$

[13]

where the shrinkage parameter $\zeta_i = \frac{\xi_i}{\xi_i + \alpha}$ is peculiar to each $i = 1, 2, \ldots, n$. In contrast, the analogous shrinkage parameter in NB1 is homogeneous (32).

We integrate out $\lambda$ from the joint posterior density in Equation [11] to obtain the density:

$$\pi(\beta, u, \alpha | y) \propto \left( \prod_{i=1}^{n} \frac{\Gamma(y_i + \alpha)}{\Gamma(\alpha)} \left( \frac{\alpha}{\alpha + \xi_i} \right)^{\alpha} \left( \frac{\xi_i}{\alpha + \xi_i} \right)^{y_i} \right)$$

$$|G|^{-\frac{1}{2}} \exp \left( - \frac{uG^{-1}u}{2} \right) \prod_{j=1}^{c} \pi(\sigma_j^2) \pi(\alpha)$$

[14]

that, as for NB1 (32), has the form of the product of $n$ conditionally independent negative binomial random densities and a multivariate normal density for $u$ with

$$E(Y_i | \beta, u, \alpha) = \xi_i.$$  

[15a]

However, unlike NB1 (32), the conditional variance of this negative binomial density is a linear and quadratic function rather than simply a linear function of the mean $\xi_i$;

$$\text{Var}(Y_i | \beta, u, \alpha) = \xi_i + \frac{\xi_i^2}{\alpha}.$$  

[15b]

Inference on Fixed and Random Effects

An empirical Bayes approach represents an approximate, yet computationally tractable, method of inference on fixed and random effects in generalized linear mixed models (7). Let $\hat{\tau}$ represent an estimate of $\tau = [\alpha \sigma_1^2 \sigma_2^2 \ldots \sigma_c^2]$ obtained from the mode of an approximation to the posterior marginal density of $\tau$. This modal estimate can be approximated using Laplacian integration, which is discussed subsequently. A set of empirical Bayes estimates of $\beta$ and $u$ is given by values that jointly maximize $\pi(\beta, u | \tau = \hat{\tau})$, the joint density of $\beta$ and $u$ conditionally on $\tau = \hat{\tau}$. The Newton-Raphson method is the most popular algorithm used for computing these empirical Bayes estimates in animal breeding (6, 7, 19, 23, 29, 32).

Letting $\theta = [\beta u]$ denote the vector of location parameters, we write the log posterior conditional density of $\theta$ given $\tau$ from Equation [14] as

$$L(\theta | y, \tau) = \text{constant} - \frac{uG^{-1}u}{2} +$$

$$\sum_{i=1}^{n} \left( - \alpha \log(\xi_i + \alpha) + y_i \log(\xi_i) - y_i \log(\xi_i + \alpha) \right).$$

[16]

Then, the vector of first derivatives can be written as

$$\frac{\partial L(\theta | y, \tau)}{\partial \beta} = Xv = \sum_{i=1}^{n} x_i v_i$$

[17a]

and

$$\frac{\partial L(\theta | y, \tau)}{\partial u} = Zv - G^{-1}u$$

[17b]

where $X = \{x_i^\prime\}$ is $n \times p$, $Z = \{z_j^\prime\}$ is $n \times q$, and element $i$ of $v$ is

$$v_i = \frac{\partial L(\theta | y, \tau)}{\partial \xi_i} \frac{\partial \xi_i}{\partial \eta_i} = \frac{y_i}{\xi_i + \alpha} - \frac{y_i}{\xi_i} = \frac{\alpha + y_i}{\xi_i + \alpha} \frac{\xi_i}{\xi_i}. $$

[18]

Similarly, the negative Hessian matrix is:

$$H_{\tau} = -\frac{\partial^2 L(\theta | y, \tau)}{\partial \theta \partial \theta^\prime} = [X^\prime RX X^\prime RZ Z^\prime RZ R^\prime Z^\prime + G^{-1}]$$

[19]

where $R = \text{diagonal matrix with diagonal element} i (r_{ii})$ written as

$$r_{ii} = \frac{\alpha + y_i}{\xi_i + \alpha} \frac{\xi_i}{\xi_i + \alpha}.$$ 

[20]

The Newton-Raphson method involves solving iteratively the set of equations

$$[X^\prime RX X^\prime RZ Z^\prime RZ R^\prime Z^\prime + G^{-1}]_{\beta \theta=[t]\rightarrow[t+1]} = [X^\prime RY]_{\theta=[t]}$$

[21]
Variance Component Estimation

Marginal inference on τ can be approximated using Laplacian integration, as suggested by Leonard (13) and by Tempelman and Gianola (30) for variance component estimation in Poisson mixed models. A Laplacian method was also used to estimate variance components in NB1 (32). Along the lines of Equation [3.13] of Tempelman and Gianola (32), the Laplacian marginal log density of τ in NB2 can be written from Equation [14] as

\[ \log \left( \frac{\pi(\theta, \tau | y)}{\pi(\tau | y)} \right) = \log(\pi(\theta, \tau | y)) - \frac{1}{2} \log(\mathbf{H}_i,l) \left[ \theta = \hat{\theta}_i \right] \]

\[ = n(\alpha \log(\alpha) - \log(\mathbf{H}_i,l)) + \sum_{i=1}^{n} (\log(\mathbf{G}) \cdot \mathbf{u} \mathbf{G}^{-1} \mathbf{u} + \sum_{i=1}^{c} \log(\pi(a_{ij}))) \]

\[ - \frac{1}{2} \log(\mathbf{G}) \cdot \frac{n}{2} \mathbf{u} + \sum_{i=1}^{c} \log(\pi(a_{ij})) - \frac{1}{2} \log(\mathbf{H}_i,l) \]

[22]

Here, \( \hat{\xi}_i = \mathbf{x}_i \hat{\beta} + \mathbf{z}_i \hat{\mathbf{u}}, i = 1,2,\ldots,n \), and \( | \mathbf{H}_i,l | \) evaluated at \( \hat{\theta}_i \) or \( \hat{\theta}_i \) given \( \tau \), using the Newton-Raphson set of equations in Equation [21]. If no covariances exist between random effects of different factors, then Equation [22] can be simplified further using \( \log(\mathbf{G}) = \sum_{i=1}^{c} q_i \log(\sigma_i^2) \) where \( q_i \) is the dimension of \( u_i \). A tractable factorization of \( \log(\mathbf{G}) \) for models involving, for example, direct-maternal genetic covariances, can be found in Meyer (18). Equation [22] can be maximized with respect to \( \tau \) with optimization methods similar to those adopted in derivative-free REML (18). The resulting modal estimates are then the Laplacian-based maximum marginal likelihood (MML) estimates of \( \tau \), with \( L(\tau | y) \) referred to commonly as the marginal log likelihood (7).

**SIMULATION STUDY**

**Design**

An animal model simulation study, similar to that in Tempelman and Gianola (32), was conducted to compare the frequentist performance of three different animal models for estimation of additive genetic variance of superovulated embryo yields in dairy cows. The models were NB1, NB2, and PAM. Embryo yields were generated from Poisson distributions under PAM assumptions with the following parameters:

\[ \lambda_{ij} = \exp(\mu_i + u_i + e_{ij}) \]

[23]

Here, \( \mu_i = \log(i) \) = mean response of herd \( i \) (\( i = 1,2,\ldots,15 \)) on the logarithmic scale. Furthermore, \( u_i = \{ u_j \} \sim N(0, \sigma_u^2) \) is a vector of random additive genetic effects, and \( e = \{ e_j \} \sim N(0, \sigma_e^2) \) is a vector of independently distributed extra-Poisson residual effects. The known matrix \( \mathbf{A} \) gives the additive genetic relationships between the base generation animals and their female progeny. The base population consisted of 64 unrelated sires and 512 unrelated dams. The base dams were superovulated and randomly mated with the base sires. After random assignment to 1 of the 15 levels of the fixed herd effect and augmentation of a residual randomly drawn from \( N(0, \sigma_e^2) \), the number of embryos collected from base female \( j \) (\( j = 1,2,\ldots,512 \)) was an independent drawing from a Poisson distribution with parameter \( \lambda_{ij} \) as in Equation [23]. The sex of the embryos collected from the donor dams was assigned at random (50% chance of obtaining a female), and the probability of a female embryo surviving to age at first breeding was 70%. The genetic value of surviving female offspring \( j \) was determined as one-half the genetic merit of each of her sire and dam plus a Mendelian sampling term randomly drawn from \( N(0, 0.5\sigma_u^2) \). After random assignment to one of the herd effects and random generation of an extra-Poisson residual, embryo yields of surviving female offspring were then generated from Poisson distributions using Equation [23]. Because of the simulation, the number of female progeny, and hence the number of records, stochastically varied from one data set to another.

Four different values of \( \sigma_u^2 \) were considered: 0.0125, 0.0250, 0.0375, and 0.0500. For all values of \( \sigma_u^2, \sigma_e^2 = 3\sigma_u^2 \) such that, using Equation [8], heritability = 0.25 in all cases. One hundred data sets were generated.
for each of the four values of \( \sigma_u^2 \), and the variance components estimated under each of the three models were \( \tau' = [\sigma_u^2 \mu, \sigma_e^2] \) for NB1 and NB2 and \( \tau' = [\sigma_u^2, \sigma_e^2] \) for PAM. As shown in Equation [2] and in Tempelman and Gianola (32), \( \alpha \) is defined differently in NB1 than in NB2. In all cases, MML estimates of \( \tau \) were computed using Laplacian integration. In NB1 and NB2, the proper prior in Equation [7] with \( z_0 = 1 \) was used for \( \pi(\alpha) \), and a flat prior on \( \sigma_e^2 \) (i.e., \( \pi(\sigma_e^2) \propto 1 \)) was used in PAM. A flat prior on \( \sigma_u^2 \) (i.e., \( \pi(\sigma_u^2) \propto 1 \)) was used in all three models. The downhill simplex method using code from Press et al. (24) was the optimization routine used for maximizing the marginal posterior density of the variance components in Equation [22]. A FORTRAN77 program based on the sparse matrix subroutines FSPAK by Perez-Enciso et al. (22) was developed and used for the analyses. Convergence of the final variance component estimates was declared when the variance of the marginal log-likelihood functions evaluated within a simplex iterate was less than 10^{-6}. Newton-Raphson estimates of \( \theta \), conditional on \( \tau \) within each marginal log-likelihood evaluation, were deemed to converge when

\[
\sqrt{\frac{(\hat{\theta}_{[t+1]} - \hat{\theta}_t)(\hat{\theta}_{[t+1]} - \hat{\theta}_t)}{p + q}} < 10^{-6}
\]  

[24]

where \( t \) pertains to the iterate number in the Newton-Raphson procedure.

Empirical relative bias (bias as a proportion of the true parameter value) and empirical relative error (square root of average squared error as a proportion of the true parameter value) were assessed for MML estimates of \( \sigma_u^2 \) over the 100 replicated data sets for each true value of \( \sigma_u^2 \) and under each of the three models. Empirical relative biases and empirical relative errors were also assessed for MML estimates of \( \sigma_e^2 \) under NB2 and PAM where \( \sigma_e^2 = \psi^{(1)}(\alpha) \) in NB2. As the extra-Poisson residual variance is heterogeneous under NB1, it was not considered in this comparison.

Results

Relative biases and errors of MML estimates \( \hat{\sigma}_u^2 \) of \( \sigma_u^2 \) under the three models are given in Figures 1 and 2, respectively. The MML inference under NB1 always lead to significantly \( (P < 0.0001) \) large relative biases on \( \hat{\sigma}_u^2 \) that ranged from 50 to 80% and tended to increase with \( \sigma_u^2 \). However, these biases were not nearly as large as those previously reported for NB1 (32), perhaps because of the stabilizing effect of the
vague, but proper, prior on \( \alpha \) in Equation [7] used in the present study. With a flat prior specification on \( \alpha \) [i.e., \( \pi(\alpha) \propto 1 \)], Tempelman and Gianola (32) noted convergence problems on \( \alpha \) for 10 of 100 replicated data sets. The empirical relative biases for \( \sigma_u^2 \) under NB2 and PAM shown in Figure 1 were not different (\( P > 0.05 \)) from 0 for all values of \( \sigma_e^2 \). Furthermore, empirical relative errors on \( \sigma_u^2 \) shown in Figure 2 were small and differed little between NB2 and PAM; empirical relative errors under NB1 were large and tended to increase with \( \sigma_u^2 \). The NB2 model reflected the simulation process more accurately than did NB1, as extra-Poisson residual variation is homogeneous under PAM and NB2 but not under NB1.

Relative biases and errors of MML estimates of \( \sigma_e^2 \) under NB2 and PAM are shown in Figures 3 and 4, respectively. Empirical relative biases on \( \sigma_e^2 \) under NB2 were consistently around 8% and always differed (\( P < 0.0002 \)) from 0, but relative biases on \( \sigma_e^2 \) under PAM were never different (\( P > 0.05 \)) from 0. Furthermore, empirical relative errors on \( \sigma_e^2 \) were smaller and declined faster with increasing values of \( \sigma_e^2 \) under PAM. Although NB2 modeled a homogeneous distribution for extra-Poisson residuals on the logarithmic scale, this log-gamma distribution became increasingly skewed negatively for smaller values of \( \alpha \) such that greater discrepancies on \( \sigma_e^2 \) between NB2 and PAM might have been expected for larger \( \sigma_e^2 \). Conversely [see also (5)], little difference would have been anticipated between a model like NB2 based on log-gamma residuals with large \( \alpha \) (or little overdispersion) versus a model like PAM with normally distributed residuals. The NB2 was preferred to PAM for empirical Bayes inference on fixed and random effects. The extra-Poisson residuals were integrated out of the joint posterior for NB2 in Equation [14] such that the dimension of \( H_y \) was reduced by \( n \), the number of observations, relative to a PAM analysis. That is, NB2 provided inference on breeding values, allowing for the uncertainty on the values of the extra-Poisson residuals. Further benefits would accrue if the true extra-Poisson residuals were more accurately characterized by a log-gamma rather than a normal distribution.

**MATERIALS AND METHODS**

**Data**

A total of 660,430 breeding receipts from October 1986 to October 1991 for AI of registered Holstein cows with full doses of semen from registered Holstein sires were received from the former Western
Ontario Breeders Incorporated (near Woodstock, ON, Canada). The Ontario Dairy Herd Improvement Corporation (Guelph, ON, Canada) and Agriculture Canada (Ottawa, ON, Canada) provided 152,286 first lactation production records for Ontario Holstein cows born between January 1, 1982 and January 1, 1990. These records included the necessary corresponding calving date and pedigree information. Furthermore, pedigrees of 32,662 sires were obtained from the Department of Animal and Poultry Science at the University of Guelph (Guelph, ON, Canada). The Ontario Dairy Herd Improvement Corporation (Guelph, ON, Canada) and Agriculture Canada (near Woodstock, ON, Canada) provided 152,286 herds in

1990. These records included the necessary corresponding calving date and pedigree information. Furthermore, pedigrees of 32,662 sires were obtained from the Department of Animal and Poultry Science at the University of Guelph (Guelph, ON, Canada).

Only virgin heifers were considered in the tabulation of NSC. Age at first calving was restricted to be within 18 to 40 mo of age. Furthermore, only heifers with gestation lengths between 263 to 299 d were considered, which corresponded to the mean plus and minus three standard deviations, respectively, based on a study of gestation lengths in Ontario Holstein cows by Nadarajah et al. (20). If the interval between two consecutive AI was less than or equal to 2 d, the latter AI was considered to be a rebreeding and not included in the NSC of that heifer. If the interval between two consecutive AI was greater than 250 d, the earlier AI was discarded and not counted, and the latter AI recorded as the first AI. This edit was used to ensure that two calvings would not be credited to the same NSC; however, this procedure did not necessarily prevent a spontaneous abortion from inflating an NSC record. Embryonic death and short-term abortions were not routinely recorded in Canadian data (27) at the time of data collection. Only calving dates on or after January 1, 1988 were considered for analysis to allow reliable tabulation of NSC from the AI receipts. The interval from October 1, 1986 to January 1, 1988 allowed time for a full gestation period plus 5 mo for NSC tabulation. Year-month seasons were assigned to the records based on the time of first AI. Fifty year-month classes ranged from October 1986 until December 1990.

All sires and paternal grandsires of heifers were known. To limit the computing dimension of the problem, the pedigree file was edited of noncontributing animals. That is, if a dam had no record in the data, she had to be genetically related to at least two individuals in the pedigree file to be identified in the pedigree. These same conditions were also applied to sires and paternal grandsires. Otherwise, these animals were treated as unknown. These pedigree edits were critical to improve the Laplacian approximation for variance component estimation by minimizing the dimension of the approximate integration in Equation [22]. These edits left a total of 22,035 identified animals, including 21 paternal grandsires, 750 sires, 1419 dams without records, and 19,845 heifers with records. Some sires also appeared as paternal grandsires in the pedigrees, and 549 heifers also appeared as dams in later records. These data were also considered in an NB1 application by Tempelman and Gianola (32).

Models

The linear model structure used for analyses was written similarly to Equation (4):

$$
\xi = f^{-1}(X\beta + Z_1u_1 + Z_2u_2 + Z_3u_3 + Z_4u_4) = f^{-1}(X\beta + Zu) = f^{-1}(W\theta)
$$

where \(\xi\) = vector of conditional means on NSC given \(\beta\) and \(u\); \(X, Z_1, Z_2, Z_3, Z_4\) = known incidence matrices; \(\beta = p \times 1\) vector of fixed effects, and \(u = q \times 1\) vector of random effects. Here, \(f^{-1}\) represents the inverse of a model dependent link function, \(Z = [Z_1, Z_2, Z_3, Z_4]\), and \(W = [X Z]\). Vector \(\beta\) included the overall mean, 50 year-month subclasses, and 21 ages at first AI (10 to 30 mo). The last level for age at first AI included both 30 and 31 mo as all records for 31-mo-old heifers were of value NSC = 1. If a separate level had been defined for 31-mo-old heifers, its posterior modal estimate under a Poisson or negative binomial mixed model would slowly converge to \(-\infty\). This situation is an example of the extreme category problem described previously by Misztal et al. (19) in the context of threshold mixed models. The fixed effects were reparameterized by expressing levels of each of the two factors as differences from the last level such that \(X\) had full column rank. The vector of random effects \(u\) included 1584 herds in \(u_1\), 232 technicians in \(u_2\), 417 service sires in \(u_3\) and 22,035 animals in \(u_4\). A problem arises in assigning levels of certain factors to records where NSC > 1. For example, if NSC = 2, a different service sire and a different technician could have been used for either AI. Therefore, service sires and technician effects were defined to be those pertaining to the first AI. Hence, it was assumed that all AI for a heifer until conception were by the same service sire and technician. The random effects were, a priori, assumed to be multivariate normal:

$$
\begin{align*}
\mu_1 &\sim N(0, I_1\sigma^2_{u_1}) \quad \text{(26a)} \\
\mu_2 &\sim N(0, I_2\sigma^2_{u_2}) \quad \text{(26b)} \\
\mu_3 &\sim N(0, I_3\sigma^2_{u_3}) \quad \text{(26c)} \\
\mu_4 &\sim N(0, A\sigma^2_{u_4}) \quad \text{(26d)}
\end{align*}
$$

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where \( A = \) additive relationship matrix among 22,035 animals, and \( I_1, I_2 \) and \( I_3 = \) identity matrices of order 1584, 232, and 417, respectively. The variance components included herd variance \( \sigma^2_{u_1} \), technician variance \( \sigma^2_{u_2} \), service sire variance \( \sigma^2_{u_3} \), and additive genetic variance \( \sigma^2_{u_4} = \sigma^2_{a} \). Covariances among levels of the four sets of random factors were assumed null, despite some evidence of small nonzero covariances between \( u_3 \) and \( u_4 \) (11).

Three different models were considered for the analysis of this data. Two models were based on the negative binomial distribution, specifically, NB1 and NB2 as in the simulation study; the third was the linear mixed model. The vector of variance components in the negative binomial models can then be written as \( \delta_{NB} = [\sigma^2_{u_1} \sigma^2_{u_2} \sigma^2_{u_3} \sigma^2_{u_4} \alpha] \) where the scale parameter \( \alpha \) is defined differently in NB1 from NB2. The vector of variance components in the linear mixed model could be similarly written as \( \delta_L = [\sigma^2_{u_1} \sigma^2_{u_2} \sigma^2_{u_3} \sigma^2_{u_4} \sigma^2_\alpha] \). For both negative binomial models, the informative prior in Equation [7] with \( z_0 = 1 \) was used for \( \alpha \). For all three models, flat priors were used on the variance components. The link function \( f \) in Equation [25] for the negative binomial models was logarithmic as in Equation [4], such that \( f^{-1} = \exp(\cdot) \), whereas an identity link function \( f = f^{-1} = \cdot \) was used in the linear mixed model. Empirical Bayes estimates of \( \theta \) were based on the MML estimate of \( \tau \), using the Newton-Raphson system of equations in Equation [21].

The counted variate analyzed under all models was NSC after the first AI (NSC1) where NSC1 = NSC - 1. An observation of NSC = 0 is not possible, and the Poisson process most likely applies to AI events immediately following the first AI.

### Variance Component Inference

The variance component estimation procedure was MML using the Laplacian approximation; in the linear model, MML is REML. The same computational strategy and convergence criteria as in the simulation study were used. Optimization was restarted at the converged values until the marginal log-likelihood function remained unchanged from one run to the next. In REML analyses of animal breeding data, the downhill simplex method may converge prematurely (12). Hence, restarts were used to ensure that convergence occurred to, at least, a local maximum.

Asymptotic posterior standard errors of the dispersion parameter estimates were computed by fitting a response surface model to the marginal log likelihood in Equation [22] as in Graser et al. (9):

\[
q_0 + q_1 \tau^r + \tau^r Q = \mathbf{L} (\tau | y) \tag{27}
\]

where \( q_0 = \) scalar, \( q_1 = \) vector, and \( Q = \) symmetric matrix. The approximate (and local) variance-covariance matrix of the variance component estimates was taken to be \( (-2Q)^{-1} \), because \(-2Q\) is an approximation to the information matrix of the marginal log-likelihood of \( \tau \) in Equation [22]. This approximation is based on the assumption that the behavior of the marginal log-likelihood function near the maximum is nearly quadratic. To ensure this behavior, only those marginal log-likelihood evaluations within 0.1 of the maximum marginal log likelihood \( L (\tau | y) \) were used in fitting the response surface model.

Marginal log-likelihood ratio tests were also used to test the hypothesis that genetic variance component was \( \sigma^2_a = 0 \) in the best fitting negative binomial mixed model. This test required deleting genetic effect \( a = u_4 \) from the negative binomial model and maximizing the marginal log likelihood of the new variance component subset \( [\sigma^2_{u_1} \sigma^2_{u_2} \sigma^2_{u_3} \alpha] \). Marginal log-likelihood ratio tests of the genetic component also required computation of the log determinant of the inverse of the relationship matrix, \( \log | \mathbf{A}^{-1} | \). This log determinant was computed using the aforementioned sparse matrix subroutines of Perez-Enciso et al. (22), although it also could have been readily determined using the principles developed by Quaas (25). The test statistic used to test the null hypothesis of \( \sigma^2_a = 0 \) was \(-2\) times the difference between the maximum marginal log likelihoods of the full model and the model excluding additive genetic effects. This likelihood ratio test statistic was compared with a chi-square distribution with one degree of freedom. As with many likelihood ratio tests, this test is approximate.

### Estimates of Fixed Effects

Estimates of fixed effects obtained with the linear and negative binomial mixed models were conditioned, respectively, on the REML and MML estimates of their respective sets of variance components. All estimates were expressed as least squares means (28). For instance, the elements of \( \beta \) were
represented by an overall mean $\mu$, 50 year-month effects $\alpha_k$, $k = 1,2,...,50$, and by 21 age at first AI effects $\gamma_l$, $l = 1,2,...,21$. For the linear mixed model, the least squares mean for year-month $k$ was computed as

$$\hat{\mu}_k = \hat{\mu} + \hat{\alpha}_k + \frac{1}{21} \sum_{l=1}^{21} \hat{\gamma}_l$$  \hspace{1cm} [28a]$$

and the least squares mean for age $l$ at first AI was computed as

$$\hat{\mu}_l = \hat{\mu} + \hat{\gamma}_l + \frac{1}{50} \sum_{k=1}^{50} \hat{\alpha}_k.$$  \hspace{1cm} [28b]$$

Because of the full rank restrictions on $X$, $\hat{\alpha}_{50} = 0$, and $\hat{\gamma}_{21} = 0$.

The least squares solutions under the two negative binomial models were similarly computed, as in Equations [28a] and [28b], but then exponentiated back to the scale of observation on a marginal mean basis (8). For example, let $x_i$ and $z_i$ represent the row incidence vectors that connect $y_i$ to $\beta$ and $u_i$, respectively. Then the expected marginal (over $u_i$) mean for response on individual $i$, based on a negative binomial or Poisson mixed model with a logarithmic link, is

$$E(y_i|\beta, G) = \exp\left(x_i^\prime \beta + \frac{z_i^\prime G z_i}{2}\right)$$  \hspace{1cm} [29]$$

(8). Likewise, the least squares means on the observed scale for the two negative binomial models were computed for year-month $k$ as

$$\hat{\lambda}_k = \exp\left(\hat{\mu}_k + \frac{1}{2} \sum_{l=1}^{4} \hat{\alpha}_l^2 \hat{\gamma}_l^2 \right)$$  \hspace{1cm} [30a]$$

and for age $l$ at first AI subclass as

$$\hat{\lambda}_l = \exp\left(\hat{\mu}_l + \frac{1}{2} \sum_{k=1}^{4} \hat{\alpha}_k^2 \right)$$  \hspace{1cm} [30b]$$

Predictive Ability

Perez-Enciso et al. (23) used a measure of cross-validation to assess the performance of linear and Poisson mixed models for the genetic analysis of litter size in pigs. Cross-validation involves splitting the data into two subsets and using parameters estimated from one subset to predict the observations in the other subset. Perez-Enciso et al. (23) gave formulas for the predictive posterior mean of a future observation given past observations $y$ and the set of variance components $\tau$. Under the linear mixed model, the predicted value of a future observation $\hat{y}_i$ for animal $i$ is given by the predictive posterior mean

$$E(\hat{y}_i|y, \hat{\tau}) = w_i \hat{\beta},$$  \hspace{1cm} [31]$$

where $w_i = [x_i, z_i]$. As shown in Equation [13] of this paper and Equation [2.10a] of Tempelman and Gianola (32), the predictive posterior mean of an observation under a negative binomial mixed model requires knowledge of a previous record $y_i$ within each subpopulation $i$. A subpopulation is defined by a particular combination of levels, one level from each of the fixed and random factors in the model. Because heifer fertility was analyzed in this study, there were no repeated records on individuals and, hence, only one record per subpopulation as defined herein. Therefore, predictions under the two negative binomial mixed models were made using the approximation

$$E(\hat{y}_i|y, \hat{\tau}) = \exp(w_i \hat{\beta}).$$  \hspace{1cm} [32]$$

This predictive mean can be further refined, as previously shown for the Poisson mixed model in the Appendix of Perez-Enciso et al. (23); however, their
procedure requires the intensive computation of many elements of $H_i^{-1}$ for each $i$.

As in Perez-Enciso et al. (23), agreement between the observed and predicted values was assessed in terms of empirical mean squared error of prediction and empirical predictive correlation between observed and expected performance. The data were split into two parts after data were deleted for year-months of first AI prior to March 1987 or later than August 1990. Also records in which age at first AI occurred at less than 13 mo or greater than 29 mo were excluded. These edits were used to avoid small subclass sizes that could lead to an extreme category convergence problem. Two subsets of equal size, each with 8799 records, were created after ensuring that observations were as evenly divided as possible among levels of the fixed factors and that overall mean NSC1 was similar in the two subsets. Location parameter estimates obtained from linear and negative binomial mixed models in the first data subset were used to predict observations in the second subset. Similarly, location parameter estimates computed from the second data subset were used to predict data in the first subset. True variance components were deemed to be REML and MML estimates computed from the entire data set.

RESULTS AND DISCUSSION

General Statistics

The overall mean and standard deviation of heifer NSC were $\mu = 1.42 \pm 0.78$, meaning that the corresponding descriptive statistics for NSC1, the analyzed variate, were $\lambda = 0.42 \pm 0.78$. These summary statistics were very similar to those reported by Raheja et al. (26) and Oltenacu et al. (21). Over 70% of the heifers in this study conceived during their first AI.

Variance Component Estimates

The REML and MML variance component estimates, their asymptotic standard errors, and maximum values $L(\tilde{\eta} | y)$ of the marginal log likelihood of the variance components under the three models are given in Table 1. The Poisson mixed model of Foulley et al. (6) is a reduced model of both NB1 and NB2 used in this study. Based upon a previous likelihood ratio test on this data (32), NB1 with a flat prior on $\alpha$ was concluded to fit the data better than a Poisson mixed model that ignored overdispersion. As neither NB1 nor NB2 is a reduced model of each other, a likelihood ratio test comparison of these two models was not possible. Yet, because both NB1 and NB2 have the same model dimension, and their marginal log likelihoods $L(\eta | y)$ have the same integration constant, a direct comparison of the two marginal log likelihoods would be justified based on model choice criteria such as Akaike's information criterion or Schwarz's Bayesian criterion [(2); p. 48]. The maximum marginal log likelihood reported for NB1 was substantially higher than that reported for NB2 in Table 1, thereby providing support that NB1 fit the data better. That is, a negative binomial model that attaches greater uncertainty to observations with low

<table>
<thead>
<tr>
<th>Variance components</th>
<th>NB1 (^2)</th>
<th>NB2 (^3)</th>
<th>Linear mixed model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_1^2$</td>
<td>$5.34 \times 10^{-3}$</td>
<td>$4.52 \times 10^{-3}$</td>
<td>$7.39 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\sigma_2^2$</td>
<td>$1.24 \times 10^{-2}$</td>
<td>$1.12 \times 10^{-2}$</td>
<td>$2.04 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\sigma_3^2$</td>
<td>$5.22 \times 10^{-2}$</td>
<td>$5.66 \times 10^{-2}$</td>
<td>$1.14 \times 10^{-2}$</td>
</tr>
<tr>
<td>$\sigma_4^2$</td>
<td>$2.57 \times 10^{-2}$</td>
<td>$2.77 \times 10^{-2}$</td>
<td>$7.65 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\sigma_e^2$</td>
<td>$2.907$</td>
<td>$1.415$</td>
<td>$0.553$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$0.188$</td>
<td>$0.088$</td>
<td>$6.64 \times 10^{-3}$</td>
</tr>
<tr>
<td>$L(\tilde{\eta}</td>
<td>y)$</td>
<td>$-18,072.78$</td>
<td>$-18,086.75$</td>
</tr>
</tbody>
</table>

\(^a\) Standard error of variance component estimate.
\(^b\) NB1 = negative binomial model with heterogeneous logarithmic scale overdispersion.
\(^c\) NB2 = negative binomial model with homogeneous logarithmic scale overdispersion.
\(^d\) $L(\tilde{\eta} | y)$ = marginal log-likelihood $L(\tilde{\eta} | y)$ (except for a constant) evaluated at the joint mode $\tilde{\eta}$.
NEGATIVE BINOMIAL MIXED MODELS

Figure 5. Least squares means for number of AI until conception by age at first AI. LMM = linear mixed model; NB1 = negative binomial model with heterogeneous logarithmic scale dispersion. Bars represent (95%) confidence intervals for least squares means based on LMM; closed lines represent confidence intervals for least squares means based on NB1.

Fixed Effects Solutions

Solutions for fixed effects were expressed on the scale of observation. For brevity, least squares solutions for year-months are not reported except to note that temporal trends existed and that least squares means were similar between NB1 and the linear mixed model. Least squares means for age at first AI under NB1 and the linear mixed model are shown in Figure 5. Under both models, age at first AI appeared to increase, and NSC decreased sharply between 10 and 13 mo of age but decreased slowly thereafter. These results agree somewhat with the estimated negative phenotypic correlations reported between age at first AI and NSC reported by Hansen et al. (10) and Raheja et al. (26). From our results, it might appear that herd managers should wait until heifers are at least 13 mo of age before inseminating them. However, the number of observations associated with the first three ages at first AI was small, being 5, 3, and 31, respectively, and was manifested in the very wide confidence intervals on the least squares means for these three age groups as shown in Figure 5. For subclasses with small numbers, confidence intervals under NB1 were generally larger than were those under the linear mixed model. Furthermore, NB1 least squares means were substantially larger at 10 and 11 mo of age but consistently lower than the linear mixed model least squares means thereafter.

Predictive Ability

Empirical mean squared errors and correlations between predictive means obtained from one data subset and the records to be predicted in the other

<table>
<thead>
<tr>
<th>Criteria</th>
<th>NB1</th>
<th>NB2</th>
<th>Linear model</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSEP1,2</td>
<td>0.620</td>
<td>0.620</td>
<td>0.621</td>
</tr>
<tr>
<td>PC1,2</td>
<td>0.151</td>
<td>0.152</td>
<td>0.148</td>
</tr>
<tr>
<td>MSEP2,1</td>
<td>0.596</td>
<td>0.596</td>
<td>0.597</td>
</tr>
<tr>
<td>PC2,1</td>
<td>0.149</td>
<td>0.146</td>
<td>0.144</td>
</tr>
</tbody>
</table>

1MSEPij = Mean squared error of prediction using estimates of fixed and random effects based on data set i to predict data in data set j; PCij = predictive correlation between actual and predicted values of data in data set j based on estimates of fixed and random effects from data set i.

2NB1 = negative binomial model with heterogeneous logarithmic scale overdispersion.

3NB2 = negative binomial model with homogeneous logarithmic scale overdispersion.
subset are given in Table 2 for both the linear and negative binomial models. The mean squared errors of prediction were virtually identical for all models, whether data set 1 was used to predict data set 2 or vice versa. However, predictive correlations were slightly greater for the two negative binomial models. Predictive abilities of linear and Poisson models were also similar for litter size in Iberian pigs, as reported by Perez-Enciso et al. (23).

CONCLUSIONS

At least two different specifications of a negative binomial mixed model are possible. The model (NB2) developed in this paper is based upon a specification of homogeneous extra-Poisson residual variance on the logarithmic scale. This specification contrasts with a previously developed model (NB1) in which this variance was heterogeneous across observations. In that context, NB2 was shown to have variance component estimation properties that were superior to NB1 when simulated data was generated from Poisson distributions with identically distributed overdispersion residuals on the logarithmic scale. Nevertheless, in an application to the analysis of NSC, NB1 had slightly superior fit and indicated that a model would be appropriate that specifies greater uncertainty on responses with low conditional means.

Based on a marginal likelihood ratio test under NB1, additive genetic variation, albeit small, appeared to be significant for heifer NSC. All random effects in total explained only a very small proportion of the total phenotypic variation in NSC, using both linear and negative binomial mixed models. Tempelman and Gianola (31) showed that small variance components and low means led to a situation in which small differences might be anticipated between estimates of location parameters obtained with linear versus Poisson mixed models. This situation may explain the small differences in predictive ability and least squares means between the linear and negative binomial mixed models. Other studies (17, 33) comparing linear and threshold mixed models for the analyses of categorical reproductive data have shown similar conclusions.

The introduction of additional modeling and methodology refinements is encouraged. For example, NSC is a response that is characterized by a great deal of right censoring (10) such that the genetic variance estimates reported earlier are plausible underestimates [e.g., see (3)]. Furthermore, the development of Markov Chain Monte Carlo methods, although computationally intensive, would facilitate fully marginal inference on all parameters in negative binomial mixed models. These methods also facilitate more reliable assessments of model choice than the crossvalidation procedures discussed earlier (2).

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