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A Redefinition of the Representation of Mammary Cells and Enzyme Activities in a Lactating Dairy Cow Model

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ABSTRACT

The Molly model predicts various aspects of digestion and metabolism in the cow, including nutrient partitioning between milk and body stores. It has been observed previously that the model underpredicts milk component yield responses to nutrition and consequently overpredicts body energy store responses. In Molly, mammary enzyme activity is represented as an aggregate of mammary cell numbers and activity per cell with minimal endocrine regulation. Work by others suggests that mammary cells can cycle between active and quiescent states in response to various stimuli. Simple models of milk production have demonstrated the utility of this representation when using the model to simulate variable milking and nutrient restriction. It was hypothesized that replacing the current representation of mammary cells and enzyme activity in Molly with a representation of active and quiescent cells and improving the representation of endocrine control of cell activity would improve predictions of milk component yield. The static representation of cell numbers was replaced with a representation of cell growth during gestation and early lactation periods and first-order cell death. Enzyme capacity for fat and protein synthesis was assumed to be proportional to cell numbers. Enzyme capacity for lactose synthesis was represented with the same equation form as for cell numbers. Data used for parameter estimation were collected as part of an extended lactation trial. Cows with North American or New Zealand genotypes were fed 0, 3, or 6 kg of concentrate dry matter daily during a 600-d lactation. The original model had root mean square prediction errors of 17.7, 22.3, and 19.8% for lactose, protein, and fat yield, respectively, as compared with values of 8.3, 9.4, and 11.7% for the revised model, respectively. The original model predicted body weight with an error of 19.7% vs. 5.7% for the revised model. Based on these observations, it was concluded that representing mammary synthetic capacity as a function of active cell numbers and revisions to endocrine control of cell activity was meritorious.
Key words: model, lactation, dairy cow, milk composition

INTRODUCTION

A model of dairy cow metabolism has been constructed (called Molly; Baldwin et al., 1987a,b,c). In that model, mammary cell numbers and enzyme activity were depicted in aggregate using the approach of Neal and Thornley (1983). In that representation, mammary enzyme activity was primarily a function of initial mammary cell numbers and DIM. The effects of milking frequency were considered, but the effects of nutritional state on enzyme activity were minimal, reflecting the state of knowledge of endocrine control at that point in time.

When simulating diets with varying nutrient content, milk yield responses are underpredicted, and BW responses are overpredicted as compared with the observed responses (McNamara and Baldwin, 2000; Hanigan et al., 2006, 2007). That is, the model underpredicts milk yield and overpredicts BW gain when dietary energy content is high and the reverse when low-energy diets are simulated (demonstrated in Figure 1). These errors suggest that milk synthesis capacity is biologically regulated in response to nutritional state, causing partitioning of more energy to milk as nutritional state improves and protecting body stores at the expense of milk production as nutritional state declines. Insulin and the somatotropin axis have been observed to play a role in regulating milk synthesis (Asimov and Krouze, 1937; McGuire et al., 1995b), allowing the animal to alter mammary metabolite use to match its nutrient supply. Because these endocrines are responsive to nutritional state (McGuire et al., 1992, 1995a), failure to consider their effects on mammary synthetic capacity in Molly likely explains at least part of the observed prediction errors with respect to observed nutritional responses.

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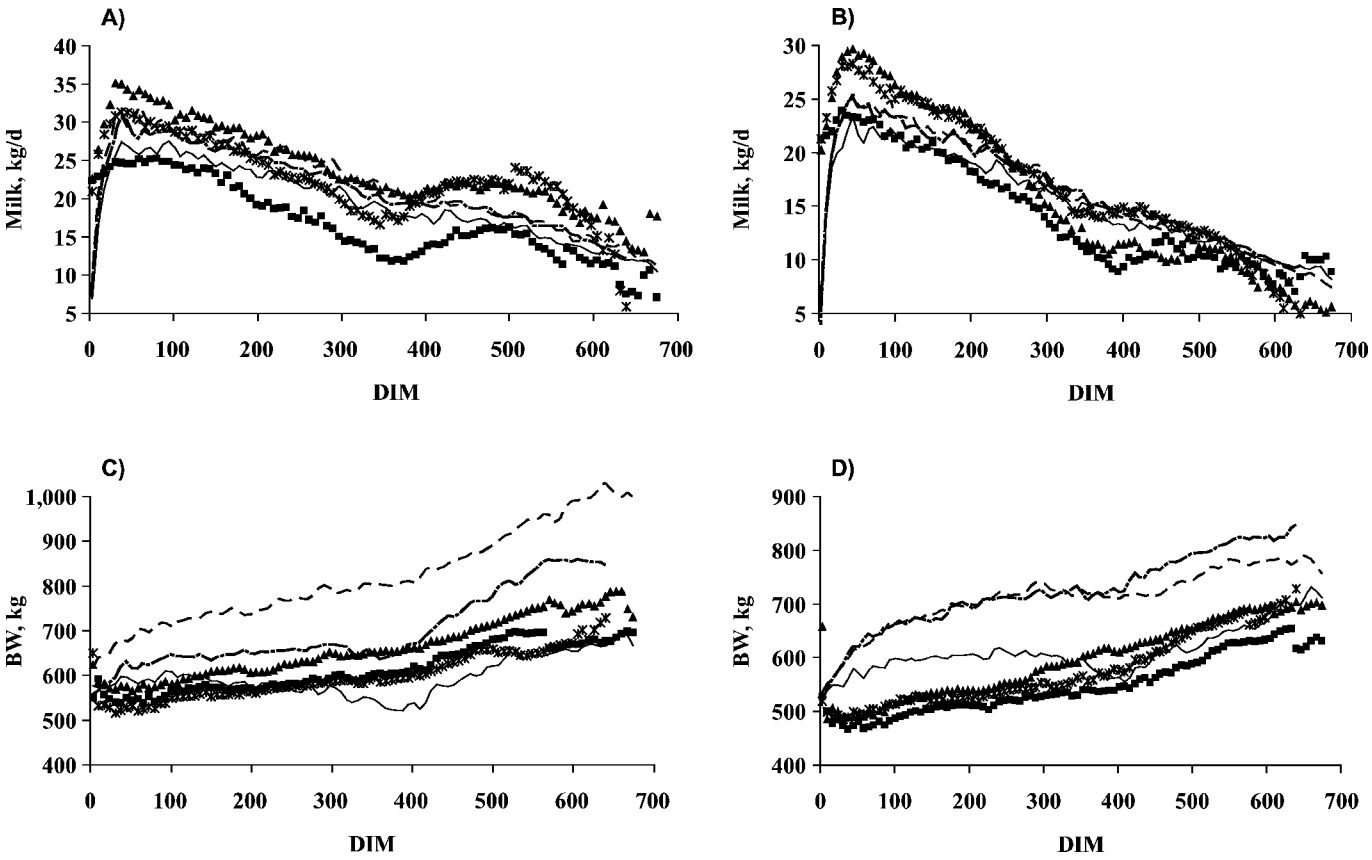

Figure 1. Predicted (line) and observed (points) milk yields and BW for cows of North American (A, C) and New Zealand (B, D) genotypes. Cows were fed 0 (■, —), 3 (✳, – · – · –), or 6 kg (▲,--) of concentrate DM per day for an extended lactation. Predictions were from Molly95.

In addition to challenges with the accuracy of milk yield and BW, milk composition predictions by Molly are inversely related to observed composition as lactation progresses (McNamara and Baldwin, 2000). Because predictions of milk composition are a desired output of the model, particularly if the model is to be used in markets with component-based pricing, this deficiency is critical. These prediction errors contribute to the prediction errors for BW change as observed by McNamara and Baldwin (2000). Because the protein and fat content of milk is underpredicted in early and late lactation and overpredicted at peak lactation, energy deposition in milk is too great at peak and too low in early and late lactation. This contributes to predictions of excessive weight loss at peak lactation and excessive gain in late lactation.

The objective of this work was to evaluate whether an alternative representation of mammary cell numbers, mammary enzyme activity, and the somatotropic axis would provide better predictions of milk and milk component yields and BW changes by Molly.

MATERIALS AND METHODS

The base model used for this work was that described by Baldwin (1995) with modifications as described by Hanigan et al. (2005). This model will subsequently be called Molly95. The revised model will be called Molly2006.

All simulations and parameter estimations were conducted using ACSL Optimize (AEgis Technologies Group, Austin, TX) using a variable-step second-order Runge-Kutta-Fehlberg numerical integrator. A maximum integration interval was set at 0.05 d. Parameters were estimated using a Nelder-Mead simplex optimization algorithm to maximize the log-likelihood function. Residual errors were assumed to be homogeneous.

Revisions undertaken in Molly2006 were as follows.

Total Mammary Cells

The aggregated representation of mammary synthetic activity originally described by Neal and Thornley (1983) and utilized in Molly95 was replaced

with a deaggregated representation of active and quiescent mammary cells based on the work of Vetharaniam et al. (2003a,b) with modifications of the representations of cells, cell division, and cell death.

In the model of Vetharaniam et al. (2003a,b), cell division was assumed to occur in binary fashion (i.e., secretory cells were derived solely from a fixed number of stem or progenitor cells), and cell division rates decayed to zero after parturition. However, the work of Dijkstra et al. (1997) demonstrated that cell division was exponential for many different species including goats (i.e., daughter cells also undergo division, generating additional secretory cells). Thus, it seems reasonable to conclude that cell division is exponential for cattle until evidence suggests otherwise.

In the model of Vetharaniam et al. (2003a,b), secretory cells were assumed to exist in active and quiescent states, with the latter subject to apoptosis. Cycling of cells to the quiescent pool was driven by reduced milking frequency, and the rate of cell senescence was affected by residence time in the inactive pool. In the Vetharaniam et al. (2003a,b) model, it was assumed that energy status of the animal did not affect cycling between the active and quiescent states or the rate of senescence; however, when the model was fitted to data derived from differing nutritional states, the rate of cell senescence was found to be significantly reduced on high-energy diets. Thus, both milking infrequently and dietary energy restriction were predicted to result in greater rates of cell death as compared with frequently milked or energy-sufficient states. These effects are manifested as a reduction in lactational persistency.

Aston et al. (1995) subjected several groups of cows to varying dietary energy inputs, which resulted in significant changes in milk yield. According to the observations of Vetharaniam et al. (2003a,b), the observed reductions in milk yield associated with low-energy diets should have resulted from greater cell death and lost productive capacity. But, cows that were energy-restricted in the first period of the study produced just as much milk in the second period as cows that were not energy-restricted. If cell apoptotic rates had been stimulated in period 1 as observed by Vetharaniam et al. (2003a,b), carryover effects should have been observed in the second period of the study. Because such effects were not observed, predicted changes in cell apoptotic rates are apparently not universally supported. As Vetharaniam et al. (2003b) observed such effects for studies conducted in New Zealand, it is unclear whether such a mechanism should be included. Given that inclusion of such a mechanism would clearly result in biased predictions for at least the observations of Aston et al. (1995), it was omitted in the current work.

Barnes et al. (1990) observed similar rates of decline in lactation yield (kg/mo) as lactation progressed for cows milked 3 times per day as for cows milked 2 times per day. These results suggest that the effects of milking frequency on cell senescence are not restricted to cells in the quiescent state as suggested by Vetharaniam et al. (2003a), because this would result in divergent lactational persistencies, which were not observed. These observations suggest that both active and quiescent cells are subject to apoptosis, with no apparent differences in the rates between the 2 pools.

Based on these observations, total mammary cells were represented as the balance of exponential cell division decaying as the animal approaches parturition and mass-action apoptosis. This balance was represented using a modified version of the model of Dijkstra et al. (1997), in which apoptosis was considered to occur in both the pre- and postparturient periods. Additionally, the equation was generalized for the entire lactation:

$$Q_{Cells} = Q_{Cells(T0)} \times e^{\left\{Sign \times \frac{\mu_{Division(T0)}}{K_{Decay}} \times \left[1 - e^{(-k_{Decay} \times |t|}\right] - (K_{Apoptosis} \times |t|)\right\}}, \quad [1]$$

where Q_{Cells} = the total number of mammary cells at time t, which was expressed as DIM with preparturient DIM assuming negative values; $T0$ = the time of parturition (DIM = 0); $Sign$ assumed a value of –1 for DIM < 0 and 1 for DIM ≥ 0; $\mu_{Division(T0)}$ = the cell division rate at $T0$; $K_{Apoptosis}$ = the rate of cell death at any point in time; and K_{Decay} = the rate of decay in μ with respect to t.

In the representation of Dijkstra et al. (1997), K_{Decay} could assume differing values pre- and postpartum. That representation was maintained herein, and the value of K_{Decay} was set by DIM using a conditional statement.

Because data for prepartum mammary growth are not currently available for cattle, the prepartum K_{Decay} was set to a value of 0.009 as observed by Dijkstra et al. (1997) for goats. Attempts were made to derive the postpartum K_{Decay} and $\mu_{Division(T0)}$, but the data were not adequate to uniquely derive both parameters. Because $\mu_{Division(T0)}$ represents the rate of cell growth, it has little influence on the shape of the postpartum cell growth curve. Therefore, it was fixed to 0.03 as observed by Dijkstra et al. (1997), and the postpartum value for K_{Decay} and $K_{Apoptosis}$ was derived from observed data herein.

The default value for $Q_{Cells(T0)}$ was arbitrarily set to 792, because this was the value derived for that parameter when Molly95 was fitted to observations from animals of the New Zealand genotype. However, this value should be set to represent the genetic potential of the

group of animals being simulated, and thus, accommodation was made for derivation of a separate value for the North American genotype.

Active and Quiescent Mammary Cells

The proportion of total mammary cells that were in the active state (P_{Active}) was defined on a fractional basis. The differential describing the change in P_{Active} with respect to t was as follows:

$$\frac{dP_{Active}}{dt} = F_{Quiescent,Active} - F_{Active,Quiescent}, \quad [2]$$

where $F_{Quiescent,Active}$ and $F_{Active,Quiescent}$ = the fractional flux of cells from the quiescent pool to the active pool and the fractional flux of active cells to the quiescent pool, respectively. These fluxes were defined as follows:

$$F_{Quiescent,Active} = K_{Quiescent,Active}K_{Fill}P_{Quiescent}, \text{ and} \quad [3]$$

$$F_{Active,Quiescent} = \frac{K_{Active,Quiescent}}{K_{Fill}}P_{Active}, \quad [4]$$

where $K_{Active,Quiescent}$ and $K_{Quiescent,Active}$ = the rate parameters for cycling between the active and quiescent states. $K_{Active,Quiescent}$ was set to 0.11 based on the observations of Vetharaniam et al. (2003a), and $K_{Quiescent,Active}$ was set to 0.3, which was less than that derived by Vetharaniam et al. (2003a). This reduction was adopted to reflect the reference state of twice-daily milking and allow for significant increases in activity in response to more frequent milking. This change is arbitrary at this point and should be derived from observational data. Additionally, K_{Fill} = the scalar for inactivity conversion associated with udder fill as originally defined by Neal and Thornley (1983) and applied in Molly95. K_{Fill} assumes a value of 1 with continuous milking and a value of less than 1 for intermittent milking.

Furthermore, P_{Active} at any point in time was determined by numerical integration of equation 2 from an initial starting proportion (iP_{Active}):

$$P_{Active} = \int_0^t dP_{Active} + iP_{Active}, \quad [5]$$

where iP_{Active} was set to 0.75, because this closely approximated the average value assumed during a lactation simulation. This value is less than that of Vetharaniam et al. (2003a), reflecting the altered setting for $K_{Quiescent,Active}$. The proportion of quiescent cells ($P_{Quiescent}$) was then derived by difference:

$$P_{Quiescent} = 1 - P_{Active}, \quad [6]$$

where P_{Active} and $P_{Quiescent}$ were used to calculate the number of active (Q_{Active}) and quiescent ($Q_{Quiescent}$) cells:

$$Q_{Active} = P_{Active}Q_{Cells} \text{ and} \quad [7]$$

$$Q_{Quiescent} = Q_{Cells} - Q_{Active}. \quad [8]$$

Mammary Enzyme Activity

Equations 1 to 8 describe the mass of active mammary cells present at any point in time. However, to maintain continuity with the original representation of mammary enzyme in Molly95, a factor relating mammary enzyme and active cell numbers was required. Because changes in cell numbers with respect to stage of lactation account for the general increase and decline in mammary capacity in the revised model, total mammary enzyme activity was related to active cells via a scalar ($P_{Enz,Cell}$) that represented the enzyme activity per active cell with modifications in activity influenced by lactation hormone (Q_{LHor}, defined below):

$$Q_{Enz,Cell} = P_{Enz,Cell}Q_{LHor}{}^{\phi}Q_{Active}, \quad [9]$$

where ϕ was used to adjust sensitivity to Q_{LHor}. Additionally, $P_{Enz,Cell}$ was initially set to 12 to yield enzyme quantities roughly equivalent to the representation in Molly95. This parameter was subsequently derived by fitting to experimental data.

It was assumed that the observed changes in milk protein and fat content with respect to stage of lactation are driven by alterations in either the osmotic balance relative to lactose concentrations or that lactose yield per mammary cell is not constant as assumed for the other milk components. To address the problem, the maximal velocity (Vm) for milk lactose synthesis ($Vm_{Gl,Lm}$) was described as a function of DIM using the equation of Dijkstra et al. (1997):

$$Vm_{Gl,Lm} = Vm_{Gl,Lm(T=0)} \times e^{\left\{\frac{k_{Vm,Syn}}{k_{Vm,Decay}}\times\left[1-e^{(k_{Vm,Decay}\times t)}\right]-(k_{Vm,Deg}\times t)\right\}}, \quad [10]$$

where $Vm_{Gl,Lm(T=0)}$ = the Vm at parturition and was initially set equivalent to the value of the original $Vm_{Gl,Lm}$ (0.0025) as described by Baldwin et al. (1987b).

Additionally, $k_{Vm,Syn}$, $k_{Vm,Decay}$, and $k_{Vm,Deg}$ were initially set to 0.005, 0.03, and 0.0005 and subsequently derived.

Lactation Hormone

Lactation hormone was altered from its original representation to reflect aspects of the somatotropin axis

(primarily IGF-I), including a representation of the effects of photoperiod. The differential describing Q_{LHor} with respect to t was:

$$\frac{dQ_{LHor}}{dt} = F_{LHor,Syn} - F_{LHor,Deg}, \quad [11]$$

where Q_{LHor} was determined from equation [11] by numerical integration starting with an initial mass of lactation hormone (iQ_{LHor}):

$$dQ_{LHor} = \int_0^t dQ_{LHor} + iQ_{LHor}, \quad [12]$$

where iQ_{LHor} was set to 1.0, which was defined as the reference state for Molly95. Synthesis ($F_{LHor,Syn}$) and catabolism ($F_{LHor,Deg}$) of Q_{LHor} were defined as follows:

$$F_{LHor,Syn} = \frac{Vm_{LHor,Syn}}{1 + \left(\frac{k_{AA}}{C_{AA}}\right)^{\chi} + \left(\frac{k_{Glc}}{C_{Glc}}\right)^{\chi} + \left(\frac{k_{Adipose}}{Q_{Adipose}}\right)^{\lambda}} \quad [13]$$

and

$$F_{LHor,Deg} = (K_{LHor,Deg} + K_{LHor,PP})Q_{LHor}, \quad [14]$$

where χ and λ were included to allow sensitivity adjustments; χ was set to a value of 2 based on an appraisal of responses of Q_{LHor} to nutrient supply, and λ was set to 2.97 based on a preliminary fit to the observed data. To derive χ would require knowledge of the independent responses of the somatotropic axis to AA and glucose, which is beyond the scope of this work. The concentrations of glucose (C_{Glc}) and AA (C_{AA}) and the mass of adipose tissue ($Q_{Adipose}$) were represented as positive affecters of lactation hormone synthesis. The first 2 terms reflect the effects of energy and AA balance on somatotropin and IGF-I secretion (Chew et al., 1984; McGuire et al., 1992; Hatfield et al., 1999; Kobayashi et al., 2002). The adipose mass term reflects the relationship among fat mass, leptin secretion, and the subsequent effect of leptin on the somatotropic axis (Chilliard et al., 2000; McMahon et al., 2001; Morrison et al., 2001). The latter element reflects the concept of a set point or homeostasis for adiposity. Such a concept is supported by observational data (Chilliard et al., 2000). Additionally when such a concept is omitted from the model, stability issues occur, including a propensity to gain or lose excessive amounts of fat mass prior to changes in milk yield (Figure 1). Because $k_{Adipose}$ essentially represents the set point for adipose mass, it must be scaled to BW, which was accomplished using a derivation of the equation of Waltner et al. (1994):

$$k_{Adipose} = (0.21 \times BW_{T0}) + (36 \times BCS_{Target}) - 122.1, \quad [15]$$

where BW_{T0} = the postpartum BW and BCS_{Target} = the target BCS. Setting the latter to a value of 3.0 was found to yield appropriate declines and recovery in fat mass as lactation progressed.

It was found that $Vm_{LHor,Syn}$ represented the maximal rate of synthesis and was arbitrarily set to a value of 4 to yield a synthesis rate of 1 in the reference state, and $K_{LHor,Deg}$ and $K_{LHor,PP}$ in equation 14 represented the basal rate of degradation of Q_{LHor} and the effects of daylength on degradation, respectively. Inclusion of the latter is supported by the observations of elevated IGF-I concentrations during long days, which were apparently associated with a reduction in clearance of IGF-I (Dahl and Petitclerc, 2003; Kendall et al., 2003). Consistent with the settings for the $Vm_{LHor,Syn}$, $K_{LHor,Deg}$ was set to a value of 1.

Additionally, $K_{LHor,PP}$ was calculated from daylength as follows:

$$K_{LHorPP} = \left(\frac{12}{Daylength} - 1\right) \times K_{Daylength}, \quad [16]$$

where $K_{Daylength}$ = a scalar for adjusting the magnitude of the effect. Daylength was calculated as:

$$Daylength = 12 + [12 \times sin(\{DayofYear - Jan1toSprEq\} \times SinDays) \times Latitude/90], \quad [17]$$

where *DayofYear* ranged from 1 (January 1) to 365 (December 31); *Jan1toSprEq* = the number of days from January 1 to the spring equinox (79 in the northern hemisphere and –101 in the southern hemisphere); and *Latitude* = the degrees of latitude at the location of the trial. *SinDays* was set to 0.017214 (calculated as $2\pi/365$) to achieve a complete sin wave during the calendar year. No accommodation was made for manual manipulation of daylength, but such effects could easily be encoded in equation [17].

Parameter Estimation and Model Evaluations

One data set was used for initial model evaluations and subsequent parameter estimations, and a second independent data set was used for evaluations of the revised model after parameter estimations were completed. The first data set consisted of observations that were collected as part of an extended lactation trial conducted in New Zealand (Kolver et al., 2006). Multiparous Holstein-Friesian cows of North American or New Zealand genotypes were fed 0, 3, or 6 kg of concentrate

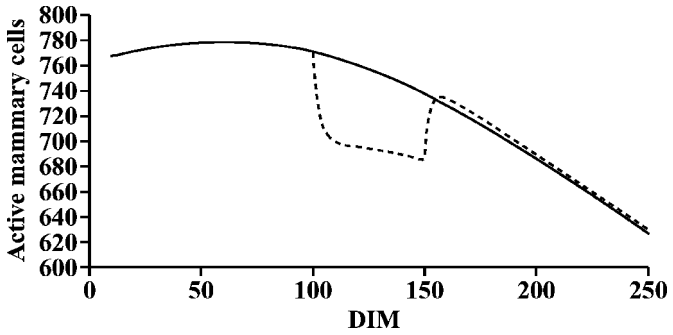

Figure 2. Predicted active mammary cells with $K_{Quiescent,Active}$ set to the default value of 0.3 (solid line) for the first 100 DIM, to 0.2 from 100 to 150 DIM, and to 0.3 after 150 DIM (dashed line).

Table 1. Parameter estimates for Molly95 derived by fitting to an extended lactation trial[1]

Parameter	Estimate	SD
Q_{UCells} (NZ)	792	2.3×10^{-2}
Q_{UCells} (NA)	1,031	6.3×10^{-3}
$Vm_{Gl,Lm}$	2.5×10^{-3}	4.8×10^{-8}
$Vm_{Aa,Pm}$	1.5×10^{-3}	3.5×10^{-8}
$Vm_{Fa,Ts}$	0.81	1.9×10^{-5}
K_{Lhor}	3.7×10^{-3}	1.4×10^{-7}
$K_{Gl,Cd}$	8.0×10^{-3}	1.5×10^{-7}
$K_{Pun,Ur}$	2,140	2.3
F_{Lm}	4.8×10^{-2}	1.1×10^{-6}

[1]Q_{UCells} was fit by genotype, in which genotypes were North American (NA) and New Zealand (NZ) Holstein-Friesians.

DM daily throughout a 600-d lactation. Feed composition, milk yield, milk composition, BW, and BCS were assessed periodically throughout the trial. Blood glucose, NEFA, and urea N were also assessed at various points during the lactation. Intake was calculated using the equation of Holmes et al. (2002). The second data set consisted of the observations of Aston et al. (1995). Observed data included DM intake, diet composition, milk yield, milk composition, and BW.

For fitting purposes, observed dietary composition and intakes were averaged by week and treatment group and used as inputs to the model. Full nutrient inputs required by the model were derived from proximate analyses as described by Hanigan et al. (2005). Where needed, observed nutrient composition of ingredients was supplemented with NRC (2001) tabular values to provide a full input data set.

Observed BW and BCS at calving were used to define initial parameters for the model directly, or in the case of BCS, using the equations of Waltner et al. (1994). The model was evaluated against weekly mean observations by treatment.

Residuals were calculated as observed minus predicted, and root mean square prediction errors (**RMSPE**) and a decomposition of those errors were calculated from the residuals as previously described (Roseler et al., 1997). Slope bias was determined as a function of DIM.

RESULTS AND DISCUSSION

Model Stability and Parameterization

The revised model code was assessed for stability by perturbing model parameters, running to steady state, resetting the parameter to the reference value, and running to steady state again. The model was found to stabilize after the initial change and to return to the original state when the input was reset to the original value, suggesting that the mammary cell subsystem was properly coded and produced stable solutions (Figure 2).

Having assessed model stability, Molly2006 was fitted to the extended lactation data to derive parameter estimates for mammary cells and enzyme activities. For reference purposes, Molly95 was also fitted to the same data. By fitting both models to the data, potential mean bias associated with prior parameter estimates in Molly95 should be removed, allowing a direct comparison of the potential benefits of the changes in model structure. Parameter estimates for Molly95 are given in Table 1, and predictions of milk yield and BW are presented in Figure 1. Predicted patterns (using Molly2006) for total cells, active cells, enzyme activity, milk yield, and milk composition are presented for 1 treatment group in Figure 3. Parameter estimates for the revised model are given in Table 2. Prediction errors for both models are summarized in Table 3. Molly2006 was fitted with and without using the fermentation stoichiometries associated with varying concentrate inclusions. Because a slight improvement in fit was observed when the simple scheme using a single set of fermentation stoichiometries was adopted, it was used for all subsequent analyses.

With the exception of $\mu_{Division(T0)}$ and the prepartum K_{Decay} for Molly2006 (previously discussed), the extended lactation data were adequate to define the parameters for both models as evidenced by standard deviations for the parameter estimates that were less than half of the estimated value.

The estimated postpartum K_{Decay} was found to be 0.44 ± 0.19. The relatively great standard deviation of the estimate is likely due to the rapidity of apparent cessation of mammary cell growth after parturition. A value of 0.44 results in cessation of cell growth by 6 DIM with a 5% increase in cell numbers after parturition. Because the difference in the growth curve is minimal for parameter estimates greater than 0.3, there is marginal ability to define the parameter (see Figure 4). Dijkstra et

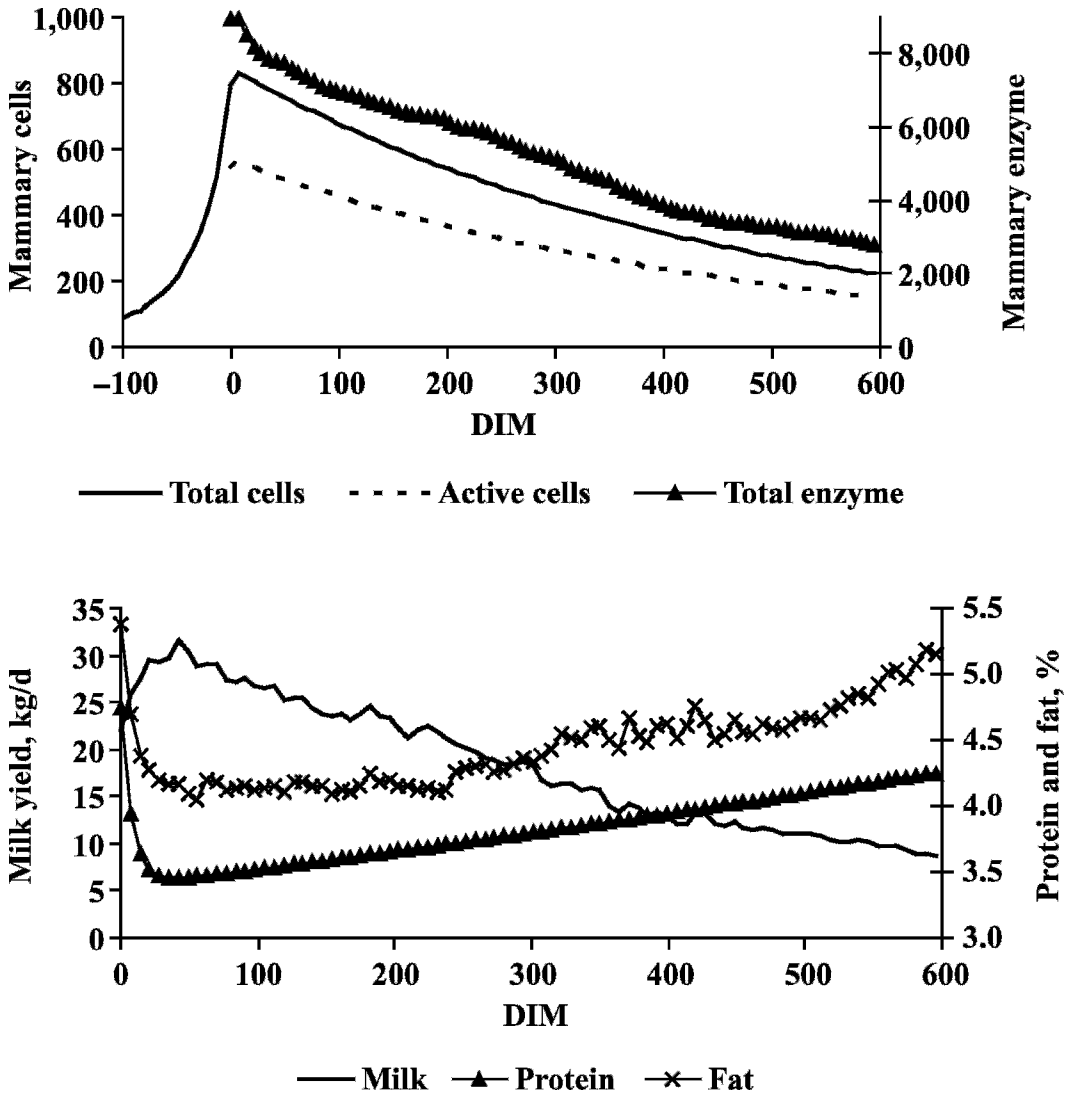

Figure 3. Predictions of total and active mammary cells, total mammary enzyme activity, milk yield, and milk composition by Molly2006 after fitting to the extended lactation data set. Inputs for the simulation were those observed for cows of the New Zealand genotype fed 3 kg of concentrate DM per day.

al. (1997) estimated a value of 0.141 for K_{Decay} when fitting to mammary DNA data derived from goats. Such a value is indicative of 18 d of postpartum cell growth and a 17% increase in total mammary DNA over that present at parturition. It is not clear whether the apparent differences in cow and goat data result from the method of derivation or reflect species differences.

The derived value for the postpartum K_{Decay} is also much greater than values derived by Val-Arreola et al. (2004) from milk production data of dairy cattle. However, when deriving such values from milk yield observations, the derived value represents a combination of cell division and cell differentiation. Because cell differentiation was accommodated in the current effort via the time-dependent description of lactose enzyme activity, the derived decay rate likely is a more accurate representation of the true decay rate and is consistent with peak protein yields occurring at or very shortly after parturition, as observed in the extended lactation trial.

It was found that $K_{Apoptosis}$ was 2.25×10^{-3} for cows of the New Zealand genotype and 1.47×10^{-3} for cows of the North American genotype. Both estimates were less than the value of 4.0×10^{-3} observed by both Val-Arreola et al. (2004) for dairy cows and Dijkstra et al. (1997) for goats.

These 4 parameters define the shape of the lactation curve and are thus the most likely to be affected by

Table 2. Mammary cell and enzyme parameter estimates for the revised model derived from an extended lactation trial[1]

Parameter	Estimate	SD
Mammary cells		
$Q_{Cells(T0)}$ (NZ)	792	—
$Q_{Cells(T0)}$ (NA)	955	14
$K_{Decay\ (Postpartum)}$	0.444	0.188
$K_{Apoptosis}$ (NZ)	2.25×10^{-3}	3.76×10^{-5}
$K_{Apoptosis}$ (NA)	1.47×10^{-3}	3.64×10^{-5}
Mammary enzymes		
$P_{Enz,Cell}$	11.8	0.4
$Vm_{Gl,Lm(T=0)}$	1.71×10^{-3}	5.13×10^{-5}
$k_{Vm,Syn}$	0.038	0.006
$k_{Vm,Decay}$	0.118	0.011
$k_{Vm,Deg}$	3.84×10^{-4}	9.59×10^{-6}
$Vm_{Aa,Pm}$	2.18×10^{-3}	4.37×10^{-5}
Endocrines		
ϕ	0.509	0.024
$K_{Daylength}$ (NZ)	0.219	0.035
$K_{Daylength}$ (NA)	0.393	0.040
Other		
$K_{Gl,Cd}$	1.07×10^{-2}	4.54×10^{-4}
$K_{Pun,Ur}$	2,163	24
$Vm_{Fa,Ts}$	0.461	0.019
K_{Bas}	2.27	0.04

[1]$Q_{Cells(T0)}$, $K_{Apoptosis}$, and $K_{LHor,PP}$ were fit by genotype, in which genotypes were North American (NA) and New Zealand (NZ) Holstein-Friesians.

parity, particularly primiparous vs. multiparous. However, because the data used for parameterization only included multiparous cows, the resulting parameter estimates should be evaluated with primiparous data to determine whether an alternate primiparous parameter set is warranted.

The changes undertaken in the representation of milk lactose resulted in milk composition that more accurately matched the patterns typically observed for dairy cattle. Milk fat and protein content were found to be elevated in early and late lactation and less at peak lactation, resulting in greater energy output in early and late lactation and reduced energy output at peak lactation when expressed per unit of milk volume. This contributed to greater accuracy of BW and BCS predictions.

Mammary enzyme activity was related to the predicted somatotropin axis as represented by the effects of ϕ in equation [9], allowing greater changes in predicted milk component yields in response to nutrition than predicted by Molly95. Although the modified representation of endocrine effects improved model accuracy with respect to the observed data (see below) and thus appear to be consistent with the hypothesized mechanism of action and observed production responses to endocrine infusions, the effects of nutritional state and endocrine profiles on enzyme activities are not generally supported by more invasive measurements of mammary enzyme activity (Sorensen and Knight, 2002; Norgaard et al., 2005).

Although the existence of a set point or homeostatic point for adiposity has not been conclusively demonstrated, the hypothesis is generally consistent with observational data, including the positive relationship between adiposity and leptin secretion and the negative effect of leptin on intake (Chilliard et al., 2000). Because leptin is positively correlated with somatotropin secretion and somatotropin influences milk yield (Chilliard et al., 2000; McMahon et al., 2001; Morrison et al., 2001), the representation seems appropriate. In support of inclusion of such a concept, removal of the affects by altering the setting for λ to a value of 0 results in a 33% reduction in the log-likelihood function derived from predictions of BW, BCS, milk yield and composition, and blood metabolite concentrations. The major changes were associated with increases in RMSPE of more than 50% for BW, BCS, and milk yield and an increase of 20% for predictions of blood glucose. Perhaps more importantly, slope bias for BW, BCS, milk yield, and blood glucose increased by 17, 32, 20, and 12 percentage units, respectively, indicating severe systematic prediction bias. And this degradation in accuracy was evident at λ settings of 2 and 1 with log-likelihood reductions of 12 and 3%, respectively, and corresponding increases in RMSPE and the percentage of prediction errors associated with slope bias for body mass, BCS, and milk yield. Additional work is required to refine the estimate for λ, which will be addressed in future work; however, assuming the current structure of Molly is appropriate, the necessity of inclusion of a set-point concept appears to be well-supported.

Photoperiod effects were also observed, as evidenced by a positive estimate for $K_{Daylength}$, which is consistent with previous observations (Dahl and Petitclerc, 2003; Kendall et al., 2003). The variable milking interval portion of the model still requires parameterization, which is planned for future work. Additionally, the mammary cell growth curves for primiparous animals could be different than for the multiparous animals used for parameterization. If so, additional parameterization work would be required to derive the appropriate settings for the younger animals, because they were not represented in the current work.

Model Prediction Accuracy

Root mean square prediction errors associated with Molly2006 were generally halved for predictions of milk yield and composition, blood metabolite concentrations, and BW and BCS relative to those from Molly95. Exceptions were milk lactose content, blood NEFA concentrations, and BUN concentrations (see Table 3). Because

Table 3. Prediction errors for Molly95 and Molly2006 after fitting to the extended lactation data and when evaluated using the data of Aston et al. (1995)[1]

	Extended lactation data set			Aston et al. (1995) data
Variable	Molly95	Molly2006[2]	Molly2006[3]	Molly2006[4]
Log likelihood	266	4,245	4,216	NA[5]
RMSPE, % of observed mean				
Milk yield	17.0	7.7	7.6	3.82
Lactose yield	17.7	8.3	8.3	3.45
Protein yield	22.3	9.4	9.4	4.45
Fat yield	19.8	11.7	11.7	7.68
Lactose, %	5.6	5.1	5.1	
Protein, %	13.4	5.4	5.4	3.46
Fat, %	15.7	9.2	9.4	8.29
Blood glucose concentration	19.8	12.4	12.7	
Blood NEFA concentration	83.3	87.5	87.4	
BUN concentration	20.7	20.8	20.8	
BW	19.7	5.7	5.7	3.66
BCS	53.2	21.7	22.2	15.97
Mean bias, % of MSPE				
Milk	0.02	0.04	0.04	0.36
Lactose	1.1	0.001	0.001	3.97
Protein	1.0	0.2	0.2	0.26
Fat	1.5	0.01	0.001	33.69
Lactose, %	32.2	16.0	16.0	
Protein, %	18.2	0.2	0.2	0.56
Fat, %	52.9	0.3	0.3	35.67
Blood glucose concentration	1.1	0.3	0.2	
Blood NEFA concentration	0.05	0.2	0.2	
BUN concentration	1.5	1.8	1.8	
BW	63.7	0.1	0.1	31.93
BCS	40.8	38.5	36.8	56.64
Slope bias, % of MSPE				
Milk	1.2	1.6	1.6	1.95
Lactose	5.1	3.0	3.0	15.22
Protein	15.4	10.1	10.0	69.33
Fat	18.7	7.7	7.0	45.20
Lactose, %	0.00006	0.00002	0.00003	
Protein, %	34.2	1.1	0.9	0.00
Fat, %	3.2	9.9	11.9	43.83
Blood glucose concentration	84.2	65.6	67.3	
Blood NEFA concentration	6.7	6.2	5.6	
BUN concentration	40.3	43.2	43.2	
BW	20.2	4.0	2.5	43.88
BCS	47.9	17.3	19.6	42.79

[1]Root mean square prediction errors (RMSPE) are expressed as a percentage of the observed mean. Mean and slope bias are expressed as a percentage of the mean square prediction error (MSPE).

[2]Model parameters were derived with stoichiometries for VFA production held constant regardless of grain supplementation.

[3]Model parameters were derived with stoichiometries for VFA production set to mixed diet values for 3 and 6 kg/d of grain supplementation and to grass forage values for 0 kg/d of grain supplementation.

[4]Predictions were from Molly2006 with fixed VFA stoichiometries and the following setting modifications: $Q_{Cells(T0)} = 799$; $Vm_{Gl,Lm(T=0)} = 1.66 \times 10^{-3}$; and $Vm_{Aa,Pm} = 1.92 \times 10^{-3}$.

[5]NA = not applicable.

both models assume constant milk lactose content, it is not surprising that those predictions did not improve. And given that both models were fitted to the data, improvements in RMSPE reflect the changes in model coding and thus suggest that the changes were beneficial. More specifically, use of equation [10] provided the desired relationship among the milk components, resulting in high milk fat and protein in early and late lactation and low contents at peak lactation (Figure 3), which significantly reduced prediction errors.

The proportions of RMSPE associated with mean bias were substantially reduced for the content of lactose, protein, and fat, and slope bias was reduced for protein content and milk protein and fat yields, reflecting the

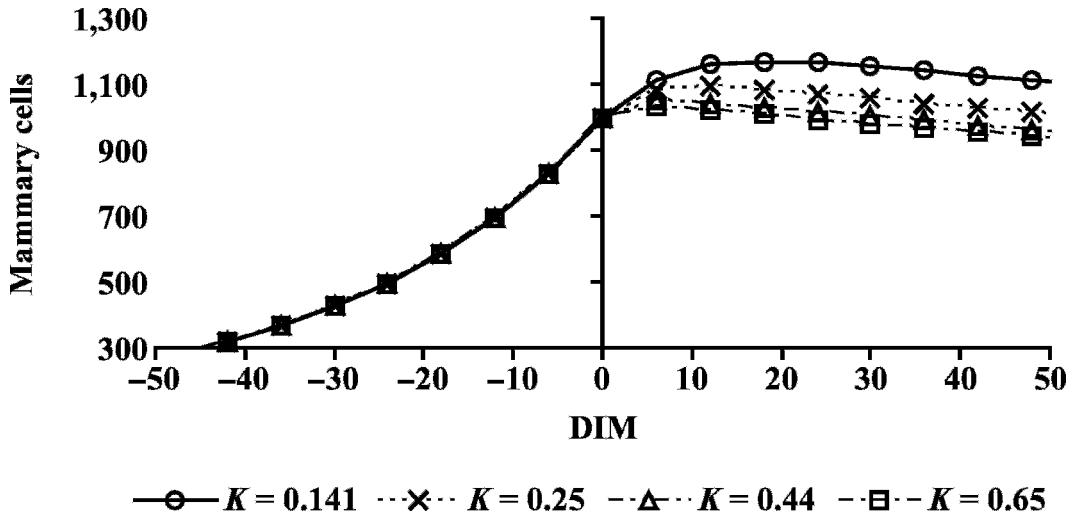

Figure 4. Predicted mammary cells using the model of Dijkstra et al. (1997) with the following settings: $Q_{Cells(T0)}$ = 1,000; $\mu_{Division(T0)}$ = 0.03; prepartum K_{Decay} = 0.009; $K_{Apoptosis}$ = 0.002; and varying postpartum K_{Decay}.

inherent problems with the Molly95 representation of milk component percentages.

Residual errors plotted against DIM for predictions of milk and milk lactose yields are presented in Figure 5, and errors for milk protein and fat yield predictions are presented in Figure 6. There were no apparent patterns to milk yield or milk component yield residuals by dietary treatment, supporting the hypothesis stated

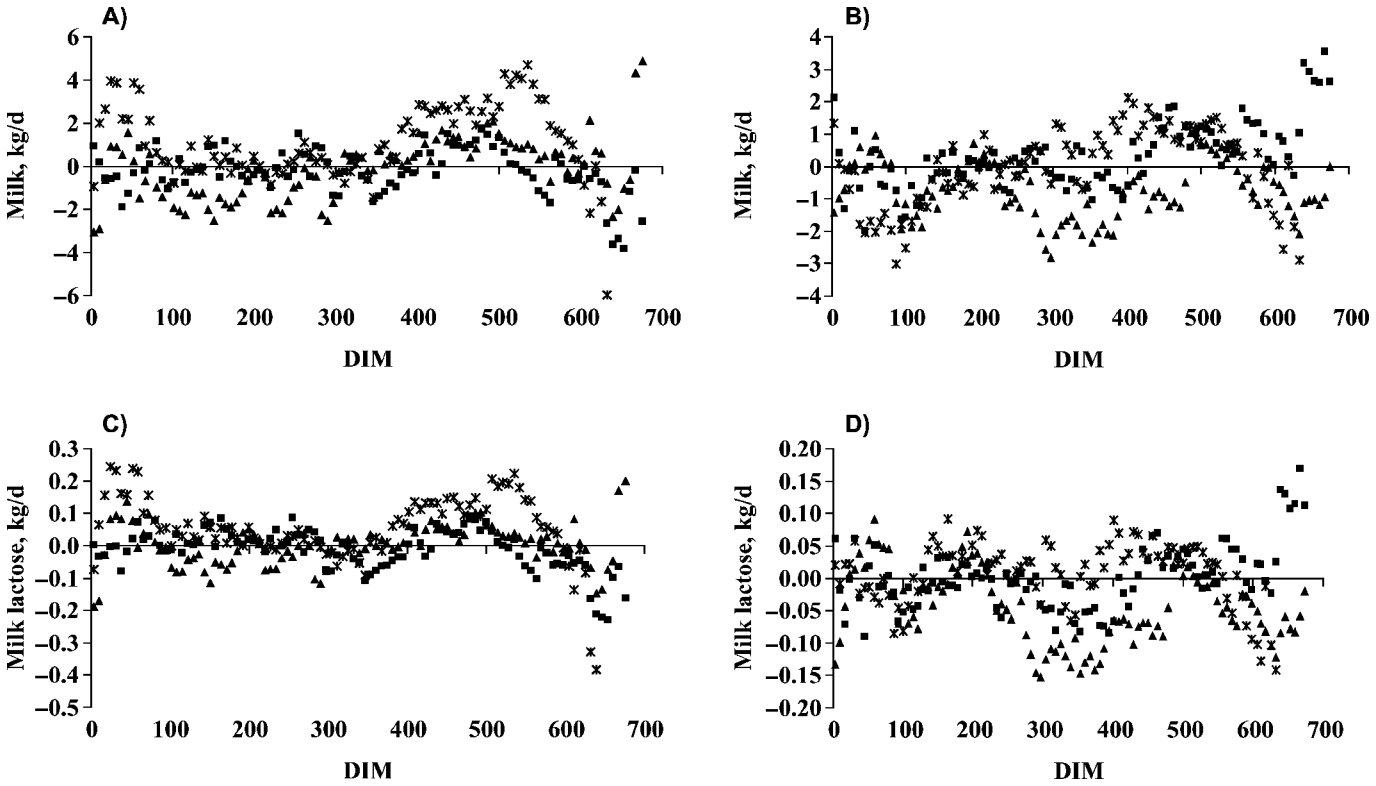

Figure 5. Residual errors for predictions of milk and milk lactose yields for cows of North American (A, C) and New Zealand (B, D) genotypes. Cows were fed 0 (■), 3 (✳), or 6 kg (▲) of concentrate DM per day for an extended lactation. Predictions were from Molly2006, with parameters listed in Table 2.

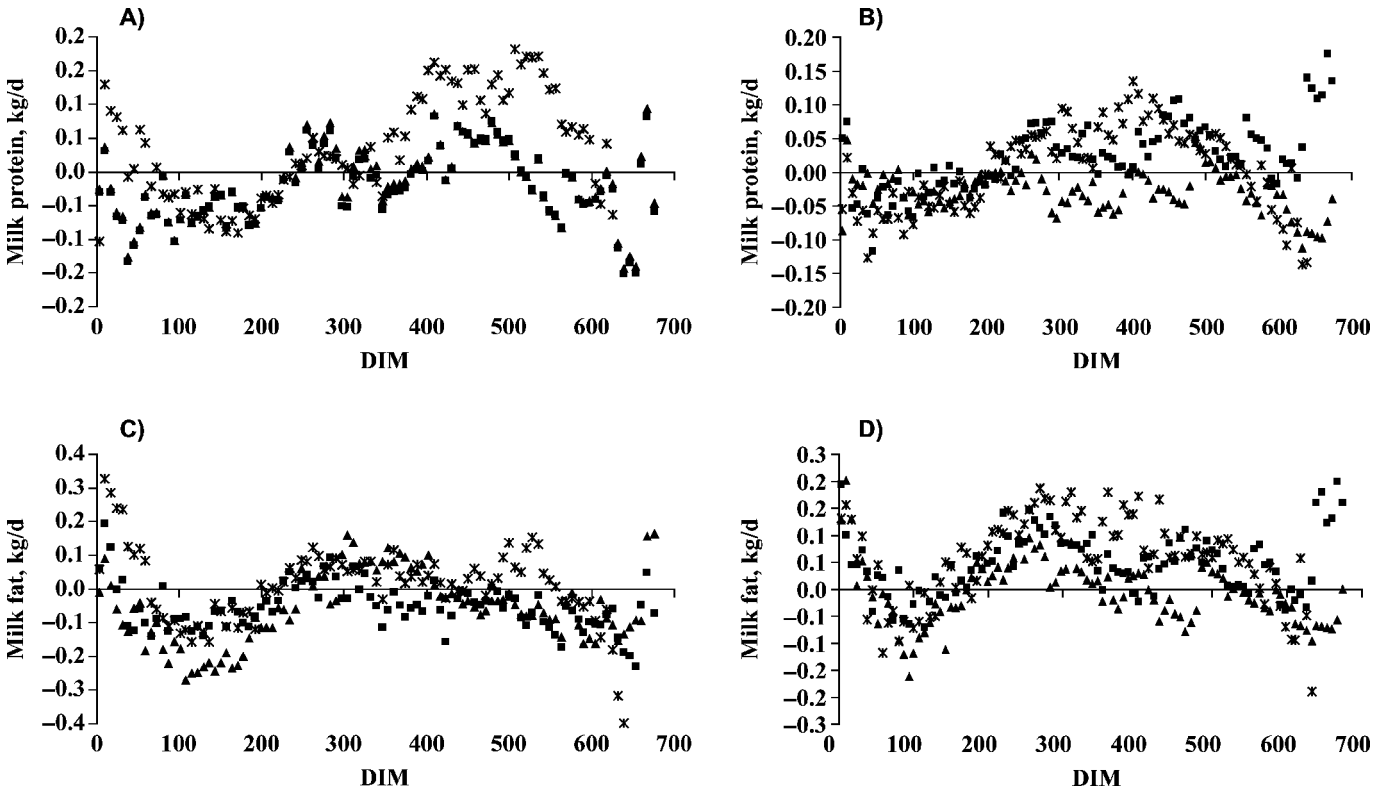

Figure 6. Residual errors for predictions of milk protein and fat yields for cows of North American (A, C) and New Zealand (B, D) genotypes. Cows were fed 0 (■), 3 (✳), or 6 kg (▲) of concentrate DM per day for an extended lactation. Predictions were from Molly2006, with parameters listed in Table 2.

previously that rates of secretory cell apoptosis were not affected by dietary status (i.e., reductions in milk yield associated with nutritional insufficiency apparently result from changes in enzyme activity per cell and not the number of mammary cells). Such a finding is consistent with the observations of Sorensen and Knight (2002) but inconsistent with the observations of Vetharaniam et al. (2003a,b). The difference in the current findings relative to those of Vetharaniam et al. (2003a,b) likely reflects the more extensive representation of mammary enzyme activity in the current model as compared with that in the model of Vetharaniam et al. (2003a,b).

Although the accuracy of predictions of all milk components and milk yield were improved, systematic deviations in residuals for milk, milk lactose, and milk protein yield predictions were apparent during the latter part of the second season for cows of both genotypes. Because the effects of gestational nutrient requirements were not considered in these simulations, it seems likely that overpredictions of milk, milk lactose, and milk protein yields at the end of the second season may at least partially emanate from nutrient use by the gravid uteris (Bell et al., 1999).

However, gestational nutrient use would only explain a portion of systematic errors in milk fat production. Rates of milk fat synthesis are underpredicted from 0 to 50 DIM, overpredicted from 50 to 200 DIM, and overpredicted again from about 550 DIM until the end of lactation. Overpredictions after 550 DIM may be due to gestational requirements, but obviously that is not the cause of errors during the first season when animals were not pregnant.

Underpredictions of milk fat yield and content in early lactation are likely due to underpredictions of blood NEFA concentrations and thus fatty acid removal and use for milk fat synthesis (Figure 7). In the model, NEFA and triacylglycerol (**TAG**) are considered as a common pool. Because TAG concentrations were not measured in the extended lactation study, it cannot be fully determined whether changes in blood fatty acids were appropriate with respect to observed values. If the representation of fatty acids was deaggregated to represent NEFA and TAG independently, the rise in NEFA in early lactation could be used to drive the elevations in milk fat. Because NEFA concentrations fall, they would represent a lesser proportion of total fatty acids due to the more stable contribution of TAG to the

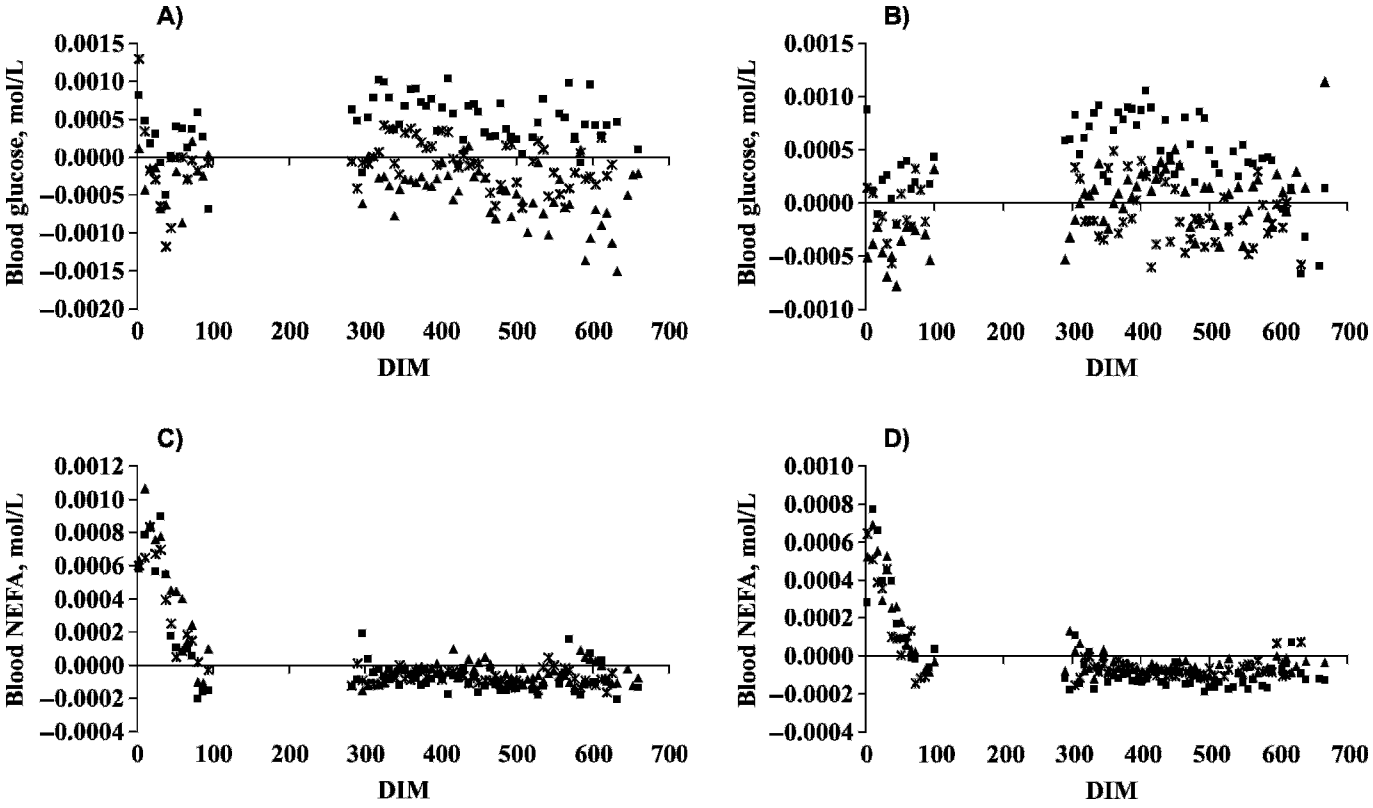

Figure 7. Residual errors for blood glucose and NEFA concentrations for cows of North American (A, C) and New Zealand (B, D) genotypes. Cows were fed 0 (■), 3 (✳), or 6 kg (▲) of concentrate DM per day for an extended lactation. Predictions were from Molly2006, with parameters listed in Table 2.

total pool. Thus, it may be possible to drive milk fat output up in early lactation without significantly reducing output at peak lactation.

Residual errors for blood glucose concentrations are presented in Figure 7. No apparent systematic prediction errors were obvious for either genotype during either season, suggesting that the mechanisms representing blood glucose metabolism and regulation in the model are appropriate in structure and have been parameterized appropriately.

As might be expected, improved predictions of milk component yields and removal of systematic bias improved the accuracy of predictions of energy partitioning, and this is reflected in reductions in RMSPE for BW and BCS (Table 3). In particular, reductions in errors of milk fat would be expected to have the greatest contribution to improvements in predictions of body energy stores. Such errors are consistent with the observation of mean and slope bias for Molly95, which was reduced for Molly2006. Although predictions were improved over those of Molly95, systematic bias in predictions is still apparent (Figure 8) for both BW and BCS. In particular, the rate of BW and BCS loss in early lactation is underpredicted, resulting in overpredictions of score at peak lactation. Although the model appears to underpredict weight recovery as lactation progresses, the prediction errors for BCS do not change, suggesting that after peak lactation, the model predicts fat metabolism with some accuracy but exhibits bias in predictions of lean mass. The latter could certainly be associated with fetal growth. Some systematic bias in both BW and BCS predictions was associated with dietary supplementation rate. Simulations of the unsupplemented animals resulted in underpredictions of BW and BCS for most of the lactation.

Because the ability to reference the model to previous settings for mammary cells (denoted as *Ucells* in Molly95) is critical to maintaining a reference point with previous work, the relationship between $P_{Enz,Cell}$ and $Q_{Cells(T0)}$ was derived by iteratively fitting $Q_{Cells(T0)}$ to the extended lactation milk yield while manually changing the setting for $P_{Enz,Cell}$. In this manner, the relationship between $Q_{Cells(T0)}$ and $P_{Enz,Cell}$ can be estimated and used for setting enzyme activities that approximate previous model settings (e.g., if previous work used a *Ucells* setting of 1,000, and this resulted

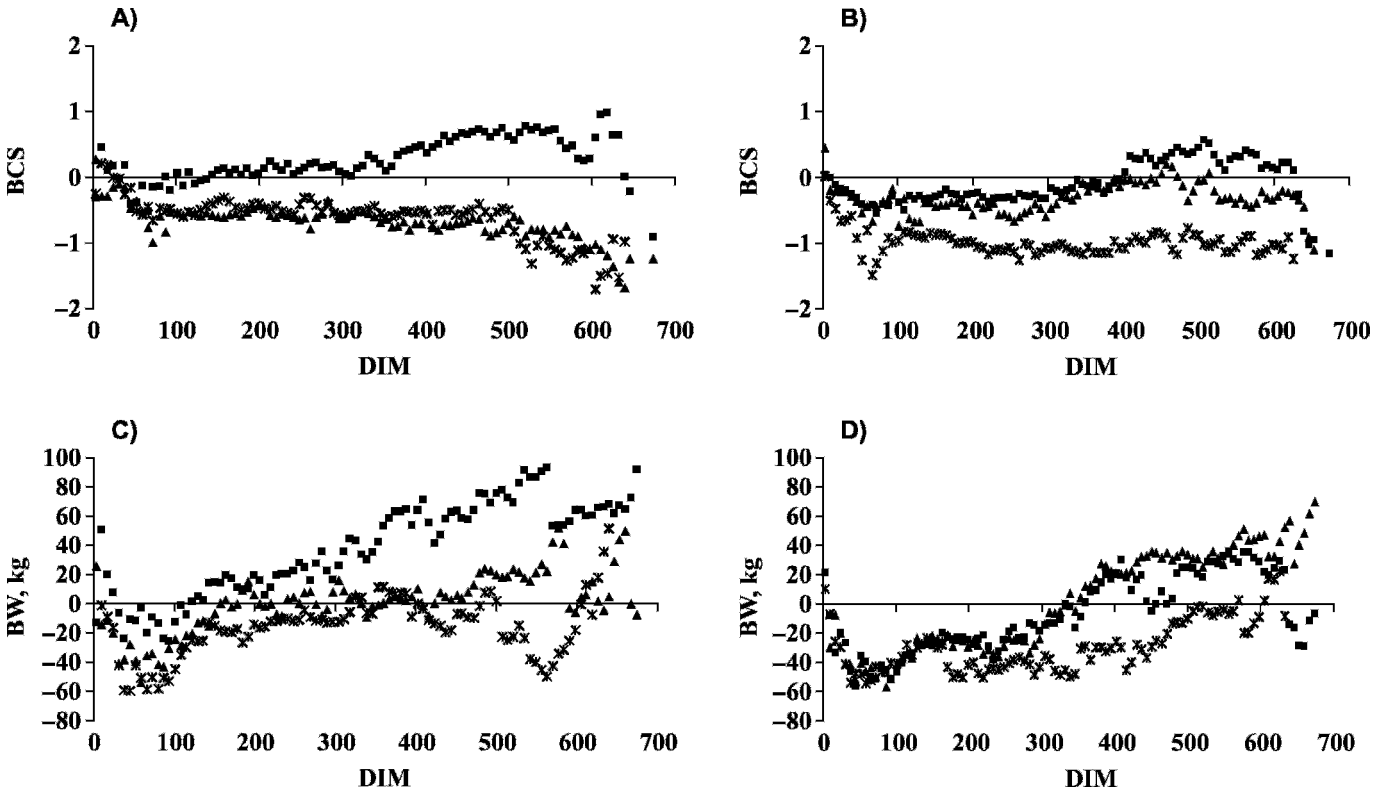

Figure 8. Residual errors for predictions of BW and BCS for cows of North American (A, C) and New Zealand (B, D) genotypes. Cows were fed 0 (■), 3 (✳), or 6 kg (▲) of concentrate DM per day for an extended lactation. Predictions were from Molly2006, with parameters listed in Table 2.

in mammary enzyme levels of 4,000, a setting for $P_{Enz,Cell}$ can be derived for the revised model that will generate the same enzyme activity when $Q_{Cells(T0)}$ is set to 1,000). The relationship is presented in Figure 9.

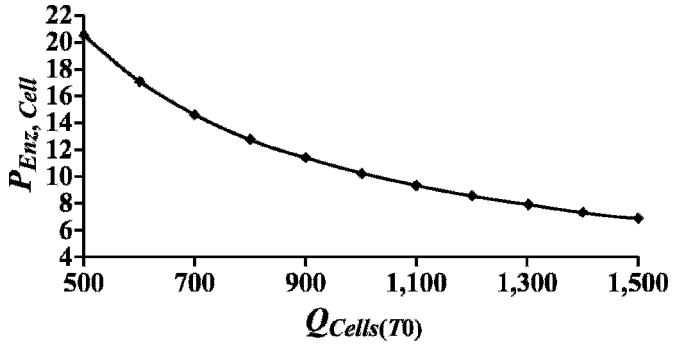

Figure 9. The relationship between $Q_{Cells(T0)}$ and $P_{Enz,Cell}$ when $P_{Enz,Cell}$ of the revised version of Molly was fitted to observed milk and milk component yields from an extended lactation study while varying $Q_{Cells(T0)}$. The equation describing the relationship was ($P < 0.0001$) as follows: $P_{Enz,Cell} = 10{,}213 \pm 0.88[Q_{Cells(T0)}]^{-1.00 \pm 0.00001}$.

Independent Model Challenge

Having assessed the fits to the extended lactation data set, the model and associated parameter estimates were tested using the data set of Aston et al. (1995). In that experiment, various supplementation schemes were evaluated for effects on milk yield, milk composition, and BW. Results of the model challenge are presented in Table 3 and Figure 10. Root mean square prediction errors for milk yield and milk components were all less than that observed for the extended lactation trial, and no apparent mean bias was observed except for milk fat yield and content. Lack of such bias for lactose and protein indicate the genetic potential of these cows was similar to the New Zealand cows. In a similar manner, the mean bias observed for milk fat may reflect different genetic potential, although model structure or parameterization errors cannot be ruled out. A significant proportion of the observed prediction errors for lactose, protein, and fat yields and milk fat content was associated with slope bias. Milk yield did not exhibit this propensity nor was there apparent slope bias when residual errors for milk yield were plotted against dietary energy concentrations, suggesting that

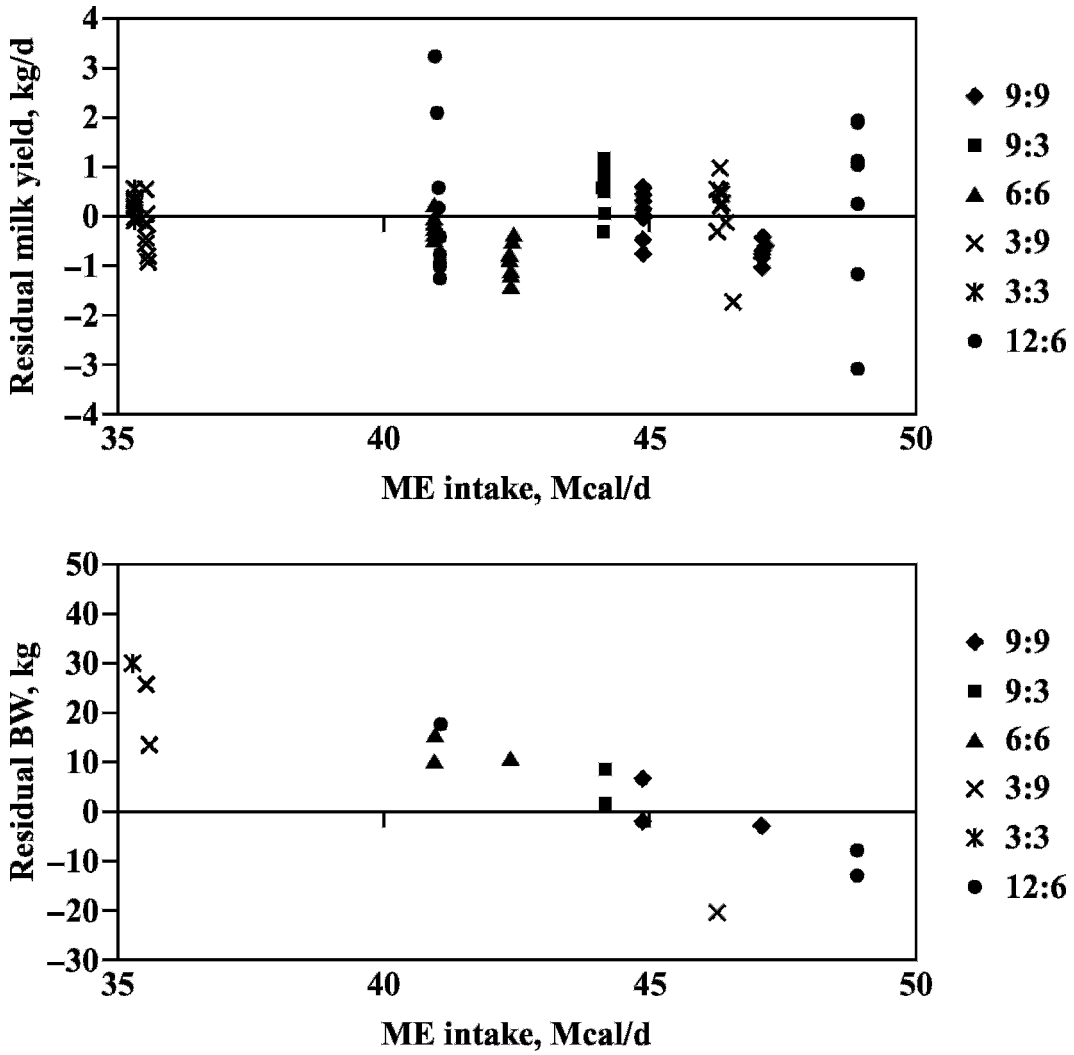

Figure 10. Residual errors for predictions of milk yields and BW of cows subjected to several strategies for concentrate supplementation. Cows were observed during 2 periods with 3, 6, 9, or 12 kg of supplement DM/d during period 1 and 3 (3:3 or 9:3), 6 (6:6 or 12:6), or 9 kg/d (3:9 or 9:9) of concentrate DM during period 2. Predictions were from Molly2006, with parameters listed in Table 2. For the evaluation with the Aston et al. (1995) data, the following setting modifications were used: $Q_{Cells(T0)} = 799$; $Vm_{Gl,Lm(T=0)} = 1.66 \times 10^{-3}$; and $Vm_{Aa,Pm} = 1.92 \times 10^{-3}$.

at least milk yield is appropriately responsive to dietary energy intputs.

Body weight and BCS were predicted with similar accuracy as for the extended lactation study, but both predictions exhibited mean and slope bias, and BW was systematically underpedicted at low dietary energy concentrations and overpredicted at high concentrations, suggesting that additional work on the representation of body energy stores is required.

In summary, predictions of milk and milk components, BW, and BCS were significantly improved by adopting a derivation of the representation of mammary cells and mammary enzymes described by Dijkstra et al. (1997) and Vetharaniam et al. (2003a,b) and by altering the representation of the somatotropin axis. Representation of mammary secretory cell loss as a function of time and unaffected by nutritional state was consistent with the observed data. The model appears to now respond appropriately to dietary energy inputs with respect to at least milk, milk lactose, and milk protein yields, and prediction errors for BW and BCS are significantly improved. Additional work on the representation of blood fatty acids and the effects of this pool on milk fat and body fat stores is required.

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