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# Interpreting experimental data on egg production—Applications of dynamic differential equations<sup>1</sup>

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**ABSTRACT** This contribution focuses on applying mathematical models based on systems of ordinary first-order differential equations to synthesize and interpret data from egg production experiments. Models based on linear systems of differential equations are contrasted with those based on nonlinear systems. Regression equations arising from analytical solutions to linear compartmental schemes are considered as candidate functions for describing egg production curves, together with aspects of parameter estimation. Extant candidate functions are reviewed, a role for growth functions such as the Gompertz equation suggested, and a function based on a simple new model outlined. Structurally, the new model comprises a single pool with an inflow and an outflow. Compartmental simulation models based on nonlinear systems of differential

equations, and thus requiring numerical solution, are next discussed, and aspects of parameter estimation considered. This type of model is illustrated in relation to development and evaluation of a dynamic model of calcium and phosphorus flows in layers. The model consists of 8 state variables representing calcium and phosphorus pools in the crop, stomachs, plasma, and bone. The flow equations are described by Michaelis-Menten or mass action forms. Experiments that measure Ca and P uptake in layers fed different calcium concentrations during shell-forming days are used to evaluate the model. In addition to providing a useful management tool, such a simulation model also provides a means to evaluate feeding strategies aimed at reducing excretion of potential pollutants in poultry manure to the environment.

**Key words:** differential equation, layer, egg production, calcium, phosphorus

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## INTRODUCTION

Differential equations are central to the sciences and act as the cornerstone of applied mathematics. They arise within biology in the construction of dynamic mechanistic models. There is a mathematically standard way of representing such models called the rate:state formalism. The system under investigation is defined at time  $t$  by  $q$  state variables:  $X_1, X_2, \dots, X_q$ . These variables represent properties or attributes of the system, such as visceral protein mass, quantity of substrate, and so on. The model then comprises  $q$  first-order differential equations that describe how the state variables change with time:

$$\frac{dX_i}{dt} = f_i(X_1, X_2, \dots, X_q; S); \quad i = 1, 2, \dots, q, \quad [1]$$

where  $S$  denotes a set of parameters, and the function  $f_i$  gives the rate of change of the state variable  $X_i$ . The function  $f_i$  comprises terms that represent the rates of processes (with dimensions of state variable per unit time), and these rates can be calculated from the values of the state variables alone, with of course the values of any parameters and constants. In this type of mathematical modeling, the differential equations are constructed by direct application of scientific law based on the Cartesian doctrine of causal determinism (e.g., the law of mass conservation, the first law of thermodynamics) or by application of a continuity equation derived from more fundamental scientific laws.

If the system under investigation is in steady state, solution to Eq. [1] is obtained by setting the differential terms to zero and manipulating algebraically to give an expression for each of the components and processes of interest. Isotope data, for example, are often resolved

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in this way, and indeed, many of the time-independent formulae presented in the animal and poultry science literature are derived likewise. However, to generate the dynamic behavior of any model, the rate:state equations must be integrated. For the simple cases, analytical solutions are usually obtained. Such cases are very often linear. A first-order differential equation is said to be linear if it is linear in the dependent variable  $X$  and the derivative, i.e.,

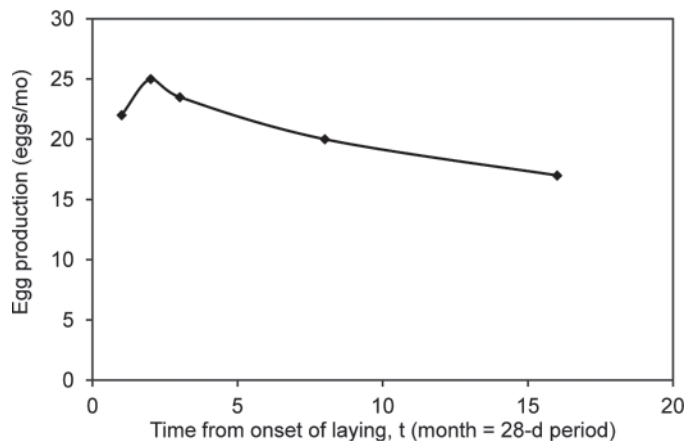
$$\frac{dX}{dt} - u(t)X = v(t),$$

where  $u$  and  $v$  are continuous functions of time. When this condition is not satisfied, the differential equation is said to be nonlinear. Linear systems are widely applied in digestion studies to interpret time-course data from marker and in vitro experiments, where the functional form of the solution is fitted to the data using a curve-fitting procedure. This enables biological measures such as mean retention time and extent of digestion in the gastrointestinal tract to be calculated from the estimated parameters. In this paper, regression equations arising from analytical solutions to linear compartmental schemes are considered as candidate functions for describing egg production curves, along with more traditional empirical equations.

For the more complex cases, only numerical solutions to the rate:state equations can be obtained. This can be conveniently achieved by using one of the many software packages available for tackling such problems. These typically employ methods such as Euler and Runge-Kutta for performing the numerical integration (Thornley and France, 2007). Such models are used to simulate complex digestive and metabolic systems. They are often used as tactical research tools to evaluate current understanding for adequacy and, when current understanding is inadequate, help identify critical experiments. This type of model is illustrated in relation to development and evaluation of a dynamic model of calcium and phosphorus flows in layers.

## FUNCTIONS FOR DESCRIBING EGG PRODUCTION CURVES

Egg production in laying birds is a reproductive activity that represents a high level of nutrient turnover. A hen's egg production when summarized generally increases to a peak and subsequently decreases gradually. A mathematical summary of the curve is useful to egg producers and poultry breeders, allowing, for example, prediction of yield from records of part of the production cycle. Such a mathematical summary will also help define economically important characteristics including persistency of lay (rate of decline in egg production from the peak). An illustrative curve, summarized on a monthly basis for brevity, is shown in Figure 1. In this



**Figure 1.** An illustrative egg production curve for a laying hen. Data pertain to a typical hen from a noncommercial flock ( $n = 28$  at onset of lay).

section, we focus on attempts to describe this curve mathematically using a single equation whose parameters can be estimated statistically by fitting it to egg production data using nonlinear regression analysis, while emphasizing single equations that can be derived from linear compartmental schemes.

### McMillan Equation

This model offers a simple compartmental description of egg production in the fruit fly, *Drosophila melanogaster* (McMillan et al., 1970a,b). The scheme, shown in Figure 2, considers egg production as a 2-stage, sequential process, consisting of a primordial stage and a developing stage. All flows between pools and out of the system obey mass action kinetics. The differential equations (one for each pool, see figure legend) are

$$\frac{dX_1}{dt} = -(k_{01} + k_{21})X_1, \quad [2]$$

$$\frac{dX_2}{dt} = k_{21}Y_{21}X_1 - (k_{02} + k_{32})X_2, \quad [3]$$

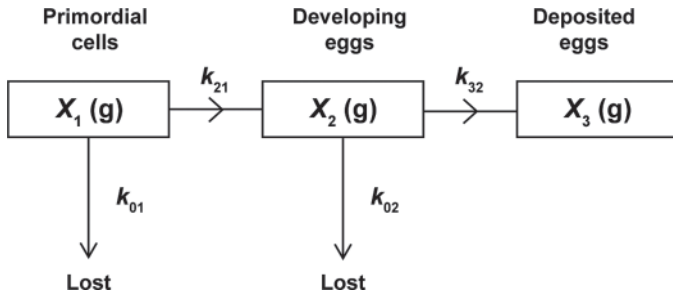
$$\frac{dX_3}{dt} = k_{32}Y_{32}X_2, \quad [4]$$

where  $t$  (d) is time since the start of egg laying, and the  $Y$  [g product (g of substrate)<sup>-1</sup>] are yield coefficients.

We are interested primarily in  $dX_3/dt$ , the rate of egg deposition, and therefore seek an analytical expression for  $X_2$ . Integrating Eq. [2] and [3] sequentially,

$$X_1 = X_1(0)e^{-\alpha t};$$

$$X_2 = \left\{ \left[ \frac{k_{21}Y_{21}X_1(0)}{\alpha - \beta} + X_2(0) \right] - \frac{k_{21}Y_{21}X_1(0)}{\alpha - \beta} e^{-(\alpha - \beta)t} \right\} e^{-\beta t};$$



**Figure 2.** Scheme for the McMillan model, comprising 3 pools (boxes) and 4 flows (arrows). The state variables  $X_1$  to  $X_3$  (all in g) represent primordial cells, developing eggs, and deposited eggs, respectively. A positive fractional rate constant  $k$  ( $d^{-1}$ ) is associated with each flow.

$$\frac{dX_3}{dt} = k_{32}Y_{32} \left\{ \left[ \frac{k_{21}Y_{21}X_1(0)}{\alpha - \beta} + X_2(0) \right] - \frac{k_{21}Y_{21}X_1(0)}{\alpha - \beta} e^{-(\alpha - \beta)t} \right\} e^{-\beta t};$$

where

$$\alpha = k_{01} + k_{21}; \quad \beta = k_{02} + k_{32}.$$

Because the physiology of egg production in insects is similar, in broad terms, to that in poultry, this leads to the extended McMillan egg production equation (McMillan, 1981; Thornley and France, 2007):

$$y = a(1 - be^{-c_1 t})e^{-c_2 t}, \tag{5}$$

where  $y$  ( $g \cdot d^{-1}$ ) is the rate of egg production,  $t$  (d) is time since onset of laying, and  $a$  ( $g \cdot d^{-1}$ ),  $b$  (dimensionless),  $c_1$ ,  $c_2$  (both  $d^{-1}$ ) are parameters ( $>0$ ). In the McMillan (1981) model, the initial value of  $X_2$  is assumed to be zero [i.e.,  $X_2(0) = 0$ ]. However, a nonzero initial condition is perhaps more reflective of actual biology in poultry because, for example, the ovarian follicles will contain synthesized yolk proteins at commencement of lay. This extended equation (Eq. [5]) does not restrict rate at time of first egg to be zero.

Formulae for useful summary statistics on a hen's egg production performance based on the extended McMillan equation are as follows (see Thornley and France, 2007, for derivations). Time to peak production,  $t_m$  (d since start of lay), and peak production,  $y_m$  ( $g \cdot d^{-1}$ ), are given by

$$t_m = c_1^{-1} \ln \left[ \frac{b(c_1 + c_2)}{c_2} \right];$$

$$y_m = a(1 - be^{-c_1 t_m})e^{-c_2 t_m}.$$

Total egg yield,  $Y$  (g), is

$$Y = a \left\{ \frac{1 - e^{-c_2 t_f}}{c_2} - \frac{b[1 - e^{-(c_1 + c_2)t_f}]}{c_1 + c_2} \right\},$$

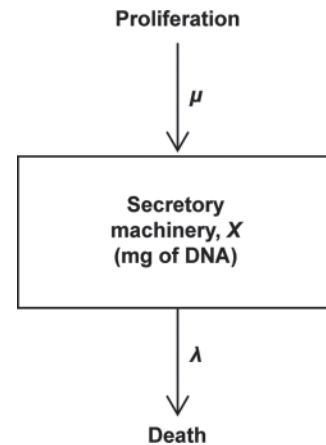
where  $t_f$  (d) is length of laying period. The relative rate of decline,  $r(t)$ , at the point midway between peak ( $t = t_m$ ) and end ( $t = t_f$ ) of laying period, which is a measure of persistency, is given by

$$r\left(\frac{t_m + t_f}{2}\right) = \frac{c_1}{b^{-1} \exp\left[c_1 \left(\frac{t_m + t_f}{2}\right)\right] - 1} - c_2.$$

### Dijkstra Equation

The scheme assumed is shown in Figure 3. The model, originally developed to describe the mammary cell population of a lactating dairy cow, comprises a single pool, one inflow and one outflow (Dijkstra et al., 1997). For current application, we tentatively define the pool as the hen's secretory machinery,  $X$  (mg), represented by DNA accumulation. The flows are secretory machinery proliferation and machinery death (both  $mg$  of  $DNA \cdot d^{-1}$ ). Time,  $t$  (d), is measured as days since onset of lay. The kinetic assumptions underlying the model are that, due to hormonal influences, the specific rate of secretory machinery proliferation,  $\mu$  ( $d^{-1}$ ), is greatest at the onset of lay and declines exponentially with time thereafter, but the specific rate of machinery death,  $\lambda$  ( $d^{-1}$ ), is constant throughout the laying period. The rate:state equation is therefore

$$\frac{dX}{dt} = \mu X - \lambda X, \tag{6}$$



**Figure 3.** Scheme for the Dijkstra model, comprising a single pool (box) and 2 flows (arrows). The state variable  $X$  (mg of DNA) represents a laying hen's secretory machinery. A nonnegative variable fractional rate,  $\mu$ , declining exponentially with time after onset of lay, is associated with proliferation, and a positive fractional rate constant,  $\lambda$ , is associated with death (both  $d^{-1}$ ).

with

$$\mu = \mu_0 e^{-kt},$$

where  $\mu_0$  is the value of  $\mu$  at onset of lay ( $t = 0$ ) and  $k$  ( $d^{-1}$ ) is a decay parameter. Substituting for  $\mu$  in Eq. [6], then integrating, yields:

$$X = X_0 \exp\left[\mu_0 k^{-1} (1 - e^{-kt}) - \lambda t\right] \quad [7]$$

where  $X_0$  (mg of DNA) is the hen's secretory machinery at onset of lay. Let  $\Omega$  [g of egg (mg of DNA) $^{-1} \cdot d^{-1}$ ], a constant, be defined as mean egg production per unit of secretory machinery per day; then, daily egg mass production by the hen,  $y$  (g of egg  $\cdot d^{-1}$ ), is given by

$$y = \Omega X. \quad [8]$$

Substituting for  $X$  in Eq. [8] using Eq. [7] yields

$$y = \Omega X_0 \exp\left[\mu_0 k^{-1} (1 - e^{-kt}) - \lambda t\right]. \quad [9]$$

Equation [9] provides a single equation for fitting to egg production data. The parameters of the equation, viz.  $\Omega$ ,  $X_0$ ,  $\mu_0$ ,  $k$ , and  $\lambda$  (all  $>0$ ), support direct physiological interpretation (albeit tentative) in terms of secretory machinery growth and death processes. Note that  $\Omega$  and  $X_0$  cannot both be defined uniquely when curve fitting because their product has to be treated as a single parameter  $y_0$ .

Formulae for useful summary statistics on a hen's egg production performance based on the Dijkstra equation are as follows (see Thornley and France, 2007, for derivations). Time to peak production,  $t_m$  (d since start of lay), and peak production,  $y_m$  (g of egg  $\cdot d^{-1}$ ), are given by

$$t_m = k^{-1} \ln(\mu_0/\lambda),$$

$$y_m = y_0 (\lambda/\mu_0)^{\lambda/k} \exp\left[k^{-1} (\mu_0 - \lambda)\right].$$

Total egg yield,  $Y$  (g), is

$$Y = y_0 \int_0^{t_f} \exp\left[\mu_0 k^{-1} (1 - e^{-kt}) - \lambda t\right] dt,$$

where  $t_f$  (d) is length of laying period. This integral is nonanalytical, but many software packages are available with procedures that yield numerical solutions easily. The relative rate of decline at time  $t_h$  (d), midway between peak production and end of laying period,  $r(t_h)$  ( $d^{-1}$ ), is

$$r(t_h) = \mu_0 \exp\left[-k(t_m + t_f)/2\right] - \lambda.$$

### Other Egg Production Equations

Table 1 lists some other time-dependent functions used in the literature to describe the egg production curve of a laying hen. Compartmental interpretations of these functions have not been offered by the publishing authors.

A typical egg production curve rises to a peak before falling away (Figure 1), which is the same trajectory mapped by the slope of a sigmoidal growth function. Therefore, growth functions (see Thornley and France, 2007), written in their differential form and expressed as a function of time, have potential application as egg production equations (Table 2).

### Parameter Estimation

Fitting of these egg production equations to data are by nonlinear regression: an iterative process generally based on ordinary least squares, aimed at minimizing the sum of squared deviations of the observed values for the dependent variable from those predicted by the model (Draper and Smith, 1998). There are several numerical optimization algorithms available for nonlinear regression [e.g., generalized least squares (Dennis et al., 1981)], though the most widely used for computing nonlinear least squares estimates is the Levenberg-Marquardt algorithm (Marquardt, 1963). In contrast with

**Table 1.** Some time-dependent egg production equations<sup>1</sup>

| Name              | Equation   | Reference                 |
|-------------------|--|---------------------------|
| Wood equation     | $y(t) = at^b e^{-ct}$  | Gavora et al. (1982)      |
| Yang equation     | $y(t) = \frac{ae^{-bt}}{1 + e^{-(t-d)}}$   | Yang et al. (1989)        |
| Adams equation    | $y(t) = \frac{a}{1 + bc^t} - d(t - g)$   | Adams and Bell (1980)     |
| Grossman equation | $y(t) = a \left( \frac{1 - e^{-t}}{1 + e^{-t}} \right) - b \left( \frac{1 - e^{-t}}{1 + e^{-(t-c)}} \right)$ | Grossman and Koops (2001) |

<sup>1</sup> $y(t)$  (g  $\cdot d^{-1}$ ) is rate of production at time  $t$ , and  $t$  (d) is time since the first egg. Parameters  $a$ ,  $b$ ,  $c$ ,  $d$ , and  $g$  are positive, and equations are listed in order of simplicity.

**Table 2.** Selected growth equations and their functional forms<sup>1</sup>

| Growth equation | Functional form   | Derivative                                  |
|-----------------|---|---|
| Schumacher      | $y(t) = y_0 \exp\left(\frac{kt_0 t}{t + t_0}\right)$            | $y'(t) = ky \frac{t_0^2}{(t + t_0)^2}$      |
| Gompertz        | $y(t) = y_0 \exp\left[\frac{k}{D}(1 - e^{-Dt})\right]$          | $y'(t) = kye^{-Dt}$                         |
| Logistic        | $y(t) = \frac{y_0 y_f}{y_0 + (y_f - y_0)e^{-kt}}$               | $y'(t) = ky \left(1 - \frac{y}{y_f}\right)$ |
| Richards        | $y(t) = \frac{y_0 y_f}{[y_0^n + (y_f^n - y_0^n)e^{-kt}]^{1/n}}$ | $y'(t) = \frac{ky(y_f^n - y^n)}{ny_f^n}$    |

<sup>1</sup> $y(t)$  (g) ( $>0$ ) is cumulative egg production,  $y'(t)$  (g·d<sup>-1</sup>) ( $>0$ ) is daily yield, and  $t$  is day of production. All parameters are positive constants, except  $n \geq -1$  in the case of Richards.

linear regression, it is not possible to solve nonlinear equations exactly, so that approximate analytical solutions are obtained by these iterative procedures, resulting in the best solution according to the convergence criterion specified.

There are alternatives to ordinary least squares estimation, such as weighted least squares or maximum likelihood. The latter is based on maximizing the log-likelihood function using algorithms such as Nelder-Mead (Lagarias et al., 1998) or quasi-Newton (Byrd et al., 1995). If all assumptions for standard regression are met, then the least squares estimation method will yield results identical to the maximum likelihood method. Maximum likelihood is to be used for nonlinear mixed models containing both fixed and random effects (St-Pierre, 2001). The weighted least squares method is required when error variances are not equal across the range of an  $x$ -variable.

Nonlinear regression may result in an unsatisfactory solution, usually when the convergence criterion is not met or one of the parameters (or its asymptotic SE) cannot be estimated. This can happen when there are too few data points available, one or more of the equation parameters is poorly defined, the model is too complicated or overparameterized, the model contains redundant parameters, or if the initial values provided for each parameter are flawed. A model that is not appropriate to fit the data will also result in unsuccessful nonlinear fitting.

Goodness-of-fit is generally assessed using the coefficient of determination ( $R^2$ ) or the mean square error, and significance of fit can be estimated using a  $F$ -test. With maximum likelihood methods, goodness-of-fit is determined from information criteria (Akaike's or Bayesian criterion), and the significance of the fit achieved can be appraised using a goodness-of-fit  $\chi^2$  statistic. Analysis of residuals is necessary to assess the suitability of a model, testing that errors are independent and normally distributed.

The validity of the parameter estimates also needs to be evaluated. The precision of each estimate can be assessed using its SE, from which confidence intervals can be derived. It is also important to check that

best-fit estimates are scientifically plausible. In certain models, parameters are biologically constrained (e.g., specific parameters cannot be  $<0$ ), but in some cases the iterative process leads to a solution violating such constraints. Clearly this should not be accepted as a realistic estimate.

Model comparisons are based on goodness-of-fit, but should also take into account model simplicity. A model with more parameters will always result in a lower residual error, but this should not be the only factor to be considered; the number of parameters should be also taken into account.

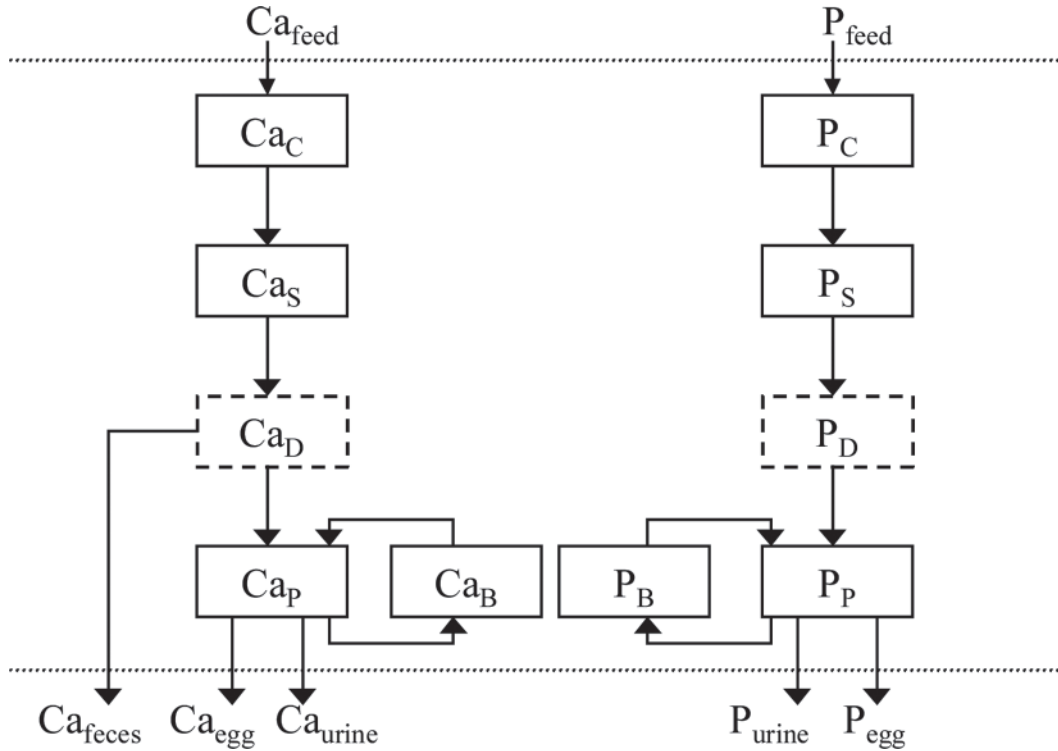
## CALCIUM AND PHOSPHORUS FLOWS IN LAYERS

Surplus minerals in excreta present an environmental pollution problem to intensive livestock operations. In laying hens, excretion of phosphorus (P) in manure is of special concern. Requirement for dietary P is mainly due to the need to store calcium (Ca) and P in bone before egg shell formation. Mobilization of stored minerals occurs if quantities absorbed from the diet are insufficient for egg formation. Mobilization of P from bone is linked to that of Ca, and the reverse. If instantaneous Ca supply (Ca absorbed into blood plasma) does not match Ca required for egg synthesis, Ca will be mobilized from bone and consequently P mobilization occurs, giving rise to P excretion in urine. Here, we give a summary account of a simulation model of Ca and P dynamics in the layer hen by Kebreab et al. (2009), developed to evaluate management strategies for reducing P excretion. The core of the model is a nonlinear system of 8 differential equations.

### Model Overview

The scheme is shown in Figure 4. The model consists of 8 state variables representing Ca and P pools in the crop (c), stomachs (proventriculus and gizzard) (s), plasma (p), and bone (b). Phosphorus is defined as absorbable P at the terminal ileum. Zero pools are assigned to Ca and P in the duodenum (d), assuming





**Figure 4.** The Ca-P model. Boxes enclosed by solid lines depict state variables, boxes enclosed by broken lines denote zero pools, and arrows depict flows. Subscripts C, S, D, P, and B indicate crop, stomachs, duodenum, plasma, and bone, respectively.

that duodenal retention time for Ca and P is small. Pools sizes are expressed in milligrams and time in days. State variables (quantities) are denoted by  $X_i$  and concentration ( $C_i$ ) is calculated as pool size divided by live weight (**LW**,  $W$ , kg). Differential equations ( $dX_i/dt$ ) describe the rate of change of state variables ( $X_i$ ) with time  $t$  (days). Rate of utilization of  $i$  in the  $j$  to  $k$  transaction is denoted by  $U_{i,jk}$  and rate of production of  $i$  in the  $j$  to  $k$  transaction by  $P_{i,jk}$ . The variable  $t'$  (h) is used for diurnal time (time within the day,  $0 \leq t' \leq 24$ ).

## Model Components

**Ca and P in the Crop.** There is one input to each of the 2 crop pools, from the feed:

$$P_{Ca_C, CafCa_C} = I_f C_{Caf}; \quad [10]$$

$$P_{P_C, PfP_C} = I_f C_{Pf}; \quad [11]$$

where  $I_f$  is feed intake ( $g$  of  $DM \cdot d^{-1}$ ) and  $C_{Caf}$  and  $C_{Pf}$  are the concentration [ $mg (g \cdot DM)^{-1}$ ] of Ca and P, respectively, in the feed. A layer is assumed to lay an egg on successive days at  $L = 1, 2, \dots$ , and 7 h after light is switched on; alternatively, on rest days, the layer will not produce an egg. The driving variable  $I_f$  was set to 101% of averaged intake when  $L = 1, 2, \dots$ , or 6 h but only 90% of averaged intake on the rest day and the day before rest day (when  $L = 7$  h). The photoperiod is set at  $16 h \cdot d^{-1}$ , light is switched on at  $t' = 0$  h, and feed intake is assumed continuous during the photope-

riod [see Kebreab et al. (2009) for further explanation]. One output of Ca and one of P from the crop are represented (i.e., to the stomachs). Fractional outflow rates are applied for Ca ( $k_{Ca_C, Cas}$ ,  $d^{-1}$ ) and P ( $k_{P_C, Ps}$ ,  $d^{-1}$ ):

$$U_{Ca_C, CafCas} = k_{Ca_C, CafCas} X_{Ca_C}; \quad [12]$$

$$U_{P_C, PfPs} = k_{P_C, PfPs} X_{P_C}. \quad [13]$$

Rates of change of pool size in the crop are

$$dX_{Ca_C}/dt = P_{Ca_C, CafCa_C} - U_{Ca_C, CafCas}; \quad [14]$$

$$dX_{P_C}/dt = P_{P_C, PfP_C} - U_{P_C, PfPs}. \quad [15]$$

**Ca and P in the Stomachs.** There is one input to each stomach pool, from the crop:

$$P_{Cas, CafCas} = U_{Ca_C, CafCas}; \quad [16]$$

$$P_{Ps, PfPs} = U_{P_C, PfPs}. \quad [17]$$

Outflows are to the duodenum:

$$U_{Cas, CasCad} = k_{CasCad} X_{Cas}; \quad [18]$$

$$U_{Ps, PsPd} = k_{PsPd} X_{Ps}, \quad [19]$$

where  $k_{CasCad}$  ( $d^{-1}$ ) and  $k_{PsPd}$  ( $d^{-1}$ ) are fractional rates of outflow of Ca and P, respectively, to the duodenum. Rates of change of pool size in the stomachs are

$$dX_{Cas}/dt = P_{Cas,CacCas} - U_{Cas,CasCad}; \quad [20]$$

$$dX_{Ps}/dt = P_{Ps,PcPs} - U_{Ps,PsPd}. \quad [21]$$

**Ca and P in the Duodenum.** The Ca and P in the duodenum are represented as zero pools, where input equals output without quantification of pool size, based on the small residence time of duodenal digesta. Inflow to the duodenum ( $\text{mg}\cdot\text{d}^{-1}$ ) equals outflow from the stomachs

$$P_{Cad,CasCad} = U_{Cas,CasCad}; \quad [22]$$

$$P_{Pd,PsPd} = U_{Ps,PsPd}. \quad [23]$$

Absorption into blood plasma ( $\text{mg}\cdot\text{d}^{-1}$ ) is represented as

$$U_{Cad,CadCap} = k_{CadCap} P_{Cad,CasCad}; \quad [24]$$

$$U_{Pd,PdPp} = k_{PdPp} P_{Pd,PsPd}; \quad [25]$$

where  $k_{CadCap}$  and  $k_{PdPp}$  are fractional absorption of Ca and P, respectively, from the duodenum. Parameter  $k_{CadCap}$  is taken as 0.7 during egg shell formation, when Ca requirements are high, and 0.4 when there is no egg shell formation. Nonabsorbed Ca is excreted in feces. As P is defined in model as absorbable P at the terminal ileum,  $k_{PdPp}$  is set at 1.

**Ca and P in the Plasma.** There are 2 inputs each to the Ca and P plasma pools, absorption from the duodenum ( $P_{Cap,CadCap}$  and  $P_{Pp,PdPp}$ ) and mobilization from bone ( $P_{Cap,CabCap}$  and  $P_{Pp,PbPp}$ ) (all  $\text{mg}\cdot\text{d}^{-1}$ ):

$$P_{Cap,CadCap} = U_{Cad,CadCap}; \quad [26]$$

$$P_{Pp,PdPp} = U_{Pd,PdPp}; \quad [27]$$

$$P_{Cap,CabCap} = U_{Cab,CabCap}; \quad [28]$$

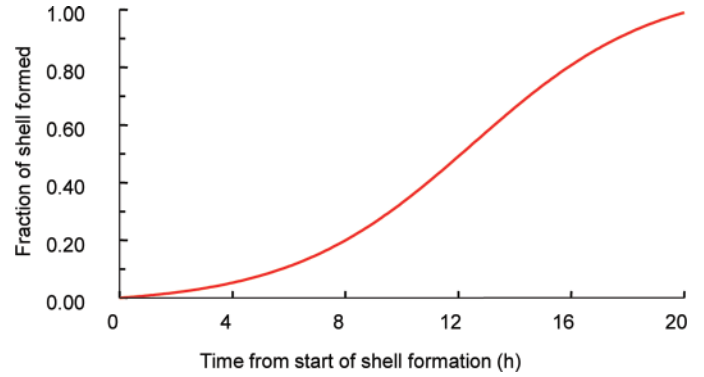
$$P_{Pp,PbPp} = U_{Pb,PbPp}. \quad [29]$$

Three outputs each from the plasma Ca and P pools are represented, i.e., utilization for egg synthesis, deposition in bone, and excretion in urine. The Ca and P for egg synthesis ( $U_{Cap,CapCae}$  and  $U_{Pp,PpPe}$ ,  $\text{mg}\cdot\text{d}^{-1}$ ) are the sum of utilization for yolk, white, and shell formation:

$$U_{Cap,CapCae} = U_{Cap,CapCayolk} + U_{Cap,CapCawhite} + U_{Cap,CapCashell}; \quad [30]$$

$$U_{Pp,PpPe} = U_{Pp,PpPyolk} + U_{Pp,PpPwhite} + U_{Pp,PpPshell}. \quad [31]$$

The shell is assumed to be formed in the 20 h before oviposition and follows a logistic pattern (Figure 5):



**Figure 5.** Time course of egg shell formation (after Van Krieken, 1996). Color version available in the online PDF.

$$y = a/[1 + e^{-b(x-c)}] - d, \quad [32]$$

where  $y$  ( $\text{g}\cdot\text{g}^{-1}$ ) is fraction of shell formed,  $x$  (h) is time from start of shell formation, and  $a$ ,  $b$ ,  $c$ ,  $d$  are parameters. Differentiating gives the fractional rate of egg formation:

$$dy/dx = abe^{-b(x-c)}/[1 + e^{-b(x-c)}]^2. \quad [33]$$

As oviposition occurs at  $x = 20$  (i.e., when  $t = L$ ) instantaneous fractional rate of egg formation,  $k_E$  ( $\text{d}^{-1}$ ), is expressed in the model as

$$k_E = 0, L < t < L + 5, \quad [34]$$

$$= abe^{-b(x-c)}/[1 + e^{-b(x-c)}]^2, \text{ otherwise.} \quad [35]$$

There are 2 exceptions to this calculation of  $k_E$ . First, when  $L = 7$  h, it is assumed that there is no ovulation on that day and, consequently, the next day will be a rest day; hence,  $k_E$  is 0  $\text{d}^{-1}$  when  $t \geq 7$  h. Second, on a rest day, there is no oviposition, but the layer is preparing for an egg at  $L = 1$  h on the next day. Hence, on a rest day,  $k_E$  is 0  $\text{d}^{-1}$  if  $t < 5$  h. The above equation for  $k_E$  applies at all other times. Albumen formation is taken to occur at the same time and rate as shell formation. Yolk formation is a continuous process. Utilization is calculated as a requirement per unit of egg component formed multiplied by the fraction of that component in the egg and by egg weight and multiplied by the laying percentage in a 100-d period. For example, Ca need of the layer for yolk formation is calculated: egg weight  $\times$  egg yolk  $\times$  Ca in yolk  $\times$  laying %/24 h, as yolk synthesis is continuous. For example, total amount of Ca needed for formation of an egg white is: egg weight  $\times$  egg white  $\times$  Ca in white, and Ca need per hour for white formation (a discontinuous process) is this total amount multiplied by  $k_E$ .

Deposition of Ca and of P in bone are represented as saturable processes:

$$U_{Cap,CapCab:Ca} = V_{CapCab} W/(1 + M_{CapCab}/C_{Cap}); \quad [36]$$



$$U_{Pp,PpPb:P} = V_{Cap,Cab} W/[f_{Ca:P} (1 + M_{PpPb}/C_{Pp})]; \quad [37]$$

$$U_{Cap,CapCab:P} = U_{Pp,PpPb:P} f_{Ca:P}; \quad [38]$$

$$U_{Pp,PpPb:Ca} = U_{Cap,CapCab:Ca}/f_{Ca:P}, \quad [39]$$

where  $U_{Cap,CapCab:Ca}$  and  $U_{Cap,CapCab:P}$  are utilization of plasma Ca for bone synthesis based on availability of plasma Ca and plasma P, respectively, and  $U_{Pp,PpPb:P}$  and  $U_{Pp,PpPb:Ca}$  are utilization of plasma P for bone synthesis based on availability of plasma P and plasma Ca, respectively (all in  $\text{mg}\cdot\text{d}^{-1}$ ). The  $V_{CapCab}$  [ $\text{mg}(\text{kg of LW})^{-1}\cdot\text{d}^{-1}$ ] is maximal rate of Ca deposition in bone,  $M_{CapCab}$  and  $M_{PpPb}$  [ $\text{mg}(\text{kg of LW})^{-1}$ ] are affinity constants,  $C_{Cap}$  and  $C_{Pp}$  [ $\text{mg}(\text{kg of LW})^{-1}$ ] concentrations of Ca and P in plasma, and  $f_{Ca:P}$  ratio of Ca to P in bone, assumed fixed. Actual deposition of Ca ( $U_{Cap,CapCab}$ ,  $\text{mg}\cdot\text{d}^{-1}$ ) and P ( $U_{Pp,PpPb}$ ,  $\text{mg}\cdot\text{d}^{-1}$ ) are therefore the minima:

$$U_{Cap,CapCab} = \text{MIN}(U_{Cap,CapCab:Ca}, U_{Cap,CapCab:P}); \quad [40]$$

$$U_{Pp,PpPb} = \text{MIN}(U_{Pp,PpPb:P}, U_{Pp,PpPb:Ca}). \quad [41]$$

Utilization of Ca and of P for maintenance ( $\text{mg}\cdot\text{d}^{-1}$ ) are

$$U_{Cap,CapCam} = R_{CapCam} W; \quad [42]$$

$$U_{Pp,PpPm} = R_{PpPm} W, \quad [43]$$

where  $R_{CapCam}$  and  $R_{PpPm}$  [both  $\text{mg}(\text{kg of LW})^{-1}\cdot\text{d}^{-1}$ ] are Ca and P maintenance requirements per unit LW.

The Ca and P excreted in urine are the sum of (i) basal requirement for Ca and P (maintenance requirement), plus (ii) amount of Ca or P in plasma that cannot be used for bone synthesis because the other mineral (P and Ca, respectively) is lacking, plus (iii) amount of Ca or P released from bone because P or Ca is required for egg synthesis, respectively:

$$\begin{aligned} U_{Cap,CapCau} &= U_{Cap,CapCam} \\ &+ \text{MAX}(0, U_{Cap,CapCab:Ca} - U_{Cap,CapCab:P}) \\ &+ \text{MAX}(0, U_{Cab,CabCap:P} - U_{Cab,CabCap:Ca}); \quad [44] \end{aligned}$$

$$\begin{aligned} U_{Pp,PpPu} &= U_{Pp,PpPm} \\ &+ \text{MAX}(0, U_{Pp,PpPb:P} - U_{Pp,PpPb:Ca}) \\ &+ \text{MAX}(0, U_{Pb,PbPp:Ca} - U_{Pb,PbPp:P}), \quad [45] \end{aligned}$$

where  $U_{Cab,CabCap:P}$  and  $U_{Cab,CabCap:Ca}$  are mobilization of bone Ca based on P and Ca needs, respectively, and  $U_{Pb,PbPp:Ca}$  and  $U_{Pb,PbPp:P}$  are mobilization of bone P based on Ca and P needs, respectively (all in

$\text{mg}\cdot\text{d}^{-1}$ ). In these equations, it is assumed that any mineral not used for bone synthesis because availability of the other mineral is not enough to support that synthesis, is excreted in urine. Equally, any mineral that has been mobilized because of necessary mobilization of the other mineral is excreted in urine.

Rates of change of pool size in the plasma are

$$\begin{aligned} dX_{Cap}/dt &= P_{Cap,CadCap} + P_{Cap,CabCap} - U_{Cap,CapCae} \\ &- U_{Cap,CapCab} - U_{Cap,CapCau}; \quad [46] \end{aligned}$$

$$\begin{aligned} dX_{Pp}/dt &= P_{Pp,PdPp} + P_{Pp,PbPp} - U_{Pp,PpPe} \\ &- U_{Pp,PpPb} - U_{Pp,PpPu}. \quad [47] \end{aligned}$$

**Ca and P in the Bone.** Inputs to the Ca and P pools in bone are from plasma:

$$P_{Cab,CapCab} = U_{Cap,CapCab}; \quad [48]$$

$$P_{Pb,PpPb} = U_{Pp,PpPb}. \quad [49]$$

Outputs from bone are to plasma:

$$U_{Cab,CabCap} = \text{MAX}(U_{Cab,CabCap:P}, U_{Cab,CabCap:Ca}); \quad [50]$$

$$U_{Pb,PbPp} = \text{MAX}(U_{Pb,PbPp:Ca}, U_{Pb,PbPp:P}), \quad [51]$$

where  $U_{Cab,CabCap:P}$  and  $U_{Cab,CabCap:Ca}$  ( $\text{mg}\cdot\text{d}^{-1}$ ) are rates of Ca utilization for bone synthesis based on availability of P or of Ca in plasma, respectively. Similarly,  $U_{Pb,PbPp:Ca}$  and  $U_{Pb,PbPp:P}$  ( $\text{mg}\cdot\text{d}^{-1}$ ) are rates of P utilization. Mobilization of Ca and P from bone is assumed to be inhibited by plasma availability of these minerals:

$$U_{Cab,CabCap:Ca} = V_{CabCap} W/(1 + C_{Cap}/J_{CabCap}); \quad [52]$$

$$U_{Pb,PbPp:P} = V_{CabCap} W/[f_{Ca:P} (1 + C_{Pp}/J_{PbPp})]; \quad [53]$$

$$U_{Cab,CabCap:P} = U_{Pb,PbPp:P} f_{Ca:P}; \quad [54]$$

$$U_{Pb,PbPp:Ca} = U_{Cab,CabCap:Ca}/f_{Ca:P}, \quad [55]$$

where  $V_{CabCap}$  [ $\text{mg}(\text{kg of LW})^{-1}\cdot\text{d}^{-1}$ ] is maximal rate of bone Ca mobilization and  $J_{CabCap}$  and  $J_{PbPp}$  [ $\text{mg}(\text{kg of LW})^{-1}$ ] are inhibition constants. Rates of change of pool size in bone are

$$dX_{Cab}/dt = P_{Cab,CapCab} - U_{Cab,CabCap}; \quad [56]$$

$$dX_{Pb}/dt = P_{Pb,PpPb} - U_{Pb,PbPp}. \quad [57]$$

The model is completely defined by Eq. [10] through Eq. [57]. At its core is a system of first-order nonlinear

differential equations; therefore, it has to be solved numerically over time subject to appropriate initial conditions and parameter estimates.

### Parameter Determination

Mechanistic simulation models, such as this, attempt to describe a complex system in terms of identifiable biological processes. Within these models, the parameters have biological and chemical significance: rate constants, initial concentrations, and so on. These parameters can, in principle, be determined independent of the particular experimental system being modeled. Such independently determined parameters should enhance the predictive power of a model, as model formulation is not tied to a specific experimental set-up. In the Ca-P model, for example, fractional outflow rates for various forms of Ca and P are based on data summarized by van der Klis et al. (1990). The higher Ca fractional absorption from the duodenum during egg shell formation, when Ca requirements are high, compared with the fractional rate when no egg shell is being formed, is adopted from Hurwitz and Bar (1965). The shell is formed over 20 h following a pattern described by Etches (1987). The ratio of Ca to P in bone is based on stoichiometric principles because most Ca and P is stored in bone as hydroxyapatite crystals,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , and mobilization of Ca and P from bone is inhibited by plasma availability of these minerals (Boorman and Gunaratne, 2001). Maximum rates of bone mobilization were set at such a rate as to sustain maximal rates of Ca utilization for egg synthesis when other Ca sources are not available. Despite the fundamental nature of the parameters of a mechanism-based simulation model, parameter values are often adjusted to improve the goodness-of-fit of model predictions to experimental data. This is achieved by applying the model as a large regression equation. Optimization routines available in simulation software for parameter estimation are frequently based on maximizing a log-likelihood function using algorithms such as Nelder-Mead or quasi-Newton. Other routines are based on nonlinear least squares approaches using algorithms such as Levenberg-Marquadt or generalized least squares. However, there are well-documented dangers to such a procedure (e.g., Hopkins and Leipold, 1996). For example, with complex simulation models characterized by a large number of parameters, it is likely that values other than the incorrect ones will be adjusted to improve the fit, and parameter values optimized for a particular set of experimental conditions might give worse model predictions than the unadjusted parameters when the attempt is made to model a different set of experimental conditions.

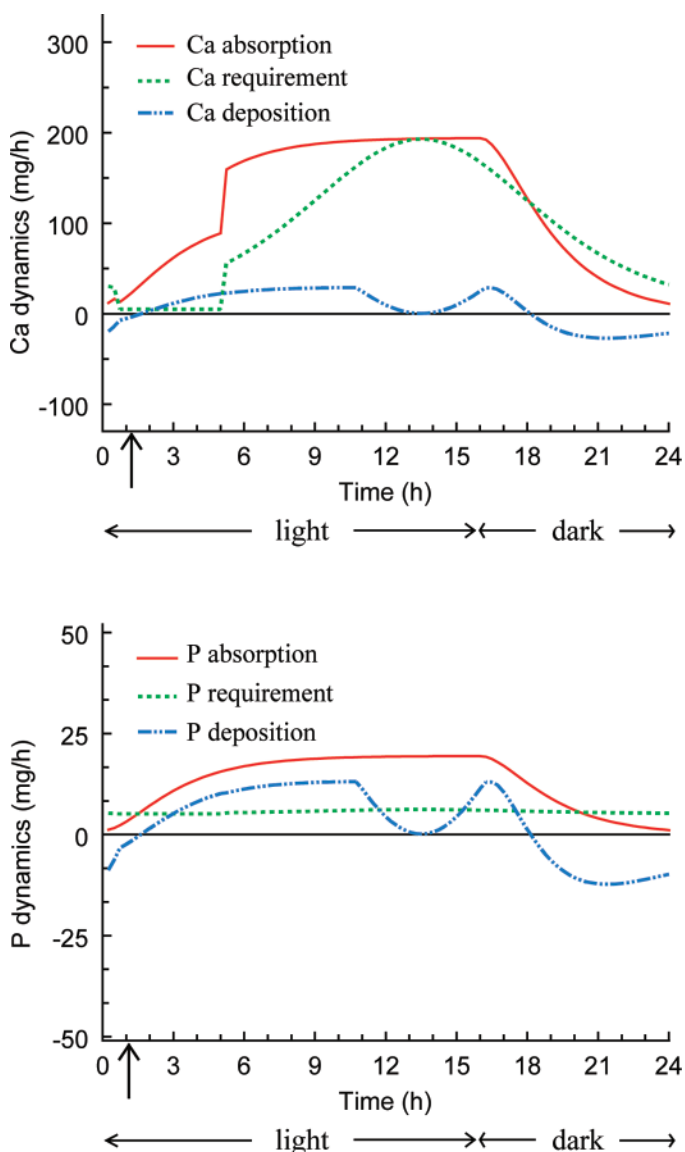
### Model Application

A summary of model application follows; full details are found in Kebreab et al. (2009). The model was pro-

grammed in the dynamic simulation language SMART (Wageningen University, Computer Science Group, Wageningen, the Netherlands). Euler's method of integration with a step size of  $\sim 1$  min was used. The programmed model was evaluated against independent data from an experiment with Single Comb White Leghorn hens fed diets that differed in Ca concentration (25, 35, and 45 mg/g).

A 24-h cycle was simulated by setting oviposition at  $t = 01:00$  h (the colon denoting diurnal time) and adjusting Eq. [34] to  $k_E = 0, 1 < t < 5$  (i.e., the model was run to simulate one particular day and one particular laying time). Quasi-steady state was achieved after 72 h of simulation. Diurnal changes in Ca and P for a layer laying an egg when  $L = 1$  h are presented in Figure 6.

Feed intake commences as soon as light is turned on (at 00:00 h) and, as a result, Ca and P absorption



**Figure 6.** Simulated diurnal dynamics of Ca (top) and P (bottom) in a layer producing an egg 1 h after light is switched on. Color version available in the online PDF.

increase. Light is turned off after 16 h and feed intake stops. The Ca and P absorption therefore decline, because the amounts of Ca and P present in crop and stomach decrease quickly and no new Ca or P enters the crop. The Ca requirements are small from the moment of egg laying (01:00 h) until 05:00 h, when formation of a new egg shell begins. The P requirements are more stable during the day, as the majority of P is required for egg yolk synthesis, which is assumed to be a continuous process. The P requirements in the first hour are higher than P absorption, and as a consequence P and Ca mobilization from bone takes place. Over this hour, all mobilized P is used for maintenance and egg production, and P excretion in urine is simply related to maintenance.

When shell formation commences, Ca requirements rise and fall in a pattern related to that of shell formation. The Ca absorption is enough to satisfy requirements until 18:00 h and P absorption is enough to satisfy requirements until 20:00 h. The surplus of P absorbed, however, cannot always be used for bone synthesis because Ca may be lacking to support this synthesis. This is the situation from approximately 11:00 to 18:00 h. Hence, some of the absorbed P is not used during these hours and is excreted in the urine. The Ca has to be mobilized from 18:00 h until the end of the day to support requirements, and P is also mobilized as a result. A large portion of this mobilized P is not required for maintenance or egg synthesis and therefore is excreted in urine. There are 2 reasons why P is not used and is excreted in the urine. From 11:00 to 18:00 h, P uptake in feed relative to Ca availability is too high to support high bone synthesis rates. From 18:00 to 24:00 h, P that is not used originates largely from bone mobilization as a result of Ca requirements.

Total net P deposition in bone is  $67.8 \text{ mg}\cdot\text{d}^{-1}$ , which would indicate dietary absorbable P can be reduced by 22% [=  $67.8/(110 \times 2.8 \times 1.01)$ ]. Layers, however, will produce eggs at different hours during the day. Evaluation of feeding strategies requires simulations at all possible hours of oviposition and assumptions about the frequency distribution within a flock of layers of those hours. Other management options to decrease P excretion evaluated using this model include feeding coarse Ca having a smaller fractional passage rate from the stomachs to the duodenum, and changes in lighting scheme that result in changes in feed intake pattern during the day. Further details of model application can be found in Kebreab et al. (2009).

## CONCLUDING REMARKS

Systems of differential equations are ubiquitous throughout much of biology. They arise through application of the rate:state formalism to construct dynamic mechanistic models. If the problem under study is in steady state, solution to the system of differential equations is found by setting the differential terms to zero and manipulating algebraically to give an expres-

sion for each of the components and processes that are of interest. Data from experiments using isotopes administered by constant infusion are usually resolved in this manner. Indeed, many of the time-independent formulae presented in the biological literature are derived likewise.

To generate the dynamic behavior of any model, the differential equations must be integrated. For linear systems, analytical solutions are usually obtained. Such models are widely applied in digestion studies to interpret time-course data from marker and in vitro experiments, where the functional form of the solution is fitted to the data using nonlinear regression. This enables biological measures such as mean retention time and extent of digestion in the gastrointestinal tract to be calculated from the estimated parameters. In this paper, we illustrated this approach with reference to the egg production curve described by layers.

For nonlinear systems, only numerical solutions to the differential equations can generally be obtained. This can be achieved by using one of several simulation software packages available for tackling such problems. Such models are used to simulate more complex digestive and metabolic systems. They are normally used as tactical research tools to identify knowledge gaps, to tease things apart before going off to the laboratory, or to make qualitative predictions. For making predictions, a mechanistic simulation model is likely to be more suitable for extrapolation than an empirical model, because its biological content is generally richer. In this paper, we illustrated the approach by simulating Ca and P flows in layers to evaluate feeding strategies aimed at reducing excretion of potential pollutants to the environment in poultry manure.

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