

# A New Approach for Calculating Metabolizable Protein Requirements by Lactating Dairy Cows

Luis E. Moraes<sup>1,2</sup>, Ermias Kebreab<sup>3</sup>, Roger Martineau<sup>4</sup>, and Helene Lapierre<sup>4</sup>

<sup>2</sup>*Department of Animal Sciences, The Ohio State University*

<sup>3</sup>*Department of Animal Science, University of California, Davis*

<sup>4</sup>*Agriculture and Agri-Food Canada*

## Abstract

This article describes a new system for calculating metabolizable protein (MP) requirements by lactating dairy cows. The system was developed with 133 treatment means from 36 scientific publications. The nonlinear relationship between total protein output (scurf + endogenous urinary + metabolic fecal + milk) and MP supply was used to construct a response model determining changes in protein output with varying supplies. The efficiency of MP utilization predicted by the model decreased nonlinearly with supply, ranging from 0.85 to 0.43 as protein supply increased from 0.78 to 3.28 kg/day (1.72 to 7.22 lb/day). The combined MP requirement (i.e., lactation plus maintenance) was defined as the MP supply needed to predict a given total protein output in the estimated response curve. A requirement function was constructed by solving the estimated nonlinear response curve in terms of the MP supply. This function directly computes the supply needed for a given total protein output while accounting for a variable efficiency at different protein supply levels. For protein outputs below 1.1 kg/day (2.42 lb/day), the calculated requirements were lower than the ones from the current Northern American feeding system for dairy cows. Conversely, for total protein outputs beyond 1.1 kg/day (2.42 lb/day), the calculated requirements were higher than predicted by current feeding systems. Finally, one example is presented with

the detailed use of the new system for calculating the combined MP requirements.

## Introduction

The current Northern American feeding system for dairy cows (NRC, 2001) assumes a constant efficiency of MP use for lactation and for most of maintenance components. The direct implication of a constant efficiency is that the supplied MP is utilized with the same efficiency, regardless of the feeding level. In the same system, the requirement of MP for lactation is determined by dividing the protein yield in milk by the constant 0.67 efficiency. As a consequence, approximately 1.5 kg of MP is required for each kg of protein outputted in milk, regardless of the level of milk production or the MP supply. Biological principles imply that cows have a genetic potential for milk production and an asymptotic potential milk production must limit protein yield when MP supply grows infinitely large. Furthermore, it is well established that the efficiency of nutrient utilization for production functions may be relatively lower at higher nutrient supplies. For instance, recent studies have shown that at higher feeding levels, MP is utilized with a relatively lower efficiency (Hanigan et al., 1998). Likewise, Metcalf et al. (2008) reported efficiencies of MP utilization decreasing from 0.77 to 0.50 with MP supplies varying from 25% below to 25% above requirement. Both Metcalf

<sup>1</sup>Contact at: 2029 Fyffe Ct., 221A Animal Science Building, Columbus, OH 43210-1095, (614) 292-6507, FAX: (614) 292-1515, Email: ferrazdiasdemoraes.1@osu.edu.



et al. (2008) and Arriola Apelo et al. (2014) suggested that the efficiency of MP utilization is not constant and decreases nonlinearly with MP supply. The use of a constant efficiency of MP utilization may be one of the underlying reasons the NRC (2001) system underestimates MP allowable milk at lower MP supplies and overestimates at relatively higher supplies (Lapierre et al., 2007).

In this context, the objective of this article is to describe a new system to calculate MP requirements by lactating dairy cows. The system is built on the principle of variable efficiency of MP utilization. Further, it relies on a nonlinear response curve to derive a requirement function determining the requirement of MP for a given level of total protein output. In the next sections, we describe the data used for the system development, the system properties, and how to use the system in practice.

### **Total Protein Output and MP Supply**

The first step in the development of the system was to define the lactating cow's protein output and MP supply. The total protein output was defined as the protein output in milk and in maintenance components. The reason for using a total protein output was to estimate a combined efficiency and a combined MP requirement rather than separate factorial requirements for maintenance and lactation. The use of combined efficiency and requirement was suggested by Lapierre et al. (2014) with the reasoning that the removal of surplus amino acids is associated with tissues having the catabolic enzymes rather than with tissues involved in protein synthesis and exportation (Lapierre et al., 2014). Maintenance protein output was composed of scurf, endogenous urinary, and metabolic fecal protein outputs. The scurf protein output was set at 0.2 g CP/kg BW<sup>0.6</sup> (Swanson, 1977), the urinary endogenous protein output set at 2.75

g CP/kg BW<sup>0.5</sup> (Swanson, 1977) and metabolic fecal protein set at 15.8 g CP/kg DMI with an average proportion of true protein/CP of 0.80 (Lapierre et al., 2014). The MP supply was defined as the calculated MP supply (NRC, 2001) minus the MP supply from endogenous sources entering the duodenum (also calculated with NRC, 2001) as described in Lapierre et al. (2014).

The data used for system development was a subset of the data from Martineau et al. (2016). The subset is composed of 133 treatment means from 36 scientific publications. In short, milk true protein yield was used as milk protein output, and when not reported, it was assumed to be 0.955 times milk CP yield. Body weight means, when not available, was assumed to be, respectively, 602 and 564 kg for North American cows and cows from Europe and other countries (Martineau et al., 2016). The relationship between total protein output and MP supply is presented in Figure 1. Treatment means are represented by solid circles and connected by a dashed line if originated from the same publication.

### **Calculation of MP Requirements**

The general strategy for determining the combined MP requirement (i.e., the MP required for maintenance plus milk production) was to construct a requirement function that calculates the MP supply required for a given level of total protein output.

#### *The relationship between protein output and MP supply*

The first step in the construction of a requirement function was to develop a model that describes the total protein output response to the MP supply. The model relies on a response curve  $f$  that represents the mean trajectory:

$$PO = f(MP) + Error$$

where  $PO$  is the total protein output (kg/day) and  $MP$  is the MP supply (kg/day). A nonlinear asymptotic curve was chosen to describe changes in total protein output as a function of MP supply. A sigmoidal curve was selected to represent  $f$  as suggested by Figure 1 and by recent studies in the literature (Doepel et al., 2004; St-Pierre and Weiss, 2012). The curve is described as follows:

$$f(MP) = PO_0 \exp \left[ \frac{MP}{MP + MP_0} \log \left( \frac{PO_{asym}}{PO_0} \right) \right]$$

where  $PO_0$ ,  $MP_0$  and  $PO_{asym}$  are the parameters in  $f$  to be estimated. The  $PO_0$  represents the estimated total protein output at zero MP supply.  $MP_0$  is a positive parameter (in MP units) associated with the specific rate of change of the curve and  $PO_{asym}$  is the asymptote, that is, the value the curve converges to as MP supply gets infinitely large. This curve is known as the Schumacher growth model [see Thornley and France (2005) for a detailed mathematical derivation].

The estimation of parameters in  $f$  with the 133 treatment means presented a few challenges. Firstly, the data comprises treatment means rather than individual level observations. Treatment means from different studies have different standard errors, consequently the traditional assumption of errors' variance homogeneity may not be valid. Secondly, because there may be intrinsic differences within studies that may not be accounted by the model structure, a meta-analysis approach should be used (St-Pierre, 2001). Further, the relationship between protein output and MP supply follows a nonlinear functional form (Figure 1), suggesting the need for a nonlinear mixed model. In this context, a Bayesian hierarchical modeling approach (Gelman et al., 2004) was used to fit the nonlinear response curve to data using

the rstan R package (Stan Development Team, 2016). This approach allows each study to have a random deviation on all parameters of the nonlinear model and accounts for possible heterogeneous errors' variances across studies. The fitted curve is presented in Figure 2 and the estimated parameters are in Table 1.

The estimated  $PO_0$  was 0.264 (SE = 0.092), suggesting that approximately 0.264 kg/day (0.58/lb/day) of protein is outputted daily when the MP supply is zero. The estimated  $PO_{asym}$  was 2.665 (SE = 0.376), suggesting that the asymptotic total protein output (i.e., limiting protein output when MP supply gets infinitely large) is 2.665 kg/day (5.86 lb/day). The model fit in Figure 2 suggests a good agreement between the treatment means and the fitted curve. However, if the model is going to be used for determining MP requirements, a formal evaluation of its ability in predicting total protein outputs is required. Therefore, we conducted a model evaluation through a cross-validation. In short, we iteratively left treatment means out of the data used for model fitting and evaluated the model predictive ability with means that were not used for model fitting. As a measure of model predictive ability, we calculated the root mean square prediction error. The estimated error (expressed as a percentage of the mean total protein output) was 14%, suggesting very good ability of the model in predicting total protein outputs with varying MP supplies.

### *The predicted efficiencies*

Once a model that precisely describes the relationship between protein output and MP supply is identified, the next step is to develop a strategy for its use in the calculation of efficiencies and requirement. One important characteristic of the selected model is that it has a variable efficiency of MP utilization for protein secretion. Understanding the changes

in efficiency determined by a model is key to a better understand of its mathematical properties and how these relate to modeling protein output responses. For instance, if the first derivative of the curve is a representation of a marginal efficiency, it changes nonlinearly at each level of MP supply. Further, if the cumulative efficiency of MP utilization is defined as the ratio of the total protein output and the MP supply, it can be predicted as the model predicted total protein output divided by the corresponding MP supply. The observed cumulative efficiencies, as well as the ones predicted by the nonlinear model, are presented in Figure 3. The predicted efficiencies decreased, as expected, nonlinearly with MP supply and ranged from 0.85 to 0.43. It is important to note that the predicted efficiencies are in good agreement with both Metcalf et al. (2008) and Arriola Apelo et al. (2014) who suggested a nonlinear decrease of the efficiency with increasing MP supplies.

#### *Determining MP requirements*

Up to this point, we have a model that properly predicts the protein output response to MP supply and is built on the principle of a variable efficiency of MP utilization. The final step in the development of the system was to develop a strategy for using this model to calculate the MP supply required for a given level of total protein output. The strategy was to construct a requirement function by inverting the response curve  $f$ . The MP requirement is therefore defined as the MP supply needed to predict a given total protein output in the response curve. The operation of inverting the curve can be seen, in this context, as solving an equation in “terms of  $x$ ”. This operation is, in fact, simple and relies on techniques that most of us learned during algebra classes in high school. For example, if we have a linear function:  $y = a + bx$  and want to “solve it for  $x$ ”, we use the following sequential steps: i)

subtract  $a$  from both sides of the equation:  $y - a = bx$  and ii) divide both sides by  $b$ , yielding:  $(y - a) / b = x$ . The result is a function that is the inverse of the original linear equation and is a function of  $y$  instead of  $x$ .

The strategy to develop the MP requirement function follows exactly the same logic: we invert the nonlinear response curve  $f$  to derive an equation that is a function of the total protein output. Inverting the nonlinear response curve is a little harder than inverting a linear equation, but the principle is exactly the same: we invert  $f$  by “solving for” the MP supply. This inverted function computes the MP supply needed to predict a given total protein output in the fitted curve. Therefore, the calculation of the MP requirement follows the principle of a variable efficiency through the requirement function, defined as  $R$ :

$$R(PO) = MP_0 \left[ \log \left( \frac{PO / PO_0}{PO_{asym} / PO} \right) \right]$$

where  $R(\bullet)$  is the requirement functions determining the MP supply required for a given level of total protein output ( $PO$ ). The requirements computed with  $R$  are presented in Figure 4. For comparison purposes, the MP requirements calculated using the NRC (2001) system are also presented in Figure 4. It is easy to see that the developed system determines MP requirements lower than the NRC (2001) system at lower protein outputs (Figure 4). Conversely, the new system determines MP requirements higher than the NRC (2001) system at relatively higher protein outputs. A protein output of approximately 1.1 kg/day (2.42 lb/day) seems to be the point at which our system coincides with the NRC (2001) and separates MP requirements that are relatively lower or relatively higher than the current feeding system. These results are in alignment with Lapierre et al. (2007) who suggested the NRC (2001) system underestimates MP allowable

milk at lower MP supplies and overestimates at relatively higher supplies.

### *Using the system in practice*

In order to demonstrate the use of the system in practice, we calculated the MP requirement for one cow in our data set using the estimated requirement function. The cow outputs 963 g/day of protein in milk, with a 602 kg (1324 lb) BW and a DMI of 18.5 kg/day (40.7 lb/day). Using Lapierre et al. (2014), the scurf protein output is 9.3 g (0.2 g CP/kg BW<sup>0.6</sup>), the urinary endogenous protein output is 67.5 g (2.75 g CP/kg BW<sup>0.5</sup>), and the metabolic fecal protein is 292 g (15.8 g CP/kg DMI). Assuming that the conversion factor of CP to true protein for milk, scurf, endogenous urinary and metabolic fecal and milk protein are 0.955, 1, 1 and 0.8 (Lapierre et al., 2014), the total true protein output (scurf + endogenous urinary + metabolic fecal + milk) of this cow is 1.23 kg/day (2.71 lb/day). Using the parameter estimates from Table 1, the estimated response curve describing the total protein output response to MP supply is:

$$f(MP) = 0.264 \exp \left[ \frac{MP}{MP + 1.177} \log \left( \frac{2.665}{0.264} \right) \right]$$

Inverting this curve, i.e., solving it in terms of the MP supply yields the estimated requirement function:

$$R(PO) = 1.177 \left[ \log \left( \frac{PO / 0.264}{2.665 / PO} \right) \right]$$

The calculated MP requirement is obtained by plugging in the *PO* in *R(PO)* above. In this example, *PO* is 1.23 kg/day (2.71 lb/day) and the calculated MP requirement is therefore 2.34 kg/day (5.15 lb/day). It is important to note that using the NRC (2001) system, the MP requirement for this cow is 2.05 kg/day (4.51 lb/day), reinforcing that requirements determined with our model are higher than the

ones calculated with a fixed 0.67 efficiency at high MP supplies.

### **Conclusions**

A new system is proposed for the calculation of MP requirements by lactating dairy cows. The system is built on the principle of variable efficiency of MP utilization and determines MP requirements for total protein output with a requirement function. The efficiencies predicted by the system decreased nonlinearly with MP supply and range from 0.85 to 0.43. At approximately 1.1 kg/day (2.42 lb/day) of total protein output, the system determines MP requirements that are similar to the ones calculated with the NRC (2001) system. MP requirements below this output level are predicted by the system as consistently smaller than requirements calculated by the current feeding system. Above 1.1 kg/day (2.42 lb/day) of total protein output, the system calculates MP requirements that are higher than the NRC (2001).

### **References**

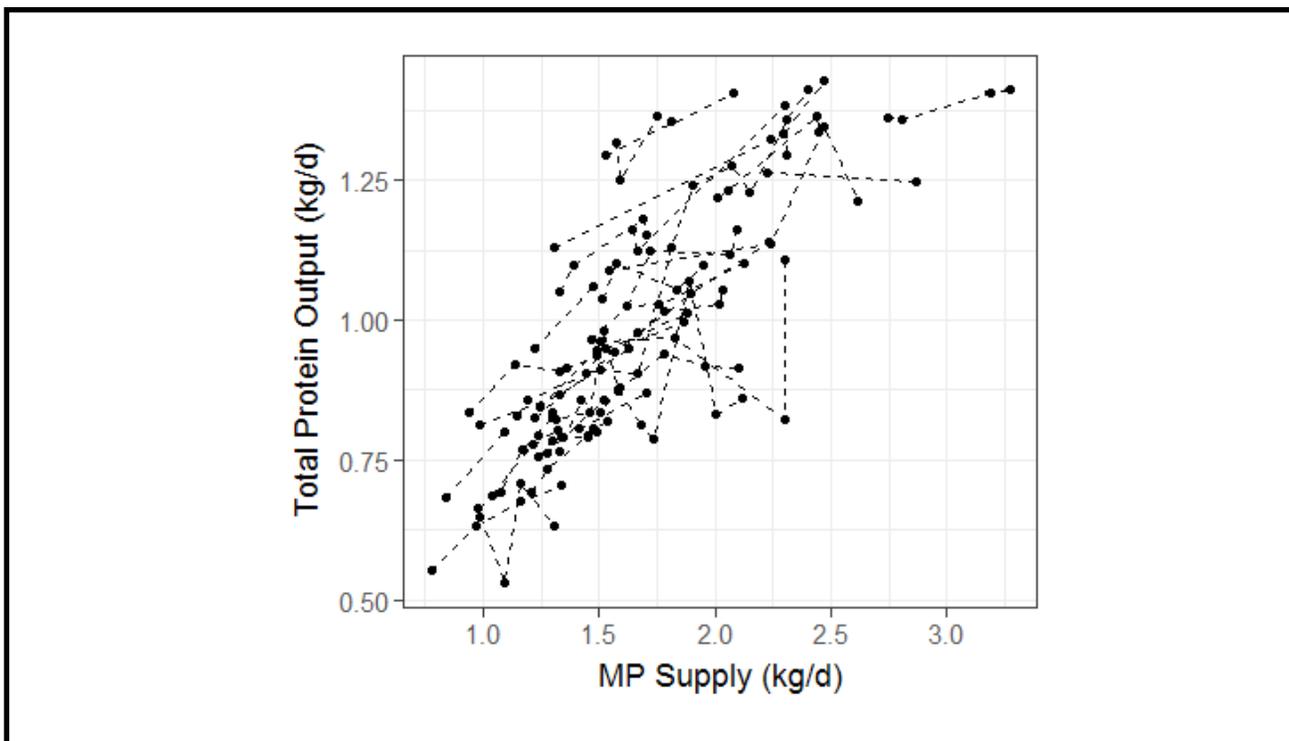
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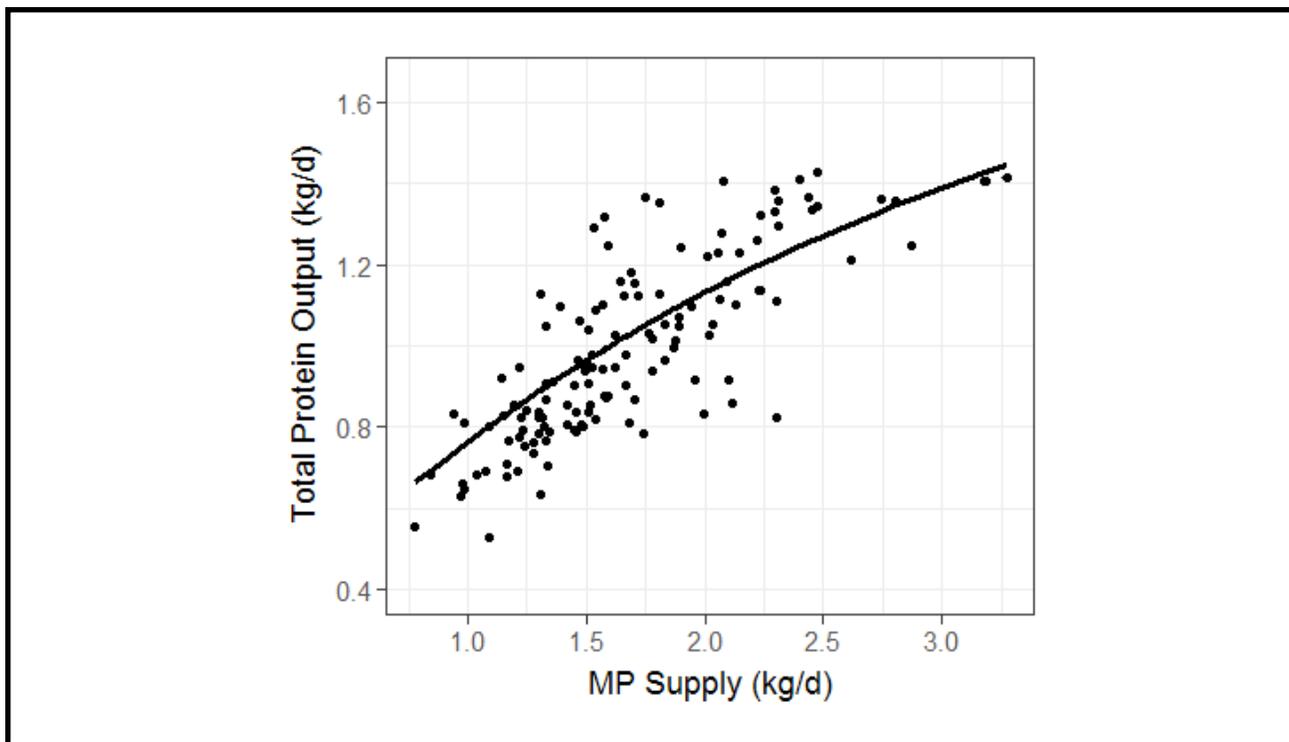
**Table 1.** Parameter estimates (Bayesian posterior means), standard errors [Bayesian posterior standard deviation (**SD**)] and 95% Intervals (Bayesian Credible Intervals) for the nonlinear response curve describing the relationship between total protein output (kg/day) and MP supply (kg/day).

Parameter <sup>1</sup>	Posterior Mean	Posterior SD	95% CrI
$PO_{asym}$	2.665	0.376	(1.754, 3.237)
$PO_0$	0.264	0.092	(0.040, 0.391)
$MP_0$	1.177	0.369	(0.278, 1.669)

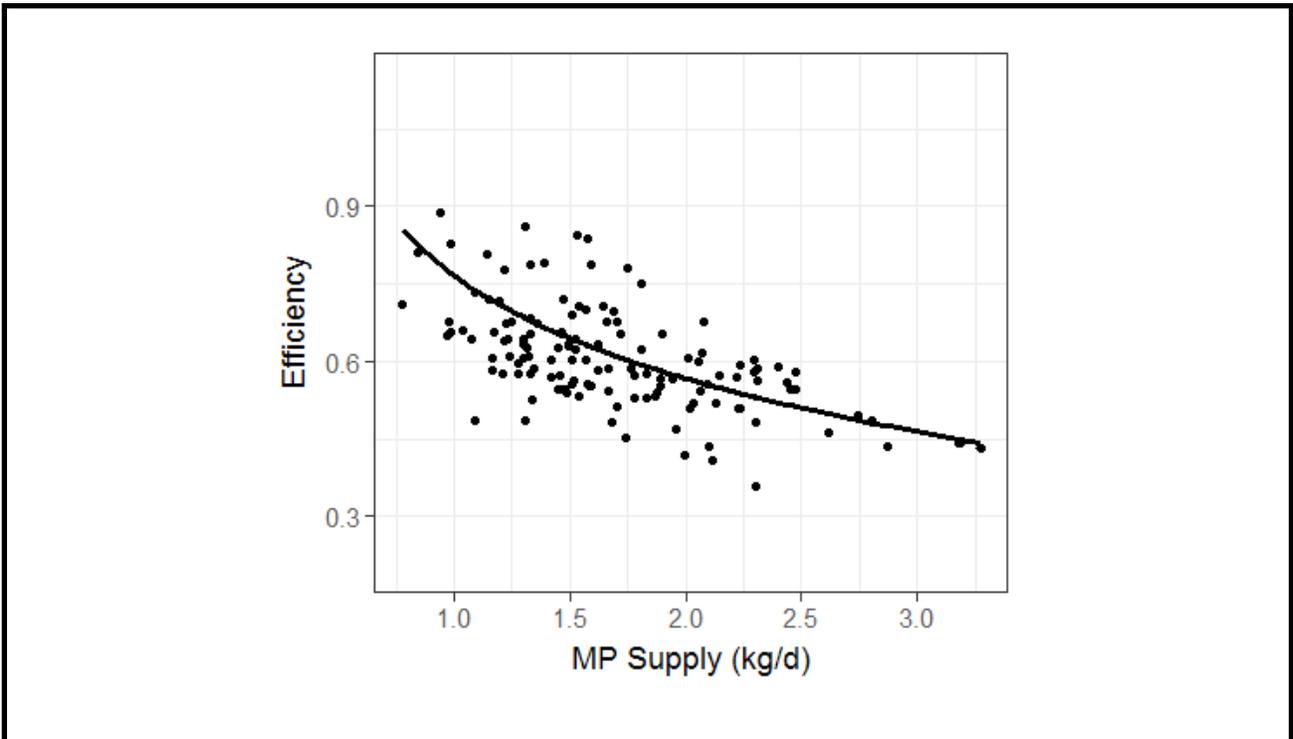
<sup>1</sup> $PO_0$  represents the estimated total protein output at zero MP supply,  $MP_0$  is a positive parameter associated with the specific rate of change of the curve, and  $PO_{asym}$  is the asymptote total protein output which the function converges to as MP goes to infinity.



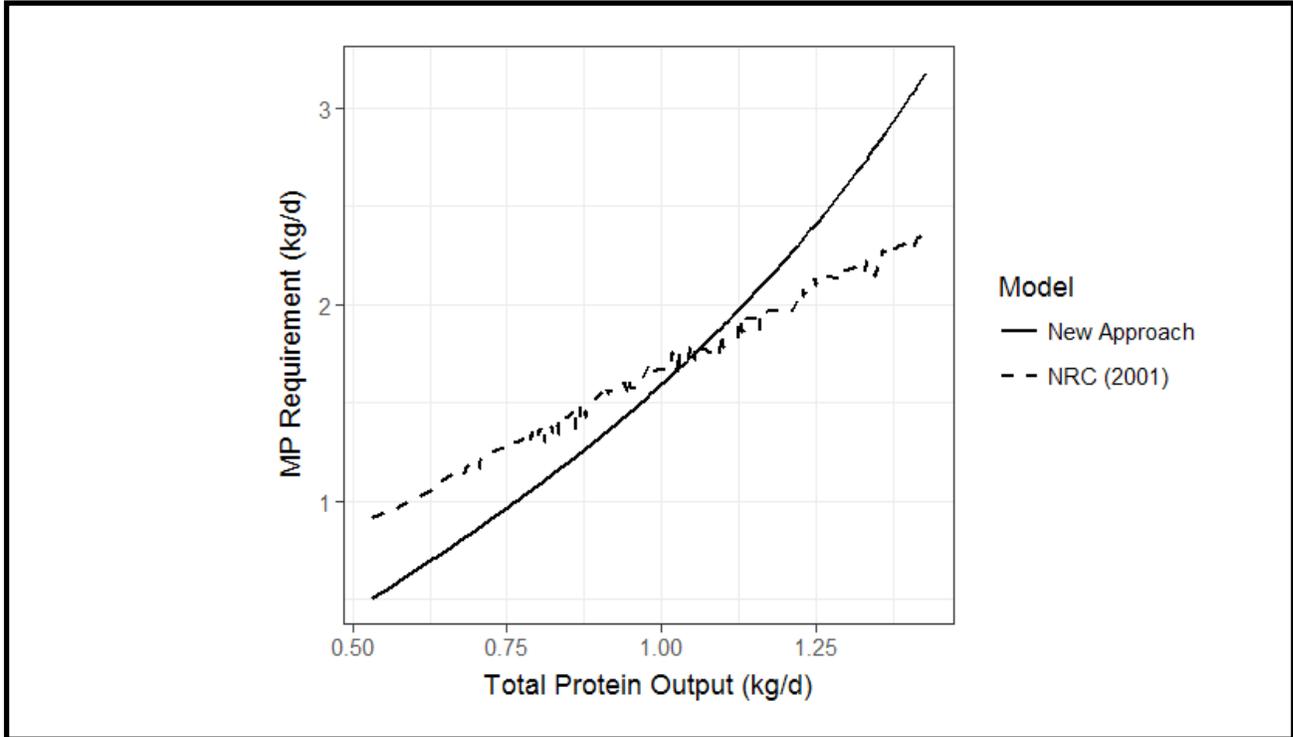
**Figure 1.** Total protein output (scurf + endogenous urinary + metabolic fecal + milk) versus metabolizable protein (MP) supply. The solid circles represent 133 treatment means from 36 publications. The dashed lines connect means from the same publication.



**Figure 2.** Total protein output (scurf + endogenous urinary + metabolic fecal + milk) versus metabolizable protein (MP) supply. The solid circles represent 133 treatment means from 36 publications. The curve is the fitted nonlinear Schumacher function using a Bayesian hierarchical modeling approach.



**Figure 3.** Combined cumulative efficiencies (total protein output divided by the metabolizable protein supply) versus metabolizable protein (MP) supply. Points are the records (Total Protein Output/MP Supply) and the curve collects the predicted efficiencies using the nonlinear Schumacher function (i.e., Predicted Total Protein Output/MP Supply).



**Figure 4.** Calculated metabolizable protein (MP) requirement (requirement for scurf + endogenous urinary + metabolic fecal + milk) for a given level of total protein output using the estimated requirement function. The dashed curve is the MP requirement calculated using the NRC (2001) system.