J. Dairy Sci. 87:1279–1297

Milk Protein Synthesis as a Function of Amino Acid Supply*

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ABSTRACT

Most prediction schemes of milk protein secretion overestimate milk protein yield from dairy cows at high protein intakes, thereby overestimating milk protein yield response to protein supplementation. This study was conducted to determine factors contributing to such an overestimation. Using published studies, a database was constructed that was limited to amino acid (AA) infusion studies, as then only the digestible amino acid of dietary origin needed to be estimated, whereas the amount infused was known exactly, thereby reducing the dependence on estimated values.

Although milk protein yield was positively related with total energy supply, and both digestible duodenal supply and infused AA, in this database there was no relationship between milk protein yield response above control treatments and the nutrient status of the cows (energy or protein). Total milk protein yield was defined as a function of individual AA supply, using a segmented-linear and a logistic model to obtain estimates of the efficiency of conversion of AA into milk protein. Except for Lys and Met supply, the segmented-linear model yielded lower root mean square error and better correlation, but both models were similar in their reliability. For both models, the estimated efficiency of conversion of AA to milk differed among AA. Estimations of the ideal profile of AA for lactating dairy cows were similar between models, with requirements for Lys and Met in line with 2001 National Research Council recommendations. The major difference is that the segmented-linear model yields a constant efficiency of conversion of an AA until requirements are met, with zero efficiency beyond this point. The logistic model allows for an estimation of the decreasing marginal efficiency of conversion of AA as the supply approaches the requirements. The use of variable efficiency factors should improve our ability to predict protein yield in response to supplemental protein.
(**Key words:** amino acid, requirement, lactation, efficiency)

Abbreviation key: AAT = total digestible AA from diet and infusion, **CPT** = total CP supply from diet and infusion, **EAA** = essential AA, **MP** = metabolizable protein, **MPT** = total MP supply from diet and infusion, **NEAA** = nonessential AA, **NELT** = total NE_L supply from diet and infusion, **PDI** = protein truly digested in the small intestine, **PY** = milk true protein yield; **PYΔ** = milk protein response; **RMSE** = root mean square error.

INTRODUCTION

With current milk pricing strategies in Canada and the United States, profit margins for producers can often be improved by maximizing milk protein yield (**PY**). However, increasing milk protein content by nutritional manipulation is difficult (DePeters and Cant, 1992). Increasing the supply of RUP does not ensure improved milk protein production (Santos et al., 1998). Substitution of RDP by RUP can lead to decreased microbial protein synthesis and therefore does not necessarily result in overall positive effects on the amount and profile of AA flowing to the duodenum, and subsequently on PY (Santos et al., 1998). Supplying AA postruminally increases the AA supply to the dairy cow by a known amount, but still the milk protein response (**PYΔ**) to such supplementation is often variable. Similarly, postruminal infusion of casein has produced inconsistent results (Clark, 1975; Hanigan et al., 1998).

Received March 17, 2003.
Accepted September 5, 2003.
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*Lennoxville Research Centre contribution number: 816; this work was partly supported by a grant from the Dairy Farmers of Canada.
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Positive responses may not be observed because the supplemental AA may not have been limiting in the basal diet. Additionally, intestinal and hepatic AA metabolism have a major impact on the amount and profile of AA supplied to the mammary gland relative to intestinal disappearance and portal absorption (Seal and Reynolds, 1993). This is evident in the study of Blouin et al. (2002), in which it was demonstrated that the liver removes from 4 (Lys) to 80% (Ser) of the AA absorbed into the portal vein, so clearly the liver is changing the profile of AA available to the mammary gland relative to the amount absorbed from the gut.

The challenge therefore remains to accurately predict PYΔ to protein or AA supplementation. The model from the National Research Council (NRC, 2001) predicts PY using fixed efficiencies of conversion of metabolizable protein (**MP**) supply for maintenance (67%), gestation (33%), and milk production (67%). The Cornell Net Carbohydrate and Protein System (CNCPS, 2000) also relates milk AA output to AA duodenal flow "devoted" to milk production using fixed efficiencies of conversion of essential AA (**EAA**) that vary among individual AA from 62 to 100% (excluding Arg). However, the recovery of postruminally infused casein into milk protein averaged only 21% across 7 studies (Hanigan et al., 1998), which contrasts markedly with the efficiencies of conversion used by NRC (2001) or CNCPS (2000). This strongly suggests that the efficiency of conversion of AA to milk protein declines as AA supply approaches estimated requirements.

The current state of knowledge in the area of ruminant AA metabolism apparently does not allow for accurate prediction of PY in response to AA supply. Prediction schemes however do exist that attempt to relate AA supply to demand (O'Connor et al., 1993), and the NRC (2001) provides recommendations for Lys and Met supply relative to total MP based on Rulquin et al. (1993) and Schwab et al. (1992b). Rulquin et al. (2001) has also proposed requirements for other EAA based on a limited number of infusion studies.

Consequently, we integrated data from studies in which AA were infused postruminally in dairy cows with the objective of defining equations to predict PY and PYΔ as functions of AA supply. Our second objective was to use this data set to test our hypothesis that the efficiency of conversion of digestible AA into milk protein is not a constant as is assumed in current prediction schemes (CNCPS, 2000; NRC, 2001). Specifically, our aim was to determine the influence of AA supply on the variation of the efficiency of conversion of AA into milk and to estimate requirements for EAA. Because of the limitations of the data set, the equations and efficiency values reported herein are not intended to be used on a practical feed formulation level, but rather to expand our perspective of how AA recommendations could be generated.

MATERIALS AND METHODS

Source of Data

Data analyzed in this study originated from 40 publications encompassing 59 trials and 217 treatments (Appendix A). Each published treatment mean corresponds to an observation in the database. Because several publications in the database reported the results of more than one experiment, the observations were classified according to experiment within publication to account for unexplained variation between experiments. Published studies involving abomasal, duodenal, or intravenous infusions of casein or free AA were selected. With these studies the supply from the infusions was known exactly, thus reducing the dependence on the NRC (2001) prediction for AA supply (see explanation later in this section). Studies in which protein intake was manipulated by dietary means were not used because estimates of digestible AA supply would then rely entirely on the NRC (2001) predictions. Similarly, trials in which rumen-protected AA were fed were excluded due to the uncertainty associated with predicting the extent of rumen-protection of the AA. Within the infusion studies, only studies with information on feed intake and diet composition sufficient to estimate duodenal AA supply were used.

Thirty-three of the trials involved abomasal infusions, 8 involved intravenous infusions, 17 involved duodenal infusions, and 1 involved intravenous and abomasal infusions. In 53 of the trials, infusions were given continuously (>20 h/d). In 4 of the trials, infusions were given over a period of less than 10 h/d, and in 2 trials, both continuous and noncontinuous infusions were used. Infusions were administered on average for 11 d (SD 4.8), with a range of 4 to 28 d. Two of the trials involved hyperinsulinemic-euglycemic clamps, and results obtained during administration of the clamp (4 observations) were excluded from the data set.

Of the 59 trials, Holstein (Friesian) cows were used in 52, Ayrshires were used in 5, and both Holsteins and Ayrshires were used in 1. In one trial, the breed of cow was not reported. The cows were multiparous in 58 of the 59 trials. Twenty-seven trials began in early lactation (<84 DIM), 29 began in midlactation (84 to 210 DIM), and 1 began in late lactation. Days in milk were not reported in 2 trials. Body weight was not given in 19 of the 59 trials. In these cases, BW was estimated using reported information from other papers by the same authors included in the database. Feed intake, BW, and milk production data are shown in Table 1. Of the 213 treatments analyzed, 57 were control treat-

Table 1. Dry matter intake, BW, and milk production of cows from all studies in the database.

	Mean	SD	Range
DMI, kg	17.7	3.3	9.1–27.6
DIM, d	98	53.5	7–220
BW, kg	566	47.8	469–675
Milk yield, kg/d	25.6	6.2	10.7–40.0
Milk protein content, %[1]	2.9	0.2	2.5–3.5
Milk protein yield, g/d[1]	738	166	318–1105
Milk protein yield response, g[2]	50.8	46.2	–66.6–184.1
Milk protein yield response, %[2]	8.37	8.5	–7.1–39.8
Milk fat content, %	3.9	0.6	2.9–6.0
Milk fat yield, g/d	974	182	530–1602

[1]Milk true protein; calculated as CP × 0.93 (NRC, 2001).

[2]Difference in protein yield between treatment and control animals.

ments, 41 were casein (sodium or potassium caseinate) infusions, 9 were casein hydrolysate infusions, and 106 were free AA infusions. The number of AA infused ranged from 1 to 20 (Appendix A).

Nutrient intakes, net energy of lactation of the diet, MP supply, and predictions of digestible duodenal AA flows were estimated for each treatment using NRC (2001) based on the reported cow and diet information. If the nutrient composition of individual ingredients was published those values were used, otherwise, default model values (NRC, 2001) were used. If total diet composition was published, but not individual ingredient composition, composition of the forages was estimated so that diet composition values agreed with published values. Intestinal digestibility of the infusates was considered to be 100%.

Data Analysis

Prediction of milk true protein yield. Principal component analysis was performed to determine which of the variables explained the majority of the variation in the data and to explore association among the variables (Jobson, 1992; SAS, 1999). The variables analyzed included production traits (PY, PYΔ, DIM); and dietary traits [AA (digestible and infused), MP supply derived from diet, MP supplied from infusion, and total supply of net energy of lactation (**NELT** = diet + infusion]. Prediction equations for true milk PY (estimated as CP yield × 0.93 (NRC, 2001) when not published) using dietary components as predictors were obtained by regressing PY against NELT (Mcal/d), total CP supply (**CPT** = CP diet + CP infusion, g/d), total MP supply (**MPT** = MP diet + MP infusion, g/d), and MPT and NELT together. Experiment within publication was included in the model as a random effect to account for variation between studies not described by other factors considered in the model. Regression analyses were performed with the MIXED procedure of SAS (1999). Intercept, linear, and quadratic terms were tested as fixed effects. A random shift on intercept and slope was modeled using experiment within publication as a subject, and a simple variance-covariance matrix for the random parameters. The PYΔ was calculated as the difference in PY between the infusion treatments and the control treatments within each experiment.

Modeling of PY as a function of AA supply. Milk true PY was modeled as a function of total supply of AA (**AAT**: dietary digestible plus infused AA). The NRC (2001) does not predict Trp or nonessential AA (**NEAA**) duodenal flows, so these terms were not included in any of the models tested. The AA in milk were estimated using the AA concentrations in milk reported by Jensen (1995). Linear and nonlinear functions were examined. The nonlinear models were fitted using a weighting of each observation based on the reported SEM of the milk protein yield in each experiment. A weighting variable was calculated for each observation by squaring the SEM and then dividing by the average of the squared SEM across all experiments (St-Pierre, 2001).

The linear function, a segmented-linear (also referred to as "broken-stick" or "bent-line") model, was fitted to the data using the model:

$$\hat{y} = a + bx \text{ (for } x \le x_b\text{) and} \quad [1]$$

$$\hat{y} = a + bx_b \text{ (for } x \ge x_b\text{)} \quad [2]$$

such that $\hat{y}$ is the predicted yield of true protein or AA in milk (g/d), x is AAT (g/d; for example, total supply of His is referred to as HisT), a is the intercept, b is the slope, and x_b is the value of x beyond which the marginal efficiency ($\Delta_y/\Delta x$) is equal to zero. This point, x_b, will be referred to as the breakpoint of the segmented-linear model. Estimates of a, b, and x_b were obtained using the NLMIXED procedure of SAS (SAS, 1999).

As the majority of the data lay below the breakpoint, a linear model was tested with those data relating PY to all AAT and NELT. Variables with the largest *P*-

Table 2. Root mean square error (RMSE) and Akaike's information criteria (AIC) for three nonlinear models tested to model AA in milk as a function of AA supply.[1]

	Michaelis-Menten		Exponential		Logistic	
AA	RMSE	AIC	RMSE	AIC	RMSE	AIC
Arg	3.61	1135	3.58	1151	3.51	1129
His	2.63	1008	2.61	1015	2.55	1002
Ile	6.48	1388	6.43	1399	6.32	1382
Leu	10.24	1581	10.07	1596	9.77	1565
Lys	8.91	1507	8.87	1526	8.79	1506
Met	2.79	1020	2.79	1037	2.79	1026
Phe	5.28	1302	5.25	1320	5.19	1299
Thr	4.47	1235	4.45	1255	4.30	1225
Val	7.60	1455	7.55	1473	7.44	1451

[1]Smaller RMSE and AIC indicate a better fit.

values were sequentially removed until the model contained only significant variables ($P < 0.10$). Residuals (observed-predicted) were then regressed against variables not used in the model to determine if additional terms would contribute to the power of the model. These additional terms included ADF intake, NDF intake, nonfiber carbohydrate intake, DIM, parity, BW, feeding frequency, and site and length of infusion. Only DIM was found to be significant and subsequently included in the model.

Final regression equations were obtained using Proc MIXED as descibed above. Michaelis-Menten, exponential, and logistic nonlinear functions were tested to predict PY as a function of AAT using the NLMIXED procedure in SAS (SAS, 1999). From the 3 models, the logistic model best fitted the observed data, based on the RMSE and Akaike's information criteria (AIC; SAS, 1999; Table 2). The logistic model selected had the form

$$\hat{y} = \frac{A}{1 + Be^{-\frac{x}{k}}} \quad [3]$$

where $\hat{y}$ and x are the same as in equation [1]; and A, B, and k are the parameters of the function defining the ceiling of the response, the amplitude and the steepness of the curve, respectively.

For the final models presented, residuals were tested for heteroscedasticity using White's and Breusch-Pagan tests in the procedure MODEL of SAS (SAS, 1999).

Calculation of efficiency of conversion of AA. The total cumulative efficiency of conversion of individual AAT to milk protein was calculated as the ratio between the milk AA yield predicted from the fitted models ($\hat{y}$: equations [1–3]) and the corresponding amount of total supply of an AA (x = AAT). Marginal total efficiency of conversion ($\Delta\hat{y}/\Delta x$) was calculated from the ratio between increment of predicted PY and increment between corresponding supply (x). For the linear model, it is equal to the slope b from equation [1], and for the logistic model, it is calculated from its first derivative [4]:

$$\frac{dy}{dx} = \frac{ABe^{-\frac{x}{k}}}{k\,(1 + Be^{-\frac{x}{k}})^2} \quad [4]$$

Calculation of the optimal total supply of AA. For the segmented-linear model, the parameter x_b is assumed to represent the value of the optimal total supply of AA, because supply beyond this point would not increase the output of y. In the logistic model, the maximal $\hat{y}$ is attained at an infinite supply of x. Thus, an alternative approach to estimate the optimal total supply of AA is outlined as follows. In the logistic function, the marginal efficiency (equation [4]; Figure 1) first increases exponentially, reaches a maximum, and decreases exponentially, giving the logistic curve its characteristic sigmoid form with point of symmetry equal to the maximum marginal efficiency. In each of these symmetrical halves it is possible to define a "critical point" in which the function either starts a faster increase or decrease (i.e., the points defining the curvature of the "S"). These critical points can be defined as the minimum and maximum values of the second derivative of the logistic function (Figure 1). Algebraically, it is possible to define these two critical points (lower: x_L and upper: x_U) as

$$x_{\mathrm{L}} = k\,\log(2B - \sqrt{3B}) \quad [5]$$

$$\text{and } x_{\mathrm{U}} = k\,\log(\sqrt{3B} + 2B) \quad [6]$$

such that x_L and x_U are the values of x (**AAT**) where the marginal efficiency increases or decreases rapidly, respectively; and B and k are the parameters for the logistic equation fitted to individual AA (Grossman,

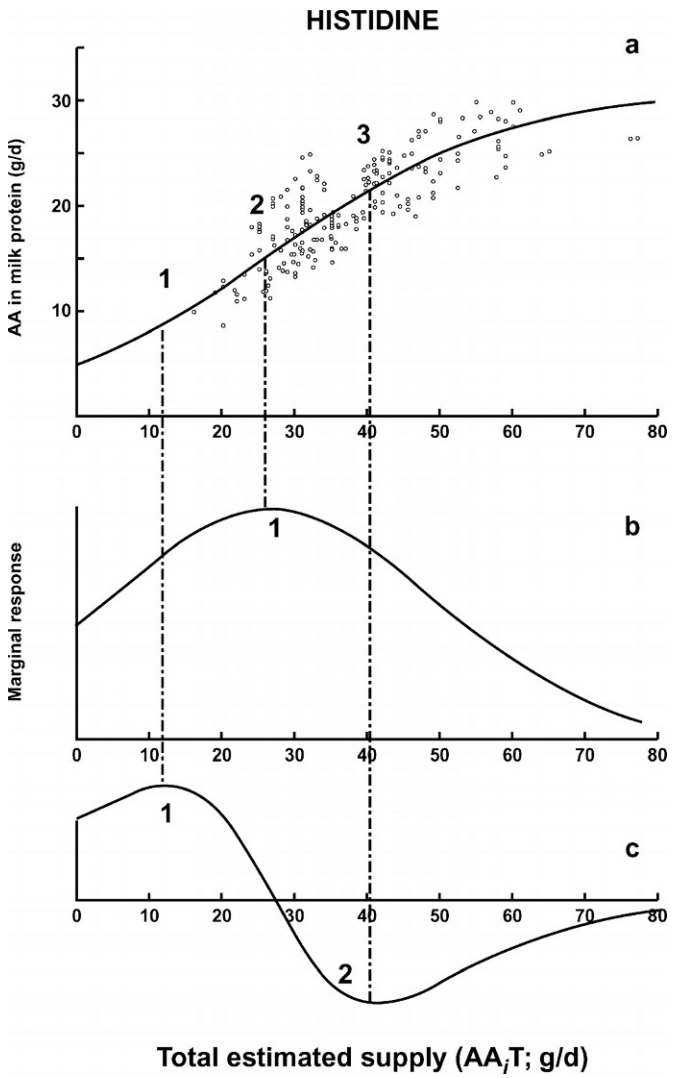

Figure 1. Visual representation of the fitted logistic function superimposed on the observed values (a); together with its first (b) and second (c) derivatives. The curve (b) represents the marginal efficiency. The maximum marginal efficiency (a2, b1) is calculated from the first derivative, and the lower (a1, c1) and upper (a3, c2) critical points are calculated from the second derivative. The upper critical point is assumed to represent the requirement for duodenal AA supply (see text for explanation).

1986). The upper critical point (x_U) was assumed to represent the optimum for AAT, as supply in excess of this value results in marginal efficiencies rapidly approaching zero. The individual AAT that corresponded to the optimum (x_b for the segmented-linear and x_U for the logistic models) were summed to give optimum supply of total EAA. The proportion of each EAA in total EAA was then calculated, with the proportion being indicative of the required relative contribution of each AA to an "ideal" pattern of metabolizable EAA. Approximate standard errors for the requirements (absolute amounts and amounts relative to EAA) were calculated using the ESTIMATE option of the NLMIXED procedure in SAS (SAS, 1999). Essential AA requirements were also determined on an MP basis assuming that EAA represent 48% of MP (average for the database).

Calculation of the efficiencies of conversion of AA for lactation. In addition to total efficiency of conversion, the efficiency for lactation was calculated using the AA supply only available for milk, with the maintenance requirement (see below for estimation of requirement) of each AA being subtracted from AAT. Therefore, the linear and the logistic functions were fitted again, this time with the x being only the AA available for milk. For the segmented-linear model, the 'no intercept' option was used with the assumption that body stores are not contributing to milk PY, therefore when AA available for milk are zero, AA yield in milk is assumed to be zero. The maintenance requirement of each AA was calculated from the estimated NRC (2001) maintenance requirement expressed as MP, with the appropriate composition of AA for each component (Table 3). The four components that comprise the maintenance MP requirement are scurf protein, urinary protein, metabolic fecal protein, and endogenous protein (NRC, 2001). To calculate the scurf requirement, the AA composition of keratin (Block and Bolling, 1951) was used. For the urinary requirement, whole empty body composition was used (Williams, 1978; Rohr and Lebzien, 1991; Ainslie et al., 1993). Metabolic fecal protein consists of various compounds including mucous secretions, bile pigments, sloughed epithelial cells, bacterial debris, and keratinized cells (O'Connor et al., 1993; NRC, 2001). According to NRC (2001), the bacterial contribution to metabolic fecal protein was calculated as 0.5 × (bacterial MP/0.8 – bacterial MP). The AA composition of endogenous protein from rumen fluid was used to estimate the AA requirement for this component (Ørskov et al., 1986). The remainder of the metabolic fecal protein requirement was calculated as metabolic fecal protein less the bacterial contribution, and was assumed to be of intestinal origin. The AA composition of porcine intestinal endogenous protein (Stein et al., 1999; de Lange et al., 1989a, 1989b) was used for this requirement. In these studies, the endogenous protein composition was determined in animals fed protein-free diets.

RESULTS

Principal Component Analysis

Principal component analysis revealed the relative dependence of PY and PYΔ on digestible nutrients arriving at the duodenum either as a result of the diges-

Table 3. Amino acid composition of tissue and endogenous proteins (g 100 g^{-1} total AA) used for the estimation of maintenance requirements.

AA	Whole empty body[1]	Abomasal isolate[2]	Ruminal isolate[3]	Intestinal isolate[4]	Intestinal isolate[5]	Keratin[6]
Arg	7.3	4.9	6.2	3.9	4.0	4.4
His	2.7	3.6	3.4	1.6	2.0	1.2
Ile	3.1	4.6	4.7	3.2	4.0	5.8
Leu	7.4	4.8	9.0	5.3	6.6	11.6
Lys	7.0	7.3	7.7	3.9	5.4	3.7
Met	2.2	1.5	1.6	1.2	2.0	1.2
Phe	3.9	4.6	5.0	4.0	7.1	4.3
Thr	4.3	6.5	6.0	5.4	7.9	8.4
Trp	0.8	1.9	1.8	1.7	1.7	1.6
Val	4.4	6.1	6.2	4.2	6.0	7.0
Ala		5.6	6.0	5.0	5.6	
Asp		9.1	3.0	7.9	9.8	
Cys		3.2	2.6	2.3	2.3	
Glu		12.4	15.0	9.5	11.4	
Gly		6.4	5.6	11.1	8.1	
Pro		6.2	6.0	21.9	5.6	
Ser		6.5	5.6	4.9	6.3	
Tyr		4.9	4.7	2.9	3.9	

[1]Average of values from Ainslie et al. (1993), Rohr and Lebzien (1991), and Williams (1978).

[2]Orskov et al. (1986), except Trp (Stein et al., 1999).

[3]Average of values from Stein et al. (1999; lactating sows and growing pigs) and de Lange et al. (1989a, 1989b; control treatments).

[4]de Lange et al. (1989b; AA treatment).

[5]Block and Bolling (1951).

tion of the diet or supplied by the infusions. The first principal component comprised the basal digestible duodenal AA flow together with NE_L intake. The second principal component comprised the supplemental AA from the infusates. The first and second principal components explained 65 and 20% of the total variance, respectively. Addition of a third principal component containing DIM and BW increased this value an additional 5%. Protein yield was positively related to both AA infused and digestible AA in duodenal flow and inversely related to DIM. The PYΔ was negatively correlated with digestible duodenal AA flow (first principal component), and poorly correlated with supplemental AA (second principal component).

Milk Protein Yield

After accounting for a significant ($P < 0.01$) random effect of experiment on slope, PY and NELT were linearly related (equation 4.1, Table 4). In contrast, the PY relationships with CPT and MPT were quadratic (equations 4.2 and 4.3, Table 4), with a significant ($P < 0.01$) random effect of experiment on intercepts. The prediction of PY was improved when MPT replaced CPT in the model (cf. equations 4.2 and 4.3). Prediction of PY was only marginally improved by including both the NELT and MPT terms (equation 4.4). From the observations of the principal component analysis, it was concluded that a regression model that included AAT and NELT would account for the majority of the variation in PY. Therefore, for data below the breakpoint determined by the segmented-linear model, PY was regressed against AAT and NELT. Removal of the nonsignificant terms and addition of the DIM term resulted in an equation containing HisT, LysT, MetT, NELT, and DIM (equation 4.5, Table 4). To determine whether any one of these 3 AA had a dominant role in determining PY, PY was regressed against NELT, DIM, and each of these three AA individually. The residual variance after fitting the models for these AA was similar (data not shown), indicating that PY is not dependent on only one AA but that the AA are highly interrelated. Regression and principal component analyses did not identify any significant relationships between PYΔ and AA infused, dietary digestible AA, AAT, or NELT. As a result, further attempts to generate prediction equations for PYΔ were not pursued.

Attempts to model the random effects of experiment for both the segmented-linear and logistic models resulted in unstable solutions due to the limited number and spread of data within each experiment. Therefore, all results presented are inferred from the fixed effects-only models. Both the segmented-linear and logistic models were similar in their ability to describe the relationship between PY and AAT, based on their root mean square error (Figure 2). Due to the different way they

Table 4. Prediction equations for milk true protein yield (PY), g/d.

Equation	Dependent variable	n	Intercept	SE	Independent variable[1]	Coefficient[2]	SE	AIC[3]
4.1	PY	213	–73.8[4]	44.8	NELT	29.04	1.57	2348
4.2	PY	213	–190.4	83.9	CPT	0.46	0.06	2346
					CPT × CPT	–0.000041	0.00001	
4.3	PY	213	39.3[4]	56.4	MPT	0.51	0.06	2315
					MPT × MPT	–0.000062	0.000016	
4.4	PY	213	–187.4	66.2	NELT	14.16	2.58	2291
					MPT	0.42	0.06	
					MPT × MPT	–0.000065	0.000015	
4.5	PY	175	–35.7[4]	46.9	HisT	4.27	1.02	1850
					LysT	0.65	0.27	
					MetT	1.68	0.75	
					NELT	20.04	2.22	
					DIM	–0.70	0.09	

[1]NELT = net energy supply from diet and infusion in Mcal/d; CPT = total dietary CP supply from diet and infusion in g/d; MPT = total metabolizable protein from diet and infusion in g/d; AAT = digestible duodenal AA flow + infusion in g/d.

[2]Coefficient for fixed effects after adjustment for the random effect of experiment.

[3]AIC = Akaike's information criteria after adjustment for the random and fixed effects of experiment: smaller value indicates a better fit.

[4]Intercept not significantly different from zero.

were defined, the optimum absolute amounts of AA estimated with the logistic model were on average 88% of the estimates from the broken stick model (Table 6). However, when the results are expressed as a percentage of EAA total supply, or as a percentage of MP, the values from the 2 models were similar (Table 6).

Efficiency of Amino Acid Use

Efficiency of conversion of dietary CP for milk protein secretion in the control treatments, calculated as PY/CPT, was 0.27. When expressed on an MP basis (PY/MPT), efficiency increased to 0.42. The efficiency of con-

Table 5. Parameters[1] (standard error[2] in parentheses) and summary of fit of a segmented-linear and a logistic model used to model milk protein yield as a function of AA total supply.

	Parameters of segmented-linear model				
AA	a	b	x_b	RMSE	Adj. R^2
Arg	80 (39)	8.7 (0.6)	107	98.5	0.65
His	135 (31)	16.4 (0.9)	53	89.9	0.71
Ile	79 (41)	7.9 (0.5)	118	102.7	0.62
Leu	156 (34)	3.9 (0.3)	211	95.0	0.68
Lys	96 (37)	5.4 (0.3)	162	102.0	0.63
Met	194 (26)	15.3 (0.8)	57	94.5	0.69
Phe	116 (38)	7.3 (0.5)	117	102.8	0.63
Thr	–30 (39)	9.3 (0.5)	113	91.9	0.70
Val	110 (40)	6.5 (0.4)	136	110.8	0.57
	A	k	B	RMSE	Adj. R^2
Arg	1100 (53)	26.8 (3.7)	7.9 (1.9)	99.1	0.65
His	1148 (70)	16.2 (2.2)	5.2 (0.7)	91.3	0.70
Ile	1127 (72)	32.4 (5.2)	6.8 (1.5)	104.7	0.61
Leu	1072 (46)	57.7 (7.5)	5.6 (1.0)	97.8	0.66
Lys	1094 (60)	44.1 (6.03)	6.7 (1.4)	101.4	0.64
Met	1194 (91)	18.0 (2.7)	4.3 (0.5)	94.5	0.69
Phe	1132 (76)	35.1 (5.9)	5.9 (1.2)	104.7	0.61
Thr	1117 (56)	26.3 (3.4)	11.3 (3.4)	92.7	0.70
Val	1171 (104)	41.7 (7.9)	5.9 (1.2)	112.7	0.55

[1]See text for explanation about each parameter.

[2]Standard errors cannot be reliably estimated for the breakpoint.

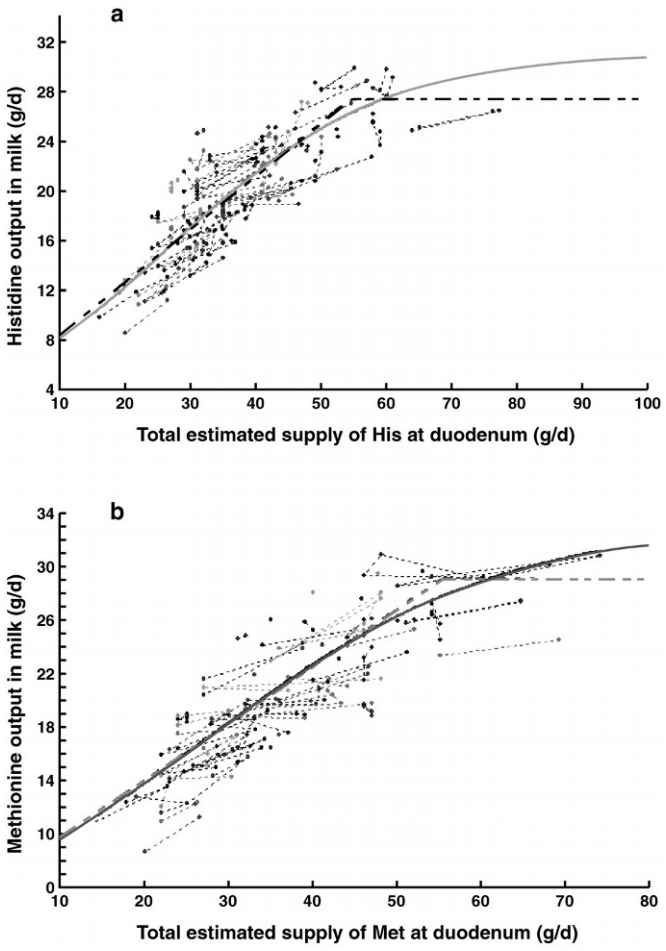

Figure 2. Graphical representation of the data sets for His and Met. Data points from the same experiment are connected by dotted lines. The logistic (solid line) and segmented linear (dashed line) fitted models are superimposed.

version for lactation of either MPT or AAT considers only the MPT or AAT that is available for milk production, i.e., the requirement for maintenance has been subtracted from the total amount available. The efficiency of MP conversion for lactation calculated using the segmented-linear model averaged 0.72, for values of total supply below the breakpoint x_b. For the logistic model, milk protein and AA in milk exhibited diminishing return behavior. The efficiency of conversion was higher at low levels of supply and decreased as AA supply increased. At 50% of the calculated optimal supply, efficiency of MP conversion for lactation was on average 0.91, and decreased to an average of 0.64 at the optimum supply.

For both the segmented-linear and the logistic models, variation in the efficiency of conversion among AA was shown (Table 7). With both models, and across all trials and milk PY, His was the most efficiently used AA, and Arg the least efficiently used. For comparison, the efficiency values used in version 4 of the CNCPS (2000) prediction model are also presented in Table 7.

DISCUSSION

Protein Yield

The relationship between PY and MPT was better than the relationship between PY and CPT. This is to be expected since MP is protein available to the animal at the intestinal level, while CP is merely a reflection of dietary protein and nonprotein nitrogen that is subject to net losses during ruminal fermentation. Total net energy of lactation was a better predictor of PY than was MPT: in ruminants, energy intake has effects on both protein and energy supplies. Energy intake directly affects the amount of energy available to the animal, which, translated into glucose and acetate supply to the mammary gland, is needed to support high levels of milk production. In addition to this, energy

Table 6. Optimal absolute and relative amounts of digestible AA supply estimated from segmented-linear and logistic curve models.

	Segmented-linear model			Logistic model		
AA	g/d	% EAA[1]	%MP[2]	g/d	% EAA	%MP
Arg	107	10.0	4.8	91	9.6	4.6
His	53	4.9	2.4	48	5.1	2.4
Ile	118	11.0	5.3	105	11.1	5.3
Leu	211	19.7	9.4	175	18.5	8.9
Lys	162	15.1	7.2	142	15.0	7.2
Met	56	5.2	2.5	50	5.3	2.5
Phe	117	10.9	5.2	108	11.4	5.5
Thr	113	10.5	5.1	98	10.4	5.0
Val	136	12.7	6.1	129	13.6	6.5

[1]EAA: essential AA, excluding Trp.

[2]MP: metabolizable protein, assuming that EAA represent 48% of MP.

Table 7. Efficiencies of utilization[1] of AA for lactation after discounting the maintenance[2,3] requirements from total AA supply.

	Efficiency of conversion of AA for milk					
	Linear model	Logistic model				CNCPS (2000)
		% of optimum supply				
AA	(fixed)	50%	75%	100%	125%	(fixed)
Arg	0.59	0.71	0.57	0.49	0.44	0.35
His	0.95	1.09	0.88	0.76	0.68	0.96
Ile	0.74	0.86	0.72	0.65	0.58	0.66
Leu	0.70	0.83	0.70	0.61	0.55	0.72
Lys	0.77	0.90	0.76	0.68	0.60	0.82
Met	0.80	0.89	0.75	0.66	0.59	1.00
Phe	0.64	0.75	0.61	0.53	0.48	0.98
Thr	0.69	0.82	0.67	0.60	0.55	0.78
Val	0.76	0.86	0.71	0.62	0.56	0.62

[1]Calculated from AA in milk as a function of AA available for milk.

[2]Maintenance requirements included scurf protein, urinary protein, metabolic fecal protein, and endogenous protein (NRC, 2001).

[3]The intestinal endogenous AA composition used to calculate maintenance requirements is the average of Stein et al. (1999; lactating sows and growing pigs) and de Lange et al. (1989a, 1989b; control treatments).

availability (from nonfat sources) in the rumen is a regulating factor of microbial protein yield, which usually accounts for more than 50% of the duodenal protein flux (Lynch et al., 1991; Schwab et al., 1992a). In the present study, estimations of bacterial MP accounted for 55% of estimated total MP supply. Indeed, energy content of the diet has had a greater impact on AA portal absorption in growing steers than the CP content of the diet (Reynolds et al., 1992).

The principal component analysis revealed that total AA supply and NELT are the primary determinants of PY. As previously discussed, energy supply is a major factor regulating MP supply. Thus, it is to be expected that these two factors would be the most influential in determining PY. The regression equation that explained most of the variation in the PY data (equation 4.5, Table 4) also agrees well with the observations of the principal component analysis. The positive coefficients of HisT, LysT, and MetT indicate that as the supply of these AA increases, PY increases. Lys and Met are often considered to be first and second limiting for milk protein synthesis (Schwab et al., 1992a; Guinard and Rulquin, 1994). Vanhatalo et al. (1999) suggested that His was first limiting when grass silage diets were fed. Although this equation suggests that His, Lys, and Met may be limiting, it must be remembered that in the majority of these studies, other AA were infused, so the possibility also exists that several AA are co-limiting.

One of the main objectives of this work was to produce equations to predict the PYΔ to supplementary AA. However, we were not able to detect a significant association between the magnitude and direction of the PYΔ and the changes in AA supply from the diets or the infusion across the studies in our database.

Requirements for Essential AA

In terms of absolute amounts, the estimation of optimum AA supply using the logistic model yielded values that were, on average, 88% of those estimated with the segmented-linear model. This is most likely the result of the different assumptions used to define the optimum supply in the two models. For the segmented-linear model, optimum supply is assumed when the efficiency is equal to zero, whereas the value for the logistic model is estimated assuming that the efficiency *approaches* zero. However, when the optimum values are represented as the amount of AA relative to total EAA and MP, both the linear and logistic models yielded fairly similar recommendations (Table 6). The recommendations for digestible Lys and Met, expressed as the percentage of total EAA are 15.1 and 5.2% and 15.0 and 5.3% for the segmented-linear and the logistic models, respectively. Both models agree with the current Lys recommendations (NRC 2001, 7.2% of MP; Rulquin et al., 1993, 7.3% of protein truly digested in the small intestine [**PDI**]) when expressed on an MP basis. Our estimates of the optimal supply of Met (2.5% of MP) are slightly greater than the 2.4% recommended by NRC, but match the 2.5% of PDI suggested by Rulquin et al. (1993).

The recommended allowance of 2.4% of MP for His is similar to that for Met. This value is considerably lower than that reported by Rulquin and Pisulewski (2000b), who suggested that the requirement for metab-

olizable His was between 3.4 and 5.6% of PDI. Considering the similarities between His and Met in terms of their hepatic uptake (Blouin et al., 2002), mammary gland uptake to output ratio (Guinard and Rulquin, 1995), and abundance in milk protein, a similar value for the recommendations of these AA seems biologically valid.

The recommendation for Leu (9.4 and 8.9% of MP for the linear and the logistic models, respectively) is consistent with Rulquin and Pisulewski (2000a), who recommended that Leu as a percentage of PDI be between 8.9 and 11.1%. These values are both in agreement with CNCPS (2000). The allowances for the other 2 branched-chain AA are also consistent with CNCPS (2000) and Rulquin et al. (2001). The Phe allowance (5.2 and 5.5% of MP for the linear and logistic models, respectively) is also in agreement with Rulquin and Pisulewski (2000c), who recommended a level between 4.6 and 5.8% of PDI.

Efficiency of Amino Acid Use

The use of the segmented-linear model is mathematically advantageous as it allows us to determine simply an estimate of AA requirements. Biologically, this model is somewhat simplistic as it suggests that below the breakpoint the efficiency of conversion of AA is constant, and beyond this breakpoint there is no increase in PY in response to increasing supplies of AA. Both Guinard et al. (1994) and Whitelaw et al. (1986) demonstrated diminishing partial efficiencies of protein use for milk protein synthesis. In the study of Guinard et al. (1994), the efficiency of converting PDI into milk protein decreased from 0.47 to 0.38 as PDI increased from 1403 to 2073 g/d as a result of casein infusions (from 0 to 762 g/d). Whitelaw et al. (1986) reported that a 200 g/d infusion of casein increased PY by 81 g/d, whereas a 600-g infusion only increased PY by 158 g/d. The use of a fixed efficiency factor in current models would at least partially explain why observed responses to supplemental protein are usually less than predicted (Bequette et al., 1998).

With current ration evaluation programs (CNCPS, 2000; NRC, 2001), increased flow of digestible AA at the duodenum is predicted to result in increased milk protein synthesis because of the assumptions that mammary gland AA supply is positively correlated with duodenal supply, that milk protein secretion is directly related to AA supply to the mammary gland, and that the efficiency of AA use is constant. The logistic model in the present study suggests that efficiency of AA use is not constant. The question then is, why would MP be used with diminishing efficiency for milk protein synthesis as its supply is increased? It may be partially attributable to changes in mammary blood flow and inefficiencies within the mammary gland. Guinard and Rulquin (1995) observed a quadratic decrease in mammary blood flow as the amount of infused Met increased from 0 to 32 g/d, with a concomitant decrease in extraction rate such that mammary gland Met uptake was unchanged. Bequette et al. (1996) reported an increased oxidation of Leu across the mammary gland as the supply of Leu increased. Diminished efficiency may also relate to metabolism in nonmammary tissues, which would reduce the AA supply to the mammary gland. Liver extraction of AA relative to portal absorption increases at higher AA supply, such that the increment in post-liver supply is much smaller than the increment in portal absorption (Guerino et al., 1991; Bruckental et al., 1997). It is still not clear if the liver is then acting to remove non-used excess AA or is having a cut on the first pass after absorption (Lobley, 2002). Therefore, as several studies have demonstrated that productive responses to AA supplementation in lactating dairy cows are not linear (Whitelaw et al., 1986; Rulquin et al., 2001), a mathematical model that allows for the changing efficiency of conversion of absorbed AA to milk protein will be a better tool to predict productive responses to dietary manipulation in dairy cows. In nonruminants, the logistic function has been used to describe the diminishing return responses to AA supplementation (Gahl et al., 1994, 1996). In our study, using the parameters from the logistic fit, this efficiency value was substantially different below and above the estimated optimum (0.91 vs. 0.56 at 50% vs. 125% of the optimum intake, respectively). These data, although applicable only to a restricted data set, clearly show that it is possible to mathematically estimate a variable coefficient for the conversion of MP to milk protein. The logistic model allows for the use of different marginal efficiencies of conversion depending on the level of AA supplied. Across AA, the marginal efficiency of conversion of digested AA into milk averaged 28% at the optimum supply and 19% for values 125% of the optimum. This marginal efficiency is in agreement with the 21% marginal efficiency reported by Hanigan et al. (1998) when casein was infused above the estimated MP requirements.

For the linear model, the efficiency of AA conversion for lactation (AA in milk/[AAT – AA for maintenance]) varied considerably among AA, ranging from a low of 0.59 for Arg to a high of 0.95 for His. A similar pattern was observed for the logistic equation. The efficiency values for the 3 branched-chain AA ranged from 0.70 to 0.76, which are in close agreement with those used in the CNCPS (2000) model (0.62 to 0.72). Likewise, the efficiency value for His and Lys are also in agreement with those of CNCPS (2000).

Although Met was the second most efficiently used AA in this study, its calculated efficiency of 80% is substantially below the CNCPS value of 100%. The difference between the 2 values may be a reflection of the methodology used to calculate the efficiency values. The CNCPS value is based on the ratio of mammary gland AA uptake to milk AA output (Overton, personal communication). The uptake of Met by the mammary gland in a 1:1 ratio with its output in milk (Guinard and Rulquin, 1994 and 1995), and its minimal catabolism in the mammary gland contribute to its high efficiency value (Mepham, 1982). The high efficiency of conversion of His can be explained by its metabolism, which in terms of its extraction by the liver (Blouin et al., 2002) and its uptake by the mammary gland relative to its output in milk, parallels that of Met (Guinard and Rulquin, 1995).

The efficiency of conversion of Phe (0.64 for the linear model, 0.53 for the logistic model) for lactation was low relative to that predicted by CNCPS (0.98; CNCPS 2000). Several possible explanations exist to account for this difference. First, it is possible that Phe was not limiting in the basal diets in the studies used in our database, and so additional Phe supplied by the infusions was not incorporated into milk protein but simply catabolized. Second, the maintenance requirement for Phe as calculated in this study may be underestimated because of the assumptions that were made regarding metabolic fecal protein requirements. Because metabolic fecal protein requirements comprise such a large proportion (~64%) of MP maintenance requirements, alterations in intestinal endogenous protein AA composition will have a major impact on the efficiency of AA use for lactation. For example, a 20% increase in Phe efficiency of use was obtained simply by changing the composition of the intestinal endogenous loss from 4.0 (Table 3) to 7.1% (obtained under AA infusion; de Lange et al., 1989b). The fact that a calculated efficiency can be increased by up to 20% so easily raises an important question—how should maintenance requirements be calculated? If the approach taken in the current study is considered reasonable and worth refining, what are the AA profiles of the constituent maintenance components that should be used? Clearly, this is an area that warrants further research.

The efficiency of Arg use, calculated to be 0.59, is considerably higher than the 0.35 used by CNCPS. This discrepancy may be attributable to endogenous synthesis of Arg from citrulline. In human adult males, the conversion of citrulline to Arg accounted for 9% of whole-body Arg flux (Castillo et al., 1993). The whole body irreversible loss rate of Arg is approximately 80% of that of Leu (Lobley et al., 1996). In the dairy cow, this would translate to a whole body irreversible loss rate of Arg of 80 mmol/h (Lapierre et al., 2002). Assuming that 9% of this is derived from de novo synthesis, then daily Arg synthesis is approximately 30 g/d. Addition of this amount of Arg to the total supply (ArgT) reduces the efficiency of conversion to 0.35.

CONCLUSION

Requirements for EAA relative to MP have been estimated using a limited number of publications. In spite of the limitations in our database, our estimations are in close agreement with currently accepted recommendations for Lys and Met, which suggests a certain reliability for our estimations for other AA. Also, demonstration has been made that the efficiency of conversion of metabolizable AA use for milk PY is not constant, but varies according to metabolizable AA supply and among individual AA. The inclusion of recommended allowances for the whole range of EAA, together with the use of variable efficiency factors, should improve our ability to predict PY in response to supplemental protein in lactating dairy cows.

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Appendix A. Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
GS, Barley	14.5	137	16.2	—	JV	23.8	771*	Metcalf et al., 1996
			16.7	TAA/400		24.4	854*	
			16.2	—		22.4	728*	
			16.7	EAA/208		23.5	867*	
AH, Corn, SBM	16.5	220	26.2	—	AB	26.5	832	Mackle et al., 1999b
			27.6	CAS/500 + BCAA/88		27.6	875	
GS, Barley, SBM	14.6	123	13.9	—	AB	17.7	516	Choung and Chamberlain, 1995a
			14.2	CAS/100		17.9	538	
			14.4	CAS/200		18.8	588	
			14.4	CAS/400		19.5	609	
			13.9	CH/110		17.7	520	
			14.1	CH/220		17.0	534	
			14.2	CH/440		18.6	587	
GS, Barley, SBM	16.6	123	13.6	—	AB	18.2	510	Choung and Chamberlain, 1995a
			14.2	CAS/160		19.5	570	
			13.4	CAS/320		20.3	618	
			13.7	TAA/146		18.8	535	
			13.6	TAA/292		20.2	615	
GS, Barley, FM	19.3	74	13.8	—	JV	17.4	487	Choung and Chamberlain, 1995b
			14.1	His/9.7, Met/9.1,Lys/30, Trp/2.6		20.0	615	
			13.6	His/9.7, Met/9.1,Lys/30, Trp/2.6		18.6	541	
GS, Barley, FM	17.3	74	13.6	—	JV	17.1	424	Choung and Chamberlain, 1995b
			13.7	His/7.0, Met/8.3,Lys/20.5, Trp/4.0		18.8	503	
			14.0	His/7.0, Lys/20.5, Trp/4.0		19.3	499	
TS, CS, Corn	14.4	207	23.8	—	AB	36.9	1107	Robinson et al., 2000
			23.1	Lys/50		35.8	1051	
			21.9	Met/15.9		34.2	1042	
			22.0	Lys/49.4, Met/16.2		34.5	1042	
TS, CS, barley	14.6	50	21.4	—	AB	33.6	902	Robinson et al., 1999
			21.3	Ile/30.7		35.1	921	
GS, barley	14.1	140	13.6	—	AB	13.9	408	Choung and Chamberlain, 1993a
			13.7	CAS/110		15.7	436	
			13.7	CAS/220		17.1	510	
			14.3	CAS/330		17.4	557	
CS, corn, SBM	14.3	133	17.9	—	AB	23.3	698	Seymour et al., 1990
			17.1	CAS/400		24.4	744	
			17.2	CH/400		23.6	698	
			16.8	Lys/30.1, Met/11.3		22.9	707	
CS, corn, SBM,CGM	14.3	133	17.1	—	AB	23.4	670	Seymour et al., 1990
			17.2	CAS/400		24.6	716	
			17.4	CH/400		24.3	716	
			16.8	Lys/30.1, Met/11.3		23.5	688	

(Continued)

Appendix A *Continued.* Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
CS, GNM, SBM	16.3	89	15.3	—	DU	24.1	660*	Guinard et al., 1994
			15.3	CAS/177		25.5	717*	
			15.2	CAS/352		25.3	718*	
			15.3	CAS/762		26.7	786*	
AH, Corn, SBM	16.6	184	20.0	—	AB	26.3	761*	Griinari et al., 1997a, 1997b
			19.6	CAS/500		28.6	831*	
AH, Corn, SBM	16.2	112	24.8	—	AB	32.3	930*	Mackle et al., 1999a
			24.3	BCAA/150		32.5	920*	
			23.7	CAS/600		33.2	976*	
			24.1	BCAA/44, CAS/600		33.4	979*	
CS, GNM, SBM	17.8	119	17.0	—	DU	24.2	674*	Guinard and Rulquin, 1995
			17.1	Met/8		23.2	660*	
			17.1	Met/16		24.3	707*	
			17.0	Met/32		24.0	689*	
GS, Barley, SBM	15.3	126	13.8	—	AB	20.2	582	Choung and Chamberlain, 1992b
			14.4	CAS/230		22.4	674	
CS, AS, Corn	16.0	28	20.4	Met/12	DU	37.4	921	Schwab et al., 1992a
			21.4	Lys/30		39.8	1010	
			21.9	Met/12, Lys/30		40.0	1056	
			20.3	CAS/400		40.0	1054	
CS, AS, Corn	16.0	70	21.1	Met/12	DU	35.1	877	Schwab et al., 1992a
			21.5	Lys/30		35.6	923	
			21.6	Met/12, Lys/30		35.0	925	
			21.9	CAS/400		37.0	980	
CS, AS, Corn	16.0	133	21.2	Met/10	DU	28.8	766	Schwab et al., 1992a
			21.3	Lys/25		29.3	777	
			21.9	Met/10, Lys/25		29.8	818	
			21.8	CAS/333		30.9	856	
CS, AS, Corn	16.0	203	18.6	Met/8	DU	21.1	584	Schwab et al., 1992a
			18.4	Lys/20		20.9	584	
			19.2	Met/8, Lys/20		21.2	617	
			17.8	CAS/266		20.8	588	
CS, AS, Corn	17.3	28	17.3	—	DU	31.7	781	Schwab et al., 1992b
			17.8	Met/10, Lys/10		32.2	908	
			18.8	Met/10, Lys/20		34.7	966	
			18.8	Met/10, Lys/30		35.5	995	
CS, AS, Corn	14.3	105	20.4	—	DU	32.5	853	Schwab et al., 1992b
			20.3	Met/10, Lys/10		32.4	870	
			21.5	Met/10, Lys/20		33.1	910	
			20.8	Met/10, Lys/30		32.8	894	
CS, AS, Corn	14.6	154	20.7	—	DU	30.4	836	Schwab et al., 1992b
			20.4	Met/10, Lys/10		31.4	886	
			20.9	Met/10, Lys/20		30.4	880	
			20.6	Met/10, Lys/30		30.7	895	

(Continued)

Appendix A *Continued.* Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
CS, AS, Corn	14.8	210	18.7	—	DU	23.0	671	Schwab et al., 1992b
			18.2	Met/8, Lys/8		20.7	622	
			19.3	Met/8, Lys/16		22.1	682	
			18.5	Met/8, Lys/24		22.1	678	
GH, WS, Barley	11.0	7	14.3	—	AB	22.1	565	König et al., 1984
			14.3	CAS/240		23.8	648	
			14.3	CAS/460		23.5	635	
GS, Barley	14.8	88	14.1	CAS/230	AB	20.1	563	Choung and Chamberlain, 1993b
			13.8	CH/260		20.2	562	
CS, GH, Corn	15.2	94	19.9	—	DU	29.1	771	Lynch et al., 1991
			19.4	Lys/24, Met/8		27.9	744	
CS, AHL, Corn	15.0	82	18.7	—	AB	25.4	716	Cohick et al., 1986
			19.0	CAS/395		27.2	800	
			12.3	—		21.5	577	
			12.7	CAS/395		24.0	688	
CS, GNM, SBM	17.7	144	17.0	—	DU	21.7	670*	Guinard and Rulquin, 1994
			16.6	Lys/9		22.4	702*	
			16.7	Lys/27		21.8	698*	
			17.0	Lys/63		21.8	683*	
CS, SBM, Corn	17.6	35	23.0	—	DU	37.5	1020*	Pisulewski et al., 1996
			22.2	Met/6		37.9	1046*	
			22.6	Met/12		36.3	1038*	
			23.1	Met/18		36.6	1076*	
			22.9	Met/24		37.1	1102*	
CS, Corn, SBM	16.8	61	20.0	—	DU	31.5	856	Aldrich et al., 1993
			19.8	Lys/24, Met/8		32.8	893	
AH, Barley, Corn	14.9	102	16.9	CAS/400	AB	23.9	718	Cant et al., 1991
	15.9		17.4	CAS/400		27.0	753	
GS, Barley, Oats	14.2	110	19.1	—	AB	24.9	743*	Varvikko et al., 1999
			19.0	Met/10		24.7	732*	
			19.6	Met/20		25.3	763*	
			19.8	Met/30		25.6	768*	
			19.2	Met/40		25.3	759*	
GS, Barley, Oats	13.5	196	17.4	—	AB	22.0	663*	Varvikko et al., 1999
			17.8	Lys/15		22.1	672*	
			17.8	Lys/30		22.2	670*	
			17.7	Lys/45		21.9	665*	
			17.6	Lys/60		22.0	654*	
GS, Barley, Oats	13.4	105	16.1	—	AB	22.9	646	Vanhatalo et al., 1999
			16.3	His/6.5		23.6	671	
			16.3	His/6.5, Met/6		23.7	677	
			16.2	His/6.5, Lys/19		24.2	667	
			16.4	His/6.5, Met/6, Lys/19		23.7	678	

(Continued)

Appendix A *Continued.* Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
CS, Corn, CGM	15.7	49	19.2	—	AB	26.4	763	King et al., 1991
			19.2	Lys/45		27.4	818	
			19.5	Lys/90		27.7	884	
CS, Corn, CGM	15.7	35	23.1	—	AB	29.7	874	King et al., 1991
			22.7	Lys/22.5		31.2	939	
			23.0	Lys/45		30.1	911	
			22.7	Lys/90		31.2	949	
			22.7	Lys/180		32.1	967	
GS, Barley	13.7	98	12.2	—	JV	15.9	447	Chamberlain and Thomas, 1982
			11.6	Met/8		15.7	426	
GS, Barley, SBM	14.7	182	14.3	—	AB	16.9	498	Choung and Chamberlain, 1992a
			14.9	CAS/230		19.5	581	
GS, Barley, SBM	14.6	56	15.4	—	JV	22.4	620	Choung and Chamberlain, 1992a
			15.1	Met/5, Phe/9.1, Trp/2.2		21.9	616	
	18.7		16.0	—		23.0	671	
GS, Barley, SBM	14.6	56	15.4	—	JV	22.4	620	Choung and Chamberlain, 1992a
			15.1	Met/5, Phe/9.1, Trp/2.2		21.9	616	
	18.7		16.0	—		23.0	671	
			15.7	Met/5, Phe/9.1, Trp/2.2		23.0	664	
BS, BP	13.6	20	9.1	—	AB	13.0	361	Whitelaw et al., 1986
			9.4	CAS/200		15.3	436	
			9.7	CAS/400		16.5	479	
			9.7	CAS/600		17.2	508	
AH, Corn	18.0	—	20.5	—	AB	30.6	970	Rogers et al., 1984
			20.4	CAS/462		32.7	1066	
GS, Barley, FM	20.0	154	17.8	—	JV	14.2	482	Kim et al., 2000
			16.9	EAA/114.4		17.1	581	
			17.3	His/6, Met/7.1, Lys/17.6		16.6	582	
			16.9	His/6		16.5	538	
GS, Barley, FM	21.2	39	18.1	—	JV	27.0	717	Kim et al., 2000
			18.2	His/9, Met/10, Lys/25.5, Trp/4.8		27.8	811	
			18.6	His/9, Lys/25.5, Trp/4.8		27.5	729	
			18.5	His/9, Met/10, Trp/4.8		27.2	755	
			19.1	His/9, Met/10, Lys/25.5		28.6	834	
GS, Barley, FM	21.1	42	16.4	—	JV	25.9	688	Kim et al., 1999
			16.7	His/9.7, Met/9.1, Lys/30, Trp/2.6		28.4	813	
			16.1	Met/9.1, Lys/30, Trp/2.6		25.2	665	
			16.7	His/9.7, Met/9.1, Trp/2.6		28.0	791	
GS, Barley, SBM	14.7	70	14.2	—	AB	14.4	432	Choung and Chamberlain, 1992c
			14.9	CAS/230		16.6	519	
GS, Barley	13.7	56	18.4	—	DU	25.2	769	Huhtanen et al., 1997
			17.4	CAS/400		24.9	734	
			17.9	—		27.4	854	
			17.9	CAS/400		26.7	829	

(Continued)

Appendix A *Continued.* Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
BS, Barley	13.1	7	11.2	—	AB	16.8	476	Ørskov et al., 1977
			11.0	CAS/300		19.5	601	
GH, Corn	11.5	70	16.3	—	AB	23.3	616	Schwab et al., 1976
			17.2	Met/7.1		23.0	604	
			17.0	Met/7.1, Lys/22.1		23.0	640	
			17.4	Met/7.1, Lys/22.1, Val/18.1		23.6	646	
			18.3	Met/7.1, Lys/22.1, Val/18.1, Ile/16.1		23.8	669	
			17.7	EAA/97.3		23.9	655	
			18.1	EAA/123.3		24.9	697	
			17.7	EAA/136.6		24.6	696	
GH, Corn	10.7	70	17.9	—	AB	29.1	738	Schwab et al., 1976
			16.9	EAA/119.8		29.8	793	
			17.6	EAA/164.8		29.4	815	
			17.7	CAS/425		30.8	830	
CS, Corn	10.7	70	19.1	—	AB	27.2	708	Schwab et al., 1976
			19.3	Lys/27.8, Met/11.3		27.1	747	
			19.2	Lys/27.8, Met/11.3, Val/25		26.4	741	
			19.3	Lys/27.8, Met/11.3, Val/25, Phe/19.6, Ile/22.3		27.0	751	
			19.4	CAS/425		27.7	777	
CS, Corn	10.9	70	20.5	—	AB	28.4	787	Schwab et al., 1976
			20.5	Lys/27.8		28.9	804	
			20.2	Lys/27.8, Met/11.3		29.2	829	
			19.9	Lys/27.8, Met/11.3, Val/25		28.7	818	
			20.5	CAS/425		29.8	864	
CS, Corn	10.7	70	18.9	—	AB	29.4	791	Schwab et al., 1976
			18.3	Lys/27.8		29.7	801	
			18.1	Lys/27.8, Met/11.3		29.8	829	
			19.2	Lys/27.8, Met/11.3, Thr/15.2		31.1	860	
			19.1	His/10.2, Arg/13.7		30.7	813	
			19.6	CAS/425		32.2	910	
AGH, Corn	17.7	—	18.5	—	AB	28.8	896	Clark et al., 1977
			18.7	CAS/450		31.0	997	
AGHL, CS, Corn	15.3	80	20.5	—		30.7	874	Vicini et al., 1988
			21.0	Arg/109	JV	31.9	846	
			20.2	Arg/178	AB	33.4	865	
CS, Corn, Barley, SBM	14.0	34	13.9	—	DU	35.0	911	Bruckental et al., 1991
			14.5	Pro/80		36.0	920	
	14.0	121	13.8	—	DU	23.6	607	
			14.7	Pro/80		24.9	693	

(Continued)

Appendix A *Continued.* Description of publications used to generate the database.

Diet[1]	CP in Diet (% DM)	DIM[2]	DMI (kg/d)	Infusate/rate[3] (g/d)	Infusion[4] Site	Milk yield (kg/d)	True protein yield[5] (g/d)	Reference
GS, Barley, SBM	13.8	210	12.7	—	AB	12.1	417*	Choung and Chamberlain, 1995c
			13.1	CAS/180		13.1	473*	
GS, Barley, FM	19.0	70	11.0	—	AB	10.7	318*	Choung and Chamberlain, 1995c
			11.3	CAS/230		13.6	416*	

[1]Primary ingredients in ration; AGH = alfalfa-grass hay, AGHL = alfalfa-grass haylage, AH = alfalfa hay, AHL = alfalfa haylage, AS = alfalfa silage, BP = beet pulp, BS = barley straw, CGM = corn gluten meal, CS = corn silage, FM = feather meal, GH = grass hay, GNM = groundnut meal, GS = grass silage, SBM = soybean meal, TS = timothy silage, WS = wheat straw.

[2]Days in milk at start of experiment.

[3]CAS = casein, CH = casein hydrolysate, EAA = essential AA, NEAA = nonessential AA, TAA = EAA + NEAA.

[4]AB = abomasal, DU = duodenal, JV = jugular vein.

[5]Calculated as published crude protein yield × 0.93 (NRC, 2001) except where indicated by *.

*Published true protein yield.