Stage of lactation and corresponding diets affect in situ protein degradation by dairy cows

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ABSTRACT

The influence of stage of lactation and corresponding diets on rates of protein degradation (k_d) is largely unstudied. Study objectives were to measure and compare in situ ruminal k_d of crude protein (CP) and estimate rumen CP escape (rumen-undegradable protein; RUP) of selected feeds by cows at 3 stages of lactation fed corresponding diets, and to determine the incubation times needed in an enzymatic in vitro procedure, using 0.2 units of *Streptomyces griseus* protease per percent of true CP, that predicted in situ RUP. Residue CP was measured after in situ fermentation for 4, 8, 12, 24, 36, 48, and 72 h of 5 protein sources and 3 total mixed rations, which were fed to the in situ cows. Two nonlactating (dry) cows and 2 cows each at 190 (mid) and 90 (peak) days of lactation were used. Each pair of cows was offered free-choice diets that differed in composition to meet their corresponding nutrient requirements. Diets had decreasing proportions of forages and contained (dry matter basis) 11.9, 15.1 and 16.4% CP and 54.3, 40.3 and 35.3% neutral detergent fiber, for dry, mid, and peak TMR (TMR1, TMR2, and TMR3), respectively. Intakes were 10.3, 21.4, and 23.8 kg of dry matter/d, respectively. Kinetic CP fractions (extractable, potentially degradable, undegradable, or slowly degradable) were unaffected by treatment. Lag time and k_d varied among feeds. The k_d was faster for all feeds (0.136/h) when incubated in dry-TMR1 cows compared with mid-TMR2 (0.097/h) or peak-TMR3 (0.098/h) cows, and no differences in lag time were detected. Calculated RUP, using estimated passage rates for each cow based on intake, differed between dry-TMR1 (0.382) and mid-TMR2 (0.559) or peak-TMR3 (0.626) cows, with a tendency for mid-TMR2 to be different from peak-TMR3. Using the average k_d and lag time obtained from dry-TMR1 to calculate RUP for

mid-TMR2 and peak-TMR3 cows using their passage rates reduced RUP values by 6.3 and 9.5 percentage units, respectively. Except for that of herring meal, in vitro residue CP at 6, 12, and 48 h of enzymatic hydrolysis was correlated ($r = 0.90$) with in situ RUP of peak-TMR3, mid-TMR2, and dry-TMR1, respectively. Although confounded within treatments, stage of lactation, diet, and intake appeared to affect CP degradation parameters and RUP. Using k_d from nonlactating cows, or the RUP calculated from them, may bias diet evaluation or ration formulation for lactating cows. In addition, enzymatic in vitro predictions of RUP should be measured using incubation times that are appropriate for lactating cows.

Key words: ruminal protein degradation, degradation rate, in situ, in vitro

INTRODUCTION

In situ techniques are the most popular methods for estimating ruminal degradation parameters in current diet evaluation models, even though estimates are influenced by numerous variations among methods. Nocek (1985) and Vanzant et al. (1998) evaluated several variations in technique, such as recipient diet, bag type, sample processing, replication, incubation procedure, bag rinsing, and microbial correction. In situ techniques also have been criticized because of lower microbial activity within bags compared with the rumen ingesta (Huhtanen et al., 1998), loss of undegraded small particles from nylon bags (Gierus et al., 2005), and the inability to measure degradation of the soluble protein fraction. Although some recommendations are not consistent, attempts to standardize the procedure have been made (NRC, 2001), but in situ results still differ among laboratories due to different methods and materials used (Madsen and Hvelplund, 1994).

Degradabilities of protein sources used in nutritional models for evaluating dairy cow rations have often been determined in situ using nonlactating animals fed near maintenance. The use of nonlactating animals is pre-

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ferred because voluntary intake and diet composition are usually less variable than those for lactating cows. Vanzant et al. (1998) suggested the use of animals fed 60 to 70% forage at near maintenance levels to standardize the in situ technique but maximize the diversity of the microbial population. However, these feeding levels and proportions of forage in the diet are not compatible with diets typically fed to high-producing dairy cows. The NRC (2001) recommends using feeding level and diets similar to the desired application, but report only one set of protein fractions and rates of digestion for each feed because data are insufficient to account for differences due to diet-related factors. Limited data are available to demonstrate the effects of production level, diets, and intakes of dairy cows on the kinetics of protein degradation.

Several in vitro procedures have been proposed to replace in situ analysis to improve accuracy and facilitate determination of rumen protein degradation parameters. Broderick (1987) and Broderick et al. (2004a) developed an in vitro method, where rumen fluid is used as the proteolytic agent. Other in vitro systems use commercial cell-free proteases (**IVenz**) instead of mixed ruminal microorganisms to avoid the need for rumen-fistulated animals. *Streptomyces griseus* protease and ficin have been used most frequently (Schwab et al., 2003). Because in situ measurements are most commonly used to estimate protein degradation, IVenz methods are typically compared with in situ data. Also, in situ analyses are used as a reference to modify IVenz conditions, such as incubation time and enzyme concentration (Poos-Floyd et al., 1985; Cone et al., 1996; Licitra et al., 1999) so they match in situ results.

Our objectives were (1) to measure and compare in situ ruminal CP degradation parameters and RUP of selected protein sources and TMR samples in dairy cows fed diets for 3 stages of lactation, and (2) to determine the hydrolysis time needed for an in vitro *Streptomyces griseus* procedure that would generate extent of undegraded CP corresponding to in situ RUP measurements.

MATERIALS AND METHODS

Cows and Diets

Six ruminally cannulated (Bar Diamond, Parma, ID) Holstein cows were used: 2 nonlactating, pregnant cows (dry), 2 cows at 190 d of lactation producing 17.3 or 24.2 kg/d (mid), and 2 cows at 90 d of lactation producing 36.4 or 42.8 kg of milk daily (peak). Nonlactating cows were within 45 d of calving. Three TMR (Table 1) were formulated to meet nutrient requirements of the cows at 3 stages of lactation according to CPM Dairy

version 3.0.8 (Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY), and these rations (**TMR1**, **TMR2**, and **TMR3**) were offered free choice to the dry, mid, and peak cows, respectively, to obtain the respective treatments varying in stage of lactation and corresponding diet (stage-diet; dry-TMR1, mid-TMR2, and peak-TMR3). For formulations, we assumed BW of 670 kg for dry, 660 kg for mid-lactation, and 640 kg for peak-lactation cows. Cows were housed at a local farm, individually offered diets ad libitum, and adapted to their diets for 2 wk before in situ incubations and sampling began. Intakes of individual cows were determined daily by weighing rations and refusals during the trial. Milk production (kg), milk fat (%), and milk protein (%) were measured daily. Milk samples were analyzed using infrared spectroscopy (MilkoScan Minor, Foss Electric, Hillerød, Denmark).

Feeds and Chemical Analyses

Feeds were selected to obtain diverse protein degradation kinetics and included the protein sources herring meal (**HM**), brewers grains (**BG**), soybean flakes (**SBF**), soybean meal (**SBM**), and sunflower meal (**SFM**), and the 3 TMR. All protein sources came from a single batch. The TMR were the actual TMR fed to the cows sampled the day before in situ incubation. All feeds were sampled twice (once for each in situ replicate) for chemical analyses when polyester bags were prepared for incubation, and analyses were performed in duplicate. Samples were dried at 60°C, and ground to pass a 2-mm screen for in situ degradation, or ground to pass a 1-mm screen for chemical analyses and IVenz degradation, using a cutting mill (SM 100, Retsch, Haan, Germany). A larger screen size was chosen for in situ analysis relative to IVenz analysis to reduce losses of undegraded small particles from the bags. Feeds were dried overnight at 105°C to obtain DM and combusted in a muffle furnace at 550°C for 4 h to obtain ash content and OM. Amylase-treated NDF (**aNDF**) was determined according to Mertens (2002) using sodium sulfite and heat stable α -amylase (Sigma-Aldrich, Steinheim, Germany), and ADF and lignin were measured according to Van Soest and Robertson (1985) using the permanganate lignin procedure. Nitrogen content was determined by a Kjeldahl procedure with Cu^{2+} as a catalyst, and multiplied by 6.25 to obtain CP. Neutral and acid detergent insoluble CP, NPN (calculated as CP equivalent), and the soluble CP fraction were determined according to the methods of Licitra et al. (1996).

Table 1. Ingredient and chemical composition (% of DM unless otherwise noted) of TMR¹, and intake and milk production of in situ cows

Item	TMR1	TMR2	TMR3
Ingredient			
Corn silage	—	25.1	30.7
Grass hay	77.8	29.3	13.7
Sunflower meal	—	5.4	6.6
Soybean flakes	—	1.6	1.9
Wheat bran	3.7	3.2	3.9
Soybean meal	4.4	10.4	12.6
Ground corn	13.4	23.0	28.2
Mix 1 ²	0.7	—	—
Mix 2 ³	—	2.0	2.4
Chemical composition ⁴			
DM (%)	85.9	55.8	51.8
OM	92.9	92.3	91.1
CP	11.9	15.1	16.4
Soluble CP (% of CP)	24.1	27.0	26.3
Amylase-treated NDF (aNDF)	54.3	40.3	35.3
ADF	27.8	19.1	15.9
Lignin	2.0	2.5	2.6
NFC ⁵	25.9	36.7	40.5
NE _L (Mcal/kg)	1.4	1.6	1.7
Daily intake ⁶ (kg)			
DM	10.3 ± 1.5	21.4 ± 1.4	23.8 ± 3.5
NDS ⁷	4.7 ± 0.7	12.8 ± 0.8	15.4 ± 2.3
Milk production and composition			
Daily yield (kg)	—	20.8 ± 3.6	39.6 ± 4.4
Milk fat (%)	—	4.2	3.6
Milk protein (%)	—	3.6	3.2

¹Total mixed rations were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0) for nonlactating (TMR1), mid-lactation (TMR2), and peak lactation (TMR3) cows. Mean values of the entire experimental period are reported.

²Mix 1 was a vitamin-mineral mix.

³Mix 2 was a vitamin-mineral mix with bicarbonate, urea, and Megalac (Church and Dwight, Princeton, NJ).

⁴Data calculated from CPM Dairy based on proportion of individual ingredients and their chemical composition.

⁵NFC = (100 – ash – CP – ether extract – aNDF).

⁶Mean ± SD of 2 cows.

⁷Neutral detergent solubles = (100 – aNDF).

In Situ Degradation

The ruminally undegraded CP residue was determined using 5 × 10 cm (for SBM, SBF, BG, and HM) and 10 × 20 cm (for TMR1, TMR2, and TMR3) polyester bags with a pore size of 50 (±15) μm (R510 bags, Ankom Technology, Fairport, NY). Approximately 1.3 g of SBM, SBF, BG, and HM, and 5.2 g of TMR1, TMR2, and TMR3 was placed in bags. Two replicates were incubated separately, with the second replicate beginning after all incubations of the first replicate were completed. Before incubation, bags were presoaked in 40°C water for 10 min. Bags were inserted in the rumen immediately before feeding, at 0800 h, and removed after 4, 8, 12, 24, 36, 48, and 72 h. After incubation, bags were put in ice water, washed with cold water for 1 h in a washing machine (water was replaced 5 times with no spin cycles), frozen until analysis, dried at 60°C, and weighed. Residues after incubation were

analyzed for DM and CP contents. Zero-time disappearance values (0 h) were obtained by washing presoaked, nonincubated samples. Small particle matter escaping from the bags was determined by filtering the washings from 5 nontreated bags of each feed through a folded filter paper (No. 595^{1/2}, Schleicher & Schuell, Dassel, Germany). The small-particulate residues were analyzed for DM and CP. The CP residues at individual incubation times were adjusted for losses of undegraded small particles escaping the bags using the equation of Weisbjerg et al. (1990), as described by Shannak et al. (2000), but were not adjusted for microbial contamination of DM or CP.

Enzymatic In Vitro Hydrolysis

Feeds were hydrolyzed with protease from *Streptomyces griseus*, Type XIV (P5147; Sigma-Aldrich) using the IVenz procedure described by Licitra et al. (1998)

with modified enzyme concentration and incubation times. Feeds were hydrolyzed with 0.2 units of enzyme per percentage of true CP in DM. True CP percentage was calculated by subtracting NPN (CP equivalent as a % of DM) from CP (% of DM). The unhydrolyzed CP in the residue was measured after 0, 4, 8, 12, 18, 24, 36, 48, and 72 h of incubation.

Estimation of In Situ Protein Degradation Parameters

Plots of the natural logarithm of undegraded CP in residues, which were adjusted for small particles escaping the bag (**ResCP**), versus time indicated potential outliers (measurements with large deviations from the linear regression line). Suspect results were detected by using the LOESS procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) and removed when the residual *t*-test for an observation exceeded that for $P < 0.0001$. The LOESS procedure develops a smooth line through curvilinear data using localized regression. The residual *t*-test ($P < 0.0001$) identified measurements whose residual error from the smooth line was more than 5 standard deviations from the average residual error. Sixteen outliers were removed from a total of 672 in situ measurements. Plots of the natural logarithm of residue CP versus time were curvilinear for some feeds, which indicated the presence of more than one CP degradation pool. Therefore, multiple kinetic models were developed and evaluated, which included either an indigestible CP pool (**iCP**) or a slowly degrading pool.

To derive feed-specific starting parameter estimates for nonlinear estimation of in situ CP degradation kinetics, the log-transform method of Mertens and Loften (1980) was used. The 72-h observation was used as an estimate of iCP for each feed and cow combination. The estimated iCP was subtracted from the residue at each time [**Res**_(t)] to obtain the potentially degradable CP (**pdCP**) at each incubation time [**pdCP**_(t)]. The natural logarithm of **pdCP**_(t) was regressed against time to obtain the slope regression coefficient, which was a starting estimate for rate of degradation (**k_d**), and a starting estimate of discrete lag time was determined by calculation (Mertens and Loften, 1980). These estimates were used as starting parameters for the NLIN procedure of SAS (version 9.1, SAS Inst. Inc.) using Model [1]:

$$ResCP_{(t)ij} = pdCP_{(0)ij} \times e^{[-kd_{ij} \times (time_{ij} - lag_{ij})]} + iCP_{ij} + e_{ij}, \quad [1]$$

where $ResCP_{(t)ij}$ was the in situ residue CP at time = *t* for the *i*th feed incubated in the *j*th cow, $pdCP_{(0)ij}$ was the potentially degradable CP at time = 0 for the *i*th feed incubated in the *j*th cow, kd_{ij} was the first-order

fractional degradation rate for the *i*th feed incubated in the *j*th cow, $time_{ij}$ was the incubation time for the *i*th feed in the *j*th cow, lag_{ij} was the discrete lag time at which CP degradation began for the *i*th feed incubated in the *j*th cow, iCP_{ij} was the undegradable CP pool for the *i*th feed incubated in the *j*th cow, and e_{ij} was the residual error for the *i*th feed incubated in the *j*th cow. Often, the iCP for a feed had small differences among cows that were not statistically different and this model was simplified to estimate a single iCP for each feed among all cows using Model [1a]:

$$ResCP_{(t)ij} = pdCP_{(0)ij} \times e^{[-kd_{ij} \times (time_{ij} - lag_{ij})]} + iCP_i + e_{ij}, \quad [1a]$$

where all variables were defined as for Model [1]. Parameter estimates for this model were obtained by using dummy variables in Proc NLIN. Model [1a] was further simplified to evaluate the effects of assuming for each feed a common lag among cows, resulting in Model [1b]:

$$ResCP_{(t)ij} = pdCP_{(0)ij} \times e^{[-kd_{ij} \times (time_{ij} - lag_i)]} + iCP_i + e_{ij}, \quad [1b]$$

where all variables were defined as for Model [1].

For some feed and cow combinations, the NLIN estimate for iCP_{ij} was not different from zero, and Model [2] was used to obtain the final k_d and lag parameter estimates for these feeds by using the results from Model [1] as starting parameter estimates for the NLIN procedure using a model with no iCP:

$$ResCP_{(t)ij} = pdCP_{(0)ij} \times e^{[-kd_{ij} \times (time_{ij} - lag_{ij})]} + e_{ij}, \quad [2]$$

where definitions were the same as for Model [1]. Model [2] was simplified to evaluate the effects of assuming for each feed a common lag among cows, resulting in Model [2a]:

$$ResCP_{(t)ij} = pdCP_{(0)ij} \times e^{[-kd_{ij} \times (time_{ij} - lag_i)]} + e_{ij}, \quad [2a]$$

where definitions were the same as for Model [1].

Some feeds appeared to have a fast (**fdCP**) and a second slowly degrading CP (**sdCP**) pool. The results for these feed and cow combinations were fit to Model [3] using the NLIN procedure using the k_d and lag results from Model [1] as starting parameter estimates:

$$ResCP_{(t)ij} = fdCP_{(0)ij} \times e^{[-kf_{ij} \times (time_{ij} - lag_{ij})]} + sdCP_{(0)ij} \times e^{[-ks_{ij} \times (time_{ij} - lag_{ij})]} + e_{ij}, \quad [3]$$

where $fdCP_{(t)ij}$ and $sdCP_{(t)ij}$ were the fast and slowly degradable CP at time = 0 for the i th feed incubated in the j th cow, kf_{ij} and ks_{ij} were the fast and slow first-order fractional degradation rates for the i th feed incubated in the j th cow, $time_{ij}$ was the incubation time for the i th feed in the j th cow, lag_{ij} was a single discrete lag time at which CP degradation began for both the fast and slowly degradable CP of the i th feed incubated in the j th cow, and e_{ij} was the residual error for the i th feed incubated in the j th cow. Model [3] was simplified to evaluate the effects of assuming for each feed a common slowly degrading pool and rate among cows, resulting in Model [3a]:

$$ResCP_{(t)ij} = fdCP_{(0)ij} \times e^{[-kf_{ij} \times (time_{ij} - lag_{ij})]} + sdCP_{(0)i} \times e^{[-ks_i \times (time_{ij} - lag_{ij})]} + e_{ij}, \quad [3a]$$

where definitions were the same as for Model [3].

Calculating In Situ RUP

Turnover time in the rumen for DM (T_{DM}) was estimated using the equation of Cannas et al. (2003) from intake of neutral-detergent solubles ($NDS = 100 - aNDF$), and $T_{DM} = 20.16 - 10.14 \times \text{natural logarithm}(\text{kg of dietary NDS intake}/100 \text{ kg of BW})$. This equation was chosen because the data set of Cannas et al. (2003) is the largest currently available for estimating turnover time based on rumen emptying. Passage rate (k_p) for the j th cow (kp_j) was assumed to equal $1/T_{DM}$.

Extents of rumen CP escape (RUP) as a proportion of the original CP were calculated for the i th feed incubated in the j th cow using the following equations:

$$RUP_{ij} = 1 - (DEG_{ij}), \text{ and}$$

$$DEG_{ij} (\text{rumen-degraded CP}) = W + pdCP \times e^{-kp_j \times lag_b} \times kd_b / (kd_b + kp_j),$$

where W was the washout = $(1 - \text{residue at 0 h incubation corrected for small particle losses})$, $pdCP$ was the potentially degradable CP = $(1 - W - iCP)$, iCP was the undegradable CP, kd_b was the fractional rate constant of degradation, lag_b was the discrete lag time, and kp_j was the estimated rate of passage for each cow. The kd_b and lag_b used to calculate DEG and RUP were obtained from the model that had the best fit for the disappearance of CP in the in situ residues for each feed and cow combination. When the best-fitting model had 2 degradable fractions, $DEG2_{ij}$ was calculated as

$$DEG2_{ij} = W + fdCP \times e^{-kp_j \times lag_b} \times kf_b / (kf_b + kp_j) + sdCP \times e^{-kp_j \times lag_b} \times ks_b / (ks_b + kp_j),$$

where $DEG2_{ij}$ was the rumen-degradable CP from 2 degradable fractions, $fdCP$ was the fast potentially degradable fraction, kf_b was the fast fractional rate constant of degradation, $sdCP$ was the slow potentially degradable fraction, ks_b was the slow fractional rate constant of degradation predicted by the best fitting model, and all other definitions were the same as for DEG.

To determine if differences occurred when using degradation parameters obtained from dry-TMR1 to calculate RUP for mid-TMR2 and peak-TMR3, RUP for mid-TMR2 and peak-TMR3 were also calculated using individual k_p of each mid-TMR2 or peak-TMR3 cow with the average degradation parameters from the dry-TMR1 treatment. The differences in RUP (**RUP DIFF**) per cow and feed were obtained by subtracting RUP calculated using the degradation and passage parameters for each cow and feed from the RUP calculated using average dry-TMR1 cow and feed degradation parameters with passage parameters of each cow.

Calculating In Vitro Enzyme Incubation Times Corresponding to RUP Estimated from In Situ Parameters

Initially, we attempted to fit the IVenz CP hydrolysis data to the in situ degradation models that are described in the previous section. However, IVenz hydrolysis data fit these models poorly, which may have been related to relatively linear degradation segments during enzymatic hydrolysis. To overcome our inability to define a model that described IVenz CP hydrolysis, an approach was used that does not require an a priori description of the model to fit the observed data. Local regression (LOESS procedure in SAS, version 9.1, SAS Institute Inc.) generates a smooth curve through data without defining a model. The LOESS procedure develops a "local" regression from consecutive segments of the data and then removes preceding data in one segment while adding data in the next segment to generate a smooth line through all of the results. This approach for deriving a smooth curve through the data assumes that data at the center of each segment is predicted most accurately by a narrow range of data before and after it on the x-axis. The center estimates from sequential local regressions were calculated by the LOESS procedure using default settings in SAS to generate a smoothed curve for each enzymatic hydrolysis curve. Linear interpolation was used to estimate the IVenz

time that corresponded to the RUP for each feed and cow combination. For example, to estimate the time of IVenz CP hydrolysis that corresponded to a RUP of 0.7009 for feed BG in cow B, the times and IVenz CP residues after hydrolysis for BG that bracketed this RUP were selected: 0.7277 at 8 h and 0.6842 at 12 h. In this example, the IVenz hydrolysis time corresponding to the RUP for BG in cow B was calculated as follows:

$$\text{IVenz time} = 8 \text{ h} + [(12 \text{ h} - 8 \text{ h}) \\ \times (0.7277 - 0.7009)/(0.7277 - 0.6842)].$$

Similar linear interpolations were used to calculate the IVenz time of hydrolysis that corresponded to the RUP of each feed incubated in each cow.

Statistical Analyses

Data for all feed and cow combinations were fitted to each kinetic model. Because the kinetic models generated different numbers of parameters and had different degrees of freedom for mean squared error (**MSE**), the reduction in MSE due to added parameters in models was tested as described by Snedecor and Cochran (1967) for comparison of regression lines. The best model was defined as that with the fewest parameter estimates that did not result in an increase in MSE using the *F*-value ($P < 0.05$) compared with the model with the next higher number of parameter estimates. For example, when Model [3] was fitted to the results for SFM for each cow, 24 parameters were estimated (4 parameters per cow $\times$ 6 cows = 24; note that fdCP (= $1.00 - W - \text{sdCP}$) was determined by difference, so only *kf*, *sdCP*, *ks*, and lag were estimated for each feed and cow combination) leaving a total of 58 df for total MSE pooled across cows. For Model [1a], 19 parameters were estimated, leaving 63 df for MSE. After the most appropriate model for each feed was selected, it was used for all cows and treatment combinations to generate parameter estimates.

To evaluate overall effects, parameter estimates for the best fitting model and the RUP or RUP DIFF calculated from these parameters were compared across treatments and all feeds using the Proc Mixed procedure of SAS (version 9.1, SAS Inst. Inc.) with the following model for a replicated split-plot design:

$$Y_{ijk} = \text{treatment}_k + \text{cow}_j(\text{treatment}_k) + \text{feed}_i \\ + \text{feed}_i \times \text{treatment}_k + e_{ijk},$$

where Y_{ijk} were the best model parameter estimates of kd_{ijk} and lag_{ijk} or calculated values for RUP_{ijk} or RUP

DIFF_{ijk} of the *i*th feed for the *j*th cow in the *k*th treatment (dry-TMR1, mid-TMR2, or peak-TMR3), and e_{ijk} was the error for the *i*th feed, the *j*th cow, and the *k*th treatment. Feed, treatment, and feed $\times$ treatment were fixed effects, and cow(treatment) was defined as a random effect with repeated measures of feed within cow. Covariance matrix used was autoregressive order 1. The significance level was set at $P < 0.05$, and differences with $0.05 \leq P < 0.10$ were considered marginally significant.

Correlation was used to evaluate the relationship between IVenz unhydrolyzed CP at selected incubation times and RUP obtained from in situ estimates.

RESULTS AND DISCUSSION

Diet Composition, Intake, Production, and Chemical Composition of Feeds

Treatment stage of lactation and corresponding diet (stage-diet) and milk production of the fistulated cows were selected to provide a wide range in characteristics that might affect CP degradation (Table 1). Milk production ranged from 0 to 40 kg over the 3 stages of lactation and we detected a >2-fold difference in DMI between nonlactating and lactating cows. Diets ranged from 78 to 44% forage and contained 54 to 35% aNDF and 12 to 16% CP on a DM basis (Table 1). Feeds were chosen to provide a wide range of CP content and degradabilities, which was indicated by their diverse composition (Table 2).

Selecting Models for In Situ Protein Degradation Parameters

In situ residues were not corrected for microbial colonization. Therefore, rates of degradation were net changes in N disappearance from the in situ bags. Krawielitzki et al. (2006) and Edmunds et al. (2012, 2013) observed a considerable proportion of microbial N in the residual CP content of selected roughages after ruminal in situ incubations. However, microbial colonization in our study may have had less effect on results compared with the cited studies, as most of our feeds were protein concentrates. Only the TMR samples contained forage and only TMR1 had a high proportion of forage. Krawielitzki et al. (2006) reported low corrections for protein concentrates and grains. Correction for microbial N is often neglected (Rotger et al., 2005; Wulf and Südekum, 2005; Foster et al., 2007; Hackmann et al., 2008), and our results would be compared most appropriately to results from the latter studies.

The feeds used in this study required different models to describe CP degradation kinetics (Table 3). We

Table 2. Chemical composition of selected feeds used for in situ protein degradation¹

Component	BG	HM	SBF	SBM	SFM	TMR1	TMR2	TMR3
OM (% of DM)	72.3	25.2	57.8	52.9	68.0	91.0	93.2	93.5
CP (% of DM)	27.7	74.8	42.3	47.1	32.0	13.4	16.0	17.6
NPN ² (% of CP)	8.0	19.9	11.0	9.9	29.8	20.0	26.3	25.0
SP ³ (% of CP)	9.3	20.5	13.7	12.5	34.6	22.7	28.1	26.8
NDIP ⁴ (% of CP)	37.0	—	20.9	9.1	12.7	27.9	29.6	20.6
ADIP ⁵ (% of CP)	7.2	—	10.8	3.5	10.2	12.0	4.6	4.2
aNDF ⁶ (% of DM)	66.0	—	32.7	19.7	42.6	57.2	40.9	37.5
ADF (% of DM)	22.6	—	13.6	9.3	28.9	28.2	23.1	19.7
Lignin ⁷ (% of DM)	5.6	—	3.3	1.4	6.4	4.1	3.4	2.7

¹BG = dried brewers grains; HM = herring meal; SBF = whole soybean flakes; SBM = soybean meal; SFM = sunflower meal; TMR1 = nonlactating cow TMR; TMR2 = mid-lactation cow TMR; and TMR3 = peak-lactation cow TMR.

²Nonprotein nitrogen (CP equivalent) by the method of Licitra et al. (1996).

³Soluble CP by the method of Licitra et al. (1996).

⁴Neutral detergent insoluble CP measured without sulfite.

⁵Acid detergent insoluble CP.

⁶Amylase-treated NDF (Mertens, 2002).

⁷Acid detergent lignin using permanganate.

found no differences in values for iCP among cows (or stage-diet treatments) for some feeds (BG, TMR1, TMR2, and TMR3) and values of zero or near zero for the iCP of other feeds. Thus, Model [1] was simplified to obtain a common iCP fraction for all cows for a given feed (Model [1a]). When protein sources HM, SBF, and SBM resulted in zero pool size for iCP (for all cows or stage-diet treatments), the model was further simplified to remove the iCP fraction (Model [2]). Model [1a] was also simplified to have a common lag time for each feed for all cows (Model [1b]) because we observed no variation in lag time among cows (or stage-diet treatments). Model [1b] was evaluated, but even though it reduced the number of parameters, it was not used, because it increased the MSE. Only one feed (SFM) appeared to have a second slowly digesting pool instead of a iCP pool, and Model [3], which contained fast- and slow-degrading pools, was derived to describe its in situ CP degradation. Because we found no differences in the slow fraction or rate among cows (or stage-diet treatments), the simplified Model [3a] was used for SFM. Although the models varied, kinetic fractions (extractable, potentially degradable, undegradable, or slowly degradable) were constant for each feed and there was no variation for testing differences among feeds or stage-diet treatments for these parameters (Table 3).

Comparisons of In Situ Protein Kinetic Parameters Among Feeds

Feeds used in this study (Table 3) had kinetic diversity in the appropriate model and in the differences in k_d and lag ($P < 0.001$). Only one analytical measurement of washout of CP from the bags (corrected for small-

particulate escape) was made for each feed, which was less than the A fraction reported by the NRC (2001) for similar feeds. Development of the models indicated no differences among cows in kinetic parameters for potentially degradable fraction, undegradable or slowly degradable fractions, or slow degradation rate. Thus, there was no replication across cows for statistical analysis of these parameters. The estimated value for each of these parameters across all cows is provided in Table 3 for a complete description of model characteristics for each feed. Feed SFM had the highest k_d (0.3396/h) and HM the lowest k_d (0.0152/h) compared with all other feeds. All other feeds had similar, intermediate k_d , ranging between 0.0612 and 0.1075/h. Average lag times ranged from 1.0 to 4.2 h and differed among feeds. Feeds HM and TMR2 had the longest and SFM and TMR3 had the shortest lag times. The lag times of the remaining sources ranged from 2.3 to 2.7 h. Although kinetic models with lag have been proposed, lag time is often neglected in the literature and is typically not used for the estimation of in situ protein degradation parameters. Hoffman et al. (1999) reported a mean lag time of 2.9 (± 2.1) h for protein degradation in legume and grass silages, Wulf and Südekum (2005) reported lag times of 3.6 and 4.6 h for treated expeller rapeseed meal and treated soybeans, respectively, and Coblenz and Grabber (2013) reported lag times of 0.7 to 3.7 h for hays and silages. Our estimates of k_d would have been lower without lag time in the models, in agreement with Coblenz and Grabber (2013), who observed that k_d were higher when lag was included in the kinetic model for hays and silages differing in tannin contents. We agree with their recommendation to include lag time in models for in situ CP kinetics. Differences in lag times among feeds when incubated in

Table 3. Kinetic models used to describe in situ protein degradability for each feed and parameter estimate

Source ¹	Model with lowest MSE ^{2,3}	Model with best fit ^{3,4}	Washout fraction ⁵	Potentially degradable CP fraction	Undegradable CP fraction	Slow rate (/h)	k _d ⁷ (/h)	Lag time ⁸ (h)	RUP ⁹	RUP DIFF ¹⁰
Probability > <i>F</i>										
Feed							<0.001	<0.001	<0.001	<0.001
Stage-diet							0.040	0.16	0.005	0.052
Feed × stage-diet							0.25	0.27	0.002	0.31
Mean contrasts for feeds										
BG	[1a]	[1a]	0.043	0.882	0.075	—	0.0612 ^c	2.42 ^{ab}	0.596 ^b	−0.094 ^{bc}
HM	[2]	[2]	0.100	0.900	0	—	0.0152 ^d	4.16 ^a	0.761 ^a	−0.038 ^a
SBF	[2]	[2a]	0.018	0.982	0	—	0.0819 ^{bc}	2.59 ^{ab}	0.524 ^{bc}	−0.055 ^{ab}
SBM	[2]	[2]	0.088	0.912	0	—	0.1016 ^b	2.69 ^{ab}	0.457 ^{cd}	−0.140 ^c
SFM	[3a]	[3a]	0.088	0.798	0.115 ⁶	0.0071	0.3396 ^a	1.30 ^b	0.301 ^e	−0.089 ^{ab}
TMR1	[3a]	[1a]	0.109	0.645	0.246	—	0.1075 ^b	2.32 ^{ab}	0.553 ^b	−0.063 ^{ab}
TMR2	[1]	[1a]	0.051	0.801	0.149	—	0.0975 ^{bc}	3.47 ^a	0.572 ^b	−0.081 ^{ab}
TMR3	[1]	[1a]	0.221	0.703	0.076	—	0.0812 ^{bc}	0.97 ^b	0.418 ^d	−0.072 ^{ab}
Feed SEM							0.0083	0.42	0.013	0.015
Mean contrasts for stage-diet										
Dry-TMR1							0.1365 ^a	2.28	0.382 ^a	0.000 ^a
Mid-TMR2							0.0972 ^b	2.09	0.559 ^{b,A}	−0.063 ^{b,A}
Peak-TMR3							0.0984 ^b	3.10	0.626 ^{b,B}	−0.095 ^{b,B}
Stage-diet SEM							0.0067	0.28	0.017	0.007

^{a-c}Means within columns for a parameter with unlike superscripts differ with $P < 0.05$ based on least squares differences between treatment pairs.

^{A,B}Means within columns for a parameter with unlike superscripts differ with $P < 0.10$ based on least squares differences between treatment pairs.

¹Feed = fixed effect for feeds; stage-diet = fixed effect for combination of stage of lactation with corresponding diet; feed × stage-diet = interaction of feed and stage of lactation with corresponding diet; BG = dried brewers grains; HM = herring meal; SBF = whole soybean flakes; SBM = soybean meal; SFM = sunflower meal; TMR1 = nonlactating cow TMR; TMR2 = mid-lactation cow TMR; and TMR3 = peak-lactation cow TMR.

²Model that fitted the protein source with the lowest mean squared error.

³See text for description of models.

⁴Model that fitted the protein source with the fewest parameters without increasing the mean squared error.

⁵Fraction extracted from time = 0 bags: Washout (corrected for small particle losses) = $(1.0 - \text{pdCP} - \text{iCP})$, where pdCP = potentially degradable CP and iCP = indigestible CP.

⁶Slow digesting pool for feed SFM.

⁷First-order fractional degradation rate for potentially digestible CP.

⁸Discrete lag time of degradation.

⁹Average ruminally undegradable CP for all 6 cows using best-fit model parameters and passage rate (k_p) estimated based on each cow's intake of neutral detergent solubles.

¹⁰Average difference in ruminally undegradable CP for the 4 lactating cows using degradability parameters obtained for nonlactating cows.

the same ruminal environments suggest that lag is not an anomaly of the in situ technique; instead, it is an intrinsic characteristic of the feed that should be considered when estimating kinetic parameters and RUP.

Comparisons of RUP Among Feeds

The calculation of RUP integrates the effects of degradation pools or fractions, rates of digestion and passage, and lag times to estimate their total effects, which is especially useful when the model for degradation varies. Substantial differences ($P < 0.001$) were detected in RUP among the feeds (Table 3) when averaged across all cows using the k_p and degradation parameters for each cow. There was more than a 2-fold difference in the highest RUP (proportion of original CP) of HM (0.761) compared with lowest RUP of SFM (0.301). Other feeds had intermediate RUP.

Nonlactating animals fed corresponding diets are often used to estimate RUP for lactating cows. To estimate the potential error in determining RUP for lactating cows using k_d and lag time obtained from nonlactating cows, RUP DIFF was calculated by subtracting the RUP for mid-TMR2 and peak-TMR3 using their respective k_d , lag, and k_p from the RUP for mid-TMR2 and peak-TMR3 using k_d and lag from dry-TMR1 with the k_p for mid-TMR2 or peak-TMR3 cows. Because the higher k_d observed for dry-TMR1 resulted in RUP that were lower than those calculated using the k_d observed for mid-TMR2 or peak-TMR3, RUP DIFF values were negative; that is, RUP calculated using dry-TMR1 k_d and lag underestimated the RUP calculated for mid-TMR2 or peak-TMR3. The RUP DIFF could only be calculated and statistically analyzed for the 4 lactating cows in the mid-TMR2 or peak-TMR3 treatments in Table 3. Feeds differed ($P < 0.001$) in RUP DIFF, with SBM having the greatest difference and differing from all feeds except BG. The lowest RUP DIFF was for HM but it was only different from SBM and BG. Differences in RUP DIFF among feeds did not appear to be related to the magnitude of RUP.

Comparisons of Protein Kinetic Parameters Among Dry-TMR1, Mid-TMR2, and Peak-TMR3 Treatments

Stage-diet treatment affected ($P = 0.04$) the fractional k_d of in situ degradation of CP across all feeds (Table 3), and we observed no interaction ($P = 0.25$) between feed and treatment. The dry-TMR1 treatment resulted in faster fractional rates (0.136/h) compared with mid-TMR2 (0.097/h) or peak-TMR3 (0.098/h). Mean contrasts were determined by least squared differences between pairs of stage-diet treatments. The higher k_d for the dry-TMR1 in situ treatment compared

with mid-TMR2 or peak-TMR3 was unexpected, given the lower energy and N content of the diet associated with the dry-TMR1 treatment. Stage of lactation was confounded with diet and intake in this study (cows within each stage of lactation received a different diet and had different intakes) so differences due to stage-diet may be due to differences in stage of lactation, diets, intake levels, or a combination of all factors. From dry-TMR1 to peak-TMR3, intake levels and dietary CP and energy contents increased, whereas dietary forage and aNDF contents decreased (Table 1).

Although results are inconsistent, studies have observed differences in protein degradability of feeds due to the forage-to-concentrate ratio of the basal diet. Broderick et al. (2004b) measured protein k_d of casein and soybean meals with an in vitro method using rumen fluid from lactating and nonlactating cows fed a lactation diet and grass silage, respectively. They observed higher protein k_d with the lactating compared with nonlactating diets, in contrast to our results. Hristov et al. (2002) reported higher proteolytic activity for casein, but not azocasein, in the ruminal fluid of heifers fed a higher versus a lower grain diet when both diets had similar CP concentrations. These discrepancies with our results may be associated with differences between the in vitro and in situ methods or with differences in substrates. Both in vitro methods used strained ruminal fluid and measured protein degradation for only a short time. Other in situ studies of similar design agree with our observations. Vanzant et al. (1998) cited several studies where dietary forage concentration increased in situ protein k_d and pointed out that in situ protein digestibility depended also on type of forage and concentrate supplement. Rotger et al. (2006) fed 2 high-concentrate diets similar in CP content, but differing in forage type, forage-to-concentrate ratio, and NDF content to heifers. Even though differences in protein k_d of individual feeds were not significant, 6 of the 7 tested feeds had higher protein k_d on the diet with the higher forage level. When Molero et al. (2004) fed 2 diets differing in concentrate level and forage type to heifers, they observed that 5 out of 6 tested feeds had higher in situ protein k_d when the low concentrate diet was fed. In a second experiment of Molero et al. (2004), heifers were fed 2 diets, similar in CP and NDF content, containing 2 levels of alfalfa hay. The rates of most feeds were not affected, but protein k_d of SBM was higher when the higher forage diet was fed.

Level of intake seems to have minor effect on protein k_d , and little information exists on the effects of stage of lactation. Both Scholljegerdes et al. (2005) and Zanton and Heinrichs (2008) investigated the effect of intake level on in situ ruminal protein k_d of feeds, restricting feed intake of heifers and a steer. Scholljegerdes et al.

(2005) observed a negative linear effect of feed intake on the protein k_d of a protein supplement, but no effect on the protein k_d of a grass hay. Zanton and Heinrichs (2008) observed a trend for a positive linear influence of intake on k_d . However, they suggested that the differences observed were of limited biological importance and cited research that indicated no effect of intake on in situ k_d of protein. Volden (1999) compared in situ degradation of protein by cows in early and late lactation, at high and low feeding levels, respectively. They reported higher in situ protein k_d of concentrate and grass silage at higher intakes in early lactation, whereas we observed no difference in rates between mid-TMR2 and peak-TMR3 treatment. However, they also reported lower fractions of potentially degradable CP for the cows in early lactation, whereas we found no difference in our study.

For typical dietary changes with stage of lactation, diet forage concentration and intake are inversely related, which complicates the application of k_d measured under conditions of diet, intake, and stage of lactation that differ from the target animal. Based on the cited literature, the difference in k_d of CP between dry-TMR1 and mid-TMR2 or peak-TMR3 observed in our study seems to be related more to diet composition than to stage of lactation or intake level. Neither stage-diet ($P = 0.16$) nor the interaction of feed with stage-diet ($P = 0.27$) affected lag time of CP degradation (Table 3).

Comparisons of RUP Among Dry-TMR1, Mid-TMR2, and Peak-TMR3 Treatments

Ruminal degradation is a function of both k_d and k_p . Therefore, the equation of Cannas et al. (2003) was used to calculate the average k_p for dry-TMR1 (0.042/h), mid-TMR2 (0.074/h), and peak-TMR3 (0.089/h). Over all feeds, stage-diet affected ($P < 0.01$) RUP, using estimated k_p for each cow (Table 3). Mean contrasts indicated that dry-TMR1 (0.382) was lower ($P < 0.01$) than mid-TMR2 (0.559) or peak-TMR3 (0.626), with a tendency ($P = 0.07$) for mid-TMR2 to be lower than peak-TMR3. Unlike k_d or lag, stage-diet had an interaction with feed ($P < 0.01$) for RUP. Examination of the stage-diet effect on each feed indicated that the interaction was not due to differences in the ranking of the stage-diet effect among feeds (for every feed, the RUP of dry-TMR1 was lower than that of both mid-TMR2 and peak-TMR3). The interaction was due to differences in the magnitude of the effect among feeds; for example, the ratio of dry-TMR1:mid-TMR2:peak-TMR3 varied from 1:1.23:1.27, for HM, to 1:1.96:2.27, for SBM.

Volden (1999) reported no difference in ruminally degraded CP of a concentrate and a grass silage between

cows in early and late lactation, at high and low feeding levels, respectively. In that study, k_d was higher in early lactation when k_p was also greater; that is, k_d and k_p were positively correlated. The negative interaction that Volden (1999) observed between the potentially degradable fraction and k_d may have contributed to the lack of an effect of lactation stage or feeding level on the extent of ruminally degraded CP. Our models suggest that the potentially degradable fraction was a constant feed characteristic across stage-diet treatments.

The difference in k_d between dry-TMR1 and mid-TMR2 or peak-TMR3 (Table 3) suggests that some of the reduction in RUP for dry-TMR1 was related to higher k_d for nonlactating cows fed diets higher in forage than those for lactating cows. However, we observed no difference in k_d between mid-TMR2 and peak-TMR3. Therefore, the tendency for differences in RUP between these 2 treatments was more likely related to differences in k_p . It is often assumed that changes in RUP with stage of lactation are due primarily to differences in intake and its effect on k_p . However, our observations suggest that these variations in RUP may be associated with changes in k_d , especially if nonlactating animals, fed their corresponding diets, are used to estimate k_d .

The higher k_d observed for dry-TMR1 compared with mid-TMR2 or peak-TMR3 raised the question about the predictive error for RUP when k_d values obtained from dry-TMR1 were applied to mid-TMR2 and peak-TMR3. By definition, RUP DIFF values calculated for dry-TMR1 cows were zero. Negative RUP DIFF values indicate that the RUP calculated using dry-TMR1 kinetic parameters (k_d and lag time) were less than those calculated using mid-TMR2 or peak-TMR3 parameters. In Proc Mixed of SAS, least squares means are compared with zero, and this test ($P < 0.007$) was used to compare the RUP DIFF of mid-TMR2 and peak-TMR3 to dry-TMR1, which was zero (different lowercase superscripts for RUP DIFF of stage-diet contrasts in Table 3). Using only nonzero data from cows in treatments mid-TMR2 and peak-TMR3, it was possible to detect a trend ($P = 0.052$) for the predictive error of RUP DIFF to be different between mid-TMR2 and peak-TMR3 (different uppercase superscripts for RUP DIFF of stage-diet contrasts in Table 3). In our study, overall RUP for mid-TMR2 and peak-TMR3 cows was underestimated by 6.3 and 9.5 percentage units, respectively, when k_d and lag time from the dry-TMR1 treatment were used for calculation compared with using the measured k_d and lag time for the respective treatments.

Our objective was to evaluate the effects of stage of lactation and corresponding diet on CP degradation kinetics using models that most accurately described in situ observations. Our models and assumptions result in RUP that are greater for lactating cows than

those reported in NRC (2001). This discrepancy results primarily from 3 factors: (1) inclusion of lag time, (2) transfer of small-particle washout CP to the degradable pool, and (3) use of different estimates of k_p . The assumption of a lag time for degradation results in potentially degradable CP passing out of the rumen before degradation begins, thereby increasing RUP. On average, this assumption increased RUP by a factor of 1.15. Measuring the washout of small-particulate CP and transferring it to the degradable pool using the equation of Weisbjerg et al. (1990) reduced the size of our washout fraction (Table 3) compared with the A fraction of NRC (2001) by an average of 0.30, which increased RUP. The ruminal turnover equation of Cannas et al. (2003) estimated different k_p from those assumed by NRC (2001). Whereas NRC (2001) assumed a k_p of 0.074/h for concentrate sources for cows with DMI of 4% of BW/d when fed a ration that is 50% concentrate, using the equation of Cannas et al. (2003), we assumed k_p of 0.042, 0.074, and 0.089/h for dry-TMR1, mid-TMR2, and peak-TMR3, respectively. Although there is no guarantee that our sources were similar to the average ingredients reported by NRC (2001), the RUP reported in this study for dry-TMR1 compared most closely to those reported in NRC (2001) for cows with DMI of 4% of BW/d fed diets containing 50% forage (this study to NRC ratio = 1.01 ± 0.20). The ratio of RUP in this study to NRC was 1.51 ± 0.25 and 1.75 ± 0.40 , for mid-TMR2 and peak-TMR3, respectively.

***In Vitro* Enzymatic Hydrolysis Times Corresponding to In Situ RUP**

Across feeds, we detected a wide distribution of IVenz times when the unhydrolyzed CP residues corresponded to the calculated RUP for each feed incubated in each cow within stage-diet. The median times of IVenz hydrolysis that resulted in residue CP corresponding to the RUP of peak-TMR3, mid-TMR2, and dry-TMR1 were 6.1, 12.5, and 56.8 h, respectively. Based on our median times, the fraction of CP remaining in residues from IVenz hydrolysis after 6, 12, and 48 h corresponded best to RUP for peak-TMR3, mid-TMR2, and dry-TMR1, respectively.

Licitra et al. (1999) calculated the times, using various enzyme concentrations, that would equal in situ RUP, which were calculated assuming a passage of 0.06/h. At the same enzyme concentration used in the present study, Licitra et al. (1999) estimated that about 15 h of IVenz hydrolysis corresponded to in situ RUP, which is less than the 48 h we estimated for dry-TMR1 and more than the 12 h selected for mid-TMR2 or 6 h selected for peak-TMR3. The intermediate IVenz time selected by Licitra et al. (1999) may be related to the in

situ results they used from 2 different research projects, the first using 3 dry cows and the second using 1 cow in mid lactation for calibration. Differences in estimated IVenz times might also be explained by the protein sources used for estimation. Half of the sources used by Licitra et al. (1999) consisted of animal proteins, whereas the only animal protein used in our study was HM. Edmunds et al. (2012) evaluated an IVenz method to predict in situ RUP of selected forages obtained from nonlactating cows fed a 78% forage diet similar to our dry-TMR1 treatment. Their IVenz method referred to that of Licitra et al. (1999) and was similar to ours, using *Streptomyces griseus*, but the enzyme concentration was about half that used in this study, and hydrolysis time was 24 h, whereas 48 h or longer was needed in our study to predict RUP for dry-TMR1. They calculated in situ RUP using a k_p of 0.04/h, which was similar to the k_p of dry-TMR1 used to calculate RUP in our study. They reported a high correlation between in situ RUP and IVenz hydrolysis, but in situ RUP was consistently overpredicted, especially for those feeds with higher RUP content. Shorter IVenz times result in higher unhydrolyzed CP residues. Thus, the shorter IVenz time selected by Edmunds et al. (2012) would result in an overprediction of RUP. This result suggests that longer enzyme times or higher concentrations than those used by Licitra et al. (1999) and Edmunds et al. (2012) are probably needed to predict RUP for nonlactating cows fed nonlactating cow diets.

Variation in the IVenz time needed to match the RUP of dry-TMR1, mid-TMR2, and peak-TMR3 in situ treatments in our study indicates that probably no single IVenz time should be used to estimate RUP for cows in different stages of lactation with their corresponding diets. If a single time were selected for an enzymatic assay, predicted RUP may need to be adjusted by any model or formulation system to account for differences in diet or physiological state of the target animal. In Figure 1, the unhydrolyzed CP residues at 6, 12, and 48 h were compared with the corresponding in situ RUP for peak-TMR3, mid-TMR2, and dry-TMR1 treatments, respectively. The HM had a large initial loss of CP at IVenz time zero, which resulted in it being an obvious outlier. Removing this outlier resulted in a correlation of 0.90 between IVenz and in situ RUP results, which suggests that the IVenz method may have potential as a laboratory assay for RUP, if the time of hydrolysis matched the stage of lactation with the corresponding diet and intake level of the target animal.

CONCLUSIONS

No single model was suitable for estimating CP degradation kinetics for all of the diverse feeds used in

Figure 1. Relationship of in vitro RUP at 6, 12, and 48 h to in situ RUP for peak-lactation, mid-lactation, and nonlactating cows when fed their corresponding diets, respectively. Correlation for all feeds except herring meal was 0.90.

this study. Some kinetic parameters, such as fractions or pools of potentially degradable or undegradable CP, did not vary among cows or stage-diet treatments. To estimate those kinetic parameters that varied, models were developed that minimized both MSE and the number of parameters. Because washout was measured, lag times could be estimated, and including lag in the model affected the estimates of k_d . To predict k_d from in situ measurements, we recommend that models are chosen with caution and include lag time. Differences in k_d and lag time were observed among the diverse feeds selected. We also observed that k_d and RUP differed with stage-diet treatments of the in situ cows. The average k_d for dry-TMR1 was greater than that of mid-TMR2 or peak-TMR3. When RUP was calculated using in situ degradation parameters and k_p based on intake, the RUP of dry-TMR1 cows was lower than that of mid-TMR2 or peak-TMR3 cows, and we observed a tendency for the RUP of peak-TMR3 to be higher than that of mid-TMR2. When degradation parameters from dry-TMR1 were used to estimate RUP for mid-TMR2 and peak-TMR3, the RUP calculated was underestimated. Because stage of lactation, diet, and intake were confounded in our study, we cannot determine which of these factors was responsible for the differences in degradation kinetics. However, we conclude that stage of lactation, diet, or intake affect CP degradation parameters and RUP, and suggest that using k_d from nonlactating cows or the RUP calculated from them may bias diet evaluation or ration formu-

lation for lactating cows. Difference in observed RUP between stage-diet treatments precluded the adoption of a single incubation time for an IVenz method using *Streptomyces griseus* protease. With the exception of HM, in vitro times of 6, 12, and 48 h could be used to estimate RUP of the feeds for peak-TMR3, mid-TMR2, and dry-TMR1, and the correlation between RUP and IVenz was 0.90. We suggest that IVenz predictions of RUP should be measured using incubation times appropriate for lactating cows.

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