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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).