Effect of rumen-undegradable protein supplementation and fresh forage composition on nitrogen utilization of dairy ewes¹

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ABSTRACT

Previous trials with dairy ewes fed stored feeds indicate a positive effect of rumen-undegradable protein (RUP) supplementation on milk yield. However, dairy sheep production in the United States is primarily based on grazing mixed grass-legume pastures, which contain a high proportion of rumen-degradable protein. Two trials were conducted to evaluate the effects of high-RUP protein supplementation and fresh forage composition on milk yield and N utilization of lactating dairy ewes fed in confinement or on pasture. In a cut-and-carry trial, 16 multiparous dairy ewes in mid-lactation were randomly assigned to 8 pens of 2 ewes each. Pens were randomly assigned 1 of 2 protein supplementation treatments, receiving either 0.0 or 0.3 kg of a high-RUP protein supplement (Soy Pass, LignoTech USA Inc., Rothschild, WI) per day. Within supplementation treatment, pens were randomly assigned to 1 of 4 forage treatments, which were applied in a 4 × 4 Latin square design for 10-d periods. Forage treatments included the following percentages of orchardgrass:alfalfa dry matter: 25:75, 50:50, 75:25, and 100:0. No interactions were observed between supplement and forage treatments. Supplementation with a high-RUP source tended to increase milk yield by 9%. Milk yield, milk protein yield, milk urea N, and urinary urea N excretion increased linearly with increased percentage of alfalfa. Milk N efficiency was greatest on the 100% orchardgrass diet. In a grazing trial, 12 multiparous dairy ewes in mid lactation were randomly assigned to 3 groups of 4 ewes each. Within group, 2 ewes were randomly assigned to receive either 0.0 or 0.3 kg of a high-RUP protein supplement (SoyPlus, West Central Cooperative, Ralston, IA) per day. Grazing treatments were arranged in a 3 × 3 Latin square design and applied to groups

for 10-d periods. Ewes grazed paddocks that contained the following percentages of surface area of pure stands of orchardgrass:alfalfa: 50:50, 75:25, and 100:0. There were no interactions between supplement and forage treatments. Milk yield, milk protein yield, and milk urea N increased linearly with increased percentage of alfalfa in the paddock. In conclusion, supplementing with high-RUP protein tended to increase milk yield and increasing the proportion of alfalfa in the diet increased dry matter intake, milk yield, and protein yield of lactating dairy ewes fed or grazing fresh forage.

Key words: dairy sheep, forage, protein degradability

INTRODUCTION

Most dairy sheep producers in North America use improved pastures as the primary source of forage and provide a supplemental concentrate in the milking parlor. Although temperate grass or mixed grass-legume pastures may be high in CP, fresh forage protein is highly degradable (>70%; Hongerholt and Muller, 1998; Bargo et al., 2003). Intake of RDP in excess of microbial utilization contributes to preduodenal N losses, increases animal energy requirements (Cannas et al., 2004), and reduces embryo survival in sheep (Bernardinelli et al., 2001; Fahey et al., 2001). In addition, the excretion of urea N contributes to environmental pollutants such as atmospheric NH₃ and nitrates in ground water (Tamminga, 1992). Therefore, balancing rations for protein degradability may improve animal performance and reduce the environmental impact of livestock production.

Although microbial protein is the primary protein source for ruminants, increasing dietary RUP can increase the flow of AA above microbial AA supply. Specific requirements are not reported for RUP in lactating ewe diets, but the NRC (2007) indicates that increasing dietary RUP from 3.4 to 9.3% of DM decreases dietary CP requirements from 17 to 15.5% of DM, indicating increased efficiency of N utilization from RUP. In trials with stored feeds, RUP supplementation increased milk yield by 13 to 27% in nondairy ewes (Robinson et al.,

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1979; Loerch et al., 1985). In dairy ewes, supplementing RUP from expeller soybean meal increased milk yield by 14% in low- and high-milk-yielding ewes (Mikolayunas-Sandrock et al., 2009). Few trials have evaluated the effect of RUP supplementation to lactating ewes on pasture. Penning et al. (1988) reported a 23% increase in milk yield and a 15% increase in lamb growth when dams were supplemented with fish meal compared with supplementation with barley or no supplement. In early lactation dairy cows, Schor and Gagliostro (2001) reported a 17% increase in milk yield and a 14% increase in milk protein yield in cows fed blood meal compared with soybean meal. Therefore, RUP supplementation may be beneficial to high-producing dairy ewes.

In addition, the inclusion of legumes in pastures increases animal performance in dairy cattle (Stiles et al., 1968), beef cattle (Yarrow and Penning, 2001), and growing sheep (Fraser et al., 2004). Dairy cattle have higher DMI on legume-based pastures compared with grass-based pastures due to lower NDF and higher digestibility of legumes compared with grasses (Kristensen et al., 2005). In grazing dairy ewes, legumes increased pasture protein availability, pasture protein intake, and DMI on pasture (Molle et al., 2008). Therefore, 2 experiments were conducted to evaluate the effect of RUP supplementation and fresh forage composition on milk production and N utilization of dairy ewes. The first experiment was conducted as a cut-and-carry trial, and the second experiment was a pasture-based grazing trial.

MATERIALS AND METHODS

Ewes and Treatments

Two trials were conducted at the University of Wisconsin–Madison, Spooner Agricultural Research Station, and all procedures were approved by the Animal Care and Use Committee of the College of Agricultural and Life Sciences.

Cut-and-Carry Trial. In May 2008, 16 third-lactation dairy ewes in mid lactation (104 DIM; SD = 8) with similar milk production (2.37 kg/d; SD = 0.22) were randomly assigned to 8 pens of 2 ewes each. Pens were randomly assigned to 1 of 2 supplementation treatments, with 4 pens receiving 0.0 and 4 pens receiving 0.3 kg of a high-RUP protein supplement per day (Soy Pass, Borregaard LignoTech, LignoTech USA Inc., Rothschild, WI), fed in 2 equal feedings in the milking parlor. Within each supplementation treatment, pens were assigned to 1 of 4 forage treatments. Dietary forage treatments were applied to pens for 10-d periods in a 4 × 4 Latin Square and balanced for carryover effects. All ewes were milked twice per day (0530 and 1700 h)

and had access to water and a free-choice mineral–salt mixture. All ewes received 0.8 kg of DM/d of an equal mixture of whole corn and soyhulls, fed twice daily in the milking parlor.

Forage treatments were composed of the following proportions of DM from orchardgrass (*Dactylis glomerata* L.) and alfalfa (*Medicago sativa*): 25% orchardgrass and 75% alfalfa (25 diet), 50% orchardgrass and 50% alfalfa (50 diet), 75% orchardgrass and 25% alfalfa (75 diet), and 100% orchardgrass (100 diet). All pens were fed the 50 diet for a 5-d adaptation period before the trial began. Forages were clipped daily at 0600 h at a height of 5 cm above the soil surface using a walk-behind sickle-bar mower (Jari Products Inc., Minneapolis, MN). Clipped forages were fed to ewes at 0800 h and stored at 7°C until feeding again at 1100 and 1800 h. Multiple feedings were designed to mimic the multiple meals consumed by ewes grazing pasture. Forage DM was determined on d 2 and 7 of each experimental period by drying forages in a 37°C forced-air oven until they reached a constant weight. As-fed forage amounts were calculated based on these DM determinations. Pens were fed forage ad libitum to allow 5% refusals. Forages were clipped to maintain similar stages of maturity.

Grazing Trial. In June 2009, 12 third-lactation dairy ewes in mid lactation (126 DIM; SD = 6) with similar milk production (2.32 kg/d; SD = 0.16) were randomly assigned to 3 groups of 4 ewes each. Groups were assigned to 1 of 3 dietary forage treatments, which were balanced for carryover effects and applied to ewes for 10-d periods in a 3 × 3 Latin square.

Forage treatments were paddocks containing the following proportions of surface area from pure stands of orchardgrass and alfalfa: 50% orchardgrass and 50% alfalfa (50 diet), 75% orchardgrass and 25% alfalfa (75 diet), and 100% orchardgrass (100 diet). Forages were planted in monoculture strips, clipped to a height of 7.5 cm, and allowed 20 d of regrowth before the start of each grazing period. No plot was grazed more than once and the maximum age of forage regrowth was 30-d. Paddocks were created using a combination of portable electric and wooden fencing. Ewes were given fresh forage daily and paddock size was based on the residual from the previous day.

Within each group, 2 ewes were randomly assigned to one of 2 supplementation treatments, receiving either 0 or 0.3 kg of SoyPlus/d, a source of RUP (West Central Cooperative, Ralston, IA), fed in 2 equal feedings in the milking parlor. All ewes were milked twice daily (0700 and 1900 h) and had access to water and a free-choice mineral–salt mixture. All ewes received 0.8 kg of DM/d of whole corn, which was fed in equal portions at each milking. All ewes were dosed twice daily with a gelatin

capsule containing 0.1 g of dotriacontane (C_{32}) at 0700 and 1900 h for a total of 0.2 g dosed/d. The dosed alkanes were used as an external marker to estimate forage DMI and forage composition (Kelman et al., 2003; Dove and Mayes, 2005).

Sample Collection, Analysis, and Calculations

In both trials, individual ewe milk yield was measured during the final 4 milkings of each treatment period using a graduated Waikato Goat Meter (Waikato Milking Systems NZ Ltd., Hamilton, New Zealand). Individual milk samples were analyzed for fat, protein, and MUN. Milk fat and protein percentages were measured using a CombiFoss 5000, and MUN was analyzed using a Foss FT6000 (both instruments from Foss Electric, Hillerød, Denmark; analyses conducted at AgSource Milk Labs, Menomonie, WI). Milk fat yield and protein yield were calculated for each ewe from daily milk yield and fat and protein percentages. In both trials, ewes were weighed at the start of the first treatment period and during the final 2 d of each experimental period, and BW change during each period was calculated as initial BW minus final BW.

Cut-and-Carry Trial. All feeds and orts were sampled on the final 2 d of each experimental period, and orts were compiled by period. All samples were dried in a 37°C forced-air oven until they reached a constant weight. Final DM was determined by drying samples overnight at 100°C in a forced-air oven. Feed and orts were analyzed according to the following methods. Neutral detergent fiber was determined using sodium sulfate and α -amylase (Sigma No. A3306, Sigma Chemical Co., St. Louis, MO) in the Ankom²⁰⁰ fiber analyzer (Ankom Technology, Macedon, NY). Nonsequential ADF was determined using the method of Goering and Van Soest (1970), adapted for the Ankom²⁰⁰. Nitrogen concentration was determined by rapid combustion (850°C), conversion of all N-combustion products to N_2 , and measurement by a thermoconductivity cell (Leco model FP-528, Leco Corp., St. Joseph, MI), and CP was calculated as N concentration $\times$ 6.25. Ash was determined according to AOAC (1990). Indigestible ADF (**IADF**) was determined by measuring ADF remaining after 12 d of in situ incubation (Huhtanen et al., 1994).

Fecal grab samples were collected at 1650 h on d 9 and 10 of each treatment period. Samples were dried in a 37°C forced-air oven until they reached a constant weight and ground to pass through a 1-mm screen in an Udy cyclone mill (Udy Corp., Fort Collins, CO). Fecal samples were analyzed for DM, ash, N, and IADF, as described earlier. Indigestible ADF was used as an internal marker to estimate total fecal output.

Spot urine samples were collected from ewes at 1750 h on d 9 and at 0650 h on d 10 by temporarily restricting breathing. Fresh urine samples were acidified by mixing 4 mL of urine with 0.08 mL of 50% H_2SO_4 , and samples were immediately frozen at -20°C until analysis. Samples were analyzed for creatinine using a picric acid assay (Oser, 1965) and analyzed for urea using an automated colorimetric assay (Broderick and Clayton, 1997); both procedures were adapted to a flow-injection analyzer. Total daily urine excretion was calculated from urinary creatinine concentration using the following equation developed for dairy ewes: daily creatinine excretion (mg/d) = $34.2 \times BW^{-0.104}$, where BW is expressed in kilograms (Faichney et al., 1995). Total urea excretion was calculated based on urea concentration and total urine output.

Grazing Trial. The orchardgrass, alfalfa, and residual forage were sampled during the final 2 d of each treatment period. Orchardgrass and alfalfa were sampled by randomly tossing 2 quadrats ($0.37m^2$) into an adjacent ungrazed, monoculture strip and harvesting the forage to a height of 2.5 cm. Pen residual forage was sampled by randomly tossing 2 quadrats into the grazed paddock and harvesting forage to 2.5-cm stubble height. Forage samples were weighed and dried in a forced-air oven at 37°C until they reached a constant weight. Final DM was determined by drying samples overnight at 100°C in a forced-air oven. All samples were analyzed for NDF, ADF, CP, and ash as described previously.

Fecal grab samples were collected at 0650 and 1850 h on d 9 and 10 of each treatment period. Samples were frozen at -20°C until dried in a 60°C oven. Fecal samples were compiled for each ewe by period and ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Fecal N was determined by combustion (Leco model FP-528), and CP was calculated as N concentration $\times$ 6.25.

Alkane concentration in forage, concentrate, and fecal samples was analyzed according to Mayes et al. (1986) with the modification that samples were initially treated with ethanolic potassium hydroxide. Following extraction, alkane concentrations were characterized using a Perkin Elmer AutoSystem gas chromatograph (Norwalk, CT), which included a glass, wide-bore capillary column (DB-1, 15 m $\times$ 0.53 mm, J&W Scientific Inc., Folsom, CA). Dietary proportions of forage species were calculated from the concentration of C_{25} , C_{29} , C_{31} , and C_{33} alkanes in forage and feces using a nonnegative least square algorithm supplied by EatWhat software (Dove and Moore, 1995). The resulting botanical composition was used to calculate C_{31} and C_{32} concentrations in individual diets. Based on the concentration

of alkanes in forage, concentrate, and feces, total DMI was calculated using the following equation (Mayes et al., 1986):

$$\text{Herbage intake} = [F_i/F_j \times (D_j + I_c \times C_j) - I_c \times C_i] / (H_i - F_i/F_j \times H_j),$$

where F_i , C_i , and H_i are the concentrations (mg/kg of DM) of C_{31} in feces, concentrate, and herbage, respectively; F_j , C_j , and H_j are the concentrations (mg/kg of DM) of dosed C_{32} in feces, concentrate, and herbage, respectively; I_c is the intake of concentrate (kg of DM/d); and D_j is the amount of dosed C_{32} (mg/d).

Spot urine samples were collected from ewes at 0730 and 1900 h on d 9 and 10 of each treatment period by temporarily restricting breathing. Fresh urine samples were acidified by mixing 2 mL of urine with 1 mL of 50% H_2SO_4 and 6 mL of H_2O , and samples were immediately frozen at $-20^\circ C$ until analysis. Samples were analyzed for creatinine using a picric acid assay (Oser, 1965) and analyzed for urea by an automated colorimetric assay (Broderick and Clayton, 1997); both procedures were adapted to a flow-injection analyzer.

Statistical Analyses

Cut-and-Carry Trial. Data were analyzed as a split-plot design. Soy Pass supplement was the main plot, with pen as the experimental unit, and forage composition was the subplot, with pen within period as the experimental unit. Soy Pass supplement was applied in a completely randomized design and forage composition was applied in a 4×4 Latin square design. Individual ewe milk production, milk composition (fat yield, fat percentage, protein yield, protein percentage, MUN), N efficiency (pen milk N/pen N intake), pen DMI, and intake of feed components were analyzed using the MIXED procedure of SAS (v. 9.2, SAS Institute Inc., Cary, NC). For all traits, the initial model included supplement (df = 1), forage treatment (df = 3), period (df = 3), forage $\times$ supplement (df = 3), and the random effect of pen within supplement. For all measurements, the interaction of forage $\times$ supplement was not statistically significant and was removed from the final model.

Grazing Trial. Data were analyzed as a split-plot design. SoyPlus supplementation was applied in a randomized design with ewe as the experimental unit. Forage composition was applied in a 3×3 Latin square design with group as the experimental unit. Individual ewe data were analyzed using the MIXED procedure of SAS (v. 9.2, SAS Institute Inc.). For milk yield, milk composition, and DMI data, the model included the

fixed effects of group (df = 2), period (df = 2), supplement (df = 1), forage treatment (df = 2), and forage $\times$ supplement (df = 2). The model also included the random effects of ewe, group $\times$ period, subgroup, and the error term for individual. The subgroup represents variation among ewes within a group fed the same supplement across periods as supplement treatments remained constant throughout the trial for each subgroup of ewes. For all measurements, the interaction of forage $\times$ supplement was not significant and was removed from the model. The association between forage composition offered and forage composition consumed was analyzed using simple correlation using PROC CORR.

For both trials, all values presented are LSM and SEM. Significant differences between LSM were declared at $P < 0.05$ unless otherwise noted, and tendencies were considered at $0.05 < P < 0.10$. Differences between LSM were calculated using a Tukey-Kramer adjustment for multiple comparisons of means.

RESULTS AND DISCUSSION

Nutrient Composition and Intake

In both trials, ewes were supplied sufficient forage DM to maximize DMI. In the cut-and-carry trial, ewes maintained at least 5% refusals. In the grazing trial, forage DM on offer averaged 2.6, 3.1, and 3.3 kg of DM/ewe per day for the 100, 75, and 50 treatments, respectively. Based on forage DMI intake levels of 2.4 to 2.6 kg of DM/ewe per day in the cut-and-carry trial, pasture allowance in the grazing trial was equal to or in excess of ad libitum daily forage intake. In both trials, there was no effect of supplementation or forage composition on BW; therefore, the data are not presented.

The nutrient composition of feeds offered in both trials is presented in Table 1. The CP concentration varied throughout the trial, but was lower for orchardgrass (9.0 to 15.2% CP in cut-and-carry; 12.8 to 14.0% CP in grazing) compared with the alfalfa (18.7 to 20.0% CP in cut-and-carry; 21.9 to 25.5% CP in grazing). Orchardgrass quality was similar to the NRC (2007) values for CP (14%) in both trials and is consistent with values for unfertilized grasses (9.1 to 15.6% CP; Bittman and Kowalenko, 2000). The CP content of alfalfa was similar to values reported in the NRC (19% CP; 2007) and previous trials (20.6% CP; Elizalde et al., 1999). The CP content of both orchardgrass and alfalfa were higher in the grazing trial than in the cut-and-carry trial. The NDF content of orchardgrass was similar to the NRC (2007) value (54% NDF) in both trials. Neutral detergent fiber was likely lower in the cut-and-carry trial compared with the grazing trial because the cut-and-carry trial started earlier in the

Table 1. Nutrient composition of forages and supplemental feeds in cut-and-carry and grazing trials

Item	Orchardgrass ¹				Alfalfa				Supplement	
	1	2	3	4	1	2	3	4	Corn and soyhulls	Soy Pass ²
Cut-and-carry trial, % of DM										
CP	10.8	11.8	9.0	15.2	20.0	19.7	19.1	18.7	9.8	45.8
NDF	45.9	54.3	49.0	49.8	29.0	36.4	32.0	36.5	35.9	31.2
ADF	25.4	28.2	27.8	27.9	23.8	28.3	23.2	28.9	23.7	8.5
Ash	6.4	12.0	25.3	13.9	9.3	10	14.2	10.6	3.6	7.6
									Corn	SoyPlus ³
Grazing trial, % of DM										
CP	12.8	14.0	13.7		22.7	25.5	21.9		9.2	44.2
NDF	59.9	57.9	56.8		37.0	32.5	37.3		12.6	18.3
ADF	35.4	34.1	33.4		30.2	27.9	30.7		3.4	9.0
Ash	11.6	11.0	12.4		10.8	10.2	10.4		1.4	6.3

¹Treatment period lasting 14 d.²LignoTech USA Inc., Rothschild, WI.³West Central Cooperative, Ralston, IA.

growing season than the grazing trial (May 23 and June 15, respectively). The NDF content of alfalfa was lower than 38.4 to 46% NDF reported by the NRC (2007) and Elizalde et al. (1999), respectively. In both trials, forages were clipped to maintain a vegetative state (cut-and-carry) or 20 to 30 d of regrowth (grazing). Therefore, alfalfa at this stage of maturity would tend to be of higher quality (low ADF and NDF, high CP) compared with more mature forages. In both trials, the ash content of the forages was generally similar to the reported ash content in orchardgrass (9%) or alfalfa (9%; NRC, 2007). However, in period 3 of the cut-and-carry trial, the ash content reached 25% of DM. This is likely due to the method of harvesting; forages were clipped with a sickle-bar mower, hand raked, and carried in large tubs. During raking, the forages may have accumulated soil, thus increasing the ash content of the feeds.

Cut-and-Carry Trial. The nutrient intake of pens of ewes in the cut-and-carry trial is presented in Table 2. Supplementation with 0.3 kg of Soy Pass/d had no effect on DMI, supporting findings by previous authors (Jaime and Purroy, 1995; Ubertalle et al., 1998). However, forage composition did have an effect ($P < 0.05$) on DMI. Ewes fed diet 100 consumed less DM ($P < 0.05$) than ewes fed 50 and 25, and the percentage of orchardgrass was negatively and linearly ($P < 0.01$) related to DMI. Molle et al. (2008) reported that in dairy ewes grazing mixed legume pastures, increasing the percentage of legume in the sward increased DMI, milk yield, and output/unit area. Penning et al. (1997) reported that ewes grazing a monoculture of white clover consumed more DM/bite, more DM/minutes grazed, and more kilograms of DM/d than ewes grazing a monoculture of perennial ryegrass. Differences in DMI may be attributed to differences in chemical composition (NDF) and pas-

sage rate. Although the reported in situ DM degradation rate of alfalfa is higher than orchardgrass at 3 stages of maturity (average of 14.7 and 8.3%/h, respectively; Hoffman et al., 1993), apparent DM digestibility was not different across treatments (Table 3).

The NDF content of the diet consumed was linearly ($P < 0.01$) related to forage composition: ewes offered 100 and 75 consumed a greater concentration of NDF ($P < 0.01$) than ewes offered 25 and 50 (Table 2). In addition, ewes on 100 and 75 consumed diets considerably greater than 38% NDF, the level recommended by Cannas (2002), which may have negatively influenced DMI and milk yield. In a trial of ewes grazing grass-legume pastures, dietary NDF ranged from 32.4 to 41.0% of DM, and percentage NDF was inversely related to milk yield (Avondo and Cannas, 2001). Cannas (2002) reported that DMI was maximized in diets with 37% NDF and milk yield was maximized when NDF intake was 1.2% of BW. Similarly, milk yield in our cut-and-carry trial was greatest when DMI was greatest (3.4% of BW) and NDF intake was 1.2% of BW (Table 4).

Consistent with dietary treatments, CP intake (% of DM) was influenced by supplementation and forage composition. Supplemented ewes consumed diets with 1% greater CP (% of DM; $P < 0.01$) compared with unsupplemented ewes; however, both groups consumed less than levels recommended for lactating ewes (16% CP; Cannas, 2002). Crude protein intake was negatively and linearly ($P < 0.01$) related to orchardgrass composition, a combined effect of the decrease in DMI and CP concentration of diets higher in orchardgrass. For every 25% increase in DM from alfalfa, dietary CP (% of DM) increased by approximately 1.4% CP. Previous trials with lactating ewes reported no effect of dietary CP% on DMI (Robinson et al., 1979; Jaime and Purroy, 1995; Cannas et al., 1998), suggesting that

Table 2. Intake of ewes in trials feeding RUP supplement and fresh forage

Item	Supplement ¹			Orchardgrass in forage, ² %					P-value		
	No SP	SP	SEM	100	75	50	25	SEM	Supp.	Forage	Forage-linear
Cut-and-carry trial											
DMI, kg/pen per day ³	5.64	5.54	0.40	4.89 ^f	5.50 ^{ef}	6.00 ^e	5.97 ^e	0.37		*	**
DMI, % of BW	3.16	3.16	0.24	2.73 ^f	3.11 ^f	3.40 ^e	3.40 ^e	0.21		*	**
Intake, % of DM											
CP	13.97 ^b	14.97 ^a	0.12	12.32 ^d	13.78 ^c	15.21 ^b	16.57 ^a	0.17	**	**	**
NDF	42.06	40.04	0.74	46.05 ^a	42.59 ^{ab}	39.37 ^{bc}	36.20 ^c	1.04		**	**
ADF	25.13	24.36	0.23	24.93	24.99	24.68	24.38	0.24	†		*
Ash	8.32	8.63	0.30	7.42 ^f	8.82 ^{ef}	9.26 ^e	8.39 ^{ef}	0.42		*	†
RUP ⁴	3.7 ^b	5.3 ^a	0.09	4.6	4.4	4.4	4.5	0.10	**		
Grazing trial											
Forage DMI, ⁵ kg/ewe per day	1.89	1.65	0.25	1.34	1.94	2.03		0.30			
Total DMI, kg/ewe per day	2.67	2.74	0.25	2.27	2.88	2.97		0.30			
Intake, % of BW	2.96	3.13	0.30	2.58	3.26	3.31		0.40			
Orchardgrass, ⁶ % of DMI	77.8	74.7	4.2	96.4 ^e	73.0 ^{ef}	59.3 ^f		5.5		*	**

^{a-d}Means within a treatment with different superscripts are different at $P < 0.01$.

^{e-g}Means within a treatment with different superscripts are different at $P < 0.05$.

¹SP = supplemented with 300 g of Soy Pass (LignoTech USA Inc., Rothschild, WI) per day in cut-and-carry trial or 300 g of SoyPlus (West Central Cooperative, Ralston, IA) per day in grazing trial; No SP = no supplement.

²Percentage of DM from orchardgrass, remaining portion from alfalfa.

³Two ewes per pen.

⁴Calculated using RUP values from Coblenz et al. (1999) and NRC (2001) and corrected for orts based on fed DM proportions of orchardgrass and alfalfa.

⁵Calculated from dosed (C_{32}) and naturally occurring (C_{31}) alkanes.

⁶Calculated using EatWhat program (Dove and Moore, 1995).

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

differences in DMI are likely due to NDF and forage degradation kinetics.

Grazing Trial. The calculated forage DMI and total DMI for ewes in the grazing trial is presented in Table 2. There were no statistical differences in DMI across supplementation or forage treatments. The levels of DMI ranged from 2.58 to 3.31% of BW in diets 100 and 50, respectively. These levels of intake were similar to the cut-and-carry trial, which ranged from 2.73 to 3.4% of BW in diets 100 and 50, respectively. Slightly lower levels of DMI in the grazing trial may have contributed to lower milk yield in the grazing trial compared with the cut-and-carry trial (Table 4).

Based on analysis of dosed and naturally occurring alkanes, ewes in the grazing trial consumed diets with botanical composition similar to those offered (Table 2). This was achieved by managing ewes in small paddocks and adjusting paddock size to reduce forage selection by ewes in each paddock. Orchardgrass accounted for 96.4, 73.0, and 59.3% of DMI in the 100, 75, and 50 treatments, respectively. The relationship between forage composition offered and consumed forage composition had a correlation coefficient of 0.772.

Milk Production and Composition

Milk yield and milk composition results are presented in Table 4. For both trials, there was no interaction of

supplementation and forage composition. Supplementing ewes with high-RUP protein tended ($P = 0.09$) to increase milk yield in the cut-and-carry trial, with an increase of 0.16 kg/d. Despite a numerical increase of 0.17 kg/d in the grazing trial, the effect of supplementation on milk yield was not statistically significant, perhaps due to the experimental design, which gave more power to detecting an effect of forage composition than supplementation. In trials with dairy cattle, previous authors suggest cows of high genetic merit (Santos et al., 1998) or those in early lactation (Schor and Gagliostro, 2001) benefit most from RUP supplementation. Therefore, the mid lactation ewes in our studies may not have had sufficient protein requirements to elicit a significant response to RUP supplementation, or the level of RUP supplementation may not have reached requirements. Based on literature values of RUP in fresh forages (Coblenz et al., 1999; NRC, 2001) and grains (NRC, 2001), RUP levels reached 3.7 and 5.3% of DM in unsupplemented and supplemented treatments, respectively ($P < 0.01$; Table 2).

In both trials, there was a tendency for forage composition to affect milk yield ($P = 0.053$ in cut-and-carry, $P = 0.055$ in grazing). Decreasing dietary orchardgrass from 100 to 50 increased milk production by 11 and 21% in the cut-and-carry and grazing trials, respectively, representing a numerical increase of 0.2 and 0.32

kg/d, respectively. Both trials demonstrated a linear increase in milk yield ($P < 0.01$ in cut-and-carry, $P < 0.05$ in grazing) as the proportion of orchardgrass decreased, emphasizing the importance of pasture composition on milk production. However, there was no additional response in milk yield when decreasing orchardgrass from 50 to 25%, or increasing alfalfa from 50 to 75%. Based on literature values of RUP, dietary RUP (% of DM) was not affected by forage treatment (Table 2). Therefore, increased milk yield from higher alfalfa intake likely resulted from increased DMI and CP intake, with little effect of RUP. Huhtanen and Hristov (2009) reported that dietary CP is a better predictor of milk yield than dietary RUP, supporting results from this trial.

Comparing the trials, ewes in the cut-and-carry had greater milk yield, which may be due to stage of lactation. Cut-and-carry ewes averaged 104 DIM compared with 126 DIM in the grazing trial. In addition, cut-and-carry ewes did not have energetic costs for grazing and were not affected by exposure to outside weather. In the grazing trial, ewes may have reduced grazing time due to high temperatures. During 1 sampling day in the second period of the grazing trial, all ewes were removed from pasture from 1200 to 1700 h when temperatures reached more than 32°C. Despite removal from pasture for 5 h, ewes had sufficient daylight hours to graze the required 8 h recommended by Penning et al. (1997).

Milk fat yield was not affected by supplementation or forage composition in either trial (Table 4). In previous trials with lactating ewes, RUP supplementation did not affect milk fat percentage or milk fat yield (Penning et al., 1988; Ubertalle et al., 1998). There was no effect of high-RUP protein supplementation on milk protein yield in either trial. In contrast, Penning et al. (1988) and Mikolayunas-Sandroek et al. (2009) reported an 11 to 13% increase in milk protein yield with RUP supplementation. Therefore, RUP levels in this trial may not have been sufficient to elicit a milk protein response.

Increasing the proportion of alfalfa increased ($P < 0.05$) milk protein yield in both trials. In the cut-and-carry trial, increasing dietary DM from alfalfa increased milk protein yield by 15% or 12.8 g/d in 25 compared with 100. In the grazing trial, increasing alfalfa increased milk protein yield by 25% or 19.7 g/d in 50 compared with 100. The increase in milk protein is likely due to increased availability of AA for milk protein production from increased alfalfa intake.

Milk urea N was greater ($P < 0.05$) in supplemented ewes in the cut-and-carry trial, reflecting differences in CP intake (Table 4). In the grazing trial, supplementation did not significantly affect MUN, but the supplemented group had a larger mean value than the unsupplemented group. Ubertalle et al. (1998) reported

Table 3. Effect of supplemental Soy Pass and forage composition on nitrogen balance of pens of dairy ewes in cut-and-carry trial

Item	Supplement ¹			Orchardgrass in forage, ² %					P-value		
	No SP	SP	SEM	100	75	50	25	SEM	Supp.	Forage	Forage-linear
N intake, g/pen per day	126.2	133.0	8.7	93.6 ^c	120.2 ^b	146.2 ^a	158.5 ^a	7.3		**	**
Milk N, g/pen per day	28.1	29.7	0.6	26.8 ^e	28.2 ^{de}	30.0 ^{de}	30.8 ^d	0.9		*	**
Milk N efficiency ³	23.9	23.8	1.6	30.4 ^a	23.6 ^b	21.2 ^b	20.1 ^b	1.6	†	**	**
Fecal N, g/pen per day	42.5	45.7	4.8	45.2	43.0	40.3	47.8	6.8			
Fecal N, % of intake	35.3	36.6	5.0	44.7	36.4	30.2	32.3	6.7			
Urinary urea N, g/pen per day	49.9	55.7	4.0	38.5 ^b	50.7 ^{ab}	59.1 ^a	62.2 ^a	4.4		**	**
Urea N, % of intake	40.9	43.8	4.0	42.1	42.7	42.3	42.2	3.7			
Total urine, L/ewe per day ⁴	3.34	2.85	0.34	2.61	2.97	3.18	3.48	0.32			*
N balance, ⁵ (% of intake N)	5.7 (5%)	1.9 (1%)	0.34	-16.9 (18%)	-1.7 (1%)	16.8 (11%)	17.7 (11%)		NA ⁶	NA	NA
Apparent DM digested, %	77.0	73.6	3.4	78.1	71.7	79.0	72.5	4.6			

^{a-c}Means within a treatment with different superscripts are different at $P < 0.01$.

^{d,e}Means within a treatment with different superscripts are different at $P < 0.05$.

¹SP = supplemented with 300 g of Soy Pass (LignoTech USA Inc., Rothschild, WI) per day; No SP = no supplement.

²Percentage of DM from orchardgrass, remaining portion from alfalfa.

³Milk N (milk protein/6.38) g/pen per day/feed N (feed CP/6.25) g/pen per day.

⁴Estimated using urinary creatinine marker.

⁵N balance = N intake - milk N - fecal N - urinary urea N.

⁶NA = not analyzed. † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

Table 4. Effect of RUP supplement and forage composition on milk and component yield in dairy ewes

Item	Supplement ¹			Orchardgrass in forage, % ²					<i>P</i> -value		
	No SP	SP	SEM	100	75	50	25	SEM	Supp.	Forage	Forage-linear
Cut-and-carry trial											
Milk, kg/d	1.79	1.95	0.06	1.74	1.85	1.94	1.95	0.06	†	†	**
Fat, g/d	121.6	123.1	3.0	117.1	121.9	124.0	126.6	4.3			
Protein, g/d	89.7	94.8	1.9	85.4 ^b	89.8 ^b	95.6 ^a	98.2 ^a	2.7		*	**
MUN, mg/dL	12.3 ^b	15.1 ^a	0.8	10.9 ^d	12.7 ^c	14.3 ^b	16.8 ^a	0.7	*	**	**
Grazing trial											
Milk, kg/d	1.65	1.82	0.16	1.55	1.78	1.87		0.11		†	*
Fat, g/d	104.9	114.9	11.0	101.5	112.8	115.6		8.2			†
Protein, g/d	84.3	93.9	7.2	78.5 ^b	90.5 ^{ab}	98.2 ^a		5.4		*	*
MUN, mg/dL	18.2	19.8	1.0	15.0 ^b	19.8 ^a	22.1 ^a		0.8		*	*

^{a-d}Means within a treatment with different superscripts are different at $P < 0.05$.

¹SP = supplemented with 300 g of Soy Pass (LignoTech USA Inc., Rothschild, WI) per day in cut-and-carry trial or 300 g of SoyPlus (West Central Cooperative, Ralston, IA) per day in grazing trial; No SP = no supplement.

²Percentage of DM from orchardgrass, remaining portion from alfalfa.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

an increase in MUN excretion in dairy ewes fed diets with increasing protein degradability. In dairy cattle, Broderick et al. (2009) reported elevated MUN with increasing RUP supplementation, suggesting the low Met content of soybean meal may have limited utilization for true milk protein production. Mikolayunas-Sandroek et al. (2009) reported that MUN was more related to CP intake than protein degradability in sheep.

Similar to milk protein yield, MUN increased linearly ($P < 0.01$) as the proportion of intake from alfalfa increased in both trials (Table 4). In the cut-and-carry trial, the MUN levels in diets 100 and 75 were lower than the level recommended by Cannas (14 to 21 mg/dL; 2002) and reflect low CP intake in these diets, which were 12.3 and 13.8% of DM, respectively. Milk urea N levels were greater in the grazing trial compared with the cut-and-carry trial, likely due to the greater CP content of forages in the grazing trial (Table 1).

Across both trials, regression of MUN on CP (% of intake), as calculated from forage composition offered, had an R^2 value of 0.45 for all ewes and 0.48 for unsupplemented ewes. Cannas et al. (1998) reported R^2 values of 0.65 and 0.43 for the regression of MUN on dietary CP% and CP intake, respectively, for ewes fed dried feeds in metabolic cages. Therefore, MUN may be used as an indicator of protein intake in ewes consuming fresh forage.

Value of RUP Supplementation

In 2009, sheep milk was valued at \$75.30/cwt in Wisconsin (USDA-NASS, 2009). Based on this price, RUP supplementation increased return/ewe by \$0.27 and \$0.28/d in the cut-and-carry and grazing trials,

respectively. If this effect is consistent throughout the 80-d period from midlactation to the end of lactation, a flock of 300 ewes may realize an increased gross return of \$6,375 to \$6,773 by supplementing an RUP source at the level in this study. Based on the current cost of Soy Pass (\$0.374/kg or \$340/ton; K. Hendricks, LignoTech USA Inc., Rothschild, WI, personal communication) and SoyPlus (\$0.328/kg or \$297.90/ton; P. Cerny, West Central Cooperative, Ralston, IL, personal communication), the total cost of supplementation of this flock during this 80-d period would be \$2,698 in the cut-and-carry trial and \$2,364 in the grazing trial. This would lead to a net return of \$3,676 and \$4,408 over 80 d for the cut-and-carry and grazing trials, respectively. Therefore, a positive economic return to producers may be realized with the supplementation of a RUP source if the increases in milk yield reported in this study are real.

Nitrogen Balance

Nitrogen balance of ewes in the cut-and-carry trial is presented in Table 3. Nitrogen consumption increased linearly ($P < 0.01$) with increasing alfalfa composition, but was not different between supplement treatments. Supplemented and unsupplemented ewes primarily excreted N in urine (43.8 and 40.9% of intake N, respectively), followed by feces (36.6 and 35.3%), and milk (23.8 and 23.9). Nitrogen excretion estimates calculated in a meta-analysis of trials with dairy cattle were 33% in feces, 33% in urine, and 26% in milk (Spanghero and Kowalski, 1997) suggesting greater N excretion in urine and lower N excretion in milk in dairy ewes compared with dairy cows.

Across forage treatments, N excretion in feces (g/d and % of intake) did not differ. However, increasing legume resulted in a linear increase ($P < 0.01$) in N excretion in grams per day in urine and milk. Urinary urea N excretion increased by 61% and milk N increased by 15% from diet 100 to diet 25. Urea N excretion, expressed as a percentage of intake N, did not differ across forage treatments, supporting the close relationship between dietary CP and urea N. In dairy cattle, urinary N excretion increased with increasing dietary CP (Broderick, 2003; Wattiaux and Karg, 2004) due to increasing urine volume or an additional increase in urinary N concentration. In this trial, the increase in urinary urea N excretion resulted from a linear increase ($P < 0.05$) in total urine volume with increasing dietary legume, with no difference in urea N concentration.

Inaccuracies in N balance (N balance = intake N – milk N – fecal N – urine N) are reported in Table 3. Among forage treatments, N excretion was overestimated by as much as 18% or underestimated by as much as 11% of intake N. Although differences between intake N and excreted N may be attributed to retention or mobilization, there was no effect of RUP supplementation or forage composition on BW. Spanghero and Kowalski (1997) reported that N balance in trials with dairy cattle tend to underestimate N excretion with errors due to volatilization from urine and feces, dermal losses, and inaccurate milk N analysis. In this trial, the N imbalance may be due to the use of markers (IADF and creatinine) to estimate total fecal and urine output. In addition, urinary urea N accounted for 58 to 82% of urinary N in wethers (Lindberg and Jacobsson, 1990) and 85 to 91% of urinary N in dairy cattle (Broderick, 2003), suggesting an underestimation of total urinary N in this trial.

Gross N efficiency was calculated as $100 \times (\text{milk N}/\text{feed N})$, where milk N = [(g of milk protein/pen per day)/6.38] and feed N = [(g of feed CP/pen per day)/6.25]. In the cut-and-carry trial, N efficiency ranged from 20.1 to 30.4% (Table 3). Whereas supplementation did not affect N efficiency, there was a linear effect ($P < 0.01$) of forage composition. Ewes consuming 100 were more efficient ($P < 0.05$) at capturing feed N in milk N compared with all treatments containing alfalfa. Although cows on diets 25 and 50 produced more milk, they also excreted more N in milk and urinary urea. While previous reports in dairy sheep indicate N efficiencies of 12.1 to 14.4%, reported N efficiencies in dairy cattle range from 26.2 to 33.8% (Wattiaux and Karg, 2004). One reason cited for the lower N efficiency in sheep is a lower efficiency of conversion of metabolizable protein to milk protein (0.58 vs. 0.67; NRC, 2007). However, this trial suggests that dairy ewes may reach

similar levels of N efficiency as dairy cattle due to high milk protein concentration.

CONCLUSIONS

Supplementation of a RUP source (Soy Pass) tended to increase milk yield in the cut-and-carry trial. Milk production tended to increase with increasing proportions of dietary alfalfa, likely a result of increasing DMI with increasing dietary alfalfa. However, no benefit in milk yield was observed above 50% alfalfa. Milk protein yield increased with increasing dietary alfalfa. The excretion of urea, in both milk and urine, was higher for ewes on higher CP diets, and milk N efficiency was greatest in the diet with the lowest forage CP (100% orchardgrass). Therefore, increased DMI, milk yield, and milk protein yield may be realized by establishing and maintaining mixed grass–legume pastures with 50% or more legume. Supplementing grazing ewes with 0.3 kg/d of a RUP source resulted in a nonsignificant increase in milk yield in lactating ewes and may result in an economic benefit to producers.

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REFERENCES

- Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Gaithersburg, MD.
- Avondo, M., and A. Cannas. 2001. NDF-intake in lactating ewes fed at pasture. Pages 502–504 in Proc. XIV Congress ASPA, Florence, Italy.
- Bargo, F., G. A. Varga, L. D. Muller, and E. S. Kolver. 2003. Pasture intake and substitution rate effects on nutrient digestion and nitrogen metabolism during continuous culture fermentation. *J. Dairy Sci.* 86:1330–1340.
- Berardinelli, J. G., J. Weng, P. J. Burfening, and R. Adair. 2001. Effect of excess degradable intake protein on early embryonic development, ovarian steroids, and blood urea nitrogen on days 2, 3, 4, and 5 of the estrous cycle in mature ewes. *J. Anim. Sci.* 79:193–199.
- Bittman, S., and C. G. Kowalenko. 2000. Within-in season grass herbage crude-protein and nitrate-N concentrations as affected by rates and seasonal distribution of fertilizer nitrogen in a high yearly rainfall climate. *Can. J. Plant Sci.* 80:277–285.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964–2971.

- Broderick, G. A., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. *J. Dairy Sci.* 92:2719–2728.
- Cannas, A. 2002. Feeding of lactating ewes. 123–166 in *Dairy Sheep Feeding and Nutrition*. G. Pulina, ed. Avenue Media, Bologna, Italy.
- Cannas, A., A. Pes, R. Mancuso, B. Vodret, and A. Nudda. 1998. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. *J. Dairy Sci.* 81:499–508.
- Cannas, A., L. O. Tedeschi, D. G. Fox, A. N. Pell, and P. J. Van Soest. 2004. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. *J. Anim. Sci.* 82:149–169.
- Coblentz, W. K., I. E. O. Abdelgadir, R. C. Cochran, J. O. Fritz, W. H. Fick, K. C. Olson, and J. E. Turner. 1999. Degradability of forage protein by in situ and in vitro enzymatic methods. *J. Dairy Sci.* 82:343–354.
- Dove, H., and R. W. Mayes. 2005. Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Rumin. Res.* 59:123–139.
- Dove, H., and A. D. Moore. 1995. Using a least-squares optimisation procedure to estimate botanical composition based on the alkanes of plant cuticular wax. *Aust. J. Agric. Res.* 46:1535–1544.
- Elizalde, J. C., N. R. Merchen, and D. B. Faulkner. 1999. In situ dry matter and crude protein degradation of fresh forages during the spring growth. *J. Dairy Sci.* 82:1978–1990.
- Fahey, J., M. P. Boland, and D. O'Callaghan. 2001. The effects of dietary urea on embryo development in super-ovulated donor ewes and on early embryo survival and development in recipient ewes. *Anim. Sci.* 72:395–400.
- Faichney, G. J., R. J. Welch, and G. H. Brown. 1995. Prediction of the excretion of allantoin and total purine derivative by sheep from the 'creatinine coefficient'. *J. Agric. Sci.* 125:425–428.
- Fraser, M. D., M. H. M. Speijers, V. J. Theobald, R. Fychan, and R. Jones. 2004. Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial ryegrass swards. *Grass Forage Sci.* 59:345–356.
- Goering, H. K., and P. J. Van Soest. 1970. *Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications)*. Agric. Handbook No. 379. ARS-USDA, Washington, DC.
- Hoffman, P. C., S. J. Sievert, R. D. Shaver, D. A. Welch, and D. K. Combs. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. *J. Dairy Sci.* 76:2632–2643.
- Hongerholt, D. D., and L. D. Muller. 1998. Supplementation of rumen-undegradable protein to the diets of early lactation Holstein cows on grass pasture. *J. Dairy Sci.* 81:2204–2214.
- Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim. Feed Sci. Technol.* 48:211–227.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232.
- Jaime, C., and A. Purroy. 1995. The response of lactating and dry ewes to energy intake and protein source in the diet. *Small Rumin. Res.* 17:17–24.
- Kelman, W., M. Bugalho, and H. Dove. 2003. Cuticular wax alkanes and alcohols used as markers to estimate diet composition of sheep (*Ovis aries*). *Biochem. Syst. Ecol.* 31:919–927.
- Kristensen, T., K. Soegaard, and I. S. Kristensen. 2005. Management of grasslands in intensive dairy livestock farming. *Livest. Prod. Sci.* 95:61–73.
- Lindberg, J. E., and K. G. Jacobsson. 1990. Nitrogen and purine metabolism at varying energy and protein supplies in sheep sustained on intragastric infusion. *Br. J. Nutr.* 64:359–370.
- Loerch, S. C., K. E. McClure, and C. F. Parker. 1985. Effects of number of lambs suckled and supplemental protein source on lactating ewe performance. *J. Anim. Sci.* 60:6–13.
- Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage alkanes for determining the composition of the diet of grazing herbivores. *J. Agric. Sci.* 107:161–170.
- Mikolayunas-Sandroch, C., L. E. Armentano, D. L. Thomas, and Y. M. Berger. 2009. Effect of protein degradability on milk production of dairy ewes. *J. Dairy Sci.* 92:4507–4513.
- Molle, G., M. Decandia, U. Solter, J. M. Greef, J. J. Rochon, M. Sitzia, A. Hopkins, and A. J. Rook. 2008. The effect of difference legume-based swards on intake and performance of grazing ruminants under Mediterranean and cool temperate conditions. *Grass Forage Sci.* 63:513–530.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington DC.
- National Research Council. 2007. *Nutrient Requirements of Small Ruminants: Sheep, Goats and New World Camelids*. Natl. Acad. Press, Washington, DC.
- Oser, B. L. 1965. *Hawk's Physiological Chemistry*. 14 ed. McGraw-Hill, New York, NY.
- Penning, P. D., J. A. Newman, A. J. Parsons, A. Harvey, and R. J. Orr. 1997. Diet preferences of adult sheep and goats grazing ryegrass and white clover. *Small Rumin. Res.* 24:175–184.
- Penning, P. D., R. J. Orr, and T. T. Treacher. 1988. Responses of lactating ewes, offered fresh herbage indoors and when grazing, to supplements containing different protein concentrations. *Anim. Prod.* 46:403–415.
- Robinson, J. J., I. McHattie, J. F. Calderon Cortes, and J. L. Thompson. 1979. Further studies on the response of lactating ewes to dietary protein. *Anim. Prod.* 29:257–269.
- Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen- undegradable protein on dairy cow performance: A 12-year literature review. *J. Dairy Sci.* 81:3182–3213.
- Schor, A., and G. A. Gagliostro. 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. *J. Dairy Sci.* 84:1597–1606.
- Spanghero, M., and Z. M. Kowalski. 1997. Critical analysis of N balance experiments with lactating cows. *Livest. Prod. Sci.* 52:113–122.
- Stiles, D. A., E. E. Bartley, G. L. Kilgore, F. W. Boren, R. L. Ott, and J. G. Clark. 1968. Comparative value of alfalfa pasture, bromegrass pasture, or alfalfa hay for lactating dairy cows. *J. Dairy Sci.* 51:1620–1624.
- Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. *J. Dairy Sci.* 75:345–357.
- Ubertalle, A., R. Fortina, L. M. Battaglini, A. Mimosi, and M. Profiti. 1998. Effect of protein degradability on urea nitrogen in sheep milk. *Scienza Technica Lattiero Casearia*. 49:67–81.
- USDA-NASS. 2009. *Wisconsin Dairy Sheep Industry Overview*. USDA National Agricultural Statistics Service, Madison, WI.
- Wattiaux, M. A., and K. L. Karg. 2004. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. *J. Dairy Sci.* 87:3492–3502.
- Yarrow, N. H., and P. D. Penning. 2001. The liveweight gain of Limousin × Friesian heifers grazing perennial ryegrass/white clover swards of different clover content and the effects of their grazing on sward botanical composition. *Grass Forage Sci.* 56:238–248.