

Effects of Forage Species on Milk Protein Production by Lactating Dairy Cows¹

P. C. HOFFMAN² and N. M. ESSER

Department of Dairy Science, University of Wisconsin, Madison, WI 53706

Abstract

Eighteen multiparous early lactation Holstein cows were assigned to a replicated 3 × 3 Latin square with 28-d periods to evaluate milk protein production potential when corn silage (CS) or small grain silage (SGS) was added to diets based on alfalfa silage (AS). Treatments consisted of all AS, a mixture of AS and CS, or a mixture of AS and SGS. Treatment diets were isonitrogenous, isofibrous, and contained maximal amounts of carbohydrates to challenge the milk protein production of cows under intensive milk production systems fed these forages. Lactation performance, milk composition, nutrient intakes, and diet digestibilities were measured. There were no significant differences in milk yield of the cows fed diets containing AS, CS, or SGS. Cows fed diets containing all AS-produced milk with a higher fat percentage than did cows fed CS, and cows fed diets containing all AS produced more milk fat and protein than cows fed CS or SGS. There

were no significant differences in the percentage of casein, whey protein, or true protein in milk when cows were fed AS, CS, or SGS. There were also no significant differences in the percentage or production of lactose or solids-not-fat when cows were fed diets containing different silages. Similarly, there were no significant differences in DMI or dietary DM digestibility by cows when fed diets containing different silages. Data suggest that adding CS or SGS to AS-based diets does not improve DMI, lactation performance, or milk protein production of lactating cows.

(Key Words: Alfalfa Silage, Corn Silage, Lactation, Milk Protein.)

Introduction

Production of milk protein is economically important to dairy producers and milk manufacturers. Numerous investigations (6, 10, 25) have been conducted to define dietary factors that influence milk protein content. Most investigations have focused on manipulation of dietary carbohydrate source and level (20). There is, however, a limited amount of information available on the effects of forage species on milk protein content. Feeding high quality alfalfa silage (AS) (low NDF) to lactating dairy cows results in

carbohydrate supplementation limits, decreasing milk protein yield (15). Hoffman et al. (12) also observed milk protein depressing effects when red clover silage replaced AS in the diets of lactating dairy cows. To alleviate potential depressed milk protein production associated with low fiber legumes, corn silage (CS) often replaces a portion of legumes in the diets of lactating dairy cows. The basis of this strategy is to feed a forage that is higher in NDF, allowing for higher levels of carbohydrate supplementation, which improves milk protein content (6). Broderick (3) replaced AS with CS in the diets of lactating dairy cows and observed a 0.2% unit increase in milk protein content. Similarly, Dhiman and Satter (5) replaced 0, 33, or 66% of the AS with CS in lactating dairy cow diets and observed increases in milk protein content.

Data (3, 5) appear to support the hypothesis that CS inclusion in legume-based diets fed to lactating cows increases fermentable carbohydrate levels in the diet, thereby increasing milk protein production. Critical examination of data, however, questions whether these observations are practical or hypothetical. The data of Broderick (3) were gathered from lactating cows fed diets that were very diverse in forage-to-

¹Supported by federal Hatch funds and the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI 53706.

²To whom correspondence should be addressed: pchoffma@facstaff.wisc.edu

concentrate ratio (or fiber content); therefore, the effect of forage species is confounded with carbohydrate feeding level. Dhiman and Satter (5) supplemented their all AS-based diet with higher levels of fat than the CS-based diets, and fat is known to depress milk protein content (13). Other authors (2, 8) also have evaluated mixtures of CS and hay crop silage compared with diets based solely on CS or hay crop silage alone and found no significant or consistent changes in milk protein percentage or yield. Because these references (2, 8) are dated, limited nutritional profiles of the diets are available for scientific inferences. Previous research questions whether CS is unique in improving milk protein content or whether the confounded nature of previous studies (3, 5) merely reflects well-known effects of carbohydrate feeding level (25) or fat supplementation (13) on milk protein production. We designed the following study to examine whether forage species has an effect on milk protein content independent of dietary carbohydrate content or fat supplementation when early lactation dairy cows are fed diets with maximal carbohydrate densities.

Materials and Methods

Forage Production and Harvest.

Treatment forages were grown at the Marshfield Agricultural Research Station (Marshfield, WI) (44° 39' N, 90° 08' W) on a Withee silt loam soil. A 5.5-ha field of spring-seeded oats (*Avena sativa* L.) was cut at the late boot stage of maturity and swathed. The herbage was allowed to wilt for ca. 30 h and then was chopped with a forage harvester to a theoretical length of 1 cm. Herbage was conserved as low moisture small grain silage (SGS) in a 4.9- × 18.3-m concrete stave silo. Similarly a 11-ha field containing summer growth alfalfa (*Medicago sativa* L.) was cut and swathed at the late bud stage of maturity. Alfalfa herbage was wilted, chopped, and conserved as AS by procedures and in a storage structure

defined previously for SGS. Finally, a 90-d relative maturity corn variety was harvested at the one-half milk line stage of maturity. Whole plant corn was chopped with a forage harvester not fitted with a kernel processor at a 0.64-cm theoretical length and was conserved as CS in a 2.6- × 32.3-m plastic silage bag. Treatment silages (AS, CS, and SGS) were stored for 235, 153, and 238 d, respectively, before feedout.

Lactation Trial. Multiparous Holstein cows (n = 18) in early lactation (61 ± 17 d; $\bar{X} \pm SD$) were assigned to a replicated 3 × 3 Latin square lactation trial. Cows were randomly assigned to squares and treatment sequences within squares. Experimental periods were 28 d. The first 21 d served as the adaptation period, and all data collection occurred during d 22 to 28.

Treatment diets consisted of a forage base of AS, a mixture of AS and CS, or a mixture of AS and SGS. Treatment diet formulation was as follows. The AS diet was formulated to contain 50.0% forage and 50.0% concentrate (DM basis), resulting in a diet with minimal levels of NDF [27.0% DM as recommended by NRC (15)]. For treatment diets containing CS and SGS, 55.0% of the NDF supplied by AS was replaced with CS or SGS NDF. Concentrate mixtures were then adjusted to provide isonitrogenous diets that contained similar levels of nonfiber carbohydrates (NFC), starch, and rumen undegraded protein (RUP). Diets were purposely formulated at maximal NFC (15) to challenge the forage source's ability to alter milk protein production under situations where maximal levels of carbohydrates are fed to lactating dairy cows.

Treatment diets were mixed and fed as a total mixed ration (TMR) at 0800 h. Amount of TMR offered was recorded, and treatment diets were sampled daily for the last 7 d of each period. Orts were weighed, recorded, and sampled according to the same procedures followed for the treatment diets. Treatment silages were sampled

once per week throughout the experiment.

Silage, TMR, and ort samples were immediately analyzed for DM by oven-drying for 48 h at 55°C. Silage, TMR, and ort samples were ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and retained for chemical analysis. All samples were analyzed for CP, ash, and absolute DM according to AOAC (1) procedures. Acid detergent fiber and NDF were determined nonsequentially according to the procedures of Goering and Van Soest (7) with modifications by Mertens (17). Starch content of silage, TMR, and ort samples were determined by Dairyland Laboratories (Arcadia, WI) using the procedures of Hessera-Saldana et al. (11). Nonfiber carbohydrate content was calculated by difference [NFC = 100 - (CP + NDF + ash + 2.0)]. Because the NDF procedure of Mertens (17) uses sodium sulfite to remove a portion of CP from NDF, the contribution of NDF CP to NFC values was not accounted for and was assumed to be similar across silages and TMR. In vitro (48 h) DM and NDF digestibilities of silages were determined according to the procedures of Goering and Van Soest (7).

Cows were housed in a free-stall barn equipped with Calan gates (American Calan, Inc., Northwood, NH) and milked twice daily at 0230 and 1430 h. Milk weights were recorded daily, and milk was sampled twice daily on d 23, 25, and 27 of each period. Milk fat, solids-not-fat (SNF) lactose, and milk urea nitrogen were determined by infrared techniques on individual milk samples (AgSource, Colby, WI). Milk protein, casein, and NPN were determined by the procedures of Rowland (23). Whey protein and true milk protein were calculated by difference.

Digestion Trial. A digestion trial was conducted simultaneously with the lactation trial. Nutrient digestibility of each treatment diet used in the lactation trial was evaluated during the collection periods using rare earth marker techniques (4).

Nutrient digestibilities in the total tract were determined by feeding 0.45 kg/d of a concentrate marked with 475 ppm lanthanum from d 18 to 28 for each period. The marked concentrate was prepared according to the procedures of Hartnell and Satter (9) and fed separately from the TMR at 0700 h to ensure complete consumption. Fecal grab samples ($n = 12$) were taken twice daily at random 12-h intervals to account for diurnal variation on d 23 to 28 of the collection period. Fecal samples were dried in a 55°C forced-air oven for 96 h, ground through a Wiley mill fitted with a 1-mm screen, and analyzed for absolute DM, CP, ADF, and NDF. The OM contents of TMR and fecal samples were also determined (1). Concentration of lanthanum of period composites of individual dry, ground fecal samples for each cow was analyzed by direct current plasma emission spectroscopy (4). All experimental procedures conducted in the digestion and lactation trial were approved by the Research Animal Resource Center at the University of Wisconsin-Madison.

Statistics. Lactation and digestion trial data were analyzed using the general linear models procedures of SAS® (25) with the model:

$$m = U + Si + Cj(S)i + Pk + Tl + e_{ijkl} \quad [1]$$

where m = dependent variable, U = overall mean of the population, Si = mean effect of square i , $Cj(S)i$ = mean effect of cow j nested within square i , Pk = mean effect of period k , Tl = mean effect of treatment l , and e_{ijkl} = unexplained residual element assumed to be independent and identically distributed. Treatment differences were determined by least significant difference when a significant treatment effect was elicited by the statistical model.

Results and Discussion

Experimental Silages. Nutrient composition and in vitro DM and NDF digestion of the experimental silages are presented in Table 1. Because no statistical analysis was

conducted on experimental silage nutrient composition or in vitro digestion characteristics, only a brief general discussion of experimental silage quality will be offered. All silages (AS, CS, and SGS) appeared to be well fermented, showed no signs of visible mold, and were aerobically stable (no heating) at feedout. The AS contained more CP than CS; SGS was intermediate. The CP values were typical of values described in feed composition tables (18). The NDF of AS and CS were similar; SGS contained more NDF than did AS or CS. The CS contained more NFC than did AS or SGS. Mineral compositions of the experimental silages differed but appeared within the normal bounds for AS, CS, and SGS (18). The in vitro DM digestibility of CS was numerically higher than that of AS or SGS, although differences were small. The in vitro NDF digestibility of CS and SGS was numerically 7.5 and 11.2% units higher than AS,

respectively. Higher in vitro NDF digestibility for CS and SGS would be expected because grass NDF is more digestible than legume NDF (14). Based on empirical evaluation, nutrient composition, and in vitro digestion measurements, treatment silages appeared to be of normal quality for their respective species (18).

Lactation Trial. The ingredient and nutrient compositions of the treatment diets are presented in Table 2. Diets were isonitrogenous, and

TABLE 1. Nutrient composition and in vitro digestion of experimental silages.

Item ^b	Experimental silage ^a		
	AS	CS	SGS
CP	20.0	7.8	14.2
ADF	35.5	25.4	40.7
NDF	41.5	42.7	61.4
NFC ^c	26.2	44.0	13.5
Starch	1.2	28.4	1.9
Ca	1.12	0.17	0.39
P	0.35	0.18	0.34
Mg	0.36	0.14	0.21
K	2.72	0.89	3.04
Ash	10.30	3.46	8.94
IVTDMD ^d	79.8	82.4	77.0
IVNDFD ^e , % NDF	51.3	58.8	62.5

^aAS = alfalfa silage, CS = corn silage, and SGS = small grain silage.

^bAll nutrients expressed on a DM basis unless specified otherwise.

^cNFC = nonfiber carbohydrate; $NFC = 100 - (CP + NDF + ash + 2.0)$.

^dIVTDMD = in vitro true DM digestibility.

^eIVNDFD = in vitro NDF digestibility.

TABLE 2. Ingredient and nutrient composition of treatment diets.

Item	TMR ^a		
	AS	CS	SGS
Ingredient, % DM			
AS	49.3	22.2	22.3
CS	—	31.1	—
SGS	—	—	18.7
Shelled corn	42.2	28.5	43.6
Soybean meal	1.8	12.5	9.8
Blood meal	2.5	1.8	1.8
Distillers grains	2.5	1.8	1.8
Trace-mineralized salt	0.36	0.36	0.36
CaCO ₃	0.36	0.87	0.80
Vitamin-mineral premix ^b	0.97	0.96	0.96
Nutrient			
DM, % as fed	51.5	44.0	51.3
CP, % as fed	17.2	17.4	17.8
RUP ^c , % CP	34.1	34.1	34.7
ADF, % DM	19.2	17.7	17.6
NDF, % DM	27.3	27.9	27.3
NFC ^d , % DM	44.3	44.3	44.0
Starch, % DM	29.9	29.0	31.2
Ca, % DM	0.80	0.81	0.80
P, % DM	0.51	0.50	0.53
Mg, % DM	0.32	0.29	0.31
K, % DM	1.66	1.33	1.62
Ash, % DM	8.19	7.37	7.94

^aTotal mixed rations (TMR) containing all alfalfa silage (AS), AS + corn silage (CS), or AS + small grain silage (SGS).

^bContained (DM basis) 15.2% Ca, 14.6% P, 600,850 IU/kg vitamin A, 162,000 IU/kg vitamin D, and 1,600 IU/kg vitamin E.

^cCalculated using NRC (19) values when forage = 50.0% intake.

^dNFC = nonfiber carbohydrate.

both CP and RUP were supplied at concentrations to support the production of ca. 42 kg/d of 4.0% FCM by a 590-kg cow (18). Diets contained similar levels of NDF ($\approx 27.5\%$), starch ($\approx 30.0\%$), and NFC ($\approx 44.0\%$). Minimal NDF and maximal NFC concentrations of the treatment diets were purposely formulated to challenge milk protein production potential of cows on the treatment silages under conditions of maximal dietary carbohydrate inclusion. Mineral and vitamin concentrations were similar among treatment diets and were at or above NRC (15) model requirements. Because AS contained more ADF per unit of NDF than did CS or SGS, ADF content of the diet containing AS had 1.6% units more ADF numerically than did the diets containing CS or SGS.

Lactation performance, milk composition, and milk component

yield of lactating cows fed diets containing AS, CS, or SGS are presented in Table 3. No significant differences ($P>0.10$) in milk yield were observed between cows fed diets containing AS, CS, or SGS. In the general context of replacing AS with CS in the diet of lactating dairy cows, our data are in contrast to the data of Dhiman and Satter (5), who observed an increase in milk yield when cows were fed a forage base of 33.0% CS and 60.0% AS compared with a forage base solely of AS. Dhiman and Satter (5) attributed increased milk yield to increases in DMI when CS was incorporated into the diet. However, Dhiman and Satter (5) incorporated more fat into their AS-based diet to create isocaloric diets. Increased fat incorporation might have depressed DMI (21) in the early lactation of cows fed AS-based

diets, confounding their conclusion. Dhiman and Satter (5) did not observe any differences in milk yield when CS replaced 66.0% AS in the diet of early lactation dairy cows. Our dietary CS inclusion rate was 58.0% of the forage base. The combined data of Dhiman and Satter (5) and our observations suggest that AS and CS may not have a positive synergistic effect on milk production in lactating cow diets when CS inclusion rates in the forage base are near 60.0%.

Cows fed diets containing all AS had a higher ($P<0.05$) milk fat percentage than did cows fed diets containing CS. Diets were isofibrous, and we did periodically evaluate (data not shown) (10) our diets for particle length and found no appreciable differences. Therefore, it is most logical that differences in milk

TABLE 3. Lactation performance, milk components, and milk component yield of lactating cows fed total mixed rations (TMR) containing all alfalfa silage (AS), AS + corn silage (CS), or AS + small grain silage (SGS).

Item	TMR				Comparison ^a		
	AS	CS	SGS	SE	AS vs CS	AS vs SGS	CS vs SGS
Milk yield, kg/d	37.2	36.1	36.0	0.63	NS	NS	NS
Milk components, %							
Fat	3.85	3.61	3.71	0.072	*	NS	NS
Total CP	3.23	3.24	3.22	0.033	NS	NS	NS
True protein	3.08	3.09	3.06	0.034	NS	NS	NS
Casein	2.27	2.41	2.37	0.071	NS	NS	NS
Whey protein	0.81	0.68	0.69	0.075	NS	NS	NS
NPN	0.14	0.15	0.16	0.003	NS	*	NS
Milk urea nitrogen, mg/dL	13.77	16.17	16.92	0.401	**	**	NS
Solids-not-fat	8.87	8.82	8.82	0.040	NS	NS	NS
Lactose	4.88	4.82	4.84	0.018	NS	NS	NS
Milk component yield, kg/d							
Fat	1.38	1.21	1.25	0.026	**	**	NS
Total CP	1.16	1.07	1.09	0.020	*	*	NS
True protein	1.15	1.15	1.14	0.013	NS	NS	NS
Casein	0.85	0.89	0.88	0.027	NS	NS	NS
Whey protein	0.30	0.25	0.26	0.028	NS	NS	NS
NPN	0.055	0.058	0.061	0.001	NS	*	NS
Solids-not-fat	3.19	2.96	2.99	0.488	**	**	NS
Lactose	1.76	1.62	1.64	0.272	**	**	NS

^aNS = not significant ($P>0.05$). * $P<0.05$. ** $P<0.01$.

TABLE 4. Nutrient intake and digestibility in lactating cows fed total mixed rations (TMR) containing all alfalfa silage (AS), AS + corn silage (CS), or AS + small grain silage (SGS).

Item	TMR			SE	Comparison ^a		
	AS	CS	SGS		AS vs CS	AS vs SGS	CS vs SGS
Intake, kg/d							
DM	23.4	23.5	22.4	0.70	NS	NS	NS
OM	21.5	21.8	20.7	0.65	NS	NS	NS
CP	4.02	4.09	4.00	0.123	NS	NS	NS
ADF	4.50	4.15	3.95	0.132	*	*	NS
NDF	6.40	6.56	6.11	0.196	NS	NS	NS
Digestibility, % intake							
DM	63.9	62.1	62.3	1.85	NS	NS	NS
OM	68.6	64.4	64.4	20.1	NS	NS	NS
CP	60.9	63.9	62.2	1.85	NS	NS	NS
ADF	42.3	38.8	41.9	1.91	NS	NS	NS
NDF	38.7	35.7	38.1	2.41	NS	NS	NS
BW	673	670	671	2.5	NS	NS	NS

^aNS = not significant. * $P < 0.05$.

fat percentage between cows fed diets containing AS and CS were because of the greater buffering capacity of AS than of CS (22). Milk fat percentage was not different for cows fed diets containing SGS compared with cows fed diets containing AS or CS.

No significant differences in the percentage of milk CP, true protein, casein, or whey protein were observed among cows fed diets containing AS, CS, or SGS. Our data are in contrast to Dhiman and Satter (5) and Broderick (3), who both observed increased milk protein percentage when cows were fed CS-based diets compared with AS-based diets. As previously stated, Dhiman and Satter (5) supplemented more fat to cows fed their AS-based diets than was observed among cows fed AS, CS, or SGS in this study. The diets of Broderick (3) were not isocaloric. Both fat supplementation (13) and diet caloric density (25) have been demonstrated to alter milk protein percentage.

Cows fed diets containing all AS had lower levels ($P < 0.01$) of milk urea nitrogen than did cows fed diets containing CS or SGS. Reasons for this observation are unclear. Theoretically, our isocaloric treatment diets contain similar levels of RUP and RDP; thus, no differences in ruminal protein utilization or subsequent differences in mammary protein (NPN) synthesis would be expected (19). We did, however, observe numerical differences in OM digestibility of the diets; cows fed diets containing all AS digested more OM than did cows fed diets containing CS or SGS (Table 4). If OM digestibility of the diet containing all AS was greater than diets containing CS or SGS, then improved utilization of RDP would be expected (19), thereby decreasing blood urea nitrogen and, subsequently, milk urea nitrogen content. Despite this logic, we cannot completely confirm this hypothesis based on our data because no significant differences ($P > 0.05$) in

OM digestion were observed among cows fed AS, CS, or SGS. There were no significant differences in milk lactose percentage or milk SNF percentage among the cows fed the different treatment diets.

Milk component yields of cows fed diets containing AS, CS, or SGS are also present in Table 3. Cows fed diets containing all AS produced more ($P < 0.05$) milk fat, CP, lactose, and SNF than did cows fed diets containing CS or SGS. These observations mirror a numerical trend of improved milk yield when cows were fed diets containing all AS. There were no significant differences in the production of true milk protein, casein, or whey protein among cows fed diets containing AS, CS, or SGS. To some extent, this observation is illogical because production of milk CP was increased when cows were fed diets containing all AS without observation of increased production of any of the major milk protein fractions (casein, whey protein, NPN). The dynamics of this query seem to center on whey protein percentage and production when cows were fed diets containing all AS. There were numerical trends of increased whey protein percentage and yield when cows were fed diets containing all AS compared with cows fed diets containing CS or SGS. The trends in increased whey protein percentage and milk yield best explain significant differences in milk CP yield among the treatment diets; however, the reader is cautioned that statistical inference did not elicit a significant difference in whey protein content among cows fed AS, CS, or SCS.

Digestion Trial. Nutrient intake and digestibility in lactating cows fed diets containing AS, CS, or SGS are presented in Table 4. There were no significant differences in DM, OM, CP, or NDF intake among cows fed the treatment diets. The ADF intake of cows fed diets containing all AS was higher ($P < 0.05$) than of cows fed diets containing CS or SGS. This was the result of the higher ADF concen-

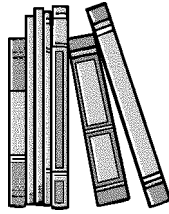
tration of the all AS-based diet. There were no significant differences in DM, OM, CP, ADF, and NDF digestibilities in cows fed the treatment diets.

Implications

Based on previous studies, a mixed forage base of AS and CS improves milk protein percentage and yield compared with diets based solely on AS. We conducted an additional evaluation of this hypothesis under the challenging conditions of minimal fiber and maximal carbohydrate inclusion in the diets. This was done to mimic diets required for high levels of milk production. We also included SGS in the experimental protocol to evaluate any uniqueness of CS. Based on our observations, there appears to be little synergy between AS and CS and their ability in lactating cow diets to enhance milk protein production. In addition, a simple mixture of SGS and shelled corn resulted in similar lactation performance measurements as in CS. In this study, we actually observed improved milk protein production when cows were fed AS-based diets.

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