

Ruminal In Vitro Degradability of Protein in Alfalfa Harvested as Standing Forage or Baled Hay¹

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ABSTRACT

Eighty-nine samples, 45 of standing forage and 44 of baled hay, were collected from alfalfa harvested at various maturities over three cuttings each during 2 yr. Alfalfa was cut and conditioned mechanically; samples of standing forage were collected by removing bunches of forage from windrows and freeze-drying them. Forage was allowed to field cure and was harvested at an average 80% DM as small rectangular bales; samples of baled hay were collected by coring bales after storing for 3 to 6 mo. Samples were analyzed for DM, ADF, total N, fractions of total N present as ADIN, N degraded at 0 h, and potentially degradable protein N. Ruminal protein degradation rates and escapes were estimated using an inhibitor in vitro system, assuming that ADIN was unavailable and that ruminal passage rate was .06/h. Standing forage contained smaller fractions of ADIN and N degraded at 0 h, contained a larger fraction of potentially degradable N, and had more rapid degradation rates and lower estimated protein escapes than baled hay. Mean degrada-

tion rates and estimated escapes were .171/h and 24% for standing forage and .075/h and 40% for baled hay. There were no differences in degradation rate or estimated escape because of harvest year, and neither was significantly related to maturity or to ADF concentration. Results indicate a significant advantage in ruminal protein escape, compared with grazed alfalfa, for alfalfa harvested and stored as hay.

(**Key words:** alfalfa protein, ruminal degradability, baled hay, standing forage)

Abbreviation key: BH = baled hay, fraction A = protein N fraction degraded at 0 h, fraction B = potentially degradable protein N fraction, SF = standing forage, UIP = undegraded intake protein.

INTRODUCTION

The protein in alfalfa forages is degraded extensively in the rumen (3, 6). Degraded protein is utilized for protein synthesis by ruminal microbes; however, wastage of forage protein caused by ammonia overflow occurs when fermentable energy is insufficient to support the microbial growth required to utilize the excess degraded protein (3). Evidence from several experiments indicates that the protein in alfalfa is utilized inefficiently by lactating dairy cows. Broderick (4) found that similar levels of forage DM from alfalfa forage and corn silage resulted in comparable production of milk and fat, but cows produced less protein and milk with depressed protein content when fed alfalfa silage or hay than when fed isonitrogenous diets based on corn silage and soybean meal. Cows fed all-alfalfa silage diets containing 21% CP produced more milk and milk protein when abomasally infused with casein (7). Compared with soybean meal or raw soybeans,

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equal amounts of CP from roasted soybeans also increased milk and protein secretion in cows receiving a diet with 50% concentrate and 50% alfalfa silage DM (8). The response in milk and protein production to formaldehyde-treated casein was substantially greater when cows grazed high protein pasture than when they ate stored forage, probably because ruminal protein escape of standing forages (SF) was low relative to stored forages (3).

A set of samples of alfalfa forages, harvested as SF or baled hay (BH) at three cuttings per year in each of 2 yr, was available from a previous experiment (15). These samples were used in the present study 1) to assess the ruminal protein degradability of alfalfa SF and alfalfa harvested as field-dried BH and 2) to determine whether ruminal degradability of alfalfa forage protein is influenced by cutting within season or maturity at harvest.

MATERIALS AND METHODS

Eighty-nine samples were prepared from alfalfa forage harvested at various maturities at three cuttings per year during each of 2 harvest yr: 54 samples from 1984 and 35 from 1985. Samples were from all trials listed by Rotz et al. (15) except for omission of the fourth cutting taken each year in October [trials 10 and 17 (15)]. In 1984, harvest trials were made at four maturities during the first cutting, three during the second cutting, and two during the third cutting (total nine trials); harvest trials at two maturities were made at each cutting in 1985 (total six trials) (Table 1). During 1984, three forage samples were harvested per windrow as SF for each single sample collected as BH, when hay was later harvested from the same windrow. The SF triplicates from 1984 were composited on an equal DM basis to give one SF sample for each BH sample. During 1985, equal numbers of replicate samples of SF and BH were taken. This sampling plan yielded three samples each of SF and BH from each trial except for trials 2 (four SF and three BH), 3 (three SF and two BH), and 16 (two SF and three BH). Alfalfa was cut and conditioned mechanically without chemical treatment. Samples of SF were collected by removing bunches of the forage from windrows, freezing them in liquid N₂ immediately after cutting,

and then storing them at -20°C until freeze-drying later. The forage was allowed to field cure and was harvested at a mean of 80% DM as small rectangular bales; samples of BH were collected by coring bales after the hay had been in storage for 3 to 6 mo. All samples were ground through a 1-mm screen using a cyclone mill. A detailed description of the alfalfa production, harvest procedures, amounts of rain that fell on drying forage, DM at harvest, and other characteristics of sample collection were published earlier (15).

Samples were analyzed for DM, total N (1), ADF, and ADIN (14). Rates of ruminal protein degradation and N fractions that are degraded at 0 h (**fraction A**) and that are potentially degradable (**fraction B**) were determined using the inhibitor in vitro system described by Broderick (5), except that incubations were conducted for 0 to 2 h in 50-ml centrifuge tubes. All SF and BH samples were incubated together in each in vitro run; incubation runs were replicated five times. Degradation rates (k_d) were corrected for unavailable N (fraction C), assuming that ADIN was equivalent to this fraction (5). Net extents of ruminal protein escape (i.e., corrected for unavailable N) were computed using the equation

$$\text{estimated protein escape (\%)} = B \times [k_p / (k_d + k_p)]$$

where $B = 100 - (A + C)$, and k_p , the ruminal passage rate, was assumed to be equal to .06/h for both SF and BH.

The general linear models procedure of SAS (16) was used for statistical analysis of data. Because the experiment was unbalanced across years, multiple statistical models were required. The model used to test for effect of harvest method included trial ($n = 15$), harvest (SF vs. BH), and harvest by trial interactions; hypothesis testing used harvest by trial as error term. The model used to test cutting and year effects included harvest (SF vs. BH), cutting and year, and interactions of harvest by cutting, harvest by year, and cutting by year; hypothesis testing for cutting and year effects used harvest by cutting and harvest by year, respectively, as error terms. Linear regression (16) of each variable on maturity and of degradation rate and estimated escape on ADF used all data and was conducted separately by har-

TABLE 1. Least squares means for alfalfa standing forage (SF) and baled hay (BH) from both cutting years.¹

Trial ²	Cutting date	Cutting no.	Maturity ³ (% Bloom)	ADF		Total N		A		B		ADIN		Rate (kg)		Est. escape	
				SF	BH	SF	BH	SF	BH	SF	BH	SF	BH	SF	BH	SF	BH
1	6/1/84	1	-10	30.8	33.0	3.00	3.49	3.65	6.18	92.6	89.5	3.72	4.31	.175	.095	23.7	34.7
2	6/4/84	1	0	34.3	34.7	2.68	2.96	4.34	5.86	91.5	89.3	4.16	4.83	.157	.101	25.4	33.2
3	6/11/84	1	10	41.1	36.5	2.52	3.12	6.08	8.23	87.9	86.9	6.01	4.91	.192	.118	20.9	30.5
4	6/14/84	1	80	41.8	41.4	2.49	2.84	7.46	7.92	86.6	85.0	5.95	7.06	.165	.098	23.4	32.3
5	7/5/84	2	30	34.2	34.6	2.57	3.17	5.05	7.25	90.0	87.8	4.93	4.94	.147	.048	26.1	49.1
6	7/5/84	2	5	33.2	32.2	2.91	3.40	5.42	7.73	90.4	88.0	4.20	4.23	.127	.044	29.0	51.0
7	7/23/84	2	25	36.4	37.2	2.94	3.50	6.74	11.78	88.8	83.1	4.42	5.11	.182	.038	22.0	51.1
8	8/14/84	3	0	25.5	28.5	3.38	3.64	7.14	7.19	89.2	88.4	3.64	4.38	.168	.092	23.5	35.3
9	8/22/84	3	10	29.8	28.4	3.06	3.52	6.46	7.00	89.2	88.2	4.35	4.79	.176	.063	22.6	43.2
11	5/28/85	1	-10	34.5	38.5	2.75	2.98	7.48	10.82	87.5	82.9	5.05	6.25	.097	.055	33.6	43.5
12	6/3/85	1	30	37.7	38.8	2.73	2.77	5.78	7.23	88.7	85.7	5.55	7.08	.169	.083	23.7	35.9
13	7/8/85	2	55	33.0	33.4	2.70	3.37	4.19	7.54	90.8	87.1	5.00	5.38	.172	.062	23.5	42.7
14	7/8/85	2	5	36.1	35.6	2.58	3.32	3.67	7.28	91.4	86.8	4.90	5.92	.169	.062	24.1	42.7
15	8/12/85	3	40	31.4	35.5	2.89	3.24	3.95	5.17	91.9	87.9	4.18	6.94	.209	.083	20.4	36.9
16	8/27/85	3	5	22.5	33.6	4.15	4.12	5.75	11.85	92.2	83.6	2.01	4.59	.259	.088	17.4	33.8
Mean				33.5	34.8	2.89	3.30	5.53	7.93	89.9	86.7	4.54	5.38	.171	.075	24.0	39.7
SD				5.2	3.6	.42	.35	1.36	2.01	1.8	2.2	1.01	1.02	.036	.024	3.7	6.9
P > F ⁴				.136		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

¹Fraction A = Fraction degraded at 0 h; fraction B = potentially degradable protein; TN = total N; Est. escape = estimated ruminal protein escape.

²Numbers correspond to trials listed by Rotz et al. (15). Samples from trials 10 and 17 (15), which were fourth cuttings taken in October of each year, were not analyzed.

³Estimated proportion bloom. Prebud and bud herbage were set arbitrarily at -10 and 0, respectively (15).

⁴Probability of a significant difference between SF and BH.

vest method to assess the effect of maturity on chemical composition and ruminal protein degradation. Because of the compositing of SF samples obtained during 1984, all models included a factor that weighted data from these samples by a factor of 3. Mean separation was by a protected ($P < .05$) Duncan's new multiple range test (18).

RESULTS AND DISCUSSION

Overall least squares means of forage composition for SF and BH samples from all 15 trials over both years are in Table 1. Significantly greater concentrations of fraction A (i.e., N in the form of ammonia and free AA before incubation) were present in BH than in SF. Proteolysis during wilting probably accounted for release of the additional protein degradation products in hay. Proteolysis of plant protein is correlated negatively to DM concentration in plant tissues, declines during wilting, and ceases when DM content rises to about 75% (12). A small but significant increase in ADIN also was found in BH. The greater amounts of N present in ADIN and in fraction A resulted in a 3.2-percentage unit reduction in fraction B in BH (Table 1). Protein in BH was degraded in the inhibitor in vitro system at 44% of the rate for SF, which resulted in an estimated ruminal escape for BH protein that was 65% greater than that in SF (Table 1). Although total N was higher in BH

than SF, probably because of losses of non-protein DM during wilting and storage (15), ADF was not different between SF and BH.

There is little quantitative data on ruminal escape for protein in alfalfa forage. The mean undegraded intake protein (UIP) value reported by the NRC (13) for alfalfa hay is 28% (SD = 7) which can be compared with our overall mean of 39.7% for BH. The UIP of alfalfa silage is set at 23% (13), which is comparable with that for SF. Presumably, autolysis in the silo by plant proteases (11) degrades the protein that likely will be degraded in the rumen, resulting in similar ruminal escape. Although the ruminal escape of SF protein of 24% was similar to the NRC (13) value for silage UIP, both our SF escape estimate and the NRC silage UIP value may be high. Ruminal degradation of silage protein actually may be greater than for SF because, unlike the soluble proteins in SF, peptides and AA in the large NPN fraction of alfalfa silage likely do not escape the rumen. Beever et al. (2) reported in vivo ruminal escape of 21% in abomasally cannulated cattle for the protein in fresh white clover herbage. Siddons et al. (17) found 18% ruminal protein escape in sheep fed alfalfa silage. When the mean escape from white clover herbage (2) and alfalfa silage (17) of 20% is compared with the SF mean, and the NRC (13) UIP value of 28% for hay is compared with the BH mean, then our inhibitor in vitro estimates of ruminal protein escape were

TABLE 2. Least squares means for the three cuttings, pooled over 2 yr for alfalfa harvested as standing forage and baled hay.¹

Cutting	n ²	ADF	Total N	Fraction		ADIN	Rate (k _d) (h)	Estimated escape (% TN)
				A	B			
		(% DM)		(% TN)				
1	37	37.0 ^a	2.84 ^b	6.98	87.5	5.53	.119	31.2
2	29	34.4 ^a	3.05 ^b	6.50	88.6	4.94	.107	35.5
3	23	29.8 ^b	3.47 ^a	6.91	88.6	4.46	.139	29.3
<i>P</i> (cutting) ³		.041	.051	.783	.195	.139	.455	.594
<i>P</i> (cutting by harvest) ⁴		.397	.155	.115	.561	.316	<.001	<.001

^{a,b}Means in columns with different superscripts differ ($P < .05$).

¹Fraction A = Fraction degraded at 0 h; fraction B = potentially degradable protein; TN = total N.

²n = Number of replicates at each cutting.

³Probability of a significant effect of cutting.

⁴Probability of a significant cutting by harvest interaction.

TABLE 3. Intercepts and slopes from linear regression of variables on maturity.¹

Variable	Intercept		Slope ²		P > F ³
	\bar{X}	SE	\bar{X}	SE	
ADF, % DM	32.6	.5	.094	.018	<.001
Total N, % DM	3.07	.05	-.005	.002	.002
Fraction A, % TN	6.2	.2	.010	.008	.230
Fraction B, % TN	89.4	.3	-.032	.010	.002
ADIN, % TN	4.5	.1	.022	.004	<.001
Rate (k_d), /h	.138	.006	.0001	.0002	.666
Estimated escape, % TN	29.2	1.1	-.025	.037	.498

¹Fraction A = Fraction degraded at 0 h; fraction B = potentially degradable protein; TN = total N.

²Dimensions of slopes are change in variable units per change in maturity units.

³Probability that slope from regression of variable on maturity is significant.

high by 23 to 42%. However, the present estimate for BH is within 2 SD of the NRC (13) value for alfalfa hay.

King et al. (9) reported greater ruminal escape of protein in alfalfa hay than alfalfa silage in abomasally cannulated sheep. Our results suggest a substantial advantage in ruminal protein escape for alfalfa hay compared with grazed alfalfa. Processing, particularly of the SF samples, may possibly have altered composition and protein degradability. Kohn and Allen (10) reported that, compared with immediate analysis, freezing fresh alfalfa in liquid N₂ and storing at -25°C reduced buffer soluble N and increased NDIN and NDF. Therefore, caution should be used when extrapolating our results to grazed forage. Determination of UIP of legume forages, particularly those of alfalfa, requires further research.

The effect of cutting within year is in Table 2. As expected, ADF content declined as season (cutting number) advanced; proportion of ADIN decreased nonsignificantly ($P = .139$) with season. Although degradation rate was slower, and estimated protein escape was greater, during the second cutting, a seasonal effect was not detected for protein degradability because neither rate nor estimated escape were different between the first and third cuttings (Table 2). Significant cutting by harvest interactions were detected for degradation rate and estimated escape (Table 2). Protein degradability was affected little by cutting for SF but was substantially different for BH. Least squares means for estimated escape for the first, second, and third cuttings were, re-

spectively, 26, 24, and 21% (SF) and 36, 47, and 37% (BH). Baled hay, but not SF, was different for second cutting, suggesting that drying or storage conditions may have influenced degradability of protein in BH samples from the second cutting during both years. Mean DM content over both years in BH put into storage was 19, 25, and 20% for the first, second, and third cuttings.

This set of samples was not designed specifically to test for effects of maturity on ruminal protein degradation. However, maturities estimated from percentages of bloom (15), including assignment of relative, negative values for prebloom herbage (Table 1), were used to assess the relationship between forage maturity and ruminal protein degradability. Regression on maturity yielded significant slopes (Table 3) for ADF, total N, and ADIN and for fraction B, which was computed by discounting for the proportion of ADIN. Changes caused by maturity were not significant (Table 3) for fraction A, degradation rate, or estimated escape. Linear regression of degradation rate and estimated escape on ADF concentration, conducted separately for SF and BH, was used as an alternative test of the effect of maturity. Regression of degradation rate on ADF was not significant for BH ($r^2 = .003$; $P = .735$); although the regression was significant ($P = .070$) for SF, the small correlation coefficient ($r^2 = .074$) suggested that this was not a strong biological relationship. Regression of estimated escape on ADF was not significant for either SF ($r^2 = .021$; $P = .340$) or BH ($r^2 = .010$; $P = .513$). We speculated that protein

TABLE 4. Least squares means from both years, pooled over three cuttings, for alfalfa harvested as standing forage and baled hay.¹

Year	n ²	ADF	Total N	Fraction		ADIN	Rate (k _d)	Estimated escape
				A	B			
		(% DM)		(% TN)			(/h)	(% TN)
1984	54	33.1	3.13	6.87	88.5	4.67	.119	32.4
1985	35	34.4	3.11	6.72	88.0	5.28	.124	31.6
<i>P</i> (year) ³		.525	.739	.849	.769	.462	.540	.417
<i>P</i> (year by harvest) ⁴		.112	.538	.106	.020	.025	.276	.391

¹Fraction A = Fraction degraded at 0 h; fraction B = potentially degradable protein; TN = total N.

²n = Number of replicates during each year.

³No significant differences because of year were detected.

⁴Probability of a significant year by harvest interaction.

degradability would decrease, and UIP would increase, with increased maturity. These results suggested that maturity alone was not an important factor in altering UIP of alfalfa forage.

Effects of harvest year on composition and degradability are in Table 4. Differences between years were not significant for any of the variables measured. The significant year by harvest interaction for fraction B and for ADIN reflected greater ($P = .015$) ADIN in BH from 1985 (6.03%) than 1984 (4.89%); neither fraction B ($P = .604$) nor ADIN ($P = .903$) was different in SF between years. Unequal replication between years required use of a conservative error term during statistical analysis and prevented strong inferences from the data. However, these results suggest that major differences in protein degradability did not occur because of crop year.

CONCLUSIONS

In vitro studies with 89 samples of alfalfa herbage harvested at three cuttings per year over 2 yr indicated that fractional degradation rates and estimated ruminal escape were 56% lower and 65% higher, respectively, for protein in BH than in SF. Although estimated escape was greater from the second cutting than from either the first or third cutting, similar degradation rates and escapes between the first and third cuttings of the year suggested that there was no seasonal trend in degradability. Neither maturity nor harvest year appeared to influence degradation rate or estimated escape of alfalfa forage protein. Results suggest a significant

advantage in ruminal protein escape for alfalfa harvested as hay compared with grazed alfalfa.

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