

Forage Protein Utilization



Improving Utilization of Forage Protein by the Lactating Dairy Cow

Glen Broderick

Introduction

Forages contribute absorbed protein to the dairy cow in two ways: 1) by providing protein that is degraded by the ruminal microbes to small nitrogen bearing compounds—peptides, amino acids and ammonia—compounds that are also used by the ruminal microbes to form new protein; and 2) by providing protein that escapes microbial breakdown in the rumen (so called rumen bypass protein). Forages contain crude protein (nitrogen x 6.25) in form of both true protein and nonprotein nitrogen (NPN). Rapid and extensive ruminal breakdown of crude protein in legume and grass forages leads to decreased protein efficiency because the ruminal microbes do not use the degraded protein as fast as it is broken down. This results in a condition called “ammonia overflow” where the excessive protein breakdown leads to excessive formation of ammonia, the final product of microbial protein breakdown in the rumen. Although ammonia can be used by ruminal microbes to form new protein, most ammonia will be absorbed from the rumen, carried by the blood to the liver, and converted there to urea and excreted in the urine. Some urea will “recycle” to the rumen, both via the saliva or directly from the blood, where it will be broken down to ammonia. In cows fed low protein diets, urea recycling is an important means of salvaging nitrogen that would otherwise be lost from the animal; however, there is little benefit from urea recycling in dairy cows fed high protein diets.

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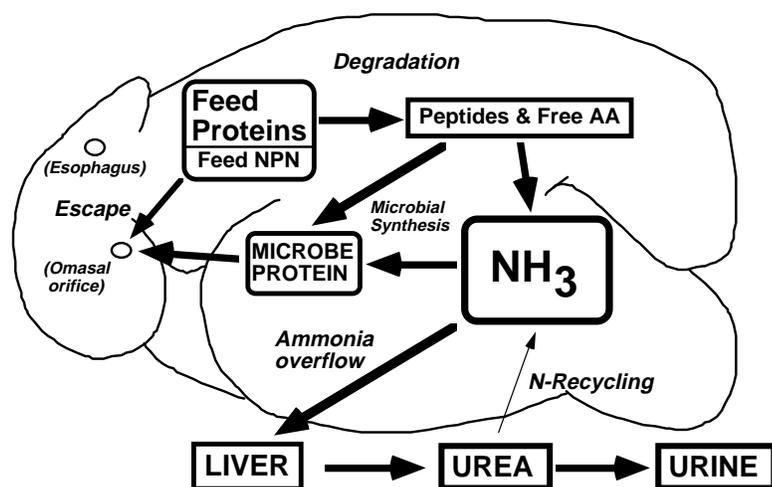


Figure 1. Protein metabolism in the rumen of the dairy cow.

The steps of the ruminal nitrogen cycle in the dairy cow are diagrammed in Figure 1.

It is often necessary to feed resistant (high bypass) proteins to dairy cows in early lactation to help meet their high requirements for absorbable (useful) protein. Although high bypass proteins are more costly, this practice is valuable in dairy cows where much of the dietary protein comes from very degradable forage proteins. There is a trend toward increased grazing of legume and grass pastures in the US; nitrogen utilization by grazing ruminants is remarkably inefficient. Poor utilization of dietary nitrogen in dairy cattle has led to increased concern about nitrogen pollution from dairy excreta (Tamminga 1992). Our challenge is to improve the efficiency of utilization of forage proteins to reduce

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the need to feed costly protein supplements and to reduce the negative environmental impact of dairy production in the US.

NPN in Alfalfa Silage

High quality alfalfa silages often contain very large amounts of NPN (Muck 1987) that is poorly used by the cow. Table 1 summarizes analyses of alfalfa silage fed in 23 lactation trials at the Dairy Forage Center research farm. Overall, 43% NDF, 0.64 Mcal NE_L/lb, relative feed value of 139, and 20.6% crude protein indicate high quality forage for silage harvested at 57% moisture. However, these silages had an average 53% NPN—that is, over half of the crude protein was not in the form of true protein when silage came out of the silo. In 1995, about 20 samples of alfalfa silage each were collected from bunker, oxygen-limiting (Harvestore), and concrete tower silos on commercial dairy farms in Wisconsin and Minnesota (Table 1). Quality of the commercial alfalfa silages, as indicated by NDF content and relative feed value, were comparable to the silages from our research farm. The NPN content also was high: NPN in silage from tower and oxygen-limiting silos was similar (55% of total nitrogen) and that from bunker silos was even higher (62% of total nitrogen). The clear message from both the research farm and the commercial samples is that NPN is a major practical problem in alfalfa silage.

Table 1.

Composition of alfalfa silage from research studies and from three different silo structures on commercial dairy farms.^{1,2}

| Item | USDFRC | Bunker | Oxygen-limiting | Tower |
|--------------------------------|--------|--------|-----------------|-------|
| Dry matter, % | 43.2 | 36.8 | 54.0 | 49.6 |
| Crude protein, % of DM | 20.6 | 19.4 | 20.7 | 19.7 |
| NPN, % of total N | 53.4 | 62.3 | 55.4 | 55.0 |
| NH ₃ , % of total N | 8.4 | 13.1 | 6.8 | 7.1 |
| ADIN, % of total N | 7.4 | 9.7 | 6.7 | 6.8 |
| NDF, % of DM | 42.5 | 45.8 | 41.5 | 41.8 |
| ADF, % of DM | 33.9 | 40.5 | 34.9 | 35.9 |
| NE _L , Mcal/lb DM | 0.64 | 0.60 | 0.65 | 0.64 |
| RFV, % | 139 | 122 | 141 | 138 |

¹DM = dry matter, RFV = relative feed value, USDFRC = US Dairy Forage Research Center.

²Analytical results from 23 trials conducted at the USDFRC research farm, and 21 bunker silos, 20 oxygen-limiting (Harvestore) silos and 19 tower silos from commercial dairy farms in Wisconsin and Minnesota.

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These high levels of NPN in alfalfa silage substantially reduce the usefulness of its crude protein. We conducted a lactation trial (Nagel and Broderick 1992) with third-cutting alfalfa that was wilted to about 65% moisture and ensiled as untreated control (C), or treated with formic acid (F) or a commercial product containing mineral acids and formaldehyde (G). Adding acids to silage drops the pH and reduces (but does not stop) NPN formation; formaldehyde is known to increase ruminal protein bypass. Reduced silage NPN indicated that treatments G and particularly F decreased breakdown of alfalfa protein in the silo (Table 2). Milking cows were fed one of three diets with most of the dry matter coming from one of these silages. Compared with silage C, yield of milk and fat was increased 7.3 lb/day and .44 lb/day on silages F and G; protein yield increased .25 lb/day on silage F and .13 lb/day on silage G (Table 2). Reducing NPN alone (F) appeared more useful than increasing protein bypass with a smaller reduction in NPN (G). These findings are interesting because Europeans recommend that formic acid be applied only to direct-cut silage, but that it is ineffective when applied to wilted silage such as was used in our feeding study (McDonald et al. 1991). In a fol-

low-up trial, replacing 5% of silage dry matter with fish meal, a high bypass protein (Broderick 1992), increased milk protein by 0.1 percentage unit on silages C and G but did not affect milk protein content with silage F. This result suggested that the protein status of cows fed silage F was better than on the other two silages. Because of its cost and corrosiveness, formic acid treatment of alfalfa silage generally is not economical in the US. Cost of formic acid has reduced its use even in Europe where the practice had been widespread. Nevertheless, our findings show that management steps that reduce NPN formation will clearly improve the protein value of alfalfa silage.

In a novel study, Makoni et al. (1994) found that protein breakdown in alfalfa silage was reduced substantially when small research silos were flushed daily with a modified atmosphere (3% O₂; 20% CO₂; 77% N₂). After 28 days, the NPN content of alfalfa silage treated with the modified atmosphere was equal to that of alfalfa silage treated with formic acid—37% of total nitrogen—compared to 51% NPN in untreated control silage. The pH of silage treated with the modified atmosphere was neutral (7.0) while that of the formic acid silage was 4.6. The modified atmosphere appears to have prevented breakdown of certain structures of the plant cells and this may have prevented much of the protein breakdown that normally occurs in the silo. Perfecting practical methods for reducing NPN content will have major benefit to dairy producers feeding alfalfa and other forage silages.

There are substantial differences among forages in the amount of NPN formation with ensiling (Albrecht and Muck 1991). Research done at the Dairy Forage Center showed that an enzyme in red clover acts to reduce silage NPN (Jones et al. 1995). We compared performance of lactating cows fed all of their diets as alfalfa or red clover silage harvested at “equivalent” maturities. Because of different growth characteristics, it is difficult to match these two forages. Although NDF contents were nearly equal (39.4% for alfalfa and 39.8% for red clover), protein levels were greater in alfalfa and the alfalfa

Table 2.

Effect of silage preservation method on alfalfa silage composition, and milk production of cows fed diets containing that alfalfa silage (Nagel and Broderick, 1992).^{1,2}

| Item | Control (C) ³ | Formic acid (F) ⁴ | Grainmax (G) ⁵ | SE |
|-----------------------|--------------------------|------------------------------|---------------------------|-----|
| Silage composition | | | | |
| Moisture, % | 61.7 | 64.8 | 64.1 | |
| Crude protein, % DM | 21.4 | 20.8 | 21.1 | |
| NPN, % total nitrogen | 43.1 | 29.1 | 35.5 | |
| NDF, % DM | 38.9 | 41.2 | 41.3 | |
| Intake and production | | | | |
| DM intake, lb/day | 40.3 | 40.1 | 43.4 | 2.4 |
| Milk, lb/day | 64.4 ^b | 71.9 ^a | 71.7 ^a | 2.0 |
| Fat, lb/day | 2.43 ^b | 2.87 ^a | 2.87 ^a | .11 |
| Protein, lb/day | 1.79 ^b | 2.03 ^a | 1.92 ^{ab} | .07 |

^{a,b}Averages with different superscripts are significantly different ($P < .05$).

¹DM = Dry matter, SE = standard error.

²Diets contained 98% of total dry matter from the experimental alfalfa silage.

³Control silage was ensiled untreated.

⁴Silage was ensiled after treatment of forage with 2.0 gal/ton of 90% formic acid.

⁵Silage was ensiled after treatment of forage with 1.5 gal/ton of Grainmax,TM a commercial silage treatment containing mineral acids and 16% formaldehyde.

Table 3.
Milk production response on alfalfa or red clover silage, with and without fish meal supplementation (Broderick, unpublished, 1995).¹

| Item | Alfalfa | Red clover | Alfalfa + fish meal ² | Red clover + fish meal ² | SE |
|------------------------------|-------------------|--------------------|-------------------------------------|--|-----|
| Diet composition | | | | | |
| Silage DM, % total DM | 72.1 | 70.3 | 72.1 | 70.3 | |
| Crude protein, % DM | 17.1 | 15.1 | 18.7 | 16.7 | |
| Intake and production | | | | | |
| DM intake, lb/day | 46.0 ^b | 46.5 ^{ab} | 50.0 ^a | 46.9 ^{ab} | 1.2 |
| Milk, lb/day | 77.7 ^a | 70.2 ^b | 78.8 ^a | 72.0 ^b | 1.2 |
| Fat, lb/day | 2.42 ^b | 2.19 ^c | 2.63 ^a | 2.35 ^{bc} | .06 |
| Protein, lb/day | 2.26 ^a | 1.97 ^b | 2.33 ^a | 2.05 ^b | .03 |
| SNF, lb/day | 6.63 ^a | 5.90 ^b | 6.67 ^a | 5.98 ^b | .10 |

^{a,b,c}Averages with different superscripts are significantly different ($P < .05$).

¹DM = Dry matter, SE = standard error.

²Diets were supplemented with 3% (dry matter basis) low-solubles fish meal.

diets contained about two percentage units more crude protein (Table 3). Milk yield was 7.5 lb/day greater on the unsupplemented alfalfa diet than on the comparable red clover diet; yield of milk components similarly was greater. Adding fish meal, a source of high bypass protein, had little effect on milk production of cows fed either silage, suggesting that the lower milk production on red clover was due to more than just lower protein intake. Ruminal ammonia concentration is an index of how much protein degradation has exceeded utilization for microbial protein formation. Very low levels of ruminal ammonia were found on both red clover diets (Fig. 2). Part of this difference likely was due to lower protein content of the red clover diets. Adding fish meal to the red clover diet made its crude protein

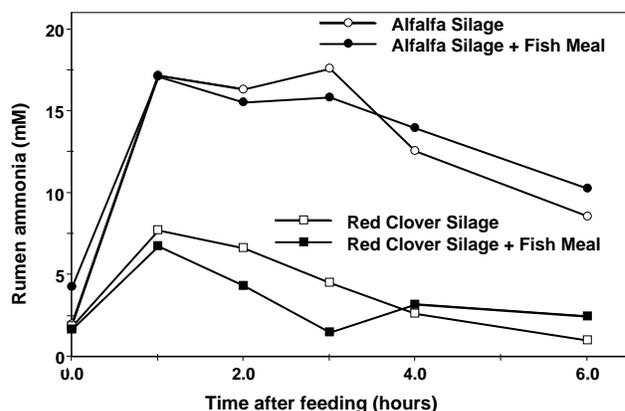


Figure 2. Ammonia concentrations for six hours after feeding in the rumens of cows fed either alfalfa or red clover silage, with or without supplementation with low-solubles fish meal (at 3% of dietary dry matter).

level similar to the unsupplemented alfalfa diet, but it still had less than half the ruminal ammonia concentration. Clearly, protein breakdown in the rumen was reduced on red clover silage but, just as clearly, lactation performance also was impaired. Work at the Marshfield Station has shown similar reduced milk yields on red clover silage (Hoffman et al. 1995). A feeding study with equalized protein levels is now being conducted to further assess the potential of red clover silage as an alternative to alfalfa.

Alfalfa Hay versus Alfalfa Silage

The long term trend toward increased harvesting of alfalfa as silage rather than hay is based on the reduced risk of weather-damage and the greater mechanization of silage harvesting. However, unlike silage, alfalfa hay has little NPN—about 10% of total nitrogen. We compared the performance of lactating cows fed all their forage as either alfalfa silage or alfalfa hay in two trials (Broderick 1995). Magnitude of the production response to high bypass protein (fish meal) was used to determine which diet was most adequate in protein before supplementation. Alfalfa was harvested from alternate windrows as either 40% dry matter silage or dry hay in small bales. Diets averaged (dry matter basis) 67% alfalfa and 30% high moisture corn. Although the silage and hay had similar levels of NDF (average 38%), the hay averaged 2.5 percentage units less protein than silage (18.1 versus 20.6% crude protein). Lower crude protein in hay was due to the greater leaf loss that typically occurs during hay harvesting (Nelson and Satter 1992). Average NPN (% of total nitrogen) content was 52% in alfalfa silage and 8% in alfalfa hay. Production data are shown in Table 4. Cows had lower dry matter intake, lost weight, and produced less milk, protein and solids-not fat on alfalfa silage without fish meal than on the other three diets.

Addition of fish meal increased protein yield 0.22 lb/day on alfalfa silage but only 0.07 lb/day on alfalfa hay. This

Table 4.

Intake, body weight change, and yield of milk and milk components on alfalfa silage or hay diets, without or with fish meal supplementation (Broderick, 1995).¹

| Item | Alfalfa silage | Alfalfa hay | Alfalfa silage + fish meal ² | Alfalfa hay + fish meal ² | SE |
|---------------------|-------------------|-------------------|--|---|-----|
| Dietary CP, % of DM | 17.1 | 15.4 | 18.6 | 17.0 | |
| DM intake, lb/day | 49.2 ^c | 52.9 ^a | 51.4 ^b | 53.4 ^a | .4 |
| BW change, lb/day | -.86 ^c | .99 ^a | .18 ^b | 1.08 ^a | .21 |
| Milk, lb/day | 77.8 ^c | 79.6 ^b | 82.5 ^a | 81.4 ^a | .6 |
| Fat, lb/day | 2.65 ^b | 2.60 ^b | 2.82 ^a | 2.69 ^b | .04 |
| Protein, lb/day | 2.29 ^c | 2.43 ^b | 2.51 ^a | 2.49 ^a | .02 |
| SNF, lb/day | 6.64 ^c | 6.81 ^b | 7.05 ^a | 7.01 ^a | .06 |

^{a,b,c}Averages with different superscripts are significantly different ($P < .05$).

¹CP = Crude protein, DM = dry matter, BW = body weight, SNF = solids not fat, SE = standard error.

²Diets were supplemented with 3% (dry matter basis) low-solubles fish meal.

means that the hay diet yielded a greater protein supply, before supplementation, than the silage diet. Ruminal ammonia was higher at all times after feeding silage than hay; ammonia averaged 15.4 and 8.1 mM on the alfalfa silage and alfalfa hay diets, respectively. When samples of these same forages were incubated in artificial rumens it was found that microbial protein formation was 29% greater on hay than on silage (Peltekova and Broderick 1996). Lower concentration of protein breakdown products in the artificial rumens indicated that hay protein was degraded more slowly—at a rate that was more in synchrony with energy digestion. This may explain the greater formation of microbial protein on hay than on silage. Protein formation by ruminal microbes (estimated from the urinary excretion of compounds, produced by the cow, from breakdown of RNA and DNA in the ruminal microbes) also was found to be greater in cows fed alfalfa hay rather than alfalfa silage (Vagnoni, unpublished). Lower concentration of ruminal ammonia in the live animal on hay diets reflected the greater microbial capture of degraded protein. Higher ruminal concentrations of ammonia on the silage diets also reflected the NPN in alfalfa silage and the 1.6 percentage unit higher crude protein in those diets. A further hay versus silage comparison was made using forage levels (50%, dry matter basis) more like that normally fed to early lactation cows (Vagnoni and Broderick, unpublished). Virtually the same results were obtained: more milk,

fat and protein were produced on alfalfa hay than on alfalfa silage. Interestingly, the increase in protein yield when fish meal supplemented the silage diet was 0.22 lb/day (the same as occurred earlier) but only 0.04 lb/day on the hay diet.

New machinery developments may help mechanize field harvesting of hay and compensate for the lack of farm labor. Koegel et al. (1988) developed an alternative hay-making process that extensively shreds herbage prior to forming it into thin forage mats for field-drying. Drying rates of the shredded alfalfa mats were three times faster than that for conventionally conditioned alfalfa. The shredded alfalfa had 15% more digestible energy than conventional alfalfa (Hong et al. 1988) and slightly greater bypass protein (Yang et al. 1993).

Concentrate Processing

Providing sufficient dietary energy to stimulate microbial protein formation in the rumen represents an important means of improving utilization of the NPN in alfalfa silage. Workers at the Dairy Forage Center have addressed this problem by studying dietary levels of corn silage (Dhiman and Satter 1994), alternative concentrate sources (Mertens et al. 1994), and moisture contents and particle sizes of the grain (Dhiman and Satter 1994) fed to lactating cows. Ekinci (1995) tested the effect of grinding of corn by using ammonia disappearance as a measure of microbial protein formation in artificial rumens. Compared to the blank (with no added grain) and unground high moisture corn, grinding high moisture corn through a 3/8" screen greatly enhanced the rate of ammonia disappearance; a finer grind (2 mm) did not further increase ammonia uptake (Fig. 3). Dry corn that was either cracked or ground was better than unground high moisture corn, but not as effective as ground high moisture corn (Fig. 3). Ekinci (1995) went on to study the effect of grain processing in a lactation experiment. Cows were fed four diets based on alfalfa silage: two with unground high moisture corn and two with ground (3/8" screen) high moisture corn; one diet with each type of corn was supplemented with high bypass protein from expeller soybean meal. Ruminal ammonia was much lower in cows fed the ground than

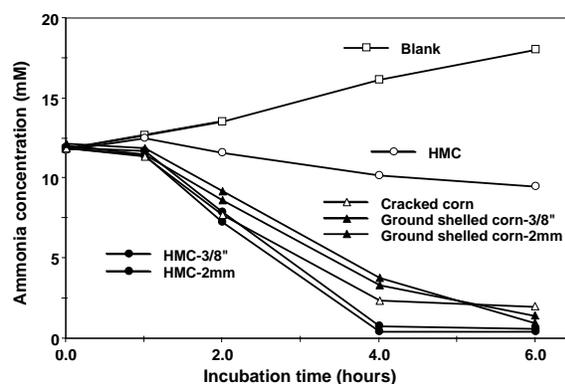


Figure 3. Ammonia concentrations in artificial rumens incubated with rumen fluid for six hours without any addition (Blank) or with addition of equal dry matter from: unground high moisture corn (HMC), high moisture corn ground through 3/8" (HMC-3/8") or 2-mm screens (HMC-2mm), cracked shelled corn, or shelled corn ground through 3/8" (3/8") or 2-mm screens (2mm).

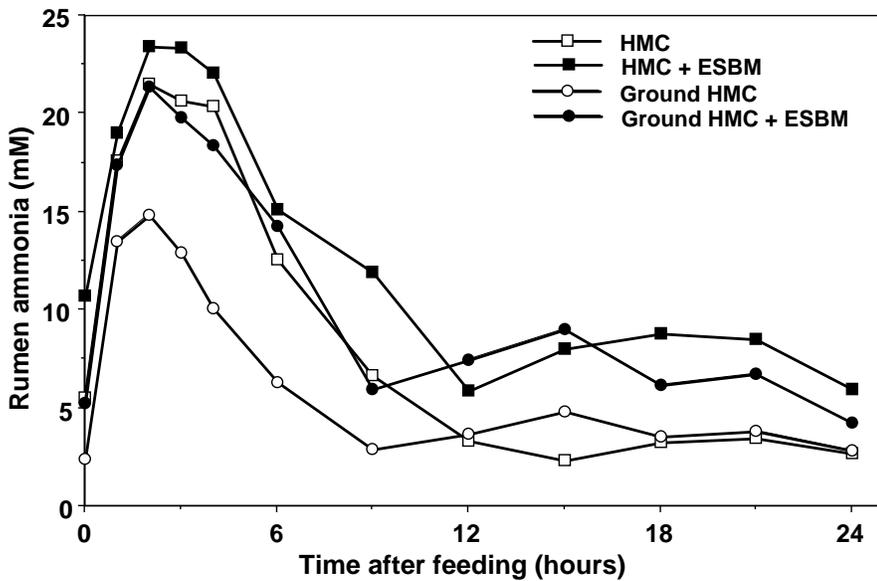


Figure 4. Ammonia concentrations for 24 hours after feeding in the rumens of cows fed once daily on alfalfa silage based diets supplemented with unground high moisture corn (HMC), unground high moisture corn plus expeller soybean meal (HMC + ESBM), high moisture corn ground through a 3/8" screen (Ground HMC), or ground high moisture corn plus expeller soybean meal (Ground HMC + ESBM).

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the unground high moisture corn, particularly during the first 12 hours after feeding (Fig. 4). Although adding expeller soybean meal increased ruminal ammonia, the supplemented diet containing ground corn was still lower than that with unground corn for the first hours after feeding (Fig. 4). Milk production also increased: yield of milk, fat, protein and solids-not fat were, respectively, 5.3, 0.31, 0.26 and 0.77 lb/day greater with the feeding of ground versus unground high moisture corn. Improvement in production of milk and milk components with grinding was nearly as great as with supplementation of expeller soybean meal; adding expeller soybean meal to the ground corn diet did not improve yield. Grinding of high moisture corn in this study clearly stimulated microbial utilization of alfalfa silage NPN. Although ruminal pH was judged not different among the four diets using statistical tests, ruminal pH was 6.24 on unground corn and 6.04 on ground corn. Also, all four diets had enough fiber (28% NDF on a dry matter basis) to help maintain rumination—the rechewing of fiber that keeps saliva flowing and the ruminal buffered from too much acid. However, if ruminal digestion is too rapid, from either too much

grain feeding or over processing of the grain, utilization of forage NPN will actually be impaired. This is because excessive ruminal digestion causes ruminal pH to drop too low. When ruminal pH falls below 6.0, microbial protein formation is depressed (Russell et al. 1992) and ruminal acidosis may result.

Potential for Genetic Selection to Reduce Protein Degradability of Alfalfa

An in vitro technique using the microbes that normally degrade protein in the rumen was used to measure ruminal protein degradability of 22 different alfalfa germplasms: 19 samples of regular alfalfa (*Medicago sativa*) and three of a related alfalfa species that has characteristic yellow flowers (*Medicago falcata*) (Broderick and Buxton 1991). There were no significant differences in crude protein, NDF and ADF content among the 22 different alfalfas. However, the protein bypass value, estimated using the ruminal in vitro method, was greater in the yellow-flowered alfalfa: ruminal bypass values averaged 17.1% for the 19 samples of *Medicago sativa* and 21.2% for the three samples of *Medicago falcata*. These differences were not related to variation in protein content because both *Medicago sativa* and *Medicago falcata* averaged 15.8% crude protein. The protein bypass value of 21% for *Medicago falcata* of course is not adequate for optimal protein utilization in lactating cows but represents a 24% improvement over the average for the regular alfalfa. Also, there were significant differences in estimated protein bypass among the 19 *Medicago sativas*. This means that conventional plant breeding techniques may be useful for developing varieties of alfalfa with reduced ruminal degradation and increased protein utilization.

Summary

Inefficient utilization of alfalfa crude protein, particularly the high amounts of NPN in alfalfa silage, and the over feeding of protein supplements that this inefficiency necessitates, leads to excessive nitrogen losses to the environment from dairy farms. Lowering protein breakdown to NPN in the silo will improve utilization of crude protein in alfalfa silage. However, practical, cost-effective methods for reducing NPN in alfalfa silage currently are lacking. Certain forages such as red clover give rise to silage with reduced NPN; although promising, red clover silage has not yet proven to be an effective substitute for alfalfa. Feeding more finely ground concentrate, if adequate effective fiber is maintained in the diet, will help maximize utilization of silage NPN for microbial protein formation in the rumen. The lower NPN content of alfalfa hay, and possibly its slower rate of protein degradation in the rumen, increases the efficiency of microbial protein formation in the rumen compared to alfalfa silage. Improved farm equipment may make hay harvesting a more practical alternative to silage harvesting in the future.

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