DEFINING QUALITY IN ALFALFA (DECONSTRUCTING THE PLANT)

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ABSTRACT

Alfalfa nutrient components play a role in the growth and development of the plant, as well as the nutrition of the ruminant animals consuming it. Despite the fact there is no symbiotic relationship between alfalfa and ruminants, the nutrient composition of alfalfa complements the nutritional requirements of ruminant animals surprisingly well. We know more about the role of some nutrient components (Neutral Detergent Fiber, NDF; Rumen Undegraded Protein, RUP; Starch, Fat, Minerals) than we do about others (Pectin; Water Soluble Carbohydrates, WSC; Rumen Degraded Protein, RDP). Lab analysis of 1070 samples of freshly cut alfalfa plants, hand-harvested from various locations across the United States from 2019-2022, demonstrates the size and range of these various nutrient fractions (expressed as a percent of dry matter).

We know the most about the largest of these fractions, NDF (33.5% ± 5.5), and its digestibility, NDFd (49.4% of NDF ± 4.9), since taken together and expressed as Ruminal Undigested NDF (RuNDF) it can have a profound impact on intake, feed passage rate through the rumen, and subsequent ruminal digestion of the entire diet. As a forage, alfalfa is well suited in this respect since its RuNDF content is relatively moderate, compared to most other forages, because of its moderate NDF content, coupled with its high rate of NDFd.

Crude protein (CP; 22.8% ± 3.2) is another of alfalfa's important nutritional contributions, comprised of RUP and RDP. RUP is a direct contributor to the essential metabolizable protein (MP), or "absorbed" protein supply to the ruminant animal and has been studied extensively (NASEM, 2021). However, no comprehensively validated laboratory method yet exists for its measurement in alfalfa. This is a significant need, since it would also allow for the calculation of alfalfa RDP (i.e., CP minus RUP) which is rich in peptides. Peptides have been shown to improve synthesis of microbial protein in the rumen, which is another important contributor to the ruminant's MP supply.

The least understood of alfalfa's carbohydrate fractions are the non-fiber carbohydrates: starch, pectin, and WSC. While starch constitutes a relatively small fraction (2.9% ± 2.2), pectin (considered by many as "soluble fiber") and WSC taken together constituted an average of 28.2% of the dry matter in this sample set. While we consider these fractions as "benign" energy sources, they warrant further study for potential beneficial effects on rumen function.

With some predictability, we can modify the nutrient composition of alfalfa through variety selection, as well as management of the crop during growth, harvesting, and storage. The key is

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possessing an understanding of how to use laboratory quality measurements to manage the alfalfa crop to the best advantage of the ultimate consumer, the ruminant animal.

Key words: Fat, Minerals, Neutral Detergent Fiber (NDF), Pectin, Rumen Degraded Protein (RDP), Rumen Undegraded Protein (RUP), Starch, Water Soluble Carbohydrates (WSC)

INTRODUCTION

Any discussion of alfalfa forage quality should be based on an understanding of the functional and nutritional components of the plant. The major constituents are crude protein, minerals (ash), fat, fibrous carbohydrates, and non-fibrous carbohydrates. While many excellent reviews discuss each of these fractions in greater detail (Hall, 2015; Mertens, 2015), this discussion will be confined to the key nutritional components in alfalfa having the greatest feeding value for ruminants.

To obtain a better understanding of the relative importance of these various fractions in alfalfa, 1070 samples of freshly cut alfalfa plants were hand-harvested from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022 and analyzed to demonstrate the size and range of these various nutrient fractions (Forage Genetics International, Gray Summit, MO, 2022). Hand-harvested plot samples were chosen to minimize confounding of nutrient profiles resulting from differential harvest losses that can occur from commercially procured samples. Samples were procured across multiple cuttings, fall dormancies, and maturities. The numbers of each nutritional assay performed on the sample set are reflected on the y-axis of the following figures, since some assays were not performed on the entire sample set.

For this alfalfa discussion, CP, ash, fat, fibrous carbohydrates, and non-fibrous carbohydrates sum to 100% (on a DM basis). The scheme laid out by M.B. Hall (2015; Figure 1) was used for identifying the carbohydrate fractions (fibrous and non-fibrous carbohydrates).

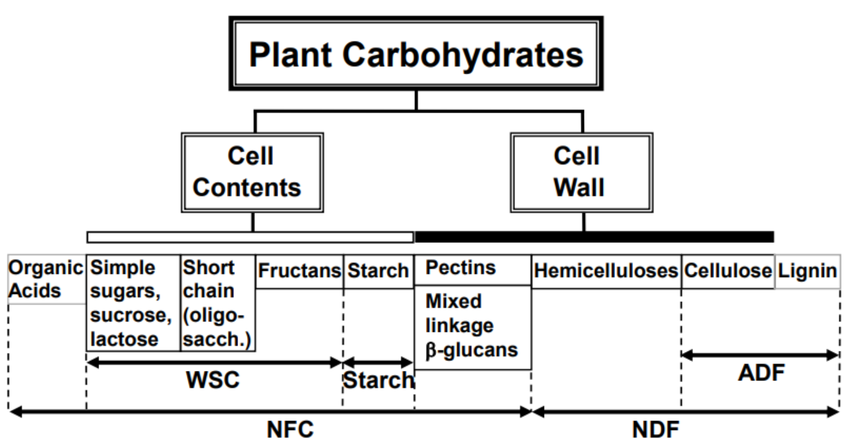


Figure 1. Plant Carbohydrates. ADF = acid detergent fiber, NDF = neutral detergent fiber, NFC = non-fiber carbohydrates, WSC = water-soluble carbohydrates. (Hall, 2015)

CRUDE PROTEIN (CP)

The distribution of crude protein in the sample set is shown in Figure 2. The variation in CP is caused not only by variety differences, but also by cutting, maturity and environmental effects.

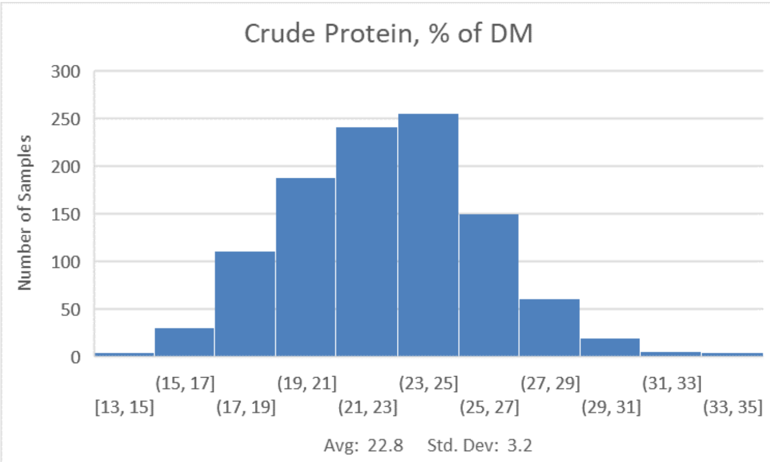


Figure 2. Crude protein from 1070 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

From a nutritional perspective, alfalfa is the highest protein containing forage available to ruminant diets. It contributes to the ruminant's metabolizable protein supply directly through a fraction that escapes ruminal digestion, known as rumen undegraded protein (RUP). Recent in vitro analysis of 71 fresh-cut alfalfa samples suggested this fraction to equal 24% ± 6.4 of CP (Forage Genetics International data, Gray Summit, MO, 2022).

The remaining 76% of the CP fraction is degraded in the rumen and known as rumen degraded protein (RDP). A portion of this fraction is captured by ruminal microorganisms in the form of peptides, amino acids, and ammonia to be used to synthesize microbial protein. Microbial protein, along with the RUP fraction, flows into the small intestine to supply the metabolizable amino acids necessary to meet the ruminant's various protein synthetic requirements.

Many consider that since the RDP fraction of alfalfa is so large, much of it cannot be captured in microbial protein synthesis and must therefore be wasted through rumen ammonia losses across the rumen wall, ultimately being excreted as urinary urea. However, some researchers have shown benefits to the diet from alfalfa's apparent RDP contribution. One such study from the Miner Institute (Grant et al., 2022) fed high producing dairy cow diets that were similar in nutrient content but contained five different ratios of alfalfa hay to corn silage in the forage portion that constituted 62% of the diet DM. Results are shown in Table 1.

Milk components

	Alfalfa-to-corn silage ratio (DM basis)				
	10:90	30:70	50:50	70:30	90:10
Fat, %	4.08	4.06	4.02	4.01	4.22
Fat, lb/d	3.9	4.0	4.0	3.9	4.0
True protein, %	3.01	3.07	3.01	3.02	3.05
True protein, lb/d[a]	2.93	3.02	3.00	2.90	2.92
MUN, mg/dl[b]	9.8	8.5	10.4	11.0	12.0
De novo FA, g/100 g FA[b]	24.76	25.86	25.82	25.22	25.58

[a]Significant cubic effect (*P* < 0.05).
[b]Significant quadratic effect (*P* < 0.05).

Table 1. Milk component yield of 105 early lactation cows fed diets varying in alfalfa hay:corn silage in 62% forage diets of similar metabolizable protein and energy content. (Grant et al., 2022)

As demonstrated by the higher milk protein yields, the lower MUN level (milk urea nitrogen, a reflection of rumen ammonia levels) and higher de novo FA levels (a reflection of milk fatty acid synthesis) shown in red in Table 1, a diet of alfalfa-to-corn silage somewhere between 30:70 and 50:50 was optimum in these diets. Presumably, this resulted from improved ruminal microbial growth and protein synthetic activity from alfalfa being present in the diet at these levels.

In vitro results (Hall, 2017) comparing two RDP sources of different ruminal availabilities would support these findings, where peptides supported greater microbial protein nitrogen synthesis than did urea (Figure 3). The RDP in alfalfa has been shown to be a rich source of peptides, derived primarily from Ribulose-1,5-*bis*phosphate Carboxylase (Rubisco) (Howarth et al., 1977), suggesting alfalfa RDP could stimulate microbial yield in the rumen.

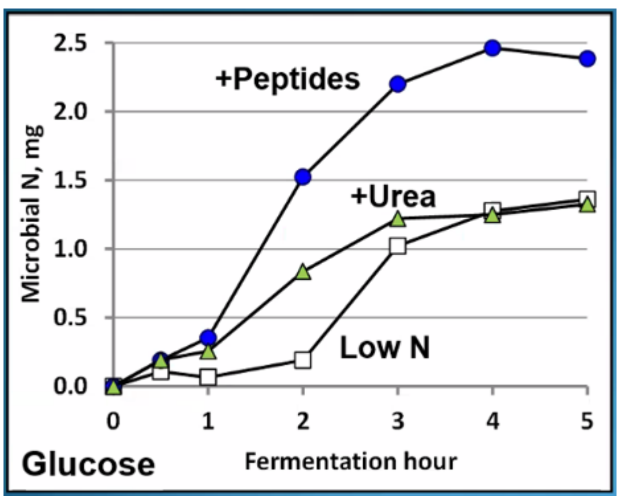


Figure 3. Effect of different rumen degradable protein (RDP) sources, urea, or peptides (tryptone), on synthesis of microbial protein nitrogen (N) in vitro when glucose was the energy source. (Hall, 2017)

While an assay for determining in vitro protein digestibility of ruminant feeds has been developed (Ross et al., 2013), its use in measuring RUP in alfalfa requires further validation.

ASH

While soil contamination can be a significant contributor to the ash content of harvested alfalfa, these samples (Figure 4) should have been relatively free of soil contamination since they were hand-harvested from research plots. Even so, the average ash content was 10.7%, with some samples as high as 17%. This ash is comprised mostly of the macrominerals calcium, potassium, phosphorus, sulfur, and magnesium (NASEM, 2021), most of which contribute to the positive cation exchange capacity (CEC) of alfalfa. This high CEC is linked to alfalfa's contribution to the diet's greater buffering capacity which promotes greater milk fat synthesis by the cow (Robinson, 2014).

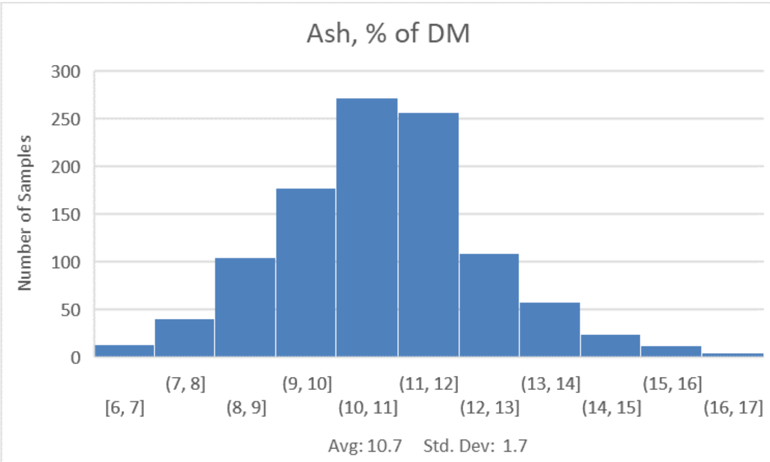


Figure 4. Ash from 1070 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

While the macrominerals in ash can have a beneficial effect on animal performance through their effect on the CEC and mineral nutrient supply, ash can also have a direct negative effect since it provides no other value to the animal and dilutes down the nutritive value of the forage. Therefore, it is important to avoid soil contamination of alfalfa during harvesting.

FAT

While fat is an energy-dense nutrient and contains about 2.25 times the energy found in carbohydrates, its content in alfalfa is relatively low (Figure 5). The average fat content, as measured after acid hydrolysis, in a subset of 72 samples from the sample set was only 3.1%, with some samples as high as 5%.

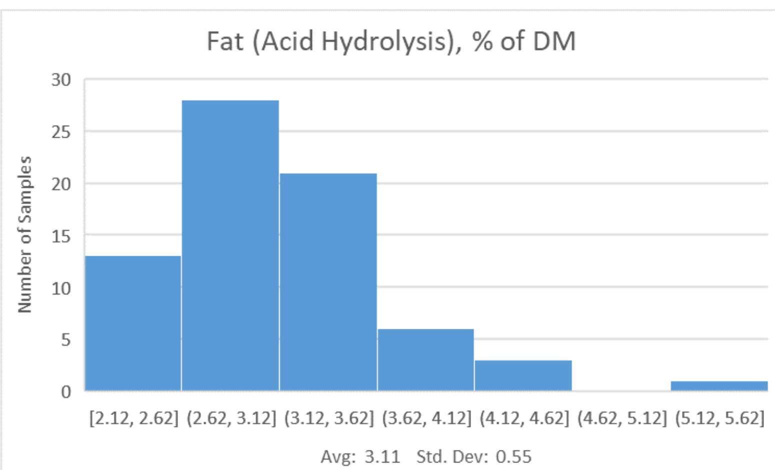


Figure 5. Fat (acid hydrolysis) from 72 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

FIBROUS CARBOHYDRATES

The largest functional and nutritional component of alfalfa is the neutral detergent fiber (NDF) fraction which represents the cell wall, or fibrous carbohydrate, portion of the plant comprising 33.5% ± 5.5 of the DM (Figure 6). Its digestibility (NDFd; 49.4% of NDF ± 4.9) shown in Figure 7 was measured following 48 hours of in vitro digestion with a buffered mixed rumen culture (Goering and Van Soest, 1970).

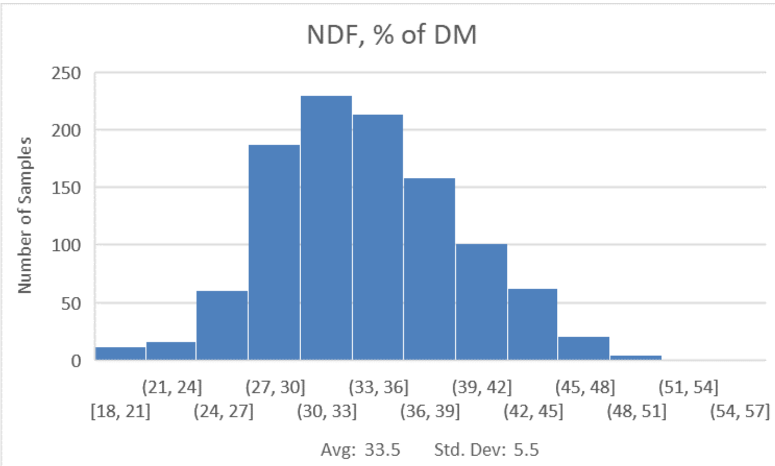


Figure 6. Neutral Detergent Fiber (NDF) from 1070 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

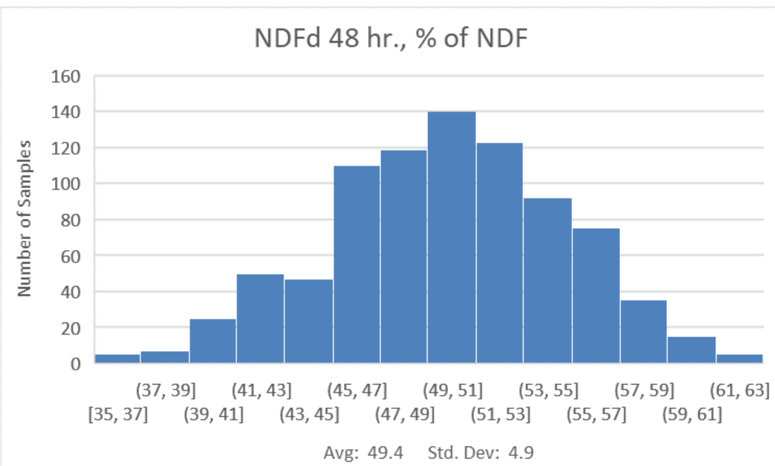


Figure 7. NDF digestibility (NDFd) from 1070 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

The importance of both these measurements rests in their contribution to the Ruminal Undigested NDF (RuNDF) content of the diet. For a particular forage, the amount of RuNDF is calculated by multiplying the undigested NDF (100% - NDFd, express on an NDF basis), by the NDF content of the forage. The sum of the RuNDF amounts from each of the forages in the diet represents an approximation of rumen fill.

The amount of rumen fill is a critical factor in controlling animal performance as shown in Figure 8. Too little rumen fill results in an increased ruminal passage rate of the diet, leading to greater intake and milk production, but at lower ruminal digestibility and poorer feed efficiency. Excessive rumen fill results in a reduced ruminal passage rate of the diet, leading to reduced intake and milk production, but at improved ruminal digestibility and feed efficiency. Most diets are formulated at an optimum compromise in rumen fill where intake and milk production are maximized at a reasonable feed efficiency. This usually occurs at an RuNDF level of approximately 11% of diet DM (Weakley, 2015). As a forage, alfalfa is well suited in this respect since its RuNDF content is relatively moderate, compared to most other forages, because of its moderate NDF content, coupled with its high rate of NDFd. Recent genetic modification of the lignin content in HarvXtra® alfalfa (Forage Genetics International) has allowed greater flexibility in fine-tuning the NDFd advantage.

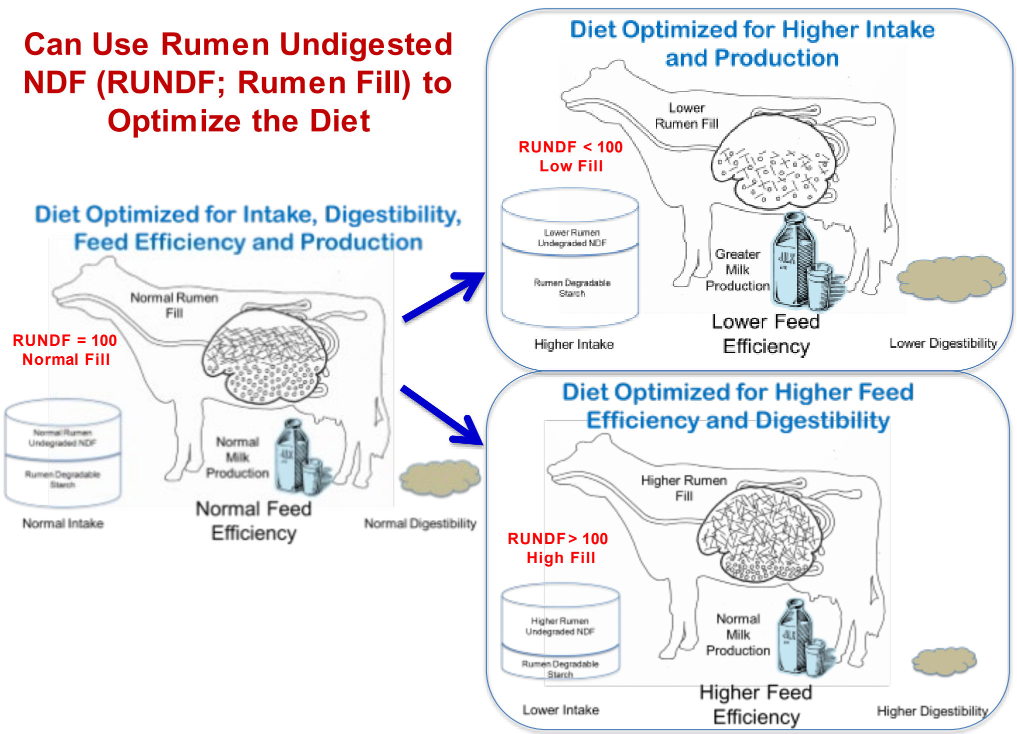


Figure 8. Influence of rumen undigested NDF (RuNDF) on intake, feed efficiency and production in dairy cows. (Weakley, 2015)

Recently, there has been increasing interest in the amount of ash in NDF (NDFash; Figure 9). While this has implications for nutritional modeling, for purposes of this discussion, it allows for the calculation of non-fibrous carbohydrates.

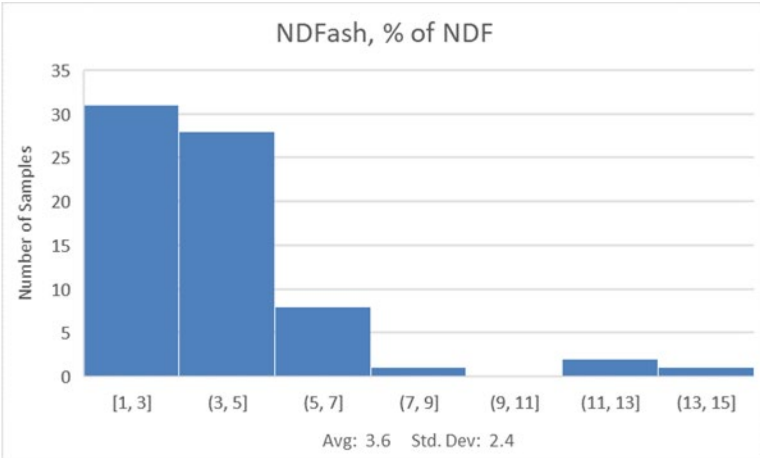


Figure 9. Ash in NDF (NDFash) from 71 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

NON-FIBROUS CARBOHYDRATES

As represented in Figure 1, the non-fibrous carbohydrate fraction is composed of many substances. For simplicity, pectin, starch, and water-soluble carbohydrates (WSC) will be the fractions discussed regarding alfalfa. As observed in Figure 10, the average starch content of a subset of 71 alfalfa samples was relatively low (2.9% ± 2.2), except for a few samples of higher content.

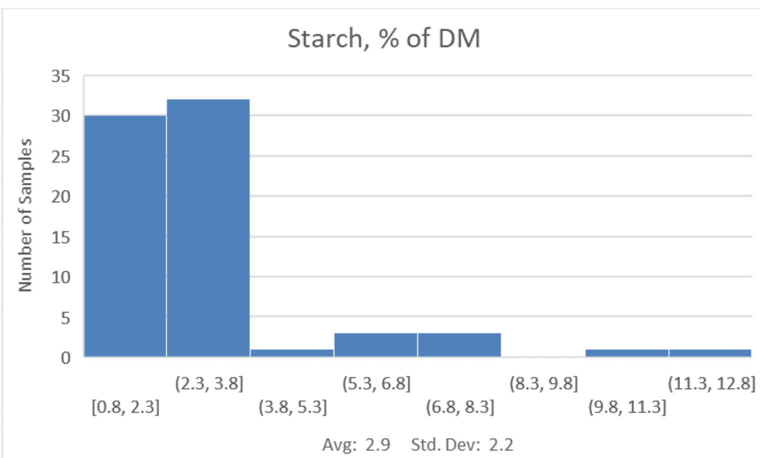


Figure 10. Starch from 71 samples of freshly cut alfalfa samples hand-harvested across multiple cuttings and maturities from test plots in WI, CA, WA, ID, KS, PA, IA, and Argentina from 2019-2022. (Forage Genetics International data, Gray Summit, MO, 2022)

Direct measurement of the pectin and WSC content of a forage is very expensive, so their content is usually determined by difference. For this alfalfa discussion, [pectin + WSC] = 100 – [CP + Fat + (Ash – NDFash) + NDF + starch]. While [pectin + WSC] cannot be calculated for each individual sample in the sample set (due to some samples missing values), it can be calculated as an average for the sample set as a whole. As such, [pectin + WSC] = 100 – [22.8 + 3.1 + (10.7 – 33.5 x .036) + 33.5 + 2.9] = 28.2%.

The reason for going to the trouble of calculating this fraction is because it is a relatively large portion of the alfalfa plant that we know relatively little about. Alfalfa has been reported to contain 10-14% pectin (Hatfield and Weimer, 1995; Jung et al., 2001), which means the remainder of the 28.2% is WSC. Pectin is rapidly degraded by rumen microbes producing acetate and propionate, but not lactate like rapidly fermented starch (Hatfield and Weimer, 1995). It can be assumed that the WSC fraction also has a high rate of ruminal digestion. Therefore, the criticism that alfalfa lacks a rapidly, ruminal digestible carbohydrate fraction like that found in corn silage, is unfounded (28.2% [pectin + WSC] + 2.9% starch = 31.1% rapidly digestible carbohydrates which will rival the starch content of an average corn silage).

IMPORTANCE OF LEAVES

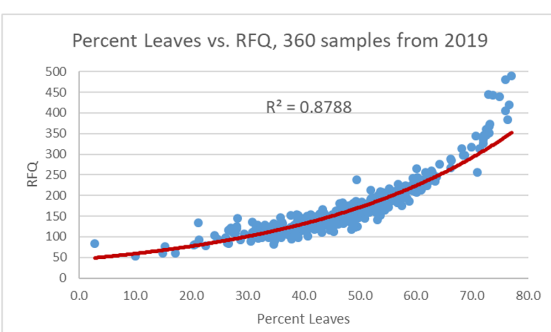
Work conducted in the Forage Genetics International Digestibility Lab, (internal data, 2021), separating alfalfa leaves from stems, demonstrated the nutritional differences between the two fractions (Table 2). As observed, leaves contain a higher concentration of protein and minerals, and less of NDF than stems. Moreover, the average Relative Forage Quality (RFQ) value, a measure of alfalfa quality, confirms most of the nutritional value of alfalfa is contained in the leaves (RFQ = 442.3 vs. 84.3 in leaves vs. stems, respectively). A study in the same lab with a different set of 200 alfalfa samples showed that every 1% improvement in leaf retention garnered a 4.6 percentage unit improvement in RFQ (Figure 11; Weakley and Rodger, 2021). This relationship became even more rewarding the greater the leaf retention. These findings emphasize the importance of retaining leaves during the growing and harvesting phases to best capture the nutritional benefits of alfalfa, as well as improve harvested yield.

Nutritional Analysis of Leaves and Stems

	CP, %DM	Ash, % of DM	NDF, %DM	NDFd, %NDF	RFQ	RFV
LEAVES						
Average	29.1	11.2	19.7	60.3	442.3	367.3
Std. Dev.	2.2	0.7	1.4	4.0	36.3	29.5
STEMS						
Average	11.8	7.4	60.5	39.4	84.3	78.9
Std. Dev.	1.0	0.8	2.5	3.5	10.4	5.8

Table 2. Nutrient profile of leaves vs. stems from 36 alfalfa samples collected from WI. CP = crude protein, NDF = neutral detergent fiber, NDFd = NDF digestibility, RFQ = Relative Forage Quality, RFV = Relative Feed Value. (Forage Genetics International internal data, 2021)

Leaves influence RFQ in a curvilinear way



% leaves	RFQ
40	132
45	150
50	172
55	196
60	224

I percentage unit leaves = 4.6 units of RFQ

Figure 11. Relationship between percent leaves and RFQ (Relative Forage Quality) from 200 alfalfa samples collected from WI, ID, and CA. (Weakley and Rogers, 2021)

CONCLUSION

While there are many factors contributing to alfalfa's nutritional value in diets, it's apparent that NDF, NDFd, RUP, RDP, and ash are important nutrient components contributing to its feeding value for ruminants. The content of NDF, and its digestibility, can have a major impact on intake, digestibility, and feed efficiency through their contribution to the RuNDF content of the diet. The amount of RUP and RDP will contribute to the metabolizable protein content of the diet both directly and indirectly, through supporting ruminal microbial protein synthesis. Knowing the proportions of RUP and RDP in the CP of alfalfa could help optimize the correct dietary balance to maximize the metabolizable protein supply to the ruminant at the greatest efficiency of CP use. Lastly, it is important to monitor ash, as levels above average amounts are likely to be of soil origin and detrimental to the overall nutrient and energy content of the alfalfa forage.

While NDF, NDFd, CP and ash are components of the RFQ quality index calculation (Moore and Undersander, 2002), the calculated value is insensitive to changes in CP, which is a concern. An improvement to RFQ (or a new quality index) could be the addition of coefficients for the concentrations of RUP and RDP in alfalfa samples.

An important aspect of optimizing the above analytical nutritional constituents is to preserve leaves in the alfalfa crop during growth, through to the point of feeding. Lastly, additional study on the large fraction of pectin + WSC in alfalfa may identify benefits for ruminant feeding beyond that as an energy source in the rumen.

REFERENCES

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (Apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379. Agricultural Research Service, USDA, Washington, DC.

Grant, R. J., S. Y. Morrison, and L. E. Chase. 2022. Varying Proportions of Alfalfa and Corn Silage for Lactating Dairy Cows. In: Proc. Cornell Nutr. Conf. for Feed Manufacturers, East Syracuse, NY.

Hall, M. B. 2015. What do today's forage analyses tell us? In: Proceedings of the Western Dairy Management Conference, Reno, NV.

Hall, M. B. 2017. Nitrogen source and concentration affect utilization of glucose by mixed ruminal microbes in vitro. J. Dairy Sci. 100:2739-2750.

Hatfield, R. D., and P. J. Weimer. 1995. Degradation characteristics of isolated and in situ cell wall lucerne pectic polysaccharides by mixed ruminal microbes. J. Sci. Food Agric. 69:185-196.

Howarth, R. E., S. K. Sarkar, A. C. Fesser, and G. W. Schnarr. 1977. Some properties of soluble proteins from alfalfa (Medicago sativa) herbage and their possible relation to ruminant bloat. J. Agric. Food Chem., 25:175–179.

Jung, H. G., J. G. Linn, J. F. S. Lamb, D. A. Samac, and D. A. Somers. 2001. Improving alfalfa fiber digestibility. In: Proceedings of the Four State Dairy Nutrition and Management Conference.

Mertens, D. R. 2015. Underlying fiber concepts and definitions. In: Proc. Cornell Nutr. Conf. for Feed Manufacturers, East Syracuse, NY.

Moore, J. E., and D. J. Undersander. 2002. Relative Forage Quality: An alternative to relative feed value and quality index. p. 16-31 In: Proc. Florida Ruminant Nutrition Symposium, January 10-11, University of Florida, Gainesville.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. Eighth Revised Edition. National Academies Press, Washington, DC.

Robinson, P. H. 2014. Are there unique features of alfalfa hay in a dairy ration? In: Proceedings, California Alfalfa, Forage, and Grain Symposium, Long Beach, CA.

Ross, D. A., M. Gutierrez-Botero, and M. E. Van Amburgh. 2013. Development of an in vitro intestinal digestibility assay for ruminant feeds. In: Proc. Cornell Nutr. Conf. for Feed Manufacturers, East Syracuse, NY.

Weakley, David. 2015. Method of calculating a feed ration for a ruminant. United States Patent 8,949,035.

Weakley, D. C., and C. Rodgers. 2021. Let LEAF be your guide to alfalfa leaves. Hay and Forage Grower, August/September, pg. 10-11.