

## PROGRESS IN DEVELOPING ALFALFA WITH IMPROVED NUTRITIONAL VALUE

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Historically, major efforts in alfalfa and other forage crops breeding programs largely have been directed toward improvement in forage yield, either directly or indirectly, through such components of yield as disease and insect resistance, response to photoperiod, to temperatures, etc. Until recently, approaches to alfalfa improvement in respect to nutritive values largely have been limited to the indirect effects from better leaf retention resulting from greater resistance to foliar diseases and to greater leafiness (or finer stems). Perhaps one of the greatest deterrents to greater emphasis on improved nutritive qualities has been the well-established fact that final hay quality can be greatly altered by management practices, including stage of maturity at harvest, curing and handling operations and perhaps other factors related to its production. And perhaps of greater importance, a major deterrent has been the difficulty of accurately measuring differences that might exist among individual plants in heterogeneous populations and in relating such differences as may be found to animal gains. The advent of improved methods for hay handling has eliminated, at least in part, many of the mechanical factors that can adversely affect quality. With the greater diversity in alfalfa utilization for both monogastric and ruminant animal feeding (including also proteins for human consumption) there is need to more critically examine the potentials that exist in breeding for improvement in nutritional quality.

In broad terms, it generally is recognized that an inverse relationship exists between yield and quality. The number, length and structure of stems contribute largely to yield of dry matter whereas leaves contribute largely to protein content and nitrogen free extract. High yields often are associated with more mature forage, which is high in fiber and lignin and low in protein. Conversely, low yields are often associated with immature forage, low in fiber and lignin and high in protein.

But irrespective of the relationships which may exist between forage yields and quality, there also are important differences among genotypes in respect to both positive and negative attributes of forage quality. It is the objective of this presentation to summarize some of these quality factors as reported in the literature and to supplement with data from our own research on alfalfa.

### Positive Factors For Quality

During the past decade a series of new techniques have been developed which make it possible to evaluate the digestibility of single plants in alfalfa. Success in plant improvement largely is dependent upon procedures which permit the examination of large populations of individual plants (or their progenies) for those characters for which positive gains are sought. Consequently, these new techniques have opened up entirely new opportunities in breeding for quality factors which heretofore were impossible to attain by direct animal feeding trials. The in vitro rumen techniques developed by Tilley and Terry and the modifications of this generalized procedure for addition of rumen extracts to forage samples under controlled laboratory conditions represents a major break-through in testing single plants for digestibility. The detergent techniques developed by Van Soest for measuring the fiber, lignin and hemicellulose components of cell walls has made it possible to utilize chemical, rather than microbiological assays, for measuring those fractions of plant materials that normally are not readily digested by ruminant animals. Research by Elliott and his group of graduate students at Michigan State University has shown that a highly significant correlation exists between in vitro digestibility values as determined by rumen extracts and by cell wall components as measured by detergent fiber analysis. But perhaps of greater importance to alfalfa breeding for improved nutritive value was the proof by Elliott that positive nutritive values were heritable characters and that population means could be changed by cycles of recurrent selection for high and low digestibility values, respectively.

Morphological relationships between digestibility values, stem structure, leafiness, height and yield of dry matter also have been measured by Elliott. Generally, alfalfa plants higher in digestibility also are higher in percent leaves, lower in cell wall quantity in the stems, shorter in plant height and usually (as a consequence of the above plant structures) are lower in yield. It is encouraging that plants have been found in

which there is no relationship between forage yield and digestibility

In summary, significant gains in digestibility in alfalfa can be achieved by breeding for higher leaf percentages and by "redesigning" stem structures, particularly in respect to a lower proportion of lignified xylem cells. Any gains made in breeding for resistance to those diseases that cause pre-harvest leaf drop to those insects that consume leaf tissue or prevent normal leaf development obviously can result in improved digestibility values.

Among other positive factors for improved nutritional values, current results would suggest that improvement in protein content may be difficult to attain. Studies by Heinrichs and others in Canada have shown that protein content may vary widely among individual plants in alfalfa populations, even when harvested at similar time schedules following a previous cutting. It also has been shown that rates of recovery leading to differences in "physiological age" as well as differences in leaf percentages may have marked effects on protein content. The major difficulty in breeding for higher protein content per se is to eliminate the secondary effects that tend to obscure true genotypic differences.

Finally, one of the potentially important positive nutritional factors that may be changed by breeding are the carotenoid pigment complexes, particularly carotene, because of its relationship to Vitamin A. Because carotene can be rather accurately determined spectrophotometrically, it has been investigated more thoroughly than other chemical constituents directly related to vitamins. Several investigators have shown that wide differences occur among individual plants in many forage species. Carotene content has been shown to be positively correlated with protein content and with chlorophyll content. Selection of plants with darker green color generally results in gains in carotene content but such visual selection cannot be used alone as a substitute for actual analyses. Carotene content is higher in young tissue than in old tissue and is higher in leaf than in green stems. Carotene content may vary among harvests during the year.

Because losses in carotene during hay curing usually are large (approximately 50% in the first day) efforts to breed for higher values are of special significance to the dehy industry. Although the amount of data on differences of xanthophyll content among individual forage plants is very limited, it is not unreasonable to assume that such genetic differences do exist. There appears to be need for more rapid and accurate procedures for measuring xanthophyll and related pigments that affect yolk and skin color in chickens before research on improvement in content of these pigment factors can be attained.

#### Negative Nutritional Factors

Although alfalfa has the highest feeding value of commonly grown hay crops, it unfortunately also contains several non-nutritive compounds. Many of these non-nutritive factors have been identified and studied and several, singly and collectively, often inhibit animal growth and thus become classified as "antimetabolites". Some of these compounds may be especially harmful in diets fed to monogastric animals. The antimetabolites in alfalfa which have been studied chemically include the enzymatic and respiratory inhibitors, antivitamin, protein fractions, saponins and hormone-like compounds. Only two of these substances will be covered in this presentation, namely saponins and the protein fraction designated as 18S.

#### Saponins

The saponin complex in alfalfa has been shown to impart physical and chemical characteristics of major importance to many biological systems. From an extensive study conducted by the USDA through the Western Regional Research Laboratory and several agricultural experiment stations from the Eastern to the Western Regions it was shown that saponin content may approach values as high as 3% and that significant differences occurred among varieties. Differences in saponin content have been shown from studies by Dr. Pedersen and co-workers at Utah State to be related to weight gains in chicks and in egg production. In the chick tests the correlation between weight gain and saponin concentration of alfalfa meal was  $-0.892$ . A highly significant negative correlation also was obtained between saponin content and egg production. For each 0.15% increase in saponin content of the diet, egg production was reduced by 6.4%. Research by Elliott and co-workers at Michigan State have shown that weanling meadow voles fed high saponin

diets fail to gain in weight and at higher levels fail to survive. There can be little question that the presence of high levels of saponin in alfalfa may be detrimental, perhaps even implicated in ruminant bloat.

Three separate bioassay procedures have been utilized for estimating differences in saponin content, namely the lysing test (hemolytic values) at Michigan State University, the *Trichoderma* test at Utah State University and the *Gambusia* fish test in our own research laboratory. Relationships between saponin values determined by the lysing and *Gambusia* tests have been excellent.

Results for selecting plants varying in saponin levels at Michigan, Utah and in our program have been in close agreement. The range in variability among plants in several alfalfa populations indicate that the frequency of low saponin genotypes is very small in winter hardy and intermediate winter hardy sources of germ plasm and more favorable from non hardy sources. Data shown below are from analyses in our laboratory of a portion of those plants also included in a study to measure differences in stable foam characteristics.

Hardiness Group	Number of Plants in Saponin Levels						Total
	Very High	High	Medium	Medium Low	Low	Very Low	
Non Hardy	84	30	26	14	9	9	172
Hardy	112	1	1	0	0	0	114

These data show the initial populations are skewed toward high saponin. Recurrent selection populations are skewed in the direction of selection but even after four cycles of selection for low saponin at Michigan State, the population consisted of some plants very high in saponin content. In our studies, mean values of plants within synthetic varieties among low saponin clones generally have exceeded the mean of the parental clones, suggesting a complex genetic system governing the expression of saponin content. But results to date strongly suggest that varieties can be produced with much lower saponin contents than those currently available. Such progress may have special significance in the nutrition of monogastric animals.

Data from our studies to date show no adverse relationships between low saponin content and resistance to spotted aphids, pea aphids, alfalfa weevil, thrips, leaf spot diseases and *Phytophthora* root rot. Consequently, this attribute (low saponin) can be incorporated into breeding materials without endangering progress in breeding for multiple pest resistance.

Relationship Between Saponin Content and Other Attributes in a Population Varying Widely in these Characters  
Mean Values

Saponin Level	No. of Plants	Weevil (1	Thrip (1	Diseases (1	Cc Foam
Very high-High	37	2.0	4.0	2.0	238
Medium	18	2.1	4.0	2.4	228
Medium Low-Low	26	2.4	4.2	1.9	218

1) Score of 0 = none, 5 = high level damage

### 18S Protein

A protein fraction designated as Fraction I (18S) Protein was first reported in 1964 by McArthur and Miltimore at the Canada Department of Agriculture Research Stations at Summerland and Pratt at Vancouver, British Columbia. From this and later research reported in 1969, these authors have shown that this fraction is the protein foaming agent in alfalfa leaves responsible for foam formation in the rumen leading to pasture bloat. It has been shown that bloat-causing legumes contain from 3.4 to 6.8% Fraction I protein compared with a trace to 1.4% Fraction I in non-bloating legumes. It also was shown that the threshold value for non-bloating was 1.8% Fraction I protein.

Extensive studies are now underway in Canada to search for alfalfa clones low in Fraction I protein as the first approach in breeding for bloat resistance. Should these

studies prove successful, a major contribution would be made for expanded uses for alfalfa in pasture programs.

Studies on the foaming properties of alfalfa and other legumes first reported by Kendall in 1964 suggest that those legumes known to be responsible for pasture bloat have higher stable foams from in vitro tests than those which do not cause bloat. Research at South Dakota by Dr. Rumbaugh showed that differences exist among alfalfa clones and that the heritability of foam characteristics was quite high. These results suggest a rapid screening procedure could be devised for measuring bloat-inducing tendencies in alfalfa.

On the basis of studies at South Dakota, we initiated in 1971 a broad survey among the full range of winter hardiness breeding materials to measure possible differences in stable foam properties. Nearly 600 clones were analyzed, using the modified procedure developed by Rumbaugh. Although a broad range from a low of 40cc to a high of 350cc of foam were obtained, the population was strongly skewed toward high foam levels. We plan to continue this survey by including additional populations. As in our saponin studies, recurrent selection will be initiated utilizing the lower portion of the distribution as parental material.

Of special interest in our studies was the possibility of obtaining recombinations of low saponin and low stable foam properties. From the results in one population in which saponin content varied from very high (Gambusia death time 20-40 minutes) to very low or none (Gambusia death time over 2000 minutes) there was a slight trend for an association between lower saponin and lower stable foam. Much more data with other populations will be needed before conclusive statements are possible.

In summary, the negative factors for nutritive value of alfalfa may be important and surely cannot be overlooked in breeding programs. Progress made in breeding for lower saponin levels amply illustrate the wide range in genotypes found within a single species and also emphasize the importance of developing analytical procedures that permit evaluation of large numbers of individual plants.

#### GENERAL SUMMARY

Patterns of plant improvement in nearly all crop plants eventually must encompass the needs of the producer, as well as that of the consumer. As many alfalfa improvement programs come closer to solving problems relating to higher yields through higher levels of disease and insect resistance and better adaptation to edaphic and climatic conditions, perhaps the next challenge will be directed toward meeting the nutritional needs of the animals consuming alfalfa in its many forms. With progress now made in evaluating potential nutritive values by in vitro procedures, this goal can be achieved.