Pectic Substances in Forages and Their Relationship to Bloat

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Factors affecting the levels of pectic substances in forages and the relationship of these polysaccharides to bloat in cattle were studied. The total pectin content varied from 1.5% in bromegrass to 7.9% in Ladino clover, and usually was about 50% water-soluble. In birdsfoot trefoil, red clover, and five varieties of alfalfa, the total pectin fluctuated from 4.7 to 6.7% during the growth period prior to first cutting. In Ranger alfalfa not subjected to grazing, the percentage of total pectin tended to increase during each growth period and also during successive growths throughout the season. The water-soluble pectin was highest in the top portion of the alfalfa herbage, whereas the residual fraction was essentially constant throughout the herbage. Levels of total pectin were somewhat higher during the day than by night. The correlation coefficients between the water-soluble and total pectins and the bloat potentials of alfalfa samples were not significant statistically.

It has been demonstrated in recent years that the pectic substances may comprise as much as 8% of the dry matter of some forages (*2*, *9*). This group of polysaccharides may, therefore, represent one of the main carbohydrates ingested by ruminants. The pectic substances take on added importance as a result of the work of Conrad *et al.* (*2*) and Head (*4*), who found that these substances may be involved in the bloat syndrome in ruminants. The high viscosity of pectin solutions suggests that they have a possible role in the formation of stable foam during bloat. This hypothesis is supported by the observation of Head (*4*) that the constituent responsible for the foam in a rumen fluid sample consisted of the sugars normally found in the pectin and the hemicellulose fractions of the plants. Moreover, Conrad *et al.* (*2*) have postulated that rapid gas formation resulting from the metabolism of pectin by rumen microorganisms is an important factor in bloat.

This investigation was undertaken to study the factors affecting the level of the pectic substances in forages and the relationship of these polysaccharides to bloat in cattle. The pectic substances studied were the water-soluble fraction (highly esterified pectin of the middle lamella) and the water-insoluble fraction (pectin of the cell wall esterified to a lesser degree) (*1*, *6*).

Materials

Plots of forage crops at the Iowa State University Agronomy Farm were the source of most of the samples used in this study. A 75- × 168-foot plot of Ranger alfalfa in its fourth year of production was used to study the variation of pectic substances through three growth periods during the summer of 1960 and the diurnal variation of these substances during a 36-hour period in early July of the same year. In addition, five varieties of alfalfa (Alfa, Culver, Ranger, Vernal, and Buffalo) in their third year of production were sampled during the growth period prior to first cutting. Each variety was replicated three times in $3^1/_2$- × 20-foot plots. Samples of birdsfoot trefoil, red clover, Ladino clover, and bromegrass also were obtained from small plots during the growth period prior to first cutting. Several additional plots of Ranger alfalfa were sampled throughout the summer at the time of actual grazing by animals under observation for bloat. Bloat scores were from data on 15 animals; incidence and severity were evaluated by the bloat rating scale proposed by Johnson *et al.* (*8*).

Except for one case, when whole herbage was studied, samples consisted of 4-inch tops and were obtained by uniform, random, manual sampling of entire plots. An aliquot of each well-mixed sample was placed in a weighed bottle for dry-weight determination, and the balance was transferred immediately to a plastic bag and frozen in crushed dry ice. Frozen samples were stored at −20° C. until analyzed. In studies concerned with variation in pectic substances as affected by stage of growth, samples generally were obtained at weekly intervals and always at 11:00 A.M.

Method of Analysis

A modification of the method of Rouse and Atkins (*11*) for pectic substances in citrus juices was adapted for the analysis of these components in forages. A 15-gram aliquot of each frozen forage sample was treated with 150 ml. of boiling 95% ethanol to inactivate the enzymes. After cooling, the sample was macerated in the ethanol for 5 minutes with a Waring Blendor and filtered. The residue was extracted three times with 100-ml. portions of 60% ethanol by heating to 70° C. These preliminary extractions with ethanol served to remove about 25% of the dry matter and resulted in white residues, free from most substances which interfere in the colorimetric method for uronic acids. The residue then was extracted three times with 150-ml. portions of water at 70° C. for a total of 20 hours. The filtrates were combined in a 500-ml. volumetric flask. The water-insoluble residue was extracted with 0.75% aqueous ammonium oxalate under the same conditions as those employed for the water extractions. These two solutions contained the water-soluble and water-insoluble pectic substances, respectively, and the sum of these two fractions represented the total pectic substances.

The solutions were analyzed by the colorimetric method for uronic acids employing carbazole (*3*) as outlined by Rouse and Atkins (*11*). The pectic substances are reported as anhydrogalacturonic acid (AGA).

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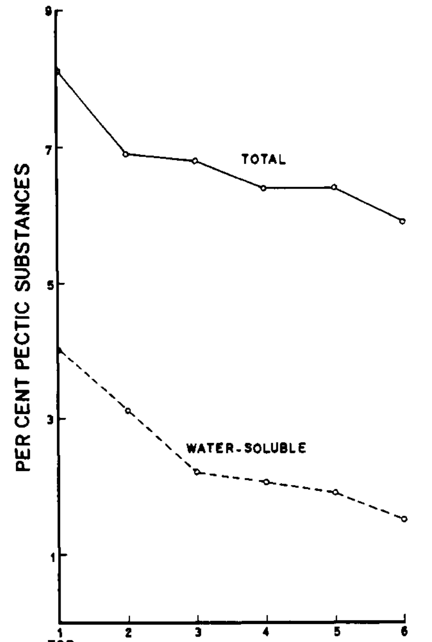

Figure 1. Variation of pectic substances in the herbage of alfalfa

Table I. Pectic Substances in Five Varieties of Alfalfa (4-Inch Tops)

Variety	Date of Sampling						
	April 27	May 4	May 11	May 23	May 31	June 6	June 14
	% Water-Soluble Pectin, Dry-Weight Basis						
Alfa	2.9	1.2	2.5	1.7	2.4	3.2	2.4
Culver	3.2	1.8	2.5	2.6	2.6	...	2.7
Ranger	3.0	1.6	2.5	2.3	2.4	2.7	2.8
Vernal	2.4	1.6	3.3	2.4	2.1	2.5	2.9
Buffalo	2.8	1.1	2.5	1.7	3.4	2.4	2.1
Av.	2.9	1.5	2.7	2.1	2.6	2.7	2.6
	% Total Pectin, Dry-Weight Basis						
Alfa	4.4	5.1	5.8	4.8	5.4	7.0	6.0
Culver	4.9	5.6	6.0	6.1	6.5	...	6.7
Ranger	4.8	5.7	5.2	6.3	5.8	7.2	6.8
Vernal	4.5	6.1	6.6	5.9	5.9	6.2	6.3
Buffalo	4.7	4.2	6.0	5.3	6.4	6.3	5.8
Av.	4.7	5.3	5.9	5.7	6.0	6.7	6.3

Results and Discussion

Variation in the Herbage of Alfalfa. Samples from a 2-foot stand of Ranger alfalfa near the bloom stage were used to study the variation of pectic substances in the whole herbage. The corresponding 4-inch sections of several plants were combined and analyzed (Figure 1). The percent water-soluble pectin was highest in the first (top) section, decreased markedly in the second and third sections, and thereafter decreased more slowly. The value for the bottom section was less than half of that for the top section. In contrast, the water-insoluble fraction remained nearly constant in all portions. The total pectin decreased gradually with a difference of 2.2% between the top and bottom sections primarily due to changes in the water-soluble fraction.

Variation among Forages. The pectic substance contents in Ranger alfalfa, birdsfoot trefoil, red clover, Ladino clover, and bromegrass during the growth period prior to first cutting are presented in Figure 2. Bromegrass was the lowest in both water-soluble and total pectins, with one half to one third the values for alfalfa. The water-soluble fraction in birdsfoot trefoil generally was higher than that in alfalfa. Of all the forages studied, Ladino clover contained the highest level of total pectin. The differences among the legumes, however, were small, especially for the total pectin. The total pectin in the legumes tended to increase until the bloom stage. Little change in either water-soluble or total pectin in bromegrass was noted during the growth period.

Variation among Several Varieties of Alfalfa. The levels of pectic substances in five varieties of alfalfa during the growth period prior to first cutting are given in Table I. Deviations from the average values for both the water-soluble and total pectins usually were small for all varieties. No variety was consistently higher or lower than the average of all the varieties. The sharp decline in water-soluble pectin values on May 4 (Figure 2 and Table I) appears to be related to a sharp retardation in growth due to cold, wet weather. However, there was no concomitant decline in the level of total pectin.

Variation with Stage of Growth. For most of the forages, the general pattern of variation of the total pectin on a dry-weight basis consisted of an increase at the bloom stage followed by a small decrease. For Ranger alfalfa, which was studied through three growth periods (Figure 3), the increase at the bloom stage was sharp. The trends in all three growth periods were similar. The same general pattern was observed for the pectic substances in the five varieties of alfalfa (Table I). The total pectin in Ladino clover, birdsfoot trefoil, and red clover varied similarly (Figure 2). Bromegrass was characterized by only slight variations in both water-soluble and total pectins.

The water-soluble pectin of the other forages (except Ladino clover) followed the same general pattern as the total pectin, but with more variability. For example, fairly sharp increases in the water-soluble fractions during the first and second growth periods of the Ranger alfalfa corresponded to the increases in total pectin, but such increases essentially were absent during the third growth period. Similarly, the average percentages of the water-soluble pectin in the five varieties of alfalfa (Table I) were rather uniform, except for the second and fourth samples. Small increases were observed for birdsfoot trefoil and red clover, but in Ladino clover a gradual decrease occurred after the second sample (Figure 2).

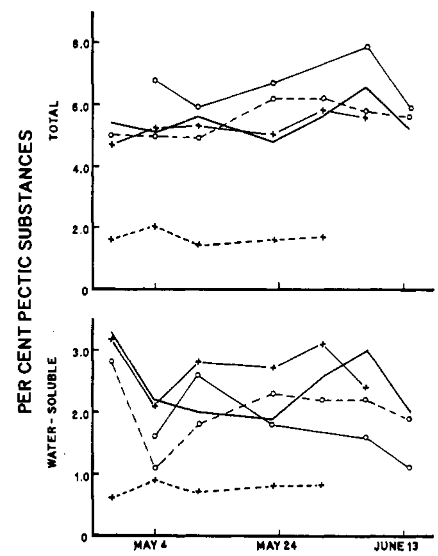

Figure 2. Variation of pectic substances in Ladino clover, Ranger alfalfa, birdsfoot trefoil, red clover, and bromegrass during one growth period

0—0 Ladino clover; X—X Birdsfoot trefoil; —Ranger alfalfa; 0···0 Red clover; X···X Bromegrass

The pattern of variation of the pectic substances (both water-soluble and total) on a fresh-weight basis was found to be quite different from that on a dry-weight basis. On a fresh-weight basis, the pectin content increased gradually as the plants approached maturity, a change which very likely was more pronounced as a result of decreasing water content of the plants with maturation. For example, the increase between the first and final samples during the third growth period of the Ranger alfalfa was only 17% on the dry-weight basis, but 78% on the fresh-weight basis.

The increase in pectic substances with

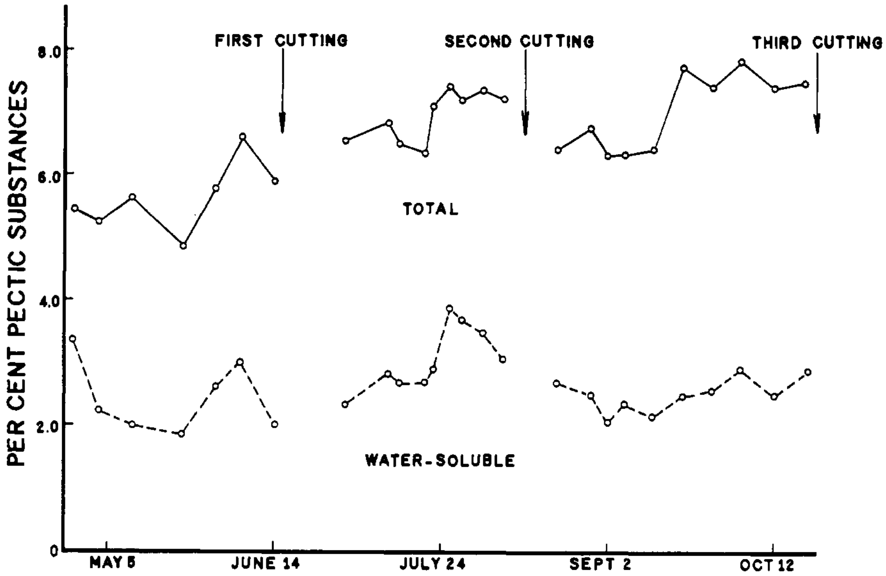

Figure 3. Variation of pectic substances in Ranger alfalfa during three growth periods of one summer

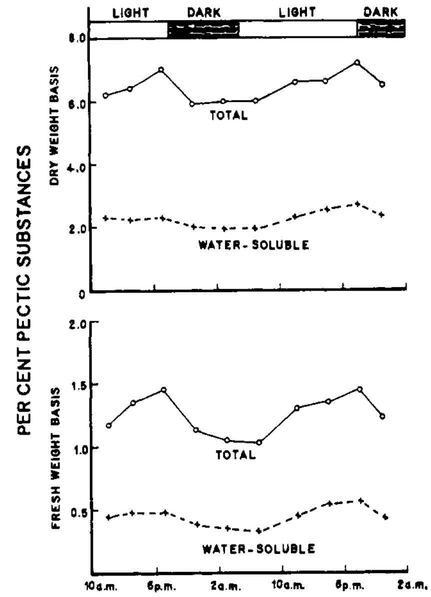

Figure 4. Diurnal variation of pectic substances in Ranger alfalfa

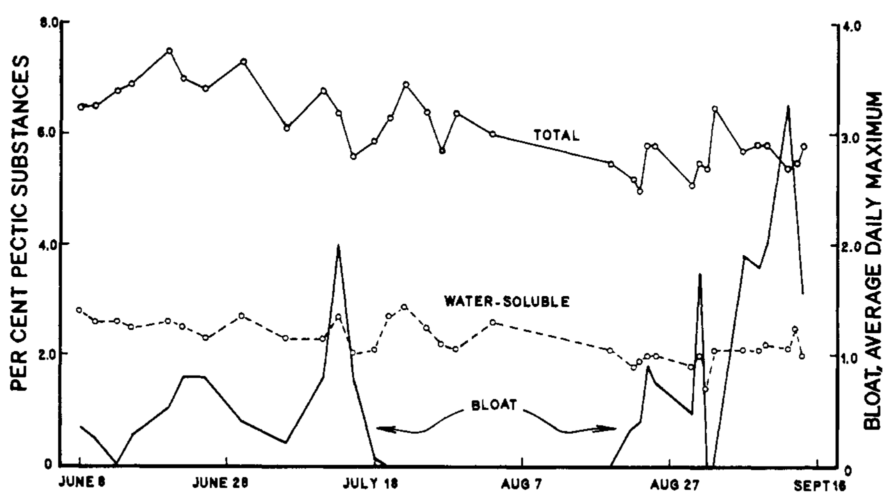

Figure 5. Relationship of pectic substances to the bloat potential of alfalfa

maturation in legumes follows the trend observed in grasses by Waite and Garrod (*12*). These workers investigated five grasses at four stages of growth and observed that the uronic acids, which constitute the pectic substances and are part of the hemicelluloses, increased appreciably with the age of the plant. The limited observations on bromegrass reported herein, however, indicated relatively little increase as growth progressed.

The sharp increase in pectic substances at the bloom stage might be explained in several ways. Depletion of other constituents without change in the pectic substances would result in a higher percentage of the latter. For example, it is known that, in grasses, flower production has a profound effect on the composition of the plant, causing depletion of the reserve polysaccharide fructosan (*10*). Another explanation for the increase may be that pectic substances are rapidly synthesized at the bloom stage. The generally observed decrease in pectic substances following bloom may be due to a constant pectic substance content associated with increasing levels of constituents like cellulose (*5*).

Seasonal Variation. The total pectic substances in 4-inch tops of Ranger alfalfa which was not subjected to grazing increased gradually during the season. The lowest and highest percentages on a dry-weight basis during the first, second, and third growth periods were 4.8 and 6.6, 6.3 and 7.4, and 6.3 and 7.8, respectively (Figure 3). In contrast, the water-soluble pectin, although fluctuating somewhat (particularly during the first and second growth periods), did not show a similar trend during the periods of study.

Diurnal Variations. Samples of 4-inch tops from an 8-inch stand of Ranger alfalfa were obtained at intervals of about 4 hours on July 1 and 2 to study diurnal changes in the content of pectic substances (Figure 4). Both the water-soluble and total pectins increased during the day and decreased during the night, but the changes were small. These variations were accentuated when the percentages were calculated on a fresh-weight basis, the moisture content being maximum early in the morning and minimum in late afternoon. For example, the total pectin on a dry-weight basis varied 22% compared with 38% on a fresh-weight basis.

Relationship of Pectic Substances to Bloat Potential of Alfalfa. The levels of pectic substances in samples from plots being grazed by experimental animals are compared with the incidence of bloat in Figure 5. Two periods of high bloat scores occurred during the season. The fluctuations of pectic substances were much smaller, however, and generally did not coincide with the fluctuations of the bloat scores. Correlation coefficients between the water-soluble and total pectins and the bloat-potential of alfalfa were −0.052 and −0.196, respectively, and were not significant statistically.

In this grazing experiment, the highest bloat scores occurred late in the season, at which time the pectic substances were low. The gradual decline as the season progressed is not in accord with seasonal trends reported earlier in this paper (Figure 3). This may be due, however, to the marked difference in harvesting procedure—i.e., frequent grazing and clipping *vs.* harvesting only three times during the summer.

Forages at all stages of growth may produce bloat (*7*), but cattle are more prone to bloat on young, succulent legumes than on mature legumes or on grasses at any stage of growth. However, the highest percentage of pectic substances occurred at and after the bloom stage. The low pectic substances in bromegrass are in accord with its low bloat potential, but birdsfoot trefoil, which is not a bloat-producing forage, was found to be similar to alfalfa in its content of pectic substances.

The results of this investigation fail to show a direct relationship between level of pectic substances and occurrence of bloat in cattle. There is still the possibility, however, that these polysaccharides may be involved, in conjunction with other animal and microbial factors, in the etiology of bloat. Under certain conditions, pectins are capable of serving as foam-stabilizing agents and, in this way, may be related to bloat. Since the level of these substances does not seem to be the important factor, perhaps the rate of their release during the early phases of rumen fermentation of bloat-producing legumes is of significance. Additional work is needed to ascertain the nature of the relationship, if any, between the pectic substances and bloat.

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NUTRIENTS IN SEEDS

Amino Acid Composition of Seeds from 200 Angiospermous Plant Species

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Amino acid compositions by ion exchange methods are reported on acid hydrolyzed seed meals from 134 plant species not previously analyzed. The data obtained, with those from 66 species reported earlier, are evaluated and interpreted together here. Lysine, methionine, arginine, proline, hydroxyproline, and glutamic acid showed the greatest variation. Less familiar amino acids and unidentified compounds were detected in many hydrolyzates. Percent of total nitrogen present as amino acids, ammonia, and unidentified nitrogen was estimated. The amino acid composition of seeds shows over-all similarities, but there are definite relationships between plant taxonomic classification and amount of each amino acid present. Detailed comparisons are given among legumes, crucifers, and composites. The results provide information on the distribution of nitrogenous constituents in a large variety of plant seeds.

Discovery and development of sources of nutritionally high-quality vegetable protein are part of an extensive screening program by the Agricultural Research Service of the U. S. Department of Agriculture which is planned to find new crops that can profitably be grown by the farmer (*33*). A literature survey (*28*) on amino acid compositions of various plant seeds showed that relatively few have been analyzed for all the common amino acids and that the nutritionally essential amino acids have been determined on seeds from 89 species of 25 plant families. The availability of rapid and accurate ion exchange, chromatographic methods to determine all the amino acids in an acid hydrolyzate offers an efficient means for such analyses. Because of the lack of published information concerning amino acid composition of plant seeds and because of the availability of suitable methods, such determinations were made a part of this screening program.

The amino acid content of seeds from previously selected species, including those of the Crucifer family, has been reported (*18*, *32*). This paper provides information concerning the amino acid composition of seeds from 134 additional species, as well as an evaluation of data obtained from the entire 200. Amino acid compositions from 14 species of *Lesquerella* of the Crucifer family determined by the same methods (*19*) were not included.

Knowledge of amino acid composition makes possible an estimate of the nutritional quality of a seed protein by comparison with known amino acid requirements (*9*, *21*, *22*). Harper (*13*) indicates that nonessential amino acids may also be important in nutrition; hence their determination provides more information for gaining insight into the role they play in nutrition and in the nutritional evaluation of each seed meal.

In addition to chemical evaluation of seed from each species for nutritional quality, amino acid compositional data from seeds of a large number of different species permit study of possible fundamental relationships. From such data, the range limits of each amino acid,