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The Carbohydrate Composition Of Two Species of Grama Grasses

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The Carbohydrate Composition Of Two Species of Grama Grasses

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One of the problems confronting animal nutritionists is a proper evaluation of the nutritive value of forage that is consumed by farm and range animals. From the chemical standpoint, this evaluation has most often been done on the basis of proximate analyses. At the present time there is some question whether these analyses are adequate to evaluate forages, thus comprehensive carbohydrate analyses are being made to give perhaps a broader picture of the composition of forages.

This bulletin reports a comprehensive carbohydrate analysis of two species of grama grasses at different stages of growth covering a two-year period; and in addition, compares these results with the conventional proximate analyses of the same samples.

The grama grasses studied are important range forages found throughout the southern Great Plains. Sideoats grama (*Bouteloua curtipendula*) is a warm-season perennial of medium growth habit with seed stalks that reach a height of 18 to 36 inches. Blue grama (*Bouteloua gracilis*) also is a warm-season grass; however, it is classed as a short grass having seed stalks 10 to 20 inches in height. Both grasses are well grazed by all classes of livestock and are reported to be highly nutritious at all stages of growth (1). Many observers rate blue grama considerably higher than sideoats in quality.

LITERATURE REVIEW

Many range grasses, including these gramas, have been extensively examined for proximate composition (crude protein, ether extract, crude fiber, nitrogen-free extract and ash) as well as for Ca and P (2, 3, 4, 5, 6). In recent years, researches on grasses have tended to seek a more detailed knowledge of the carbohydrate composition of grasses; however, only limited studies have been made of western range grasses.

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A good review covering the subject of carbohydrate constituents of roughages is found in the bulletin by Hansen *et al.* (10). A recent paper by Dodd and Hopkins (11) reported the percentages of sugars, starch and fructosans found in the roots, rhizomes and crowns of blue grama grass as affected by clipping. At certain stages of growth, they reported as much as 10 percent starch and 13 to 19 percent of fructosans in these organs.

Recent work which formed a background for the studies reported in this bulletin was reported in the *Journal of the Science of Food and Agriculture* (7, 8, 9). These papers treat in great detail the carbohydrate composition of alfalfa, ryegrass, timothy and orchardgrass grown in Great Britain. Detailed analyses such as these are not practical when a large number of samples are to be considered; consequently, less detailed analyses covering groups of compounds rather than single carbohydrates are reported in this bulletin.

SAMPLING TECHNIQUES

Forage samples of the two species were collected bimonthly from a pasture seeded in 1942 on the Southern Plains Experimental Range near Woodward, Oklahoma. The pasture was winter grazed and summer deferred during the years that the forage samples were collected. The soil type is a Pratt loamy fine sand, gently rolling phase. The samples were collected by hand plucking a total of about 200 g. forage (from numerous individual plants). Usually 50 or more plants were sampled to secure the needed amount of material. The hand plucking simulated the grazing habits of cattle on the experimental range. At each sampling date, the collectors studied the current grazing habits of cows on the experimental range and tried to duplicate in the sample the kind of forage being eaten. Admittedly, such a sampling procedure does not precisely emulate the cow, but the resultant sample is more representative of the cow's diet than would be forage obtained by clipping plants with scissors to a predetermined stubble height.

On each sampling date, two collectors secured the forage samples and a composite sample was made for each species. The purpose of this procedure was to minimize individual differences that might exist between collectors. After the samples were plucked, they were placed in plastic bags and transported by car to the laboratory in Woodward where they were immediately weighed, oven-dried in kraft paper bags at 70° C for 24 hours, reweighed, and shipped to the Oklahoma Agricultural Experiment Station at Stillwater. After reaching Stillwater, the samples were dried to constant weight at 105° C and then were ground through the medium mesh screen of a Wiley mill. After grinding, the samples were stored in the dark in air-tight sample bottles until they were needed for analysis.

ANALYTICAL PROCEDURE

MOISTURE: All results are reported on a moisture-free basis;

however, the moisture content of the original samples is reported so that results can be calculated back to field growth if desired. All samples were dried at 105° C until constant weight was reached.

PROXIMATE ANALYSIS values were determined essentially as directed in the A.O.A.C. Methods (12) and include results for moisture, ash, crude protein, ether extract, crude fiber and nitrogen-free extract (N.F.E.). The protein method used was the Kjeldahl-Gunning modification. The detailed carbohydrate methods that follow deal with a breakdown of the proximate analyses values, (crude fiber and nitrogen-free extract).

SUGARS: These values were determined on solutions prepared by extracting samples of the forage with 80 percent ethanol for at least 36 hours. For the actual determinations, aliquots of the ethanol extract were evaporated to near dryness and then taken up in water, after which the samples were clarified with neutral lead acetate and finally deleaded with potassium oxalate. **Reducing sugars** were run on the clarified extracts according to the Shaffer-Somogyi method as described by Heinze and Murneek (13). This method does not differentiate between various reducing sugars such as glucose, fructose, etc. Results in the tables are expressed as percentages of glucose. **Sucrose** percentages were calculated from results secured by running reducing sugars on solution aliquots after they had been hydrolyzed overnight with HCl (sugars after inversion — reducing sugars $\times$ .95). No effort was made to determine if any other oligosaccharides were present other than sucrose, although it is recognized that they would be included in this fraction. **Total sugars** is a value calculated by adding together the reducing sugars and sucrose values. Fructosan results were run on the extracted residues through the use of a relatively specific method adapted from McRary *et al.* (14). Results are calculated as fructose which values do not make any allowance for water added in hydrolysis. Since the values are low, this does not introduce any appreciable error.

MILD ACID HYDROLYSIS values were secured by boiling the extracted residues for 20 minutes with O.2N H_2SO_4, and then determining the reducing value of the cleared hydrolysates and calculating the results as glucose (15). This method has been used for the determination of fructosans; however, tests by Billings (16) have shown that much more than fructosans are included in this value. Most of the total is probably accounted for by the hydrolysis of short chain pentosans to give pentose sugars. Since the proportion of the various sugars is unknown and since the reducing values of pentose sugars approximate that of glucose, the results are calculated as glucose.

STARCH (by diastase) was determined as directed in the A.O.A.C. (12), except Takadiastase was substituted for malt diastase. Results are reported as 0.9, the glucose values. These percentages undoubtedly represent much more than true starch values and include all hot-water soluble polysaccharides. *Starch* (specific method) was determined by a modification of the method described by Pucker *et al.* (17). In this

method the starch was extracted with perchloric acid, precipitated with iodine, and recovered as starch which was then hydrolyzed and the reducing value determined as glucose. This value $\times$ 0.9 gave the starch equivalents. The small amounts of starch recovered by this method indicates that the starch-by-diastase includes much more than true starch as was expected. The great disadvantage of the second method is the length of time required for the determinations.

STRONG ACID HYDROLYSIS values are the results secured by hydrolyzing the samples with HCl according to the A.O.A.C. method for pure starch (12). The results are calculated as glucose $\times$ 0.9, and the results include at least starches, dextrins, fructosans and those hemicellulose fractions extracted by 24 percent KOH. This is a composite value and gives results covering most of the carbohydrates in plants that are not included in the cellulose plus sugar fractions. In these samples, since starch and fructosan values are low, the results measure chiefly the hemicellulose fraction.

CELLULOSE was determined essentially as directed by Patton (18), except the analyses were run on holocellulose samples which seem to give more consistent and probably more nearly absolute values as compared to running the determination on the ground forage directly (19). **HOLOCELLULOSE** was determined essentially as directed by Binger and Sullivan (20). The defatted samples were extracted with ammonium oxalate and then delignified with sodium chlorite. Results reported in the table are corrected for ash, lignin and protein content. This value includes chiefly cellulose and hemicelluloses. **HEMICELLULOSE** values are reported in two columns: One is for the combined values secured by adding together the percentages extracted with 4% and 24% potassium hydroxide essentially as directed in the *Modern Methods of Plant Analysis,* by Paech and Tracey (21). This method is long and costly and was used only one year. The other column percentages are calculated values secured by substracting cellulose percentages, run on holocellulose, from the percentages of holocellulose after they were corrected for impurities. The two values differ somewhat; however, they follow the same general trend, and the second value can be secured from data already run and probably is as true a picture of the actual percentages as is the specific method extraction method mentioned previously.

LIGNIN was determined essentially as proposed by Thacker (22). While lignin is not a carbohydrate, its close association with the nutritive value of plants justifies its inclusion here.

In addition to the fractions mentioned previously, some other analyses are included for comparative purposes and to help complete the study of the nutritive value of the grasses as measured by chemical analyses. **TOTAL NITROGEN** (protein) was determined by the Kjeldahl-Gunning method (11), as was **alcohol-soluble** nitrogen. **Insoluble nitrogen** which measures chiefly the structural forms or proteins was calculated by difference.

SOLUBLE SOLIDS was run on the 80 percent alcohol extract, and is composed of the sugars, some soluble proteins, organic acids and extractives. These components are assumed to be readily mobile and percentages vary greatly with the growth stages of the plant.

RESULTS AND DISCUSSION

The data in the tables and figures will be discussed in relation to the various components, taking into account seasonal changes, species differences and the interrelationships between the various fractions.

Proximate Analysis and Related Values

Table I gives the data for proximate analysis, nitrogen fractions and alcohol-soluble solids. Most of these components do not cover carbohydrate analysis; however, their inclusion makes it possible to compare several of the carbohydrate fractions with long established fractions used in estimating the nutritive value of grasses.

These data, with two exceptions, indicate that there is little difference in the proximate composition of the two species. One exception was in protein nitrogen and the nitrogen fractions, in which blue grama was somewhat higher at all seasons of the year. This was particularly true when the grasses were immature. Associated with this higher nitrogen content was a concomitant reduction in N.F.E. values. While it is doubtful that the higher protein content of the blue grama is nutritionally significant, it is consistent and chemically significant.

The other exception was in the alcohol-soluble solids which showed very wide seasonal fluctuations. Sideoats grama was considerably higher, on the average, in this fraction. Since this fraction contains a variety of substances, most of which were not determined, it is not possible to evaluate nutritionally this fraction. One might postulate that since this fraction is soluble and contains the sugars, sideoats grama with a higher total percentage might be slightly more palatable to cattle grazing this grass.

Marked seasonal differences were noticeable in most fractions with crude fiber and protein values showing the greatest variations. Particularly noticeable was the low protein content of the winter samples as compared to the early spring growth.

Simpler Carbohydrates and Starch

The data presented in Table II again indicate that in most constituents there were only small differences between the two species of grasses. The differences that did exist were in the two fractions that are not well characterized; namely, mild acid hydrolyzable values and starch by diastase. Both of these values were appreciably lower in the sideoats species and thus indicate a lesser amount of the short chain polysaccharides. Very probably these values duplicate each other to a

Table I.—Proximate Analysis and Related Values (Dry Weight Percentages)

Harvest Date	Water	Ether Extract	Crude Fiber	Ash	N.F.E.	Protein	Nitrogen		Soluble Solids
							Soluble	Insoluble	
				Bouteloua curtipendula (Sidcoats grama)					
5/15/57	59.92	2.48	24.29	11.07	49.72	12.44	.43	1.56	15.62
6/20/57	61.37	2.03	29.68	10.27	49.29	8.63	.31	1.11	15.86
9/17/57	42.09	2.23	33.93	10.45	48.95	4.44	.14	0.61	10.54
10/28/57	39.69	2.09	34.57	10.01	47.64	5.69	.20	0.74	10.38
1/16/58	12.53	2.46	31.14	9.40	52.56	4.44	.14	0.57	7.84
4/ 1/58	7.23	2.25	33.14	8.64	51.66	4.31	.12	0.57	5.20
5/27/58	47.21	2.33	28.20	9.52	49.45	10.50	.32	1.36	15.74
6/19/58	51.85	2.36	29.90	9.24	50.12	8.38	.32	1.02	15.20
9/15/58	40.13	1.87	32.20	11.44	50.24	4.25	.14	0.54	11.35
10/20/58	16.86	1.87	33.56	9.64	52.12	2.81	.07	0.38	8.50
1/28/59	8.91	1.96	32.44	9.57	53.58	2.75	.06	0.38	6.23
4/21/59	11.05	1.33	36.41	9.27	50.36	2.63	.05	0.37	4.33
5/18/59	62.58	2.64	26.80	10.53	47.09	12.94	.53	1.54	18.84
Avg.	**35.49**	**2.15**	**31.25**	**9.93**	**50.21**	**6.48**	**.22**	**.83**	**11.20**
				Bouteloua gracilis (Blue grama)					
5/15/57	67.82	3.06	28.60	10.81	41.47	16.06	.92	1.77	15.48
6/20/57	60.15	2.32	33.51	10.90	45.93	7.44	.49	0.79	9.44
9/17/57	40.55	2.38	34.97	9.78	46.68	6.19	.22	0.78	7.58
10/28/57	33.02	2.39	33.64	11.32	47.34	5.31	.16	0.74	7.62
1/16/58	16.02	1.96	32.18	10.83	49.84	5.19	.16	0.67	6.91
4/ 1/58	10.34	2.19	34.30	10.37	48.08	5.06	.13	0.68	4.36
5/27/58	37.77	2.75	28.78	7.74	48.54	12.19	.47	1.48	13.33
6/19/58	46.97	2.63	30.09	6.97	50.68	9.63	.42	1.12	13.79
9/15/58	27.17	1.72	34.40	8.82	50.81	4.25	.13	0.55	8.17
10/20/58	14.72	1.52	33.87	8.49	52.43	3.69	.10	0.49	6.69
1/28/59	9.47	1.40	34.64	6.99	54.22	2.75	.06	0.38	4.89
4/21/59	14.08	1.23	33.14	8.91	53.09	3.63	.07	0.51	4.73
5/18/59	61.31	2.53	29.35	8.63	45.49	14.00	.56	1.68	16.37
Avg.	**33.80**	**2.16**	**32.42**	**9.27**	**48.82**	**7.34**	**.30**	**.90**	**9.18**

Table II.—Simpler, well defined carbohydrate fractions (Dry weight percentages)

Harvest Date	Sugars			Mild Acid Hyd.	Fructosans	Starch	
	Reducing	Sucrose	Total			Diastase	Specific Activity
			Bouteloua curtipendula (Sideoats grama)				
5/15/57	.48	1.73	2.21	4.02	0.22	5.92	0.16
6/20/57	.56	1.80	2.36	3.70	0.22	5.02	0.35
9/17/57	.54	1.43	1.97	4.52	0.19	8.12	0.54
10/28/57	.61	1.51	2.12	4.27	0.17	7.79	0.48
1/16/58	.74	0.53	1.27	4.49	0.14	6.97	0.31
4/ 1/58	.23	0.09	0.32	4.37	0.13	7.12	0.35
5/27/58	.57	1.34	1.91	6.33	0.16	7.41	0.26
6/19/58	.42	2.45	2.87	4.47	0.14	2.61	0.25
9/15/58	.66	1.68	2.34	6.45	0.18	3.36	0.18
10/20/58	.57	0.60	1.17	7.30	0.16	4.91	0.07
1/28/59	.46	0.25	0.71	8.37	0.11	4.49	0.09
4/21/59	.26	0.04	0.30	7.97	0.17	4.55	0.28
5/18/59	.71	1.34	2.05	6.19	0.16	4.49	0.78
Avg.	**.52**	**1.14**	**1.66**	**5.57**	**0.17**	**5.60**	**0.32**
			Bouteloua gracilis (Blue grama)				
5/15/57	0.47	2.78	3.25	3.43	0.18	9.27	0.45
6/20/57	0.36	1.08	1.44	5.03	0.20	10.89	0.20
9/17/57	0.33	0.64	0.97	4.14	0.12	10.40	0.20
10/28/57	0.41	0.85	1.26	5.24	0.17	9.11	0.42
1/16/58	0.73	0.83	1.56	4.08	0.12	5.16	0.39
4/ 1/58	0.17	0.00	0.17	3.70	0.13	6.69	0.33
5/27/58	0.60	0.54	1.14	7.99	0.19	7.34	0.15
6/19/58	0.57	1.55	2.12	5.71	0.11	3.22	0.33
9/15/58	0.51	1.25	1.76	8.46	0.11	3.48	0.07
10/20/58	0.53	0.90	1.43	9.41	0.17	5.12	0.10
1/28/59	0.33	0.14	0.47	8.45	0.15	4.24	0.08
4/21/59	0.30	0.07	0.47	10.34	0.20	5.52	0.08
5/18/59	0.18	3.55	3.73	6.58	0.20	4.76	0.48
Avg.	**.42**	**1.18**	**1.51**	**6.35**	**.16**	**6.55**	**0.27**

considerable extent. Certainly the specific values for fructosans and starch indicate that neither of these fractions make up a very large percentage of these two grasses. The values for fructosans were very low and indicate a minimum content of this fraction in the forages. These results were in great contrast to the relatively large amounts of fructosans reported in the roots, rhizomes, and crowns of blue grama as reported by Dodd and Hopkins (11). Possibly the method used in our tests is more specific for this fraction or perhaps fructosans do not accumulate in the forage. Tests on ten western grasses (unpublished) in our laboratories indicated that rarely if ever was there more than two percent of fructosans present in the stubble.

Starch also is a minor storage fraction in these grasses, and there was no apparent difference between the two species. Differences in sugars also were seemingly not significant. Sucrose is the fraction that showed great seasonal variations, in that it practically disappeared during the winter months. In any circumstance, sugars make up only a very small fraction of the energy portion of these forages.

Other Carbohydrate Fractions and Lignin

These components are those that in the overall make up a large part of the carbohydrate bulk in a forage, Table III. Results for strong acid hydrolysis were quite similar for both species and showed some seasonal fluctuation, with the largest amounts occurring in the winter months. These results are comparable to those for hemicelluloses, which substances undoubtedly make up the bulk of this fraction (see methods page 6). Fluctuations in the hemicellulose values ranged in much the same way as the acid hydrolysis values, although fluctuations on individual dates were greater. Hemicellulose values secured by extraction were appreciably higher than those secured by difference; however, they varied in a manner analogous to the calculated values. It is apparent that from one-quarter to one-third of the dry matter is found in this fraction and this is a fraction about which very little is known. Hemicelluloses should be very well utilized by livestock, in that our samples were completely hydrolyzed by relatively dilute acid in qualitative tests.

Holocellulose values were relatively similar for the two species and showed their highest values during the dormant winter season. The high value during the winter season indicates a high total energy value for these grasses when used for winter grazing. Cellulose values showed a fluctuation from season to season of a maximum of around 40 percent; however, the two species fluctuated in much the same direction, and very little or no final differences were apparent.

Lignin values were variable from season to season and showed a great percentage range from the minimum to maximum value (Figure 1). In no instance were the values excessive as compared to other range grasses (unpublished data). The species difference was considerable in this fraction, with blue grama averaging only about 75 percent as much lignin as sideoats grama. From a feeding stand-

Table III.—Complex and less well characterized carbohydrate fractions and lignin (Dry weight percentages)

Harvest Date	Strong Acid Hydrol.	Holo-Cellulose	Hemicellulose		Cellulose[1]	Lignin
			4 + 24% KOH	By difference[1]		
		Bouteloua curtipendula (Sideoats grama)				
5/15/57	24.06	51.98	22.47	26.27	25.71	5.51
6/20/57	24.60	54.12	25.35	23.38	30.74	4.87
9/17/57	26.68	59.85	28.77	26.65	33.20	6.92
10/28/57	26.78	59.35	26.85	24.26	35.09	7.60
1/16/58	26.73	60.71	26.91	25.75	34.96	7.72
4/ 1/58	27.77	60.20	27.22	24.94	35.26	8.64
5/27/58	26.82	53.14		25.22	27.92	6.95
6/19/58	25.40	55.98		27.51	28.47	7.82
9/15/58	26.91	59.17		28.00	31.17	8.02
10/20/58	28.91	59.06		25.90	33.16	7.41
1/28/59	30.11	47.01		15.54	31.47	6.85
4/21/59	29.08	54.94		19.10	35.84	6.62
5/18/59[2]	23.29					
Avg.	**26.70**	**56.29**	**26.26**	**24.38**	**31.92**	**7.08**
		Bouteloua gracilis (Blue grama)				
5/15/57	23.57	47.15	22.52	20.90	26.25	3.26
6/20/57	30.47	57.53	26.51	27.94	29.59	4.30
9/17/57	28.33	64.76	30.02	32.26	32.50	6.83
10/28/57	30.26	62.94	29.78	31.29	31.65	6.40
1/16/58	28.04	62.70	28.39	32.33	30.37	6.73
4/ 1/58	26.78	61.70	28.08	30.19	31.51	5.90
5/27/58	27.47	54.68		25.52	29.16	6.03
6/19/58	25.82	58.61		28.88	29.73	6.42
9/15/58	27.75	65.60		31.50	34.10	6.40
10/20/58	29.06	50.76		17.17	33.59	5.93
1/28/59	30.29	55.66		20.78	34.88	5.98
4/21/59	29.21	50.36		15.90	34.46	4.58
5/18/59[2]	23.89					
Avg.	**27.76**	**57.70**	**27.55**	**26.22**	**31.48**	**5.73**

[1] Hemicellulose and cellulose run on holocellulose samples.
[2] Holocellulose samples lost.

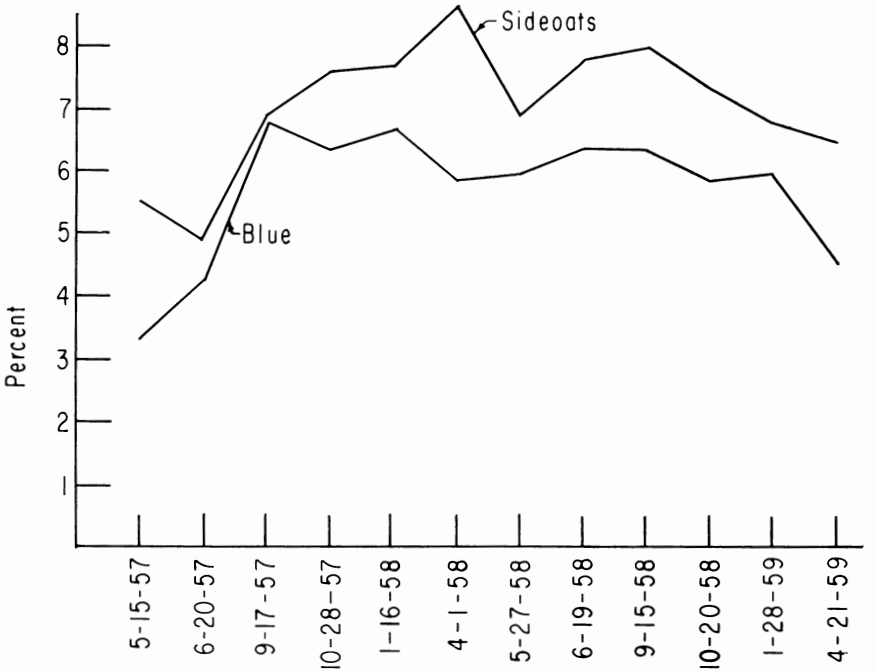

Figure 1. Dry weight percentages of lignin at various harvest dates for sideoats and blue grama.

point this is probably the greatest difference between the two species, since the effect of lignin on the digestibility of forages is widely accepted (23).

A Comparison of Crude Fiber and Cellulose Plus Lignin

Since by definition crude fiber is chiefly lignin plus cellulose, it is interesting to compare the sum of cellulose plus lignin with the crude fiber values secured on these grasses. The results presented in Figure 2 indicate a marked reduction in average crude fiber results as compared to the total of the two main constituents. With the blue grama the fiber results were approximately 15 percent of the total lower, and for the sideoats species, 25 percent. From these results, it is apparent that for these species as well as for many others, crude fiber percentages fall much below the theoretical value. In evaluating the nutritive value of sideoats grama by proximate analysis, this difference of 25 percent could be significant.

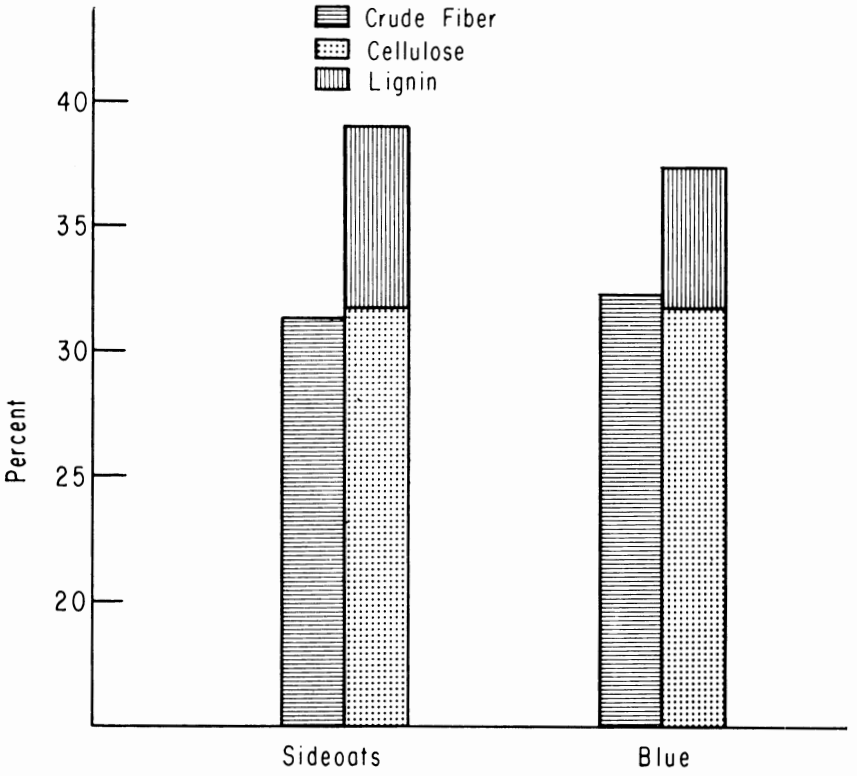

Figure 2. Comparison of crude fiber and cellulose plus lignin averages for sideoats and blue grama.

SUMMARY

1. As might be expected, marked seasonal changes were found in all constituents for which analyses were secured. These changes were similar for the two species.

2. The overall composition of the two species are quite comparable, with the exception of four components wherein there may be significant difference.

3. Blue grama was consistently higher in protein content, although the overall advantage was only about 13 percent of the total.

4. Blue grama usually had a higher content of starch by diastase and mild acid hydrolysis values (they measure much the same fractions); although here the results, sampling by sampling, were not so consistent. The overall difference was 19 and 14 percent, respectively.

5. Sideoats grama consistently showed a higher percentage of soluble solids, and the overall difference exceeded 20 percent. This is a fraction about which little is known, and the difference probably

has little nutritional significance. Since the soluble sugars are similar for the two species, the difference probably is in the organic acid fraction.

6. Probably the greatest nutritional difference is in the lignin content where there was a consistent difference. Blue grama contained only about 76 percent as much lignin as sideoats grama. This should make the blue grama significantly more digestible.

7. The data indicate a very low content of both fructosans and starch when specific methods are used for their determinations. These values are so low that little or no nutritional significance can be attributed to these fractions.

8. Some comparison of methods has been possible from this study. One that is of interest is a comparison of strong acid hydrolyzable values with hemicellulose results. The comparison of extracted hemicellulose (4 + 24% KOH) values with the strong acid hydrolysis values show a very close correlation, and a fair correlation is shown between the acid hydrolysis and calculated values. In this particular study where fructosans and starch were present only in very small amounts, the acid hydrolyzable values are probably a very satisfactory estimate of the total hemicellulose content of the plants, and certainly they are much simpler to determine.

9. It was apparent that crude fiber values do not nearly equal the theoretical crude fiber sum of lignin plus cellulose for these grasses.

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OKLAHOMA'S WEALTH IN AGRICULTURE

Agriculture is Oklahoma's number one industry. It has more capital invested and employs more people than any other industry in the state. Farms and ranches alone represent a capital investment of four billion dollars—three billion in land and buildings, one-half billion in machinery and one-half billion in livestock.

Farm income currently amounts to more than $700,000,000 annually. The value added by manufacture of farm products adds another $130,000,000 annually.

Some 175,000 Oklahomans manage and operate its nearly 100,000 farms and ranches. Another 14,000 workers are required to keep farmers supplied with production items. Approximately 300,000 full-time employees are engaged by the firms that market and process Oklahoma farm products.

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