Distribution of Sulfur In Alfalfa[1]

By A. F. Kingsley[2]

Alfalfa hay is used extensively as an animal feed and is considered one of the most valuable roughages grown in the United States. The proteins of alfalfa serve as an important raw material for the production of meat in the cattle and sheep industry, and for the production of milk in the dairy industry. Ground alfalfa is used as a protein supplement, especially in the feeding of swine and poultry.

Information from the Agricultural Marketing Service, U.S.D.A., shows that during 1950 there were 364,000 acres of alfalfa grown in North Dakota with an average yield of 1.5 tons of hay per acre, making a total production of 546,000 tons. For the period 1950 to 1954 the acreage of alfalfa was increased to 918,000 acres with an average yield of 1.54 tons of hay per acre, and a total production of 1,412,000 tons. In the United States 22,996,000 acres of alfalfa were grown in 1954 with an average yield of 2.1 tons of hay per acre, with a total production of 49,328,000 tons.

In spite of their widespread use, very little is known regarding the nutritional qualities of alfalfa proteins. Although the literature contains adequate information on the quantity of crude protein in different varieties of alfalfa, very little information on the quality of alfalfa protein had been published prior to a survey of the amino acid content of alfalfa made in 1948 (1). In this survey, 12 varieties of alfalfa were analyzed microbiologically, and all found to be low in methionine and cystine content. The other nutritionally important amino acids were present at levels that are presumably satisfactory for good animal nutrition.

The discovery that standard varieties of alfalfa are deficient in the sulfur-containing amino acids prompted the writer's attention to the role of sulfur in the chemical composition of alfalfa. Sulfur occurs in plants as inorganic sulfates and in organic combinations. It is a constituent of many compounds of importance in nutrition and metabolism. These include cystine and methionine, amino acids which are present in most proteins; thiamin and biotin which are vitamins of the B-complex, and possibly other compounds which may affect the general metabolism of the plant. With the exception of cystine and methionine, these compounds are present in minute amounts or are not present at all.

Cystine (or its reduced form cysteine) and methionine, on the other hand, are widespread and carry most of the organic sulfur

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in the proteins of our common food plants. It has been reported (2) that the organic sulfur content makes up more than half of the total sulfur content of prairie hay, sweet clover, corn fodder, and brome grass, and that nearly all of the sulfur found in cereal grains is in organic combinations. Methionine and cystine sulfur make up approximately 20 per cent of the total sulfur in alfalfa (1). Woodman and Evans (3) reported the following values for alfalfa hay:

	Total Sulfur % dry basis	Sulfate Sulfur % dry basis	Organic Sulfur % dry basis
Alfalfa (pre-budding growth)	0.72	0.16	0.56
Alfalfa (in bud)	0.58	0.11	0.47
Alfalfa (in flower)	0.55	0.05	0.50

Thomas and Hendricks (4) reported the following values for alfalfa leaves:

	Total Sulfur	Inorganic Sulfur	Organic Sulfur	Volatile Sulfur	Organic Sulfur-Volatile Sulfur
Alfalfa leaves	0.57	0.218	0.352	0.097	0.255

Volatile sulfur is an estimation of the cystine sulfur that occurs in the plant. If expressed as per cent cystine, then the volatile sulfur, 0.097%, corresponds to 0.40% cystine. Likewise, the remainder of the organic sulfur fraction, (0.255%) can be expressed as per cent methionine, 1.19%.

Converting the organic sulfur content of lucerne (alfalfa) as reported by Evans into cystine and methionine (assuming cystine accounts for one-third of the total organic content and methionine the remainder of the organic sulfur fraction) would give values of 0.79% cystine and 1.54% methionine.

If the above organic sulfur values for alfalfa were only cystine and methionine sulfur, then alfalfa would be nutritionally complete as a protein feed. However, it is well known that the protein of alfalfa is low in the sulfur-containing amino acids, especially methionine (1, 5). Therefore, since very little has been done to completely identify the organic sulfur fraction of feeding stuffs, this investigation was undertaken.

Experimental

Two replicated samples of the first and second cuttings of three standard varieties of alfalfa—Grimm, Ladak, and Ranger—were supplied by the Agronomy Department from plots at the Fargo Station and when available, from plots at the Langdon and Williston Branch Stations. The freshly cut alfalfa plants were dried at 50°C. for 24 hours in an oven equipped with a blower, ground in a Wiley Mill, and stored in sealed jars until analyzed.

Nitrogen determinations were made by the Kjeldahl procedure (6). Moisture was determined by weight loss following drying at 105°C. for six hours with a residual pressure of 20 mm. of mercury. Sulfur fractionations were made by the procedure of Thomas and Hendricks (4). Inorganic sulfurs were determined by the method of Woodman and Evans (3). Total sulfurs were determined gravimetrically as barium sulfate (7). The microbiological assay techniques were adapted from published methods (8,9).

Results and Discussion

In Table I the sulfur fractionation of the three varieties, Grimm, Ladak and Ranger, are compared for the first and second cutting for hay at the three designated locations. The organic sulfur fraction makes up approximately half of the total sulfur content of the three varieties of alfalfa for the first cutting at the Fargo Station. However, for the second cutting the organic sulfur content makes up approximately 40 per cent of the total sulfur (Table I). It can be seen that the three varieties grown at the Langdon and Williston Branch Stations had organic sulfur contents which make up more than half of the total sulfur contents. There is little to choose between the three varieties for sulfur fractionation at any one location for a particular cutting even though the three varieties varied from station to station and cutting to cutting. The data support the findings of Evans and Greaves (10) who studied the total sulfur content of alfalfa and found that it varied with the amount of available sulfur in the soil and the variety of alfalfa, but neither of these factors seemed to affect the organic sulfur content. The time when harvest was made was still a greater factor.

The cystine and methionine results are shown in Table II and are expressed as milligrams per gram of nitrogen. If the amino acids values obtained on entire animals can be regarded as a measure of "nutritionally complete" protein, alfalfa is deficient in cystine and methionine as reported by Block (11) since the values obtained on entire animals for cystine and methionine are 138 and 188 mg. per gram of nitrogen, respectively. Grimm and Ranger at the Fargo Station for the first cutting contained 73 and 70 mg. of cystine and 79 and 80 mg. of methionine per gram of nitrogen, respectively, or approximately 52 per cent of the cystine and 43 per cent of the methionine requirements for whole animal protein.

The first cuttings at the Fargo Station for the three varieties of alfalfa are higher in cystine and methionine than the second cuttings at the same station. The second cuttings at the Langdon and Williston Branch Stations are higher in cystine and methionine than the second cuttings at the Fargo Station. However, there is little to choose between these varieties for cystine and methionine content at any one station for a particular cutting, though the three varieties varied in amino acid content from station to station and cutting to cutting. One wouldn't expect a change in protein

TABLE I.—**Total, Inorganic, Organic, Volatile and Non Volatile Sulfurs in Standard Varieties of Alfalfa.**

(Per cent dry basis)

Location	Total Sulfur F	Total Sulfur L	Total Sulfur W	Inorganic Sulfur F	Inorganic Sulfur L	Inorganic Sulfur W	Organic Sulfur F	Organic Sulfur L	Organic Sulfur W	Volatile Sulfur F	Non Volatile Sulfur F
Grimm+	.257			.132			.134			.050	.084
Ladak+	.242			.120			.121			.044	.077
Ranger+	.253			.132			.120			.041	.079
Grimm++	.371	.332	.307	.206	.115	.147	.165	.218	.160	.063	.102
Ladak++	.370	.321	.340	.209	.114	.156	.160	.208	.184	.058	.102
Ranger++	.346	.333	.316	.206	.128	.141	.140	.205	.175	.052	.088

\+ 1st cutting for hay
++ 2nd cutting for hay
F—Fargo Station
L—Langdon Branch Station
W—Williston Branch Station

TABLE II.—**Crude Protein and the Cystine and Methionine Content of Standard Varieties of Alfalfa.**

Location	Cystine (mg. per gram nitrogen) F	Cystine (mg. per gram nitrogen) L	Cystine (mg. per gram nitrogen) W	Methionine (mg. per gram nitrogen) F	Methionine (mg. per gram nitrogen) L	Methionine (mg. per gram nitrogen) W	Crude Protein (per cent dry basis) F	Crude Protein (per cent dry basis) L	Crude Protein (per cent dry basis) W
Grimm+	73	----	----	79	----	----	15.1	----	----
Ladak+	61	----	----	74	----	----	14.4	----	----
Ranger+	70	----	----	80	----	----	14.3	----	----
Grimm++	51	56	71	63	72	72	18.4	20.8	18.2
Ladak++	51	63	70	62	68	68	18.7	21.1	19.8
Ranger++	60	67	72	63	76	74	16.7	20.7	17.9

\+ 1st cutting for hay
++ 2nd cutting for hay
F—Fargo Station
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structure of one variety of alfalfa grown under normal conditions, but only the quantity of protein produced by the plant as influenced by season, environment and stage of growth at harvest.

In Table II the nitrogen results are expressed as per cent of crude protein. It can be seen that all three varieties, Grimm, Ladak, and Ranger, contained higher crude protein in the second cuttings than in the first cuttings at the Fargo Station. The second cuttings at Langdon and Williston Branch Stations are higher in crude protein than the second cutting of alfalfa at the Fargo Station. Using crude protein as an index of quality there is little to choose between the three varieties at any one station for a particular cutting.

It is of interest to note the cystine and methionine content of alfalfa grown at the Fargo Station as calculated from the organic sulfur and volatile sulfurs shown in Table I (Table III). Volatile sulfur is expressed as cystine, and organic sulfur minus volatile sulfur is expressed as methionine.

TABLE III.—**The Cystine and Methionine Content of Standard Varieties of Alfalfa Calculated from Organic Sulfur Content (Fargo Station, Table 1)**

	Cystine (Volatile Sulfur) (mg. per gram of nitrogen)		Methionine (Organic Sulfur Minus Volatile Sulfur) (mg. per gram of nitrogen)	
	1st cutting	2nd cutting	1st cutting	2nd cutting
Grimm	87	88	161	160
Ladak	78	80	157	157
Ranger	74	79	162	154

It can be seen that the cystine values are approximately 30 per cent higher than the values obtained by microbiological assay (Tables II and III). Also, the methionine values are 125 per cent higher than the corresponding methionine values. The average value for methionine, 159 mg. per gram of nitrogen, compares favorably with 188 mg. per gram of nitrogen, the methionine value obtained on entire animal protein. However, at present we do not know if this organic sulfur fraction can be expressed as such. We do know that the sulfur-containing vitamins of the B-complex, thiamin and biotin, contribute very little to the total organic content of alfalfa. For example, the organic sulfur contributed by thiamin in good alfalfa hay (20 per cent crude protein, sun cured) is 0.000041 per cent. Biotin would contribute less. This amount of organic sulfur would not affect the total organic sulfur content in relation to the cystine and methionine content of organic sulfur.

Since the microbiological values obtained for cystine and methionine do not account for all of the organic sulfur found in alfalfa, it must be assumed that alfalfa contains an unknown organic sulfur fraction or that the accepted values for cystine and methionine are reported too low. Much more work will be required

before the complete distribution of the organic sulfur fraction in alfalfa is known.

References

1. Kingsley, A. F. Master's Thesis, Purdue University, 1948.
2. Painter, E. P. North Dakota Sta. Bimo. Bul. 5, 20-22 (1943).
3. Woodman, M. A. and Evans, R. E. J. Agri. Sci. 23, 459-462 (1933).
4. Thomas, M. D. and Hendricks, R. H. J. Biol. Chem. 153, 313-325 (1944).
5. Tisdale, S. L., Davis, R. L., Kingsley, A. F., and Mertz, E. T. Agron. J. 42, 221-225 (1950).
6. Paul, A. E. and Berry, E. H. J. Assoc. Offic. Agr. Chemists 5, 108 (1921).
7. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Washington, D. C., 6th Edition (1945).
8. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A. J. Biol. Chem. 177, 533-544 (1949).
9. Stokes, J. L., Gunness, M., Dwyer, I. M., and Coswell, M. C. J. Biol. Chem. 160, 35-49 (1945).
10. Evans, R. J. and Greaves, J. E. Proc. Utah Acad. Sci. 14, 17-22 (1937).
11. Block, R. J. and Bolling, Diana. The Amino Acid Composition of Proteins and Foods. Chas. C. Thomas, Publisher, Springfield, Ill. (1945).

SHARP INCREASE IN RYE ACREAGE

Rye acreage seeded in North Dakota in the fall of 1954 was placed at 600,000 acres, according to the USDA Agricultural Marketing Service. This is an increase of 75 per cent from the 343,000 acres sown in the fall of 1953 and is the largest acreage since the fall of 1941 when 1,001,000 acres were sown. Average acreage for the 10 years 1943-52 was 278,000. Acreage increases occurred in nearly all sections of the state and part of this increase comes as a measure to control weeds and wild oats.

For the United States, rye acreage sown for all purposes in the fall of 1954 is the largest in 12 years. An estimated 5,052,000 acres were sown in the fall of 1954 which is one-fourth more than the 1953 acreage of 4,023,000 acres, and nearly a third more than the 10-year average of 3,831,000 acres. This is the second year in which rye acreage has shown a sharp increase.

This increase in rye acreage is due to reduced wheat acreage allotments and an increased need for rye as supplemental pasture in areas which had dry weather during summer and fall months. Seedings were generally made under favorable conditions. Nearly one-third of the United States seedings was made in North Dakota, Illinois, Nebraska and South Dakota. By areas, the largest increase, 38 per cent, occurred in the north central states, while the western states were only three per cent above 1953.

North Dakota's cattle population continued its upward trend for the fourth successive year during 1954, according to the USDA Agricultural Marketing Service. On January 1, 1955 cattle numbers in North Dakota were at a record peak of 1,937,000 head. Hog and sheep numbers were both higher than in 1953 while horses continued to disappear from farms. More chickens were on hand but fewer turkeys. The state's cattle inventory on January 1 was put at 1,937,000 head, three per cent higher than in 1953 and one per cent above the previous record of 1,915,00 head on January 1, 1945. The increase was in beef cattle, with dairy animals remaining about the same.

Don't blame bad luck for all your troubles. Bad luck usually is the outcome of bad judgment.—Hoard's Dairyman.