Results and Discussion

As shown in Table I, the supplement of lysine lessened the body weight loss obtained when the wheat flakes were consumed without the milk and sugar. However, the animals were still unable to grow, even with the lysine supplement.

When the milk and sugar were fed along with the wheat flakes, *t* tests showed that no significant differences (p = >0.05) in growth or protein efficiency resulted from the added lysine. The casein diet gave a significantly better (p = <0.05) protein efficiency than the lysine-supplemented milk-sugar-wheat flakes diet. No other significant differences were shown in protein efficiency or growth when the cereal and milk diets were compared to the casein diet (p = >0.05).

Under the conditions of this experiment, no positive response in growth or efficiency in protein utilization was shown when the milk-sugar-wheat flakes diet was supplemented with 0.5% DL-lysine hydrochloride. The lysine supplement would be equivalent to 0.2% L-lysine. The DL form was used because L-lysine was not available commercially at the time the experiment was performed. The lack of growth response from the lysine may be explained by the relatively high level of this amino acid in the milk alleviating the lysine deficiency of the wheat flakes protein. Amino acids other than lysine would probably be necessary to give a positive growth response.

Table II gives the amino acid contents of the basal wheat flakes and the milk-sugar-wheat flakes mixture. These data were calculated from microbiological analyses of wheat flakes and the basic mixture used in a different feeding test at this laboratory. The methionine, histidine, lysine, and phenylalanine contents of the basic mixture were below the levels which may be required for the rat (Table II). Therefore, low levels of various essential amino acids may have limited the effectiveness of the lysine. Growth patterns obtained from supplementation of cereal proteins with combinations of amino acids calculated from microbiological amino acid analyses in unpublished work at this laboratory show that such analyses as a basis for calculating amino acid deficiencies in various food proteins are not completely reliable.

Gessert and Phillips (*4*) showed that a lysine supplement to a low protein ration containing skim milk and cereal grains retarded growth in young dogs. In the range of protein levels of 10 to 15%, the amino acid balance is very critical. The positive effect from the supplementation of wheat flakes alone with lysine might be expected, as lysine is the limiting amino acid of wheat protein, and it is the amino acid which is considered to be most adversely affected by cereal processing.

Although the cereal component of the milk-sugar-wheat flakes mixture supplied about 40% of the protein of the diet, the body weight gain and protein efficiency were similar to those obtained from the casein diet.

Table II. Amino Acid Composition of Wheat Flakes, with and without Milk and Sugar

Amino Acid	Calculated Amino Acid Composition of Basal Test Diets: In wheat flakes diet, %[a]	In wheat flakes, milk, and sugar diet, %[a]	Requirement for Growing Rat as of Diet[b]
Arginine	0.26	0.53	0.2
Histidine	0.15	0.29	0.4
Isoleucine	0.42	0.50	0.5
Leucine	1.00	1.34	0.8
Lysine	0.13	0.76	1.0
Methionine	0.17	0.18	0.4
Phenylalanine	0.34	0.59	0.7
Threonine	0.30	0.51	0.5
Tryptophan	0.09	0.17	0.2
Valine	0.66	0.67	0.7

[a] Calculated from microbiological amino acid analyses of wheat flakes and a wheat flakes, milk, and sugar mixture without added lysine. Values adjusted to allow for supplements of corn oil, vitamins, and minerals.

[b] Requirements suggested by Almquist (*2*).

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FORAGE NUTRIENTS

Free Reducing, Acid-Hydrolyzable, and Total Sugars and Total Available Carbohydrates in Ladino Clover, Nutritionally Significant Chemical Components of Forage Legumes

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Although legumes are a major forage crop in the United States, relatively little is known about their content of such biochemical constituents as carbohydrates, saponins, and organic acids.

The readily available carbohydrates of legumes are an important source of energy for ruminants, and as carbohydrates have been designated as one of the possible causative factors in bloat (*2, 3, 6*), the carbohydrate content of Ladino clover as affected by such factors as plant part, season, and time of day was determined.

Materials

Dormant Ladino clover stolons were obtained during the dormant seasons of 1947–48 and 1948–49 as described under plant parts.

Whole herbage and plant parts samples were taken in 1954 from five

The free reducing, the 0.4N hydrochloric acid–hydrolyzable, and the total sugar contents, were determined in whole herbage samples of Ladino clover, and in some separated parts such as leaf blades, petioles, stolons, and press juices. On some dormant stolons, only total available carbohydrates were determined. Many of these samples were from the same plots on which the experimental animals were grazed, or from which the herbage was harvested for use in the bloat studies conducted at the Agricultural Research Center, Beltsville, Md. The effects of season of the year, time of day, fertilizer treatments, and frost injury on the sugars in these samples were some of the variables studied.

fields of Ladino clover at the Beltsville Research Center. Each Ladino clover field was given a different fertilizer treatment in the spring of 1954 (Table I), and, during May and June, was irrigated when necessary with a sprinkler system providing $1^1/_2$ inches of water on each occasion. Because of the severity of the drought, it was not possible to irrigate after June 29, 1954.

All of the 1954 whole herbage samples were cut with a reel-type power lawn mower equipped with a grass catcher and all of the material was used as collected.

Growing Ladino clover stolons, while herbage, and plant parts samples were taken in 1956 from a fenced area in a cattle pasture at the research center.

All of the samples were rapidly dried in a portable forced–hot air apparatus especially designed for use in the field. In all cases, most of the drying was finished in 15 minutes after the samples were cut. With this machine, about 3 pounds of green material could be dried at one time.

Methods of Analysis

Crude fiber, moisture, crude protein, and nitrogen-free extract determinations were made by the methods of the Association of Official Agricultural Chemists (*1*).

Total available carbohydrates were determined by the method of Weinmann (*11*).

The extraction of the sugars from the dried samples was done by a modification of the method of Thomas, Melin, and Moore (*10*). A weighed portion, 3.000 grams, of air-dry, at least 40-mesh material was soaked in 5.0 ml. of water per gram of sample for 30 minutes with stirring at 3- to 4-minute intervals. The mixture was transferred quantitatively to the blender cup (the semimicro size preferably), using 4.0 ml. of 100% ethyl alcohol per ml. of the soak water used, and the transfer was completed with small portions of 80% ethyl alcohol. The charge was then blended at full speed for 10 minutes, the extract was filtered through a sintered-glass funnel of medium porosity into a 250-ml. wide-necked volumetric flask, using a minimum of vacuum, and the blender cup was rinsed into the funnel twice. The residue was returned to the cup with about 80 ml. of 80% ethyl alcohol, blended as before, and filtered through the same funnel into the same flask. The residue was transferred to the funnel quantitatively with 80% ethyl alcohol and washed first with the 80%, and finally with three small portions of 100% ethyl alcohol. The combined filtrates were made to volume with 80% ethyl alcohol and stored in the refrigerator. The residue was dried and kept for the determination of dextrin, starch, and other components.

The transfer of the sample from alcoholic to aqueous solution and the removal of nonsugar reducing substances were done largely by the usual methods. An aliquot of the ethanolic solution was evaporated on the steam bath to a depth of about 7 mm., water was added to a depth of about 14 mm. and evaporated as before, and the process was repeated once more after the odor of ethyl alcohol had gone. The residue should never be brought to dryness. This solution, together with as much of the precipitated solids as possible, was transferred with the aid of a policeman to a 50-ml. volumetric flask and made to volume. The contents of this flask were drained directly into a 125-ml. flask containing a suitable amount, 200 mg., of dry neutral lead acetate. The flask was stoppered and shaken vigorously 2 to 3 times in 5 minutes, and the contents were filtered as soon as possible through a dry paper into a 125-ml. flask containing a suitable amount, 400 mg., of dry sodium oxalate. In both filtrations the receiving flasks were shaken occasionally to help dissolve the dry reagents in the filtrates. These flasks were stoppered, shaken, stored overnight in the refrigerator, brought to room temperature, and filtered through a dry paper into a small dry flask. Preferably, this solution should be used promptly, but it may be stored in the refrigerator if necessary after adding a preservative—i.e., 1 mg. of mercuric iodide per 50 ml. of solution. *Caution.* Mercuric iodide is very poisonous.

Table I. Effect of Fertilizers and Lime on Sugar Content of Ladino Clover

Plot No.	Fertilizer per Acre[a]: P_2O_5, lb.	K_2O, lb.	Lime, tons	Cutting	Date, 1954	Sugars as Dextrose, Moisture-Free Basis: Free reducing	Hydrolyzable	Total
69	0	0	0	1st	5-6	6.41	4.79	11.20
				1st	5-27	5.18	4.63	9.81
				2nd	5-27	3.98	4.81	8.79
				3rd	6-29	2.03	4.44	6.47
				Av.		4.40	4.67	9.07
48	50	100	0	1st	5-5	6.32	3.31	9.63
				1st	5-26	4.43	2.48	6.91
				2nd	5-26	4.01	3.19	7.20
				3rd	6-29	2.24	3.32	5.56
				Av.		4.25	3.07	7.32
68	100	200	0	1st	5-6	6.56	3.22	9.78
				1st	5-27	4.54	3.24	7.78
				2nd	5-27	3.87	3.50	7.37
				3rd	6-29	2.25	3.90	6.15
				Av.		4.30	3.47	7.77
47	100	200	4	1st	5-5	5.17	4.82	9.99
				1st	5-26	6.37	2.31	8.68
				2nd	5-26	5.19	2.71	7.90
				3rd	6-29	2.11	2.56	4.67
				Av.		4.71	3.10	7.81
70	200	0	0	1st	5-5	8.18	4.06	12.24
				1st	5-27	7.71	4.42	12.13
				2nd	5-27	5.78	3.94	9.72
				3rd	6-29	3.50	3.19	6.69
				Av.		6.29	3.91	10.20

[a] Applied in March 1954.

The above cleared solution was hydrolyzed substantially as by Weinmann (11). An appropriate aliquot, 15 ml., of the solution was taken and, hydrochloric acid was added to a normality near 0.4. The mixture was heated in the boiling water bath for 30 minutes, cooled in running water, neutralized to alizarin red indicator, made acid with 1 to 2 drops of approximately 3N hydrochloric acid, made to a volume of 50 ml., and refrigerated overnight. After the hydrolyzate had come to room temperature, it was filtered through a dry paper into a dry flask, and used, preserved, and stored like the cleared solution.

The copper-reducing power of both of these solutions was determined by the method of Somogyi (8), modified by the use of phosphoric acid to help stabilize the end point in the titration mixture. The exactly optimum amount was not found, but about 0.5 ml. of approximately 10N acid was usually effective in final volumes of 25 to 50 ml., when added just before the titration. Since this paper was first submitted, Manohin and Kakabadse (4) have published their recommendation to use phosphoric acid instead of sulfuric acid in all iodometric titrations, when acidity is required, to stabilize the end points. The dextrose equivalence of the thiosulfate solution was determined by running Bureau of Standards dextrose samples and blank analyses concurrently with the unknowns.

Definitions

The copper-reducing power of the lead acetate–sodium oxalate cleared aqueous solution prepared from the 80% ethyl alcohol extracts of the samples was considered to represent the free reducing sugars. The copper-reducing power of an aliquot of the same cleared solution, after hydrolysis with 0.4N hydrochloric acid for 30 minutes in a boiling water bath, represented the total sugar. The difference between these two gave the hydrolyzable sugars.

As in all of these samples there may be more than one sugar present in each of these preparations, all results were reported as if only dextrose were present.

Results on Whole Herbage

Fertilizer Treatment. The fertilizer treatments, plot numbers, cutting dates, and certain chemical analyses of a group of samples taken on several occasions in 1954, when all five of the plots were sampled within a 24-hour period, are given in Table I. Unfertilized plot 69 had the most grass, while plots 47 and 70 had the least. Of these variously treated fields, plot 70 treated with a phosphorus carrier only, but at the highest level employed, had the highest average percentage of free reducing sugars. The other treatments appeared to have no effect. Earlier, Tesar and Ahlgren (9) found that Ladino clover did not respond well to fertilizer treatments with respect to either survival after severe cutting treatments or to production of dry matter.

Maturity. The first two lines of each group in Table I contain data on the changes due to a difference in maturity. Both sets of samples were first cuttings, but one set was allowed to grow 20 days longer than the other. The average sugar contents of the herbage from the five plots at the early and later harvests were, respectively: free reducing sugars, 6.53 and 5.64%; hydrolyzable sugars, 4.04 and 3.42%; and total sugars, 10.57 and 9.06% (moisture-free basis). The additional growth period thus resulted in percentage decreases in all three classes of sugars in the more mature forage.

Seasonal Effects. The cuttings which were taken from plot 47 at intervals during the growing season provide data relative to the effect of seasonal factors on composition. The results for five different growth periods are given in Table II. The free reducing sugars were more than twice as high in the forage of the May cuttings as in the later ones. After this drop, there was very little change for the rest of the season. The hydrolyzable sugar fraction was high in the first cutting, then fell to about half of this value for the next three cuttings, but at the September 28 and October 7 cuttings, rose to a level intermediate between the early and the midseason ones. This

Table II. Changes in Composition of Ladino Clover with Season, Plot 47

(All percentages reported on moisture-free basis; sugars as dextrose)

Date of Cutting, 1954	Cutting No.	Days Growth	Height, Inches	Free Reducing Sugars, %	Hydrolyzable Sugars, %	Total Sugars, %	Crude Protein, %	Crude Fiber, %	Nitrogen-Free Extract, %
5-5	1st	20[a]	7	5.17	4.82	9.99	26.06	10.84	45.70
5-26	2nd	21	4.5	5.19	2.71	7.90	27.36	10.98	47.45
6-29	3rd	34	7	2.11	2.56	4.67	21.95	16.40	45.48
8-30	4th	62	12	2.06	2.46	4.52	24.56	15.98	43.71
9-28	5th	29	3	2.02	3.94	5.96	25.67	12.72	46.74
10-7	5th	38	6	1.91	3.90	5.81	27.18	14.69	42.88
10-8	5th[b]	39	6	1.98	5.71	7.69	25.67	13.92	43.21

[a] Assuming April 15 as start of growing season.
[b] Sample freshly frosted.

Table III. Diurnal Studies on Ladino Clover

(Sugars reported as dextrose, moisture-free basis)

Date and Time of Cutting	Free Reducing Sugars, %	Hydrolyzable Sugars, %	Total Sugars, %	Weather Notes
	Ladino Clover, Plot 47, Swine Area, A.R.C.			
5-6-54				
6:00 A.M.	5.48	4.41	9.89	Partly cloudy
10:00 A.M.	6.62	4.04	10.66	Partly cloudy
2:00 P.M.	7.77	3.52	11.29	Partly cloudy
6:00 P.M.	7.97	3.06	11.03	Partly cloudy
10:00 P.M.	7.85	2.21	10.06	Partly cloudy
	Ladino Clover, Field 14, A.R.C.			
5-18-56				
6:30 A.M.	6.36	4.12	10.48	Clear, heavy dew
8:30 A.M.	5.48	4.00	9.48	Clear
11:30 A.M.	5.12	3.80	8.92	Partly cloudy
2:45 P.M.	6.15	4.10	10.25	Partly cloudy
5:30 P.M.	6.78	4.43	11.21	Clear, warm
8:30 P.M.	6.52	4.44	10.96	Clear, light dew
6-13-56				
7:02 A.M.	5.13	2.19	7.32	Hot, clear
10:14 A.M.	4.93	2.81	7.74	Hot, clear
1:20 P.M.	7.06	2.65	9.71	Hot, clear
4:05 P.M.	3.92	2.87	6.79	Hot, clear
6:45 P.M.	3.82	2.64	6.46	Hot, clear
8:55 P.M.	3.86	2.17	6.03	Hot, clear
7-16-56				
7:00 A.M.	3.50	2.27	5.77	Partly cloudy, medium dew
9:15 A.M.	2.72	2.69	5.41	Clear, warm, no dew
12:00 noon	3.86	2.66	6.52	Overcast, warm, humid
3:15 P.M.	3.60	3.27	6.87	Mostly cloudy, variable wind
5:55 P.M.	2.85	3.42	6.27	Mostly cloudy, hazy
8:55 P.M.	3.44	3.08	6.52	Medium overcast, dew?

fraction was the highest for the season in the October 8 cutting, which was taken early on the morning of a heavy frost.

Diurnal Effects. The results of some diurnal studies are given in Table III. In the series of samples taken May 6, 1954, the percentage of free reducing sugars increased to about a maximum by early afternoon and held for the rest of the 16-hour period, while the hydrolyzable sugars decreased at each sampling. The resulting total sugars increased till early afternoon and then decreased to nearly the same value as the first sample. In 1956, three series of diurnal samples were taken. These were from a different field and were cut at later dates than the 1954 series. These differences may account for the lack of trends in the 1956 data. Diurnal factors are not always observable in Ladino clover herbage as harvested.

Results on Plant Parts

Dormant Stolons. Dormant Ladino clover stolons were taken in both the 1947-48 and 1948-49 seasons from replications II, III, and IV of the orchard grass–Ladino clover mixture plots in a grass-legume association study at the Plant Industry Station, Beltsville, Md. This experiment involved four grass-legume mixtures, two frequencies of cutting, and nine scheduled forage-harvesting periods—all replicated five times, thus requiring 360 plots. Three of the nine harvesting schedules were started in April, three in May, and three in June; and one of each group of three schedules was closed in September, one in October, and one in November of each year, thus fixing both the length of the periods and the portion of the growing season which each one included (Table V).

The weight of sample from an area of 4 square feet, and the percentage of total available carbohydrates—both on the air-dry basis—were determined on the stolons.

When the total available carbohydrate values for the nine cutting schedules are averaged for each stolon harvesting date and arranged as in Table IV, the total available carbohydrates are shown to reach a maximum value during the fall or early winter, as was also later shown by Tesar and Ahlgren (*9*) and Moran and Sprague (*5*). The values were much lower by late winter or early spring. This decline in stolon carbohydrates was also reported for total polysaccharides by Wood and Sprague (*12*), and recently for total available carbohydrates by Ruelke and Smith (*7*).

The data from 52 pairs of these stolon samples were arranged in Table V, with respect to both the weight of stolons obtained from an area totaling 4 square

Table IV. Total Available Carbohydrate Content of Ladino Clover Stolons in the Dormant Season

(As dextrose, air-dry basis)

Replication No., Forage Expt.	Stolon Harvest Dates	Averages of 9 Forage-Harvesting Periods, %: 6-inch series	12-inch series
	Dormant Season, 1947–1948		
II	Nov. 28	28.0 (7 peroids only)	27.7
III	Dec. 30, Jan. 9	26.3	(No samples)
IV	Feb. 18	20.9	19.5
	Dormant Season, 1948–1949		
II	Dec. 8, 9	25.7	24.7
III	Dec. 10	26.3	25.8
IV	Mar. 9, 10, 11	17.7	16.3

Table V. Total Available Carbohydrates and Dry Matter in Ladino Clover Stolons from 6-Inch vs. 12-Inch Forage-Harvesting Series

(Carbohydrates, as dextrose, and dry matter; both on air-dry basis)

Forage Harvesting Schedules		Stolon Harvest Dates	Total Available Carbohydrates, %			Dry Matter Yields, G., from 4-Sq. Ft. Area		
Started	Ended		6-inch series	12-inch series	Difference	6-inch series	12-inch series	Difference
April	Sept.	11-28-47	27.8	28.6	−0.8	6.8	7.3	− 0.5
		12-9-48	25.2	26.1	−0.9	6.2	4.5	+ 1.7
		3-9-49	16.1	14.5	+1.6	6.4	5.1	+ 1.3
		12-10-48	26.3	26.9	−0.3	9.3	8.1	+ 1.2
		3-10-49	16.5	13.8	+2.7	9.4	5.5	+ 3.9
		2-18-48	20.1	17.3	+2.8	8.3	1.3	+ 7.0
		12-13-48	28.5	27.2	+1.3	17.5	7.2	+10.3
		3-11-49	16.5	16.0	+0.5	11.7	6.6	+ 5.1
	Oct.	11-28-47	27.1	25.4	+1.7	5.1	5.0	+ 0.1
		12-9-48	29.4	25.7	+3.7	7.9	4.0	+ 3.9
		3-10-49	18.6	18.2	+0.4	5.3	2.8	+ 2.5
		12-10-48	28.7	25.6	+3.1	17.9	11.5	+ 6.4
		3-10-49	18.1	17.2	+0.9	14.1	9.5	+ 4.6
		2-18-48	22.3	20.8	+1.5	13.5	6.5	+ 7.0
		12-13-48	29.3	28.9	+0.4	16.1	11.6	+ 4.5
		3-11-49	18.9	17.4	+1.5	12.9	9.2	+ 3.7
	Nov.	11-28-47	27.6	28.6	−1.0	7.1	3.5	+ 3.6
		12-9-48	24.5	24.3	+0.2	11.8	7.9	+ 3.9
		3-9-49	16.7	16.8	−0.1	8.1	4.0	+ 4.1
		12-10-48	26.8	24.5	+2.3	15.9	9.8	+ 6.1
		3-10-49	17.4	16.4	+1.0	13.2	9.2	+ 4.0
		2-18-48	19.8	22.6	−2.8	16.8	10.1	+ 6.7
		12-13-48	29.5	29.0	+0.5	21.5	15.4	+ 6.1
		3-11-49	20.2	16.2	+4.0	17.6	16.3	+ 1.3
May	Sept.	11-28-47	29.3	30.4	−1.1	12.4	5.5	+ 6.9
		12-8-48	26.4	25.2	+1.2	10.4	7.7	+ 2.7
		12-10-48	27.9	27.0	+0.9	5.1	8.0	− 2.9
		2-18-48	21.7	20.8	+0.9	17.7	14.4	+ 3.3
		12-13-48	28.1	27.0	+1.1	12.2	14.8	− 2.6
	Oct.	12-8-48	27.3	23.8	+3.5	11.7	7.7	+ 4.0
		12-10-48	25.1	24.5	+0.6	8.4	10.2	− 1.8
		2-18-48	22.3	20.1	+2.2	13.5	9.9	+ 3.6
		12-13-48	28.3	27.3	+1.0	15.3	14.4	+ 0.9
	Nov.	12-8-48	24.5	25.4	−0.9	11.7	9.6	+ 2.1
		12-10-48	23.3	26.1	−2.8	4.4	3.0	+ 1.4
		2-18-48	18.3	18.2	+0.1	14.3	7.6	+ 6.7
		12-13-48	28.2	25.6	+2.6	8.3	9.8	− 1.5
June	Sept.	11-28-47	31.2	30.4	+0.8	9.5	7.3	+ 2.2
		12-8-48	24.1	24.9	−0.8	8.1	5.6	+ 2.5
		12-10-48	24.9	25.1	−0.2	13.8	10.9	+ 2.9
		2-18-48	23.5	19.8	+3.7	3.9	2.5	+ 1.4
		12-13-48	30.2	29.3	+0.9	15.6	8.0	+ 7.6
	Oct.	11-28-47	25.5	24.1	+1.4	3.4	1.7	+ 1.7
		12-9-48	26.3	25.0	+1.3	5.9	6.8	− 0.9
		12-10-48	25.4	26.7	−1.3	14.0	12.8	+ 1.2
		2-18-48	18.2	18.5	−0.3	5.6	10.4	− 4.8
		12-13-48	25.3	25.5	−0.2	9.9	10.2	− 0.3
	Nov.	11-28-47	27.3	27.3	±0.0	3.2	1.4	+ 1.8
		12-9-48	24.0	21.7	+2.3	7.6	4.3	+ 3.3
		12-10-48	27.9	25.4	+2.5	18.6	12.5	+ 6.1
		2-18-48	21.9	17.4	+4.5	3.3	4.8	− 1.5
		12-13-48	28.5	26.3	+2.3	13.4	11.8	+ 1.6

Table VI. Sugar Content of Separated Parts of Ladino Clover Plants

(As dextrose, moisture-free basis)

Date Cut	Growth Data, Weather	Free Reducing Sugars, %	Hydrolyzable Sugars, %	Total Sugars, %	Plant Parts
	Ladino Clover, Plot 47, Research Ctr.				
May 18, 1954	9 to 12 inches, 1st cut	6.69	3.95	10.64	Leaf blades
	Early bloom	11.40	1.52	12.92	Petioles
	Cloudy, rainy	5.88	3.35	9.13	Whole herbage[a]
	Ladino Clover, Pasture, Research Ctr.				
May 18, 1956	1st cutting	5.81	5.82	11.63	Leaf blades
	Mostly cloudy	11.00	4.63	15.63	Petioles
		3.75	5.46	9.21	Stolons
		6.15	4.10	10.25	Whole herbage[a]
June 13, 1956	2nd cutting	3.12	3.07	6.19	Leaf blades
	6 inches high	6.64	3.69	10.33	Petioles
	Hot, clear	8.25	5.82	14.07	Stolons
		3.92	2.87	6.79	Whole herbage[a]
July 16, 1956	3rd cutting	5.11	4.15	9.26	Leaf blades
	6 to 9 inches high	3.79	4.08	7.87	Petioles
	Clear, breezy	3.16	5.25	8.41	Stolons
		3.60	3.27	6.87	Whole herbage[a]

[a] Ladino clover herbage as either harvested or grazed, usually includes very few stolons.

Table VII. Sugars in Press Juice from Ladino Clover

(Reported as dextrose)

Date, 1954	Material	Free Reducing Sugars, G./L.	Hydrolyzable Sugars, G./L.	Total Sugars, G./L.
Oct. 7	Ladino clover	13.27	2.28	15.55
Oct. 8	Ladino clover (frosted)	16.31	4.89	21.22

feet and the percentage of total available carbohydrates in them, so that those relating to the plots from which the herbage had been cut as often as it reached a height of 6 inches could be compared with those relating to the plots which had been cut only as often as herbage reached a height of 12 inches. The 6-inch series had higher percentages of total available carbohydrates than the 12-inch series in 37 cases, and lower in 14 cases with one tie. The 6-inch series had higher stolon weights in 43 cases and lower ones in nine. These results are apparently not in very good agreement with those of Tesar and Ahlgren (*9*) or of Wood and Sprague (*12*), but the plots were managed differently so that the data are not strictly comparable.

Stolons in the Growing Season. Three times in 1956, Ladino clover stolons were obtained at the same time as the samples of whole herbage and other parts. The resulting data are presented in Table VI, where these stolons appear to have a nearly constant, rather high content of hydrolyzable sugars, but are variable in free reducing sugars.

In Ladino clover, the stolons are the principal structures in which reserve carbohydrates—mostly starch—are stored. As these reserves are used to initiate new growth in the spring or after any severe defoliation, appropriate management practices should be observed to conserve these structures.

Petioles and Leaf Blades. In the May cuttings of both 1954 and 1956, the percentage of free reducing sugars in the petioles was very much higher than in either the leaves or whole herbage (Table VI). In these young petioles, this fraction contributed more than 70% to the total sugars in their respective samples.

Press Juice. On several occasions the juice was pressed from green Ladino clover and used in connection with the experimental production of bloat in sheep (*3*). Portions of these were mixed with four volumes of 100% ethyl alcohol, boiled a few minutes, and refrigerated until analyzed. Two of these were prepared from the same material as the herbage samples of these dates in Table II, and the results given in Table VII indicate that the effects of frosting show in the juice as well as in the whole herbage.

Bloat Causing Pasturage. Pastures in which animals were bloating were visited on two dates in 1954. On one farm, the pasturage consisted mostly of Ladino clover from 3 to 10 inches high, grazed at the rate of 26 dairy cows on 20 acres. Two cases of bloat were seen from 1:00 to 3:00 P.M., May 25, 1954. This herbage contained 4.85% free reducing sugar, 3.26% hydrolyzable sugar, and 8.11% total sugar (moisture-free basis). On another farm visited from 11:00 A.M. to 2:30 P.M., May 28, 1954, where two animals had died from bloat (one only 2 days previously) on a pasture predominantly of closely grazed second growth Ladino clover 3 to 6 inches high, the herbage contained 4.51% free reducing sugars, 3.79% hydrolyzable sugar, and 8.30% total sugar (moisture-free basis).

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