THE EFFECT OF FERTILIZERS ON THE SUGAR CONTENT, BUFFER CAPACITY AND ACIDITY OF RED CLOVER AND ALFALFA BEFORE AND AFTER ENSILING

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THE EFFECT OF FERTILIZERS ON THE SUGAR CONTENT, BUFFER CAPACITY AND ACIDITY OF RED CLOVER AND ALFALFA BEFORE AND AFTER ENSILING

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INTRODUCTION

The factors involved in the production of high quality silage are not well clarified. In general, unsatisfactory silage is produced when the forage is wet and cold or high in protein and low in sugar. Yet frequently silages are unexplainably good--or poor. Even corn or sudan grass silage, if too wet, sometimes becomes highly offensive in spite of the high sugar, low protein content of the crop. Conversely, clover silages are sometimes excellent even when wet, high in protein and comparatively low in sugar.

The legumes are well recognized to be high in buffering capacity and somewhat low in sugars. This combination of factors does not appear to favor the rapid development of the low pH that is necessary to inhibit the activity of proteolytic bacteria or of those that consume lactic acid at higher pH ranges.

Many experiments have shown that manipulation of the nutrient conditions of a plant can change the buffer capacity, the sugar content and the proportions of minerals in the plant. It is evident, therefore, that the silage-making characteristics of a plant are not inalterable. These experiments were designed to investigate the effect of various fertilizers upon the sugar content, buffer capacity and pH of red clover and alfalfa plants and the silages made from them.

REVIEW OF LITERATURE

In a highly buffered plant, it would appear that the acids produced by fermentation in silage would be relatively ineffective in lowering the pH. Wilson and Webb (18) (20) found that leguminous materials required more acid to bring about a change of one pH unit than did non-leguminous. As many legumes were found to be low in soluble carbohydrates and, therefore, presumably unable to produce much acid in fermentation, they concluded that this lack of sugars is probably the main reason why many attempts to make legume silage are failures. In a study of nitrate in plants, Wilson (19) found that the nitrate in plants being ensiled may have a strong effect on the buffers. The nitrate ion may be reduced to basic ammonium. If the vegetation contains much nitrate, he indicates there can be enough base developed during fermentation to have a significant neutralizing effect.

Dunne (5) states that the principal types of substances responsible for the buffer effects of plant saps are organic acids, amides, amino acids, phosphates and sugars. A low phosphate or a low potassium supply increases the hydrogen ion concentration and the buffer capacity of the sap. He states that calcium plays a minor role in the buffering system.

Brown (4), Benedict and Brown (3), Mortimer (9), Vinall et al. (15), MacGillivray (8), Wallace (17) and Nightingale, et al. (10), are some of the many that have done research in an effort to determine the effect of the mineral fertilizers upon plant composition. Careful study of the data in these papers suggests that the relationships between carbohydrates and proteins, and the ordinary mineral fertilizer elements vary widely under different conditions and with different treatment. Turchin (14) found that potassium has a strong effect on both the protein and the carbohydrates in plants. He stated that the function of potassium in plants is to take part in processes whereby simple carbohydrates and organic nitrogenous compounds are converted into complex substances.

Bear (2) has emphasized the cation and anion relationships in plants. He shows that, in any plant, the sum of the milli-equivalents of the cations is in constant ratio with the sum of the milli-equivalents of the anions; that is, $\frac{K + Ca + Mg + Na}{N + P + S + Cl + Si} = \text{Constant}$.

Bear further states, "Absorption of N in the NH_4 form should result in a reduction in the K + Ca + Mg + Na content of plants and this has been found to occur. It should also result in an increase in the content of P + S + Cl + Si and this has been demonstrated." Such substitution would be expected to alter the buffer capacity appreciably and to affect the quality of silage produced.

Many investigators have used small experimental silos. Garber and Odland (6); Odland, et al. (11); and Autrey, et al. (1), have used 2-quart Mason jars as miniature silos, mostly with satisfactory results. The three authors listed above all found that the chemical composition of silage from small and large silos was approximately the same.

EXPERIMENTAL PROCEDURE AND RESULTS

All silage was made in large test tubes (75cc). In filling the test tubes, the green material was cut into 1/8 - 1/4-inch pieces with a paper cutter, then rammed tightly with a stick to give a specific gravity of about 0.80, or eight-tenths gram of silage per cubic centimeter.

In Experiment I the tubes of silage were stoppered and sealed with scotch tape. However, the gas produced was sometimes sufficient to expel the stoppers and allow spoilage at the surface. Subsequently, all tubes were inverted in a shallow oil bath with the oil not deep enough to allow it to be in contact with the silage. This allowed free discharge of gases but did not permit the entrance of air.

The majority of the fresh plant materials and silages was analyzed for total sugars, pH and buffer capacity. Chopped, 5-gram portions of the material were extracted with eighty percent ethanol, using a modified Waring blender. This method of extraction has been used by Waldron, et al., (16); Thomas, et al., (13); and others and was found to give approximately the same values as other methods. Following the extraction process, the extracts were clarified with lead acetate and hydrolyzed with hydrochloric acid; and total sugars were evaluated by the gravimetric method of the Association of Official Agricultural Chemists (21). In the tables total sugar is expressed as invert sugar.

The pH of the material was determined by using a Beckman pH meter with extension glass electrodes. In determining buffer capacity, a 5-gram sample of silage or green material was ground for five minutes in 50 ml. of water in a modified Waring blender. To this suspension 0.25 N NaOH was added to increase the pH to 11, then 0.25 N HCl was used to lower the pH from 11 to 3.5. In this paper buffer capacity is expressed as the number of ml. of 0.25 N HCl needed to bring the sample from pH 11 to pH 3.5. Consequently, a high buffer capacity would mean that a large amount of acid was used in titrating--a low buffer capacity, the opposite.

During the summer of 1951, the Dairy Department at Michigan State College made some grass silage. As a test of the quality of the test tube silage, three tubes were filled with the cut green material while the silo was being filled. Two 1-quart Mason jars were also filled. Seven months later the silo silage was fed, and samples were taken to compare with the tube silage as to pH. Each reading below is an average of three samples:

Silo - pH 4.25   Test tubes - pH 4.65   Mason jars - pH 4.55

All three silages had a pH and an odor within the range to be expected with the material ensiled.

Experiment I

Roots of 1-year-old alfalfa plants were transplanted from the field to sand with a pH of 6.0 in the greenhouse in November, 1950. Seven roots were put into each of twelve glazed pots. One-half the cultures were given what was called the nitrate solution, consisting of $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and $FeSO_4$. The others were given a similar solution, in which the $Ca(NO_3)_2$ was replaced by $(NH_4)_2SO_4$ to give an equal amount of nitrogen. The plants were cut at about three-fourths bloom and analyzed for sugars, buffers and pH. Some of the material was made into silage and analyzed after intervals of two weeks, six weeks and five months. This experiment was repeated at a later date. NO_3 indicates the plants received nitrate nitrogen, NH_4 that ammonium nitrogen was used.

Table 1 shows how the use of two different forms of nitrogen affected greenhouse alfalfa plants and silage. In

Table 1. Effect of NO_3 and NH_4 in Nutrient Solutions on Several Constituents of Green and Ensiled Alfalfa Plants

Const.	Green		2-wk. silage		6-wk. silage		5-mo. silage	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
pH	5.98	5.82	5.95	5.90	4.95	5.84	4.80	4.40
Sugars*	4.30	4.42	5.43	6.09	tr.	5.80	--	--
Buffers	9	9	16	20	24	24	17	22

*In all cases where the term, "sugars," is used, it means percent total sugars on a dry matter basis.

the green plants, there seemed to be little difference between the two treatments. When ensiled the reducing sugars increased at first in both cases. During the first two weeks of fermentation, both lots of silage built up a large buffer system without appreciable change in pH. The buffer capacity increased still further in the silage at six weeks. The silage from the plants which received nitrate apparently reached its maximum degree of acidity much faster than that from those fed ammonium. After five months the silage made from plants receiving ammonium nitrogen was of higher quality than that from plants which had been fed nitrate. It also had a higher buffer capacity.

This experiment was repeated, the results being similar with two exceptions: The sugars had all been used in five weeks in both cases, and the quality of the final product was lower.

Experiment II

After the plants used in Experiment I were cut, the sand was washed and the plants were given new nutrient solutions. Six different nutrient solutions were used and each treatment was duplicated except that one duplicate had nitrate nitrogen and the other ammonium nitrogen. The nutrient solutions were the same as before but with certain deletions or additions as follows (with pot designations in parenthesis):

Solution 1 - the normal amount of calcium (Ca)
" 2 - the addition of sodium (Na)
" 3 - the quantity of potassium was doubled (2K)
" 4 - the quantity of calcium was doubled (2Ca)
" 5 - no calcium was used at all (-Ca)
" 6 - the quantity of potassium was reduced by half ($\frac{1}{2}$K)

When the plants were in three-fourths bloom, they were cut and the material treated as in Experiment I.

Tables 2 and 3 show the effect of the various nutrient levels upon the composition of these alfalfa plants and the quality of silage made from them. As can be seen from

Table 2. Effect of Various Nutrient Elements upon the Total Sugars in Greenhouse Alfalfa Plants and the pH of Ensilage made from Them

Treatment	Sugar		pH			
	Green plants		9-wk. silage		29-wk. silage	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
Ca	5.09	4.46	5.11	5.81	6.55	6.85
Na	5.05	3.75	5.10	5.11	5.52	6.60
2K	7.03	3.37	7.70	7.60	7.21	7.50
2Ca	4.36	3.08	5.42	5.20	7.20	4.65
-Ca	3.79	4.71	5.20	6.20	7.05	5.70
½K	4.68	3.90	5.23	6.67	6.35	4.93

Table 3. Effect of Various Nutrient Element Levels upon the Buffer Capacity of Green and Ensiled Greenhouse Alfalfa

Treatment	Green		9-week silage		29-week silage	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
Ca	9	8	21	24	23	23
Na	9	9	23	23	20	21
2K	10	8	28	20	23	19
2Ca	10	8	22	16	22	24
-Ca	9	10	21	22	22	24
½K	9	9	18	22	24	19

Table 2, the nutrient level appeared to have a large modifying effect on the amount of sugars in the plant and the pH of the silage. High sugar content in the plants was not often associated with low pH in the silage. In fact, sometimes the poorer silages came from plants with high sugar content. By the end of a 29-week period, only the combination of high calcium and ammonium nitrogen treatment had produced an acceptable silage. The poorest silage was that which had received the excess potassium in all cases. Since the calcium treatment may be considered as the check, the sodium treatment was the only treatment that consistently produced a better quality of silage.

The effect of various mineral nutrient levels on the buffer capacity of the plants or silage was neither large nor consistent (as is shown in Table 3). There appeared to be no significant difference in plant or silage composition that could be attributed to either nitrate or ammonium forms of nitrogen. Within nine weeks most of the silages were at or near their maximum buffer capacity. After twenty-nine weeks most buffers were about the same.

Experiment III

In this experiment nine 16' x 16' plots were laid out in an established 1-year-old stand of alfalfa and red clover. In the spring (May 18) these plots were fertilized, at the rate of 1,000 pounds per acre, with different materials as follows: 3-12-12, 2-16-8, 0-20-0, 0-20-20, 4-24-12, NH_4NO_3, $(NH_4)_2SO_4$ and hydrated lime. One plot was left as a check. Samples of first cutting clover and first and second cutting alfalfa were taken by cutting about two inches from the ground when the plants were in three-fourths bloom. These plants were analyzed and ensiled as in previous experiments.

From Table 4 it is apparent that red clover plants contained several times as much total sugar as did the alfalfa.

Table 4. Effect of Fertilizers on the Percent Sugar (dry matter) of Green Red Clover and Alfalfa Plants

Treatment	Red clover	Alfalfa	
		1st cut.	2nd cut.
3-12-12	12.5	3.45	3.44
2-16-8	12.6	2.08	2.37
0-20-0	14.0	1.99	2.37
0-20-20	*	3.32	3.04
4-24-12	*	2.61	1.38
NH_4NO_3	*	3.94	*
$(NH_4)_2SO_4$	12.3	4.60	3.62
Hyd. lime	11.4	3.76	4.20
No tmt.	14.1	4.95	4.20

*The percent of sugar was not obtained due to experimental error.

This may explain why red clover appears to be easier to ensile than alfalfa. The fertilizers did not have the same effect on red clover as on alfalfa, some increasing the sugar in alfalfa but decreasing it in red clover. By the end of a 7-week silage fermentation period, all the sugars had been used in all cases except for 1.8 percent in the case of the lime-treated red clover.

The effect of the fertilizers on the buffer capacity of the plants or silages (Table 5) was not pronounced except in the long-term silage. Alfalfa was more uniform in buffer capacity under the various treatments than was red clover.

Most of the silages listed in Table 6 with a pH of below 4.5 had the odor and appearance of good silage while some of that with a pH of 4.5 to 4.7 could be classed as fair. As usual, the effect of the use of a complete fertilizer varied considerably with the individual fertilizer and with the type of plant. In both cuttings the alfalfa check plants had the second highest pH silage.

Experiment IV

During July and August of 1951, samples of forage and soil were collected from many points in Ingham County, Michigan. The forage samples consisted of 300 grams of red clover or alfalfa plants. These were analyzed for sugar and made into silage as described previously. The soil on which

Table 5. Effect of Various Fertilizers on the Buffer Capacity of Fresh and Ensiled Alfalfa and Red Clover

Treatment	Red clover			Alfalfa			
	1st cut.			1st cut.			2nd cut.
	Green	7 wks.	16 wks.	Green	7 wks.	17 wks.	Green
3-12-12	8	17	19	6	16	18	7
2-16-8	6	17	16	6	17	17	7
0-20-0	7	16	25	7	16	18	10
0-20-20	8	18	20	7	17	20	7
4-24-12	6	18	19	6	16	18	8
NH_4NO_3	7	17	14	6	14	17	9
$(NH_4)_2SO_4$	7	16	20	6	16	16	7
Hyd. lime	7	17	16	7	19	19	8
No tmt.	8	*	*	7	17	18	8

*Broken

Table 6. Effect of Fertilizers on the pH of Silage Made From Alfalfa and Red Clover

Treatment	Red clover		Alfalfa	
			1st cutting	
	7 wks.	16 wks.	7 wks.	17 wks.
3-12-12	5.50	5.10	5.13	4.65
2-16-8	4.35	4.10	5.22	5.95
0-20-0	4.65	5.40	4.76	4.45
0-20-20	5.58	4.87	5.02	4.70
4-24-12	4.80	6.50	4.50	4.60
NH_4NO_3	4.70	4.50	4.90	4.55
$(NH_4)_2SO_4$	4.70	4.75	4.65	4.32
Hyd. lime	4.30	4.05	5.55	4.60
No tmt.	--	--	5.35	5.23

they were growing was sampled and tested for pH. The results are shown in Table 7.

Table 7. Effect of Soil pH upon Percent Total Sugars in the Green Material and on the pH of Silage made from Alfalfa or Red Clover

Soil pH	Alfalfa		Red clover	
	Silage pH	% sugar	Silage pH	% sugar
5.50	5.60	2.18	5.12	5.62
5.65	5.40	4.47	--	--
6.11	5.25	5.09	--	--
6.20	4.60	--	--	--
6.45	4.67	2.05	4.78	5.36
6.55	5.10	3.20	--	--
6.60	4.59	3.00	4.30	4.61
6.61	--	--	5.00	5.65
6.65	4.60	--	4.30	4.83
6.65	4.72	2.12	--	--
6.81	5.82	1.47	6.42*	4.10
6.85	6.25	1.11	5.10	5.81
6.86	6.85	2.49	4.35	4.35
6.91	4.69	1.50	--	--
6.94	5.20	3.95	--	--
6.96	5.40	2.98	--	--
7.10	--	--	4.25	2.75
7.22	5.25	3.63	--	--
7.25	--	--	5.60*	4.27
7.30	--	--	4.38	4.43
7.34	4.90	3.00	--	--
7.61	4.70	--	5.85	3.65
7.68	5.10	3.89	--	--
7.80	4.95	3.55	--	--

*Air may have entered these silages due to movement during ensiling period.

There was apparently no correlation between the soil pH and either the percent of sugars in the plant or the quality of the ensilage. Though the best alfalfa silages were made from plants grown on soils below pH 6.66, this was not at all true of the red clover silage, the best being from soil with a pH of 7.1. The same can be said for the effect of soil pH on the percent sugar. The plants having the most sugars were mostly produced on soils of low pH.

DISCUSSION

Apparently satisfactory grass silage can be made in test tubes. In all cases where the material was packed tightly and air excluded, silage free from mold was obtained. Grass silage of a low pH (4.1) was produced in some cases. The results of this experiment agree with Huffman (7) that although most investigators emphasize the importance of a pH of 4.2 or below for good silage, satisfactory alfalfa silage was produced when the pH ranged as high as 4.7. One noticeable result was that the material from the field-fertilized plots generally produced a much better grade of silage than did greenhouse plants, due perhaps to a higher dry matter content.

The results of the first experiment were erratic and no conclusions could be drawn. The only relatively consistent part of the experiment was the buffer capacity, the buffer of the silage from nitrate-treated plants being slightly higher in each case, up to six weeks. After that the buffer capacity of these silages declined to a point below that of the ensilage from ammonium-treated plants. The differences, however, were not sufficiently consistent to conclude whether or not there may have been a substitution in the plant of ammonium nitrogen for nitrate or of poor buffering elements for good.

In Experiment II nitrate or ammonium nitrogen was used in solutions high or low in calcium, potassium or sodium. Although most of the literature agreed that a high rate of potassium fertilization was accompanied by a smaller percent of sugar in the plant, the combination of high potassium and nitrate nitrogen in this experiment produced the highest percentage of sugars in the plants. Silage from these plants, however, was some of the poorest obtained. Conversely, one of the best silages obtained in this experiment was that made from plants receiving low potassium and ammonium nitrogen where the percent of sugar was low.

The various elements seemed to have a small effect on the buffer capacity of the green plants. From the data in Table 3, it appears that there may have been some slight substitution of ions. When NH_4 rather than NO_3 was used in nutrient solutions, there was, on the average, a slightly reduced buffer capacity in both the plants and the silage from them. No large differences in pH of plants or silages can clearly be attributed to either NH_4 or NO_3 in any combination with other nutrient elements.

In Experiment III there seems to be some correlation between the buffer capacity of a silage and its pH and quality. As is shown in Figure I, most of the silages with a low buffer capacity also had a low pH. This would seem to indicate that if a small buffer system were developed in the silage, conditions would be better for the production of a higher quality product. Not so much acid would be needed to lower the pH to the desired 3.5 - 4.0 range. It is apparent that sometimes a strong buffer system develops in silage. This might be caused by proteolysis and by the formation of weak acids and would indicate an undesirable cycle in the silage fermentation process. The finding of a low buffer capacity in the green plant did not necessarily mean that the buffers of the silage would also be low.

The accumulation of a comparatively large amount of sugar in the green plant was no assurance that a high quality silage would be produced. Samples containing only a small amount of sugar in some cases produced higher quality silage than similar plants with twice the sugar content. The red clover and alfalfa plants growing in soil treated with hydrated lime produced the highest quality long-term silage obtained, though not the highest percent of plant sugars. From the results of this experiment, it seems that the use of nitrogen and calcium fertilization might help to produce better silage. The use of any fertilizer at all seemed better than no treatment.

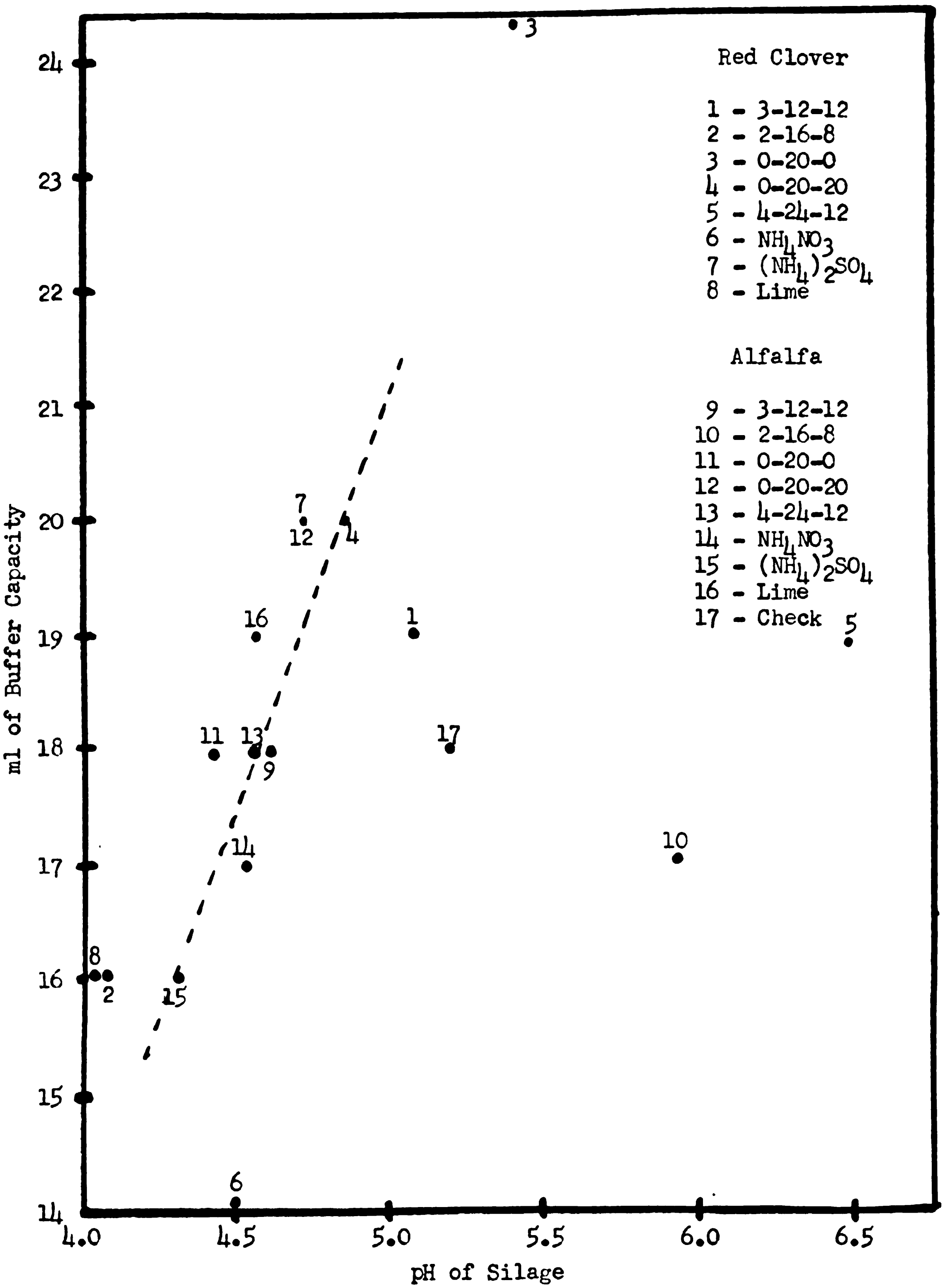

Figure I. The relationship of buffer capacity to the pH of silage 16 to 17 weeks old

Note that when the buffer capacity is low, the pH also is low.

Shönfeld (12) found that the average sugar content of sugar beets varied inversely with the pH of the soil. The results from Experiment IV seem to indicate that this is not strictly true of alfalfa and red clover plants. The plants with the highest percent of sugar were found in the lower ranges of soil pH. Shönfeld's findings seemed to apply more to the alfalfa than to the red clover in this case. Actually, no direct correlation could be found between soil pH, silage quality and the quantity of reducing sugars in the plants.

It must be noted that most of this material is the result of one year's experimental work only and cannot be considered conclusive.

SUMMARY

Alfalfa and clover plants were grown in greenhouse cultures or collected from field soils of various pH's or to which various of the ordinary fertilizer nutrients had been added. Analyses for sugar content, pH and the buffer capacity of the plants were made, both before and after making into silage.

Little effect on silage quality could be attributed to the abundance or scarcity of any nutrient element, or combination of them. Neither did there seem to be any clear correlation between sugar content of the plant and the pH of the silage.

In the silages as a whole, a rather definite relationship appeared between the buffer capacity (ml. 0.25 N HCl from pH 11 to pH 3.5) and the pH of silage. Silages with high pH generally had high buffer capacity, and those with lower buffer capacity usually were found to be comparatively low in pH. Offensive silages were uniformly high in pH and high in buffers, which suggests that proteolysis and the production of weak acids were large factors in the lack of successful fermentation.

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