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BACITRACIN AS A PRESERVATIVE FOR LEGUME-GRASS SILAGE

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The use of meadow crop mixtures of alfalfa and grasses has increased tremendously in the past ten years. In Ohio the tonnage increased from 162,000 tons in 1949 to 1,008,000 tons in 1957 (2). If this crop is wilted to a dry matter content of 35 percent the resulting silage usually is acceptable and has a low content of butyric acid. Most farmers, however, prefer to cut direct and ensile without wilting. Such high moisture silage needs a preservative to avoid development of relatively high amounts of butyric acid and spoilage.

REVIEW OF LITERATURE

Bacitracin became available in large quantities quite recently but few experiments have been conducted with it as a silage preservative.

Dexter (7) used five antibiotics individually, mixed, and also at varying concentrations as a preservative in making alfalfa silage in glass jars in 1954. Jars were opened successively on the 3rd, 7th and 21st days after ensiling. "On each of the three dates, great differences existed between the silages made from untreated forage and that treated with antibiotic. The forages treated with antibiotic were green and similar to freshly chopped alfalfa, while the untreated samples had the brownish green color characteristic of silage. When the jars were opened, the treated silages smelled fresh and grassy, while the untreated were fermented as expected." However, when the experiment was repeated in the two following years these findings were not confirmed.

In 1954 the alfalfa was ensiled on June 29 and in 1956 on June 26. Even at the latitude of East Lansing, Michigan the alfalfa could be expected to be in an advanced stage of maturity and the percentages of soluble sugars and starch low. This probably accounts for the relatively high pH's found.

Andrews and Stob (1) ensiled alfalfa in full bloom in small plastic bags. They found that 4 grams zinc bacitracin per ton did not affect pH at either 14 or 42 days after ensiling. At either 40 or 400 grams of bacitracin per ton the pH was higher for both periods of fermentation. When ground corn and molasses were added individually with and without bacitracin the pH was lowered materially. Molasses and bacitracin together lowered pH more than ground corn and bacitracin. At the 400 gram level bacitracin definitely inhibited acid formation. These silages were not fed.

Rusoff *et al.* (12) used zinc bacitracin at the rate of 10 grams/ton as a preservative on white clover in comparison with molasses (80 pounds/ton), metabisulfite (8 pounds/ton) and an untreated control. The effect of the silages on milk production was determined with 12 milking cows in a 63-day reversal trial. Average values were determined for each silage for pH, dry matter eaten, F.C.M. production and total dry matter consumed per pound of F.C.M. produced. The only significant differences ($P = 0.05$) were in silage consumption. The amounts eaten were 67 pounds bacitracin silage, 73.9 pounds molasses silage, 63.3 pounds bisulfite silage and 86.2 pounds of the untreated control. The pounds of milk produced per pound of total dry matter intake was 1.05 for bacitracin silage and 0.97 for each of the other three.

Rusoff (10) determined lactic acid and volatile fatty acid content of the four above mentioned silages and found that bacitracin resulted in higher lactic and propionic acids, high acetic acid and low butyric acid. The resultant pH was comparable to that produced by metabisulfite. The N.F.E. content of the four silages indicates that bacitracin controls fermentation of N.F.E. with a resulting low pH. Zinc bacitracin, like molasses and metabisulfite, reduced protein decomposition and ammonia formation.

Miles (9) fed young cattle with silages made by preserving Johnson grass with molasses, metabisulfite and zinc bacitracin as well as an untreated control. Digestion trials showed coefficients of 60.95 percent, 54.42 percent, 56.04 percent and 55.73 percent for molasses, metabisulfite, zinc bacitracin and untreated respectively. Rusoff (11) concluded "the silage doesn't affect the odor, quality or quantity of milk.

The antibiotic eliminates itself during the preservation process and is gone when the silage is ready for feeding."

OBJECTIVES OF THIS RESEARCH

To get additional information on the effects of bacitracin on chemical composition and feeding value of low dry matter silage the following experiments were planned.

The material was ensiled in the low dry matter range since that is the material needing a preservative. When the crop is wilted to a dry matter content of 30 to 40 percent a desirable silage usually results even though no preservative is used.

Experiment I

An alfalfa-brome mixture, treated with bacitracin was ensiled in four small laboratory silos holding nine pounds each, at four dry matter levels ranging from 20.5 to 26.8 percent in comparison with untreated and metabisulfite treated crop from the same lot of alfalfa-brome mixture at the same dry matter levels. The following determinations were made on the resulting silages:-pH, total acidity, lactic acid, volatile organic acids, amines, ammonia, nitrates and nitrites, crude protein, reducing sugars, carotene, dry matter by the toluene method and proximate analysis.

Experiment II

To determine the effects of bacitracin on milk production and body weight changes a feeding experiment was conducted using three silages made as described above and fed to cows arranged in a 3x3 Latin square design with the third period repeated to make possible determination of carry-over or residual effects.

Dry matter analyses of all feeds were made. Butterfat determinations and milk recordings made calculation of four percent fat-corrected milk (4 percent F.C.M.) possible.

The experimental cows were weighed for three consecutive days at the beginning and close of each experimental period.

The cows were fed a minimum allowance of hay, grain in proportion to milk production and silage *ad lib.* The same chemical analyses were made on the three silages as described under Experiment I.

Experiment III

To test further the effects of bacitracin-treated silage in comparison with untreated and metabisulfite treated silage on milk production and body weight changes a continuous feeding experiment was conducted.

The same feeding methods as outlined in Experiment II were used and the same chemical analyses as for Experiment I were made.

EXPERIMENTAL METHODS AND RESULTS

Experiment I. Laboratory Silos

METHOD of ENSILING: About 150 pounds of alfalfa-brome meadow crop were harvested on June 7, 1960 and spread on a plastic sheet to wilt. Enough material was removed to provide nine pounds (4082 grams) for each of three silos and 400 grams for duplicate samples of 200 grams each for determination of percentage dry matter by drying in an electric oven at 100° C.

One 9 pound sample was placed in a cylinder (6" diameter and 21" tall) with baked-on glass lining without a preservative.* The crop was well packed and covered with a polyethylene poultry bag and a round 1" follower that fitted tightly to the walls. Five cement blocks were then placed on the top to give a density equal to that found in a 30' or 40' silo. The amount of weight per square inch of surface area of silage varied between 8.3 and 8.6 pounds in the 12 silos involved.

A second silo was similarly filled with 9 pounds of alfalfa-brome treated with Silotracin** at the rate of 5 pounds per ton by mixing in a polyethylene turkey bag. Five pounds of Silotracin contain not less than 5 grams of zinc bacitracin.

A third silo was also similarly filled with material treated with a commercial sodium metabisulfite ("Stafresh") at the rate of 8 pounds per ton.

This procedure was repeated at intervals to give four sets of three silos each, at varying dry matter levels.

The remaining crop which had been spread on the plastic sheet was thoroughly mixed each time before removing the material for ensiling and for dry matter analysis.

CHEMICAL ANALYSIS: The ensiled material had a proximate analysis on a dry basis of 10.22 percent ash, 23.82 percent crude fiber, 1.70 percent ether extract, 17.04 percent protein and 47.22 percent

Twelve such cylinders were provided through the courtesy and cooperation of the A. O. Smith Corporation, Milwaukee, Wisconsin.

***The Commercial Solvents Corporation kindly supplied the Silotracin and provided a Grant-in-Aid in partial support of this project.*

Table 1.–Weights of Alfalfa-Brome Ensiled and of Silage and of Dry Matter Recovered after Ensiling

Silo No.	Treat-ment*	Crop Ensiled	Dry Matter	Dry Matter	Silage Recovered	Silage Dry Matter	Silage Dry Matter	Silage Recovered	Silage Dry Matter Recovered	Date Opened
		(g.)	(%)	(g.)	(g.)	(%)	(g.)	(%)	(%)	
1	U	4082	20.5	837	3732	22.2	829	91.4	99	9-26-60
2	M	4082	20.5	837	3887	23.0	894	95.2	107	9-26-60
3	B	4082	20.5	837	3982	21.0	836	97.7	100	9-26-60
4	U	4082	23.0	939	3992	23.0	918	97.8	98	12- 7-60
5	B	4082	23.0	939	3959 1/	23.0	911	96.9	97	12- 7-60
6	M	4082	23.0	939	3838	25.8	990	94.0	105	12- 7-60
7	U	4082	25.0	1021	3978	23.5	935	94.0	92	1-18-61
8	M	4082	25.0	1021	3958	25.1	993	97.0	97	1-18-61
9	B	4082	25.0	1021	3891	23.0	895	95.3	88	1-18-61
10	B	4082	26.8	1094	3842 1/	24.8	953	94.1	87	1-31-61
11	M	4082	26.8	1094	3854	25.8	994	94.4	91	1-31-61
12	U	3594	26.8	963	3483	24.8	864	96.9	90	1-31-61
Average	U		23.8			23.4		95.0	95	
	M		23.8			24.9		95.1	100	
	B		23.8			23.0		96.0	93	

* U = Untreated
M = 8 lb. sodium metabisulfite per ton.
B = 5 g. zinc bacitracin per ton.
1/ Limited top spoilage.

N.F.E. The dry matter analyses of the crop at ensiling appear in Table 1.

The silages were thoroughly chopped and mixed in a Hobart chopper in preparation for sampling. That bacitracin does not prevent oxidation of dry matter while metabisulfite does is suggested by the average dry matter content of the three silages. The average per cent of dry matter recovered likewise indicates that metabisulfite reduces dry matter oxidation while bacitracin does not. The silage analyses appear in Table 2.

Table 2.–Analyses of Silages and Silage Juices

Silo No.	Silage	** pH	** Total Acidity	** Amino Acids	** Total V.F.A.	** Fatty Acids: Acetic	Propionic	Butyric	Lactic	* Total Nitrogen (Wet Basis)	** NH_4^+	** NH_2^-	** NO_2^- + NO_3^-	* Reducing Sugars	* Carotinoids
			ml 0.1 N NaOH/10 ml juice			ml 0.01 N NaOH/0.5 ml juice				(%)	ml 0.01 N H_2SO_4/10 ml juice			(%)	p.p.m.
	Untreated														
1	9-26-60	4.6	16.7	7.9	14.1	5.1	0.2	0	7.2	0.58	47.0	4.6	1.9	0.019	41.8
4	12-7-60	4.7	18.1	10.4	20.8	6.9	0.1	0.1	0	0.64	72.8	7.5	1.7	0.031	23.3
7	1-18-61	4.9	18.3	11.9	25.3	9.4	0.3	0.2	4.6	0.65	80.6	11.7	2.1	0.034	31.8
12	1-31-61	4.8	20.7	12.7	25.6	11.3	0.3	0	8.1	0.68	82.4	17.6	1.3	0.106	33.6
Av.			18.5	10.7	21.5	8.2	0.2	0.1	5.0	0.64	70.7	10.4	1.8	0.048	32.6
	Bacitracin														
3	9-26-60	4.7	18.9	8.6	20.5	9.1	0.4	0	3.5	0.57	32.2	4.0	2.7	0.026	40.2
5	12-7-60	4.7	19.4	10.5	20.5	7.2	0.1	0.2	5.3	0.63	69.8	9.4	1.8	0.029	28.8
9	1-18-61	5.8	5.0	22.5	45.7	6.6	1.3	9.9	0	0.68	167.3	21.9	2.7	0.023	43.7
10	1-31-61	5.9	5.2	19.6	48.5	8.8	1.4	12.6	0	0.70	265.9 1/	11.5	1.4	0.021	45.7
Av.			12.1	15.3	33.8	7.9	0.8	5.9	2.2	0.65	133.8	11.7	2.2	0.025	39.6
	Metabisulfite														
2	9-26-60	4.8	10.2	7.8	9.0	1.1	0	0.1	2.1	0.58	30.9	3.9	1.6	0.027	39.3
6	12-7-60	4.7	14.6	10.6	12.5	3.0	0.1	0.1	5.6	0.64	64.5	7.1	2.1	0.093	17.8
8	1-18-61	4.8	15.8	11.5	12.9	4.5	0.1	0.4	6.4	0.65	66.9	8.3	2.6	0.222	29.1
11	1-31-61	4.8	18.2	14.3	16.3	5.1	0.2	0.7	8.4	0.67	77.8	9.1	1.9	0.040	25.6
Av.			14.7	11.1	12.7	3.4	0.1	0.3	5.6	0.64	60.0	7.1	2.1	0.096	28.0

*Analyses made on wet silage.
**Analyses made on silage juice.
1/ Sample was over heated by steam pipe.

Analyses other than nitrogen partition were made by standard procedures. The following procedure was used for the separation of the nitrogen fractions of silages into three groups: ammonium (NH_4^+); amide (NH_2-); and nitrite-nitrate ($NO_2^- + NO_3^-$). It is a modification of the procedure described by Varner, *et al.* (13).

PROCEDURE: A solution of freshly-pressed silage juice in 80 percent ethyl alcohol is allowed to stand under refrigeration for 20 minutes. The precipitated protein is removed by centrifugation. Three 50 ml. aliquot samples were taken from the supernatant and delivered into Kjeldahl distilling flasks.

NH_4^+: Addition of 1 drop of 50 percent NaOH and 5 ml. of borate buffer (a saturated solution of $Na_2B_4O_7 \cdot 10H_2O$ in water, adjusted to pH 10 with sodium hydroxide) makes the solution sufficiently alkaline for the removal of the ammonium fraction. The solution is then distilled for 10-15 minutes (until the ethanol is removed), the distillate being collected in 35 ml. of 1 percent boric acid solution, which is then titrated with 0.01 N H_2SO_4. The number of milliters of acid is recorded.

NH_2 –: After adding 15 ml. of 50 percent NaOH and 60 ml. water, antifoaming compound* is sprayed into the Kjeldahl flasks. Then the distillation is carried out as above, for 10-15 minutes.

$NO_2^- + NO_3^-$: A few grains of granulated zinc are added to the reaction flasks to prevent bumping. Approximately 0.5 grams of powdered zinc is added along with 5 ml. of 20 percent $FeSO_4$, 5 ml. Ag_2SO_4 (saturated) and 60 ml. water. Distillation for 10-15 minutes removes nitrites and nitrates.

The silage in silo 5 was covered with a circular follower that did not fit as closely as the others and a small amount of spoilage occurred due to admittance of air. Silo 10 was located where the atmospheric temperature was increased by a heat pipe during the autumn. The circular follower dried and admitted some air. The pH and ammonia of the silage from silo 5 indicated that no appreciable protein degradation occurred while those for silo 10 indicate marked degradation.

The pH's for silos other than 9 and 10 were those expected for legume silages. The ammonia formed by protein degradation maintained the pH at a high level and favored activity by the butyric acid forming organisms. Silos 9 and 10 are the only ones in which appreciable amounts of butyric acid were produced. The silage prepared with metabisulfite and the untreated silage had a greater amount of lactic acid than that prepared with bacitracin, however, there was less acetic acid when metabisulfite was used. The concentration of amides other than in bacitracin silage in silos 9 and 10 appears to be within a normal range.

Dow-Corning antifoam A spray was used here.

That metabisulfite decreased the oxidation of reducing sugars is apparent. That bacitracin furthered such an oxidation appears probable.

The concentration of carotenoids suggests a protective action by bacitracin and a failure of metabisulfite to prevent carotenoid breakdown.

Experiment 2. Feeding Trial – Latin Square Design

ENSILING: Harvest of legumes (principally alfalfa) and grasses began on June 9 and continued with interruptions through June 17. Each load was weighed and sodium metabisulfite at the rate of 8 pounds per ton was spread on each load. Sufficient was ensiled to to permit feeding the herd as well as experimental cows thus insuring fresh silage. Untreated and bacitracin-treated silage were to have been ensiled immediately but rainy weather prevented and those silages were ensiled from second cutting on July 14 and 15. This difference in origin of the silages is reflected in the protein as determined by Kjeldahl on both wet and dried silage samples.

The crop ensiled for untreated and bacitracin-treated silages was placed by loads alternately on one side and then the other of a plastic sheet which divided a 10' x 40' silo. Silotracin was spread over weighed loads at the rate of 5 pounds per ton.

Samples of the ensiled material for untreated silage averaged 18.8 percent dry matter while that treated with bacitracin averaged 18.9 percent. Because of the high moisture content of the crop effluent poured from the silo for three days. Since the pressures would be equal on both sides of the plastic sheet, which divided the silo at the center of the door, the juice movement would be outward around the door rather than to the other side of the sheet. The extremes in dry matter content of the finished silage were 17 percent at the top to 22 percent near the bottom of the silo.

SPOILAGE: The use of bacitracin definitely reduced top spoilage. The top spoilage on the untreated silage was 1367 pounds while that on the bacitracin-treated was 833 pounds or only 61 percent as much.

RECOVERY: The edible untreated silage recovered was 56 percent of the "as ensiled" weight while the bacitracin-treated was 65 percent. The high moisture content of the ensiled material and large amount of effulent are responsible for the low recovery of both silages. The increased percentage recovery on the

Table 3.–Chemical Analyses of Silages Used for Feeding Trials. 1/

Treatment Sampled on	** pH	** Total Acidity	** Amino Acids	** Total V.F.A.	** Fatty Acids: Acetic	Propionic	Butyric	Lactic	* Total Nitrogen (Wet Basis)	** NH_4^+	** NH_2^-	** NO_2^- NO_3^-	* Reducing Sugars	* Carotinoids
		ml 0.1 N NaOH/10 ml juice			ml 0.01 N NaOH/0.5 ml juice				(%)	ml 0.01 N H_2SO_4/10 ml juice			(%)	p.p.m.
10-26-60														
Bacitracin	5.4	9.56	9.56	29.85	9.98	0.75	0.80	0.76	0.629	47.32	16.55	5.80	0.0072	26.1
Untreated	6.2	1.46	18.34	35.68	4.91	0.90	6.73	0.0	0.637	116.90	17.35	3.73	0.0166	36.4
Metabisulfite	4.2	14.68	6.20	3.55	0.44	4.76	3.90	3.40	0.389	14.15	3.57	1.71	0.2971	33.2
11-2-60														
Bacitracin	5.0	13.79	9.08	25.94	9.46	0.73	0.05	1.12	0.619	43.85	3.62	1.44	0.0228	34.7
Untreated	5.4	9.04	12.67	30.28	9.19	1.08	1.54	0.32	0.598	75.50	3.86	1.35	0.0260	20.4
Metabisulfite	4.8	13.06	5.85	17.32	2.65	0.20	2.23	2.82	0.377	38.49	3.13	1.04	0.1272	22.4
11-15-60														
Bacitracin	5.2	11.32	9.68	26.78	10.27	1.20	0.12	0.52	0.664	44.58	4.53	1.50	0.0150	29.6
Untreated	5.5	7.00	13.14	31.07	8.83	0.78	0.56	0.28	0.656	60.93	4.32	1.42	0.0214	23.5
Metabisulfite	4.3	11.81	4.81	5.60	1.10	0.00	0.00	4.82	0.396	17.75	2.80	1.03	0.8348	20.4
11-29-60														
Bacitracin	5.0	13.03	8.90	25.00	4.79	0.47	0.12	0.00	0.659	63.04	5.28	1.06	0.0194	29.4
Untreated	5.4	7.93	11.41	30.06	1.74	0.56	1.01	0.10	0.627	93.88	5.40	1.21	0.0194	33.2
Metabisulfite	4.9	9.18	4.94	15.18	1.39	0.00	0.28	3.02	0.366	29.26	2.91	0.85	0.2020	10.8
12-13-60														
Bacitracin	5.2	9.65	9.95	24.03	9.50	0.72	0.12	0.30	0.638	66.39	4.91	3.19	0.0280	38.0
Untreated	5.0	12.22	9.74	24.68	8.66	0.58	0.02	0.72	0.620	63.44	4.96	1.67	0.0219	39.2
Metabisulfite	4.3	12.17	4.95	6.02	0.86	0.00	0.00	3.44	0.372	26.12	2.81	1.26	0.6080	34.4
12-19-60														
Bacitracin	5.5	8.49	10.78	34.40	8.63	1.58	2.44	0.00	0.628	115.20	7.79	2.02	0.0193	37.4
Untreated	5.5	8.05	12.34	38.40	7.68	1.17	3.62	0.00	0.627	127.70	6.20	1.28	0.0182	36.8
Metabisulfite	4.4	12.17	3.15	4.30	0.78	0.05	0.00	4.18	0.373	23.86	3.33	0.77	0.4576	36.0
1-3-61														
Bacitracin	4.9	16.09	9.22	24.15	11.52	0.73	0.00	2.64	0.662	62.31	8.05	3.59	0.0418	43.3
Untreated	5.7	5.80	13.65	32.92	8.80	0.84	2.89	0.00	0.633	130.40	9.10	3.77	0.0304	47.0
Metabisulfite	4.4	11.38	4.88	5.10	0.97	0.00	0.00	3.38	0.341	24.72	3.15	1.08	0.2078	40.8
1-9-61														
Bacitracin	5.5	7.24	11.84	30.04	8.46	0.72	1.42	0.38	0.618	85.96	15.52	1.22	0.0284	53.5
Untreated	5.8	4.88	14.49	36.24	9.13	0.21	3.49	0.00	0.657	131.00	18.29	2.15	0.0250	49.3
Metabisulfite	4.4	10.96	5.13	5.85	1.24	0.00	0.00	4.13	0.342	23.21	5.67	0.73	0.3082	30.9

1/ Metabisulfite-treated silage was made from June 9 through June 17 while the untreated and bacitracin-treated were made on July 14 and 15.

* Analyses made on wet silage.

** Analyses made on silage juice.

basis of four experimental (laboratory type) silos was only four percent from bacitracin treatment (Experiment 1). While the recovery of usable silage from the bacitracin-treated silage was 73.4 percent as compared with 62.4 percent for the untreated silage or 11 percent greater.

CHEMICAL ANALYSIS: The chemical analyses (Table 3) of the silages used for the feeding trial were made at eight times during the feeding trial which will be described in the succeeding section of this paper.

An analysis of variance of the chemical analyses of untreated and bacitracin-treated silages reveals some interesting differences. The analyses of metabisulfite silage were not included in the analysis of variance as the ensiled material was of an earlier cutting and had a different proximate analysis than that used for the other silages. The effectiveness of both bacitracin and metabisulfite in curbing the proteolytic bacteria may be seen in the lowered concentration of ammonia in seven of the eight analyses. The effect of bacitracin was significant ($P = 0.05$).

Bacitracin inhibited protein hydrolysis significantly ($P = 0.01$).

The amine content of the three silages was variable and the differences were not significant.

Bacitracin had a measurable effect on the development of steam volatile fatty acids ($P = 0.05$) but metabisulfite appears to have a greater effect.

The consistently high values for total acidity of metabisulfite silage reflect its direct effect by acidification and its indirect effect through prevention of ammonia formation. The average values for bacitracin silage and metabisulfite silage are nearly the same. The value for total acidity of bacitracin silage is significantly higher ($P = 0.05$) than for untreated silage.

The content of butyric acid of the two treated silages is less than that of the untreated silage, the difference for the bacitracin silage being significant ($P = 0.05$).

The differences in concentration of acetic, propionic and lactic acids between treated and untreated silage are not significant.

These data indicate that the action of bacitracin may not be in directing the fermentation of carbohydrate but rather in preventing protein hydrolysis and deamination. Reduction in butyric acid from the action of bacitracin and metabisulfite may be due to prevention of deamination of those amino acids capable of giving rise to butyric acid.

DESIGN of FEEDING EXPERIMENT: Six Holstein and six Jersey cows were placed upon a 3 x 3 Latin square design with the last period repeated. A second Latin square was used so that a different sequence of silages could be used. The combination of the two provided every possible sequence of one silage following another. Repetition of the third period without change of silage gave each silage followed by itself. This combination made possible the determination of of the significance of residual effects. The experimental design is described by Cochran and Cox (3). The experimental design and assignment of silages to groups appear in Table 4.

Table 4.--The Assignment of Cow Groups to Silages*

	Cow Groups					
Experimental Period	1	2	3	4	5	6
Oct. 18-31, I	U	B	M	U	B	M
Nov. 1-14, II	B	M	U	M	U	B
Nov. 15-28, III	M	U	B	B	M	U
Nov. 29-Dec. 13, IV	M	U	B	B	M	U

*U = Untreated
M = Metabisulfite
B = Bacitracin

Holsteins were fed five pounds of alfalfa hay and Jerseys three. All cows were fed grain ratio to milk production according to the following rule:-- Holsteins were fed 0.4 pound grain per pound of milk over 20 and Jerseys 0.5 pound per pound of milk over 12. The grain mixture was composed of 600 pounds ground shelled corn, 300 pounds oats, 100 pounds soybean oil meal, 10 pounds salt and 10 pounds steamed bonemeal. They also had access to steamed bonemeal and salt separately in the exercise yard. Silage was fed to the limit of appetite and refusal was weighed back.

The cows were grouped according to the data appearing in Table 5. The grouping is of less importance than in most experimental designs since every cow was assigned each silage at some time during the experiment.

The experimental periods were of 14 days each. One cow (1427) in group 6 went off feed near the end of the 3rd period and had to be removed from the experiment. The average feed intake and milk production of this cow for the part period were used to calculate the production for that entire period. For analysis of covariance, the missing data for the 4th period were estimated by the missing plot technique of Coons (6).

Table 5.--Data Used in Grouping the Cows

Gr.	Cow	Age	Days fresh	Lb. milk before expt.	Previous Lact.	4% M.E.	Body wt.
I	H 1196	7-5	188	43	15,509	15,664	1112
	J 1317	4-8	47	33	7,165	8,260	801
II	GH 1206	7-3	46	35	8,714	9,537	1248
	J 1393	4-5	26	33	8,503	9,949	901
III	H 1212	7-0	158	49	10,937	11,967	1277
	J 1379	3-9	41	34	8,279	9,935	807
IV	H 1290	5-1	30	39	8,112	10,302	1108
	J 1235	6-7	43	34	10,385	10,187	872
V	H 1428	3-0	36	41	6,723	8,942	1001
	J 1100	9-7	26	33	10,893	11,050	962
VI	H 1427	3-0	83	29	8,047	10,542	1077
	J 1008	11-8	27	32	9,448	9,598	965

The data were totaled by periods according to the type of silage fed and appear in Table 6.

The analysis of variance shows no direct effect of bacitracin on milk production while there is a significant (P = 0.05). decrease in production due to metabisulfite. There is, however, a significant reduction in production in the period following the feeding of either bacitracin-treated or metabisulfite-treated silage as compared with the untreated silage. The direct effects were significant at P = 0.025 while the residual effects were significant at P = 0.05.

BODY WEIGHTS: The cows were weighed for three consecutive days at the beginning of each experimental period and at the close of the experiment to determine the effects of the silages on body weight changes.

The percentage gain or loss of body weight has been analyzed for variance with the following results:-

Table 6.--Dry Matter Intake and Milk Production

	Period					
	1	2	3	4	Combined 1-2-3	4% F.C.M./lb. D.M.
	lb.					
Silage						
Untreated						
Silage D.M.	932.7	843.8	1124.6	1145.0		
Hay D.M.	192.6	196.6	196.6	207.1		
Grain D.M.	476.6	505.6	367.4	387.5		
Total D.M.	1601.9	1546.0	1688.6	1739.6	4836.5	1.21
4% F.C.M.	1903.0	2150.8	1799.8	1858.3	5853.6	
Silotracin						
Silage D.M.	873.1	1053.8	1059.6	1040.7		
Hay D.M.	192.6	196.6	196.6	198.1		
Grain D.M.	437.3	407.0	504.3	519.5		
Total D.M.	1503.0	1657.4	1760.5	1758.3	4920.9	1.21
4% F.C.M.	1967.1	1842.9	2140.1	2122.0	5950.1	
Metabisulfite						
Silage D.M.	993.7	1084.1	1048.2	1144.7		
Hay D.M.	192.6	196.6	196.6	199.4		
Grain D.M.	454.5	428.5	465.8	483.5		
Total D.M.	1640.8	1709.2	1710.6	1827.6	5060.6	1.11
4% F.C.M.	1841.5	1932.9	1856.6	1691.5	5631.0	

Analysis of Variance
4% F.C.M.

	Means			
Treatment	Direct Effect	Residual Effect	Period	Group
U	502.1	508.6	1	476.0
S	494.6	476.1	2	493.9
M	460.8	472.8	3	483.1
			4	490.2
L.S.D. 0.05	29.5	30.8	All	485.8

Dry Matter Intake

	Means			
Treatment	Direct Effect	Residual Effect	Period	Period Means
U	418.9	438.2	1	395.4
S	416.9	411.6	2	409.5
M	429.5	415.3	3	430.1
			4	451.7
L.S.D. 0.05	N.S.	19.4	All	421.7

Analysis of Variance
Body Weight Changes

Treatment	Means: Direct Effect	Means: Residual Effect	Period	Mean
U	- 0.3	+ 2.1	1	+ 1.2
B	+ 0.5	+ 1.3	2	+ 1.1
M	+ 2.3	- 0.9	3	+ 0.4
			4	+ 0.7
L.S.D. 0.01	1.6	1.8	All	+ 0.8

There is a significant difference at the 1 percent level in direct effects of metabisulfite-treated silage in comparison with untreated and bacitracin-treated on body weight changes – the cows increasing in weight while on metabisulfite silage. These residual or carry-over effects on body weight changes are also significant at the 1 percent level of probability between metabisulfite silage and the other two silages. In this relationship, however, the effect of metabisulfite has been to reduce body weight, while the residual effect of untreated and of bacitracin-treated silages has been an increase in body weight. Thus it appears that this particular metabisulfite silage had two specific physiological effects: 1) to abruptly depress milk production and 2) to increase body weight gains. Since the forage source differed from the other two silages, it is impossible to tell whether these effects arose primarily from the nature of the ensiled forage or the metabisulfite treatment. Based on previous findings it was judged to have resulted from the forage quality (5).

Experiment 3. Continuous Feeding Trial

To further test the effects of the three silages on milk production a continuous feeding trial was started immediately after the close of the Latin square design experiment previously described. Four cows were used in addition to those used in experiment 2. The cows were assigned to three groups to obtain similarly balanced groups and these in turn were assigned at random to the three silages as shown in Table 7.

They were fed hay at the rate of 5 pounds daily to Holsteins and three to Jerseys. The same grain mixture was fed at the beginning to all groups at the same rate as in the previous experiment and was then decreased at the rate of 2 percent each week during the experiment.

The experiment was concluded at the end of 36 days when the experimental silages were fed out.

Table 7. – Grouping of Cows Used on Continuous Silage Feeding Experiment

No.	Age	Days Fresh	Milk: Daily	Milk: Previous Lactation	Body wt.
Metabisulfite					
J 1008	11-8	89	34	9,598	935
1379	3-9	98	31	9,935	837
1405	3-4	30	32	7,756	793
H 1212	7-0	215	46	11,967	1265
H 1290	5-1	87	37	10,302	1167
Av.	6-2	104	36	9,912	999
Bacitracin					
1100	9-7	83	26	11,050	1013
1317	4-8	104	23	8,260	877
1406	3-4	56	34	9,568	852
H 1206	7-3	103	32	9,537	1253
H 1291	5-3	35	58	11,685	1205
Av.	6-0	76	35	10,020	1040
Untreated					
J 1184	7-10	51	40	9,957	835
1235	6-7	100	32	10,187	929
1393	4-5	83	34	9,949	886
H 1196	7-5	245	36	15,664	1185
1428	3-0	93	36	8,942	1040
Av.	5-10	114	36	10,940	975

The dry matter intake from silage, hay and grain and milk production are summarized in Table 8. That the variability of the animals within groups is too great for differences between groups to be significant with respect to either dry matter intake or milk production is shown by statistical analysis of the data. When both dry matter and milk production were related to a 1000 pound weight basis the significance of the data remained unchanged. When the 36-days data are divided into three 12-day periods there appear to be trends. Perhaps a longer continuous trial might have resulted in significant results.

Table 8.--Dry Matter Intake and Milk Production on the Continuous Feeding Experiment

Groups	(lb.)
Untreated	
Silage D.M.	3439
Hay D.M.	606
Grain D.M.	1450
Total D.M.	5459
4% F.C.M.	6982
lb. 4% F.C.M./lb. D.M.	1.27
Bacitracin	
Silage D.M.	3830
Hay D.M.	606
Grain D.M.	1268
Total D.M.	5704
4% F.C.M.	6221
lb. 4% F.C.M./lb. D.M.	1.09
Metabisulfite	
Silage D.M.	3256
Hay D.M.	600
Grain D.M.	1483
Total D.M.	5339
4% F.C.M.	5806
lb. 4% F.C.M./lb. D.M.	1.09

DIGESTION TRIAL

A digestion and nitrogen balance trial was conducted during experiment 3 using one Jersey cow of each group for excreta collection and using the technique described by Conrad *et al.* (4). The cows were fed twice daily as described under experiment 1. The essential data of the digestion trial appear in Table 9. The coefficients of digestibility of dry matter were the same within experimental errors confirming the findings of Miles (9). The digested dry matter per 1000 pound body weight was 24.5 pounds, 24.9 pounds and 23.8 pounds for the cows on untreated, bacitracin-treated and metabisulfite-treated silages respectively. The differences in per cent of protein digested appear to be too great to be explained as experimental error. The coefficients are 74.0 percent for untreated, 70.2 percent for bacitracin-treated and 67.6 percent for metabisulfite-treated silage. The nitrogen balances appear to reflect the effect of milk production, the losses of nitrogen being 22 grams daily for untreated silage and 11.7 grams for bacitracin while the cow on metabisulfite-treated silage produced less 4 percent F.C.M. and gained 8.4 grams nitrogen daily. The actual milk production shown in Table 9 was converted to 4 percent F.C.M. which was 43.1 pounds, 40.6 pounds and 32.0 pounds for the cows receiving untreated, bacitracin-treated and metabisulfite-treated silages respectively. All three cows were loosing body weight at about the same rate.

The yield of 4 percent F.C.M. per pound of dry matter eaten was 1.34, 1.39 and 1.14 pounds for cows on untreated, bacitracin-treated and metabisulfite-treated silages, respectively. The difference in efficiency between untreated and bacitracin-treated silages is not considered a real difference while the difference in efficiency between these two and the efficiency factor for metabisulfite-treated silage is considered an important difference.

DISCUSSION

Use of a 3 x 3 Latin square design experiment, replicated so that there was every possible sequence of one silage following another, made possible a determination of significance of the effects of three silages on milk production and on dry matter intake. Repetition of the third period made possible the determination of carry-over or residual effects by having each silage followed by itself.

A 36-day continuous feeding trial, with three groups balanced with respect to breed, age, weight, previous production and milk yield at the beginning of the feeding trial, was not a satisfactory experimental method for determining the effects of the silage upon

Table 9.--Digestion Trial Data (Daily)

	Silage			Alfalfa Hay		Grain Mix		Actual	D.M.	Protein	Nitrogen
Cow	Kind	Amount	D.M.*	Amount	D.M.	Amount	D.M.	Milk	Digested	Digested	Balance
		(lb.)							(%)	(%)	(g.)
J 1184	Untreated	80.0	17.2	3.0	2.6	14.2	12.3	36.1	68.4	74.0	−22.0
J 1406	Bacitracin	84.0	18.3	3.0	2.6	9.4	8.2	29.9	67.0	70.2	−11.7
J 1405	Metabisulfite	66.0	16.5	3.0	2.6	10.4	9.0	29.8	69.0	67.6	+ 8.4

*Dry matter

milk production and dry matter intake. The variability of the animals within groups was too great for significance of the differences between groups. Perhaps a longer experimental period might have resulted in significant differences.

The failure to use the dry matter content of the metabisulfite ration efficiently for milk production during the digestion trial was not due to lack of starch for utilization of the protein, since 32 percent of the dry matter of that ration came from the grain mixture while only 28.2 percent of the dry matter of bacitracin-treated silage ration came from grain. Furthermore, the cow fed untreated silage had 38.3 percent of her dry matter intake supplied in the form of grain and she produced less milk per pound of dry matter than the cow fed bacitracin-treated silage. Apparently some carbohydrate constituent other than starch and necessary for efficient use of nitrogen may be missing in silages. Another alternative interpretation would be that there was a deficiency of protein. The former suggestion seems more probable.

The cow receiving metabisulfite-treated silage was fed 109 percent of her requirement of digestible protein according to Morrison's (8) standard. The cow fed bacitracin silage was fed 150 percent of her protein requirement and that fed untreated silage was fed 155 percent of her requirement. There is some doubt then, whether 109 percent of the theoretical liberal allowance of digestible protein is adequate when most of it is supplied by fermented legume-grass silage.

The ammonia determinations made on the silage juices showed a consistent and appreciable increase with progression of time from ensiling to the time of analysis. This observation holds true not only in both treated silages but also in the untreated silage. The increase with time occurs both in the laboratory size silos and in the 10' x 40' silo. Thus it appears that the effectiveness of both bacitracin and metabisulfite decreases with lapse of time after ensiling.

Total nitrogen determinations on wet silage showed the same protein content of the untreated and bacitracin-treated silages (18.8 and 18.7 percent respectively on a dry matter basis) while the ammonia content of the respective juices show a marked effect of bacitracin in reducing protein deamination. Decreased ammonia formation in the metabisulfite silage is even greater, the ammonia of the juice being less per gram of protein — this may be due either to the effect of the metabisulfite or to the higher dry matter content of the material ensiled.

The lower protein content of the silages as determined by proximate analysis as compared to the analysis made on wet silage might be expected as the ammonia is volatilized at 100° C.

Only a small amount of the total nitrogen occurred as nitrate and nitrite nitrogen. Nitrate and nitrite determinations show great variability and tend to be higher in bacitracin-treated silage but the differences are not statistically significant.

1. Alfalfa-bromegrass was ensiled in laboratory silos at four moisture levels varying from 20.5 to 26.8 percent. (a) untreated, (b) treated with Silotracin at the rate of 5 pounds per ton (containing 5 grams zinc bacitracin) and (c) treated with sodium metabisulfite at the rate of 8 pounds per ton.

Twenty-one different analyses were made in duplicate or triplicate on each of the 12 lots of silage.

Moisture was determined by oven-drying and by Toluene distillation. Total nitrogen was determined by Kjeldahl on the wet silages. Sugar and carotenoids were determined on extracts of wet silage. The following determinations were made on silage juice:— pH, total acidity, amino acids, ammonia, amides, nitrates and nitrites, total steam volatile fatty acids, acetic acid, propionic acid, butyric acid and lactic acid. A proximate analysis was made on dried silages.

Metabisulfite depressed formation of total steam volatile fatty acids while bacitracin did not. The total acidity was greater in bacitracin than in metabisulfite silage except where excessive drying and resultant ammonia formation interferred. pH was in the same range for both silages except where excessive ammonia formation occurred. Acetic acid was significantly higher ($P = 0.05$) in bacitracin or untreated silages than in metabisulfite-treated silage. Carotenoids were significantly higher ($P = 0.10$) in bacitracin-treated than in metabisulfite-treated or untreated silage.

II. Untreated, zinc bacitracin-treated and sodium metabisulfite-treated silages were fed on a 3 x 3 Latin square design experiment with 14-day periods and with the third period repeated. This design was duplicated and the silages assigned to the cows represented all possible sequences of one silage following another and of each following itself permitting determination of direct and carry-over effects of silages on dry matter intake, milk production and body weight.

The direct effect of metabisulfite silage was significant ($P = 0.05$) reducing milk production below that of cows fed untreated or bacitracin-treated silage. There were carry-over effects ($P = 0.05$) by both bacitracin and metabisulfite in reducing milk production in the succeeding period while milk production rose in the period following the feeding of untreated silage.

Dry matter intake was greater (but not significantly so) when the animals were on metabisulfite silage. There was a significant (P = 0.025) depressing carry-over effect of both bacitracin and metabisulfite on dry matter intake in the succeeding period. Recoveries of the dry matter of ensiled material for the silages fed were 73.4 percent for bacitracin-treated and 62.4 percent for untreated silage.

Metabisulfite treatment of silage resulted in a significant (0.01) increase in body weight as compared to both untreated and bacitracin-treated silage.

Study of the chemical analyses of eight samples of silage collected during the feeding trial and digestion trial reveal the following facts:— Both bacitracin and metabisulfite reduced ammonia formation. Metabisulfite was a better inhibitor of protein hydrolysis than bacitracin resulting in a lowered concentration of amino acids. The effect of bacitracin was highly significant (P = 0.01). Bacitracin definitely inhibited production of steam volatile fatty acids (P = 0.05) while metabisulfite appeared to have a greater effect. Both bacitracin and metabisulfite reduced ammonia production and butyric acid—the effect of bacitracin being statistically significant (P = 0.05).

III. A continuous feeding trial was conducted for a 36-day period. Three groups of five cows each were assigned to the three silages. Neither dry matter nor milk production were significantly different due to source of silage. The feeding period was believed too short.

A digestion trial showed no marked differences in digestibility of dry matter from untreated and bacitracin-treated silages but did show marked differences in nitrogen balances reflecting level of milk production.

CONCLUSIONS

It is concluded that bacitracin may provide an effective preservative for reducing top spoilage in silos and may result in recovery of a higher per cent of the dry matter of the remainder of the silage; however, the dry matter recovered has no greater feeding value than that of untreated silage. Metabisulfite is likewise valuable in reducing top spoilage. The effectiveness of both will be increased by the use of an air barrier.

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