unknown. It seems that the inhibition is not due to a heat-labile substance. Kendall and Touchberry (*9*) showed that the inhibition of trypsin by soybean forage extracts increases as the seed begins to form. Borchers and Ackerson (*4*) reported the presence of a trypsin inhibitor in the seed of alfalfa; therefore, it seems possible that the inhibitory effect may be one of the causes of slow growth, obtained when high levels of alfalfa meal are included in chick rations.

A different result may be obtained from in vitro studies than under in vivo conditions. Hence, in vivo techniques will be required before the final answer to this problem is obtained.

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SILAGE EVALUATION

Polyunsaturated Fatty Acids in Legume-Grass Silage

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The effects of time after ensiling, presence or absence of preservative, and of crop ensiled on the linoleic, linolenic, and total long-chain fatty acids in legume-grass silage were studied employing laboratory silos (glass jars), concrete miniature silos, and a bunker-type silo. There appeared to be no major differences between silages with and without added preservatives as regards the polyunsaturated and total long-chain fatty acids in silages made in the three types of silos. The silage fermentation process caused no major change in the amount of polyunsaturated fatty acid in the total dry matter. However, the percentage of linoleic acid in the total fatty acid of silages was distinctly lower than that in the original forage at the time of ensiling.

THE HIGHLY UNSATURATED FATTY acids are major constituents of the saponifiable fraction of lipides extracted from green plants (*8, 10, 11*). Moreover, the presence of polyunsaturated fatty acids in alfalfa leaf meal (*6*) and in buckwheat leaf meal (*7*) has been established. As linoleic and linolenic acids are the principal polyunsaturated fatty acids in these green plants and meals, the consumption of such materials helps provide the fatty acids considered to be essential in the nutrition of farm animals. Increasing use of silages made from legumes and grasses emphasizes the need for data on the composition of this type of livestock feed. The paucity of information concerning the levels of the polyunsaturated fatty acids remaining in the silage after the fermentation has taken place led to the present investigation.

Experimental

Three types of silos, as described below, were employed in this study.

One-gallon glass jars were used as miniature laboratory silos for comparing silages treated with sodium nitrite (8 pounds per ton), calcium acetate (8 pounds per ton), and dried beet pulp (160 pounds per ton) with silage to which no preservative was added. Samples of freshly cut legume-grass forage (mostly clover) were mixed with appropriate preservatives and packed into jars which were then sealed with screw-cap lids and paraffin. Each treatment was replicated eight times and the bottles were stored at 75° to 80° F. Samples were taken for analysis at the time of ensiling and at 1, 4, 7, 14, 21, 28, 56, and 84 days thereafter.

Miniature concrete silos, 6 feet high and 2 feet in diameter, were used to study the effects of various silage preservatives added to chopped, nonwilted, legume-grass mixture. Sodium metabisulfite (8 pounds per ton), calcium formate (20 pounds per ton), cane sugar (10, 20, and 30 pounds per ton), dried beet pulp (160 pounds per ton), and ammonium acetate (8 pounds per ton) were tested in this experiment. Each preservative was employed in at least two silos and, for each trial, duplicate silos were filled with forage to which no preservative was added. The forage in each silo was tramped thoroughly during the filling operation and subsequently at daily intervals for 3 days, after which each silo was sealed with a plastic cover. Samples for analysis were taken at the time of filling and when the silos were emptied at times ranging from 4 to 10 weeks after ensiling.

A bunker-type silo (24 × 100 foot concrete slab with a 4-foot wall along one side) was filled with approximately 450 tons of chopped, nonwilted forage (mostly alfalfa with some brome grass), half of which was treated with ground corn (100 pounds per ton) as a preservative. The forage was packed thoroughly with a tractor during the filling operation and for an hour or more for each of 6 days after the silo was filled. Samples for analysis were taken at the time of ensiling and from various areas of the stack 22 and 31 weeks later.

Each sample taken was 1 to 2 pounds of forage or silage and was a composite representing either an entire miniature

silo or a particular area within the bunker silo. To help reduce sampling error, the particle sizes within each sample were reduced by chopping the silage with a large paper cutter. After thorough mixing and subdividing, duplicate 5-gram portions of each sample were transferred to Waring Blendor cups and were blended for 5 minutes with 180 to 200 ml. of a 3 to 1 alcohol-ether mixture. The contents of each cup were transferred to 600-ml. beakers, brought to a boil in a 70° C. water bath, and allowed to cool for approximately 5 to 10 minutes. The heating process was repeated and, after cooling to room temperature, the mixture was filtered with suction, the residue washed with alcohol-ether (3 to 1), and the filtrate diluted to 250 ml. with alcohol-ether (3 to 1). This extraction procedure removed 94 to 98% of the total lipides in silage.

Fifty-milliliter aliquots of the alcohol-ether extract were concentrated to approximately 5 ml. and then saponified with potassium hydroxide in a 60° C. water bath for 45 minutes. After cooling, the nonsaponifiable components were removed by three successive extractions with Skellysolve A. The remaining solution was acidified with 25% sulfuric acid (neutral red indicator) and the total fatty acids (long-chain types) were extracted with Skellysolve A. The extraction was repeated twice and the extracts were combined and diluted to 50 ml. Total fatty acids were estimated in 20-ml. aliquots (2 to 6 mg. of fatty acid) of the extract by the micro-oxidative technique of Boyd (*2*).

The polyunsaturated fatty acids were estimated by alkali conjugation by heating the acids from 10-ml. aliquots (1 to 3 mg. of polyunsaturated acids) with 6.5% potassium hydroxide in ethylene-glycol reagent for 30 minutes at 180° C. under nitrogen. The isomerization reagent was prepared by a slight modification of the method of O'Connor, Heinzelman, and Dollear (*9*). A Beckman Model DU spectrophotometer was employed to measure absorbance at wave lengths recommended for estimating dienoic, trienoic, and tetraenoic acids (*3*). The linoleic and linolenic acid contents were calculated from the equations developed by Brice and coworkers (*4*).

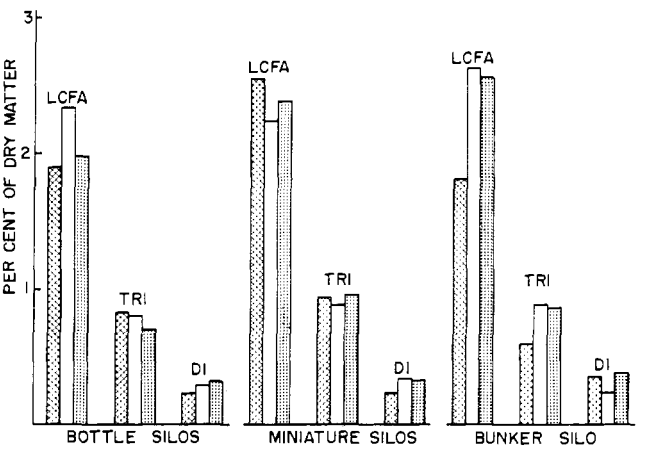

Figure 1. The long-chain fatty acid (LCFA), linolenic acid (TRI), and linoleic acid (DI) content of forage (as ensiled) and of silages (with and without added preservatives) in bottle, miniature, and bunker silos

Cross hatch graph: forage, as ensiled
Open graph: silage, no preservative
Dotted graph: silage, added preservative

Results and Discussion

As there appeared to be no appreciable differences in trends between the four treatments used in the bottle silo experiment, the data at each time interval for all treatments were averaged and are presented in Table I. Although trends in the levels of the acids studied were somewhat erratic, the long-chain fatty acid and linoleic acid contents tended to increase slightly. In the latter case, this was true both on a percentage-of-fatty-acid basis and a dry-matter basis. The apparent increases might be explained, at least in part, by the loss in fermentable dry matter during the fermentation process. There was considerable variation in the linolenic acid values, with values of most samples being somewhat below those for the samples as ensiled.

The linoleic, linolenic, and long-chain fatty acids decreased after 1 day in the jars and then increased after 4 days. The significance of the trends observed in this experiment is not clear.

Table II summarizes data relative to the percentages of linoleic and linolenic acids in the total long-chain fatty acids in the crops as ensiled and in the silages from the three classes of silos. The greatest variation appeared in the percentages of linoleic acid in the crops as ensiled; the values for the miniature silos were less than 50% those for the bunker silos. Moreover, the percentage of linoleic acid in the bunker silage (no preservative) was substantially lower than that for the other silages. As the various preservatives tested had no consistent effect on the polyunsaturated fatty acid content in the silage, all the preserved silage data for each class of silo were averaged. Ground corn was the preservative employed in the bunker silage and, as the lipide of corn is quite high in linoleic acid (*1*), the silage with added corn contained more linoleic acid than the silage to which no preservative was added.

Mean values for linoleic, linolenic, and total long-chain fatty acids for forages before ensiling and for the silages (with and without preservatives) from the three types of silos are shown in Figure 1. These data indicate the variability observed among the three types of silos. There appeared to be no consistent effect of preservative on the content of the acid fractions studied in the silages. However, in each type of silo, the linoleic acid content of the silage with added preservative was slightly higher than that for the forage as ensiled.

In the experiment with concrete miniature silos, a comparison of first and second cuttings of forage was made and these data are presented in Table III. Although a relatively small number of samples of forage (as ensiled) and of silage with no preservative were analyzed, the data indicate higher total long-chain fatty acid and linolenic acid values in the second-crop forage and silages. Whether these higher values might be attributed to the ratio of legume to grasses or to the specific cutting is not known as no accurate estimate of the

Table I. Effect of Time on Fatty Acid Contents of Forage Ensiled in Bottle Silos

Time in Silos, Days	Samples	Linoleic Acid		Linolenic Acid		Long-Chain Fatty Acids,
		DM[a], %	LCFA[b], %	DM[a], %	LCFA[b], %	DM[a], %
0	4	0.22	11.6	0.83	43.2	1.90
1	4	0.19	11.0	0.63	35.5	1.77
4	4	0.25	14.4	0.66	37.0	1.79
7	4	0.27	12.7	0.74	37.0	2.03
14	4	0.27	13.4	0.81	41.0	1.98
21	4	0.29	16.4	0.75	41.3	1.81
28	4	0.41	19.4	0.77	37.0	2.09
56	4	0.32	14.1	0.77	34.9	2.25
84	4	0.44	16.3	0.68	24.9	2.43

[a] DM, dry matter.
[b] LCFA, long-chain fatty acids.

Table II. Percentage of Linoleic and Linolenic Acids in Total Long-Chain Fatty Acids of Forage as Ensiled and in Silages

Material Analyzed	Type of Silo	No. Samples	LCFA[a], % Linoleic acid	LCFA[a], % Linolenic acid
Crop as ensiled	Bottle	4	11.6	43.2
	Miniature	3	8.8	37.0
	Bunker	5	19.4	32.8
	Mean		14.1 ± 1.0[b]	37.3 ± 2.4[b]
Silage, no preservative	Bottle	8	11.5	36.8
	Miniature	4	14.7	40.4
	Bunker	11	8.9	35.3
	Mean		10.8 ± 1.0[b]	36.7 ± 1.0[b]
Silage, added preservative	Bottle	24	15.8	35.8
	Miniature	20	13.0	39.0
	Bunker	11	14.2	34.6
	Mean		14.5 ± 0.8[b]	36.7 ± 1.3[b]
All silages		78	13.4 ± 0.7[b]	36.7 ± 1.0[b]

[a] LCFA, total long-chain fatty acids.
[b] Mean value ± standard error.

Table III. Comparison of Acid Contents of Forages in Concrete Miniature Silos

Crop	Material Analyzed	No. Samples	Dry Matter, % Linoleic acid	Dry Matter, % Linolenic acid	Dry Matter, % Long-chain fatty acids
First crop	Crop as ensiled	4	0.22	0.83	1.90
	Silage, no preservative	2	0.32	0.82	1.97
	Silage, added preservative	10	0.25	0.80	2.14
Second crop	Crop as ensiled	3	0.23	0.92	2.54
	Silage, no preservative	2	0.36	1.05	2.78
	Silage, added preservative	10	0.42	1.07	2.99

Table IV. Summary of Fatty Acid Content in Forage as Ensiled and in Silages

Material Analyzed	No. Samples	Dry Matter, %[a] Linoleic acid	Dry Matter, %[a] Linolenic acid	Dry Matter, %[a] Long-chain fatty acids
Crop as ensiled	12	0.28 ± 0.02	0.75 ± 0.06	2.03 ± 0.10
Silage, no preservative	23	0.26 ± 0.02	0.87 ± 0.04	2.47 ± 0.14
Silage, added preservative	55	0.33 ± 0.02	0.82 ± 0.03	2.30 ± 0.07
All silages	78	0.31 ± 0.02	0.83 ± 0.02	2.35 ± 0.07

[a] Mean values ± standard error.

percentage of legume in the mixture was made.

As there appeared to be no major differences among the various factors studied on the content (dry-matter basis) of the polyunsaturated fatty acids and total long-chain fatty acids in forages as ensiled and in silages with and without added preservatives, all of the data in this investigation were combined according to type of material and the results are summarized in Table IV. The total long-chain fatty acids (percentage of dry matter) were approximately 15% higher in silages (total of 78 samples) than in the original forages at the time of ensiling. This may be attributed primarily to a decline in fermentable dry matter during the fermentation period.

It is not clear, however, to what extent, if any, destruction of these acids (present as esters in the material analyzed) might have occurred. The linolenic acid content of silages with or without added preservative was higher than that in the crop as ensiled. The mean value for the 78 samples was about 11% higher than that in the forage. Although the silages without added preservative appeared to contain somewhat less linoleic acid than the crop when ensiled, the mean value for all silages combined indicated an increase of approximately 11% over the starting material.

As the total long-chain fatty acids showed a greater increase than the polyunsaturated fatty acids, it might be inferred that some degradation of these unsaturated acids occurred during the fermentation. Such a hypothesis is supported in part by the data in Table II which show, on the basis of percentage of total long-chain acid, a distinct decrease (compared to the original ensiled) in linoleic acid and a slight decrease in linolenic acid in the silages to which no preservative was added. There was a tendency for the percentage of linoleic acid to be higher in the silages with added preservatives. Moreover, the data (Table II) indicate a very slight decrease in the mean percentage of linolenic acid in silages with and without preservatives when compared to the crop as ensiled.

The percentages of linoleic (13.4%) and linolenic acids (36.7%) in the total long-chain fatty acids of silages reported herein are in rather close agreement with the content of the same acids in the triglyceride fraction and also the phospholipide fraction of dehydrated alfalfa leaf meal (*6*). These data also confirm the early observation by Hilditch and Jasperson (*5*), that the triethenoid carbon-18 acids exceed the diethenoid carbon-18 acids in mixed pasture grasses.

The data presented herein show that legume-grass silages of acceptable quality provide substantial amounts of polyunsaturated fatty acids to ruminants consuming these silages. In most instances, these products are at least as rich (on a dry-matter basis) in linoleic and linolenic acid moieties as are mixtures of fresh legume and grasses. Thus the fermentation process did not result in a drastic reduction in the polyunsaturated fatty acids in the silage lipides.

Further work is needed before the nature of the changes in the percentages of the polyunsaturated fatty acids that do occur during the fermentation process can be established. Also, it is not clear whether the changes are more pronounced in the triglyceride fraction or in the phospholipide fraction of the lipides.

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