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Effectiveness of an Aspergillus Oryzae Culture on the Preservation Efficiency and Feeding Value of Alfalfa-Brome Haylage Stored in Gas-Tight and Concrete Stave Silos

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BY

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

High-quality forage is essential in feeding dairy cattle if dairymen intend to increase milk production or to maintain a high level of production. Problems of preserving high-quality forage for the winter feeding of livestock occur each year. The preservation of legumes and grasses as hay is governed by a number of factors: (1) curing weather, (2) stage of maturity of the crops, and (3) other factors such as equipment available and methods of handling the crop. The inability of dairymen to control these factors results in heavy nutrient losses in the hays during the curing process due primarily to leaching and shattering of leaves.

The development of a more satisfactory process of preservation has been the objective of investigators for many years. In recent years artificial dehydration of these crops has been investigated as a method of preservation. However, the large capital outlay and high operating costs nearly eliminate it from consideration of the average dairymen.

Preservation of these crops in silos seems to be the most logical solution to the problem. Direct-cut silage has not been entirely satisfactory due to low quality and rather high preservation losses. If these crops can be preserved as low-moisture silage and stored irrespective of the weather, the common haymaking losses will be greatly reduced because the crop is exposed to less weathering during the curing process. With the trend toward farm mechanization, a feeding program utilizing low-moisture silage appears quite popular. Therefore, it was desirable to investigate the possibility of storing low-moisture silage in concrete stave silos and to determine the effects of an enzymic mixture in the preservation and feeding value of such ensilage.

REVIEW OF LITERATURE

Difficulties in obtaining silages of consistent high quality from direct-cut, high-moisture hay crops have led to the wilting of such crops prior to ensiling. Many investigators (16, 18, 23, 37) have reported that silage quality and preservation can be improved by wilting immature hay crops to 65 to 70 per cent moisture. Wilting is now one of the generally accepted ensiling procedures. Reduction of the moisture content below 65 to 70 per cent has not been generally recommended because of the common conception that there will be greater heating and molding due to the difficulty of air exclusion from the more porous silage mass. In Italy forage containing only 35 to 45 per cent moisture was successfully ensiled using the Crema Process (24). The Crema Process excludes the air by weighting the silage surface with 600-2000 lbs. per square yard. Perkins (20), on his dry matter scale, designated the range between 40 and 60 per cent dry matter as "no man's land." In this region he noted the possibility of making good silages but pointed out the difficulties of air exclusion. Woodward and Shepherd (37) reported success with 40 per cent dry matter ensilage. Hayden <u>et al</u>. (13) successfully made silages ranging from 17 to 35 per cent dry matter. Generally their results showed that the qualities of silage increased as the percentage dry matter increased within the limits of their studies. With the advent of gas-tight, glass-lined silos, the possibility existed of storing forage having less than 65 to 70 per cent moisture but without the inconveniences of the Crema Process. The term "haylage" is used to describe this type of ensilage having a moisture content of less than 55 per cent and usually between 35 to 50 per cent moisture.

Little information is available concerning the effect of the crop moisture content on preservation efficiency and silage quality when gas-tight silos are used. Gordon _et al._ (11) reported in 1951 that storage of haylage and slightly wilted silage appeared practical in gas-tight silos. Shepherd _et al._ (25) reported a favorable comparison of wilted silage stored in gas-tight silos and in concrete stave silos. Haylage was compared with wilted silage stored in gas-tight silos, also. Storage losses of dry matter were less than that occurring in wilted silages, and the losses were no higher than expected from dried, field-cured hay. Voelker (32), in a later report, observed no visible loss from spoilage in haylage stored in gas-tight silos and a total weight loss of 2 to 7 per cent. However, Shepherd _et al._ (25) reported a loss of dry matter in the field during wilting of the forage that was made into wilted silage and haylage of 6 and 13 per cent, respectively. This led to their conclusion that there was no particular advantage of extending the field-curing period beyond the slightly wilted stage when the differences in field losses are considered. A later report by Gordon _et al._ (10) indicated that weight loss during storage of haylage tended to be less than the dry matter loss, as any water formed by fermentation or respiration remained in the haylage.

Information concerning the effects of wilting hay crops to less than 65 per cent moisture and storage in concrete stave silos is extremely limited. It has been generally accepted that anaerobic conditions must be maintained to obtain silage of high feeding value (15). Due to the difficulty in obtaining an anaerobic medium in concrete stave silos, with silage below 65 per cent moisture, this level was considered the minimum moisture content at which high-quality silage could be obtained (4). Below 65 per cent moisture it was believed that any beneficial results obtained would be offset by increased spoilage and nutrient loss due to mold growth and heating. Investigators (5, 15, 30) have demonstrated that maximum temperatures, ammonia nitrogen, and butyric acid are higher in aerated silages than in nonaerated silages. Langston <u>et al</u>. (15) reported that silage which was aerated, by the introduction of air into the silo, had an abnormally high butyric acid content. Silage that was sealed immediately after ensiling did not develop butyric acid and was of superior feeding value. Differences in the oxygen content of silages may be produced by variations in such factors as density, length of cut, moisture content, and speed of filling the silo (4). Experiments have indicated the difficulty of separating oxygen effects from those of density, moisture, and other factors which may affect exclusion or infiltration of oxygen into silage (30). The presence of oxygen in the silage mass, following ensiling, is usually accompanied by increased silage temperatures. Olson and Voelker (19) reported a maximum temperature of 122°F. when alfalfa silage was stored in a concrete stave silo. Bender <u>et al</u>. (5) reported temperatures as high as 160°F. in haylage of 23 to 55 per cent moisture while direct-cut silage reached temperatures of 109°F. They recommended that partially dried grass be ensiled first where leachings from the greener material and greater pressure will exclude the air and preserve the silage. However, Rogers and Bell (23) reported that dry matter limits do not seem to exist as they stored haylage at 86.6 per cent dry matter. Such haylage had a fragrance not associated with hay at the same dry matter content stored in the mow.

An improvement in silage feeding value by wilting as compared to direct-cut silage has been reported by many workers (9, 18, 23, 37). Such improvement has not been invariably obtained (36). Many investigators have reported dry matter consumption to be lower while feeding wilted silage than when feeding hay from the same crop (12, 14, 29). However, Shepherd _et al._ (26) ensiled alfalfa at 40 per cent dry matter and compared it with alfalfa hay from the same stand. The wilted alfalfa silage was more palatable and sustained milk production and body weight better than did the hay. Substitution of wilted alfalfa silage for alfalfa hay in a ration on an _ad libitum_ basis has, in most cases, resulted in reduced milk production and live weight gains (6, 14). Shepherd _et al._ (26) reported that equal production resulted when the displacement was made on an equalized dry matter basis.

Many reports are available demonstrating the improvement of silage chemical quality by wilting high-moisture hay crops to 65 to 70 per cent moisture. The applicability of these reports to lower moisture levels is not known. Archibald and Kuzmeski (2) reported that a high-moisture content is undesirable because of its association with butyric acid formation. Shepherd _et al._ (25) reported that wilted silage had a lower pH, a more complete utilization of reducing sugars, and a more general decrease in nitrogen-free extract than did haylage. This was interpreted as indicating less fermentation with a lower moisture content. Woodward and Shepherd (37) had previously noted that the extent and character of fermentation was affected by the moisture content of the forage. With an increased moisture content, the fermentation becomes more extensive and the greater the likelihood that objectionable odors will develop.

It was generally believed that a pH of 4.2 was necessary for inhibition of the butyric acid bacteria to obtain a palatable silage (1, 35). Mikkin et al. (17) noted that green silage with a moisture content of 50 per cent developed only one-half to one-third the acidity normally concentrated in direct-cut silage. Archibald (1) reported that butyric acid develops at approximately pH 5.0 and at this pH lactic acid development is very slow. Investigators (10, 21, 37) have obtained high-quality haylage, with practically no butyric acid, in which the hydrogen ion concentration had increased to only 1×10^{-5}M. Gordon et al. (10) reported that the chemical evaluation of silages having a broad range of moisture contents requires more than pH measurements. They also noted that the pH as such gave little indication of differences in silage quality.

Some workers concluded that the growth inhibition of butyric acid bacteria in haylage is not caused by the hydrogen ion concentration but by the soluble compounds in the forage which are concentrated during wilting (8, 35). Richard (22) found that it was not the concentration of hydrogen ions but the concentration of undissociated lactic acid that was responsible for the growth inhibition of the butyric acid bacteria. Stolk (27) demonstrated, in experiments with pure cultures of clostridia, that the dry matter content as such had a growth-inhibiting effect. He found that when the cellulose content of the media reached 60 per cent, the growth of the clostridia was inhibited. DeMan (7) indicated that the osmotic pressure may be a factor in suppressing butyric acid bacteria. Wieringa (35) demonstrated that at a pH of fresh grass (6.5) a decrease in the freezing point of 3°C. suppressed the growth of the butyric acid bacteria. Butyric acid was produced in all silages in which the freezing point was decreased less than 3°C. This led to their conclusion that in wilted grass silage the osmotic pressure may play a considerable role in preventing butyric acid formation in the initial period of fermentation.

Rather consistent relationships have been noted between direct-cut silage and haylage with respect to other quality criteria (10, 33). Haylage contained about one-half as much ammoniacal nitrogen as was present in direct-cut silage indicating a lesser degree of protein breakdown. Each organic acid, except lactic acid, was less in haylage than in direct-cut silage. Acetic acid was predominant in direct-cut silage while lactic acid was the predominant acid in haylage. Archibald and Kuzmeski (2) have correlated a high-moisture level with a higher retention of carotene, increased butyric acid, low lactic acid, and a high-fiber content of the ensilage. They stated that the increased butyric acid and fiber content more than offset the advantage of carotene retention.

Early investigators (4, 13) proposed that the low content of fermentable carbohydrates, stage of maturity, and moisture content of alfalfa were contributing factors in the putrid, objectionable silage often obtained. It was generally believed that, in order to obtain high-quality silage, precautions must be taken to stimulate the formation of lactic acid as compared to the formation of butyric acid. Since many measures for promoting lactic acid development also assist the development of butyric acid, the best ensiling methods seemed to be those which inhibited the growth of butyric acid bacteria (8). Wieringa (35) listed the factors which are more harmful to the clostridia than to the lactic acid bacteria. These factors are: (1) low temperature (maximum 20 to 25°C.), (2) low pH (maximum 4.2), (3) high lactic acid content, and (4) high osmotic pressure. All of the methods of preservation are either directly or indirectly related to one or more of the above factors. Bender and Bosshardt (4) listed six methods which have been attempted and used to alleviate any deviation from the above factors and improve the quality of the silage. It is not known what effect these methods of preservation have on haylage. The recommended methods of preservation of silages were:

(1) The addition of sulfuric and hydrochloric acids to increase the acidity of the ensilage to a pH where undesirable bacterial action is checked.

(2) Addition of fermentable carbohydrates which favor lactic acid development and give a natural rise in acidity.

(3) Inoculation of the ensiled material with a culture of lactic acid bacteria permitting a more rapid fermentation.

(4) The partial wilting to alter bacterial activity.

(5) The elimination of plant cell respiration by the replacement of entrapped air with carbon dioxide, thus the establishment of anaerobic conditions.

(6) The killing of the bacterial flora by sterilization.

Bender and Bosshardt (4) listed the variables which determine the quality of silage. These included: season, kind of crop, maturity, moisture, protein, length of cut, packing, and type of silo. The correlation of these variables with the quality of haylage is not known.

EXPERIMENTAL PROCEDURE

The objectives of this experiment were: (1) to determine the effects of an addition of an *Aspergillus oryzae* culture in the preservation of low-moisture silage (haylage); (2) to compare gas-tight storage with storage in an upright concrete stave silo for the preservation of haylage; and (3) to compare the relative feeding value of haylage from different storage methods and the effects of the addition of an enzymic mixture on milk production, dry matter consumption, and body weight changes of comparable animals receiving haylage from the different treatments.

Forage Used and Filling Procedure

First-cutting, immature alfalfa and brome were used as the forage for ensiling. This mixture contained 60 per cent alfalfa and 40 per cent brome. The alfalfa was in the early bud stage and the brome had just begun to head at the time of cutting. The forage was crushed with a rubber-roll conditioner soon after it was cut and left to wilt in the swath.

Two gas-tight silos and two concrete stave silos were filled on June 17, 1961. Each of the silos was filled as rapidly as possible with haylage from the same field. All of the haylage was chopped with a forage harvester to a 3/8-inch theoretical length, using sharp cutter knives. No manual or mechanical method of distribution was employed. All of the silos were filled until they contained at least 30 tons of haylage. One concrete stave silo and one gas-tight silo received the enzyme-culture plus its carrier at the rate of 10 pounds per ton of haylage. This addition was hand-fed into the blower to permit a more even distribution among the ensiled haylage. The average moisture percentage of the haylages as they were ensiled was as follows: gas-tight (enzyme-treated), 36.5; gas-tight (untreated), 40.7; concrete stave (enzyme-treated), 46.0; and concrete stave (untreated), 38.2. Both concrete stave silos were refilled on July 19, 1961, with haylage of comparable quality, with the enzyme-culture added to one silo again, in order to provide enough haylage for both feeding trials and to remove enough feed daily to prevent spoilage.

Enzyme-Culture

The culture which was added to haylage of one gas-tight silo and one concrete stave silo was composed of the following ingredients: *Aspergillus oryzae* culture on wheat bran, lactic acid forming bacteria cultured and dried on alfalfa, active yeast, dried citrus meal, and dextrose. This culture contained a mixture of enzymes, in which amylase and protease were predominant. Lactic acid forming bacteria had a potency of three billion per pound and 10 billion live yeast cells were present per pound of culture. This culture was carried in wheat red dog, so that 5 per cent of the finished product was made up of the culture. The finished product (culture and red dog) was hand-fed into the blower at the time of ensiling at the rate of 0.5 per cent by weight.

Chemical Samples

Samples were taken for chemical analysis on every third truckload of haylage before ensiling. A grab sampling technique was used to obtain a representative sample of the loads. During the out-feeding period a similar technique was employed and samples were obtained daily for the first two weeks and weekly thereafter. These samples were analyzed for the following constituents: dry matter, crude protein, ether extract, crude fiber, ash, carotene, and hydrogen ion concentration expressed as pH. The pH measurements were made on a water-haylage mixture and were determined by a Beckman pH meter. The dry matter constituents were analyzed by the Station Biochemistry Laboratory. Temperature changes of the haylage stored in the concrete stave silos were recorded daily for the first two weeks and weekly thereafter until they approached the environmental air temperature. These changes were obtained by inserting a sensitive dial thermometer at a depth of 2 feet below the surface in the center of the concrete stave silo.

Feeding Trial

The feeding trial for the milking cows was conducted for 84 days starting June 19, 1961. The trial for the heifers was conducted concurrently with the milking cow trial for the first 81 days. Milk production and body weight changes were observed for one month prior to the trial.

The milking cow trial consisted of 24 Holstein cows which were divided into four comparable groups based on initial milk production and body weight. Each group of six cows received the same haylage treatment throughout the entire experiment because shorter periods would probably not give adequate production and weight responses. Each cow was fed a concentrate mixture at the rate of 1 pound for each 3 pounds of milk produced. The concentrate mixture was composed of the following ingredients: ground shelled corn, 1700 lbs.; rolled oats, 1500 lbs.; soybean oil meal, 400 lbs.; linseed oil meal, 200 lbs.; wheat bran, 200 lbs.; steamed bone meal, 50 lbs.; and trace mineral salt, 50 lbs.

Body weights were obtained on all the cows at the end of the preliminary period and at the close of the experiment. An average initial and ending weight was obtained by weighing the animals for three consecutive days and using this three-day average. This reduced the chances of differences in relative amount of fill and allowed greater precision of this measurement.

The amount of haylage was limited so that the weighback would not exceed 20 per cent. Haylage was weighed for each group of cows and the cows were exposed to haylage at all times except during milking and grain feeding.

Milk production was recorded for each cow daily in each group. The butterfat percentage of the milk produced was obtained from the regular HIR testing program. All milk produced was corrected to 4 per cent butterfat. Production persistency was calculated every four weeks as a per cent of the previous four-week production.

The heifer trial consisted of 40 heifers which were divided into four groups according to breed and body weight. Each group was composed of 10 heifers and the average initial body weights were as follows: Group A, 1116 lbs.; Group B, 923 lbs.; Group C, 677 lbs.; Group D, 471 lbs. All groups of heifers were fed haylage free choice with approximately a 20 per cent weighback. Groups A and B received haylage as their sole feed. Groups C and D received haylage plus 3 and 5 pounds of the herd concentrate mixture, respectively. All groups received haylage twice daily, at which time weighback from the previous feeding was determined and the dry matter content adjusted to the original dry matter content of the haylage. Each group of heifers received each of the four types of haylage using a switch-over design because of the great differences in the initial body weights of the heifers. All heifers were weighed at the beginning and end of each three-week period. The weighings were made at approximately the same time each period which reduced the changes in relative amount of fill.

A mineral mixture of 25 per cent dicalcium phosphate and 75 per cent trace mineral salt was provided free choice to each group of heifers. All four groups of heifers were housed outside in large pens with constant access to water. They were checked each day for heat periods, injuries, and sickness.

RESULTS AND DISCUSSION

The results and discussion of this research will be presented in the sequence of chemical evaluation, temperature changes, pH changes, heifer feeding trial, and milk production trial.

Chemical Evaluation

Eighty samples were collected at the time of ensiling and as the haylage was removed during the feeding period. After collection, these samples were taken immediately to the Station Biochemistry Laboratory where they were stored until analyzed. The proximate analysis values for the dry matter constituents as ensiled and as removed are presented in Table 1.

The total difference in preservation efficiency of the dry matter constituents was very slight, irrespective of treatment or storage. A general decrease in N-free extract occurred during storage. This decrease probably represented a decrease in the simple sugar content of the haylage. However, absolute agreement between a decrease in N-free extract and a concomitant decrease in the sugar content is probably fortuitous. The crude fiber and ash values of each haylage increased during storage. This trend was probably only relative due to the decrease in N-free extract and other dry matter constituents. A decrease in N-free extract probably does not represent a total loss, as conversion of the simple sugars to short-chained fatty acids may have taken place. The fatty acids obtained from such a conversion may appear in the increased ether extract values of the haylage removed

Table 1. Average Chemical Composition of Haylage As Stored and As Removed

Type of silo	% Dry matter	% Ether extract	% Crude fiber	% Crude protein	% Ash	% N-free extract	Carotene (mcg/g)
Concrete stave (enzyme-treated)							
As stored	50.4	1.46	27.67	20.22	9.26	41.39	73.4
As removed	50.4	1.92	29.68	20.41	9.48	38.91	41.6
Change	0.0	+0.46	+1.61	+0.19	+0.19	-2.48	-21.8
Concrete stave (untreated)							
As stored	62.6	1.80	31.57	20.99	8.29	37.35	32.7
As removed	63.0	2.20	32.26	20.82	8.76	35.96	22.8
Change	+0.4	+0.40	+0.69	-0.17	+0.57	-1.49	-9.9
Gas-tight (enzyme-treated)							
As stored	65.0	1.51	29.28	20.44	8.61	40.16	38.9
As removed	65.6	1.96	30.26	20.24	8.94	38.60	31.2
Change	+0.6	+0.45	+0.98	-0.20	+0.33	-1.56	-7.7
Gas-tight (untreated)							
As stored	66.7	1.60	30.61	18.61	8.81	40.37	41.0
As removed	66.9	2.10	30.84	19.36	9.16	38.54	35.8
Change	+0.2	+0.50	+0.23	+0.75	+0.35	-1.83	-6.2

during the feeding period.

The gas-tight silos had a slight advantage in carotene retention. In the haylage stored in gas-tight silos, 82.5 per cent of the original carotene was recovered, whereas only 60.4 per cent of the original carotene was recovered in the haylage stored in concrete stave silos. Carotene is readily destroyed by heat and oxygen. The absence of oxygen and high storage temperatures in gas-tight silos probably contributes to the higher retention of carotene.

The enzyme-culture addition appeared to have little, if any, effect upon preservation efficiency. The enzymatic activity of *Aspergillus oryzae* has been utilized for many years in the commercial fermentation of sake and other liquors in the Orient. While the enzymatic activity of *A. oryzae* has been established (28), the effect of an addition of these enzymes to ensilage is not well understood. It seemed reasonable that the presence of anylase in the enzymic mixture may promote a more rapid breakdown of starch, thereby enhancing the growth of lactobacilli which would result in haylage of high quality. It appears that the addition of this enzymic mixture to haylage may not result in increased preservation efficiency of alfalfa. The limited content of fermentable carbohydrates in alfalfa, approximately 5 per cent, may be a limiting factor in the usefulness of an addition of this enzymic mixture. However, it is the opinion of the silage investigators (30) that the limited quantity of fermentable carbohydrates does not appear to be the limiting factor in fermentation of alfalfa silage as considerable amounts of carbohydrates remain after fermentation. Since haylage appears to undergo a mild fermentation, the effects of any addition to enhance fermentation would be reduced because the low-moisture content appears to be a limiting factor. Investigators (30) have demonstrated that the important carbohydrates involved in the fermentation of grass silages are fructose, glucose, and sucrose, either as such or in their osan form. They also believe that fructosan is the most important reserve carbohydrate and plays the dominant role in fermentation of grass silage. The difficulty in obtaining high-quality alfalfa silage may be linked with the absence of fructosan in this crop. It is not known whether fructosan is present in brome-grass or, if present, if the enzymic mixture obtained from _A. oryzae_ is capable of hydrolyzing this compound. In a preliminary experiment in the laboratory, it was found that the enzyme-culture addition which was added to the haylage was capable of hydrolyzing a 1 per cent solution of corn starch. However, the weight of the enzyme mixture plus its carrier approximately had to equal the weight of corn starch in solution in order to reduce Benedict's solution in one hour. At the rate of 10 pounds of this enzyme-culture mixture per ton of haylage, this amount may not have been sufficient to obtain any noticeable results in the dry matter constituents analyzed. If it is assumed that a sufficient quantity of amylase were present, the activity of amylase may have been retarded because optimum conditions for activity are not always obtained. While the addition of enzymic mixtures to ensilage offers many interesting possibilities, the results obtained concerning their effect on preservation efficiency did not show a conclusive, positive response in this research.

The enzyme-culture which was added to the haylage also contained lactic acid bacteria from silage sources. It is the opinion of investigators (30, 34) that the addition of cultures of lactic organisms has never been accompanied by tangible improvements in alfalfa silage quality, as the freshly chopped forage usually has a sufficient number of natural lactic acid organisms. The number of these organisms increases tremendously during the initial period of fermentation. Also, the bacterial flora in haylage may be of less importance than in silage as the fermentation is limited and the likelihood of objectionable odors and products to develop is also decreased. Bender et al. (5) reported lower bacteria counts due to the low-moisture content and that the types present were not typical of fermented silage but resembled types found on partially dried grasses.

The efficiency of preservation in gas-tight storage is of considerable economic importance as the high initial cost of these silos must be offset by reduced storage losses and/or increased feeding value of the haylage. Gordon et al. (10) reported that a loss of 4 per cent dry matter is about the minimum to be expected in haylage stored in gas-tight silos. In this experiment ample time probably was not provided to obtain any great differences in dry matter constituents. Any dry matter loss that occurred was probably more than total weight loss as any water formed by plant cell respiration and fermentation would probably remain in the haylage. However, it should be emphasized that these values represent the average composition for the initial period of fermentation and any advantage that may be assessable to gas-tight storage would be reduced because of the short period of storage.

Each of the haylages had a pleasant aroma. Since the feeding in this experiment was begun immediately after ensiling, spoilage was not a problem. It must be emphasized, however, that a sufficient quantity of haylage was removed daily from the concrete stave silos and top spoilage was nearly eliminated. There was no visible spoilage in gas-tight storage.

Temperature Changes

The changes in temperature of the treated and untreated haylage stored in the concrete stave silos are presented in Table 2. The untreated haylage reached a maximum temperature of 125°F. two days after ensiling while the treated haylage reached a maximum temperature of 115°F. in the same period of time. During the first seven days, after ensiling, the untreated haylage averaged 2°F. higher per day than the treated haylage. The temperature of the haylage stored in the gas-tight silos did not appear to rise above the environmental air temperature.

It is doubtful that the lower temperature of the treated haylage was due to any effects of the enzyme-culture addition. It is known that high temperatures persist for a longer period of time in porous, aerated ensilage (30). At the time of ensiling, the average moisture content of the treated haylage was somewhat higher than in the untreated haylage. This higher moisture content of the treated haylage probably enhanced compaction which initiated a more rapid exclusion of oxygen and decreased cell respiration. Measures were employed to promote compaction and reduce aeration. These measures included: (1) chopping

Table 2. Temperature Changes of the Haylage Stored in Concrete Stave Silos

Date	Enzyme-treated	Untreated
6/16/61	80°F.	80°F.
6/17/61	105°F.	110°F.
6/18/61	115°F.	125°F.
6/19/61	112°F.	115°F.
6/20/61	110°F.	110°F.
6/21/61	105°F.	110°F.
6/22/61	105°F.	100°F.
6/23/61	105°F.	98°F.
6/24/61	103°F.	95°F.
6/25/61	101°F.	98°F.
6/26/61	100°F.	100°F.
7/3/61	103°F.	95°F.
7/10/61	95°F.	98°F.
7/18/61	95°F.	95°F.
7/23/61	90°F.	91°F.

the haylage as short as possible, (2) rapid filling of the silos, and (3) other factors which provided a more nearly anaerobic medium, such as sealing the silo doors. It appears that the lower temperatures of the treated haylage stored in the concrete stave silo were the result of the higher moisture content of this haylage at the time of ensiling.

An effort was made to ensile all of the haylage at the 35 per cent moisture level. It was found that this was quite difficult to accomplish because forage at 35 per cent moisture dehydrates rather rapidly on good drying days, and the resulting forage may contain considerably less moisture. If the moisture content decreases to below 30 per cent, the field losses increase, and the advantage of making haylage is greatly reduced.

pH Changes

The pH changes for the haylage stored in the gas-tight and concrete stave silos are presented in Table 3. The pH was measured with a Beckman Model H2 pH meter. All of the pH measurements were made on a water-haylage mixture. Dilution blanks (99 c.c.) of distilled water were added to 11 grams of haylage and mixed in a Waring blender. A portion of this solution was then measured for hydrogen ion concentration. The treated haylage in the concrete stave silo tended to increase in hydrogen ion concentration at a slightly more rapid rate than the other haylages. This may have been due to some effect of the enzyme-culture addition in presenting the acid-forming bacteria with a more utilizable form of substrate. However, all of the haylages attained a pH of approximately 5.6 at the end of the five weeks, irrespective of storage or treatment. On July 24, 1961, the two concrete stave silos were refilled and the pH measurements again followed a similar trend.

The pH may be of less importance in the preservation of haylage than in silage. It has been assumed that a pH of 4.2 was necessary for inhibition of butyric acid bacteria to obtain high-quality ensilage (1, 35). However, high-quality haylage, with practically no butyric acid present, has been obtained at a pH of 5.5 (33). Investigators (10, 21, 37) have demonstrated that a high pH in haylage is less objectionable than in high-moisture silage, this concept being supported mainly by animal intake data. The pH per se probably gives less indication of haylage quality than the pH and moisture content combined. This quality criterion of high-moisture silage may not be applicable to haylage.

Table 3. pH Changes of the Haylage

Date	Gas-tight silos		Concrete stave silos	
	Enzyme-treated	Untreated	Enzyme-treated	Untreated
6/16/61 (filled)				
6/17/61	6.40	6.90	6.40	6.70
6/18/61	6.35	6.20	5.75	6.45
6/19/61	6.6	6.15	5.70	6.15
6/20/61	6.3	5.45	5.65	5.80
6/21/61	6.35	6.20	5.48	5.80
6/22/61	6.35	5.95	5.50	5.68
6/23/61	6.25	5.85	5.55	5.87
6/24/61	6.05	5.90	5.55	5.75
6/25/61	6.10	6.20	5.70	5.75
6/26/61	6.05	5.90	5.50	5.75
6/27/61	6.20	5.85	5.50	6.20
7/3/61	5.95	5.87	5.46	5.50
7/10/61	5.85	5.85	5.70	5.40
7/19/61	5.55	5.60	5.65	5.65
7/24/61	5.70	5.75	6.80	6.90
7/28/61	5.60	5.50	5.90	5.70
8/4/61	5.35	5.60	5.50	5.65
8/11/61	5.50	5.60	6.10	5.60
8/18/61	5.50	5.40	5.50	5.90

It seems logical that the great risk involved in the preservation of silage is that lactic acid conversion caused by the clostridia may keep pace with its production during the initial period of fermentation. This fermentation trend, in which clostridia convert lactic acid, probably occurs to a much lesser degree in haylage. Since the hydrogen ion concentration of haylage does not increase sufficiently to exert an inhibitory effect upon the clostridia, it is probable that other factors may cause this inhibition.

There still needs to be a better understanding of the effect of the dry matter content of the forage to be ensiled upon the fermentation processes occurring in the silage. Different degrees of fermentation may occur depending on the presence or concentration of the many variables, both known and unknown (1, 15). Wieringa (35) reported that grass silages having a high dry matter content inhibit the growth of various species of bacteria. Hence, any forage ensiled at a high dry matter content would tend to minimize the losses caused by bacteriological conversion. But this would only be true if the dry matter content is sufficiently high. Dykstra (8) reported that, in order to obtain these results, the forage should be homogenously wilted until the dry matter content has risen to at least 40 per cent. The concentration of dry matter probably can be adduced in explanation of the preservative effect. However, Rogers and Bell (23) reported that the degree of wilting is less of a factor in the preservation of silage than is the stage of maturity when wilting began.

It may be important that during wilting there is an increase in the concentration of readily fermentable carbohydrates, when concentration is expressed as a percentage in the original material. As this percentage increase occurs, haylage may have more favorable conditions for lactic acid development than direct-cut silage. This increased lactic acid content may not lead to a lower pH because there is also a percentage increase in protein. There could also be an increase in lactic acid with a corresponding decrease in other acids and the pH would not be altered.

__Heifer Trial__

Forty heifers were divided into four comparable groups and fed haylage free choice. To insure the presence of haylage before the heifers at all times, each group was offered an excess of haylage so that there was approximately a 20 per cent weighback. The weighback was similar in proportion of stems to the haylage fed and appeared edible. Each group of 10 heifers received each of the four types of haylage, using a switch-over design, because of the great differences in initial weights of the heifers. The average initial and final weights per heifer of each group are given in Table 4.

Table 4. Weight Gains of the Heifer Groups

Heifer group	Days	Grain per day	Initial weight	Final weight	Gain	Average daily gain
				Pounds		
A	81	0	1116	1276	160	1.98
B	81	0	923	1065	142	1.75
C	81	3	677	849	172	2.12
D	81	5	471	630	159	1.96

The growth rates for all the groups are above the normal expected gains as given in growth standard tables. Group A heifers were heavy springing heifers, which explains their relatively good gains without grain. Group C heifers were not as fat as the other heifers at the beginning of the trial, which may explain their relatively large gains. However, it should be emphasized that the feeding periods were too short to give a true indication of growth, but an indication of consumption and short-term gains from haylage was obtained. It is recognized that great variation may occur in the short-time gains in heifers. However, the differences in the ages of the heifers were so great that the groups could not be subdivided differently. Also, by feeding each type of haylage to each animal, differences between animals in gaining ability were not confounded with the treatment effects.

The dry matter consumption and weight gains of the heifers receiving the different types of haylage are presented in Table 5. The average daily gain of heifers while receiving the enzyme-treated haylage was 1.99 lbs. per day and 1.92 lbs. per day while receiving the untreated haylage. While all the heifers received a preliminary adjustment period, all of the groups made their largest weight gains the first period. A percentage of this gain was probably due to the differences in the relative amount of fill. The total differences between the enzyme-treated and untreated haylage were very slight and within the magnitude of experimental error. The average daily gain for the heifers while receiving haylage from the gas-tight silos was 1.87 lbs. per day and 2.01 lbs. per day while receiving haylage from the concrete stave silos. This difference is more likely an expression of the amount of fill of the Group A heifers during the first period of feeding rather than a difference in preservation efficiency.

The acceptability of haylage by the heifers was very satisfactory. The average dry matter consumed per pound of gain was 10.3 lbs.

Table 5. Dry Matter Consumption and Weight Gains of the Heifers

	Gas-tight silos		Concrete stave silos	
	Enzyme-treated	Untreated	Enzyme-treated	Untreated
		Pounds		
Weight gains:				
Group A	45	44	71	0
Group B	11	32	49	50
Group C	48	33	22	69
Group D	43	47	34	35
Total weight gained	147	156	176	154
Average daily gain	1.81	1.93	2.17	1.90
Dry matter consumed:				
Group A	4452	5072	4383	4620
Group B	4645	4386	4137	4123
Group C	3906	4216	4136	4158
Group D	3757	3024	3253	3626
Total consumed	16760	16698	15909	16527
Daily per heifer	20.7	20.6	19.6	20.4
D.M. per lb. gain	11.4	10.7	9.1	10.7

and 10.7 lbs. for the treated and untreated haylage, respectively. There appeared to be about 4 per cent greater feed efficiency of the haylage that contained the enzymic mixture than the untreated haylage which may have been due to slightly better preservation of this haylage. The average dry matter consumed per pound of gain was 9.9 lbs. for haylage stored in the concrete stave silos and 11.0 lbs. for haylage stored in the gas-tight silos. A slight advantage may be assessed to the haylage stored in the concrete stave silos as the usual advantages of gas-tight storage were not obtained in this experiment. However, it must be emphasized that the haylage was fed immediately after ensiling

and any advantage of gas-tight storage would be reduced.

At the present time it appears that haylage is an excellent feed for developing large body capacity in heifers because they consume it in large quantities. Several experiments indicate that a positive relationship exists between the dry matter content of the silage and dry matter consumption by dairy animals (13, 23, 37). It is generally conceded that wilted alfalfa silage has limitations as a feed for dairy cattle. Most alfalfa silages contain some factor(s) that reduces the live weight gain and voluntary intake. This effect is positively related to the moisture content of the silage, but it is not the water content per se (30). However, it is accepted that the water content of the crop at the time of ensiling does have considerable to do with acceptability (23, 30). Therefore, the factor(s) involved must develop during the fermentation process which takes place following the ensiling of the crop. The severity of these factors is probably related to the extent and/or type of fermentation that occurs in the silo. Since the acceptability of alfalfa silage increases as its characteristics approach that of hay (10, 11, 23), the explanation may be adduced that partial drying alleviates the effect of the reduced voluntary intake factor(s). In essence, this is one of the most important advantages of making alfalfa haylage as compared to alfalfa silage.

<u>Milk Production Trial</u>

Twenty-four Holstein cows were divided into four comparable groups based on body weight and initial milk production. Each group of six cows received the same haylage throughout the entire feeding trial. The cows were fed 1 pound of grain for each 3 pounds of actual milk produced. The same haylages were tested with the cows as with the heifers. The results of the milk production trial are summarized in Table 6.

The acceptability of the haylage by the cows was very satisfactory. The total differences in dry matter consumption were very slight, irrespective of treatment or storage. The cows which were fed the enzyme-treated haylage from the gas-tight silo consumed slightly more dry matter per day than any of the other cows on the trial. It is not known whether this was due to any effects of the enzyme-culture addition or if it was a summation of individual cow differences.

There was a tendency for all the cows to gain weight, regardless of the type of haylage fed. The cows on the enzyme-treated haylage gained an average of 18.5 lbs. more weight in 12 weeks than did the cows on the untreated haylage. Although haylage seemed to stimulate weight gains among the cows, it did not appear to stimulate milk production.

All milk produced was corrected to 4 per cent butterfat to permit a more accurate evaluation of the haylage fed. Milk production was maintained remarkably well in all the groups as indicated by the relatively high persistency values. The actual milk production trends are presented in Figure I. The cows on enzyme-treated haylage produced approximately 6 per cent more actual milk than did the cows on the untreated haylage. However, this may have been due to the higher initial production which was maintained throughout the trial. Also,

Table 6. Dry Matter Consumption, Body Weight Changes, and Milk Production of the Cows While Fed Haylage for 12 Weeks

Items compared	Gas-tight silos		Concrete stave silos	
	Enzyme-treated	Untreated	Enzyme-treated	Untreated
Feed dry matter consumed (lb./cow/day)				
Haylage	26.9	24.9	23.9	25.5
Concentrate	11.7	10.6	11.3	10.5
Total	38.6	35.5	35.2	36.0
Daily F.C.M. per cow (lb.)				
Initial production	40.5	39.6	39.9	38.9
Average production	34.7	32.4	34.1	32.1
Decline (12 weeks)	12.4	12.7	13.6	10.6
Decline (%)	27.0	30.0	30.6	25.4
Average persistency (%)	89.8	88.0	89.2	89.2
Dry matter per lb. F.C.M.	1.11	1.09	1.03	1.12
Average live weight (lb.)	1479	1466	1453	1440
Average daily live weight change (lb.)	+0.99	+0.71	+1.06	+0.73

cow number 2232 contacted mastitis and had to be replaced by cow number 2309 which accounts for the decreased daily production, during the eighth week of the trial, of the cows receiving the untreated haylage from the concrete stave silo. In a final evaluation, there was probably little, if any, advantage of the enzyme-treated haylage over the untreated or any great differences between gas-tight and concrete stave storage in this trial.

It is interesting to note that there was a tendency for the cows to reduce the butterfat content in their milk during the course of this trial. The monthly butterfat percentage of the milk produced is given

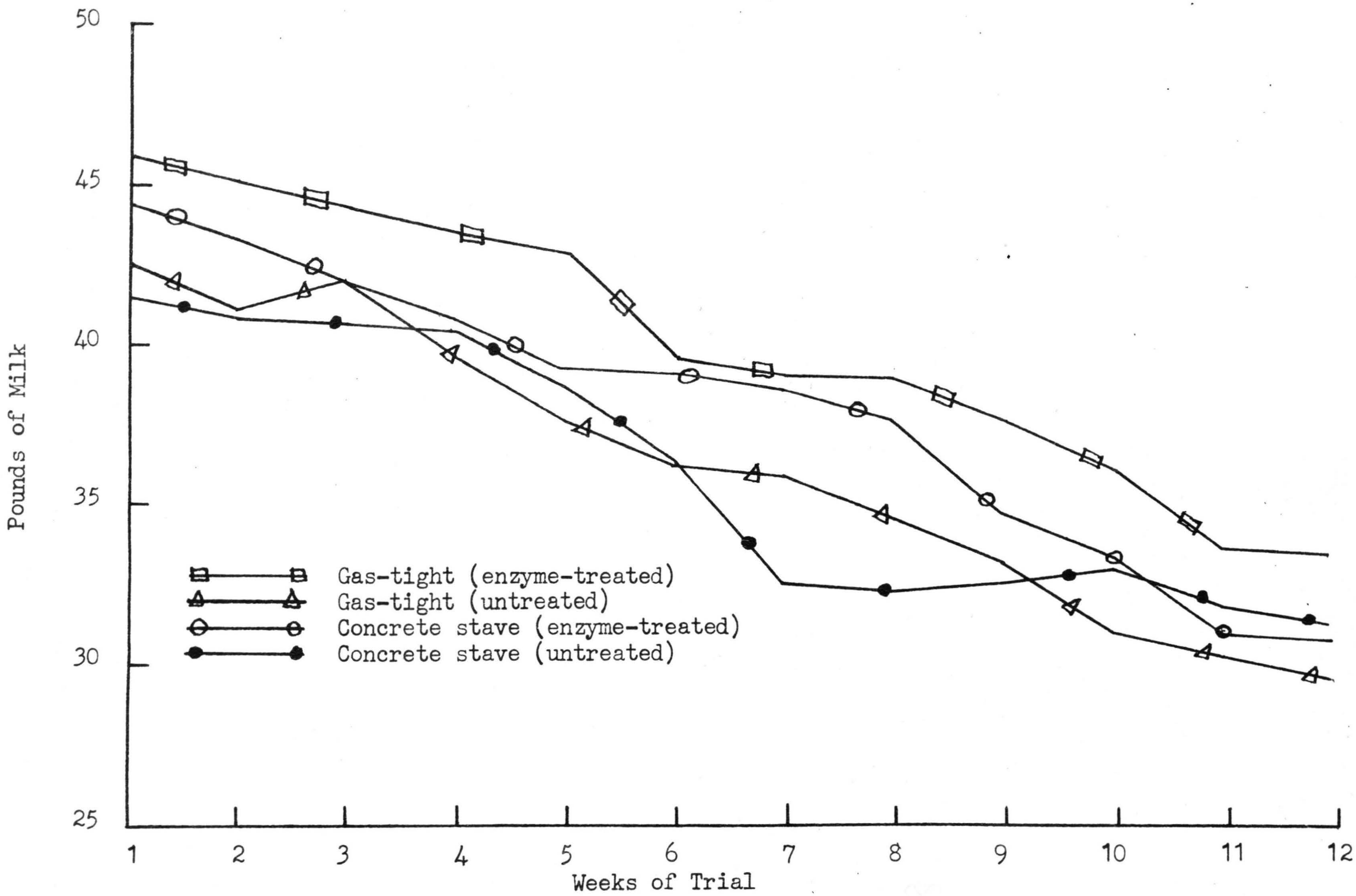

Figure I. Daily milk production unadjusted for butterfat content in Figure II. The remainder of the herd in 1961 and the same cows in the summer of 1960 were used for comparison. The butterfat content in the milk of the cows on trial during May (pretrial) was 3.61 per cent and it reached a minimum of 3.13 per cent during the month of July. In September (post trial), the butterfat content in the milk of the cows on trial was 3.95 per cent. The other groups did not appear to follow a similar trend. It is likely that high environmental temperatures during the month of August would have a tendency to decrease the butterfat content of the milk. However, this trend was not nearly so evident with the remainder of the herd which did not receive haylage. It is known that finely ground or restricted roughage rations, when fed to cows, tend to decrease the fat content of the milk (3, 31). Chopping the haylage as short as possible to obtain the advantage of air exclusion may have been a contributing factor in decreasing the butterfat content of the milk produced.

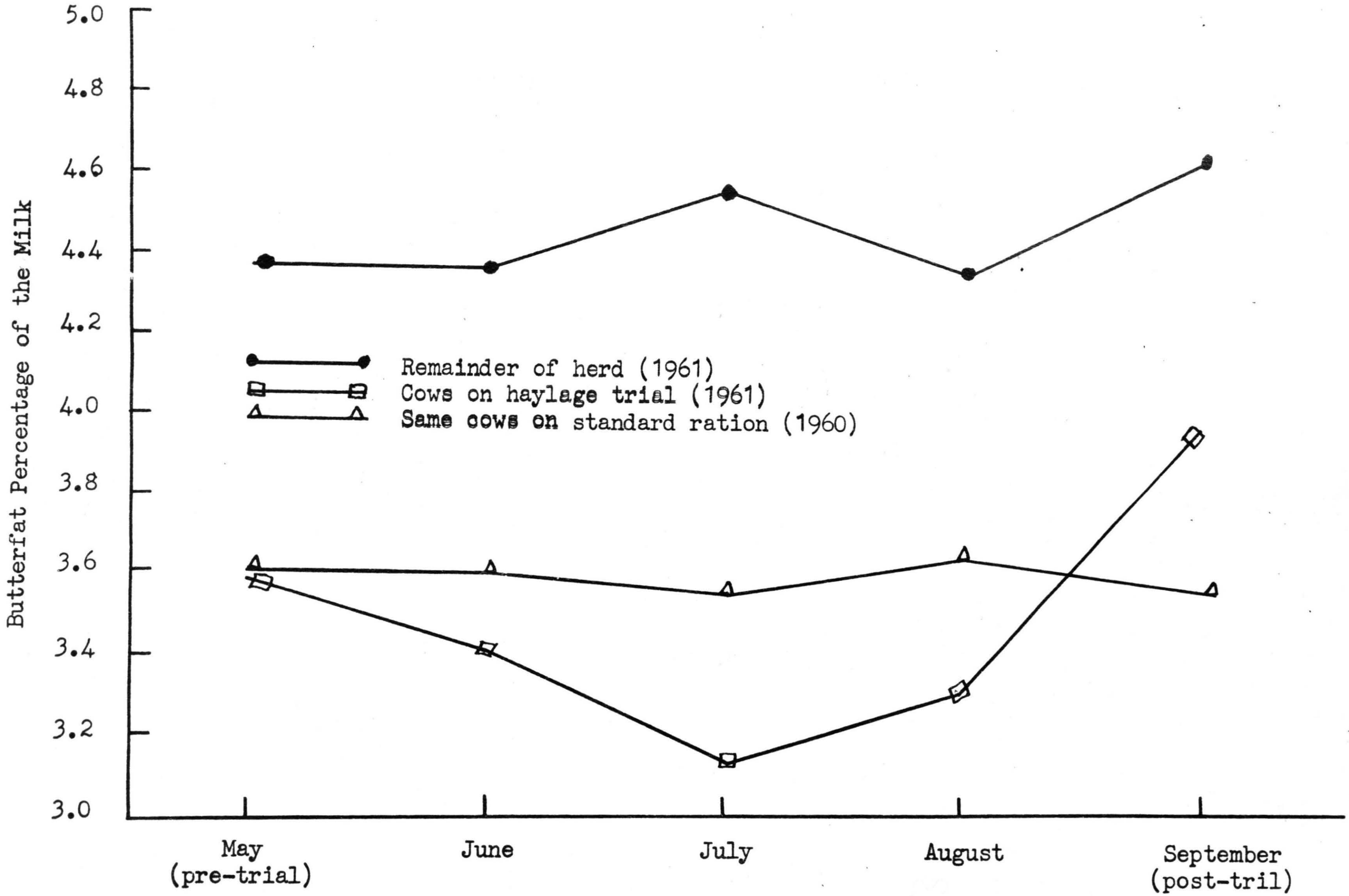

Figure II. Monthly alteration of the butterfat percentage of the milk produced

SUMMARY

Alfalfa-brome haylage, 33.3 to 49.6 per cent moisture, was preserved in two gas-tight silos and two concrete stave silos. One gas-tight silo and one concrete stave silo received an enzymic mixture obtained from *Aspergillus oryzae*. The enzymic mixture plus its carrier was hand-fed into the blower at the time of ensiling at the rate of 10 pounds per ton of haylage. The haylage was fed for 84 days to four groups of six cows each. Four groups of 10 heifers each were fed each of the haylages using a switch-over design.

Upon chemical evaluation, it appeared that the addition of the enzymic mixture had little, if any, effect on preservation efficiency. The preservation of all the haylages was very satisfactory, irrespective of storage or treatment. The pH trends were similar in each of the four haylages. All of the haylages attained a pH of 5.6 five weeks after ensiling. The untreated haylage stored in the concrete stave silo reached a maximum temperature of 125°F. The enzyme-treated haylage stored in the concrete stave silo reached a maximum temperature of 115°F. two days after ensiling.

The acceptability of all the haylages by the heifers and cows was very satisfactory. The average daily dry matter consumed from haylage was 25.4 lbs. and 25.2 lbs. by the cows receiving the enzyme-treated and untreated haylage, respectively. The average daily dry matter consumed from haylage by the cows receiving haylage from gas-tight storage was 25.9 lbs. and 24.7 lbs. for the cows receiving haylage

from the concrete stave silos. Milk production trends were similar for all the groups.

The heifers did not show any preference for any one haylage, regardless of storage or treatment. The average daily gain of the heifers while receiving the enzyme-treated haylage was 1.99 lbs. and 1.92 lbs. while receiving the untreated haylage. The average daily gain of the heifers while receiving haylage from the gas-tight silos was 1.87 lbs. and 2.01 lbs. while receiving haylage from the concrete stave silos. The daily dry matter consumed per heifer was similar, irrespective of storage or treatment.

CONCLUSIONS

1. While the addition of Aspergillus oryzae enzymic mixtures to alfalfa-brome haylage offers many interesting possibilities, such an addition did not give a conclusive, positive response in this research.

2. Alfalfa-brome haylage can be stored successfully in concrete stave silos if measures are employed to enhance a rapid exclusion of air and to prevent air infiltration.

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