THE INFLUENCE OF NITROGEN FERTILIZATION OF BROMEGRASS ON THE FLAVOR AND NITROGEN COMPOSITION OF MILK

by

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INTRODUCTION

The importance of feed flavor as a factor influencing per capita consumption of milk is recognized by the dairy industry. However, due to the nature of flavor and the lack of adequate tools to measure either its quantitative or qualitative character, little is known about its origin or chemical composition. In addition to these problems, the ill-defined nature of "feed flavor" makes it a very convenient classification to cover any questionable flavor defect in milk.

It was felt that there was a need for fundamental study of feed flavors in milk. This phase of the general study was an investigation of the flavor of milk produced on bromegrass with different levels and types of fertilization. Bromegrass was investigated because it is a widely used, economical pasture and induces a highly objectionable off-flavor in milk. Nitrogen fertilization levels were studied for two reasons. First, the nature of the flavor of milk produced on bromegrass can be described most accurately as being "fishy". This characteristic odor suggests that possibly amines, a nitrogen bearing class of organic chemical compounds, may be responsible. If this were so, the level of nitrogen in bromegrass could influence the composition of the milk. Second, workers at the Ohio State Experiment Station (3) reported that amines contributed to feed flavors in milk.

The objectives of this study were to establish:

1. Whether the types and level of nitrogen fertilization have any effect on the flavor of milk.

2. Whether there is a relationship between nitrogen fertilization and bromegrass composition and subsequent milk composition.

3. Whether there is a relationship between ammonium ion, amide, total nitrogen of bromegrass and the intensity of milk flavor.

LITERATURE REVIEW

Trout et al. (22) reported that cows on bromegrass pasture yielded milk with an objectionable flavor. This milk could not be mixed with other milk for marketing purposes. It was found that pasteurization did not improve the flavor of the milk. These workers reported that alfalfa-bromegrass pasture also produced an unpleasant flavor in milk. In this study, cows were milked three times a day. The interval between the morning and the noon milking was shorter than the interval between noon and the evening milking. The cows were removed from the pasture at night. It was suggested that the longer interval accounted for the more intense off-flavor of the evening milk. Bromegrass pasture by itself did not impart consistently the same level of feed flavor to the milk.

In another study, Foreman et al.(7) reported that milk produced from cows on bromegrass had an undesirable, unclean flavor. However, they could not detect an off-flavor in the milk immediately after it left the udder of the cows.

Colson (4) indicated that bromegrass produced a feed flavor in milk to which consumers objected. He stated that, according to a trained test panel, the flavor of milk produced on bromegrass pasture was not improved by vacuum treatment. Removing the cows from bromegrass pasture four hours before milking did improve the flavor, according to the taste panel.

Consumer reactions to bromegrass milks indicated an improvement in milk flavor with various treatments. Colson reported differences between control milk and milk produced on bromegrass pasture, as measured by the reactions of both the taste panel and consumers.

Analysis of Milk for Low Molecular Weight Nitrogen Compounds

Choi et al. (2) reported that ammonia was present in fresh milk and its products. They stated that ammonia could be determined quantitatively in milk by a colorimetric method after deproteinizing the milk with sodium tungstate in sulfuric acid solution. The levels of ammonia were found to vary from .3 - 1.3 µm. per hundred ml. of fresh milk.

Cole et al. (3) reported the presence of ammonia and volatile amines in milk possessing a feed flavor. These workers employed gas chromotography to analyze the solvent extract of acid whey from milk with feed flavor. The separation of ammonia and amines was accomplished by temperature programming on an undecanol liquid paraffin column. The identification of amines was accomplished by comparing the retention time and retention volume for the unknown material with that of known amines. The confirmative results obtained by infra-red spectroscopy revealed that milk contained ammonia, methylamine, ethylamine, n-propylamine, n-butylamine, and n-hexylamine. The quantitative determination of amines was accomplished by collecting the eluted amines from the column in distilled water which was adjusted with .01 N sodium hydroxide to the phenol red end point. As the amines were collected in distilled water, continuous titrations were made with .01 N hydrochloric acid and burette readings were taken at one minute intervals to determine the amount of amines eluted. They found that the ammonia content ranged from 0.3 to 3.0 µm/10ml in good flavor milk and from 0.9 to 2.5 µm/10ml in feed flavor milk.

Hexylamine ranged from 0.5 to 1.7 μm/10ml in good flavor milk and from 0.7 to 2.4 μm/10ml in feed flavor milk. Methyl and ethyl amines ranged from 1.7 - 2.4 μm/10ml for each in feed flavor and .5 to 1.7 μm/10ml in good flavor milk. Butyl amine ranged from 1.6 to 2.2 μm/10ml in feed flavor milk and from .5 to 1.7 μm/10ml in a good flavor milk.

Effect of Fertilizers on Bromegrass

Eggleton (6) found that ammonia salts could be utilized directly by bromegrass when applied to the soil in the salt form. He stated that the nitrogen in the plants was increased when the plants were treated with nitrogen. At the end of the experiment he observed that the proportion of amide nitrogen in the plant on all of the treated plots was substantially lower than in the plants on the control plot.

Carey et al. (1) reported that the total nitrogen of bromegrass was increased readily by applying ammonium salts to the soil. They observed that there was a steady decline of the ammonia, amide, and protein as the bromegrass matured. In early stages of bromegrass growth, the total nitrogen increased in direct proportion to the ammonium salts which were applied to the soil. The ammonium content of bromegrass varied with the kind of nitrogen fertilizers which were utilized. Amide content of bromegrass also showed an increase when nitrogen fertilizers were applied to the soil.

Kohler (12) reported that young grasses which were richer in protein than mature grasses were also richer in carbohydrates, carotene, vitamin C, thiamine, riboflavin and other nitrogenous compounds. Pirie (17) reported that the amount of nitrogen in the plants was affected by such climatic conditions as temperature, humidity, and rainfall.

Steward and Pollard (21) observed that heavy rainfall leached some of the water soluble nitrogenous compounds from the plants and from the soil.

Flavor Analysis

Westfall et al. (24) reported that the motivation of people towards marketable products depended mostly upon the psychological behavior of the individual and the traditional ways of life. They observed that the structured interview* was not efficient enough to obtain good results from the interviewers to produce good results, but they gave different results. He observed that, in comparing the two techniques, the structured interview produced higher proportion of responses about a given subject area than the unstructural questionnaires, because the former forced an answer and the latter did not.

Harrison and Elder (9) reported the panel should be trained before starting the judgment, so as to give enough time for the taster to become acquainted with the quality of the products. They observed that the psychological effect was an important factor in the taste panel to produce good results.

Peryam (15) observed that a trained panel was able to discriminate the quality of a food much better than an untrained panel. He stated that it was easy to conduct the analysis with few people on the panel. It was found that hunger made the individual much more sensitive towards the odor of the food

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*Structured interview utilizes a form questionnaire compelling responses to direct questions, whereas unstructured interview utilizes essay responses of general areas in question.

products. Krum (13) confirmed that a trained panel produced satisfactory results in judging food products. He used the paired comparison and the t-test to analyze his data and to determine its significance.

Peryam and Swartz (16) reported that the triangular taste test was psychologically difficult, but they found it gave a greater precision in many instances. They observed that the test had an advantage of being easier to conduct and requiring less time and that when detectable differences existed, few judgments were required to obtain statistically significant results. Roessler et al. (19) reported that the triangular taste test alone was reliable to predict the consistency of a taster for certain quality of food product.

Crist and Seaton (5) reported that results of taste tests alone were not satisfactory to measure the quality of a product. They observed that accurate results, increased sensitivity and repeatability were obtained in combining taste, scent, and inspections. They found that a rank correlation was much better than a linear correlation in the analysis of the taste panel.

Ishler et al. (10) reported that organoleptic testing was characterized by a diminishing ability of the taster to evaluate small differences in flavor as testing was prolonged. It was observed that fatigue was prominent in testing of some types of food while it was not prominent in others.

In another study, Ishler et al. (11) reported that bias in coding could be minimized in either of two ways:

1. By reversing codes between halves of each test in order to cancel out the bias.

2. By using only codes for which no appreciable bias has been found.

EXPERIMENTAL PROCEDURES

A study was designed to establish the effect of different kinds and amounts of nitrogen fertilizers on certain properties of milk. Four groups of cows which were randomly selected were placed on separate and fenced bromegrass pastures, which were fertilized with different levels and kinds of commercial nitrogen fertilizers. Chemical analyses were made weekly on the soil, bromegrass plants and milk in order to follow the distribution of the nitrogen fractions in these systems. The flavor of the milk obtained from groups of cows on the pasture was evaluated daily. Climatic conditions, as rainfall, temperature, humidity were obtained from the University Weather Station in the Department of Physics.

Preparation of the Pasture

In the early spring of 1961 a selection of the University Farm bromegrass pasture was divided into four 1.26-acre plots. Each plot was fenced and fitted with a gate. A chemical analysis was made of the soil of the experimental area. The results of this analysis are shown in Table 1.

Table 1. Soil analysis for the chemical composition of the experimental area.

Organic matter	pH	Lime ppm	Phosphate ppm	Potassium ppm
2.2	5.7	400-500	63	500

Fertilizers were applied with a mechanical spreader on the experimental plots on April 1. The levels and types of fertilizers which were applied were as follows:

1. Plot 1 was not fertilized.
2. Plot 2 was treated with 750 pounds of ammonium nitrate per 1.26 acre, at the rate of 200 pounds of nitrogen per acre.
3. Plot 3 was fertilized with 560 pounds of urea per 1.26 acres, at the rate of 200 pounds of nitrogen per acre.
4. Plot 4 was fertilized with 1500 pounds of ammonium phosphate per 1.26 acres at the rate of 200 pounds of nitrogen per acre.

Selection of Cows

Four cows from each of the three breeds (Jersey, Guernsey, Ayrshire) were randomly selected to make up four groups. Each cow of each group was clearly marked with a number which corresponded with a plot number. Throughout the study each cow was assigned to one of the plots. Cows were placed in these plots immediately after milking and kept on pasture until just prior to milking.

Soil

Samples of soil were obtained from each plot once a week, starting on April 25 and continuing until May 22. Both ammonium nitrogen and nitrate nitrogen were determined from the soil samples. Ammonium nitrogen was measured by the Kjeldahl method and nitrate nitrogen was established by nesslerization of soil extracts by a modification of the method presented by Gilbert, Eppson, Bradley and Beath (8). Both ammonium and nitrate nitrogen are expressed in parts per million. These analyses were made in the Agronomy Department at Kansas State University.

Bromegrass

About five pounds of bromegrass was collected once a week on the same day that soil samples were taken. The bromegrass from each plot was sampled by plucking about the same amount of the bromegrass that the cow tends to eat. The bromegrass samples were cut into pieces less than four inches in length and autoclaved at four pounds pressure for five minutes to inactivate enzymes. They were then dried for four hours at 65°C in a circulating air oven. The dry samples were ground to pass through a 20 mesh screen and stored in the dark until needed. Ammonium nitrogen and amide nitrogen were determined by nesslerization of extracts from the dried grass samples by the method of Pucher, Vickery and Levenworth (18). The results were expressed in terms of mg. of either ammonium or amide nitrogen per gram of dry weight. The nesslerized solutions were determined with a Beckman Model DU spectrophotometer at 420 mu.

Milk for Organoleptic Analysis

Milk samples were collected from evening milkings from May 2 to June 1, 1961. Milk from the cows on each plot was collected in one can, mixed well in the can and sampled. On the week days, these samples were taken immediately to the laboratory, tempered to 60°F. and analyzed organoleptically. On weekends, the samples were collected in the same manner, but were refrigerated until the following Monday morning at which time they were analyzed. Milk samples were analyzed organoleptically by four trained judges. The range of flavor scores was 1 through 4 with the following interpretation: 1, no feed flavor; 2, slight feed flavor; 3, definite feed flavor; 4, pronounced feed flavor. Each sample was given randomly to the judges three times for evaluation and scoring in order to minimize chance of faulty judgment and to establish the reliability of the analyst. After the cows had been on separate plots for 23 days, all cows were placed on the same plot in order to determine differences among groups of cows. The milk was collected separately by groups as previously described and organoleptically analyzed. The results of these analyses were compared to the results of the previous analyses to establish groups of cows differences. Five days out of 23 days were randomly selected to be compared with the data obtained when cows were placed on one plot. Both data obtained from the organoleptic study were analyzed statistically according to the methods described by Snedecor (20). Samples of milk were withdrawn from each composite of pooled milk from each of the groups. These samples were taken on the same day that soil and plant samples were collected and were analyzed for total nitrogen (TN), non-casein nitrogen (NCN), and non-protein nitrogen (NPN). Milk was analyzed for total nitrogen, non-casein nitrogen and non-protein nitrogen by the method employed by Whitnah and Bassette (25). The results were expressed in grams of nitrogen per hundred grams of milk.

RESULTS

Soil Analysis for Ammonium Ion

The results of the analysis for ammonium ion of soil from the four experimental plots are presented in Fig. 1. The soil ammonium ion for April 25 varied from 116.1 ppm on plot 4 treated with $(NH_4)_3PO_4$ to 4.4 ppm on the unfertilized plot, 1. The soil treated with NH_4NO_3, plot 2, was comparatively

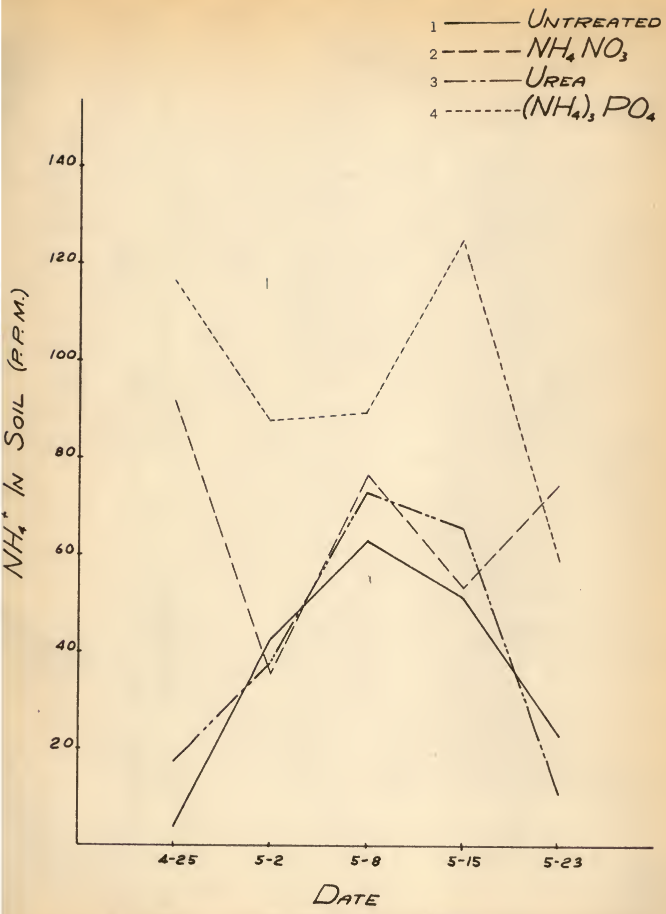


Fig. 1. Effect of nitrogen fertilization on ammonium content of the soil.

high with 91.6 ppm and the urea treated plot, 3, contained 17.3 ppm ammonium ion. The area treated with $(NH_4)_3\ PO_4$ remained relatively high in concentration of ammonia throughout the five-week experimental period. Soils of plots 1, 2 and 3 followed a similar trend with respect to ammonia concentration during the second, third, and fourth weeks. Soil from these three plots attained a peak in concentration of ammonia during the third week. It dropped sharply on all plots except plot 2, which increased abruptly from 53.3 ppm to 74.0 ppm during the fifth week of the study.

Soil Analysis for Nitrate

The results of the analysis of soil for nitrate from the four plots are presented in Fig. 2. The soil nitrate concentration varied from 13.19 ppm on the plot treated with urea to 1.86 ppm on the unfertilized plot. The soil treated with NH_4NO_3 was comparatively high with 7.34 ppm, whereas the soil treated with $(NH_4)_3PO_4$ contained only 3.85 ppm nitrate on April 25. The untreated area remained relatively low in soil nitrate throughout the five weeks of the experiment. The soils from the four plots decreased in concentration of nitrate on the second week. On the third week, soils of plots 3 and 4 decreased in nitrate concentration whereas soil of plots 1 and 2 increased in it. Soils of the four plots decreased on the fourth week but increased shraply in nitrate concentration on the fifth week. Throughout the study, the soil of plot 3 had the highest and that of plot 1 had the lowest nitrate concentration. The soil of plot 2 had a higher concentration than that of plot 4. The soil of plot 2 increased sharply from 1.1 to 5.9 ppm of nitrate on the fifth week of the study.

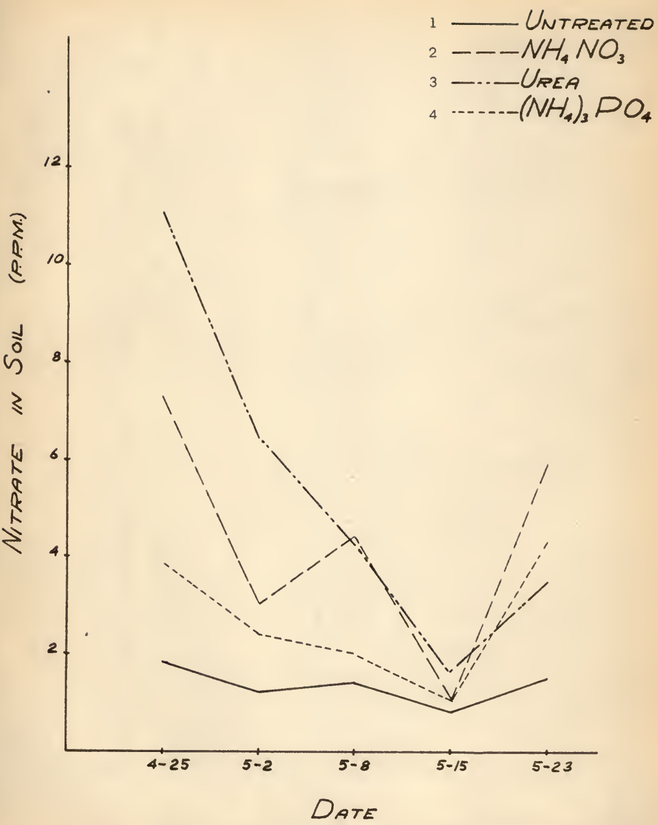


Fig. 2. Effect of nitrogen fertilization on nitrate content of soil.

Plant Analysis for Ammonia

The results of the analyses of bromegrass ammonia in terms of mg/gram dry weight on each of four plots are presented in Fig. 3. The ammonia concentration of bromegrass varied from 0.44 mg/g on plot 1 to 0.59 mg/g on plot 4. The ammonia concentration of samples from plots 2 and 3 each contained 0.57 mg/g on April 25. The bromegrass on plots 3 and 4 had a similar trend during the second, third, and fourth weeks of the experiment. The concentration of ammonia in samples from plot 1 did not change from the second to the third week, but it increased steadily during the fourth and fifth weeks of the study. There was a sharp decline in ammonia concentration of bromegrass on plot 2 from 0.28 mg/g in the third week to 0.16 mg/g in the fourth week. The concentration of ammonia in the sample from plot 3 declined in the fifth week from 0.29 to 0.25 mg/g, whereas it increased in bromegrass of plots 1, 2 and 4.

Bromegrass Analysis for Amide

The results of the analyses of bromegrass for amides on the four plots in terms of mg/g dry weight are presented in Fig. 4. The bromegrass amide concentration varied from 0.60 mg/g on plots 2 and 4 to 0.55 mg/g on plot 1, on April 25. Generally, throughout the study, there was a decline in concentration of amide in bromegrass on plots 3 and 4. There was a sharp decline in its concentration in samples from plots 1 and 2 in the second week, but an increase in its concentration on these plots in the third week. While the concentration of bromegrass amide from plot 1 decreased to 0.04 mg/g in the fourth week, it increased to 0.47 mg/g in bromegrass on plot 2. When amide concentration of

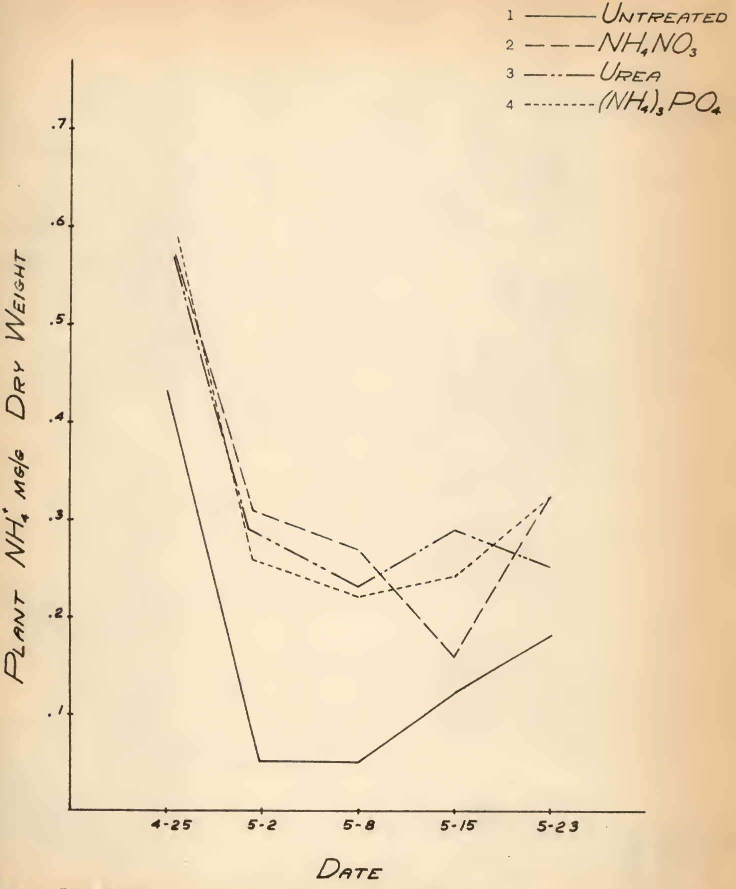


Fig. 3. Effect of nitrogen fertilization on ammonium content of bromegrass.

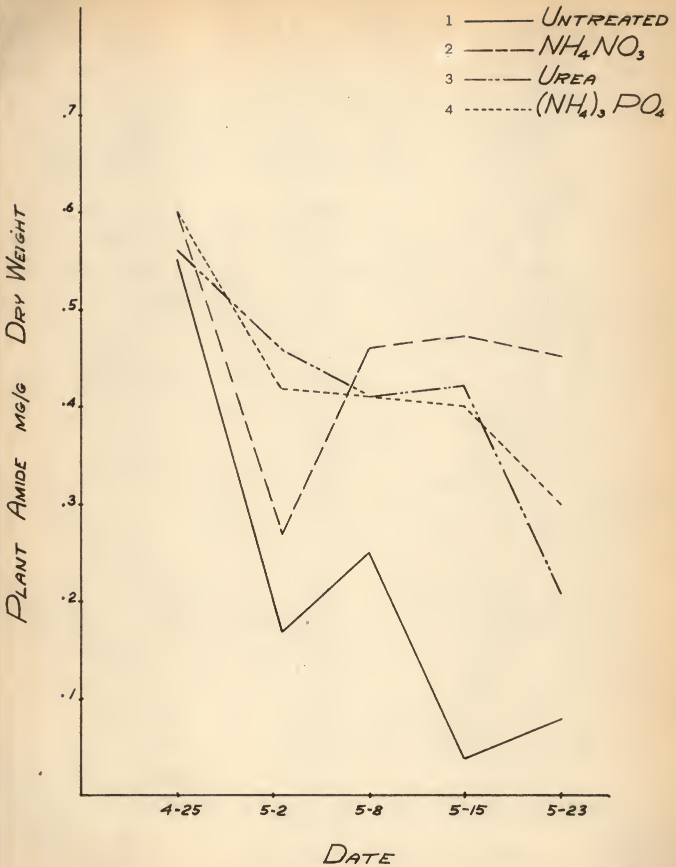


Fig. 4. Effect of nitrogen fertilization on amide content of bromegrass.

bromegrass on plots 2, 3 and 4 decreased in the fifth week of the study, it increased slightly on bromegrass on plot 1.

Plant Analysis for Total Nitrogen (TN)

The results of the total nitrogen analyses of bromegrass expressed as percent of dry weight on the four plots are presented in Fig. 5. For the first week, it varied from 31.06 percent on plot 4 to 21.88 percent on plot 1. On plot 3 it was comparatively high with 29.31 percent and on plot 2, 26.88 percent. There was a steady decline in the total nitrogen of bromegrass on plot 1 from 21.88 percent on the first analysis to 8.13 percent in the fifth week. TN of bromegrass on plot 4 remained relatively high throughout the five weeks of the study. A similar trend for TN of bromegrass on plots 2, 3, and 4 was observed during the third, fourth and fifth weeks of the experiment.

Climatic Conditions

The daily average of temperatures summarized as weekly average are presented in Fig. 6. The weekly average of temperatures was 51.85° for the week ending April 25. It increased slightly in the second week but increased sharply to 65.85°F. in the third week. It decreased in the fourth week but increased to 62.14°F. in the fifth week. The weekly totals of rainfall are presented in Fig. 7. The weekly total of rainfall was 0.259 inches for the week ending April 25. It was 2.807, 0, 4.655, and 0.112 for the second, third, fourth and fifth weeks, respectively.

The daily relative humidity summarized in a weekly average is presented in Fig. 8. The relative humidity was 95.57 when averages were summarized from 6 a.m. data for the week ending April 25. It was a 100, 96, 71, 100 and a 100

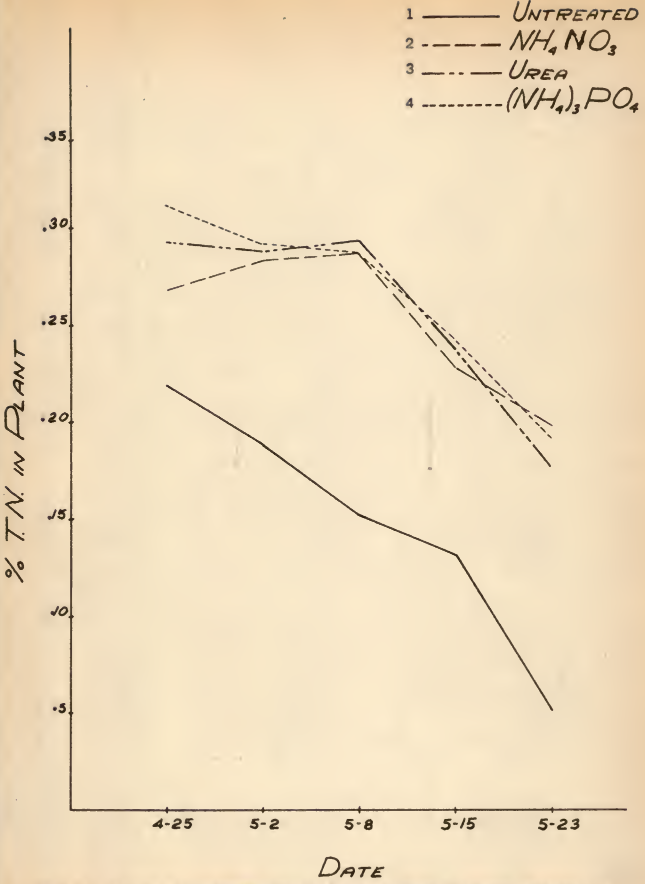


Fig. 5. Effect of nitrogen fertilization on total nitrogen content of gromegrass.

Fig. 6. Daily average temperatures summarized weekly from April 18 through May 23.

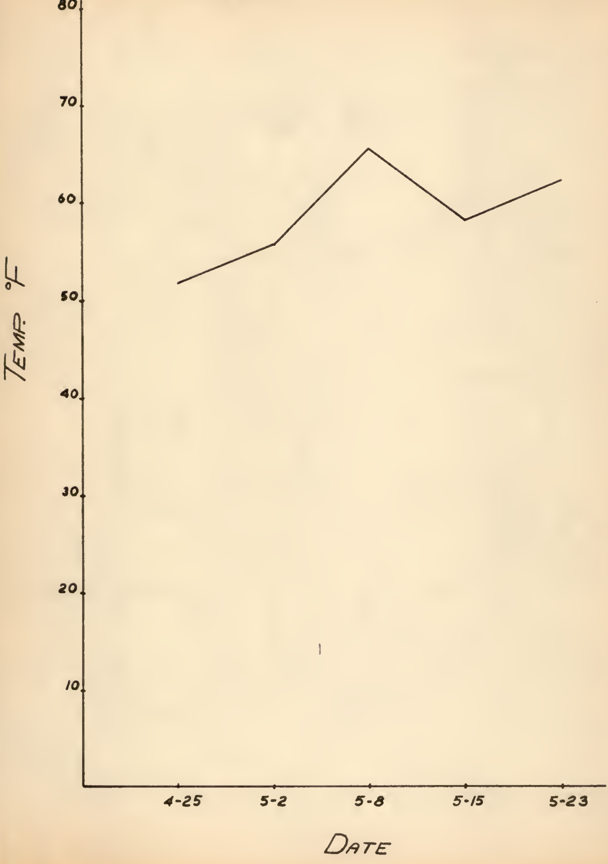


Fig. 7. The weekly total precipitation from April 18 through May 23.

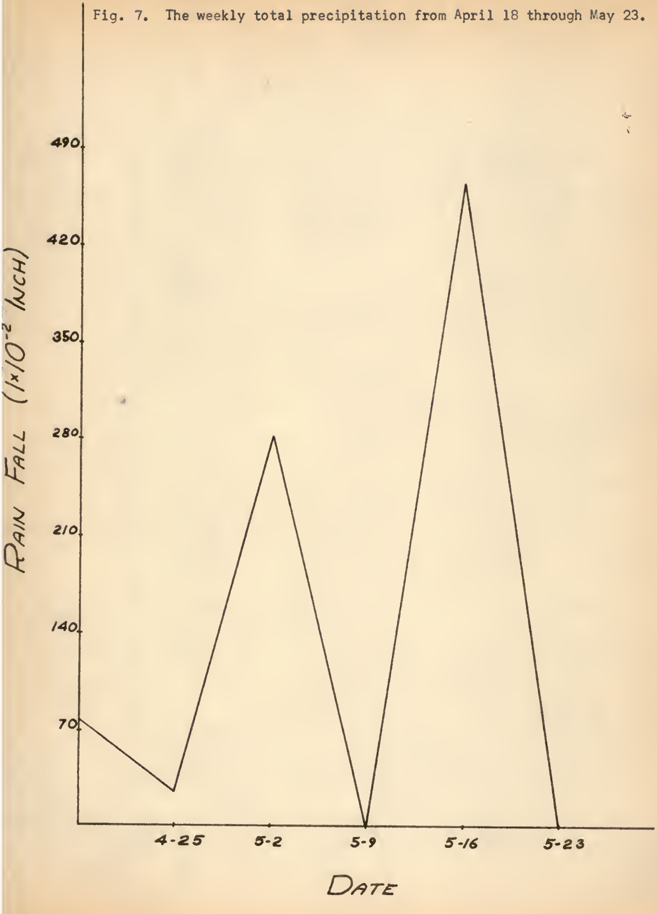
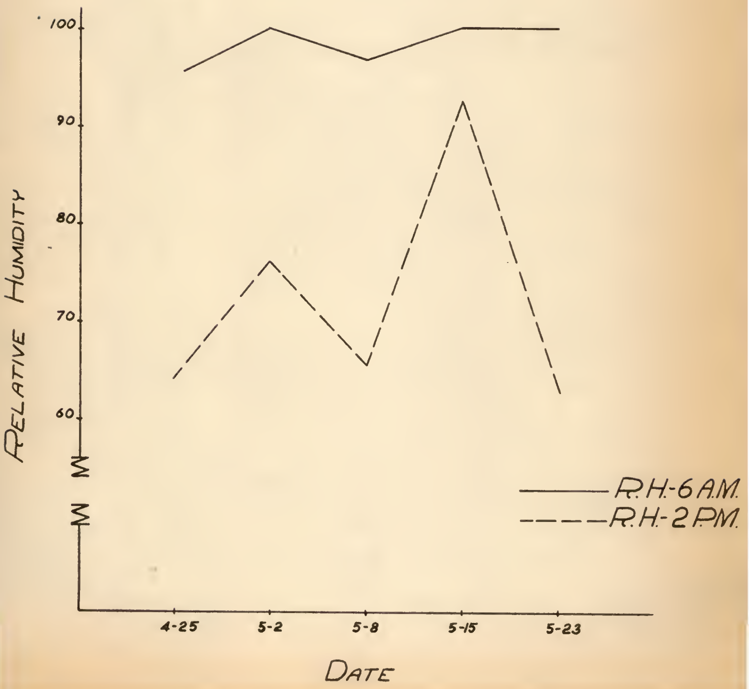


Fig. 8. Daily average relative humidities summarized weekly from April 18 through May 23.



for the second, third, fourth and fifth weeks, respectively, while it was 64.28 when averages were summarized from 2 p.m. data for the same date. It was 76.28, 65.57, 92.85, and 62.85 in the second, third, fourth and fifth weeks, respectively.

Milk Analysis for Non-casein Nitrogen (NCN)

The results of the analyses of milk for NCN from the four groups of cows are presented in Fig. 9. The NCN varied from 0.151 percent in the milk which was collected from cows on plot 2 to 0.131 percent in the milk from cows on plot 4, and from 0.149 percent in milk from cows on plot 1 to 0.133 percent in milk from cows on plot 3 on May 2. In the second week, the NCN in milk from cows on plot 2 decreased while it increased in milk from cows on plots 3 and 4. From groups of cows on plots 1 and 3, it decreased through the third and fourth weeks of the study. In milk from cows on plot 4, it increased steadily throughout the experimental period. Milk from cows on plot 2 increased abruptly in the NCN concentration from 0.146 percent in the second week to 0.179 percent in the third week, while in the fourth week it decreased sharply to 0.098 percent, then increased in the fifth week to 0.139 percent. The NCN concentration in milk from groups of cows on plot 3 decreased in the second and fourth weeks. There was an increase in NCN of milk from each group of cows in the fifth week of the study.

Milk Analysis for Non-protein Nitrogen (NPN)

The results of the analyses of milk for non-protein nitrogen from the four groups of cows are presented in Fig. 10. On May 2, NPN varied from 0.048 percent for milk from cows on plot 3 to 0.038 percent for milk from cows

Fig. 9. Concentration of non-casein nitrogen in milk produced from bromegrass on soil treated with different sources of nitrogen fertilization.

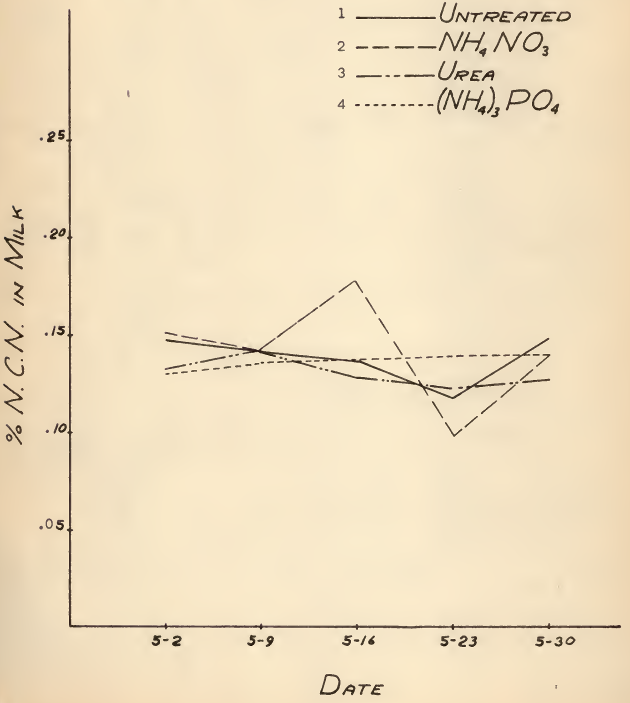
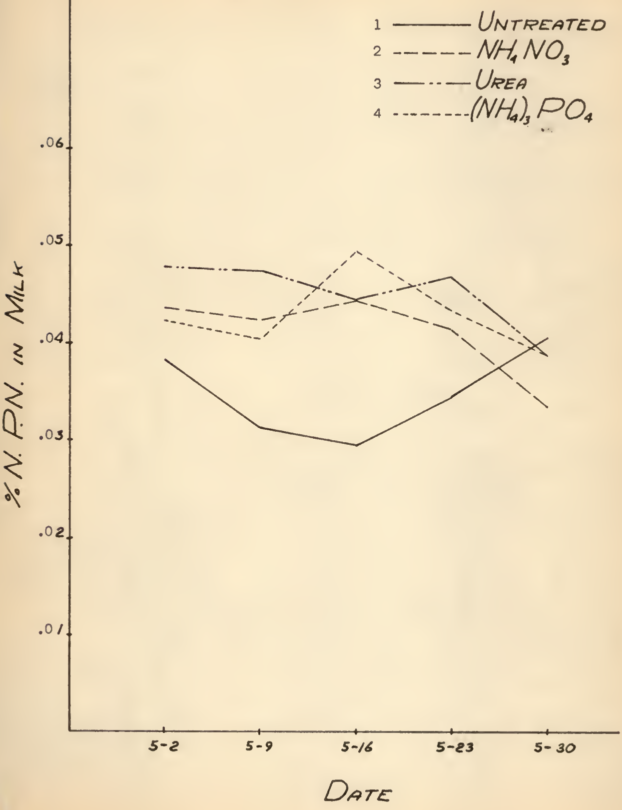


Fig. 10. Concentration of non-protein nitrogen in milk produced from bromegrass on soil treated with different sources of nitrogen fertilization.



on plot 1. NPN for milk from cows on plots 2 and 4 were 0.044 percent and 0.042 percent, respectively, on May 2. In general, NPN for milk cows on plots 2, 3 and 4 followed the same general trend through the five weeks of the study. NPN for milk from cows on plot 1, the unfertilized area, was relatively low throughout the four weeks, but increased in the fifth week to 0.040 percent.

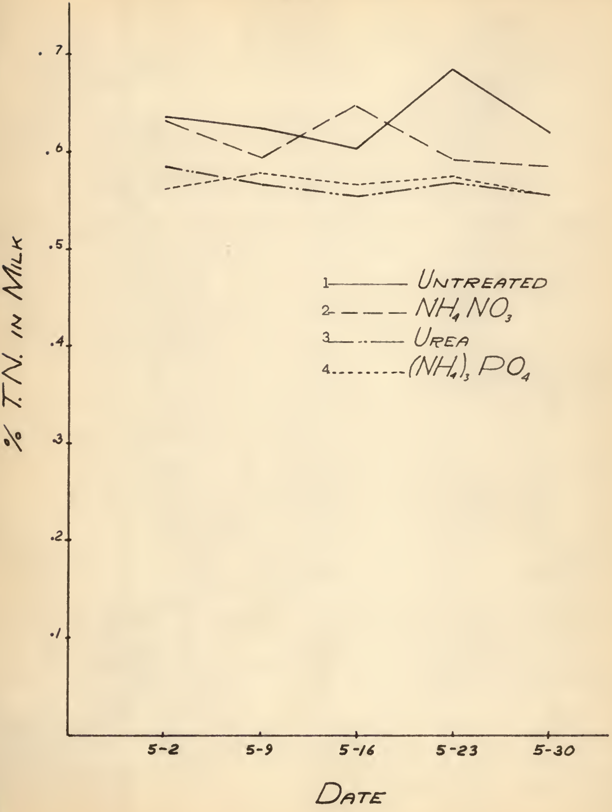
Milk Analysis for Total Nitrogen (TN)

The results of the analyses of milk for total nitrogen from the four groups of cows are presented in Fig. 11. The concentration of TN, on May 2, varied from 0.635 percent in milk from cows on plot 1, to 0.561 percent in milk from cows on plot 4. The TN concentration of milk from cows on plot 2 was comparatively high with 0.632 percent, whereas on plot 3 it was 0.585 percent. Milk from cows on plots 3 and 4 did not vary markedly during the five weeks of the study; and remained consistently lower in TN than that from cows on plots 1 and 2. The TN concentration of milk from cows on plot 1 decreased steadily in the second and third weeks of the study, then increased abruptly to a maximum of 0.685 percent in the fourth week, and then decreased again to 0.620 percent in the fifth week. Milk from cows on plot 2 declined in TN in the second week and increased to 0.648 percent in the third week and decreased steadily to 0.584 percent in the fifth week.

Organoleptic Analysis of Milk

The results of the organoleptic analyses for milk flavor are presented in Table 13 of the Appendix. Table 13 shows the raw data for the analyses of the milk produced on each plot of the bromegrass pasture throughout the 23 days of

Fig. 11. Concentration of total nitrogen in milk produced from bromegrass on soil treated with different sources of nitrogen fertilization.



the study. The mean flavor scores which were assigned to milks from each group of cows during the 23 days appear in Table 14 of the Appendix.

Table 2. Analysis of variance of flavor scores during 23 days, among judges, pastures and days.

Source of variation	D/F	Sum of squares	Mean square	F	Sig.
Dates	22	32.74	1.49	6.24	*
Judges	3	36.35	12.12	50.80	*
Pastures	3	2.89	0.96	4.04	*
Pastures x dates	66	9.89	0.15	0.63	n.s.
Residual	273	65.11	0.24		

The F-test following the analysis of variance in Table 2 shows significant differences among judges, pasture, and dates at the five percent level. There is no significant interaction of pastures and dates at the five percent level.

Table 3. The least significant difference (LSD) of the mean flavor scores among plots.

Ordered array of plots	Mean flavor scores	Difference and their significance		
		Judge 1 LSD	Judge 2 LSD	Judge 3 LSD
2	2.97	0.88*	0.43*	0.36*
4	2.61	0.51*	0.06 n.s.	
3	2.54	0.45*		
1	2.09			

LSD = 0.14

The five percent level LSD tests among mean flavor scores of judges revealed that judge 2 was significantly different from judges 1, 3 and 4. Judges 3 and 4 were significantly different from judge 1, but not different from each other.

The LSD test of the mean flavor scores for the 23 days appear in Table 15 of the Appendix.

The Development of the Flavor as a Function of Time

In order to determine how the flavor developed throughout the study, the average flavor scores of milk obtained from cows on all plots were averaged for each day. These averaged scores throughout the entire 23 days showed the development of bromegrass flavor in milk as a function of time. A mean bromegrass flavor score was obtained by averaging all of the daily averages for the 23 days. By comparing the number of daily flavor scores that were above and below the summary average (for 23 days) it was possible to observe a trend. During the first week the daily average bromegrass milk flavor scores increased from the first through the seventh days as follows: 1.71, 2.1, 2.2, 2.7, 2.8, 2.8, 2.7 (see Table 14 in Appendix). The mean for the 23 days was 2.55. During the first week, scores on three days were above the mean and four were below it. In the second and third weeks five were above the mean score and only two were below it. It appeared that the off-flavor developed with time.

Analysis of Variance for Data Collected When Cows Were on the Same Feed

An analysis of variance was performed on the data which were collected, from May 24 to May 29, when the four groups of cows were placed on the same plot. The raw data of the analysis for five days which were evaluated by the three judges appear in Table 17 of the Appendix.

Table 5. Analysis of variance among groups of cows, judges, and treatments.

Source of variation	D/F	Sum of squares	Mean squares	F	Sig.
Groups of cows	3	2.64	0.88	3.04	*
Treatments	1	6.08	6.08	20.95	*
Groups x treatments	3	2.14	0.71	0.25	n.s.
Judges	2	8.32	4.16	14.34	**
Groups x judges	6	3.07	0.51	0.18	n.s.
Treatments x judges	2	1.77	0.88	3.05	n.s.
Groups x treatments x judges	6	1.42	0.24	0.08	n.s.
Residual	96	27.84	0.29		
Total	119	53.26			

The F-tests in Table 5 show that variances among groups of cows, judges, and treatments were significant at the five percent level. There were no significant interactions at the five percent level. Five percent level LSD tests of the mean flavor scores among groups of cows revealed that group of cows on plot 1 was significantly different from all other groups of cows.

Table 6. The least significant difference of the mean flavor scores among groups of cows.

Ordered array of groups of cows	Mean flavor scores	Difference and their significance Group 2 LSD	Group 4 LSD	Group 3 LSD
1	2.78	0.37*	0.31*	0.12 n.s.
3	2.66	0.25 n.s.	0.19 n.s.	
4	2.47	0.06 n.s.		
2	2.41			

LSD = 0.28

DISCUSSION

Analyses of soil, pasture and milk in order to study the passage of nitrogen salts from the soil through the plant and into milk was only partially successful. It was possible to observe certain relationships between the nitrogen composition in the soil and the plant; or between that in the bromegrass plant and the milk. However, most of the relationships are based upon some speculation or interpretations.

The level of ammonia in the soil over the five week experimental period was undoubtedly influenced considerably by rainfall, temperature and humidity (14). The climatic effects were difficult to assess; however, there are certain innate characteristics of the nitrogen salts which allow for speculation. The relatively high ammonia content of the soil treated with $(NH_4)_3PO_4$ throughout the study was probably due in part to the more acid condition of the soil. Ammonium phosphate is not pure 16-20-0 but a blend of the ammonium salts of phosphoric and sulfuric acids. The decrease in soil pH as a result of this tends to delay nitrification by soil bacteria. In addition to the pH effect, the relative insolubility of $(NH_4)_3PO_4$ makes it less available to the plant. Since it is not as soluble as the other nitrogen compounds, it probably was not leached out as readily and appeared at relatively high levels throughout the study (14,17,21).

A tendency for the other three plots to attain a fairly high ammonia concentration about midway through the study suggests that the excretions by the cows contributed to the soil ammonia.

The ammonia concentrations of the soil could not be observed as directly translated into any of the plant nitrogen fractions measured. The unfertilized plot was considerably lower in each of the plant nitrogen fractions analyzed, but there was little else that could be demonstrated. Trends in the concentration of plant nitrogen fractions will be discussed later.

Soil nitrate was influenced by the rate of conversion of the reduced ammonia to nitrate. Urea is apparently converted rapidly. Ammonium nitrate with half of its nitrogen in the nitrate form was initially high in nitrate (14). It is interesting that the curves illustrating soil ammonia and soil nitrate on plot 2 are very similar. One might anticipate this from knowledge of the composition of the salt.

Urea appears to be converted primarily to nitrate since the ammonia concentration of the soil of plot 3 is very similar to the unfertilized soil; whereas $(NH_4)_3PO_4$ appears to go principally into ammonia since the nitrate concentration throughout the study of this plot is similar to the unfertilized soil.

Except for a nitrogen deficiency on the unfertilized soil which showed up as low amide, ammonia and total proteins in the bromegrass throughout the study, no well-defined relationship between soil composition and plant composition could be established. The young growing plants reflected an initial high composition of nitrogen compounds. As the plants matured, they increased in cellulose and fiber content and the nitrogen fractions were diluted (12). The nitrogen chemical composition (amide, ammonia and total nitrogen) of the bromegrass from the different fertilized plots was not very different.

Attempts to relate one or more of the bromegrass nitrogen fractions to any of the milk nitrogen fractions were fruitless. Milk from cows on the unfertilized nitrogen deficient pasture contained a relatively high total nitrogen. A possible explanation of this fact could be that as production of milk was reduced on the semi-starvation ration (this pasture was lacking in vegetative growth), the solids content of the milk increased. Although no total solids were run, it was established that milk production from cows on this unfertilized pasture was 1538 lbs/month, whereas it was 2130, 2370 and 2220 pounds for group of cows on plots 2, 3, and 4, respectively. The nonprotein nitrogen concentration of milk produced on the unfertilized pasture was considerably lower than this fraction from the fertilized plots.

There was an abrupt increase followed by a sharp decrease in the NCN concentration of milk produced on the pasture fertilized with $(NH_4)NO_3$ in the third and fourth weeks. One cow on this plot demonstrated symptoms of nitrate poisoning and had to be temporarily taken off the experiment.

The relationships between either milk nitrogen or plant nitrogen fractions and the flavor of milk as analyzed by the taste panel are not established from this study. Although it is apparent that bromegrass produces an undesirable flavor, none of the nitrogen fractions of the plant that were measured could be directly related to flavor intensity.

The statistical analysis of the organoleptic data reveal that the flavor differences observed were due to differences among groups of cows. When all of the cows were placed on the same feed, the group of cows that throughout the study were on the unfertilized plot produced significantly poorer flavored milk.

In retrospect it would be advisable, if this study were to be repeated, to guard against evaluating the experimental cows at the end of the study. The cows that were on the unfertilized plot were poorly nourished. When these cows were placed with the other cows on lush fertilized bromegrass, it is quite probable that they consumed much more than cows that had been well-nourished. An evaluation of the groups of cows should have been made at the beginning of the experiment.

If differences in the flavor of milk produced on the various experimental plots actually did occur, they were small. They were not the marked differences that one would anticipate if nitrogen fertilizers were a primary cause or contribution to the flavor of milk.

CONCLUSION

On the basis of the results the following conclusions can be drawn:

1. The level of nitrogen fertilizations which were applied to the soil of bromegrass pasture affected the nitrogen composition of bromegrass plant.

2. Ammonia, amide, and total nitrogen concentrations in bromegrass were affected by the stage of growth of bromegrass.

3. There was no definite relationship between the nitrogen composition of bromegrass and the nitrogen composition of the milk.

4. There was no definite relationship between the nitrogen concentration (amide, ammonia, and total nitrogen) of bromegrass and the flavor intensity of the milk.

5. The LSD tests at the five percent level indicated that flavor differences that occurred were due to differences among groups of cows and not to the effect of fertilization.

6. Objectionable flavors were developed in milk produced from bromegrass as indicated by the panel flavor scores.

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LITERATURE CITED

1. Carey, V., H. L. Mitchell, and D. Anderson.
   Effect of nitrogen fertilization on the chemical composition of bromegrass. J. Agron. 44:467-469. 1952.

2. Choi, P. P., C. M. O'Malley, and C. W. Fairbanks.
   Colorimetric determination of ammonia in milk and dry products of milk. J. Dairy Sci. 29:645-649. 1946.

3. Cole, D. D., W. J. Harper, and C. L. Hankinson.
   Observations on ammonia and volatile amines in milk. J. Dairy Sci. 44:171-173. 1961.

4. Colson, T. J.
   Correlation between consumer reaction and organoleptic analysis of feed flavored milk. Master's Thesis, Kansas State University, 1961.

5. Crist, J. W. and H. L. Seaton.
   Reliability of organoleptic tests. Food Res. 6:529-536. 1941.

6. Eggleton, W. G. E.
   The assimilation of inorganic nitrogenous salts, including sodium nitrate, by grass plant. Biochem. Jour. 29:1389-1397. 1935.

7. Foreman, C. F., E. W. Bird, F. E. Nelson, and W. S. Rosenburger.
   Observations regarding an unclean flavor in milk produced by feeding bromegrass. J. Dairy Sci. 42:936. 1959. (Abst.)

8. Gilbert, C. S., H. F. Eppson, W. B. Bradley, and O. A. Beath.
   Nitrate accumulation in cultivated plants and weeds. Wyo. Agr. Expt. Sta. Bul. 227. 1946.

9. Harrison, S. and W. L. Elder.
   Some applications of statistics to laboratory taste testing. Food Tech. 4:434-439. 1950.

10. Ishler, N. H., E. A. Laue, and G. A. Bullman.
    Reliability of taste testing and consumer testing methods. I. Fatigue in taste testing. Food Tech. 8:387-388. 1954.

11. Ishler, N. H., E. A. Laue, and A. J. Janish.
    Reliability of taste testing and consumer testing methods. II. Codes bias in consumer testing. Food Tech. 8:389-391. 1954.

12. Kohler, G. O.
    The effect of stage of growth on the chemistry of the grasses. J. Biol. Chem. 152:215-223. 1944.

13. Krum, J. K.
Methods and analyzing results in sensory panel testing. Food Engg. 27(7)74. 1955.

14. Millar, C. E.
Soil Fertility. John Wiley and Sons, Inc., New York. 1955.

15. Peryam, D. R.
Panels, etc., for quality and organoleptic tests. Food Industry 22(12)42. 1950.

16. Peryam, D. R. and V. W. Swartz.
Measurement of sensory differences. Food Tech. 4:390. 1950.

17. Pirie, N. W.
Leaf proteins. Annual review of plant physiology 10:33-77. 1959.

18. Pucher, G. W., H. B. Vickery, and C. S. Leavenworth.
Determination of ammonia and amide in plant tissue. Industry and Engg. Chem., Anal. Ed. 152-154. 1935.

19. Roessler, E. B. and J. F. Suymon.
Significance of triangular taste tests. Food Res. 13:503-505. 1948.

20. Snedecor, G. W.
Statistical Methods. 5th Ed. Iowa State College Press, Ames. 1957.

21. Steward, F. C. and J. K. Polland.
Nitrogen metabolism in plants. Annual review of plant physiology 8:65-106. 1957.

22. Trout, G. M., C. R. Meger, and C. M. Harrison.
Effect of alfalfa-bromegrass pasture on the flavor of milk when the cows are milked three times daily. Mich. Expt. Sta. Qtr. Bul. 22:163-174. 1940.

23. Walters, J. H.
Structured and unstructured techniques. J. Marketing 25:58-62. 1961.

24. Westfall, R. L., H. W. Boyd, and D. T. Campbell.
The use of structured techniques in motivation research. J. Marketing 22:134-138. 1957.

25. Whitnah, C. H. and R. Bassette.
Nitrogen fractions in centrifuge preparations from milk. J. Dairy Sci. 43:1731-1735. 1960.

APPENDIX

Table 7. Ammonium ion concentration, expressed in ppm, in soil with different levels of nitrogen fertilization.

Plot :	Dates				
	April 25	May 2	May 8	May 15	May 23
	ppm				
1	4.4	43.1	62.5	51.2	23.3
2	91.6	35.9	76.1	53.5	74.0
3	17.3	36.7	72.4	65.8	10.6
4	116.1	87.9	89.3	124.5	58.4

Table 8. Nitrate concentration, expressed in ppm, in soil with different levels of nitrogen fertilization.

Plot :	Dates				
	April 25	May 2	May 8	May 15	May 23
	ppm				
1	1.86	1.20	1.40	0.80	1.70
2	7.34	3.07	4.40	1.10	5.90
3	13.19	6.26	4.30	1.60	3.90
4	3.85	2.42	2.00	1.20	4.30

Table 9. Climatic conditions for five weeks started on April 25 through May 29.

	April 25 - May 1	May 2-8	May 9-15	May 16-22	May 23-29
Week	1	2	3	4	5
Weekly average[1] temperature F°	51.85	55.71	65.85	58.28	62.14
Weekly total precipitation in inch	0.259	2.807	0.00	4.665	0.112
Relative humidity:[1]					
6 a.m.	95.57	0.00	96.71	100.00	100.00
2 p.m.	64.28	76.28	65.57	82.85	62.85

[1]Weekly averages compiled from daily averages.

Table 10. Percent total nitrogen in bromegrass obtained from four plots with different level of nitrogen fertilization.

Plot :	April 25	May 2	May 8	May 15	May 23
	Percent total nitrogen				
1	21.88	18.94	15.06	12.63	8.13
2	26.88	28.13	28.63	22.69	20.31
3	29.31	28.56	28.75	23.63	18.13
4	31.06	29.00	28.56	24.06	19.56

Table 11. Ammonium concentration in bromegrass obtained from four plots with different levels of nitrogen fertilization, expressed in mg/g dry weight.

Plot :	April 25	May 2	May 8	May 15	May 23
	Milligram per gram dry weight				
1	0.43	0.05	0.05	0.12	0.18
2	0.57	0.31	0.28	0.16	0.32
3	0.57	0.29	0.23	0.29	0.25
4	0.59	0.26	0.22	0.24	0.32

Table 12. Amide concentration in bromegrass obtained from four plots with different level of nitrogen fertilization, expressed in mg/g dry weight.

Plot :	April 25	May 2	May 8	May 15	May 23
	Milligram per gram dry weight				
1	0.55	0.17	0.25	0.04	0.08
2	0.60	0.27	0.46	0.47	0.45
3	0.56	0.47	0.41	0.42	0.21
4	0.60	0.42	0.41	0.40	0.29

Table 13. Flavor scores for milk from four groups of cows placed on different plots with different level of nitrogen fertilization.

	Plot no.	Dates																							
		2			3			4			5			6			7			8			9		
		Flavor scores*																							
Judge 1	1	2	2	1**	2	1	1	3	1	2	3	3	3	3	3	2	3	2	3	3	3	3	1	2	2
	2	1	1	1	1	1	2	1	2	1	2	2	3	2	2	2	3	2	2	2	2	2	2	1	1
	3	1	1	1	1	1	1	2	1	2	1	2	2	2	2	2	2	2	2	2	2	1	2	2	2
	4	1	1	1	2	2	1	2	1	1	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1
Judge 2	1	2	2	2	3	2	3	2	3	3	4	3	3	4	4	3	4	3	4	4	3	3	3	3	3
	2	2	2	2	3	3	3	2	2	3	4	3	3	3	3	3	4	3	3	3	3	3	3	3	2
	3	2	2	2	2	2	2	2	2	2	3	2	3	3	3	4	3	3	4	4	3	3	3	3	3
	4	2	2	2	3	3	3	3	2	3	3	3	3	3	4	4	3	3	3	3	3	3	3	2	2
Judge 3	1	1	2	1	2	3	3	2	3	3	3	2	2	3	2	3	2	3	3	3	2	3	3	3	3
	2	2	2	2	3	2	2	2	2	2	2	2	2	3	3	3	2	3	3	3	2	3	2	2	2
	3	2	3	2	2	2	3	2	2	2	3	2	2	3	2	2	2	2	2	2	2	3	2	2	2
	4	3	2	2	2	2	2	3	3	3	2	2	2	2	2	2	2	3	2	3	3	2	2	2	2
Judge 4	1	2	1	1	2	3	2	2	2	3	2	3	3	4	3	4	4	3	4	4	3	4	3	2	2
	2	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	4	3	3	3	2	3	1	1	2
	3	1	2	2	2	2	2	3	3	2	2	2	2	3	3	3	3	3	3	3	2	2	1	2	2
	4	2	2	2	2	2	2	3	3	3	3	2	3	3	4	3	3	3	4	3	3	3	2	2	2

Table 13 (Cont.).

	Plot no.	Dates																							
		10			11			12			13			14			15			16			17		
		Flavor scores																							
Judge 1	1	3	2	2	2	2	2	2	2	2	3	2	3	3	3	3	2	2	3	3	2	2	1	1	1
	2	3	2	2	2	1	1	3	2	2	2	2	2	3	2	2	2	2	3	2	2	3	1	2	1
	3	2	3	3	2	1	2	3	3	3	3	3	2	3	3	2	2	3	2	2	2	2	2	2	2
	4	1	1	1	1	1	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
Judge 2	1	4	3	3	4	3	4	4	4	3	2	3	3	4	4	4	3	3	3	4	4	4	2	3	3
	2	3	3	3	4	2	3	4	4	2	3	3	3	4	4	4	3	3	3	4	4	3	3	3	3
	3	4	3	3	4	4	4	4	4	4	3	4	4	4	4	4	3	3	3	4	3	4	4	4	3
	4	3	2	3	3	4	4	4	4	3	3	3	3	4	3	4	3	3	3	3	3	2	2	3	2
Judge 3	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	2	3	2	2	3	3	3	3
	2	2	2	2	2	2	3	3	2	3	3	3	3	2	2	3	2	3	3	2	3	3	3	3	2
	3	2	1	1	2	2	2	3	2	3	2	2	2	3	3	3	2	2	2	2	2	2	2	3	3
	4	2	2	2	3	2	3	2	3	3	3	2	3	2	3	2	2	3	2	2	2	3	3	2	3
Judge 4	1	3	4	3	4	3	2	3	2	2	3	1	3	4	3	4	3	3	3	2	3	3	3	3	3
	2	3	3	3	3	3	3	3	2	2	2	2	2	3	2	4	2	3	2	2	2	2	2	2	2
	3	4	3	3	3	4	4	2	3	3	1	2	2	3	3	3	2	2	4	2	2	3	3	2	3
	4	3	3	3	2	3	3	2	2	3	2	3	3	2	4	3	4	3	4	2	3	3	2	3	3

(continued)

Table 13 (Cont.).

	Plot no.	Dates 18			19			20			21			22			23		
		Flavor scores																	
Judge 1	1	2	2	2	2	2	2	2	2	3	3	2	3	2	2	2	4	3	3
	2	2	3	3	2	2	2	2	3	2	3	3	3	2	3	3	3	2	3
	3	4	3	3	3	3	3	2	3	4	2	2	2	3	3	3	3	4	3
	4	2	2	2	2	2	2	3	3	3	1	1	1	2	3	3	2	2	2
Judge 2	1	3	2	2	3	2	2	2	2	2	3	3	3	4	4	3	3	3	3
	2	3	3	3	3	2	2	3	2	2	3	3	3	4	4	4	3	3	3
	3	4	3	4	2	1	2	2	2	2	2	3	3	4	4	4	2	2	3
	4	3	2	3	2	2	2	2	2	2	2	3	2	3	3	4	2	3	2
Judge 3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3
	2	3	3	3	3	3	3	3	3	3	2	3	3	2	3	2	3	2	3
	3	2	3	2	2	2	2	3	3	3	3	3	3	3	3	2	3	3	3
	4	3	3	3	2	3	3	3	3	3	2	3	3	3	3	4	3	3	3
Judge 4	1	3	3	3	3	2	2	2	2	2	3	3	3	2	2	2	3	3	3
	2	2	2	2	2	2	3	2	3	2	3	3	2	3	3	3	3	2	2
	3	2	3	3	2	3	3	3	3	3	2	3	2	2	3	3	2	3	2
	4	3	2	3	2	3	3	2	3	3	2	2	2	3	2	3	2	3	3

* Criticisms are designated as follows:

No feed flavor	1
Slight	2
Definite	3
Pronounced	4

** Each sample was given three times for evaluation.

Table 14. Mean flavor scores of three evaluations by each judge for milk from four groups of cows on different plots with different nitrogen fertilization, over a period of 23 days.

Days	1				2				3			
Pastures	1	2	3	4	1	2	3	4	1	2	3	4
Judge	Mean flavor scores											
1	1.7	1.0	1.0	1.0	1.3	1.3	1.3	1.3	2.0	1.3	1.7	1.3
2	2.0	2.0	2.0	2.0	2.7	3.0	3.0	2.0	2.7	2.0	2.3	2.7
3	1.3	2.0	2.3	2.3	2.3	2.3	2.3	2.0	2.3	2.0	2.0	3.0
4	1.3	1.7	1.7	2.0	2.3	2.0	2.0	2.0	2.3	2.7	2.7	3.0
Total	6.3	6.7	7.0	7.3	9.0	8.6	7.6	8.7	9.7	7.6	8.4	10.0
Av. all judges	6.8				8.7				8.9			
Av. per judge	1.7				2.1				2.2			
Days	4				5				6			
1	3.0	2.3	1.7	2.0	2.7	2.0	2.0	2.0	2.7	2.3	2.0	2.0
2	3.3	3.3	2.7	3.0	3.7	3.0	3.3	3.7	3.7	3.3	3.3	3.0
3	2.3	2.0	2.0	2.0	2.7	3.0	2.3	3.0	2.7	2.7	2.0	2.3
4	2.7	2.0	2.0	2.7	3.7	3.0	3.0	3.3	3.7	3.3	3.0	3.3
Total	11.3	9.6	8.7	9.7	12.8	11.0	10.6	11.0	12.8	11.6	10.3	10.6
Av. all judges	9.8				11.1				11.2			
Av. per judge	2.7				2.8				2.8			
Days	7				8				9			
1	3.0	2.0	1.7	1.7	1.7	1.3	2.0	1.0	2.3	2.3	2.7	1.0
2	3.3	3.0	3.3	3.0	3.0	2.7	3.0	2.3	3.3	3.0	3.4	2.7
3	2.7	2.7	2.3	2.7	3.0	2.0	2.0	2.0	2.0	2.0	1.3	2.0
4	3.7	2.7	2.3	3.0	2.0	3.3	3.0	3.3	3.3	3.0	3.3	3.0
Total	12.7	10.4	9.6	10.4	10.0	7.3	8.7	7.3	10.9	10.3	10.6	8.7
Av. all judges	10.8				8.3				10.1			
Av. per judge	2.7				2.1				2.5			
Days	10				11				12			
1	2.0	1.3	1.7	1.3	2.0	2.3	3.0	2.3	2.7	2.0	2.7	2.0
2	3.7	3.0	4.0	3.7	3.7	3.3	4.0	3.7	2.7	3.0	3.7	3.0
3	2.0	2.0	2.0	2.7	2.3	2.7	2.7	2.7	3.0	3.0	2.0	2.7
4	3.0	3.0	3.7	2.7	2.3	2.0	1.7	2.7	2.3	2.0	2.7	2.3
Total	10.7	9.6	11.4	10.4	10.3	10.6	12.4	11.0	10.7	10.0	10.1	10.4
Av. all judges	8.3				11.1				10.3			
Av. per judge	2.6				2.8				2.6			

(continued)

Table 14 (Cont.)

Days	13				14				15			
Pastures	1	2	3	4	1	2	3	4	1	2	3	4
Judge :	Mean flavor scores											
1	3.0	2.3	2.7	2.0	2.3	2.3	2.3	3.0	2.3	2.3	2.0	2.0
2	4.0	4.0	4.0	3.7	3.0	3.0	3.0	3.0	3.7	3.7	2.3	2.3
3	3.0	2.3	3.0	2.3	2.7	2.7	2.0	2.3	2.7	2.7	2.0	2.3
4	3.7	3.0	3.0	3.0	3.0	2.3	2.7	3.7	2.7	2.0	2.3	2.7
Total	11.0	10.3	10.0	11.0	11.7	10.7	10.0	9.7	11.7	10.7	10.0	9.7
Av. all judges	10.6				10.5				10.5			
Av. per judge	2.6				2.6				2.6			

Days	16				17				18			
1	1.0	1.3	2.0	1.7	2.0	2.7	3.3	2.0	2.0	2.0	2.0	2.0
2	2.7	3.0	3.7	2.3	2.3	3.0	3.7	2.7	2.3	2.3	1.7	2.0
3	3.0	2.7	2.7	2.7	3.0	3.0	2.3	3.0	3.0	3.0	2.7	3.0
4	3.0	2.0	2.7	2.7	3.0	2.0	2.7	2.7	2.3	2.3	2.7	2.7
Total	9.7	9.0	11.1	9.4	10.3	10.7	12.0	10.4	9.6	9.6	9.1	9.7
Av. all judges	9.8				10.8				9.5			
Av. per judge	2.4				2.7				2.6			

Days	19				20				21			
1	2.3	2.3	3.0	3.0	2.7	3.0	2.0	1.0	2.7	2.7	3.0	2.7
2	2.0	2.3	2.0	2.0	3.7	4.0	4.0	3.3	3.7	4.0	4.0	3.3
3	3.0	3.0	3.0	3.0	3.0	2.7	3.0	2.7	3.3	2.3	2.7	3.3
4	2.0	2.3	3.0	2.7	3.0	2.7	2.3	2.0	2.0	3.0	2.7	3.0
Total	9.3	9.9	11.0	10.7	11.7	11.4	10.0	8.0	11.0	12.0	12.4	12.0
Av. all judges	10.2				10.3				11.8			
Av. per judge	2.5				2.6				2.9			

Days	22				23			
1	3.3	2.7	3.0	2.0	2.3	2.3	2.0	2.0
2	3.0	3.0	2.3	2.3	2.7	2.3	2.0	2.7
3	3.0	2.7	3.0	3.0	3.0	3.7	2.3	3.0
4	3.0	2.3	2.7	3.0	3.0	2.7	2.7	2.7
Total	12.3	10.7	10.9	10.0	11.0	11.0	9.0	10.4
Av. all judges	10.8				10.5			
Av. per judge	2.7				2.6			

Table 15. LSD of mean flavor scores during 23 days of the study.

Ordered array of days	Mean flavor score	Differences and their significance						
		Days, LSD						
		1	8	2	3	18	16	4
		x-1.71	x-2.08	x-2.12	x-2.33	x-2.31	x-2.45	x-2.46
13	3.06	1.35	0.98	0.94	0.83	0.75	0.61	0.60
21	2.96	1.25	0.88	0.84	0.73	0.65	0.51	0.50
5	2.84	1.13	0.76	0.72	0.61	0.53	0.39	0.38
6	2.83	1.12	0.75	0.71	0.60	0.52	0.38	0.37
11	2.77	1.06	0.69	0.65	0.54	0.46	0.32	0.31
22	2.74	1.03	0.66	0.62	0.51	0.43	0.29	0.28
17	2.71	1.00	0.63	0.59	0.48	0.40	0.26	0.25
7	2.69	0.98	0.61	0.57	0.46	0.38	0.24	0.23
14	2.64	0.93	0.56	0.52	0.41	0.33	0.19	0.18
10	2.63	0.92	0.55	0.51	0.40	0.32	0.18	0.17
15	2.63	0.92	0.55	0.51	0.40	0.32	0.18	0.17
23	2.59	0.88	0.51	0.47	0.36	0.28	0.14	0.17
12	2.58	0.87	0.50	0.46	0.35	0.27	0.13	0.13
20	2.57	0.86	0.49	0.45	0.35	0.26	0.12	0.10
19	2.56	0.85	0.48	0.44	0.33	0.25	0.11	0.07
9	2.53	0.82	0.45	0.41	0.30	0.22	0.08	
4	2.46	0.75	0.38	0.34	0.23	0.15	0.01	
16	2.45	0.74	0.37	0.33	0.22	0.14		
18	2.31	0.60	0.23	0.19	0.08			
3	2.23	0.52	0.15	0.12				
2	2.12	0.41	0.04					
8	2.08	0.37	0.00					
1	1.71	0.00						

Table 15 (Cont.).

Differences and their significance							
Days, LSD							
9	19	20	12	23	15	10	14
x-2.53	x-2.56	x-2.57	x-2.58	x-2.59	x-2.63	x-2.63	x-2.64
0.53	0.50	0.49	0.48	0.47	0.43	0.43	0.42
0.43	0.40	0.39	0.38	0.37	0.33	0.33	0.32
0.31	0.28	0.27	0.26	0.25	0.21	0.21	0.20
0.30	0.27	0.26	0.25	0.24	0.20	0.20	0.19
0.24	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N.S.	N	N	N	N	N	N	N
N	N	N	N	N	N	N	N

(continued)

Table 15 (Cont.)

Differences and their significance							
Days, LSD							
7	17	22	11	6	5	21	13
x-2.69	x-2.71	x-2.74	x-2.77	x-2.83	x-2.84	x-2.96	x-
0.37	0.35	0.32N	0.29	0.13	0.12	0.10	
0.27	0.25	0.22N	0.19	N	N	N	
N	N	N	N	N	N	N	
N	N	N	N	N	N		
N	N						
N	N						
N	N						

LSD = 0.33
Arithmetic mean = 2.55

Table 16. Flavor scores for milk from four groups of cows placed on one of the fertilized plots.

	Plot no.	Dates 24			25			26			27			28			29		
		Flavor scores*																	
Judge 1	1	3	2	2**	3	3	3	3	2	3	3	3	3	3	2	3	2	1	1
	2	2	2	3	3	2	2	2	2	2	3	2	2	2	2	2	2	1	1
	3	2	2	2	2	3	2	2	2	2	4	3	3	4	4	3	2	2	2
	4	2	2	2	3	2	2	2	2	2	2	2	2	4	4	4	2	2	1
Judge 3	1	3	3	3	4	4	4	3	3	4	4	3	4	4	4	4	3	3	3
	2	4	3	4	3	3	3	4	3	3	4	3	4	3	4	3	3	2	2
	3	3	2	2	2	3	2	2	2	3	4	3	3	3	3	3	4	4	4
	4	3	3	3	3	3	3	3	4	3	3	3	3	4	4	4	3	3	3
Judge 4	1	3	3	3	3	3	3	3	3	2	3	2	3	3	3	3	3	3	3
	2	3	2	3	2	2	2	2	2	3	3	2	2	2	2	2	3	2	2
	3	2	3	3	3	3	3	3	3	3	3	3	3	3	4	3	4	4	4
	4	3	3	2	2	2	3	3	2	3	2	3	3	2	3	3	3	3	3

* Flavor scores ranging from 1 - 4 as follows:

No feed flavor	1
Slight	2
Definite	3
Pronounced	4

** Each sample was given three times for evaluation.

Table 17. Mean flavor scores (of three evaluations) by each judge for milk from four groups of cows on different plots with different nitrogen fertilization, over a period of five days.

A. Cows on one of the fertilized plots

Days	1				2				3				4				5			
Pastures	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Judge	Mean flavor scores																			
1	1.3	1.3	1.3	1.7	2.3	2.3	2.7	1.0	2.3	2.3	2.0	2.0	2.0	2.7	2.3	2.0	2.7	3.0	2.0	1.0
2	2.7	2.3	2.3	2.0	2.0	2.0	1.3	2.0	2.7	2.7	2.0	2.3	3.0	3.0	2.7	3.0	3.0	2.7	3.0	2.7
3	2.3	2.0	2.0	2.0	3.0	3.0	3.3	3.0	2.7	2.0	2.3	2.7	3.0	2.0	2.7	2.7	3.0	2.7	2.3	2.0
Total	6.3	5.6	5.6	5.7	7.6	7.3	7.3	6.0	7.7	7.0	6.3	7.0	8.0	7.7	8.3	7.7	8.7	8.4	7.3	5.7
Av. all judges	5.8				7.1				7.0				7.9				7.5			
Av. per judge	1.8				2.7				2.3				2.6				2.5			

B. Mean flavor scores for five days selected at random from 23 days of mean flavor scores.

Days	1				2				3				4				5			
Pastures	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Judge	Mean flavor scores																			
1	3.0	2.3	2.5	2.3	2.7	2.0	2.0	2.0	3.0	2.0	3.3	2.0	2.7	2.0	3.7	4.0	1.3	1.3	2.0	1.7
2	4.0	3.0	2.3	3.0	3.3	3.3	2.3	3.3	3.7	3.7	3.3	3.0	4.0	3.3	3.0	4.0	3.0	2.3	4.0	3.0
3	3.0	2.0	3.0	2.7	2.7	2.7	3.0	2.3	2.7	2.3	3.0	2.7	3.0	2.0	3.7	3.0	3.0	2.0	4.0	3.0
Total	10.0	7.3	7.8	8.0	8.7	8.0	7.3	7.6	9.4	8.0	9.6	7.7	9.7	7.3	10.4	11.0	7.3	5.6	10.0	7.7
Av. all judges	8.3				7.9				8.7				9.6				7.6			
Av. per judge	2.7				2.6				2.9				3.2				2.5			

Table 18. Percent total nitrogen in milk obtained from four groups of cows.

Plots	Dates: May 2	May 9	May 16	May 23	May 30
	Percent total nitrogen				
1	.635	.626	.603	.685	.620
2	.632	.594	.648	.591	.584
3	.585	.568	.558	.569	.555
4	.561	.579	.565	.573	.554

Table 19. Percent non-protein nitrogen concentration in milk obtained from four groups of cows.

Plots	Dates: May 2	May 9	May 16	May 23	May 30
	Percent non-protein nitrogen				
1	.038	.031	.030	.034	.040
2	.044	.042	.044	.041	.033
3	.048	.047	.044	.047	.039
4	.042	.040	.049	.043	.036

Table 20. Percent non-casein nitrogen in milk obtained from four groups of cows.

Plots	Dates: May 2	May 9	May 16	May 23	May 30
	Percent non-casein nitrogen				
1	.149	.141	.137	.121	.149
2	.151	.146	.179	.098	.139
3	.133	.140	.129	.127	.131
4	.131	.137	.138	.141	.140

THE INFLUENCE OF NITROGEN FERTILIZATION OF BROMEGRASS ON
THE FLAVOR AND NITROGEN COMPOSITION OF MILK

by

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A study was designed to establish first, how nitrogen fertilizations affect the flavor of milk. Second, how nitrogen fertilizations and bromegrass composition affect the subsequent milk composition. Third, how the ammonium, amide and total nitrogen concentration of bromegrass affect the intensity of milk flavor. In order to make this study, four separate fenced plots of bromegrass pasture were employed. Three plots were fertilized with different kinds of commercial nitrogen fertilizers and one plot was left unfertilized. Soil samples were collected from each plot once a week, starting on April 25 and continuing until May 22. Samples of bromegrass were obtained from each plot once a week, on the same day that soil samples were taken. Milk samples were collected from four groups of cows representing three breeds, Jersey, Guernsey and Ayrshire. These cows were assigned to each plot by random and were given numbers corresponding to the plot on which they pastured. Samples of milk were collected daily from May 2 until June 1, for organoleptic analysis. Additional samples of milk were collected weekly, on the same day that soil and grass samples were taken, for chemical analysis. Soil samples were analyzed for ammonium to establish the effect of nitrogen fertilization on ammonia content of the soil. Soil samples were analyzed also for nitrate to see the effect of nitrogen fertilization on the nitrate content of the soil. It was found that both ammonia and nitrate concentrations were affected to some extent by the kind of nitrogen fertilization. Bromegrass samples were analyzed for ammonium, amide and total nitrogen. It was found that the concentrations of these constituents were affected by nitrogen fertilization as well as by the stage of growth of the bromegrass.

Weekly collected milk samples were analyzed for NPN, NCN, and TN. These three constituents were found to vary with age of bromegrass, and to some extent with the level of nitrogen fertilization.

Analysis of variance of the organoleptic panel flavor scores indicated significant differences among judges, pastures and days at the five percent level. LSD tests at the five percent level indicated that plot 1 was significant for the mean flavor scores. An analysis of variance was performed on data which was collected when all cows were placed on one plot. This analysis of variance showed significant differences among groups of cows and judges at the five percent level.

LSD tests at the five percent level indicated that group of cows on plot 1 was significantly different from groups on the other plots. These differences could be related more or less to group of cows on plot 1.