

EFFECT OF THE SURFACE ACTIVE AGENT POLOXALENE
ON MILK FLAVOR WHEN FED TO COWS

by

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INTRODUCTION

Flavor is one of the most important characteristics of milk governing its market value. Innumerable experiments have been conducted and many research reports published on causes and corrections of off-flavors in milk. In spite of this many of the off-flavors still are encountered.

It is generally accepted that feeds and feeding practices contribute to milk flavor; therefore a great deal of research has been directed towards understanding how the flavors are transmitted to milk. From such research it eventually may be possible to eliminate feed associated off-flavors.

Of the chemical substances involved in milk flavors methyl sulfide (Me_2S) has been shown recently to be of considerable importance. It has been suggested that the normal concentration of Me_2S in milk could result from the catabolism of methionine (20) and this concentration in conjunction with the excess coming from particular feeds could give rise to feed flavor defects.

The possibility that the condition of gases in the rumen might contribute to flavors was considered by several researchers here. In conjunction with a study of bloat in dairy cattle, two producers cooperating with Kansas State University dairy scientists in testing polyoxypropylene polyoxyethylene block polymer (poloxalene) as a bloat preventive agent believed that the treatment improved milk flavor. Controlled studies at the University and at some commercial dairy farms demonstrated no adverse effect of poloxalene on animal health or production (27). A logical extension of this research was to establish the effect of the surface agent on milk flavor.

The research presented in this thesis was designed to:

- (a) determine the effect of the surface active agent, poloxalene, on milk flavor under controlled conditions with cows fed bloat provoking alfalfa and also rye and bromegrass pasture.
- (b) observe the effect of this agent on the type of volatile materials found in the milk.
- (c) determine changes in some of the physical-chemical properties of rumen ingesta upon treatment with poloxalene.

REVIEW OF LITERATURE

Importance of Flavor in Milk

Maintenance of a uniform milk flavor free of disagreeable taints is of major importance to the dairy industry. Consumers accept or reject milk more on the basis of flavor uniformity than for any other reason. Therefore a great deal of research has been directed towards understanding and controlling milk flavors. Taints caused by feeds are the most common type of flavor defect observed in milk. As early as 1757 (60) in England it was reported that feeding turnips to cows produced a bitter flavor in milk. According to Jensen in 1960 (31) the most prevalent milk flavor defect was of feed origin and was observed in 72.7% of the milk samples analyzed. Although the widely used closed pipeline milking system avoids the incorporation of many feed and barn odors into milk, there is still a problem with flavor defects. In 1963 Shipe (59) reported a New York milk flavor survey in which 39% of the samples tasted from 120 dairies had feed flavor defects.

The continued existence of feed flavors in milk may be due partially

to the dairyman's ignorance of available information regarding its occurrence, prevention and commercial importance. However, more fundamental knowledge and research is necessary before the problem will be brought under control. One additional consideration regarding this problem is the lack of an objective measure of the feed flavor. Many off-flavors not otherwise characterized are classed as feed.

Factors Responsible for Feed Flavor

During the past two centuries there have been numerous papers published indicating that ingestion of certain feeds and inhalation of strong odors were responsible for some of the off-flavors in milk. In addition to the kind of feed, feeding habits and type of housing were found to influence milk flavor.

Bradfield et al. (8) in 1960 observed that flavor scores of milk were lowest when cows were fed silage or breathed the odor from the silage 2 1/2 hr before milking. In 1957 Potts et al. (53) observed that feeding silage 2 hr before milking produced a strong off-flavor in milk. Numerous papers have been published in support of these statements. Shipe (59) reported that Babcock in 1928 gave some practical recommendations for preventing feed flavors in milk. Babcock suggested feeding all materials likely to cause off-flavors just after milking and keeping cows and barn clean and free of odors.

Different kinds of feeds have been incriminated in causing off-flavors in milk (60). Strobel (60) reported that Roadhouse in 1937 showed that 15 lb alfalfa hay or 25 lb green cut alfalfa when fed prior to milking produced a distinct feed flavor in milk. A soda or alkaline-like off-flavor

was noticed by Trout et al. (62) in 1940 in milk from cows grazing on alfalfa-bromegrass pasture. It was also found that alfalfa contributed a more distinct off-flavor than bromegrass. Colson and Bassette (17) studied the effect of sorghum silage, rye pasture and bromegrass pasture on flavor of the milk. Milks from cows fed the above feeds were evaluated by a trained taste panel and by the percentage of milk returned by consumers. These results indicated that rye pasture produced a stronger off-flavor than bromegrass and sorghum silage, whereas the sorghum silage produced least taint in milk. McDowall in 1957 (36) found that both animal fats and vegetable oils produced off-odors in milk when fed to cows. Some of the concentrates and feed additives were also found to produce disagreeable taints in milk (36, 37, 40, 23).

Since feeds have been shown to be one of the sources of off-flavors in milk, research has been directed to identify the actual chemical compounds in feeds or in milk responsible for the flavor defect.

Chemical Compounds Responsible for Feed Flavors in Milk

Morgan et al. (43) in their study of volatile constituents of grass and corn silage found mixtures of methyl sulfide, lower aldehydes, ketones, alcohols and simple methyl, ethyl and propyl esters. They suspected methyl sulfide and esters as principal contributors to the off-flavors; these occurring from inhalation of the silage odor. Potts and Kessler in 1957 (53) reported that there was no apparent relationship between the levels of acetone bodies in the milk of cows fed with grass silage and the flavor of the milk. Loney et al. (34) in 1963 observed greater concentrations of acetone and 2-butanone in milk of cows fed silage shortly before milking.

Shipe et al. (58) reported that introduction of acetone, 2-butanone, methyl sulfide and Cis-3-hexane-1-ol through rumen or lung imparted flavors that closely resembled those that could be found in commercial milk. In 1956 Patton et al. (50) detected methyl sulfide (Me_2S) in normal milk and suggested that this might contribute to characteristic flavor of raw milk. From their data the threshold level of Me_2S was calculated to be 12 ppb. in distilled water. At higher concentration in milk the flavor was described as malty or cowy. Wynn et al. (65) in 1960 observed Me_2S in raw milk. Day et al. (20) in 1964 identified Me_2S in butter and reported that high concentrations could be characterized as feed flavor. Toan et al. (61) in 1965 reported the threshold level of Me_2S in homogenized milk as 115 ppb. which could be detected by gas liquid chromatography. The flavor above this threshold level was described as methyl sulfide-like, molasses-like or cowy.

Since Me_2S appears to be associated with the flavor of milk and milk products, information about its origin in the animal body should be helpful in controlling the level in milk.

Day et al. (20) suggested two possible precursors of Me_2S , each originating from plant materials. These are dimethyl- β -propiothetin and methyl methionine sulfonium salt which have been isolated from algae and asparagus respectively, by Challenger et al. (11). Kiribuchi and Yamanishi in 1963 (33) reported that the sulfonium salt was being converted to Me_2S and homoserine when green tea was heated. Samuelson (57) detected S^{35} in the Me_2S fraction of milk volatiles after administering methionine- S^{35} through the left pudic artery of a lactating cow. McRorie (39) suggested that certain organisms were responsible for the release of methyl sulfide

from the methyl sulfonium derivative of methionine occurring in cabbage. In this investigation homoserine and a characteristic odor of Me_2S were identified as products of decomposition of both the naturally occurring and synthetic methyl sulfonium derivatives when heated in aqueous solutions. Ruzicka (56) isolated 3.5 gm of Me_2S from 1000 kg of dried bovine blood. Normal concentrations of Me_2S encountered in milk were reported to result from metabolic product of methionine. The high concentrations of Me_2S which contributed to feed flavors may develop from particular feeds (20).

Physiological Transmission of Flavors to Milk

Peterson et al. (52) reported that inhalation of some odors produces volatile substances in the blood and milk which were responsible for flavor defects in milk. Bradfield and Allen in 1960 (8) observed a more intensive flavor when the cows ate the feed than when they only breathed the odor from it. In 1960 Dougherty et al. (21) conducted a series of experiments at Cornell to obtain information on the physical process involved in imparting feedy flavors to milk. Their observations led to the speculation that eructed gases could be a contributing factor in the production of off-flavors in milk. In 1962 (22) they studied the depth of penetration of eructed gases into the lungs and the effect of these on the flavor of milk. Two cows with ruminal and tracheal fistulas were used in this research to facilitate introduction into the lungs or rumen of substances suspected of influencing milk flavor. Odors were introduced into the lungs by pumping air through a chamber containing odoriferous material. Test substances were introduced into the rumen through a ruminal cannula while the lungs were being supplied with fresh air. The milk samples were collected at

regular intervals and judged by a panel of six experienced judges. The results indicated that ethyl and amyl acetates appeared in milk in 15 min when introduced through the lungs, as against about 60 min when introduced through the rumen. Likewise when the gases from the rumen were permitted to enter the lungs they caused off-odors more rapidly than when they were blocked from entering the lungs. This supports the hypothesis that excreted gases can increase the rate of flavor transmission. The same group conducted experiments to determine the pathway of transmission of the onion flavor to milk. Their observations indicate that macerated onion by itself could not cause a flavor defect in milk unless it is incubated at least half an hour with rumen ingesta. This is in agreement with Peterson and Brereton (52) who reported that onion odor failed to impart a typical onion flavor to milk. Honkanen et al. (27) in 1964 conducted experiments to determine the compounds that could be transferred to milk through the digestive route. A series of aliphatic alcohols, aldehydes, ketones and esters were fed and variations in the rate of their transmission into the milk through the rumen was observed. Experiments in this study were performed with cows that were fed an odorless purified diet. Pure chemical compounds under investigation were dissolved in acetone or ethanol and fed through armored plastic tube into the rumen. Milk samples were collected before feeding, and at 1 to 2 hr intervals after feeding and analyzed later by gas chromatography. Most of the substances tested were transferred to the milk. The maximum concentration was reached in 2 hr after feeding. Higher molecular weight compounds reached the maximum concentration somewhat later. The concentration then rapidly decreased so that 8 to 10 hr after administering the material only about 10% of the maximum concentration was present.

Since the flavor compounds are transported through the blood to the mammary gland the time necessary to impart a detectable off-flavor depends upon the concentration of flavor compounds in the blood. The concentration in the blood in turn depends on the amount being supplied to the cow's lungs or rumen. As the supply is removed it is assumed that there is a reversal of flow of flavor components. They are transported by the blood away from the mammary gland and are eventually excreted or exhaled. Thus the lungs and rumen act not only as points of absorption for flavor compounds but also as points of elimination. This explains why feeding after milking provides a means of avoiding feedy flavor (59).

With this concept of transmission of flavors to the milk, it can be speculated that accumulation of gases in the rumen as in bloat or drenching of the animal with some of the medicaments may result in off-flavor.

Effect of Bloat and Surface Active Agents on Milk Flavors

Bloat in ruminants is not a new malady. It was described by a Roman author in 60 A.D. He recommended as a treatment pouring vinegar through the left nostril and putting 2 oz of grease in the jaws. Johns (32) reported that a writer in 1807 recommended drenching with sweet oils, raw linseed, or melted hog lard to relieve a bloated animal. Some of these remedies are still in use today.

It is believed that bloat is caused by excessive frothing of the rumen digesta and accompanied by probable inhibition of eructation. There are differences of opinion regarding factors responsible for foam formation. Nichols in 1954 (47) stated that the amount of reduction in buoyancy of rumen material is directly related to quantity of gas formed. As legumes

quickly gravitate to the lower portions of the rumen, bubbles of gas formed on food particles become entrapped in ruminal juice and the level of fluid in the rumen is raised, thereby preventing eructation. Different compounds present in the plant such as saponins, proteins and pectins were incriminated in bloat production because they are foam formers. Much work has been done in attempting to correlate legume saponins and bloat (19, 16, 32). It does not seem probable that saponins are responsible for the viscous foam formation in the rumen. The rumen pH is higher in legume bloated animals than in the saponins extracted from legumes (38). Furthermore, in the pH range of saponin foam, rumen stasis develops (18) whereas in bloat, ruminal contractions are not inhibited except in terminal stages (15). The fact that foamed rumen liquor cannot be foamed again (Mangan 1959, 42) indicates that the foaming agent is protein and not saponin. Therefore, the causative agent would appear to be cytoplasmic protein of legumes or salivary mucoprotein. Gutierrez (25) in 1959 isolated strains of microorganisms which produced significant amounts of slime from the degradation of saponins. The production of slime from the plant compounds by bacteria suggests it as a significant factor in the bloat syndrome in animals on legume pasture. The bloat producing ability of legumes seems to vary with the condition of the feed. Boda in 1958 (7) observed a lower intraruminal gas pressure in cows fed dehydrated alfalfa compared to those fed fresh alfalfa. The quantity of dehydrated alfalfa fed was limited so that the amount of dry matter was equal to the amount present in fresh alfalfa consumed previously. The suggested explanation for this reduction of bloat associated with dehydration may be denaturation of water soluble proteins. Meyer et al. (41) in 1965 observed a decrease in the nitrogen content of

the whole alfalfa plant and its fractions with increasing maturity. A general decrease in the incidence of bloat was also observed with increasing plant maturity. Since the formation of foam has been shown to be a surface active phenomenon and bloat is produced by excessive foaming of rumen ingesta following consumption of legume, efforts were made by various workers to control foaming by using surface active agents. Substances which lower the surface tension and control foam formation like mineral and vegetable oils, animal fats and non-ionic antifoaming agents, all are included in the category of surface active agents.

Nichols et al. (48) in 1957 tried silicones and lecithins to influence ruminal surface tension. They found lecithins to be more effective than silicones in surface tension reduction. Brown et al. (9) in 1958 reported that the addition of 1 to 2% water dispersible oil to drinking water markedly reduced the incidence and severity of bloat in steers grazing alfalfa pasture. Oils sprayed on fresh alfalfa silage indicated that a level of 0.25 lb of oil per animal per day greatly reduced bloat. They also reported that penicillin reduced bloat when first administered, but after late summer rains, when the bloat producing potential of the forage increased, a higher incidence of bloat was observed in animals fed penicillin than in those that were not fed penicillin. Reid (54) in 1958 noticed that pasture spraying with emulsified peanut oil, emulsified tallow, cream of Avlinox (a ricinoleic acid derivative) provided a high degree of protection from bloat. Sprays containing polymerized dimethyl silicone at rates of 1 to 4 g per cow were ineffective. For sheep Avlinox sprayed on pasture gave protection from bloat for several hours. Colvin et al. (18) in 1959 observed that the administration of vegetable oils or animal fat, before

alfalfa feeding, prevented the development of bloat by increasing the rate of eructation.

The effect of some of these surface active agents on milk flavor was studied by various workers. McDowall et al. (36) in 1957 studied the effect of ingestion of linseed oil, soybean oil, mixed beef and mutton tallow and peanut oil on production and properties of milk and butterfat. For treatment of bloat drenching cows with 300 ml linseed oil twice daily had no appreciable effect on the flavor of milk, cream and butter during the first two days of treatment. On the third day milk and cream had a sweetish flavor. This foreign flavor was observable in milk and cream for several days after cessation of treatment. Soybean oil drenched at the same rate and dose caused a hay-like flavor in cream on the first day of treatment. On the following two days of drenching this became apparent also in the milk and butter. The taint was present for three days after cessation of treatment, but was not considered objectionable by the graders. No effect on yield, composition, or flavor was observed when animals were drenched twice daily with 125 g tallow emulsion. Drenching with 300 ml peanut oil twice daily caused a slight sweetish flavor in the milk and cream at the end of drenching period. The off-flavor was not sufficiently strong to be commercially significant. There was no evidence of any foreign flavor in the milk or cream, throughout the period of treatment, when cows were treated with lower dose rates of any of these oils. The flavor of the milk from cows that were on pastures sprayed with any of the oils mentioned above were normal throughout the grazing period.

McDowall et al. (37) in the same year reported that drenching of cows with 300 ml of whale oil twice daily for 3 days caused objectionable fishy

or oily flavors in the milk and cream. The off-flavor persisted in these products for several days after cessation of drenching. Because of its marked effects on the flavor of milk, cream and butter and on the other properties of butter, whale oil was considered to be unsuitable for treatment of bloat. McDowall et al. (37) stated that any material used for the treatment or the prevention of bloat in dairy cows should not have undesirable effects on milk production, quality or composition or on butterfat properties. According to Johns (32) surface active agents, to be effective and practical, should maintain a concentration in the rumen over the whole period during which the animal is eating the bloat producing herbage. Oils appeared to lose their effect quickly because of their low water solubility. The animals were protected for only 2 to 4 hr (1, 16, 32).

A search was made for antifoaming agents that are more water soluble than oils and that remain in the rumen for a longer period of time. In 1965 Bartley (1) used a nonionic surfactant to prevent legume bloat. The surface active agent used was a polyoxypropylene polyoxyethylene block polymer (poloxalene). This material is a liquid with a high molecular weight. Some of its other physical and chemical properties are presently under investigation. Four trials were conducted in which two pairs of dry fistulated, identical twins were employed. In the first and second trials the experimental animals were pastured from 7 A.M. to 11 A.M. and 4 P.M. to 7 P.M. on good quality alfalfa pasture that was in the early stages of growth. In trial I, one member of each twin pair was treated with poloxalene and the other served as control. In this trial 20 g of poloxalene effectively prevented bloat for at least 12 hr. In trial II, 20 g of

poloxalene introduced via rumen fistula prevented froth formation and bloat during the first day. The incidence of bloat was slightly greater on the second day than the first day but still lower than that observed during a three day pretreatment control period, indicating a significant carryover effect. In trials III and IV, animals were fed freshly cut alfalfa at 7 A.M. and 4 P.M. In trial III, 10 g and 20 g levels of poloxalene were administered orally and were proven to be equally effective. The introduction of poloxalene into the rumen by fistula was found to be more effective than feeding with grain. In trial IV, 5 g level was administered with grain and found to prevent bloat during the first 12 hr after its administration, however with little carryover effect into the next day. It was observed during this study that no animal treated with poloxalene had a bloat score above 1.5 (a definite bloat condition scores between 3 and 5) during the 12 hr period following treatment. This surface active agent had been effective in numerous field trials (1). Helmer et al. (26) studied the effect of poloxalene on milk production, feed intake, health, reproduction and rumen fermentation. It was found that this surface active agent had no deleterious effect on milk production, milk fat, body weight, feed consumption, conception rate or animal health. Ten grams of the agent daily did not affect rumen ammonia concentration, rumen pH or rumen lactic and volatile fatty acid concentration. Helmer et al. (26) concluded that feeding the bloat preventive agent in quantities up to 40 gm per day does not affect dairy animals unfavorably. Prior to the research reported herein the effect of this surface active agent on milk flavor has not been studied.

Methods of Analysis of Flavor Compounds

In grading milk and milk products, organoleptic analysis has been used extensively even from early days of flavor research. Patton and Josephson (51) used organoleptic analysis to derive the flavor thresholds of some pure chemical compounds and considered that concentrations in excess of their threshold range produced a direct contribution to flavor. Colson and Bassette (17) in 1962 employed a consumer group and taste panel to evaluate feed flavors in milk. Organoleptic analysis, however, has limited use in flavor research. It can be used to characterize the flavors but not to quantitate them. Many chemical analytical methods have been utilized to identify and measure chemical compounds responsible for different flavors in foods in hopes that an objective quality control can be realized.

Paper chromatography and column chromatography were used to identify volatile compounds in milk and milk products (49). James and Martin in 1952 (29) used gas chromatography in the analysis of mixtures of volatile fatty acids. Gas chromatography combined with mass-spectrometric analysis was used by Patton et al. (50) to detect methyl sulfide in raw milk. Bassette et al. (2) used mass spectrometry coupled with gas liquid chromatography (GLC) in study of flavors of milk. GLC methods of analysis have developed rapidly and are applied extensively in dairy as well as in non-dairy industries for flavor research and quality control. According to a review by Chou (12) there were more than 1600 publications on the application of gas chromatography in food flavor research in the year 1961. In most of these methods low-temperature reduced-pressure distillation techniques together with various trapping procedures were employed to recover volatile compounds from foods for chromatographic analysis.

Nawar et al. (44) collected volatiles from 500 g of milk fat by means of recycling vapors and employing a trapping system. They detected eight components in small quantities in samples heated up to 120° C; whereas heating for two hours at 130°-185° C produced up to 19 components. Wong and Patton (63) used as many as 5 traps which included one wet ice, two dry ice-ethanol traps and two liquid nitrogen traps after a cold water condenser to increase the trapping efficiency. The distillates subsequently were analyzed. Wong in 1963 (64), by using the same method compared volatile compounds of fresh and decomposed cream. Bavisotto et al. (5) used a dual column, low temperature operated GLC to study a number of volatile materials of fermented milk products. Nawar et al. (45) stated that the development of hypersensitive detectors has provided a means of detecting flavor compounds at the parts per billion level and analysis is rapid, accurate and reproducible. They suggested that by GLC methods sufficient information may be obtained by transmission of head space gas sample directly to the instrument. Wynn et al. (65) vaporized volatile distillates of milk with different feed flavors by surrounding the container of the distillates with oil at 100° C. The vapors were then sampled directly into the helium, passing through the GLC column.

Nawar in 1962 (46) analyzed cheese volatiles and Jennings et al. (30) used a dual column instrument with ionization detectors, and nitrogen as the carrier gas to analyze milk volatiles. Vapor samples were prepared by heating the milk for 1 hr at 80° C in a stoppered flask. One ml of gas sample was withdrawn from above the liquid through the stopper for chromatographic analysis. They observed an increased peak height of methyl sulfide in the case of milk with alfalfa feed flavor when compared to

rancid milk, milk exposed to germicidal lamp, fluorescent lamp for 6 hours, etc. Bassette et al. (3) employed a somewhat similar but improved technique for analysis of dilute aqueous solutions. Two ml aqueous test solutions in a 5 ml serum vial was saturated with 1.2 g of sodium sulfate to increase volatiles in head space vapors to be analyzed. The sample was warmed to 60° C for 8 min to accumulate head space gas above the liquid for chromatographic analysis. With this technique and employing a hydrogen flame detector and a modified electrometer, they were able to detect some organic compounds at less than 0.1 ppm. These workers (4) later could analyze with the same method the volatile components present at and below part per million level in biological fluids such as milk, blood and urine. In 1965 Toan et al. (61) used the same method and could detect Me_2S even at parts per billion levels. With such a high sensitivity, this technique proved successful for direct chromatographic analysis of flavor compounds and was employed in the author's research.

MATERIALS AND EXPERIMENTAL PROCEDURE

A predetermined number of dairy cows in mid-lactation was selected to be fed each of the rations employed for the milk flavor portion of this experiment. Two pairs of fistulated, dry, identical twin cows were employed in the study of pH and surface tension of rumen fluid. Half the cows involved in each of the feeding trials were administered the surface active agent, polyoxypropylene polyoxyethylene block polymer (poloxalene) to determine its effect on milk flavor. The other half were not treated. The feeds employed were bromegrass, rye, and alfalfa forages. Milk samples were collected during the evening milking and brought to the laboratory.

About 10 ml of milk from each sample was refrigerated for GLC analysis. The remainder of each milk sample was pasteurized and stored in the refrigerator for organoleptic analysis. The concentration of methyl sulfide in experimental samples as measured by GLC was compared with the flavor scores of the milk samples reported by a judging panel. Samples of rumen fluid were collected from the fistulated cows one time before and twice after feeding the grain ration and analyzed for pH and surface tension.

Feeding of the Cows and Collection of Milk Samples

Procedure for Feeding of the Cows: In the first feeding trial bromegrass pasture was studied. Six cows in mid-lactation were selected from the Kansas State University dairy herd for use in this experiment. Three of the cows were treated and three untreated. Of two pairs of fistulated twins employed, one from each pair was treated with poloxalene. The bromegrass pasture was about 4 in. high at the start of the trial. The cows were turned out to graze every day at 11 A.M. and brought in for milking at about 2:30 P.M. After milking, the cows were allowed to graze the pasture again until 4 A.M. They were brought in then for milking. The fistulated twin dry cows also were turned out to graze bromegrass at 11 A.M. and brought into the lot by 3 P.M. for feeding the grain ration. After being fed grain, they were again kept on pasture until 7 A.M. at which time they were brought into the dry lot for their morning grain ration. Other than during pasture feeding and milking, the cows were kept in a dry lot where only water and a mineral mixture were provided. At milking time each cow was fed 2 lb grain mixture. Only 2 lb grain mixture were fed in order to increase the animal appetite such that she might

consume more pasture. The cows were fed according to the above program for four days prior to actual collection of milk samples for examination. For a few days in the beginning of this trial the cows were fed at the rate of 1 g poloxalene per 100 lb weight, with the grain mixture. Subsequently the dosage was increased to 2 g per 100 lb body weight and continued at this rate throughout this feeding trial.

Rye pasture was investigated in the second trial. Since there was limited rye pasture only four cows were used, of which two were treated and the other two were not. The study of pH and surface tension of ruminal fluid was discontinued in this trial to conserve forage. The program for grazing, milking time of the cows and collection of samples were the same as those in the first trial. Again 2 lb grain mixture was fed at milking time. However, the poloxalene dosage was 2 g per 100 lb body weight from the beginning of this feeding trial.

In the third trial, chopped green alfalfa was fed. Ten cows in early to mid-lactation with good production records were selected. Five were treated with poloxalene and the other five were untreated. Two additional cows were also selected for production of control milk. The control cows were fed only hay and grain and did not receive poloxalene. The same two pairs of fistulated identical twin dry cows which were used in the bromegrass trial were employed in this trial. One cow from each twin pair was treated with poloxalene and the other not treated. The pH and surface tension of rumen fluid was determined as in the first trial. All the treated and untreated cows were fed chopped green alfalfa ad libitum but the quantity was adjusted so that they could finish all of it within 2 hr. In the beginning of the trial the feeding started at about 1 P.M. and the

cows were brought in for milking by 3 P.M. Later when the evening milking time was changed to 4 P.M. the feeding time also was changed to about 2 P.M. The cows were fed alfalfa green chop again at 7 A.M. after the morning milking. Dried alfalfa hay was provided to the control cows throughout the trial. Grain ration was fed at the rate of 1 lb for every 3.5 lb of milk yield for Holsteins and 1 lb for 2.5 lb of milk yield for Jerseys at milking time. The twin dry cows were fed only 2 lb of grain ration at the same time that the milking cows were fed. Control cows were fed the grain ration at this same rate. Poloxalene was administered orally in liquid form by capsule at the rate of 1 g per 100 lb body weight at the time of feeding the grain ration.

Collection of Milk Samples: Milk samples were obtained from bucket milkers daily except on weekends. The udder was cleaned and dried thoroughly before milking. Milk samples were collected from individual cows in quart bottles during the bromegrass and rye pasture feeding trials, whereas in the alfalfa feeding trial milk samples from individual cows were collected in separate Whirl-Pack plastic bags. Composite milk samples from treated, untreated and control cows also were collected in quart bottles.

Preparation of Milk for Organoleptic and Gas Chromatographic Analysis

Milk samples were brought to the laboratory immediately after collection. A small portion of each cow's milk as well as one sample from the mixed herd milk was refrigerated in Whirl-Pack plastic bags for gas chromatographic analysis. The remaining portion of each sample was

laboratory pasteurized by heating the milk at 61.5° to 62° C for 30 min in milk bottles with regular shaking at 5 min intervals. The level of water in the water bath was kept well above the milk level in the bottle. During the bromegrass and rye pasture feeding trials, control milk was collected from the University farm bulk tank.

Since there were more cows in the alfalfa feeding trial than in previous trials, milks from all treated cows were combined and compared organoleptically with the milk from untreated cows instead of with individual milk samples. Small quantities of each of the composite milk samples were saved in addition to small aliquots of each of the individual cow milk samples for gas chromatographic analysis. The remaining composite milk samples were laboratory pasteurized and refrigerated until time for tasting.

A trained panel of 8 to 10 persons, all with some experience in judging milk flavor, was employed to examine the samples. The panel was not informed about the experiment. During the first week prior to administration of poloxalene normal and feed flavored milk samples were arranged at random for tasting. This procedure was adopted in order to familiarize the judges with the feed flavor in the milk and to be sure that they could differentiate between normal and feed flavors. The milk samples were presented in brown bottles to avoid the identification of the experimental milk by difference in color between normal milk and more highly pigmented experimental milk. Those persons who were unable to make consistent judgments on replicates of the same sample during the week training period were dismissed from the panel. The pasteurized experimental milks were refrigerated and organoleptic analysis with the judging panel of 8 to 10

judges was completed within 24 hours of milk collection. The milks were distributed randomly in duplicate in coded brown colored bottles and fore-warmed to 60° F before arranging for tasting. Two milks from the previous day's samples, one having the maximum and the other the minimum flavor score, were also included every day at the time of judging the milks. The flavor scores of those two milk samples (their average panel flavor scores for that day) were displayed on the bottles in order to serve as references at the time of scoring the fresh samples.

The flavor score range was from 1 to 10. Number 1 indicated an excellent flavor and 10 denoted a very bad flavor. The average flavor score of each sample was calculated. The results were statistically analyzed and related to the treatments.

Gas Chromatographic Analysis. The instruments used for gas chromatographic analyses were: Aerographs Model 600-B (A) and Model 500-C (B), each with a hydrogen flame ionization detector. A 1.05 mv Brown-Honeywell recorder for instrument A and a 1 mv Brown-Honeywell recorder for instrument B were used. Matched 10 ft x 1/8 in. stainless steel columns packed with 20% carbowax 20 M on 60 to 80 mesh acid washed HMDS treated chromosorb P were employed in each instrument. The operating conditions of the two instruments were:

	(A)	(B)
Column temperature	100° C	100° C
Nitrogen out flow	15.4 ml/min	17.4 ml/min
Hydrogen out flow	24.04 ml/min	26 ml/min
Chart speed	1/3 in./min	1/3 in./min

Apparatus and material used in sample preparation were as follows:

Sampling bottles - serum vials, 15 mm diameter x 52 of 5 ml capacity with self-sealing rubber caps

Mechanical shaker - A Fisher clinical shaker operated at a rate of 275 to 285 oscillations per min

Syringe - 1 ml gas tight syringe, Hamilton No. 1001

Reagents - sodium sulfate, anhydrous, ACS grade; mercuric chloride, anhydrous, ACS grade

Samples of treated, untreated and control milks were analyzed within 24 hr by preparing headspace gas according to the procedure used by Toan et al. (61). In this procedure a 2 ml milk sample was saturated with sodium sulfate by adding the sample to a vial containing 1.2 g of sodium sulfate. The vial was then heated at 60° C for 2 min in a water bath, mixed on the shaker for 5 min, a new dry serum cap was introduced and the sample was heated again for 8 min at 60° C in a water bath. One milliliter of headspace vapors was drawn from above the sample (by puncturing the rubber serum cap with the syringe needle) and injected into the chromatograph.

Identification of Me_2S in the milk sample was accomplished by the simple chromatographic reaction technique of Bassette et al. (3). The methyl sulfide peak could be eliminated by treating the 2 ml milk sample with 0.2 g mercuric chloride (HgCl_2) in the self-sealing rubber capped serum vial. The HgCl_2 treated milk was placed on a mechanical shaker for 1 hr after which it was analyzed as outlined above.

The same method was adopted for gas chromatographic analysis of laboratory pasteurized composite milk samples from poloxalene treated and untreated cows daily during alfalfa feeding trial. At the end of this trial the composite milk samples from one day including those from treated and untreated cows were subjected to vacuum pasteurization. This milk

was analyzed according to the above method to determine how effectively Me_2S was removed by vacuum pasteurization.

To analyze quantitatively for Me_2S , heights of Me_2S peaks in chromatograms obtained from milk collected during different feeding trials were compared with those of a standard curve. The curve was prepared from the chromatographic analyses of Me_2S standard solutions, prepared by W/V, in pasteurized-homogenized milk.

Determination of Taste Threshold of Methyl Sulfide: The method developed by Patton and Josephson (51) for determining significance of volatile flavor compounds in feed was employed to establish the taste threshold of methyl sulfide in commercially pasteurized homogenized milk. A panel of six judges composed of students and university staff members was employed for the threshold determination. Tasting was conducted in the product evaluation laboratory in the Dairy Science Department. Only one analyst was tested at a time. The tasters were told that Me_2S was the substance under investigation. A dilution well above the expected threshold, and a control sample (without Me_2S) were first presented to acquaint the judges with the flavor. Then control samples and different dilutions of Me_2S were presented randomly to the judges in 3/4 oz cups. Each analyst was asked to indicate, without guessing, whether or not Me_2S was present in the sample by saying "yes" or "no." With a six-member panel and five replications per concentration, a total of 40 to 45 judgments was obtained for a given concentration. The threshold level was, as defined by Patton et al. (51), the 50% correct response level.

Collection of Rumen Fluid and Estimation
of pH and Surface Tension

Three times daily during the bromegrass feeding trial rumen fluid samples were collected through the ruminal fistulae with a syringe and placed into marked screw capped bottles. Samples were collected at 7 A.M., again at 3 P.M. just before feeding grain ration and finally at 1 P.M., between the two feedings. During the alfalfa feeding trial, samples of rumen fluid were collected at these same hours.

Estimation of pH and Surface Tension: The rumen fluid samples were brought to the laboratory as soon after collection as possible. A potentiometer was used to measure the pH. Surface tension of the samples was determined after filtering them through four layers of cheese cloth, at body temperature. An early model Cenco-Du Nouy Tensiometer was employed for the determination. The results obtained from the samples of treated and untreated cows were summarized and recorded.

RESULTS AND DISCUSSION

Analysis of Milk for Flavor

Milk collected during a conditioning period prior to the bromegrass feeding trial had a definite feed flavor. This was evidenced by an average higher flavor score of 5.7 as determined by taste panel for milk produced from 6 cows on bromegrass pasture for five days as compared to 3.2 for milk produced by the University herd in dry lot over the same period. In the numerical range employed for organoleptic analysis, 10 represents a strong off-flavor whereas 1 indicates excellent milk flavor.

During each of the experimental periods, following conditioning, organoleptic analysis of milk indicated that an off-flavor was produced in milk from cows fed each of the experimental feeds. The average flavor score of milk from cows on bromegrass pasture was higher than that of milk from cows on rye pasture but slightly lower than that of milk from cows on alfalfa. All of these milks scored higher (poorer flavor quality) than corresponding control milks except for a few days during rye pasture feeding when control milk was of poor quality. A gradual improvement in milk flavor towards the end of each of the feeding trials was apparent from a study of the average daily flavor scores. This improvement was most pronounced during the alfalfa feeding trial (Appendix).

The average scores of milk from cows treated with poloxalene during the bromegrass feeding trial were slightly higher (poorer flavor quality) than those from untreated cows. During rye and alfalfa feeding trials, however, milk from untreated cows scored slightly higher than that from treated cows. It is likely that these small differences between treated and untreated were due to chance. The average flavor scores of milk from treated and untreated cows and control milk obtained from the University dairy herd during bromegrass and rye pasture feeding are presented in Table 1. The flavor score of control milk produced from cows fed alfalfa hay and grain during alfalfa pasture feeding trial is also presented. Higher alcohol and acetone peaks for control milk during bromegrass and rye pasture studies reflect silage in rations of control cows compared with hay and grain during alfalfa. This probably also is reflected in flavor scores of control milks.

Table 1. Flavor score of milk collected during different feeding trials.

Pasture fed	No. of days	No. of cows*	No. of observations †	Treated	Untreated	Control ‡
Bromegrass	8	3	48	4.3	4.0	3.6
Rye	9	2	36	3.8	4.0	3.8
Alfalfa	12	5	24	4.2	4.3	2.4

*Number of cows in each of the treated and untreated groups.

†Number of cows x days x 2 during bromegrass and rye feeding trials. Composite samples were examined during alfalfa feeding.

‡Milk from University dairy bulk tank was used during bromegrass and rye pasture feeding trials. Milk from two cows maintained on hay and grain was used as control during alfalfa feeding.

It would be appropriate here to consider the ways in which surface active agents when fed to cows could influence milk flavors. It is felt that reducing the surface tension of the rumen fluid and breaking the froth would increase the concentration of available gases for absorption in the rumen. The free gases then might be absorbed quickly through the rumen wall and excreted rapidly from the animal system. Surface active agents likewise could influence the milk flavor by increasing the rate of eructation. Dougherty et al. (21) stated that eructed gases helped in rapid transmission of flavor compounds from the rumen to the milk. These eructed gases enter the lungs through the trachea where they are absorbed into the blood and transmitted to the milk. Shipe (59) reported that lungs also can act as points of excretion of volatile materials. Honkanen et al. (27) reported that flavor compounds could be absorbed

through rumen wall, so some compounds present in oils like whale oil, linseed oil, and peanut oil which are surface active agents might be absorbed into the blood and enter the milk. It is possible that the surface active agents themselves or the metabolic products from the agents could contribute to flavor.

Gas Chromatographic Analysis. Chromatograms of milks from treated and untreated cows in each feeding trial contained three easily recognized peaks in addition to air peak. In each trial these peaks were designated as A, B, C, corresponding to the order of their emergence from the chromatographic column (3, 4, and 6 min). Representative chromatograms of milk collected during each of the three feeding trials from treated, untreated, and control groups are presented in Figs. 1, 2, 3. Throughout the study, chromatograms from control milk showed only two prominent peaks, B and C. Peak A in the chromatogram was identified as methyl sulfide (Me_2S) by its response to treatment with mercuric chloride (3), and its agreement with Me_2S retention time. Peak B was shown to be acetone by qualitative prechromatograph reaction with acid hydroxylamine and comparative retention times. Previous work conducted in this laboratory on feed flavor of milk proved peak C to be ethanol by the method using a boric acid column (28). During the bromegrass study where the University herd milk was used as control milk, the concentration of ethanol (peak C) was relatively large for control milk.

The Me_2S (peak A) concentration of milk collected during alfalfa feeding was greater than that found in milk produced on rye pasture, which was greater in turn than that from bromegrass pasture. There was considerable interest in Me_2S concentration because other workers (22, 50) have

Fig. 1. Chromatographic pattern of: control, treated and untreated milk collected during the bromegrass feeding trial.

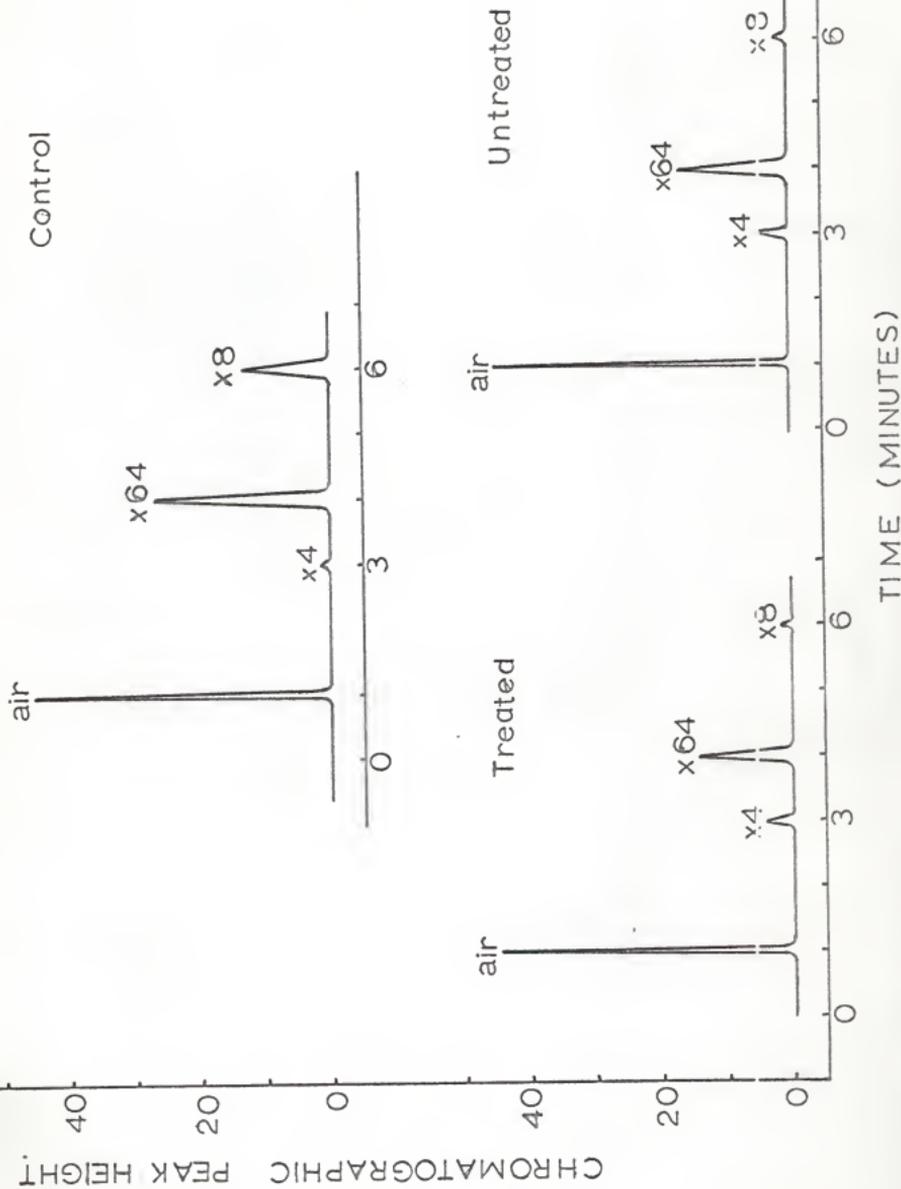


Fig. 2. Chromatographic pattern of: control, treated and untreated milk collected during the rye pasture feeding trial.

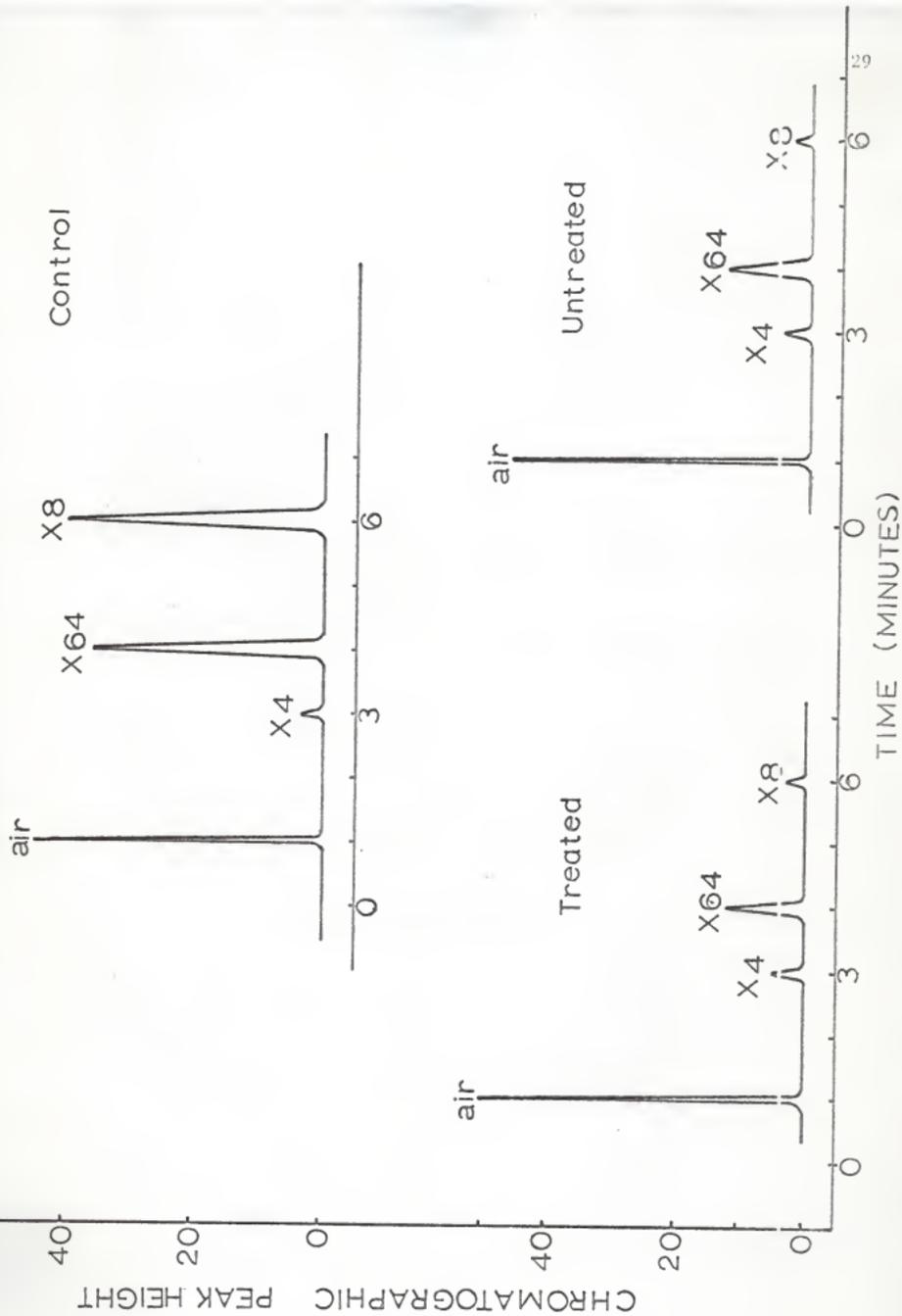
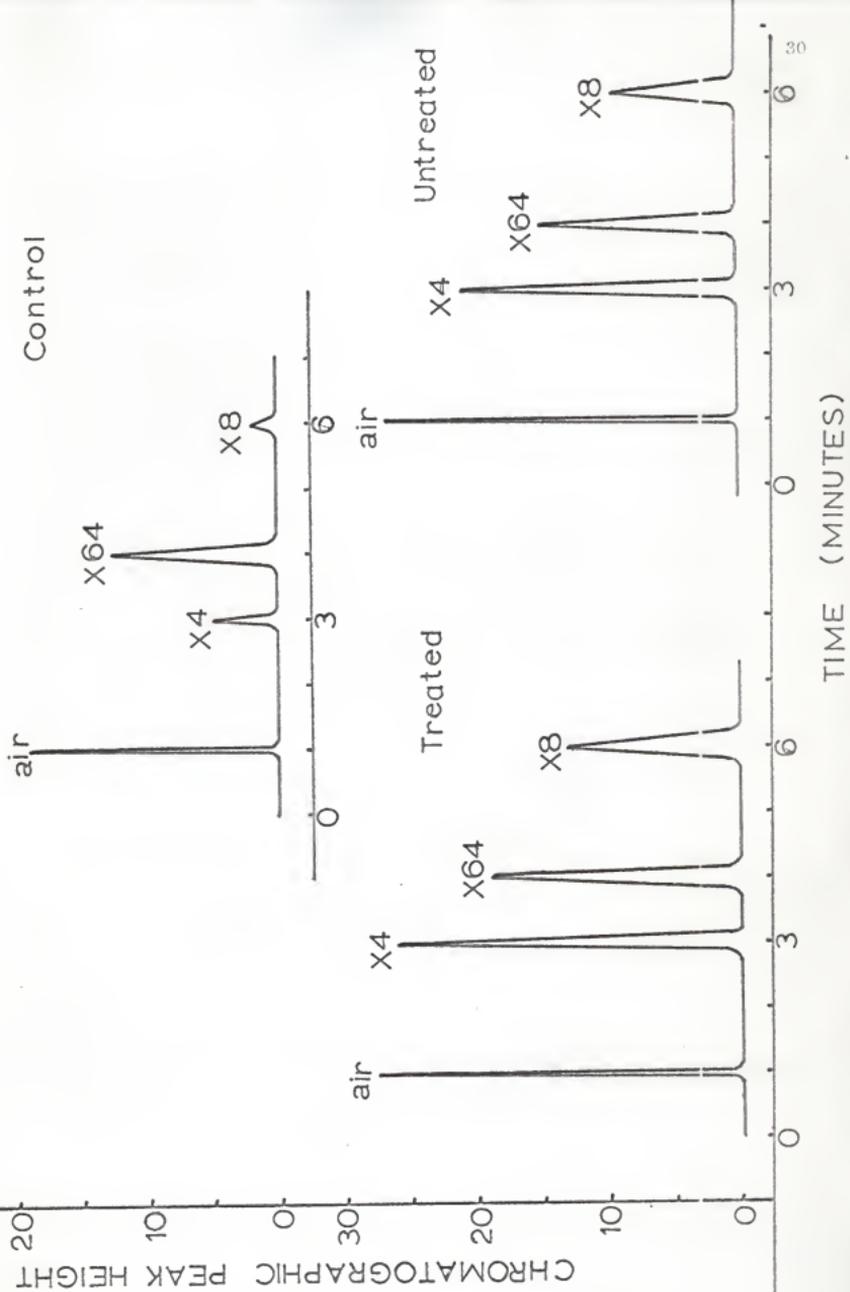


Fig. 3. Chromatographic pattern of: control, treated and untreated milk collected during the alfalfa pasture feeding trial.



implicated it as a cause of off-flavor in milk. It was interesting to observe a gradual reduction in Me_2S as each of the pasture plants matured. Me_2S concentration in milk from untreated cows was found to be less than that in milk from treated cows but definitely greater than that in control milk.

Quantitative Measurement of Me_2S . All peak heights in chromatograms from both instruments were adjusted by establishing an expected response for 1 ppm acetone (1 ppm acetone = peak height x attenuation, 1600) and correcting each peak by a factor based upon actual response of 1 ppm acetone for that day. Difference in sensitivities between the two instruments could be eliminated in this manner.

For quantitative analysis of Me_2S in experimental milk a standard curve of Me_2S in pasteurized milk with 3.6% fat was prepared from the chromatographic analysis of different standard solutions of added Me_2S ranging from 5 ppb to 400 ppb. These data are presented in Fig. 4.

Each point on this standard curve in Fig. 4 represents a duplicate determination with variation in peak heights being less than 10%. To determine the significance of the Me_2S concentration in experimental milk, the threshold level of Me_2S in milk was determined and the average results are presented in Fig. 5.

A threshold level of approximately 19 ppb in the pasteurized homogenized milk was deduced from the curve. It is evident from Table 2 that the Me_2S concentration in milk samples collected during bromegrass or rye pasture feeding are below the threshold level and probably do not influence the flavor of the milk. But concentrations of 35 ppb and 27 ppb in milk of treated and untreated cows respectively fed alfalfa were above the

Fig. 4. Standard curve and regression line equation relating to GLC peak heights to concentrations of methyl sulfide in pasteurized, homogenized milk.

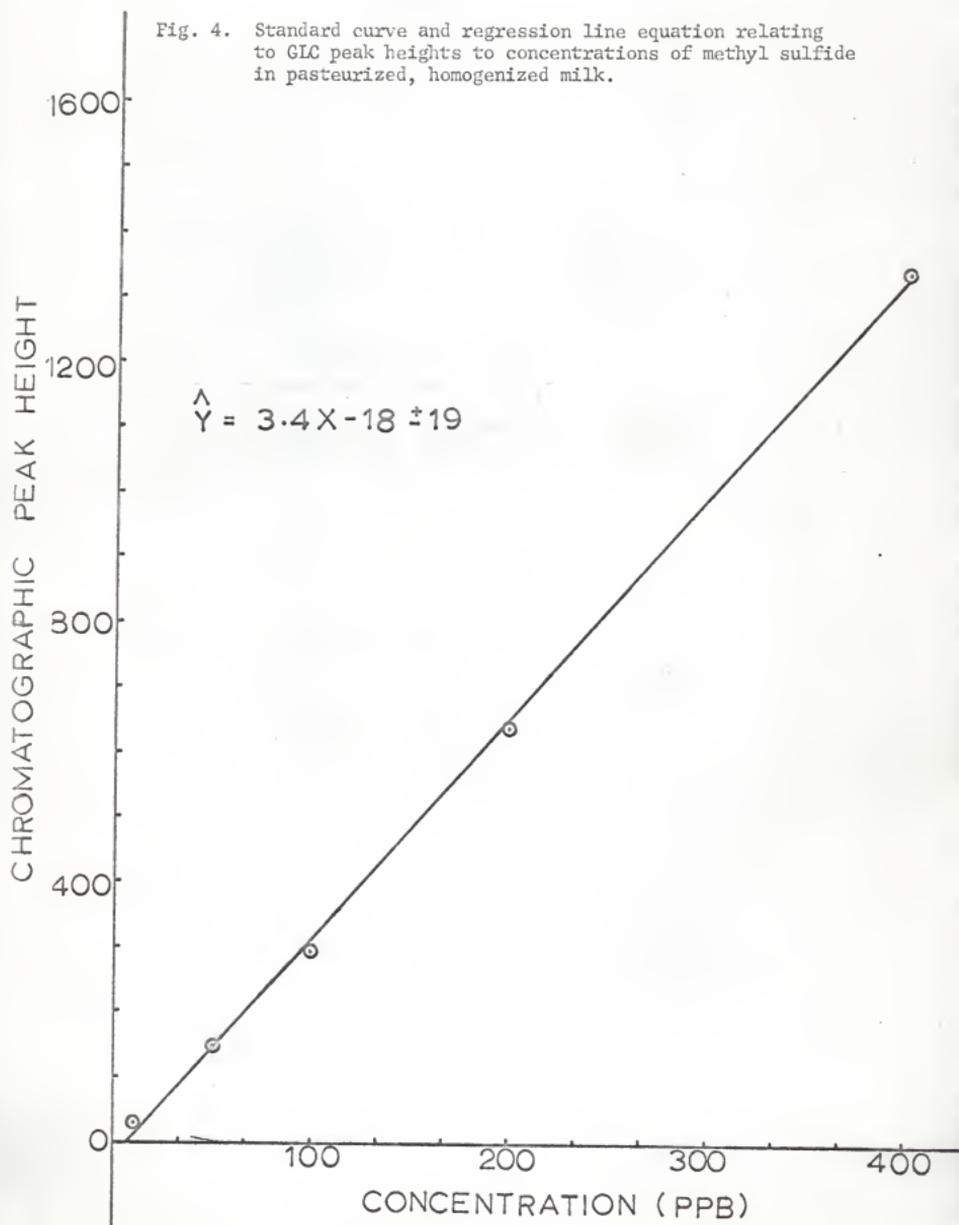
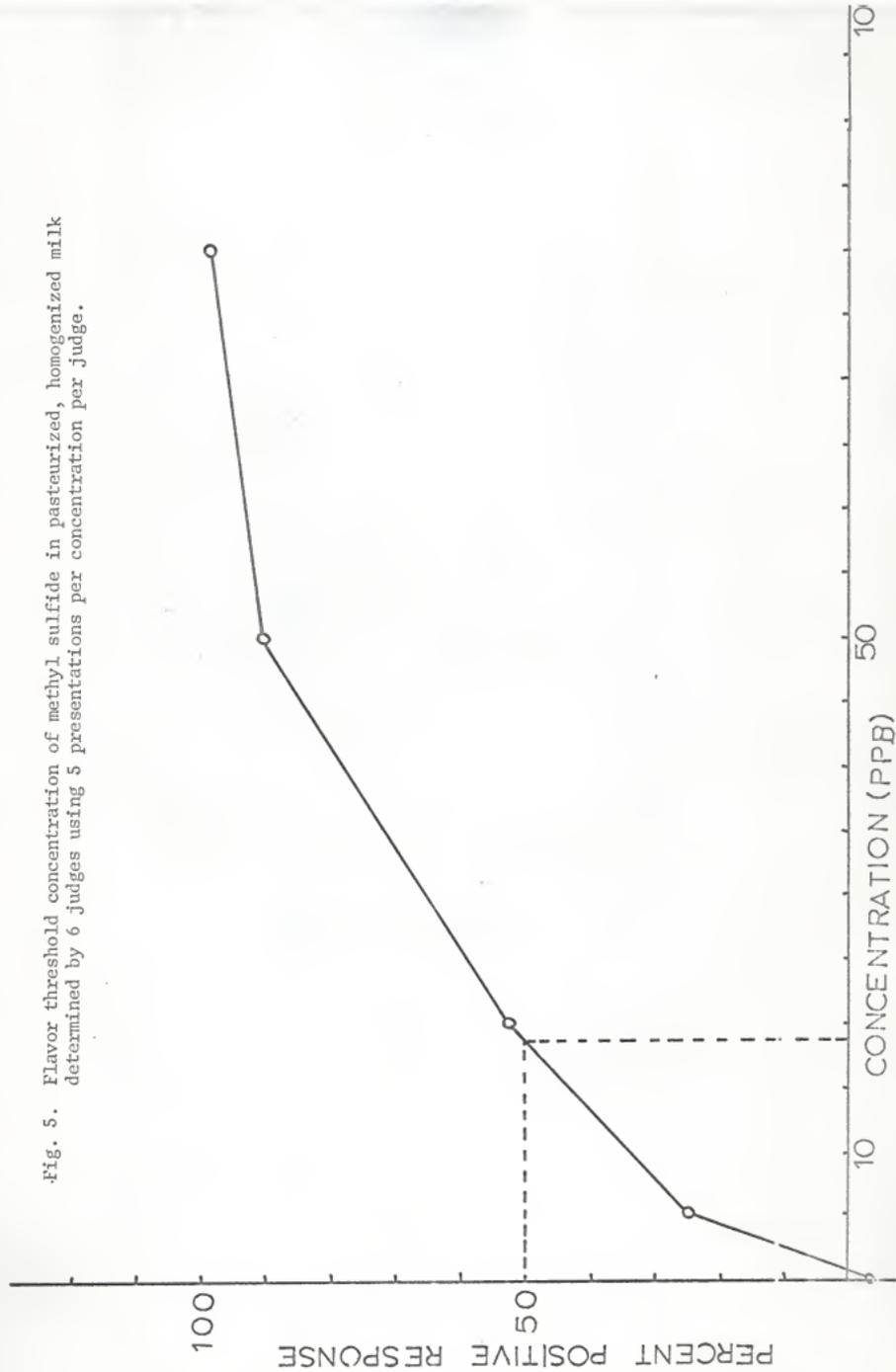


Fig. 5. Flavor threshold concentration of methyl sulfide in pasteurized, homogenized milk determined by 6 judges using 5 presentations per concentration per judge.



threshold level and probably do influence the flavor score.

Table 2. Methyl Sulfide peak heights and flavor scores of milk from treated and untreated cows in different feeding trials.

Pasture fed	Milk sample	Average flavor score	Average Me ₂ S peak height ^t	Concentration of Me ₂ S in ppb
Bromegrass	Treated	4.5	18.1	< 10
	Untreated	4.1	17.4	"
Rye	Treated	3.8	18.9	< 10
	Untreated	4.0	16.4	"
Alfalfa	Treated	4.2	101.3	35
	Untreated	4.3	84.0	27

Some speculations have been made regarding differences in Me₂S concentration of milk from cows on different feeds. Day et al. (20) suggested two possible precursors of Me₂S, dimethyl-β-propiothetin and methyl methionine sulfonium salt, both originating from plant materials. Kiribuchi and Yamanishi in 1963 (33) reported that the sulfonium salt was being converted to methyl sulfide and homoserine when green tea was heated. The difference in Me₂S concentration of milk collected during different feeding trials reported in this thesis could be attributed to difference in the chemical compounds present in plant materials of different pastures. Chemical composition of plants may be a reflection of variation in the mineral and chemical compounds in the soil and are characteristic of species. In this study the effect of soil was minimized by cultivating all three pastures in similar soils. The decrease in Me₂S with maturity of the plant could be attributed to variation in the chemical composition of the

plant during its growth or differences in the amounts of gases produced in rumen from plant at different stages of growth. Young and succulent feeds may produce more gases than mature and fibrous ones. Meyer et al. in 1965 (40) observed a decrease in the nitrogen content of the whole alfalfa plant and its fractions with increasing maturity. A general decrease in the incidence of bloat was also observed with increasing plant maturity. A complicating factor in analyzing these differences is encountered in variation among cows on the same ration.

The threshold level for methyl sulfide varies with the medium used. Toan et al. (60) reported the threshold value for Me_2S in water to be 9 ppb compared with 12 ppb reported by Patton et al. (50). Toan also reported that the threshold concentration of Me_2S in vacuum treated pasteurized-homogenized milk was approximately 10 times as high as that in distilled water (60). The Me_2S threshold value in pasteurized-homogenized milk obtained in this study was approximately 19 ppb; only twice as high as that in distilled water. This fact illustrates the importance of medium in detection of volatiles by organoleptic analysis. There is no definite explanation for differences between threshold levels obtained here and those reported by Toan. It is possible that an error was made in preparation of his Me_2S standard solution. Sensory testing is of basic importance in flavor identification research. However, with highly subjective nature of organoleptic analyses the reliability of such determinations is limited. The threshold level of Me_2S in pasteurized-homogenized milk found in this study, the difference in threshold levels in water and pasteurized-homogenized milk reported by Toan et al. (60) and in skim milk as reported by Patton et al. (51) indicates variation in its solubility in different

solvents. This reaffirms the observation by Bassette et al. (3) on the effect of solubility characteristics on the release of different volatile chemical compounds into the vapor phase.

Relationship between Flavor Score,
Methyl Sulfide Concentration and Poloxalene Treatment

The correlation coefficient between methyl sulfide concentrations and the flavor scores of milk from cows fed bromegrass, rye and alfalfa pastures are presented in Table 3.

Table 3.

Pasture employed	Correlation coefficient between Me ₂ S concentrations and flavor score	
	Treated	Untreated
Bromegrass	0.39	0.49
Rye	0.67	0.11
Alfalfa	0.73	0.57

The data indicate a closer correlation between flavor and Me₂S among treated cows than among untreated cows with the exception of bromegrass pasture. Daily changes in average Me₂S peak heights and flavor scores presented in Figs. 6, 7, 8, 9 illustrate the tendency of the scores to follow in the direction of peak height changes. Results of the individual cows during bromegrass and rye pasture feeding are presented in Appendix A. A positive correlation between methyl sulfide concentration and flavor score was observed in most of the cases except one cow in bromegrass and one cow

Fig. 6. Daily changes in methyl sulfide concentration (peak heights) and flavor score of milk collected from treated and untreated groups during the bromegras feeding trial.

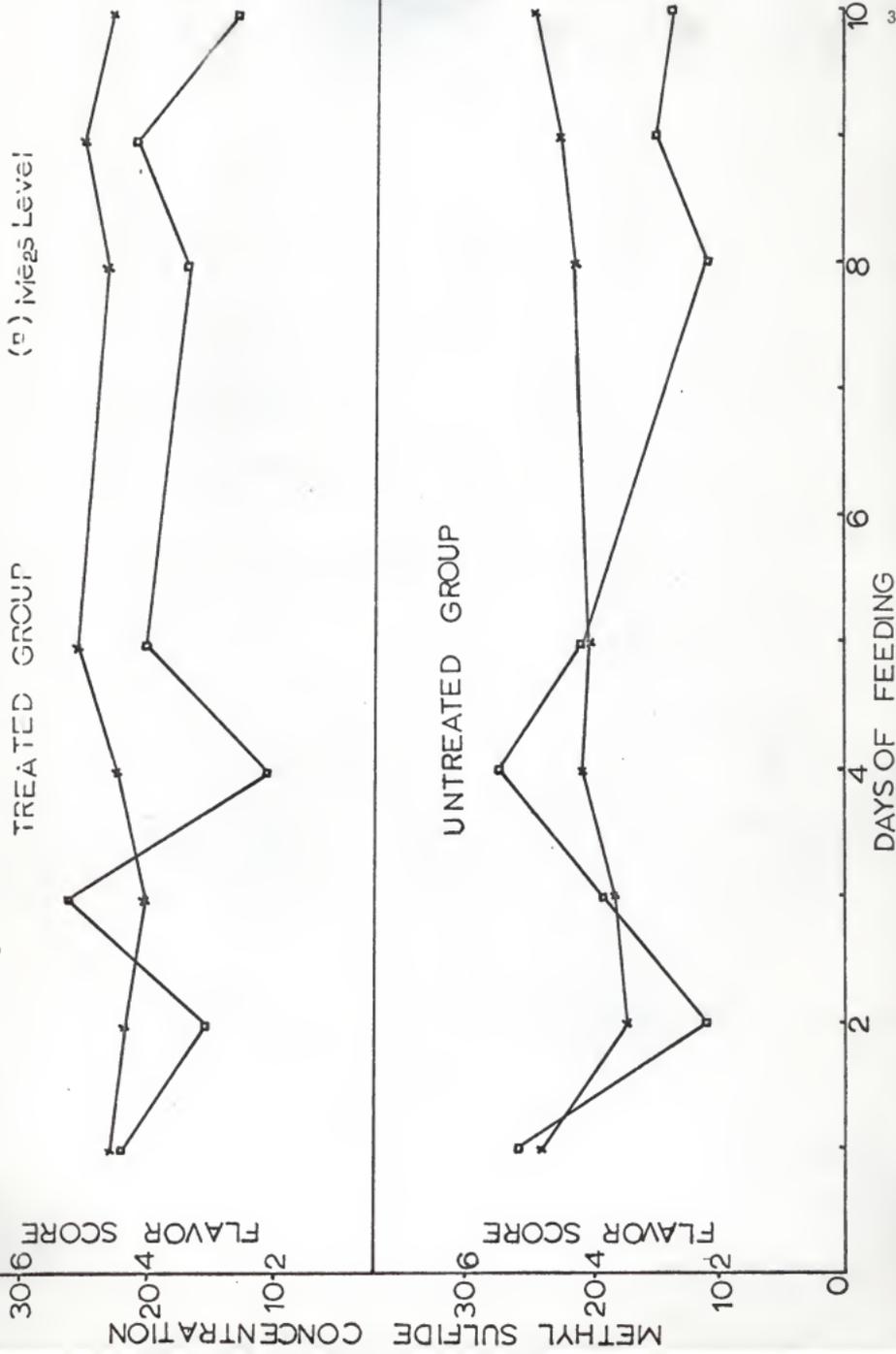


Fig. 7. Daily changes in methyl sulfide concentration (peak heights) and flavor score of milk collected from treated and untreated groups during the rye pasture feeding trial.

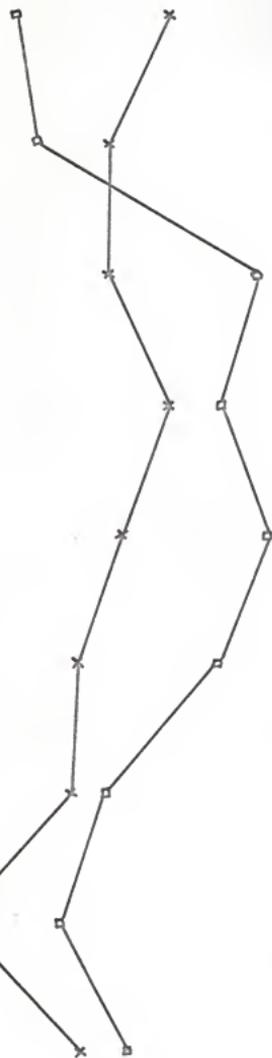
(x) Flavor Score
(□) Me₂S Level

TREATED GROUP

FLAVOR SCORE
30.6
20.4
10.2

30.6

METHYL SULFIDE CONCENTRATION



UNTREATED GROUP

FLAVOR SCORE

30.6
20.4
10.2

30.6

METHYL SULFIDE CONCENTRATION



DAYS OF FEEDING

3

6

9

Fig. 8. Daily changes in methyl sulfide concentration (peak heights) and flavor score of milk collected from treated group during the alfalfa pasture feeding trial.

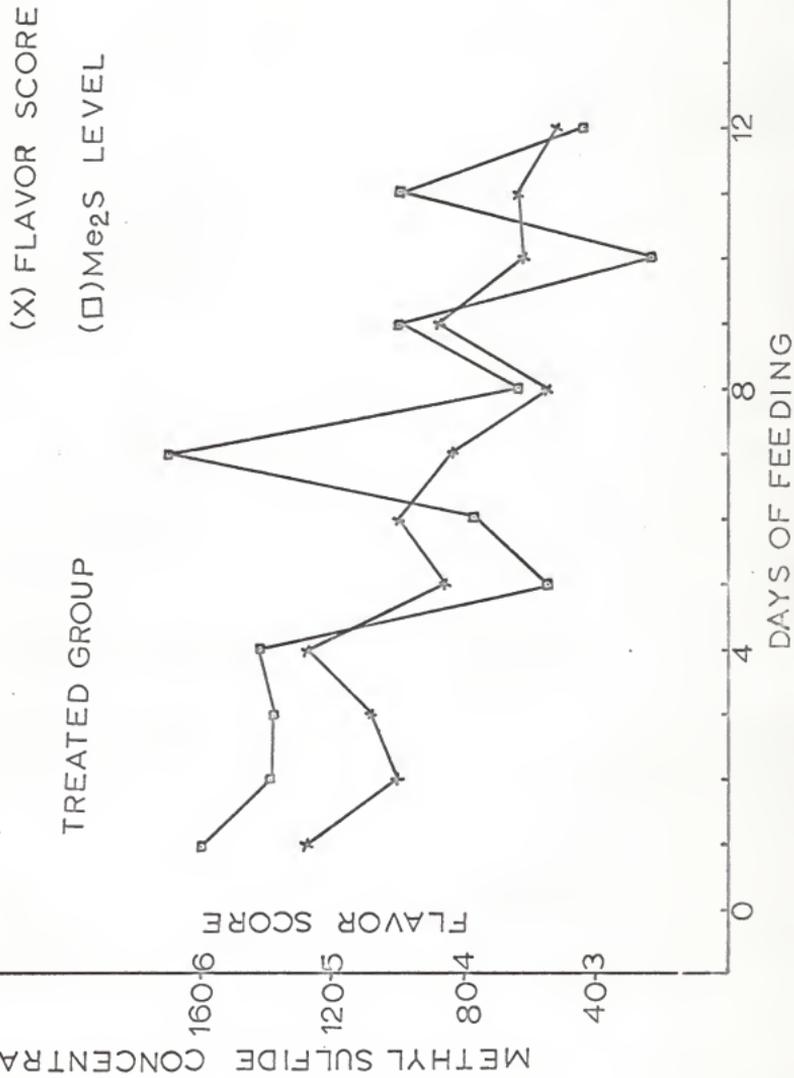
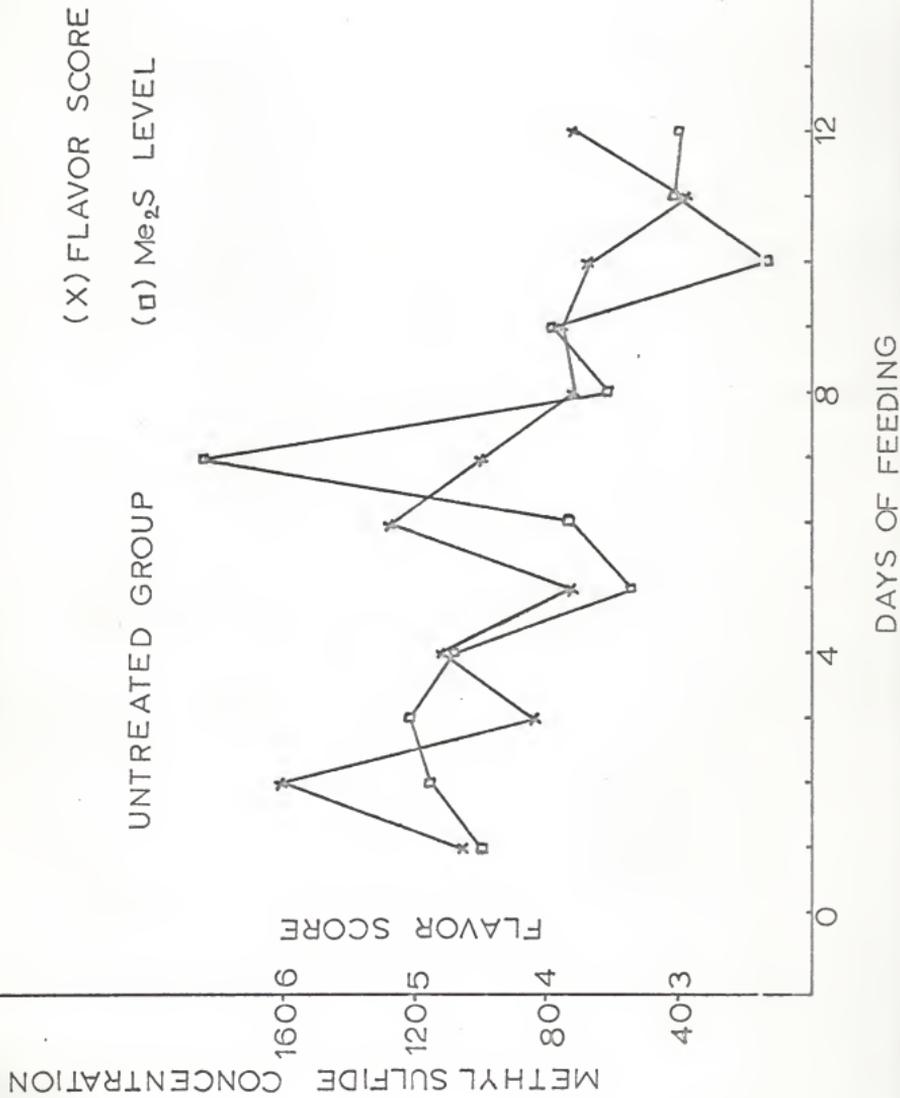


Fig. 9. Daily changes in methyl sulfide concentration (peak heights) and flavor score of milk collected from untreated group during the alfalfa feeding trial.



in rye pasture feeding trials. The results of the JLC and organoleptic analysis reveal no significant difference in volatile constituents or flavor score between milks collected from cows treated with poloxalene and untreated cows. A better correlation between Me_2S and flavor score was observed in milk from treated cows than from untreated cows.

References to Me_2S in milk and dairy products are numerous. Some studies have implicated Me_2S as characteristic flavor of normal milk and good quality dairy products, while others have suggested that it is associated with the feed flavor. Day et al. (20) characterized high concentration of Me_2S in butter as feedy. Toan et al. (60) reported that milk possessed a methyl sulfide-like, molasses-like, or covey flavor at concentrations above threshold levels. Based on these statements it could be assumed that the flavor of milk from cows fed alfalfa, in which Me_2S concentrations were higher than threshold, was influenced by methyl sulfide. During the bromegrass and rye pasture feeding trials, even though there is a correlation between Me_2S and flavor, the concentration of Me_2S , as such, is not sufficient to influence the milk flavor. It is possible, however, that the small amount of Me_2S might parallel the production of other components that could influence flavor.

The Effect of Laboratory and Vacuum Pasteurization on Milk Flavor

The chromatograms of milk before and after laboratory pasteurization and vacuum heat treatment are presented in Fig. 10. Almost 95% of the Me_2S and half of the acetone was removed after vacuum heat treatment of milk. However, no significant change in Me_2S and acetone was observed by laboratory

pasteurization where heat alone was employed. The observation of the effect of vacuum pasteurization on Me_2S and acetone are in agreement with results of Brunner et al. (10). They indicated that 95% of the volatile components were removed from the milk by the vacuum heat treatment. On the other hand, no appreciable change was observed in ethyl alcohol concentration from either of the treatments. The insignificant change in Me_2S concentration observed in laboratory pasteurized milk verified that this procedure could be employed in preparing the milk for flavor judgment and related to the original raw milk.

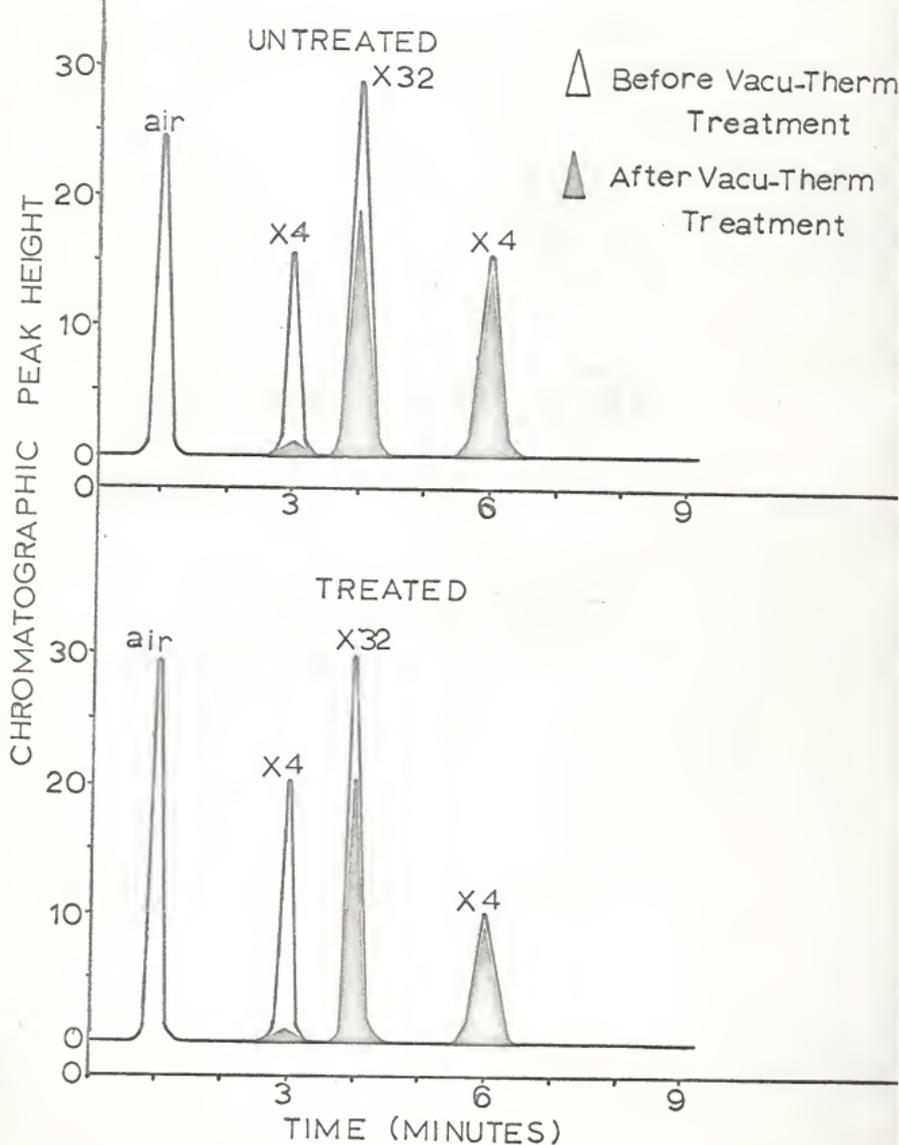
Analysis of Rumen Fluid for Surface Tension and pH

Two pairs of fistulated, identical twin dry cows were used to represent the rumen conditions of milking cows. These fistulated cows were maintained on the same feed as the other experimental cows. From the results obtained by the analysis of rumen fluid of these cows (Table 4) it is evident that the surface active agent, poloxalene, reduced surface tension of rumen fluid and produced a slight reduction in the pH.

Table 4. Averages of surface tension and pH of rumen fluid from treated and untreated cows at different intervals during the day.

Pasture fed	Sample	No. of animals	No. of days	Surface tension in dynes			pH		
				11 am	1 pm	3 pm	11 am	1 pm	3 pm
Bromegrass	Treated	2	11	43.5	43.4	44.1	6.8	6.9	6.7
	Untreated	2	11	53.8	53.1	54.8	7.0	6.9	6.8
Alfalfa	Treated	2	8	44.8	43.7	43.7	6.8	6.5	6.4
	Untreated	2	11	53.2	53.3	53.8	6.9	6.7	6.6

Fig. 10. Chromatographic pattern of milk from cows on alfalfa pasture before and after vacu therm treatment.



The results of a study to determine the effective length of time of poloxalene activity on surface tension and pH of rumen fluid are presented in Table 5.

Table 5. The averages of surface tension and pH of rumen fluid from treated cows indicating the duration of poloxalene activity.

Sample	Trial no.	No. of animals	Date	Surface tension in dynes			pH		
				7 am	1 pm	3 pm	7 am	1 pm	3 pm
Treated	1	2	6-17-65			42.9			6.4
			6-18-65	42.9	46.1	46.8	7.1	6.6	6.3
			6-21-65		52.8			6.6	
Treated	2	2	6-22-65			43.9			6.4
			6-23-65	44.3	46.4	47.1	6.8	6.5	6.3
			6-24-65	46.4	53.9	50.7	6.9	6.6	6.4

These results obtained during the alfalfa trial indicated that the effect of a single dose (2 g per 100 lb body weight) of poloxalene was effective for 24 to 36 hours. There was no significant change in pH of the rumen fluid during this period.

It is generally agreed that bloat results from a stable froth formation, which might allow entrapped gases to be absorbed over a longer period through the rumen wall into the blood and eventually into the milk. A change in rumen pH should affect the flavor of milk through its influence on bloat. A reduction in pH of the rumen fluid to 5.4 in severe and 6.4 in moderate bloat was observed (37). Occasionally a mild condition of

bloat was noticed in the untreated experimental cows during alfalfa feeding. The untreated cow in one pair of identical twins used in this experiment showed evidence of constant moderate bloat during alfalfa feeding.

Froth formation is a surface active phenomenon, and a decrease in surface tension may reduce the incidence of bloat. The surface active agents, by reducing the surface tension, may prevent the formation of gas bubbles present in the frothy rumen ingesta and leave only free gas in the rumen. This free gas can be absorbed quickly into the blood and excreted from the animal system rapidly. From the results obtained in this study it is apparent that poloxalene has a marked reducing effect on the surface tension of the rumen fluid which lasted for 24 to 36 hr. From the results of this experiment it is apparent also that poloxalene itself did not impart any flavor to milk nor did it alleviate off-flavors induced by pasture forage. From the results of GLC analysis, the remarks by the flavor judges distinguishing a Me_2S characteristic in experimental milk, especially in milk collected during alfalfa feeding trial, it is apparent that some flavor defects in milk could be attributed to Me_2S .

CONCLUSIONS

The results obtained during this investigation established that:

1. Poloxalene treatment of cows before feeding exerted no significant effect on milk flavor.
2. The surface tension of rumen fluid was reduced by the poloxalene treatment for 24 to 36 hr. The treatment exerted no significant effect on pH of rumen fluid.
3. The threshold concentration of methyl sulfide was 19 ppb in

commercial pasteurized homogenized milk.

4. The concentration of methyl sulfide detected in milk from alfalfa fed cows was sufficient to influence milk flavor. Levels found in milks from cows fed bromegrass and rye pasture were below threshold concentrations.

5. There was positive correlation between flavor scores and peak heights of methyl sulfide in all three feeding trials.

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APPENDIX A

சென்னை
சென்னை மாநகராட்சி
சென்னை மாநகராட்சி

Figure 1. Daily changes in methyl sulfide concentration (peak heights) and milk flavor score, from cows A149 and 177C during bromegrass feeding trial.

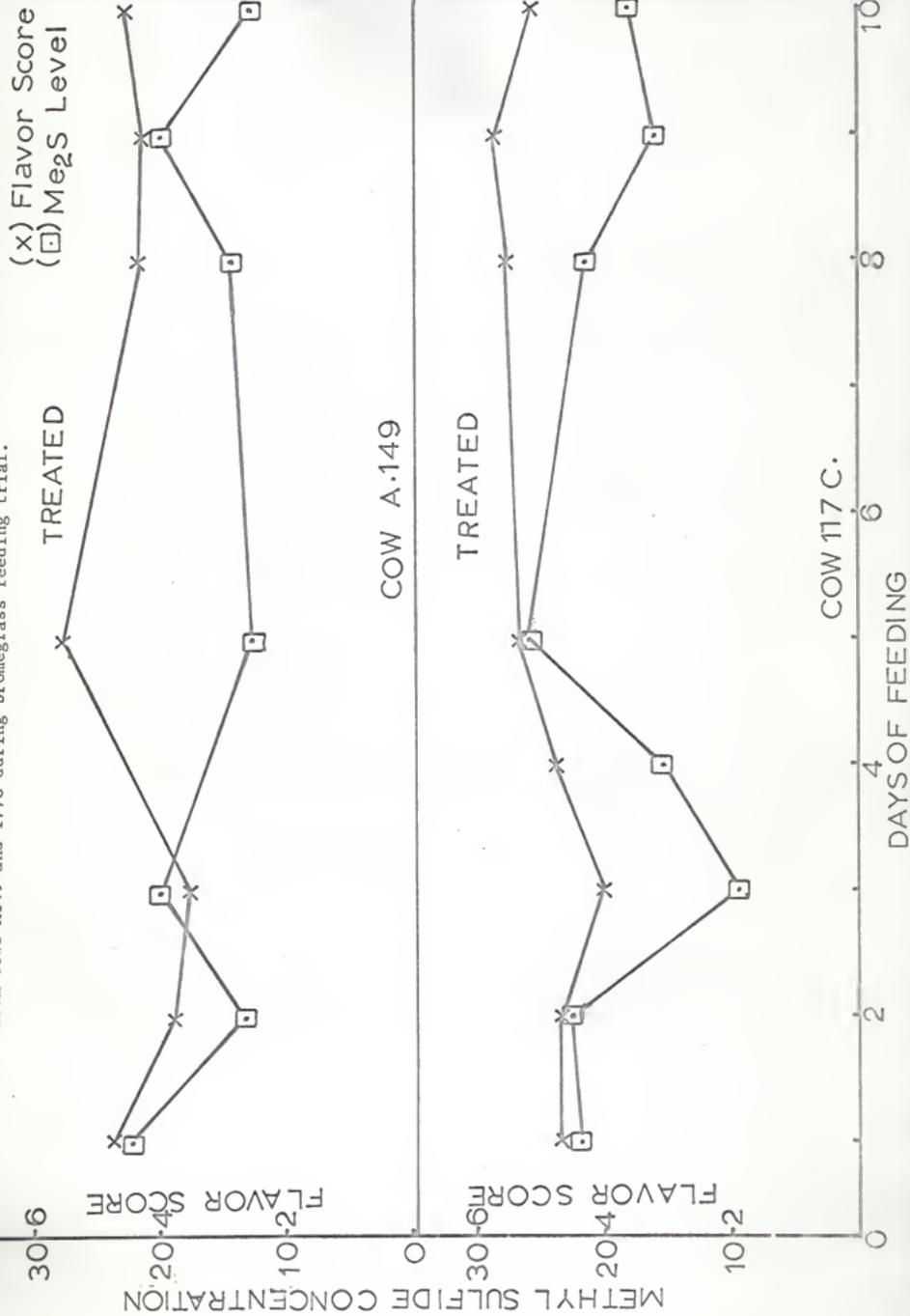


Figure 2. Daily changes in methyl sulfide concentration (peak heights) and milk flavor score from cows 109D and 140D during bromegrass feeding trial.

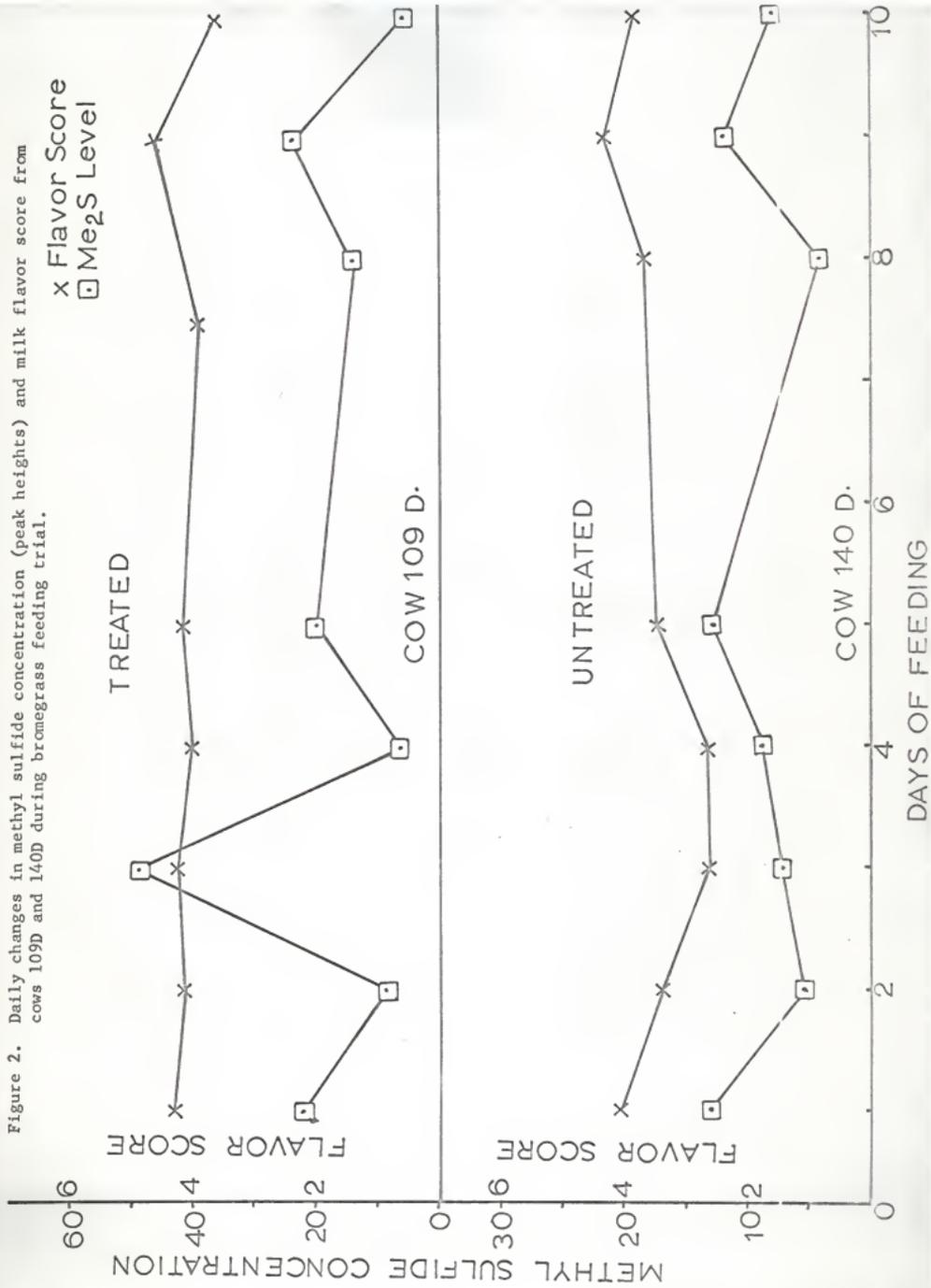


Figure 3. Daily changes in methyl sulfide concentration (peak heights) and milk flavor score from cows 111D and 141D during bromegrass feeding trial.

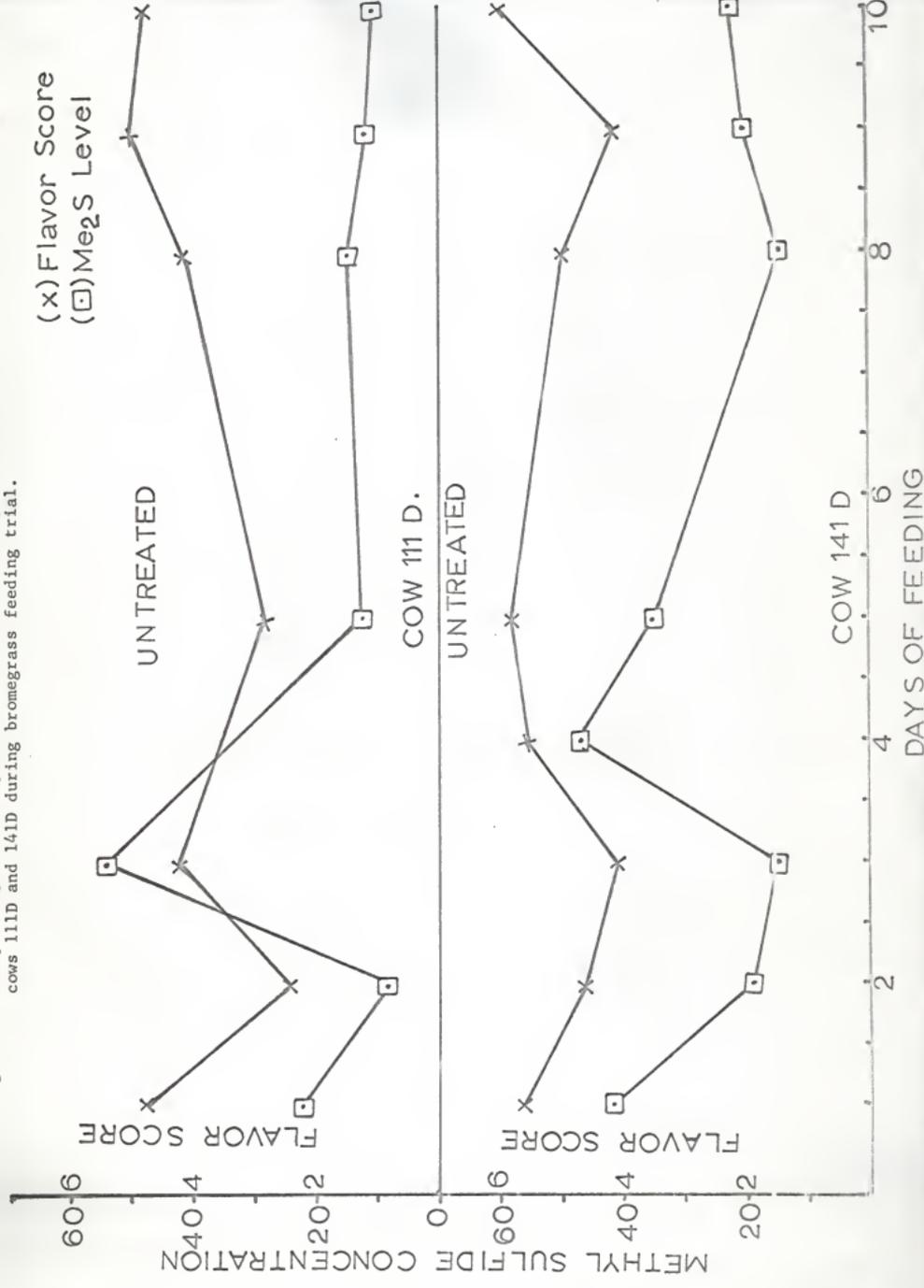


Figure 4. Daily changes in methyl sulfide concentration (peak heights) and milk flavor score from cows 383C and 275C during rye pasture feeding.

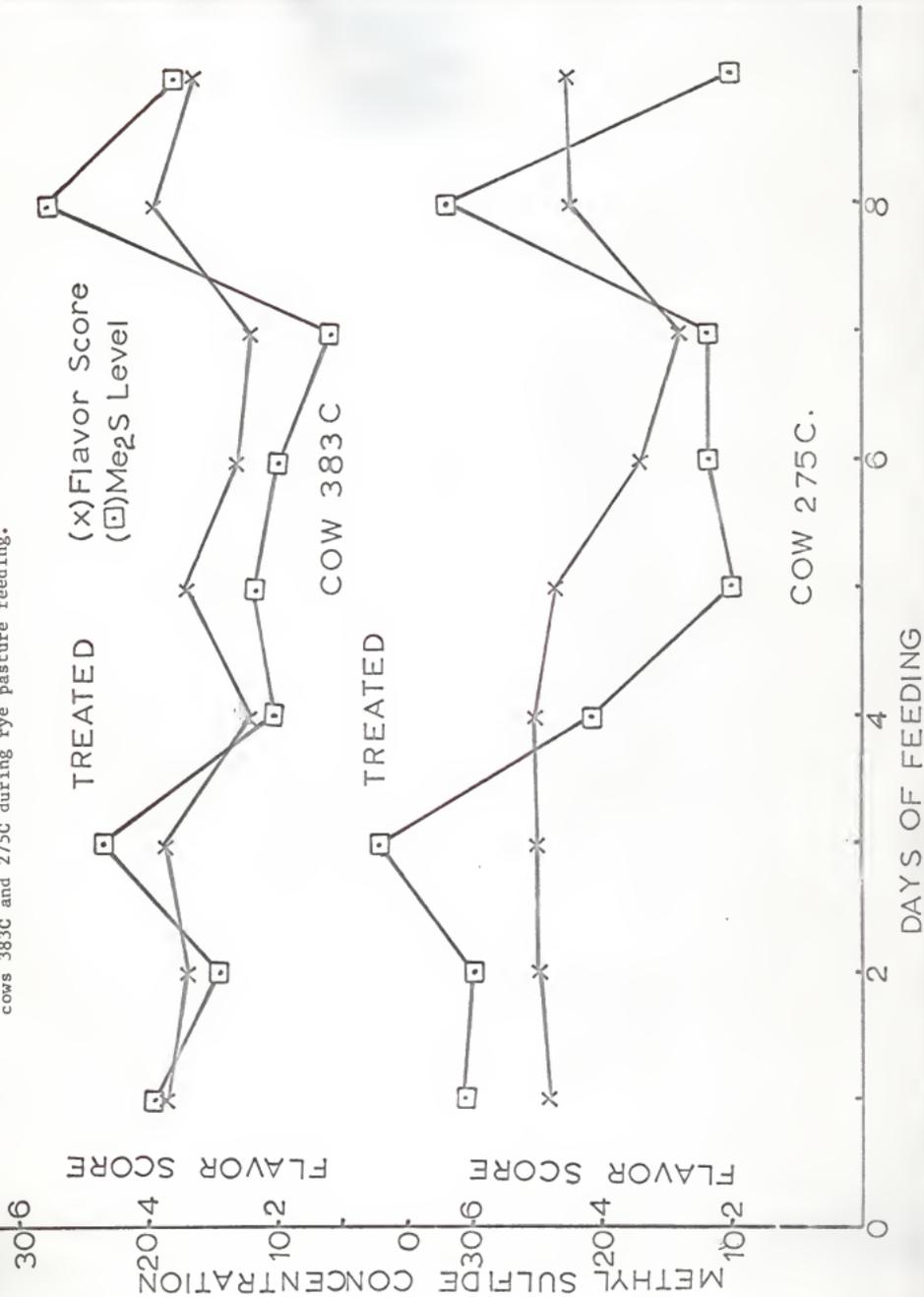
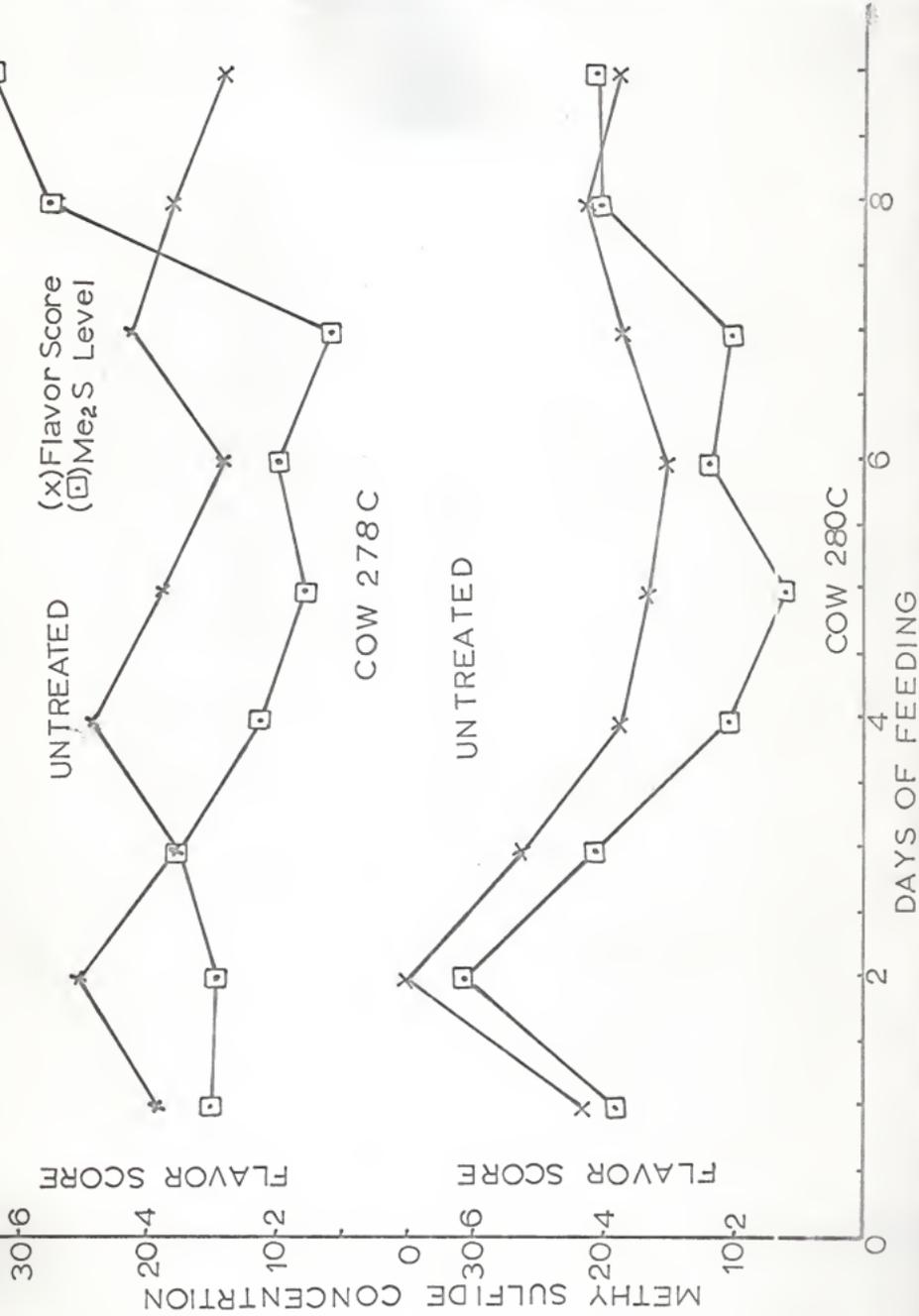


Figure 5. Daily changes in methyl sulfide concentration (peak heights) and milk flavor score from cows 278C and 280C during rye pasture feeding.



EFFECT OF THE SURFACE ACTIVE AGENT POLOXALENE
ON MILK FLAVOR WHEN FED TO COWS

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The purpose of this study was to determine: (a) the effect of the surface active agent poloxalene on milk flavor when administered to cows on bloat provoking alfalfa pasture and also bromegrass and rye pasture, and (b) the effect and duration of the effect of poloxalene on surface tension and pH of rumen fluid. An attempt also was made to relate chromatographic analyses of milk to organoleptic responses.

The procedure adopted to establish the effect of poloxalene on milk flavor consisted of pasturing cows on a specific forage for a 7-10 day experimental period, some receiving poloxalene and others not and collection of milk for gas chromatographic (GLC) and organoleptic analysis. Cows in mid-lactation with good milk production records were pastured on either bromegrass or rye or alfalfa pasture during the experimental period. During bromegrass and rye pasture trials half of the cows were treated with 2 g poloxalene per 100 lb body weight added to the grain ration. During the alfalfa trial, poloxalene was administered in liquid form in a capsule.

Cows were conditioned to the specific feed 5 days before starting the collection of the experimental milk. They were milked within 2 1/2 hr after taking them off pasture. Milk samples from individual cows were collected during the bromegrass and rye pasture trials. Both individual and composite milk samples were collected during the alfalfa trial, but only composite milk samples were used for organoleptic analysis. During the bromegrass and rye pasture trials control milk was collected from the university dairy bulk tank, whereas, during the alfalfa trial, milk from two cows maintained on hay and grain served as control. Bulk tank control milk was found to possess some off-flavor due to silage. This flavor could be avoided when control cows were fed hay and grain. An aliquot of raw milk was used for

GLC analysis. For organoleptic analysis laboratory pasteurized samples were used.

Laboratory pasteurization had no effect on volatile components in milk, whereas commercial, "Vacu-Therm" pasteurization virtually eliminated methyl sulfide (Me_2S) and reduced other volatile materials.

GLC analysis was used in qualitative and quantitative analysis of Me_2S and acetone in milk samples. Previous findings in this laboratory established the presence of ethanol. The concentration of Me_2S in milk collected from treated and untreated cows during bromegrass and rye pasture trial was found to be less than 10 ppb, whereas, during the alfalfa trial, its concentration was 27 ppb to 35 ppb. Since the Me_2S flavor threshold level in pasteurized homogenized milk was 19 ppb, it can be assumed that concentrations in milk collected from cows grazing alfalfa influenced the flavor, whereas during bromegrass and rye pasture trials, the concentration of Me_2S was not sufficient to influence milk flavor. Careful observation of Me_2S concentration and flavor score revealed that high Me_2S correlated with poor milk flavor in most cases. Organoleptic analysis of milk established that poloxalene treatment of cows exerted no significant effect on milk flavor.

The effect of poloxalene on surface tension and pH of rumen fluid was established by maintaining two pairs of fistulated, identical twin dry cows on the same forage and according to the same feeding schedule as the milking cows during the bromegrass and alfalfa trials. The rumen samples were collected three times a day.

Analysis of samples revealed that poloxalene reduced the surface tension of rumen fluid for periods of 24 to 36 hr and had no significant effect on pH of the rumen fluid.