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Effect of nitrogen fertilization on the nutritional value of corn silage as determined by feedlot performance of beef heifers, digestion trials, and in vitro studies

Garlin Ray Wilson

To the Graduate Council:

I am submitting herewith a thesis written by Garlin Ray Wilson entitled "Effect of nitrogen fertilization on the nutritional value of corn silage as determined by feedlot performance of beef heifers, digestion trials, and in vitro studies." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

C.S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

O.G. Hall, L.L. Christian, C.C. Chamberlain

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 13, 1964

To the Graduate Council:

I am submitting herewith a thesis written by Garlin Ray Wilson entitled "Effect of Nitrogen Fertilization on the Nutritional Value of Corn Silage as Determined by Feedlot Performance of Beef Heifers, Digestion Trials, and In Vitro Studies." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S Hobbs

Major Professor

We have read this thesis and recommend its acceptance:

O. Glen Hall

C C Chamberlain

L L Christian

Accepted for the Council:

Dean of the Graduate School

EFFECT OF NITROGEN FERTILIZATION ON THE NUTRITIONAL VALUE OF CORN SILAGE AS DETERMINED BY FEEDLOT PERFORMANCE OF BEEF HEIFERS, DIGESTION TRIALS, AND *IN VITRO* STUDIES

A Thesis

Presented to

the Graduate Council of

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Garlin Ray Wilson

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CHAPTER I

INTRODUCTION

Beef cattle production in the southern United States and especially in Tennessee has increased at a phenomenal rate during the past decade. This increase in cattle numbers has resulted in a greater demand for forage crop production. No other area of this country has enjoyed a more favorable opportunity for intensive grassland beef production.

Greater demands for winter feeds have been accompanied by an increase in forage production. Many areas of Tennessee and especially the eastern portion, which is less favorable for hay production, have met this increased demand for cheap forages with silages. Because of the high energy content and palatability of the corn silage, it has been of increasing importance for beef cattle.

More emphasis has been placed on intensive forage production in this area as the available labor supply has been decreasing along with a narrowing margin in farm operations. Therefore, the total pounds of digestible nutrients produced by a forage crop has received more attention. The cost of silage operations accompanied by the increased labor costs per unit and decreased margin in cattle operations has necessitated the cattle farmer to make optimum use of his available land, labor, feeds, and cattle.

As in row crop production of all kinds, silage production has been directed toward highest possible yields per acre. This increase in yield has been due primarily to the introduction of more productive varieties, better management in silage production, and the extensive use of more fertilizers per acre. Increased plant population per acre and the use of more productive varieties have created a greater demand for available plant food. Since the prices of fertilizer have remained relatively stable, the cattle farmer has been able to increase silage yields more economically with fertilizer than through any other means.

Nitrogen has been the element most commonly increased in use for silage production. Some people have postulated that this increase in nitrogen application in silage production may result in a detrimental build-up of nitrates and nitrites in the silage. The first report in recent years of a vitamin A deficiency in beef cattle being fed on a conventional ration has led investigators to examine several possible causative agents. Some workers have postulated that the nitrate accumulation in plants causes an interference in carotene conversion to vitamin A. Inadequate data have been reported to substantiate this theory.

One of the purposes of this research was to determine the effect of high levels of nitrogen fertilization on the nitrate build-up in corn silage. Another objective of this work was to determine the effects of this build-up, if indeed it did occur, on subsequent performance of beef cattle fed these silages with and without vitamin A supplementation as the major component of the wintering ration in addition to determining any carry-over effect during the finishing period. Realizing that the high level of nitrogen (300 pounds per acre) is not recommended nor economical, it was the objective of this study to allow for possible detrimental levels of nitrate to accumulate. The low level of nitrogen (100 pounds per acre) is the level generally recommended. However, with increasing plant population per acre and more productive varieties the recommended economical level of nitrogen application may increase in the future. Therefore, these data may answer some questions as to the effect of these higher levels of nitrogen application on cattle performance.

A comparison of some other techniques for forage evaluation was made. Another objective was to determine the digestibility of these silages with cattle and sheep. An *in vitro* technique was employed to measure possible differences in the silages that might be reflected in rumen microbial activity. Cellulose breakdown was used as a measure of microbial activity in these studies.

An objective evaluation was made of these silages as determined by cattle performance, digestibility, and cellulose digestion by rumen microbes. Further, the various techniques were evaluated as measures of forage nutritive value.

CHAPTER II

REVIEW OF LITERATURE

I. FEEDING TRIALS

Corn silage has been shown by various workers to be an acceptable and economical roughage in wintering rations for beef heifers. Snapp and Neumann (1960), after reviewing numerous feeding trials utilizing corn silage as the major component of the ration, concluded corn silage to be superior to all other types of roughages for wintering cattle.

Hall and Felts (1963) fed corn silage to beef heifers, averaging 449 pounds in weight, for 120 to 140 days during three wintering trials. These heifers gained an average of 1.67 pounds per head per day. In their work the heifers were fed at a level of about 20 pounds per head per day plus 1.3 pounds of mixed hay, 1.1 pounds of cottonseed meal, and 3.8 pounds of ground ear corn. The total pounds of air-dry feed required per hundred pounds of gain during the winter was 732 and the increase in slaughter grade was one-third of a grade. These workers indicated corn silage to be superior to the other silages tested.

Wilson (1964) reported production data on beef heifers fed a similar corn silage. In three trials, heifers weighing 506, 461, and 388 pounds were fed from 112 to 140 days during the winter. The average daily gains of these heifers by weight groups were 1.35, 1.41, and 1.61, respectively. The heifers were fed silage *ad libitum* plus 4.0 pounds of corn, 1.0 pound of cottonseed meal and approximately 2.0 pounds of alfalfa-orchardgrass hay per head daily. The total pounds of air-dry feed required per hundred pounds of gain were 909, 913, and 751 for the three weight groups, respectively.

Anderson et al. (1964) reported the performance data of 149 beef heifers of various weights and grades when fed corn silage as the major constituent in the wintering ration. The cattle were fed 143 days on a full feed of corn silage plus about 3.0 pounds of alfalfa-grass hay, 1.0 pound of cottonseed meal, and 5.0 pounds of shelled corn per head daily. The wintering period was followed by a 42-day full feed of ground shelled corn, limited silage, and the same level of hay and cottonseed meal as fed during the wintering period. The average daily gain of these heifers ranged from 1.81 to 2.00 pounds per day during the wintering period and 1.76 to 1.95 pounds per day when both periods were included. The average daily gains, including both periods for the 350 to 400 pound heifers were 1.89 and 1.76 for the good and choice grading cattle, respectively, and 1.86, 1.95, and 1.79, respectively, for medium, good, and choice 450 to 500 pound heifers.

These recent reports strongly substantiate the conclusions of earlier workers as to the feeding value of good quality corn silage. However, in recent years some doubt has been shown regarding the vitamin A bio-potency of silage carotenes.

Research interest was renewed in ruminant vitamin A nutrition as the result of a study reported by Mitchell et al. (1960). These workers reported data on thirty, 400 pound, steers fed corn silage ad libitum plus hay and protein supplement for 112 days. The silage phase was followed by a 56-day full feed of either dry or high moisture corn. During the third feeding period, a complete mixed ration was fed containing carotene sources to allow for intakes of 4,000 I. U. of vitamin A equivalent per head per day. This intake level of vitamin A for this weight cattle is generally thought to be well above the required level (N. R. C., 1958). After 56 days on the latter ration, several steers were reported to show classical vitamin A deficiency symptoms. Several postulations have been offered as to why these symptoms should occur on such a ration.

Nitrate was implicated as a result of work reported earlier by Muhrer et al. (1955) where vitamin A deficiency symptoms occurred simultaneously with nitrate toxicity in a group of cattle. Jordan et al. (1961) reported average daily gains of steers fed corn silages grown on soils receiving varied nitrogen fertilization and varied seeding rates. The four treatments were: 16,000 plants per acre with no fertilization, 32,000 plants per acre with no fertilization, 16,000 plants and 200 pounds of NH_4NO_3, and 32,000 plants and 200 pounds of NH_4NO_3 per acre. Per cent nitrate as KNO_3 equivalent for the silages on a dry matter basis were: 0.16, 0.18, 0.75, and 0.63, respectively. The average daily gains for the four lots of steers on these silages were: 1.13, 1.33, 1.24, and 1.40 pounds, respectively. Liver biopsy samples obtained from these steers showed the liver to contain 30 mcg. of vitamin A per gram of fresh liver. After 88 days feeding of these silages, liver vitamin A values had dropped to 17.6, 16.0, 7.6, and 5.7 mcg. per gram of fresh liver for the four groups. However, after 133 days all lots were equally low and averaged 3.0 mcg. of vitamin A per gram of fresh liver.

Weichenthal et al. (1961) reported decreased gains on cattle fed ground shelled corn and alfalfa hay when nitrate was added to the ration as 1 per cent $NaNO_3$. They concluded that supplemental $NaNO_3$ had no effect on plasma vitamin A. Subsequent efforts to exemplify the effects of nitrate fertilization upon the nutritional value of corn silage were reported by Jordan et al. (1963). Ten tons of feed lot manure plus 300 pounds of NH_4NO_3 per acre were added as fertilizer to corn silages. A second corn silage was produced with no fertilization. The fertilized silages contained 0.67 and 0.75 per cent KNO_3 equivalent while the unfertilized corn contained 0.18 and 0.16 per cent on a dry matter basis. Forty-eight crossbred, Angus x Hereford, steers fed these silages failed to show any significant differences among the silages as measured by average daily gains. Following the silage phase, the same steers were fed mixed hay, soybean meal, and cracked corn (full feed) for 104 days. One-third of the steers were fed supplemental vitamin A (8,000 I. U. per head per day), and one-third received stabilized beta-carotene in amounts equivalent to 8,000 I. U. vitamin A per head per day while the other one-third received no supplementation. Average daily gains for the treatment groups of steers were 2.40, 2.40, and 2.43 pounds, respectively, and were not significantly different.

Research reported by Smith et al. (1964) and in preliminary reports by Zimmerman et al. (1962, 1963) and Smith et al. (1963) yielded similar results from corn silages grown with three levels of fertilization. Corn was fertilized in late spring with either no fertilization, 670 pounds of ammonium nitrate per acre, or 670 pounds of ammonium nitrate plus 400 pounds of superphosphate (53 per cent P_2O_5). The nitrate content of the silages was 0.18, 0.31, and 0.28 per cent, respectively, on a dry matter basis. Yearling, Hereford, steers averaging 706 pounds fed these silages showed no significant differences between the control silage and the silage receiving 670 pounds of ammonium nitrate per acre. However, the steers receiving the silage fertilized with ammonium nitrate plus superphosphate gained significantly ($P < 0.05$) faster than the steers fed the silage grown without fertilization. There were no significant differences among the feed efficiency data for these steers. Essentially the same results were reported on a second similar trial with silages containing 0.14 and 0.45 per cent nitrate, KNO_3 equivalent on a dry matter basis, grown with no fertilizer and with 670 pounds of ammonium nitrate per acre. These authors concluded that neither nitrate nor other factors directly associated with the soil fertilization treatments appeared to be responsible for the apparent poor silage carotene utilization determined by liver biopsies from the experimental animals.

II. DIGESTION STUDIES

Results from metabolism studies, involving three years and nine trials, with steers fed corn silage were reported by Garrigus (1951). He reported the following average digestibility coefficients for corn silage: dry matter, 67.3; crude protein, 56.9; crude fiber, 58.8; ether extract, 83.4; nitrogen-free extract, 74.4; and total digestible nutrients, 69.5.

Utilizing steers and wethers, Wilson (1964) found digestion coefficients for a corn silage ration supplemented with concentrate to be slightly higher than those reported by Garrigus.

In an extensive series of digestion trials with cattle, Alexander et al. (1962) found corn silage digestibility coefficients for dry matter, crude protein, crude fiber, ether extract, nitrogen-free extract, and total digestible nutrients to be 63.8, 53.5, 75.1, 66.9, 63.0, and 64.6, respectively. The corn silages used in these studies were grown with two levels of fertilization and two plant populations. The levels of fertilization used were not reported, but the authors stated that the level of fertilization had no influence on the digestibility coefficients obtained.

Various workers have reported on the relationship of digestibility coefficients obtained with steers and wethers. Cipolloni et al. (1951) in data calculated from Schneider (1947) indicated that steers digested all nutrients in silages better than sheep. Watson et al. (1948) reported crude protein digestibility coefficients for silages to be higher for cattle than for sheep. Swift and Bratzler (1959) found no significant differences between the digestion efficiencies of cattle and sheep. Wilson (1964) obtained slightly higher digestion coefficients with steers than with wethers when both were fed corn silage supplemented with concentrate.

In recent years, *in vitro* technique has become a useful and accepted tool for the evaluation of forages. Various reports have been published related to the usefulness and adaptation of the technique (Burroughs *et al*., 1950a; Burroughs *et al*., 1950b; Arias *et al*., 1951; Hall, 1955; Cheng *et al*., 1955).

An extensive review of the literature revealed no studies utilizing the *in vitro* technique for comparing the effect of various rates of nitrogen fertilization on the cellulose digestibility of corn silage. However, the *in vitro* technique has been used to study the effect of nitrogen fertilization on cellulose digestibility of other forages. Hall *et al*. (1964) reported results from *in vitro* digestion of three varieties of Bermudagrass fertilized with 0, 60, 120, and 180 pounds of nitrogen per acre. The *in vitro* cellulose digestibility of the first cuttings from each grass variety was improved with the addition of up to 120 pounds of nitrogen per acre. However, the addition of 180 pounds of nitrogen per acre resulted in decreased cellulose digestibility in grass samples obtained at first cutting.

Perez and Story (1960) reported less *in vitro* gas production from hays fertilized with 200, 400, and 800 pounds of nitrogen per acre than from hays receiving no nitrogen fertilization. Hopkins *et al*. (1960) found nitrate to be responsible for the decreased *in vitro* gas production of brome grass, orchardgrass, and alfalfa forages fertilized at levels of 400 and 800 pounds of nitrogen per acre.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. SILAGES

These data were collected on two silages produced during the 1963 growing season. Corn silage (Dixie 29) was produced on the University of Tennessee Tobacco Experiment Station in Greene County in northeast Tennessee. The corn was grown on uniform Class I Hunington silt loam soils located in the same area. One silage (control) was fertilized at the rate of 100 pounds of nitrogen per acre as NH_4NO_3, the amount usually recommended for corn silage production in this area. The second silage (nitrated) was produced from corn fertilized at the rate of 300 pounds of nitrogen per acre in the same form. Other management practices were kept uniform. Accepted corn production practices were employed throughout the growing season. The two silages were cut at the same time when the corn was in the early dent stage and stored in identical small upright concrete silos of 10 ton capacity. The nitrated corn had a greener appearance at the time of ensiling. Samples of these silages were obtained for chemical analyses by A.O.A.C. (1960) methods and will be subsequently discussed.

II. FEEDING TRIAL

Forty 470 pound Hereford heifer calves which graded high good in type, part purchased in the Tennessee Demonstrational Feeder Calf Sales and part produced on the experiment station from purebred cows, were used as experimental animals. Each heifer was graded on type and condition by two competent graders of the University of Tennessee Animal Husbandry Department staff. Individual weights were taken on two consecutive days and the average of the two weights was used as the initial weights. Five heifers were assigned to each lot in order to make the lots as uniform as possible on the basis of origin, weight, type, and condition. Two lots were then assigned to each of the four treatments and the lot averages used as the experimental unit. The four treatments were: control corn silage, control corn silage plus 20,000 I. U. of vitamin A per head daily, nitrated corn silage, and nitrated corn silage plus 20,000 I. U. of vitamin A per head daily. The animals received all the corn silage they would consume without excessive waste plus 4 pounds of ground ear corn, 1 pound of cottonseed meal, and 2 pounds of good quality alfalfa hay per head per day. Fresh water, salt, and dicalcium phosphate were available *ad libitum*. Each heifer was implanted with 24 milligrams of stilbestrol at the beginning of the feeding period. The animals began the winter feeding period on October 11, 1963 and were fed for 113 days. They were fed in covered barn lots adjacent to the experimental silos.

The heifers were fed twice daily and the silage was removed from the silo at the time of feeding to insure freshness. The vitamin A supplement was fed to two treatment groups. A quantitative measure of the supplement containing 100,000 I. U. of vitamin A was sprinkled over the concentrate once per day. It was assumed that each of the five animals received an equivalent amount of the supplement (20,000 I. U.). Feed weights were recorded at each feeding.

Individual weights were taken every 28 days and each animal was weighed on two consecutive days at the end of the phase. The average of the two days weights was used as the final weight for the silage phase and as the initial weight for the terminal full feed phase. Each heifer was scored on condition or slaughter grade at the end of the silage phase.

Following the silage phase, all lots were kept intact and continued on a finishing ration for 80 days. The finishing ration consisted of a full feed of a 7:1 ground ear corn:cottonseed meal mixture plus 2.0 pounds of good quality alfalfa hay. The lots receiving vitamin A during the silage phase were continued on vitamin A at the same level. The heifers were fed twice daily all they would clean-up without excessive waste. The vitamin A supplement was fed to the designated lots in the same manner as during the silage phase. Individual weights were taken every 28 days and on two consecutive days at the end of the finishing period. The average of the two days weights was used as the final weight. The heifers were scored at the end of the full-feeding period for slaughter condition by two graders similar to those who performed the initial grading.

The animals were sold to a packing company in Knoxville for $21.35 per hundred pound. The heifers were transported by truck to Knoxville, a distance of approximately 75 miles, and a transportation cost of $3.75 per head was charged against each animal. Individual weights were obtained at the packing company and this weight was used as the slaughter weight. Each animal was tagged for identification as they were slaughtered. Individual warm carcass weights were obtained immediately after the carcass was washed. This weight was decreased by 2.5 per cent to allow for cooler shrink in order to calculate the dressing per cent on a chilled carcass weight basis. After the carcasses were chilled for approximately 48 hours, subsequent carcass data were taken. Loin-eye measurements were obtained by tracing the area between the twelfth and thirteenth ribs of the left side of each carcass on acetate paper. The tracings were then measured with a compensating planimeter. Fat thickness over the rib was measured at a point three-fourths the length of the loin-eye from the chine. Slaughter grade, marbling score, and estimated per cent kidney fat were obtained from the local USDA grader. From these data USDA Yield Grades were calculated. All trait differences in production and carcass data were statistically analyzed by analysis of variance procedure, Snedecor (1956).

III. METABOLISM STUDIES

Cattle

Experimental animals and design. Twelve steers averaging 558 pounds, of similar age, type, condition, and breeding were used as experimental animals. All the steers were halter broke and fed a corn silage ration for two weeks prior to the seven-day preliminary period. The steers were weighed at lotting time and were paired according to weight. The animals were then randomly assigned to two rations giving a randomized block design.

Silages and feeding method. The silages for the metabolism studies were transported to Knoxville at the onset of the preliminary period. Sealed boxes of 1,500 pounds capacity were used to haul and store the silages during the trial. Two boxes of each silage were obtained from the silos, near the end of the wintering trial, for use in the metabolism studies. The boxes were not sealed at the top. Due to an unusual siege of warm temperatures some spoilage occurred. Because of this problem, the collection period was shortened to five days instead of the customary seven. Care was taken to avoid feeding spoiled silage and hence the trial was necessarily terminated two days early.

Two 500 gram samples of each fresh silage were obtained from the boxes at the initiation of the metabolism trial and another such sample, to be used for chemical analysis, was obtained near the mid-point of the collection period. The samples were stored in air-tight containers until the end of the trial. Two 500 gram samples of each silage sample were placed in aluminum cake pans and dried at 70°C. for 72 hours. The dried samples were allowed to reach a moisture equilibrium with the air before weighing, and were ground in a Wiley Mill with a 20 mesh screen.

The samples from each silage were kept separate and held in air-tight jars until they were chemically analyzed.

The analyses shown in Table I for dry matter, crude fiber, ether extract, ash, and nitrogen-free extract were determined by A.O.A.C. (1960) methods. Crude protein was calculated from nitrogen determinations by the Kjeldahl method using slight modifications. Per cent nitrate was determined as KNO_3 equivalent using a method obtained from O. E. Olson (1964) of the South Dakota State College Biochemistry Department. The method utilizes Devarda's alloy for the reduction of nitrate and nitrite to ammonia. The per cent cellulose was determined by using the method of Crampton and Maynard (1938) with minor modifications.

During the preliminary and collection periods the steers were fed twice daily at regular intervals all the silage they would consume with minimum weigh-backs. In addition each steer was fed a 4:1 mixture of ground shelled corn:cottonseed meal at a calculated level so that the level of concentrate fed on an air-dry basis would approximate the ratio of concentrate:silage fed to the heifers during the wintering period. Weigh-backs were taken once a day and were subtracted from the silage fed.

<u>Equipment and sampling</u>. The metabolism stalls used in this work were similar to those described by Hobbs <u>et al</u>. (1950). All gross fecal and feed weights were taken on beam scales, accurate to 0.5 pound. Fecal aliquots were weighed on triple-beam balances, accurate to 5 grams.

TABLE I

PROXIMATE COMPOSITION OF SILAGES AND CONCENTRATE MIXTURE
(AS FED BASIS)

	Control Corn Silage	Nitrated Corn Silage	Concentrate Mixture[1]
	%	%	%
Dry matter	28.32	25.08	89.16
Crude protein	2.30	2.10	16.00
Crude fiber	7.14	5.94	4.00
Nitrogen-free extract	16.72	15.04	63.35
Ether extract	0.84	0.68	3.58
Ash	1.25	1.25	2.23
Nitrate (KNO_3 equivalent)	0.07	0.12	-
Cellulose	6.71	6.17	4.60

[1]Concentrate mixture consisted of a 4:1 mixture of ground shelled corn:cottonseed meal.

The fecal pans were weighed, sampled, emptied, and cleaned at each collection time. All pans were weighed initially while clean and empty. The difference between the full weight and the empty weight was used as the fecal excretion for the 24-hour period. A 3 per cent aliquot was obtained from the weighed and thoroughly mixed sample. These aliquots from each steer were placed in a separate wide-mouth jar and sealed. The jars were stored in a refrigerator at 35°F. during the trial. At the end of the five-day collection period, the total of the five aliquots was used for chemical analyses and calculating digestion coefficients.

Analysis. The fecal samples were prepared for analysis and analyzed for air-dry matter according to the procedure reported by Wilson (1964). All chemical analyses of the feces were conducted in accordance with the A.O.A.C. (1960) procedures with the exception that nitrogen was determined by the Kjeldahl method using slight modifications. Digestibility coefficients were statistically analyzed according to analysis of variance procedures of Snedecor (1956).

Sheep

Experimental animals and design. Metabolism studies were conducted with 12 wether sheep of similar type, breeding, and condition. The wethers, with an average weight of 115 pounds, were paired according to weight. One animal from each pair was then randomly assigned to one of two treatment silages.

Silages and feeding method. Silages fed to the sheep were from the same source as that fed the cattle. The metabolism studies using sheep were conducted concurrently with the cattle studies. During the preliminary period, the sheep were fed twice daily all the silage they would consume. This intake determined the amount fed during the collection period. Water was available at each feeding. A 4:1 mixture of ground shelled corn:cottonseed meal was fed in the same proportion to silage as fed to the heifers in the feeding trial. Weigh-backs were taken before each feeding and subtracted from the amount fed. The trial consisted of a six-day preliminary period and a five-day collection period.

Equipment and sampling. The metabolism stalls as described by Briggs and Gallup (1949) were used in this study. A triple-beam balance, accurate to 1 gram, was used for weighing feed and fecal collections. A graduated cylinder was used to measure urine excretion. Fecal and urine collections were taken at regular 24-hour intervals. The total weighed fecal excretion was placed in paper bags and these individual daily collections were placed in heavy plastic bags. All fecal excretions were stored in refrigeration at 35°F. until the termination of the collection period. A 10 per cent sample of urine was taken each day from each wether, placed in wide-mouth jars, and refrigerated at 35°F.

Analysis. At the end of the collection period, the five-day collections were mixed thoroughly and two 500 gram composite samples were taken for each animal for subsequent analysis. The total of the urine composites of each animal was sampled for nitrogen determination according to the procedure used for the cattle metabolism studies. The feed and feces samples were processed and analyzed in a similar manner to that employed in the cattle studies.

IV. *IN VITRO* STUDIES

Technique and Design

Rumen microbial *in vitro* studies were conducted using whole rumen liquor as the inoculum. A factorial experimental design involving two levels of nitrogen (100 pounds N per acre and 300 pounds N per acre), three sampling times, and two levels of concentrate addition (none and 0.376 gram) was used. One sample of the corn silages was obtained during the latter part of the feeding trial and the other two during the metabolism studies. The fresh samples were dried according to the procedure discussed previously.

Prior to collecting the rumen liquor, a 0.4 gram air-dry aliquot from each of the three samples of each silage was weighed into four separate 50 milliliter low actinic, Erlenmeyer flasks. The concentrate, composed of a 4:1 mixture of ground shelled corn:cottonseed meal, was added to half of the flasks containing aliquots from the same silage sample. This was an attempt to measure the effect of concentrate supplementation upon cellulose breakdown by rumen microbes. Each determination was made in duplicate to test the repeatability of the technique. The amount of concentrate added (0.376 gram) was in the same ratio to silage-dry matter as that fed in the feeding trial and metabolism studies.

Whole rumen liquor was used as the inoculum from two fistulated steers to measure animal source differences. Each trial was duplicated with rumen liquor from each steer. The fistulated steers were purebred Herefords and were fed twice daily a ration of 10 pounds of good quality alfalfa hay, plus 3 pounds of a 4:1 mixture of ground shelled corn: cottonseed meal per head daily. One steer was approximately eight years of age and weighed 1,500 pounds. Steer number two was approximately 20 months of age and weighed about 1,000 pounds.

Procedure

The silage samples were weighed into the flasks about four hours prior to collecting the rumen liquor and 15 milliliters of a 0.8 per cent sodium bicarbonate solution were added to each flask. The substrate and sodium bicarbonate mixture was then agitated on a magnetic stirrer for one minute, in order to suspend the substrate. The duplicate flasks were then connected with a glass tubing train and placed in a water bath at 39.5°C. Carbon dioxide was bubbled through the flask contents at the rate of one bubble per second to insure that anaerobic conditions were present at the time of inoculation.

Approximately five hours after the morning feeding rumen contents were obtained from the fistulated steers and strained through eight layers of cheese cloth. Approximately 2,000 milliliters of rumen liquid were strained into a previously warmed thermos bottle. The rumen liquor was taken to the laboratory and strained again through eight layers of cheese cloth into a one liter Erlenmeyer flask. Carbon dioxide was bubbled briskly through the liquor for 20 minutes prior to inoculating the substrate.

Fifteen milliliters of the whole rumen liquor were added to each flask. The flasks were then re-connected to the carbon dioxide inlet and placed in the water bath at 39.5°C. for a 21 hour fermentation period. Cellulose determinations were made on the contents of each flask at the end of the fermentation period according to the procedure of Crampton and Maynard (1938), with slight modifications. Using the amount of cellulose remaining in the substrate and the cellulose in the original sample, digestibility coefficients were calculated for each flask. Differences were statistically analyzed using the analysis of variance procedures of Snedecor (1956).

CHAPTER IV

RESULTS AND DISCUSSION

I. FEEDLOT PERFORMANCE

Winter Period

During the 113-day wintering period of the feeding trial, no significant differences were noted in the gains of the heifers on the four treatments (Table II). The range in average gain per head during this period was only four pounds. Heifers fed the control corn silage averaged gaining 1.77 pounds per day and those fed the control silage plus 20,000 I. U. of vitamin A per head per day averaged gaining 1.78 pounds per day. The heifers fed the nitrated corn silage gained 1.75 pounds per day and those fed the nitrated silage plus 20,000 I. U. of vitamin A per head per day gained 1.74 pounds per day.

As shown in Table II feed consumption of the four heifer groups was almost identical. Although the silage was fed free choice, there were no significant differences between treatment groups with a range in average daily silage consumption of only 1.1 pounds. Similarly, the amount of feed required per hundred pounds of gain was similar for the four treatment groups. The average amount of feed required per hundred pounds of gain was 764 pounds of air-dry feed for all treatments with a range of 34 pounds of air-dry feed. The heifers fed the control corn silage without vitamin A required 782 pounds of air-dry feed and those

TABLE II

EFFECTS OF SUPPLEMENTAL VITAMIN A ON PERFORMANCE OF BEEF HEIFER CALVES FED CONTROL AND NITRATED CORN SILAGES
(October 11, 1963 to February 1, 1964 - 113 Days)

	Control Corn Silage		Nitrated Corn Silage	
	Without Vit. A	20,000 I.U. Vit. A/Head Da.	Without Vit. A	20,000 I.U. Vit. A/Head Da.
No. of an./lot	5	5	5	5
No. of an./treatment	10	10	10	10
Av. wt. and gn./head, lb.				
Initial wt.	470	473	469	468
Final wt.	670	674	667	665
Total gain	200	201	198	197
Daily gain	1.77	1.78	1.75	1.74
Av. daily feed, lb.				
Corn silage	21.4	20.6	21.0	21.7
Concentrates[1]	5.0	5.0	5.0	5.0
Alfalfa hay	2.0	2.0	2.0	2.0
Feed req./cwt. gain, lb.				
Corn silage				
Fresh basis	1209	1157	1200	1247
Air-dry basis	387	370	348	362
Concentrates[1]	282	281	286	287
Hay	113	112	114	115
Total[2]	782	763	748	764
Feed costs/lb. gain[3]	13.1¢	12.8¢	13.1¢	13.4¢
Grades				
Initial type	G+	G+	G+	G+
Initial slaughter	H Std.	H Std.-	H Std.-	H Std.-
Final slaughter	LG-	LG-	LG-	LG-

[1]Four parts ground ear corn and 1 part cottonseed meal.

[2]Air-dry basis.

[3]Feed costs based on following prices: corn silage, $8/ton; alfalfa, $40/ton; ground ear corn, $1.25 bu.; and cottonseed meal, $80/ton.

fed vitamin A required 763 pounds of air-dry feed per hundred pounds of gain. The group of heifers fed the nitrated corn silage without vitamin A averaged 748 pounds while the animals with vitamin A supplementation averaged 764 pounds of air-dry feed per hundred pounds of gain.

As shown in Table II, the feed costs per pound of gain were nearly identical with only a 0.6 cent range. The average final USDA slaughter grade at the end of the winter was low Good for all four treatments.

Finishing Period

Table III gives the performance data for these heifers in the subsequent 80-day full-feed period. The average initial weight of the four treatments was 670 pounds and the average final weight was 799 pounds. As in the wintering period, the average daily gains were similar for the treatment groups. The treatment means for average daily gains ranged from 1.58 to 1.66 pounds and were not significant.

The heifers consumed similar amounts of feed per day and the treatment differences were not significant. The average daily concentrate consumption for the treatment groups ranged from 15.7 to 16.1 pounds per day. Due to the nearly identical gains and feed intake, there were no differences among treatments in feed efficiency. The averages of the four treatments ranged from 1,098 to 1,152 pounds of air-dry feed per hundred pounds of gain during this period. Consequently, the feed costs per pound of gain were very similar with a range of only 0.7 cent.

TABLE III

SUBSEQUENT PERFORMANCE OF THE HEIFERS ON A FINISHING RATION
(February 1, 1964 to April 21, 1964 - 80 Days)

	Previous Treatment			
	Control Corn Silage	Control Corn Silage + Vit. A	Nitrated Corn Silage	Nitrated Corn Silage + Vit. A
No. of an./lot	5	5	5	5
No. of an./treatment	10	10	10	10
Av. wt. and gain/hd., lb.				
Initial wt.	670	674	667	665
Final wt.	800	800	800	795
Total gain	130	126	133	130
Daily gain	1.62	1.58	1.66	1.62
Av. daily ration, lb.				
Concentrates[1]	15.7	15.9	15.9	16.1
Alfalfa hay	2.0	2.0	2.0	2.0
Silage	1.1	1.1	1.1	1.1
Feed req./cwt. gain, lb.				
Concentrates[1]	969	1006	958	994
Hay	123	127	120	123
Silage (air dry)	19	19	18	19
Total	1111	1152	1098	1136
Feed costs/lb. gain	21.2¢	21.9¢	20.9¢	21.6¢
Grades				
Initial slaughter	LG-	LG-	LG-	LG-
Final slaughter	HG-	G+	HG-	HG-
Av. sale price/cwt.	$21.35	$21.35	$21.35	$21.35

[1]Concentrates consisted of 7 parts ground ear corn and 1 part cottonseed meal.

The control plus vitamin A group had an average final USDA slaughter grade of average Good and the other three treatment groups averaged high Good. However, this difference was not statistically significant. The actual sale price of all treatment groups was identical, $21.35 per hundred pounds.

It should be noted that the gains of these heifers during the full-feed period were not as high as are often obtained on this type of ration. Part of this may have resulted from the winter gains being higher than those obtained on a similar wintering ration reported by Hall and Felts (1963) with heifers and Benson et al. (1961) with steers. A few of the heifers in several lots went off feed during the last two weeks which may account for the low full-feed period gains. The wintering gains of these heifers also exceeded those reported on similar cattle and ration by Wilson (1964). Wilson reported gains during the wintering period of 1.35 and 1.38 pounds per day on 500 pound heifers in a 1961-1962 wintering trial and 1.41 and 1.40 pounds per day on 460 pound heifers in a 1962-1963 trial. The silages used by Wilson were similar to the control silage used in this study, but he fed cracked shelled corn instead of the ear corn fed in this study.

However, other workers (Anderson et al., 1964) have reported winter gains of heifers similar to those obtained in this study followed by subsequent daily gains in the feedlot of nearly 2.0 pounds per head per day. Therefore, other factors undoubtedly contributed to the somewhat lower, than expected, gains made during the full-feed period.

As shown in Table IV, the average daily gains of the heifers for both experimental periods were 1.71 pounds for those fed the control silage, 1.69 pounds for those fed the control silage plus vitamin A, 1.72 pounds for the nitrated silage fed group, and 1.69 pounds for the nitrated silage plus vitamin A fed heifers. These differences in average daily gains were not statistically significant. Likewise, the feed costs per pound of gain were not statistically different. The average feed cost for all treatments was 16.35 cents per pound of gain with a range of 0.40 cent. Returns per head over feed and transportation to market costs are also shown in Table IV. These differences were not statistically different.

Carcass Data

Carcass data obtained on the heifers are reported in Table V. There were no significant differences due to treatment for any of the carcass traits studied, including slaughter weight taken at the packing plant. The treatment groups had dressing percentages, based on adjusted warm carcass weights, ranging from 60.8 to 62.0 per cent. Although not significant, the heifers on the control corn silage had a slight advantage in marbling score, and the heifers receiving vitamin A had slightly more fat over the rib. The USDA yield grades by treatment group ranged from 3.2 to 3.5 for these heifers.

TABLE IV

SUMMARY OF THE PERFORMANCE OF THE HEIFERS FOR BOTH EXPERIMENTAL PERIODS
(October 11, 1963 to April 21, 1964 - 193 Days)

	Control Corn Silage		Nitrated Corn Silage	
	Without Vit. A	20,000 I. U. Vit. A/Head Da.	Without Vit. A	20,000 I.U. Vit. A/Head Da.
No. of heifers	10	10	10	10
Av. wt. and gain/head, lb.				
Initial wt.	470	473	469	468
Final wt.	800	800	800	795
Total gain	330	327	331	327
Daily gain	1.71	1.69	1.72	1.69
Feed cost/lb. gain	16.2¢	16.4¢	16.2¢	16.6¢
Initial type grade	G+	G+	G+	G+
Initial slaughter grade	H Std.	H. Std.-	H Std.-	H.Std.-
Final slaughter grade	HG-	G+	HG-	HG-
Initial value/cwt.	$22.75	$22.75	$22.75	$22.75
Final value/cwt.	$21.35	$21.35	$21.35	$21.35
Returns/head over feed costs[1]	-$0.72	-$1.56	-$1.69	-$3.36

[1]Calculated on basis of weight at Knoxville. A transportation charge from Greeneville to Knoxville of $3.75/head is included in the costs.

TABLE V

CARCASS DATA OF HEIFERS

	Previous Treatment			
	Control Corn Silage	Control Corn Silage + Vit. A	Nitrated Corn Silage	Nitrated Corn Silage + Vit. A
No. of an./lot	5	5	5	5
No. of an./treatment	10	10	10	10
Slaughter wt., lb.	766	764	761	755
Slaughter grade	HG-	G+	HG-	HG-
Federal carcass grade	HG	HG	HG-	HG-
Warm carcass wt., lb.	478	482	480	480
Dressing per cent[1]	60.8	61.4	61.5	62.0
Marbling score	Slight+	Slight+	Slight	Slight
Loin-eye area,[2] sq. in.	10.17	9.80	10.48	10.05
Fat thickness, in.[3]	0.49	0.54	0.52	0.53
Per cent kidney fat	2.80	2.85	2.70	2.70
USDA Yield Grade	3.30	3.50	3.20	3.35

[1]Calculated on the basis of warm carcass weight less 2.5%.

[2]Loin-eye area measured between the 12th and 13th ribs.

[3]Taken at the 12th rib.

II. METABOLISM STUDIES

Silages

Chemical composition. As shown in Table I, the control corn silage used in the metabolism studies contained 28.32 per cent air-dry matter as compared to 25.08 per cent for the nitrated corn silage. This substantiates the observation that the nitrated corn appeared greener and more moist than the control corn at harvest. Therefore, on a fresh basis, the control corn silage also had a slightly higher per cent crude protein, crude fiber, nitrogen-free extract, and ether extract than the nitrated corn silage--2.30 vs. 2.10, 7.14 vs. 5.94, 16.72 vs. 15.04, and 0.84 vs. 0.68, respectively. The per cent ash was the same for both samples--1.25. The per cent nitrate, as KNO_3 equivalent, was 0.07 for the control and 0.12 for the nitrated corn silage. Slightly more nitrate might have been expected to accumulate; however, these amounts were similar to the per cent reported by Smith et al. (1964) in corn silage grown with 670 pounds ammonium nitrate per acre. The 670 pounds of ammonium nitrate, or 235 pounds of nitrogen per acre, resulted in levels of 0.11 and 0.13 per cent nitrate equivalent on a fresh basis in two trials. Nitrate represented 0.31 and 0.45 per cent of the silage on a dry matter basis in Smith's study as compared to 0.40 per cent in this study with 300 pounds of nitrogen.

Digestibility Studies

Digestibility with steers. As noted in Table VI, the control silage fed group of steers averaged consuming 19.03 pounds of silage per

TABLE VI

DIGESTIBILITY OF CONTROL AND NITRATED CORN SILAGES SUPPLEMENTED WITH CONCENTRATES BY STEERS

	Control Corn Silage	Nitrated Corn Silage
No. of calves	5	6
Av. wt. of calves, lb.		
Initial	566	551
Final	546	525
Av. daily feed consumption, lb.		
Silage		
Fresh basis	19.03	19.53
Air-dry basis	5.86	5.39
Concentrate[1]	3.00	3.00
Digestibility of rations, %		
Dry matter	68.15	69.41
Crude protein	54.87	56.57
Crude fiber	58.12	58.74
Ether extract	75.52	75.72
Nitrogen-free extract	74.17	75.63
Total digestible nutrients		
Air-dry basis	68.87	70.31
Fresh basis	21.21	19.41

[1]Concentrate mixture fed was a 4:1 mixture of shelled corn: cottonseed meal.

head per day on a fresh basis and those fed nitrated silage consumed 19.53 pounds, or 5.86 and 5.39 pounds, respectively, on an air-dry basis. The difference between treatments in feed consumption was not statistically significant. Feed intake was intended to be regulated at a maintenance level, but the steers lost an average of 20 and 26 pounds on the control and nitrated groups, respectively, during the 12-day period.

There was no significant differences between treatments in any of the digestion coefficients. Digestion coefficients for the dry matter in the control silage averaged 68.15 as compared to 69.41 for that in the nitrated silage. The digestion coefficients for the crude protein, crude fiber, ether extract, and nitrogen-free extract for the control and nitrated silage rations were: 54.87 vs. 56.57, 58.12 vs. 58.74, 75.52 vs. 75.72, and 74.17 vs. 75.63, respectively.

These digestion coefficients indicate that the level of nitrate fertilization did not effect the efficiency with which the steers digested the silages. Except for crude protein, these digestion coefficients are in close agreement with those obtained by Garrigus (1951) with similar silages. The digestibility coefficient for crude protein reported by Garrigus was about 3.0 per cent higher than those obtained in this study. The digestibility coefficients reported in Table VI for dry matter, crude fiber, and nitrogen-free extract are each within 1.0 per cent to those reported by Garrigus.

These digestibility coefficients are slightly lower than those reported by Wilson (1964) for corn silages similar to the control silage used in this study. The digestibility coefficients obtained in this study for dry matter were about 2.5, crude protein 3.0, crude fiber 5.0, and nitrogen-free extract 1.8 per cent less than those reported by Wilson.

Per cent total digestible nutrients in the control silage ration was 68.87 while the nitrated silage ration contained 70.31 per cent on an air-dry basis or 21.21 and 19.41 per cent on a fresh basis, respectively. These differences were not statistically different and were similar to the per cent total digestible nutrients reported by Wilson (1964) for a similar corn silage.

Digestibility with wethers. The digestibility coefficients obtained using, 114 pound, 3-year-old wethers are shown in Table VII. The feed intake was at about a maintenance level as indicated by the initial and final test weights. The average daily silage consumption was 4.87 and 4.52 pounds for the wethers fed the control and nitrated silage rations, respectively. The wethers fed the control silage ration consumed slightly more silage on a dry matter basis than did the wethers fed the nitrated silage ration, but not significantly more.

The digestibility coefficients of the silage rations obtained with wethers were not significantly different for dry matter, crude protein, crude fiber, ether extract, or nitrogen-free extract. The actual coefficients obtained for dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract for the control and nitrated silage rations were: 69.36 and 70.54, 62.66 and 63.66, 42.52 and 39.73, 83.25 and 81.52, and 79.18 and 80.43, respectively.

TABLE VII

NITROGEN BALANCE AND DIGESTIBILITY OF CONTROL AND NITRATED CORN SILAGES SUPPLEMENTED WITH CONCENTRATES BY WETHERS

	Control Corn Silage	Nitrated Corn Silage
No. of wethers	4	4
Av. wt. of wethers, lb.		
Initial	112	117
Final	112	112
Av. daily feed consumption, lb.		
Silage		
Fresh basis	4.87	4.52
Air-dry basis	1.50	1.26
Concentrate[1]	1.00	1.00
Digestibility of rations, %		
Dry matter	69.36	70.54
Crude protein	62.66	63.66
Crude fiber	42.52	39.73
Ether extract	83.25	81.52
Nitrogen-free extract	79.18	80.43
Total digestible nutrients		
Air-dry basis	70.73	71.80
Fresh basis	21.78	19.82
Av. nitrogen intake, gm.		
Silage	8.13	6.93
Concentrate	11.61	11.61
Total	19.74	18.54
Av. nitrogen output, gm.		
Urine	8.17	8.08
Feces	7.37	6.75
Total	15.54	14.83
Nitrogen balance, gm.	4.20	3.71

[1]Concentrate mixture fed was a 4:1 mixture of ground shelled corn: cottonseed meal.

The nitrogen intake data in Table VII show that the wethers fed the control silage ration consumed 19.74 grams as compared to 18.54 grams for the wethers fed the nitrated silage ration. However, this difference was not significantly different. The difference in nitrogen intake is probably due to the difference in crude protein on a fresh basis and the slight difference in silage intake of the two silages. This slight difference in nitrogen intake is reflected by the slightly higher fecal nitrogen output. The urinary nitrogen was essentially the same for both groups of wethers and the difference in total nitrogen output was not statistically different. The nitrogen balance of these mature wethers was 4.20 grams for the control and 3.71 grams for the nitrated silage fed group. This difference was not statistically significant.

The metabolism studies with wethers indicated no significant differences between total digestible nutrients for the silages. The average per cent total digestible nutrients were 21.78 and 19.82, on an air-dry basis, for the control and nitrated silage rations, respectively.

Table VIII gives the mean squares for the differences between the silages and between species found in this trial. There were no significant differences between the silage digestibility coefficients obtained within either species for dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract. Data from both wethers and steers showed that the two silages were equally digestible. The mean square obtained for the difference between species for dry matter digestibility was not statistically significant. However, wethers gave significantly ($P < 0.01$) larger average digestibility coefficients for crude fiber,

TABLE VIII

MEAN SQUARES FOR DIGESTIBILITY COEFFICIENTS OF SILAGES OBTAINED WITH WETHERS AND STEERS

	Source of Variation			
	Silages	Species	Silages X Species	Residual
d.f.	1	1	1	15
Dry matter	6.50	5.72	0.58	6.87
Crude fiber	5.39	250.99**	4.40	15.68
Crude protein	0.01	1391.85**	16.57	22.01
Ether extract	3.95	211.24**	2.15	23.29
Nitrogen-free extract	6.35	108.26**	2.64	5.19

**$P < 0.01$.

ether extract, and nitrogen-free extract. Whereas, steers gave a significantly larger ($P < 0.01$) digestion coefficient for crude protein.

Alexander _et al_. (1962) obtained significantly larger digestibility coefficients for crude protein with steers than with wethers when fed several corn silages. No other species differences in digestibility coefficients with corn silages were reported by Alexander.

Wilson (1964) reported slightly lower coefficients with wethers than with steers for digestible dry matter, crude protein, crude fiber, ether extract, nitrogen-free extract, and total digestible nutrients on a similar control corn silage. Except for crude protein, the digestibility coefficients obtained with wethers as compared to those obtained with cattle reported herein disagree with those reported by Wilson. However, the actual coefficients obtained with wethers reported by Wilson were similar to those reported herein.

Although the differences between per cent total digestible nutrients for the silage rations were not significant, both steers and wethers yielded slightly higher total digestible nutrient coefficients for the nitrated silage ration on an air-dry basis. Generally, the differences were from 1.5 to 2.0 per cent more for the nitrated silage.

III. _IN VITRO_ STUDIES

Table IX shows the chemically determined per cent cellulose of the two test silages and the per cent cellulose digestion by rumen microbes _in vitro_. The control silage contained 21.83 per cent cellulose on an air-dry basis or 6.71 per cent on a fresh basis as compared to

TABLE IX

CELLULOSE CONTENT AND *IN VITRO* CELLULOSE DIGESTIBILITY OF CONTROL AND NITRATED CORN SILAGE BY RUMEN MICROBES

	Control Corn Silage	Nitrated Corn Silage	Average
Cellulose, %[1]			
Fresh basis	6.71	6.17	6.44
Air-dry basis	21.83	22.37	22.10
Cellulose digested, %[2]			
With concentrate			
Steer 1	15.26	16.42	15.84
Steer 2	23.45	24.18	23.82
Average	19.35	20.30	19.82
Without concentrate			
Steer 1	29.03	32.66	30.84
Steer 2	29.43	34.51	31.97
Average	29.23	33.59	31.41
Average of two steers	24.29	26.94	25.62

[1]Each figure represents three samples in duplicate or six determinations.

[2]Each figure represents three samples in duplicate in two trials or twelve determinations.

22.37 and 6.17 per cent for the nitrated silages on an air-dry and fresh basis, respectively. The difference between cellulose content of the silages was not significant.

As shown in Table IX, rumen microbial activity as indicated by cellulose digestion was generally lower than expected. Wilson (1964) using a washed suspension of rumen microbes reported cellulose digestibility figures of about 40 per cent when corn silage was used as a substrate. Since whole rumen liquor was used in these studies, even higher values could be expected. From visual observations, the samples of whole rumen liquor obtained appeared to have a low level of microbial activity. In studies utilizing Solka-floc and a complete nutrient solution, only 60 per cent of this available source of cellulose was digested. This also indicated that only a medium level of microbial activity was present in the rumen liquor.

The mean squares obtained by analysis of variance of the in vitro data are presented in Table X. Despite the low level of microbial activity, the results obtained were very consistent between trials and between samples. Also, the differences between duplicates were within 2.0 per cent error in all cases. Even though the actual difference in per cent cellulose digestibility obtained with the control and nitrated silages was small (24.29 vs. 26.94), the difference was highly significant ($P < .01$).

The difference in cellulose digestibility coefficients observed between the substrates when concentrate was added was also highly significant ($P < .01$). Over 31 per cent of the cellulose in the silages

TABLE X

MEAN SQUARES FOR IN VITRO CELLULOSE DIGESTIBILITY DATA

Source	d.f.	Mean Squares
Nitrogen level (N)	1	84.40**
Concentrate (C)	1	1609.74**
Animal (A)	1	248.52**
Trial (T)	1	0.18
Sample (S)	2	10.26
N x A	1	0.77
N x T	1	5.27
N x C	1	34.85
N x S	2	11.55
A x T	1	1.36
A x C	1	140.94**
A x S	2	0.54
T x C	1	5.94
T x S	2	0.74
S x C	2	4.78
Residual	27	8.34

$^{**}P < 0.01$.

was digested when no concentrate was added as compared to only 19.82 per cent when concentrate was added. This represented a 37 per cent depression in cellulose digestibility due to concentrate addition.

The difference between animal rumen liquor source was highly significant ($P < .01$). As indicated by cellulose disappearance during a 22-hour fermentation period, the microbes in the rumen liquor samples obtained from steer one were less active than those from samples obtained from steer two (23.30 vs. 27.90). In addition, the concentrate by rumen liquor source interaction was highly significant ($P < .01$). The interaction indicated that the concentrate addition had a different effect upon cellulose digestibility when the rumen liquor inoculum came from steer one than it did when the sample came from steer two. Inoculum from steer one, the older steer, yielded a cellulose digestibility value of 15.84 per cent with concentrate addition and a value of 30.84 per cent when no concentrate was added to the flasks. The inoculum from steer two gave values of 23.82 and 31.97 per cent with and without concentrate addition, respectively.

The significant interaction may have been due, in part or all, to the different ages of the two fistulated steers. Also, the older fistulated steer, number one, had developed a sizeable air leakage around the top edge of the fistula. This opening, even though the cover was kept closed, allowed for escaping of gases from the rumen as well as the intake of atmospheric oxygen. This possibly altered the flora of the rumen. Thus, the flora could have developed into one preferring the concentrate to the cellulose as a source of energy.

It can also be noted in Table X that the two rumen liquor sources gave similar cellulose digestion values when only those flasks without added concentrate are considered. Thus when microbes from steer one were used, 29.03 per cent of the cellulose in the control corn silage was digested as compared to 32.66 per cent for the nitrated silage. When bacteria from steer two were used 29.43 and 34.51 per cent of the cellulose in the control and nitrated silages, respectively, was digested.

Despite the significant mean square of the interaction, the mean square for concentrate additions was significantly higher than the interaction. This would indicate that the addition of concentrates to the silage in this study caused a marked and significant depression in *in vitro* cellulose digestibility (19.82 vs. 31.41 per cent for the control and nitrated silages, respectively). This agrees with work reported by Burroughs *et al*. (1950), Arias *et al*. (1951) and later confirmed by Hunt *et al*. (1954). These workers found that the sizeable additions of various energy sources, including starch, to fermentation flasks containing cellulose significantly depressed cellulose digestion. They also found that introductions of readily available sources of energy in small amounts significantly enhanced *in vitro* cellulose utilization by rumen microbes.

Highly significant differences ($P < .01$) in per cent cellulose digestion (24.29 and 26.94 for control and nitrated silages, respectively) by rumen microbes were also found between silages (Tables IX and X). This difference was possibly due to the differences in nitrate content of the silages as was shown in Table I. The higher content of the nitrated silage would indicate more available nitrogen for the microbes. However, there was no significant difference in the crude protein content of the two silages either on a fresh or air-dry basis. Even though both silages were cut at the same chronological age, the nitrated silage had a greener color at harvest and a higher moisture content upon analysis which indicated it was at a younger physiological stage of maturity. As a result, the cellulose was probably more readily available to microbial degradation.

CHAPTER V

SUMMARY

This study was designed to evaluate the nutritional value of silages fertilized with 100 (control) and 300 (nitrated) pounds of nitrogen per acre. Feeding trials, metabolism trials, and *in vitro* studies were the techniques employed in this evaluation. Also, proximate chemical composition was determined on all feedstuffs.

The feeding trials included a 113-day wintering trial and an 80-day finishing period involving 40, 470 pound, heifer feeder cattle. Two similar lots of five heifers each were randomly assigned to each of four treatments during the winter. The four silage treatments were: control silage, control silage plus 20,000 I. U. of vitamin A per head per day, nitrated silage, and nitrated silage plus 20,000 I. U. of vitamin A per head per day. The silages were fed *ad libitum* and each animal received in addition five pounds of a 4:1 mixture of ground ear corn:cottonseed meal, and 2.0 pounds of alfalfa hay per head per day. The finishing period consisted of a full feed of concentrates with the same lots receiving equivalent vitamin A supplementation as was fed during the wintering trial. The heifers were slaughtered by a packing company. Performance data, feed costs, and carcass data were obtained.

Metabolism studies were conducted with steers and wethers fed these silages with concentrate supplementation. Six steers and four wethers were assigned to each silage utilizing a randomized block design.

The ration consisted of *ad libitum* silage plus three pounds (steers) and one pound (wethers) of a 4:1 mixture of ground shelled corn:cottonseed meal with the restriction that the intake level established during the seven-day preliminary period was maintained during the five-day collection period. Digestion coefficients for dry matter, crude protein, crude fiber, ether extract, nitrogen-free extract, and total digestible nutrients were calculated for these rations with both species. Nitrogen balance was determined with the wethers only.

In vitro studies with whole rumen liquor were conducted according to a factorial experimental design utilizing the two silages (control and nitrated), three samples (taken at different times), two rumen liquor sources (two fistulated steers), and two concentrate levels (0 and 0.376 gram). Two trials were conducted with rumen liquor from each steer. Each determination was duplicated in order to check the experimental technique. The per cent cellulose digested by the rumen microorganisms was determined and used for nutritional evaluation.

The results of these investigations were:

1. The nitrated silage was greener at harvest and had a higher moisture content. The nitrated silage contained 0.12 per cent nitrate, as KNO_3 equivalent, as compared to 0.07 per cent in the control silage on a fresh or as-fed basis.
2. The performance data on the heifers during the wintering and finishing trials revealed no significant differences between any treatments. Subsequent carcass data of the heifers revealed no treatment effects.

3. Metabolism trials demonstrated the silage rations to be equally digestible by both steers and wethers as measured by the digestibility coefficients. Steers gave significantly ($P < .01$) higher digestibility coefficients for crude protein on both rations than did wethers. The wethers gave significantly ($P < .01$) higher digestibility coefficients on both rations for crude fiber, ether extract, nitrogen-free extract, and total digestible nutrients.
4. The in vitro studies revealed a highly significant difference ($P < .01$) in per cent cellulose digestibility between silages in favor of the nitrated silage. However, the actual difference between the two silages was very small (24.29 vs. 26.94) and the practical significance of the difference is questionable. The difference due to concentrate addition and rumen liquor source was highly significant ($P < .01$), but the concentrate X source interaction was also highly significant.

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LITERATURE CITED

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