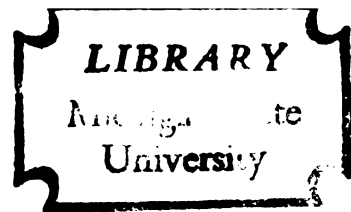


RELATIONSHIP OF SILAGE FERMENTATION  
AND ADDITIVES TO DRY MATTER  
CONSUMPTION BY RUMINANTS

Thesis for the Degree of Ph. D.  
MICHIGAN STATE UNIVERSITY  
ERSKINE HAMILTON CASH  
1972



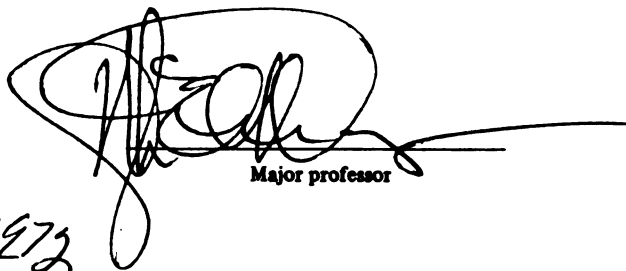
This is to certify that the  
thesis entitled  
Relationship of Silage Fermentation  
and Additives to Dry Matter  
Consumption by Ruminants

presented by

Erskine Hamilton Cash

has been accepted towards fulfillment  
of the requirements for

Ph.D. degree in Animal Husbandry

A large, stylized handwritten signature in black ink, likely belonging to the major professor.

Major professor

Date

July 24, 1972

O-7639



~~SEP 8 1975~~ 90

## ABSTRACT

### RELATIONSHIP OF SILAGE FERMENTATION AND ADDITIVES TO DRY MATTER CONSUMPTION BY RUMINANTS

By

Erskine Hamilton Cash

Six experiments were conducted to investigate the relationship of corn silage fermentation and additives to dry matter consumption by ruminants. A common objective of all six experiments was to vary the level of unidentified water soluble nitrogen fraction in corn silage and determine its effect on ruminant nutritional parameters.

Experiment I was designed to measure the effects of maximizing fermentation of corn silage with limestone treatment and minimizing fermentation with formic acid treatment on steer performance, silage nitrogen and acid fractions, and steer metabolic parameters. The formic acid and limestone treated silages were compared with a control silage receiving no treatment. All three silages were fed to steer calves in a 161 - 238 day feeding trial and the silages were compared on both an all silage ration and a 60% corn silage and 40% high moisture shelled corn ration on a dry matter basis. Lactic acid levels were significantly ( $P < .01$ ) increased and decreased with limestone and formic



acid treatments, respectively (control - 9.44% of DM, formic acid treated - 2.61% of DM, and limestone treated - 14.94% of DM). Formic acid treated silage contained only about 25% as much lactate as the control silage and the limestone treated silage resulted in a 74% increase in lactate content compared to the control. The unidentified nitrogen levels were not significantly different for the three treatments. The unidentified nitrogen compounds made up 41% of the total nitrogen in the control silage and 34% of the total nitrogen in the formic acid and limestone treated silages; therefore, varying the extent of fermentation did not greatly affect the silage unidentified nitrogen levels. Average daily gain was essentially identical for cattle fed the three treated silages (.80 kg). Eighty-five percent dry matter consumption for the three silages was control - 7.88 kg, formic acid treated - 7.78 kg and limestone treated - 7.58 kg. The decreased consumption for the limestone treated silage fed group was offset by an improvement in feed efficiency (control - 9.88, formic acid treated - 9.87 and limestone treated - 9.50). Feed cost per 100 kg gain was elevated for the formic acid treated silage fed cattle compared to the other two silages due to the cost of the formic acid. Carcass grade averaged middle to high Choice for all groups of cattle with small differences being significant ( $P < .01$ ). Small differences in marbling (moderate - to slightly abundant) were also significant ( $P < .05$ ) and differences in other carcass traits were not significant. The nitrogen

digested (g/day), nitrogen retained (g/day) and nitrogen retained as a percent of nitrogen intake were significantly higher ( $P < .01$ ) for the control silage compared to the two treated silages. Other metabolic parameters were not significantly different (as shown in Experiment III).

Experiment II was designed to measure the effects of stimulating fermentation with NPN additions on steer performance, nitrogen balance parameters and silage nitrogen and organic acid fractions. Five silage treatments were studied: control untreated silage, Pro-Sil supplemented and treated silages, urea-mineral treated silage and urea-mineral plus formic acid treated silage. The silage treatments were compared on an all silage ration and a 60% corn silage and 40% high moisture shelled corn ration on a dry matter basis. The neutralizing effect of Pro-Sil and urea-mineral without formic acid resulted in stimulated fermentation and bacterial activity, yielding significantly ( $P < .01$ ) greater lactic acid levels (control - 7.75% of DM, Pro-Sil and urea-mineral treatments - 10.79% of DM). The urea-mineral plus formic acid treated silage resulted in significantly ( $P < .01$ ) less lactic acid than the control (5.36% of DM and 7.75% of DM). Total nitrogen was significantly ( $P < .01$ ) increased approximately 50% by NPN additions compared to the control untreated silage. Water insoluble nitrogen levels were significantly ( $P < .01$ ) higher for all NPN treated silages compared to the control; however, unidentified nitrogen levels were not significantly altered by stimulating fermentation with NPN

additions (range of unidentified nitrogen, .52% - .69% of DM). Average daily gains were significantly higher for the NPN treated silages (.88 kg) than for the control silage supplemented with soy-mineral (.80 kg) ( $P < .05$ ) or Pro-Sil (.75 kg) ( $P < .01$ ). Feed consumption varied from 7.57 kg to 8.05 kg on 85% dry matter basis. Feed efficiency followed average daily gain and feed cost favored the NPN treated silages. Carcass grade for all groups of cattle averaged between low and middle Choice; however, the Pro-Sil supplemented silage fed cattle were significantly ( $P < .05$ ) lower in carcass grade and marbling than the control silage fed group. The Pro-Sil supplemented silage fed group had significantly ( $P < .05$ ) less fat thickness than the control and Pro-Sil treated silage fed groups. Fat thickness was also significantly ( $P < .05$ ) lower for the urea-mineral plus formic acid treated silage than the control silage fed cattle. Percent kidney, heart and pelvic fat significantly favored the Pro-Sil supplemented ( $P < .01$ ) and the urea-mineral plus formic acid treated ( $P < .05$ ) silage fed groups, compared to all other treatments. Other carcass traits were not significantly different. There were no significant differences in nitrogen balance parameters in this study (as shown in Experiment IV).

Results of Experiments I and II were summarized across level of silage contained in the ration. Average daily gain was significantly ( $P < .01$ ) higher for the 60% corn silage and 40% shelled corn fed cattle than for the all silage fed

cattle (.92 kg vs. .73 kg). Dry matter intake (8.13 kg vs. 7.40 kg) and feed efficiency (8.84 vs. 10.14) paralleled average daily gain. The all silage ration produced 51% more beef per hectare of corn grown (1730 kg vs. 1149 kg) and, consequently, returns were greater per hectare of corn grown (\$1191 vs. \$803). All cattle graded middle to high Choice; however, cutability significantly ( $P < .01$ ) favored the all silage fed cattle due to less fat thickness.

Experiment V was designed to monitor changes occurring during fermentation in untreated control and Pro-Sil treated corn silage. Samples were taken when fresh and on days 1 through 10, 15, 20, 30, 60 and 90 after ensiling. The pH of the control silage decreased rapidly from 5.72 in fresh material to 4.65 on day one. This was apparently due to the large increase in lactate from 0 to 1.60% of silage dry matter. Most of the initial increase in soluble nitrogen in the control silage (fresh - day one) was due to an increase in unidentified nitrogen which increased from .29% in the fresh material to .43% of dry matter on day one. The majority of the proteolytic activity occurred early in the fermentation process (fresh - day one). The insoluble nitrogen level in the Pro-Sil treated silage increased to a maximum of 1.23% of silage dry matter on day 5 when the soluble nitrogen as a percent on total nitrogen had decreased to a low level. The unidentified nitrogen was slightly higher in the Pro-Sil treated silage than in the control silage. Therefore, the increase in insoluble nitrogen was not due to a reduction in proteolysis and evidence suggests that soluble nitrogen

compounds in corn silage may be incorporated into microbial protein. The lactate content of the Pro-Sil treated silage was higher than in the control silage after day two on all but one sampling time. The pH on day 90 was 4.08 and 4.05 for the Pro-Sil treated and control silages, respectively.

Experiment VI was designed to vary the resulting level of unidentified water soluble nitrogen compounds occurring in fermented silage and to determine the effects of these compounds on silage dry matter intake and metabolic and blood parameters. The four silage treatments utilized to vary the unidentified nitrogen levels were untreated control - 31.68% DM, autoclaved and reinoculated - 32.85% DM, sun dried - 52.13% DM and air dried - 84.51% DM. The unidentified nitrogen ranged from 15.95% to 33.89% of total nitrogen for the air dried and control silages, respectively. Dry matter intake ranged from 63.47 to 68.48 g/kg body weight<sup>.75</sup> and was not significantly affected by altered unidentified nitrogen levels. Nitrogen balance parameters were not significantly different for the four silage treatments. In vitro studies indicated that the unidentified nitrogen was converted to volatile base ( $\text{NH}_3$ ) within 12 hours.

RELATIONSHIP OF SILAGE FERMENTATION  
AND ADDITIVES TO DRY MATTER CONSUMPTION BY RUMINANTS

By

Erskine Hamilton Cash

A THESIS

Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Husbandry

1972

1000

1000

1000

1000

1000

5

1000

1000

C77568

Erskine Hamilton Cash  
candidate for the degree of  
Doctor of Philosophy

DISSERTATION: Relationship of Silage Fermentation and Additives to Dry Matter Consumption by Ruminants

OUTLINE OF STUDIES:

Major Area: Animal Husbandry (Ruminant Nutrition)

Minor Subject: Biochemistry

BIOGRAPHICAL ITEMS:

Born: June 21, 1947; Spottswood, Virginia

Undergraduate Studies: Virginia Polytechnic Institute,  
1965-1969

Graduate Studies: Michigan State University, 1969-1972

EXPERIENCE: Graduate Teaching Assistant, Michigan State  
University, 1969-1972

MEMBER: American Society of Animal Science  
Alpha Zeta  
Phi Kappa Phi  
National Block and Bridle Club



## ACKNOWLEDGMENTS

The author extends his appreciation to Dr. Hugh E. Henderson for his advice, guidance and patient counsel throughout his graduate program. His encouragement and enthusiasm have been greatly appreciated.

Appreciation is also extended to Dr. Werner G. Bergen for his advice and assistance in interpretation of laboratory results. The author is further indebted to the other members of his graduate committee, Dr. J. T. Huber and Dr. Richard W. Luecke, for their advice and participation in the writer's graduate program.

The author also wishes to thank Dr. Ronald H. Nelson for making the facilities of Michigan State University available for this research.

The author extends his appreciation to Dr. William T. Magee for his assistance in the statistical analysis of data and to Mrs. Phyllis A. Whetter for her assistance in laboratory analysis.

Appreciation is also extended to Mrs. Susan B. Steiner for her assistance and typing of this manuscript.

The author is grateful to his wife, Wilhelmina, for her understanding patience and encouragement throughout the author's career. Also, the author is grateful to his parents for their support.

11-11-11

11-11-11

11-11-11

11-11-11

11-11-11

11-11-11

## TABLE OF CONTENTS

	<u>Page</u>
LIST OF TABLES . . . . .	.viii
LIST OF FIGURES . . . . .	xi
I. INTRODUCTION . . . . .	1
II. LITERATURE REVIEW . . . . .	3
Silage Fermentation . . . . .	4
Carbohydrate Breakdown . . . . .	7
Protein Breakdown . . . . .	10
Silage Additives . . . . .	15
Urea . . . . .	16
Limestone . . . . .	20
Pro-Sil . . . . .	23
Formic Acid . . . . .	25
Mineral Acid and the AIV Method . . . . .	30
Other Additives . . . . .	30
Control of Voluntary Intake . . . . .	30
Voluntary Silage Intake . . . . .	38
Summary . . . . .	46
III. MATERIALS AND METHODS . . . . .	47
Experiment I - Steer Feeding Trial 1 . . . . .	47
Design . . . . .	47
Harvesting of Silages . . . . .	47
Feeding Trial . . . . .	50
Silage Analysis . . . . .	50
Experiment II - Steer Feeding Trial 2 . . . . .	52
Design . . . . .	52
Harvesting of Silages . . . . .	52
Feeding Trial . . . . .	55



	<u>Page</u>
Experiment III - Metabolism Study with Corn Silage Varied in Extent of Fermentation .	55
Design . . . . .	55
Feeding Regime . . . . .	56
Sample Collection . . . . .	59
Laboratory Analysis . . . . .	60
Experiment IV - Nitrogen Balance Study with NPN Silage Additives . . . . .	60
Design . . . . .	60
Experiment V - Silage Fermentation Study . . .	62
Design . . . . .	62
Silage Analysis . . . . .	64
Experiment VI - Intake, Metabolic and <u>In</u> <u>Vitro</u> Studies of Corn Silage Containing Varying Levels of Unidentified Soluble Nitrogen	64
Design . . . . .	64
Forage Preparation . . . . .	65
Experimental Silos . . . . .	67
Feeding Regime . . . . .	67
Sample Collection . . . . .	67
Intake Period . . . . .	67
Collection Period . . . . .	69
Laboratory Analysis . . . . .	70
Procedures for <u>In Vitro</u> Fermentation . . . .	71
Statistical Analysis . . . . .	72
IV. RESULTS AND DISCUSSION . . . . .	73
Experiment I - Feeding Trial 1 . . . . .	73
Chemical Analysis of Silage . . . . .	73
Nitrogen Fractions . . . . .	73
Organic Acids . . . . .	75
Effect of Maximizing and Minimizing Silage Fermentation on Performance of Feedlot Cattle . . . . .	77

	<u>Page</u>
Experiment II - Feeding Trial 2 . . . . .	81
Chemical Analyses of Silage Containing NPN Additions . . . . .	81
Nitrogen Fractions . . . . .	82
Organic Acids . . . . .	84
Effect of Additives on Feedlot Performance .	86
All Silage Ration vs. 40% Shelled Corn and 60% Silage Ration . . . . .	91
Experiment III - Metabolism Study with Corn Silage Varied in Extent of Fermentation .	92
Chemical Analysis of Silage . . . . .	92
Rumen Ammonia and Blood Urea Concentrations.	94
Rumen VFA Concentrations . . . . .	94
Dry Matter Intake and Dry Matter Digestibility . . . . .	96
Nitrogen Balance . . . . .	99
Experiment IV - Nitrogen Balance Study with NPN Silage Additives . . . . .	99
Chemical Analysis of Silage . . . . .	99
Dry Matter Intake and Dry Matter Digestibility . . . . .	101
Nitrogen Balance . . . . .	101
Experiment V - Silage Fermentation Study . . .	101
Control Silage Fermentation Parameters . . .	103
Pro-Sil Treated Silage Fermentation Parameters . . . . .	107
Experiment VI - Intake, Metabolic and <u>In</u> <u>Vitro</u> Studies of Corn Silage Containing <u>Varying</u> Levels of Unidentified Soluble Nitrogen . . . . .	112
Pilot Experiment . . . . .	112
Chemical Analysis of Silage . . . . .	116
Dry Matter . . . . .	116
Nitrogen Fractions . . . . .	117
Organic Acids . . . . .	120
<u>Studies In Vivo</u> . . . . .	121
Water Balance Trial . . . . .	121
Dry Matter Intake . . . . .	124

	<u>Page</u>
Dry Matter Digestibility . . . . .	125
Nitrogen Balance . . . . .	127
Rumen Ammonia and Blood Urea . . . . .	127
Rumen pH and VFA Concentrations . . . . .	131
Rumen Dry Matter . . . . .	134
Plasma Amino Acid Analyses . . . . .	134
Studies In Vitro . . . . .	136
Correlation Coefficients . . . . .	137
V. SUMMARY . . . . .	141
BIBLIOGRAPHY . . . . .	147
APPENDIX - Correlation Coefficients . . . . .	166

## LIST OF TABLES

<u>TABLE</u>		<u>Page</u>
1	Silage Treatments Used in Experiment I - Feeding Trial I . . . . .	49
2	Formulation of Soy-Mineral Supplement (45% CPE on DM Basis) . . . . .	49
3	Silage Treatments Used in Experiment II - Feeding Trial II . . . . .	53
4	Formulation of Pro-Sil . . . . .	54
5	Formulation of Urea-Mineral Silage Additive . .	54
6	Experiment III - Metabolic Study Design of Experiment . . . . .	57
7	Experiment III - Metabolic Study Treatments Utilized . . . . .	57
8	Experiment III - Metabolic Study Formulation of Mineral Supplement . . . . .	58
9	Experiment IV - Metabolic Study Treatments Utilized . . . . .	61
10	Experiment IV - Metabolic Study Design of Experiment . . . . .	61
11	Experiment VI - Metabolic Study Design of Experiment . . . . .	68
12	Experiment VI - Metabolic Study Formulation of Mineral and Vitamin Supplement .	68
13	Average Chemical Analysis on Dry Matter Basis of Silages and High Moisture Corn Fed . . . . .	76
14	Corn Silage Additives Compared . . . . .	79
15	Corn Silage Additives Compared . . . . .	80



<u>TABLE</u>		<u>Page</u>
16	Average Chemical Analysis on Dry Matter Basis of Silages and High Moisture Corn Fed . . .	85
17	Corn Silage NPN Additives Compared . . . . .	88
18	Corn Silage NPN Additives Compared . . . . .	90
19	All Silage vs. 40% Shelled Corn and 60% Silage Ration . . . . .	93
20	Mean Rumen Ammonia Values . . . . .	95
21	Mean Blood Urea Values . . . . .	95
22	Mean Rumen Acetate Concentrations . . . . .	97
23	Mean Rumen Propionate Concentrations . . . . .	97
24	Mean Rumen Butyrate Concentration . . . . .	98
25	Effects of Silage Additives on Digestion Parameters . . . . .	100
26	Effects of Silage NPN Additives on Digestion Parameters . . . . .	102
27	Means of Control Silage Fermentation Para- meters by Days . . . . .	105
28	Means of Pro-Sil Treated Silage Fermentation Parameters by Days . . . . .	109
29	Cell Wall Constituents and ADF Nitrogen Values . . . . .	113
30	Pilot Experiment Altering Unidentified Nitrogen Levels . . . . .	115
31	Average Chemical Analysis on Dry Matter Basis of Silages . . . . .	122
32	Cell Wall Constituents and ADF Nitrogen Values . . . . .	123
33	Means for Water Balance Study . . . . .	126
34	Means for Dry Matter Intake and Digestibility .	126
35	Means for Nitrogen Balance Study . . . . .	128
36	Mean Rumen Ammonia Values . . . . .	130

<u>TABLE</u>		<u>Page</u>
37	Mean Blood Urea Values . . . . .	130
38	Mean Rumen Acetate Concentrations . . . . .	132
39	Mean Rumen Propionate Concentrations . . . . .	132
40	Mean Rumen Butyrate Concentrations . . . . .	133
41	Mean Rumen pH . . . . .	133
42	Means for Rumen Dry Matter Determinations . .	135
43	Mean Plasma Amino Acid Concentrations . . . .	135
44	Means of Cellulose Digestibility for <u>In Vitro</u> Studies of Corn Silages . . . . .	138

## LIST OF FIGURES

<u>FIGURE</u>		<u>Page</u>
1	Barnett - General scheme of fatty acid formation in silage . . . . .	9
2	Barnett - Diagram of protein breakdown in silage . . . . .	14
3	Experimental Silo Unit . . . . .	63
4	Changes in water soluble nitrogen and lactic acid concentrations during fermentation . . . . .	111
5	Changes in water soluble nitrogen and volatile bases during <u>In Vitro</u> digestion . .	139
6	Changes in water soluble nitrogen and volatile bases during <u>In Vitro</u> digestion (urea added) . . . . .	149

# I

## INTRODUCTION

Research conducted has demonstrated that no other crop will equal corn silage in energy production per acre of crop fed. However, silage rations have been shown to have a depressing effect on voluntary dry matter intake when compared to the same crop harvested in another manner. Generally, feed efficiency is improved on silage rations, thus partially offsetting the reduction in voluntary dry matter intake.

Assuming that the reduction in dry matter intake of silage was eliminated and feed efficiency was unchanged, it can be readily seen that animal production would be enhanced.

It appears that products or a combination of products produced or activated during fermentation may be responsible for decreased dry matter consumption. Much research has been reported attempting to relate levels of organic acids produced in silage during fermentation to the depression in the dry matter intake. These results have been inconsistent. Little is known relative to the effect of unidentified water soluble nitrogen compounds on dry matter intake.

Therefore, the objectives of this study were:

(1) To compare and evaluate the effects of maximizing and minimizing fermentation without the confounding effect

of ad

reter

ottel

plant

param

and m

the s

trea

and

mid

stil

of added nitrogen on silage proteolysis, metabolic parameters, dry matter consumption and performance of feedlot cattle.

(2) To compare the effects of treating green corn plant material with NPN and minerals on silage fermentation parameters, metabolic parameters and evaluating the protein and mineral adequacy of the treated silages.

(3) To define and compare the changes taking place in the silo during the fermentation process in untreated and treated silages.

(4) To determine the cause of proteolysis in silage and evaluate the effect of experimentally altered levels of unidentified water soluble nitrogen compounds in silage utilizing both in vivo and in vitro systems.

## II

### LITERATURE REVIEW

A review by Coppock and Stone (1968) refers to ancient methods of storing grain and ensiling crops. The practice of storing grain in pits or underground trenches dates back to the Greeks and Egyptians, as pointed out by Jenkins (1884). In 1843, Johnston described and recommended a German method for harvesting green fodder by packing the direct cut material into trenches, which were then covered with boards and earth to facilitate sealing. Miles (1918) gave Reihlen, a German, credit for being the first to preserve the whole corn plant utilizing a silo.

Goffart (1877), of Burtin, France, conducted silage experiments as early as 1852 and was the first to describe in a practical way the important aspects of making corn silage. He stressed the importance of reducing the length of cut from four centimeters to one centimeter and the basic need for air exclusion from the silage mass.

Silage production and preservation has undergone many changes, and many questions have been answered but an abundance of questions remain unanswered.

Today the economic significance of silage as a method of preserving and storing feed crops is recognized in most countries of the world. Since silage is a perishable

fermentation product, storage conditions are critical to minimize loss of nutrients and assure a high quality feedstuff. Many factors affect the establishment and maintenance of an anaerobic atmosphere and the development of sufficient acid to preserve the ensiled material.

There has been an increasing interest in additives to alter fermentation products and correct the nutritional deficiencies of the ensiled feedstuff. An excellent review on silage additives has been written by Owen (1971).

One of the major problems encountered in feeding ensiled material is a reduction in voluntary dry matter consumption as compared to companion forage preserved as hay or other dry forms of storage. Thomas et al. (1961) and McCullough (1962) suggested that the problem is due to compounds formed during the fermentation process. This can explain the increasing interest in additives that alter resulting fermentation products.

The purpose of this review is to evaluate the effect of silage additives on silage fermentation products and the resulting performance of feedlot cattle. Another objective is to consider factors affecting voluntary dry matter consumption by identifying products of silage fermentation that reduce silage dry matter intake.

### Silage Fermentation

It is necessary to have an understanding of the silage fermentation process and the resulting products before one can identify changes in this fermentation process that may



to call

of fax

possib

tion.

1

change

refue

tarbo

prote

sugar

farme

four

prod

baby

bioc

crop

by c

and

too

lac

whi

of

be caused by silage additives. A knowledge of the origin of fermentation products is required to understand their possible relationship with a reduction in dry matter consumption.

Annett and Russell (1907) concluded that the major changes which take place during fermentation are a large reduction in nitrogen free extract (later found to be soluble carbohydrate), an increase in nonprotein nitrogen (due to protein breakdown) and an almost complete disappearance of sugars; however, they found little change in fiber content.

Barnett (1954) summarized the changes occurring during fermentation in crop material ensiled without additives as a four- or possibly five-phase process.

Phase 1. Respiration of the plant cells results in the production of carbon dioxide, the utilization of simple carbohydrates and a flow of water from the mass due to these biochemical processes and the mechanical compression of the crop are accompanied by these events.

Phase 2. Acetic acid is produced in small quantities by organisms of the coliform group. This phase is short and merges into the third phase.

Phase 3. Lactic acid organisms, lactobacilli and streptococci, supported by adequate carbohydrate, initiate a lactic acid fermentation.

Phase 4. The mass reaches a period of quiescence during which the lactic acid accumulation peaks resulting in a pH of less than 4.2.

with

is n

app

the

res

res

mi

sol

for

for

and

res

the

in

es

si

re

co

ti

c

t

The four phases require about three weeks for completion with the first 3 being complete after three days. If there is not a sufficient amount of lactic acid to give a pH of approximately 4.2 or less or if air is allowed to penetrate the mass then the fifth phase may result.

Phase 5. Butyric acid-producing organisms attack both residual soluble carbohydrate and the lactic acid which has been formed. In extreme cases, there may be deamination of amino acids with the formation of higher volatile fatty acids and ammonia as well as decarboxylation leading to the formation of amines and carbon dioxide.

Peterson, Hastings and Fred (1925) noted that after four to five hours, oxygen disappeared and carbon dioxide increased at a rapid rate for about forty-eight hours. They reported large numbers of lactic acid producing bacteria twenty-four to forty-eight hours after ensiling.

Utilizing antiseptics, Russell (1907) demonstrated that the living maize cell and plant enzymes are primary and essential during Phase 1 of the ensiling process. He considered microorganisms secondary and nonessential during Phase 1.

Peterson, Hastings and Fred (1925), utilizing sterilized corn inoculated with bacteria to make silage, demonstrated that plant cell respiration was nonessential. They concluded that bacteria are mainly responsible for the production of acids from sugar and starches.

Kempton (1958) found that less than 0.1% of the bacteria

on the

lactob

ship b

silage

Wisem

teris

lactob

lactob

was d

that

featu

actio

to be

Thas

do r

alco

lati

cont

mat

car

aft

of

bat

on the crop at time of ensiling were capable of growing on lactobacillus selection medium. He also found no relationship between the initial number of bacteria on the fresh silage crop and final silage quality. Kroulik, Burkey and Wiseman (1955) found few organisms on green plants characteristic of those in silage and none were typical of silage lactobacilli.

### Carbohydrate Breakdown

Hunter (1921) proved that the carbohydrate breakdown was due to bacterial action. Watson and Nash (1960) stated that the formation of organic acids is the most striking feature of the silage making process. They considered the action of the microorganisms on carbohydrates of the crop to be primarily responsible for the organic acid formation. These researchers also pointed out that some organic acids do result from respiratory activity which gives rise to alcohol.

Geasler (1970) reported a significant positive correlation between soluble carbohydrate content and organic acid content of corn silage. He also reported that increasing maturity of the corn plant significantly reduced soluble carbohydrate content of a silage sample taken at 12 days after ensiling and significantly reduced lactic acid content of the silage.

Hawkins also reported (1969) a negative correlation between total organic acid production and silage dry matter.

When studying the degradation of carbohydrates, it is

02

73

13

11

10

12

27

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

11

necessary to divide organic acids into volatile and non-volatile acids. The major nonvolatile acid, lactic acid, is present in the largest quantity (Barnett, 1954) and is more important in silage fermentation than the volatile acids (Watson and Nash, 1960). The volatile acids in well-fermented silage include acetate with traces of propionic and butyric acids (Barnett, 1954).

A general scheme of the formation of organic acids in silage was put forth by Barnett (1954) as shown in Figure 1.

The fate of starch during the ensiling process is unclear. Dox and Yoder (1920) reported a 10 percent loss in starch during fermentation and Peterson, Hastings and Fred (1925) obtained a loss of 26 to 29 percent. Other work showed little degradation of starch (Dexter, Huffman and Benne, 1959).

Morgan and Pereira (1962) showed that steam distillates of grass-legume and corn silages contained  $C_2 - C_6$  isobutyric,  $\alpha$  and  $\beta$  methyldehydroxybutyric acids, furfural, phenylacetaldehyde, benzaldehyde, butanone, acetone,  $C_2 - C_6$  aldehydes, 2 and 3 methylbutanal and 2 methylpropanal.

Generally a pH of less than about 4.5 is indicative of a desirable silage fermentation. Kroulik et al. (1955a) found a single pH determination at the end of the storage period to be unreliable as a measure of acid production because he obtained erratic pH values in normal alfalfa silage during storage. Watson and Nash (1960) disagree and report that pH is a satisfactory index of the course of fermentation process.



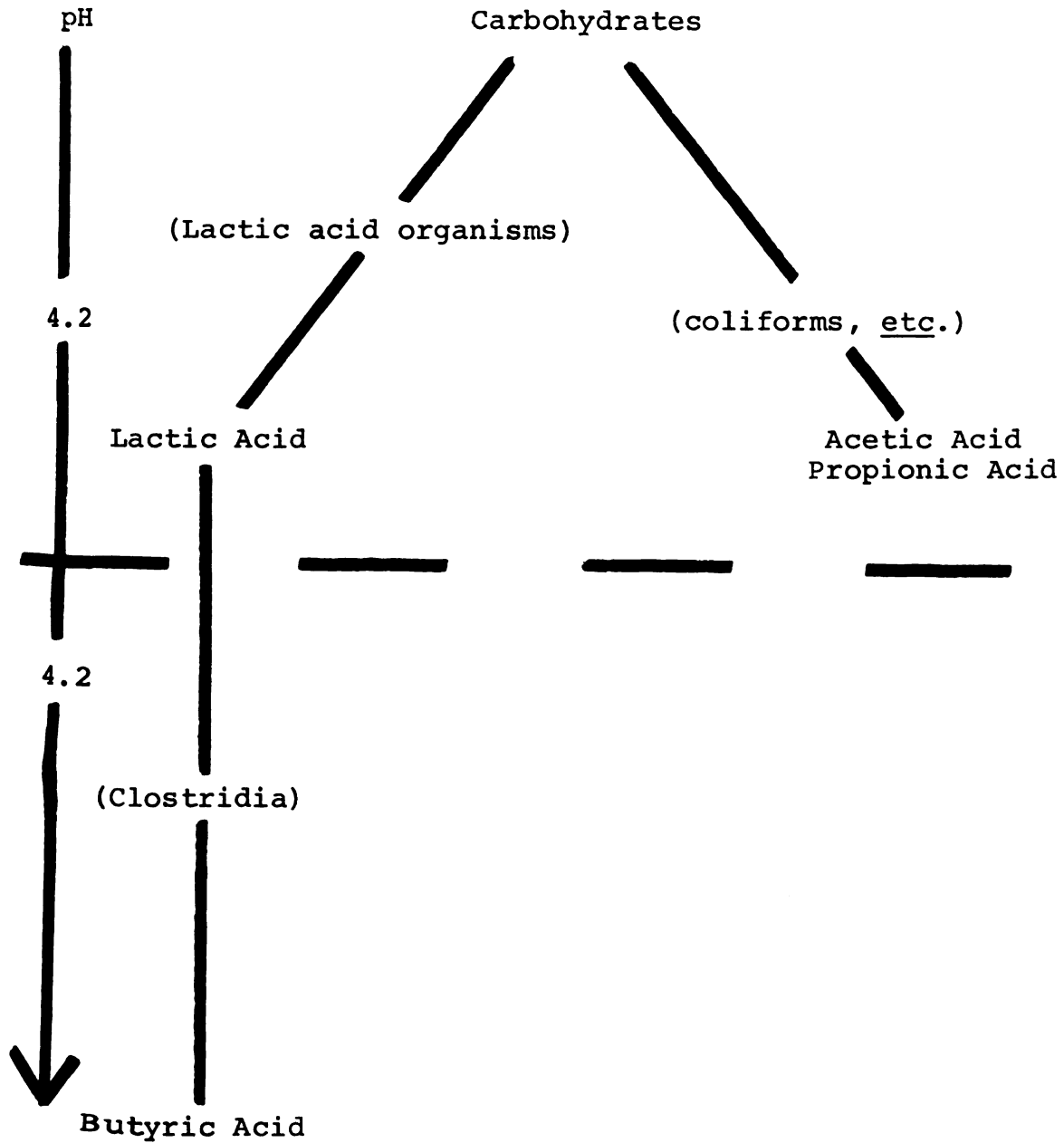


Figure 1. Barnett - General scheme of fatty acid formation in silage.

### Protein Breakdown

The occurrence of proteolysis during the ensiling process has been well established, but its extent varies greatly. Brody (1965) showed that from 18% to 29% of the total nitrogen underwent proteolysis depending upon the dry matter of the ensiled crop. Others (Bentley et al., 1955 and Henderson, et al., 1970a and 1971d) have demonstrated that nearly half of the crude protein of the fresh corn plant was degraded to ammonia and other NPN during fermentation. Ensiling of grass resulted in 65 percent of the total nitrogen appearing as soluble nonprotein nitrogen compared to 20% in the fresh grass (Hughes, 1970a). Hawkins (1969) reported that 54% of the total nitrogen was soluble nonprotein nitrogen in 22% dry matter alfalfa silage. According to Fatianoff et al. (1966), proteolysis during ensiling is generally very extensive and irregular.

Russell (1908), using maize, reported that tryptic enzymes are responsible for the breakdown of protein to amino acids. His work indicates that these changes in protein are functions of living cell protoplasm and plant proteolytic enzymes. The microorganisms may attack the nitrogenous products and carry them beyond the stage reached by the enzymes of the cell (Russell, 1908).

Kirsch (1930) repeated the work of Russell using red clover. He reported protein breakdown did not occur in autoclaved silage, a process that denatures proteins, even after inoculation with lactic acid bacteria. In silage treated with toluene, at levels to inhibit any possible



bacterial activity, degradation was the same as in the control silage. Kirsch (1930) concluded that proteolysis to the stage of amino acids is due to plant enzymes and further changes are not necessarily due to plant enzymes but may be caused by bacteria. Hunter (1921) and Mabbitt (1951) also reported plant enzymes were the cause of proteolysis.

Hughes (1970a) working with grass silage reported the percent of nonprotein nitrogen made up by amino acids, volatile basic nitrogen (ammonia), and nonvolatile amines to be 63.0, 17.2 and 14.4, respectively. This was from silage ensiled for two months. Amino acids decreased to 50.3 percent and ammonia increased to 25.8 percent by 18 months. The water soluble nitrogen fraction in the initial grass contained only 7.3 percent amino acids.

Hughes (1970b) reported that high pH spoiled silage resulted in a loss of amino acids and a proportionate increase in volatile basic nitrogen (ammonia). In overheated perennial ryegrass-cocksfoot silage, the ammonia nitrogen was high with complete destruction of certain amino acids. No putrefaction products appeared in this overheated silage (Hughes, 1970b).

Earlier work at Jealott's Hill reviewed by Watson and Nash (1960) indicated that grassland silage in the pH range of 4.0 to 4.49 contained 21.26 percent of the crude protein as amino acids. Hawkins (1969) reported that  $\alpha$  amino nitrogen represented approximately 20% of the total nitrogen in 22% dry matter alfalfa silage. Amino acid expressed as a percent of total nitrogen decreased as dry matter content increased.

Other research with corn silage (Bergen and Henderson, unpublished) indicates that less than .1 percent of the silage dry matter was recovered as amino acids.

Geasler (1970) reported that total nitrogen (expressed as a percent of dry matter) in corn silage decreased with maturity as did water soluble nitrogen (expressed as a percent of total nitrogen).

Protein degradation during ensiling of alfalfa measured by the increase in water soluble NPN (expressed as a percent of total nitrogen) was increased with decreasing dry matter content (Hawkins, 1969). Comparing 40% and 51% dry matter ryegrass wilted silage, Brady (1965) reported less proteolysis and amino acid metabolism in the 51% dry matter silage.

Proteolysis is very rapid when sufficient moisture is present and the degree of proteolysis is influenced by the time taken in the removal of the moisture (MacPherson, 1952b). Protein degradation in direct cut silage and a slow dried crop is very similar with one major exception. Amides, especially asparagine, accumulate rapidly during wilting but a low level of amides and a high ammonia content is observed in silage (MacPherson, 1952b).

MacPherson (1962) suggested specific bacterial decarboxylases form amines. Gale (1941) demonstrated that bacterial decarboxylases could produce  $\gamma$  amino - n - butyric acid from glutamic acid. Neumark (1962) showed that many plants may contain tyrosine decarboxylase, therefore, the presence of tyramine in silage may be only partially due to

bacterial activity; however, tyramine recovered in corn silage is not present in the corn plant (Neumark, 1961). According to Hughes (1970a), Voss (1966) reported that plant decarboxylases were active during early stages of ensiling and bacterial decarboxylases became active during later stages. Hughes (1970a) reported that observations of Voss (1966) could imply that losses of amino acids were principally due to the action of bacterial rather than plant decarboxylases.

Nonvolatile amines accounted for some 14 percent of the nonprotein nitrogen in grass silage (Hughes, 1970a). Putrescine and cadaverine were the main amines present and histamine formed only a minor portion. The concentrations of cadaverine, tyramine and putrescine were sufficient to account for a high proportion of the losses of the respective parent amino acids while the amounts of ethanolamine,  $\beta$  phenylethylamine, tryptamine and histamine were not.

Watson and Nash (1960) state that, "Protein breakdown to simpler nitrogenous substances is obtained from all types of silage. The degree of proteolysis is variable, and cannot be used by itself as a guide to the quality of the silage since the nature of the breakdown products is also of supreme importance."

Barnett (1954) has a comprehensive diagram of protein breakdown as shown in Figure 2.

Virtanen (1934) showed that protein degradation is almost inhibited below pH 4.0 and that ammonia nitrogen increases with the pH value. MacPherson (1952a) reported the breakdown of protein is extremely rapid but slows down at a pH of

2

12

14



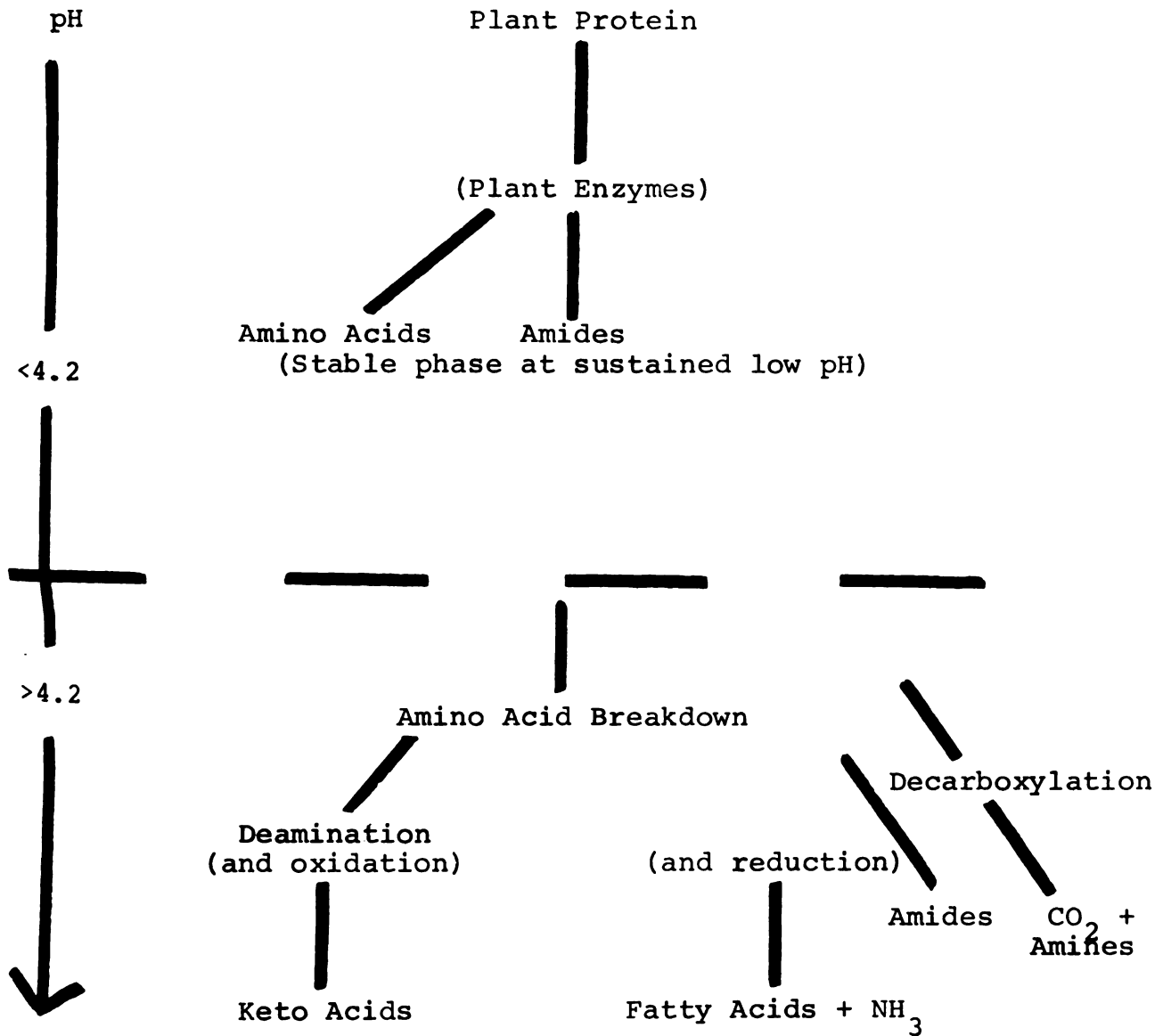


Figure 2. Barnett - Diagram of protein breakdown in silage.



about 5.

Proteolysis appears difficult to control except by treatment with mineral acids. The theory behind the Virtanen (A.I.V.) process of ensiling is that adding mineral acid should immediately decrease the pH, thus preventing protein breakdown without using up the carbohydrates to produce organic acids (Peterson et al., 1936). Virtanen (1934) showed respiration of the plant cells to be suppressed as acidity rose, being only 20 percent of normal at pH 3.5 and ceasing completely at pH 3.0. The A.I.V. process will be discussed in more detail later.

### Silage Additives

In this section attention will be given to additives directed toward controlling the rate and level of endproducts of fermentation and to those designed to improve the nutritive value of corn silage, particularly, by elevating the crude protein content. Major consideration will be given to those that are considered to have the greatest potential and economic significance. Other reviews covering silage additives were written by Watson and Nash (1960), Barnett (1954), Coppock and Stone (1968), and Owen (1971).

Many additives contribute to the nutritive value and directly or indirectly affect the products of fermentation. Legume forages normally contain excess levels of protein, carotene, and calcium. Legume forage nutrient additives are generally high in fermentable carbohydrates. The high carbohydrate containing additives serve as a supplemental

energy source and stimulate lactic acid fermentation which is often inadequate in high moisture legume silages. Grain crop silages are high in available energy and fermentable carbohydrate, but low in protein and minerals. Therefore, the addition of nitrogen and mineral containing additives at time of ensiling has become widely studied and practiced.

### Urea

Urea added to corn silage is hydrolyzed by urease contained in the fresh chopped corn plant resulting in higher levels of ammonia than the control untreated corn silage (Hastings, 1944 and Karr et al., 1965). The later workers reported that 28 to 50 percent of the urea added to corn silage at ensiling was hydrolyzed. Most of the hydrolyzed urea is recovered in the form of ammonia (Huber et al., 1968b; Henderson et al., 1970a; and Lopez et al., 1970). Urea recovery from treated silage has varied from 4% to 84% (Bentley et al., 1955; Hatfield et al., 1966; and Huber et al., 1968b); however, it is generally agreed that about 50% of the urea applied remains as urea for silage in the dry matter range of 28 to 40 percent.

Urea treated silage was found to have a higher pH than untreated silage as reported by Davis (1944) and Cullison (1944). Klosterman et al. (1963b) reported that the buffering capacity of ammonia arising from urea hydrolysis produced a higher pH and increased levels of lactate and acetate in urea treated silage. Formation of ammonium salts resulting from the combination of ammonia and organic acids

produced in the silage has been suggested by Bentley et al. (1955), Henderson, et al. (1969) (1970a), (1971d) and Johnson et al. (1967).

In the rumen the ammonia from ammonium salts is released more slowly than ammonia from urea and this may facilitate rumen microbial growth (Wetterau and Holzchub, 1960 and 1961), as reported by Coppock and Stone (1968). Belasco (1954), utilizing in vitro studies, showed that the nitrogen utilization from ammonium salts is greater than that from urea. Ammonium lactate and ammonium succinate were especially higher than urea. According to Belasco, ammonium salts have a stimulatory effect on nitrogen fixation by rumen micro flora.

Varner et al. (1971) reported no difference in digestibility of dry matter, organic matter, cellulose or crude protein when rations were supplemented with either ammonium salts, urea or soybean meal. Higher rumen ammonia levels were obtained with urea and ammonium salts. Steers fed soybean meal or ammonium salts either in mixtures or separately retained more of their dietary nitrogen daily as compared to steers fed urea. Using feedlot cattle, Varner et al. (1968) found that mixtures of ammonium salts high in ammonium propionate were superior to urea and ammonium acetate, and equal to soybean meal as measured by cattle performance. High levels of acetate in the mixture of ammonium salts resulted in performance that approached or equaled urea.

Allen et al. (1971) compared ammonium acetate, ammonium lactate, urea and soybean meal as sources of supplemental nitrogen for feedlot steers receiving a high concentrate

ration. The ammonium lactate supplemented group gained significantly ( $P < .05$ ) faster than the urea and soybean meal supplemented steers. The ammonium acetate supplemented group gained significantly faster than the urea supplemented group. Feed efficiency followed average daily gain. Allen et al. (1972) observed no significant increase in gain when ammonium salts were compared to soybean meal and urea as sources of supplemental nitrogen for feedlot steers.

It is well documented that urea treated silage results in higher levels of water insoluble protein than the control (Rayetskaya et al., 1964; Johnson et al., 1967; and Henderson et al., 1971d). Rayetskaya et al. (1964) utilizing N<sup>15</sup> labeled urea reported that higher true protein content of urea treated silage is due to both a protein sparing effect and increased bacterial synthesis.

A comparative feeding trial with urea treated corn silage and untreated corn silage utilizing lambs indicated that apparent dry matter digestibility was similar and apparent crude protein digestibility was improved (Bentley, Klosterman and Engle, 1955, and Wetterau, 1959). Bentley et al. (1955) also reported increased in vivo cellulose digestibility for the urea treated silage. Gorb and Lebedinsky, 1960, (as reported by Owens, 1968) found small increases in apparent digestibility of dry matter and crude protein for urea treated silage as compared to control corn silage when fed to lambs.

Schmutz (1966) utilizing wethers reported that urea treated corn silage when compared to urea-limestone treated

silage resulted in a slight depression in digestibility of dry matter, crude protein, and ash, with a significant reduction in crude fiber digestibility at 0.5 percent urea.

Lambs were reported to retain more nitrogen when urea was added at time of ensiling (Karr et al, 1965). Hatfield et al. (1966) concluded that supplemental energy was more effectively utilized when urea was added at ensiling. Henderson and Purser (1968) reported reduced dry matter consumption of beef heifer calves when fed urea additions added to silage at time of feeding versus urea added at ensiling and control supplemented with soybean meal, the latter two were similar. Differences in gain were small for heifers receiving urea treated and urea supplemented silages. They also reported reduced daily gain of beef heifers receiving urea treated silage in comparison to control silage supplemented with soybean meal. Urea reduced feed cost, however. McClure et al. (1972) observed similar daily gain and feed efficiency values for steers fed urea treated and urea supplemented silages. Grain was fed at approximately 1 percent of body weight.

Early work with urea showed reduced ration acceptability but no reduction in milk production (Woodward and Sheperd, 1944, and Wise, 1944). Huber, Thomas and Emery (1968a) found no difference in average production of cows fed urea treated silage as compared to untreated silage. These authors suggest that high heat of fermentation may render nitrogen unavailable since the persistency of lactation was lower for cows on urea treated high dry matter corn silage (44 - 45%).

When corn silage is treated with 0.5 percent urea, a

concurrent reduction in the natural protein content of the concentrate from about 18 to 13 percent did not depress milk yield, whereas yields are reduced without the urea (Huber et al., 1967). Huber et al. (1967) reported that it may be possible to increase the level of urea in silage to 0.85 percent when urea is not present in the grain ration; however, when the ration contained 0.5% urea treated silage and a 1% urea grain mixture, milk yields were depressed (Owen, 1968).

### Limestone

Klosterman et al. (1961a) suggested that the high feeding value of corn silages might be due to their organic acid content. They also found that the amount of acetic and lactic acids in these silages could be markedly increased by the addition of neutralizing material at the time of ensiling.

Treating whole plant corn silage of about 30 percent dry matter with 1 percent high-calcium limestone (36.7% Ca and 0.29% Mg) or 0.5 percent high-calcium limestone and 0.5 percent urea produced increases up to 100 percent in the acetic and lactic acid content (Klosterman et al., 1961b, and Klosterman et al., 1963b). Johnson et al. (1967) confirmed the previous findings with limestone-urea treated silage and also reported that the amount of acids (lactic and acetic) produced, decreased as dry matter content of the silage increased.

In eight experiments utilizing 600 steers and heifers, Klosterman et al. (1963b) reported that the addition of 1 percent high-calcium limestone or 0.5 percent limestone and

0.5 percent urea to either whole plant corn silage or ground ear corn silage increased feed efficiency. Cattle fed the treated silages gained significantly faster in four of the eight experiments.

Immature corn silage (20% dry matter) treated with 1 percent high-calcium limestone was higher in pH and lactate content but resulted in lower intake and rate of gain in growing heifers (Nicholson and Cunningham, 1964).

Harvey et al. (1963) found no difference in feed efficiency or gain of beef calves fed corn silage treated with 0.5% limestone and those fed the control corn silage.

Schmutz (1966), using 28 percent dry matter corn silage, compared silages containing (a) no additive, (b) 0.5 percent urea, (c) 0.75 percent urea, (d) 0.5 percent limestone, (e) 0.5 percent urea plus 0.5 percent limestone, and (f) 0.75 percent urea and 0.5 percent limestone. All rations were isonitrogenous. Significantly higher gains were reported for heifers fed a, b and c as compared to those fed d and f.

Limestone treated corn silage fed to lactating dairy cows has given inconsistent results with respect to intake and milk production. There was no difference in silage dry matter intake or in milk yield of cows fed either untreated silage, silage treated with 0.5 or 1.0% limestone, or untreated silage plus 1 percent limestone added at feeding (Byers, 1964; Huber, 1966; and Schmutz, 1966). Kesler et al. (1964), McCullough et al. (1964) and Simkins (1965a) reported greater dry matter consumption by cows fed untreated silage as compared to the limestone treated silage. McCullough

et al. (1964) also reported a decrease in milk production for cows receiving limestone treated silage.

A summary of urea and limestone treatments prepared by Essig (1968) and reviewed by Owen (1971) reached the following conclusions:

1) Limestone addition between 0.5% and 1.0% increased the total organic acid production especially lactic acid. Limestone additions greater than 0.5% reduced total titratable acidity and increased pH as the level of limestone increased. It appears necessary to limit limestone to 1% or less in order to maintain a pH of 4.5 or less.

2) Limestone generally increased acceptability of the silage or had no effect, and urea addition improved intake when compared with untreated silage fed without added protein. Compared with natural protein supplements; silages treated with high levels of urea were consumed in lower amounts but usually had no effect when a level of no more than 0.5% was included.

3) Average daily gain was not affected by silage treated with 0.5% to 1.0% limestone.

4) The addition of up to 1.0% of limestone generally improved feed efficiency.

5) Limestone additions had no effect on digestibility of organic matter, cellulose or protein of whole plant or ground ear corn silage.

6) Ensiling losses of dry matter were increased by the addition of 0.5% to 1.0% urea to corn at ensiling time. Increased gas production and lactate levels were associated



with these losses. Ammonia levels were elevated six to nine times the amount in untreated silage.

7) A combination of urea and limestone addition produced effects similar to those produced when each was added separately.

8) The combination of urea and limestone addition did not affect gain, but feed efficiency was improved by about 5% over the control.

### Pro-Sil

Pro-Sil is a suspension of ammonia, minerals, and molasses formulated to supply all supplemental protein and minerals needed to make corn silage a balanced ration for feedlot cattle (Henderson et al., 1970a), non-lactating dairy cattle or cows producing below 40 pounds of milk (Huber et al., mimeo D-236).

Henderson et al. (1971e) reported that corn silage treated with Pro-Sil significantly ( $P < .01$ ) increased total nitrogen, water insoluble nitrogen, nonprotein nitrogen, ammonia nitrogen, lactic acid and pH when compared to the control silage. Acetic acid content in Pro-Sil treated silage decreased significantly ( $P < .01$ ). This is in agreement with other work (Beattie et al., 1971).

Beattie et al. (1971) reported that nitrogen fractionization of the Pro-Sil treated silage revealed 21% of the increase in total crude protein was in the form of water insoluble protein, 58% in the form of ammonium salts, and 21% remained as unidentified nitrogen compounds. Approximately

95% of the nitrogen added as Pro-Sil was accounted for by the increased crude protein content of the Pro-Sil treated silage.

Pro-Sil treated corn silage has given results equal to control silage supplemented with soybean meal and urea treated silage for feedlot cattle receiving an all silage ration and a 60% corn silage - 40% shelled corn ration on a dry matter basis (Beattie et al., 1971; Henderson et al., 1971e; and Henderson et al., 1971b). Henderson et al. (1971c) and (1971d) reported no significant difference in daily gains due to nitrogen source when yearling steers and steer calves were fed varying concentrate levels in combination with either Pro-Sil treated silage or control silage supplemented with soybean meal. Little difference in feed consumption was reported for the various protein sources. Feed cost favored the NPN treated silages in all cases.

Pro-Sil treated rye silage fed steers gained faster and more efficiently than steers receiving urea supplemented silage and feed costs were lower for the Pro-Sil silage fed steers (Henderson et al., 1970b).

Higher milk yields, less average change per day and greater milk persistencies have been noted for high producing cows fed Pro-Sil treated silage than those receiving control silage or silage treated with urea or urea plus minerals (Huber et al., 1971a; Huber et al., 1971b; and Huber et al., mimeo D-236). Their data also indicates that Pro-Sil will maintain production even on silage of high dry matter content (40% DM) which is an advantage over higher dry matter corn silage treated with urea. Huber et al. (1968b) reported poor results with



high dry matter, urea treated silage (40% dry matter).

Beattie (1970), using steers, reported no significant difference in apparent dry matter digestibility, nitrogen digestibility or nitrogen retention when comparing control silage supplemented with soybean meal, Pro-Sil treated silage and urea-mineral treated silage. Henderson et al. (1970a) reported dry matter digestibility to be essentially identical in sheep fed Pro-Sil, urea plus minerals and urea alone treated silages. The nitrogen from the ammonia treated silages was used as effectively as nitrogen from urea treated silage and the inclusion of minerals at ensiling appeared to be advantageous to the utilization of silage nitrogen. Lichtenwaler (1971) observed only small differences in dry matter digestibility and nitrogen digestibility with lactating cows fed control, Pro-Sil treated and urea treated silages.

### Formic Acid

Much of the work using formic acid as a silage additive has been done in Norway where weather conditions make it difficult to wilt grasses and legumes to a dry matter level capable of undergoing a desirable fermentation. Also, wilting often results in heat damage which depresses protein digestibility, consequently there was a need for a silage making alternative other than wilting. The addition of formic acid to grass direct cut, high moisture grass and legume silage has markedly influenced fermentation and improved nutritive quality (Saue and Breirem, 1969a). Norwegian experiments demonstrated that formic acid treated silage was equal to

1

artificially dried grass and better than untreated silages or hay when fed in mixed rations for milk production and growth (Saue and Breirem, 1969a and Saue and Breirem, 1969b).

Waldo et al. (1969) conducted experiments with Holstein heifers fed formic acid treated (0.5%) unwilted silage. Daily gain and digestibility of energy of the formic acid treated unwilted silage were increased by 12% and 14%, respectively, when compared to control untreated hay. The crops utilized were orchardgrass and alfalfa. The formic acid treated silages were lower in pH, butyric acid, acetic acid and ammonia nitrogen but higher in lactic acid level when compared to untreated unwilted silages. More rapid gains were made on the formic acid silage from equal digestible energy intake, which indicated greater efficiency of utilization of digestible energy.

Waldo et al. (1971a) compared both direct-cut alfalfa and sudan-sorghum hybrid ensiled untreated or treated with 0.5% of a 90% solution of formic acid. Formic acid treatment reduced silo storage losses of dry matter, energy, nitrogen and sugar. The reduced dry matter loss is consistent with results of Derbyshire et al. (1969). The formic acid treated silage was lower in pH and butyric acid content and similar in acetate level when compared to untreated silage. Although lactate in formic acid treated silages was only about half that in untreated silages; in one experiment, formic acid treated alfalfa silage contained twice as much as its control. Holstein heifers fed formic acid treated silage gained significantly more than those fed untreated silage. The intake of

dry matter or energy was generally greater when the silage was treated with formic acid. Feed efficiency was improved in all experiments (Waldo et al., 1971a).

In two experiments using wilted orchardgrass silage, formic acid treatment increased silage intake and dry matter preservation and reduced ammoniacal nitrogen and pH (Derbyshire et al., 1969 and Derbyshire et al., 1970). The treated silage had a higher lactate content and gave a slight increase in milk yield when compared to the control silage in the second experiment.

Derbyshire et al. (1971) treated orchardgrass and alfalfa-orchardgrass silage with formic acid. Formic acid treated silages were significantly lower in pH, ammoniacal nitrogen as a percent of total nitrogen, acetic acid and lactic acid. When a ratio of 60 : 40 forage to grain was fed, there was a slight reduction in milk yield on the formic acid treated silage; however, the average daily change in fat corrected milk favored formic acid addition. No effect of formic acid on milk yield was noted on a 70 : 30 ratio. These workers also report an increase in daily gain and greater feed efficiency for the treated silage when fed to heifers.

Waldo et al. (1970) compared a 19% dry matter alfalfa silage preserved with formic acid with the same forage wilted to 35% dry matter and untreated. Storage losses were 20% for the direct cut treated forage and only 4% for the wilted untreated silage; however, sugar losses were greater in the wilted untreated material. The low dry matter treated silage was fed to Holstein heifers and resulted in lower digestibility,

similar intakes and greater gains and feed efficiency when compared to the wilted untreated material.

Results of Thomas et al. (1969) indicated that milk yields, intake and digestibility of formic acid treated silage was similar to a control hay ration.

Lessard et al. (1970) found dry matter intake, digestibility and milk fat test were reduced when direct cut, formic acid treated sudan-sorghum silage was fed, but milk yields were maintained slightly better with the treated silage. Wilted, untreated silage resulted in performance similar to the direct cut formic acid treated silage.

Recent work (Fisher et al., 1970) indicated that wilting sudan-sorghum increased dry matter intake when fed to lactating cows. The addition of formic acid to the direct cut material resulted in lower dry matter digestibility, but the efficiency of energy utilization in terms of milk yield and body weight change was improved.

Castle et al. (1970a) and (1970b), using Ayrshire cows, reported increased digestibility, intake and milk production with formic acid treated direct cut grass silage. In the first experiment, lactate levels were increased 2 to 3 times by treating a mixture of direct cut timothy and ryegrass with formic acid. No such increase in lactate occurred in the latter experiment when the formic acid treated silage consisted of meadow fescue and meadowgrass.

There does not seem to be a consistent trend in lactate content in formic acid treated silage. Wilkins and Wilson (1970) reported that lactic acid content of formic acid treated



silage is directly related to the water-soluble carbohydrate level in the plant. Formic acid apparently increased lactic acid concentrations in high moisture grass silages according to Saue et al. (1969a).

Huber (Mimeo D-235) treated 44% dry matter corn silage with formic acid and received an increase in milk yield which he attributed to the increase in silage intake. Formic acid treatment resulted in reduced organic acid levels, NPN concentrations and ammonia levels. A smaller increase in performance was noted with a 28% dry matter silage; however, the organic acid levels, NPN concentrations and ammonia levels were reduced. Huber (Mimeo D-235) suggested that the reduced NPN concentrations in the formic acid treated silage may have been related to higher intakes of the treated silage.

Waldo et al. (1971b) reviewed research relating to formic acid treatment of hay crop silage and concluded that formic acid treated direct cut grass or legume silage will reduce silo storage loss, proteolysis, total organic acid levels, ammoniacal nitrogen, and pH when compared to untreated wilted silages, untreated direct cut silage and hay. Performance traits that are usually improved with formic acid treatment when compared to untreated wilted silage, direct cut silage and hay include: gain, milk production, feed intake, feed efficiency and dry matter digestibility.

Additional work needs to be done before definite conclusions can be drawn concerning formic acid treatment of corn silage.

### Mineral Acid and the AIV Method

Archibald et al. (1960) summarized the effects of acidifying silage additives over a number of silage parameters. Lactic acid levels were higher, but butyric acid and volatile bases were lower compared to control silages. Owen (1971) reported that the corrosive nature of mineral acids is a serious problem which tends to limit their use.

### Other Additives

The use of bacterial cultures, molasses, whey, sterilants and etc. as additives to properly ensiled corn silage appears to be of little or no value. For a review of these additives, see Owen (1971) and Watson and Nash (1960).

### Control of Voluntary Intake

The voluntary intake of feed is often the main factor limiting animal production. Consequently, it is of considerable interest to researchers and has been under investigation for some time. Factors affecting voluntary feed consumption were reviewed by Balch and Campling (1962) and Baile (1968d) and will be of primary concern in this review. Physical limitations of the digestive tract and the physiological processes of ruminant digestion and metabolism will be considered as factors controlling intake. Components of silage that affect consumption will also be considered.

A quantitative similarity between sheep and cattle in relation to their voluntary consumption of roughages of different apparent digestibilities was reported by Blaxter and Wilson (1962). Differences between sheep and cattle in

fermentation, digestion, and utilization of a ration of dried grass and oats were minor (Blaxter and Wainman, 1961a). Therefore, this review will not make reference to the species of ruminant from which the data were obtained.

Balch and Campling (1962) report that voluntary intake of diets consisting mainly of roughages was limited by the capacity of the reticulorumen and by the rate of disappearance of digesta from this organ. The reticulorumen contains approximately 70% of the total gut contents (Coombe and Kay, 1965); therefore, its role in control of intake appears to be significant.

The addition of materials (digesta or chopped roughage) given intra-uminally has been shown to reduce oral intake (Campling and Balch, 1961b and Weston, 1966). Intra-uminal addition of water in adult sheep and cattle did not affect intake since water rapidly leaves the rumen (Campling and Balch, 1961b). Water bladders placed in the rumen decreased intake (Campling and Balch, 1961b).

Pregnancy has been shown to decrease dry matter intake especially during late gestation (Campling, 1966a) and Graham and Williams (1962) observed an increase in passage through the digestive tract as the gestation period progressed.

Hulton (1963) as reported by Campling (1966b) used monozygotic twin cattle and obtained a 47 percent increase in intake with the lactating twins. Taylor (1959) observed an inverse relationship between internal fat and fill weight in steers.

Differences in physiological volume of the reticulorumen

in sheep was reported to affect voluntary intake on an individual basis (Purser and Mois, 1966). Another factor responsible for differences between animals in intake is variation of retention time between animals (Campling et al., 1961a). Differences in retention time may be due to variation in the efficiency of chewing during eating and ruminating or in the amount of movement of the digestive tract or both.

The rate of disappearance of digesta depends on its rate of breakdown in the reticulorumen by microbial and mechanical processes (Campling, 1969). The rate of outflow through the reticulo-omasal orifice is dependent upon a reduction in particle size and possibly particle density (Montgomery and Baumgardt, 1965a).

Campling and Freer (1962a) found the mean retention time of particles is inversely related to their specific gravity within the range 1.02 to 1.21 g/cm<sup>3</sup> and directly related to particle size within a range of 4.8 to 3.2 mm in diameter. King and Moore (1957) reported particles of approximately 1.2g/cm<sup>3</sup> in density and 20 to 30 x 10<sup>-3</sup> cm<sup>3</sup> in size resulted in maximum rate of passage. Pearce and Moore (1964) observed an increased retention time of particles in the rumen due to restricting rumination.

Digestibility affects the rate of breakdown to small particle size. Conrad et al. (1964) demonstrated a positive relationship between dry matter digestibility and voluntary intake in predominantly roughage diets ranging in digestibility from 52 percent to 80 percent; however, above 65 percent, intake decreased with increasing digestibility of the

diet. When cell wall constituents compose 50 to 60 percent of the forage dry matter, they appear to limit intake (Van Soest, 1965).

Alterations in the physical form of roughages has gained popularity as a method of reducing particle size thus reducing retention time in the digestive tract and thereby increasing voluntary intake. Three experiments conducted with dried grass, hay and oat straw fed as such or after grinding and pelleting resulted in an appreciable increase (26%) in voluntary intake of straw only (Campling et al., 1963).

Grinding a roughage may increase voluntary dry matter intake up to 50% (Weston and Hogan, 1967b), increase daily gain up to 100% (Beardsley, 1964), increase the rate of passage, decrease the retention time (Keith et al., 1961), and decrease dry matter digestibility (Rodrigue and Allen, 1960). The inclusion of grain in the diet will remove the effect on dry matter digestibility due to modification of physical form of the roughage (Johnson et al., 1964).

Passage through the intestines is rapid compared to the rumen (Castle, 1956a and 1956b). Campling (1969) reports that fill in the abomasum and intestines seems unlikely to restrict intake of long roughages; however, ground and pelleted hay may reduce voluntary intake. On pelleted hay, large amounts of digesta in the abomasum and intestines inhibit the flow of digesta from the reticulorumen (Campling et al., 1966b and Campling et al., 1963).

In order to account for intake differences due to

physical form of diets and thus to refine the relationship between ration nutritive value and intake, Baumgardt (1970) proposed including density in addition to energy measures in the description of ration nutritive value. The term caloric density has unit of kcal/ml.

Palatability is often considered to be a factor influencing intake; however, it is difficult to assess. Greenhalgh and Reid (1967) equalized dry matter digestibility and showed a significant difference in intake which they concluded was due to palatability. Their results indicate that palatability and digestibility are of approximately equal magnitude in influencing voluntary dry matter intake.

An important factor in the control of voluntary intake that is often overlooked is the nitrogen status of the animal. Egan (1965b) increased dry matter intake of a low nitrogen chaffed oaten hay by 42% and 12% with duodenal infusions of casein and urea, respectively. Egan (1965a) reported elevated blood urea levels, ruminal ammonia levels and increased cellulose digestion from duodenal infusion of casein when a low protein cereal hay was fed. Weston (1967a) increased voluntary feed consumption of a wheaten hay by infusing protein abomasally or increasing the crude protein content of the diet to 7% or 15% with gluten.

Henderson et al. (1971f) and Beattie et al. (1971) using corn silage rations not supplemented with protein for negative control rations received significantly lower average daily gains by steers on these rations due to reduced daily dry matter consumption, when compared to protein

supplemented rations.

There is evidence that ruminants will eat to a constant dry matter rumen fill (Blaxter et al., 1961b; Ulliyatt et al., 1967; and Freer et al., 1963). Other work suggests that on some diets ruminants do not eat to a constant fill (Campling et al., 1961b and Montgomery et al., 1965a). Waldo et al. (1965) and Campling (1966b) found a higher percent of dry matter in ruminal digesta in the animals fed hay than silage.

The inclusion of concentrate in roughage rations has been shown to increase the dry matter digestibility (Montgomery and Baumgardt, 1965b and Bloom et al., 1957) and decrease cellulose digestibility (Montgomery and Baumgardt, 1965b and Conrad et al., 1963). Increased retention time for the roughage portion of a concentrate roughage ration was reported by Montgomery and Baumgardt (1965b) and Eng et al. (1964). Utilizing isonitrogenous rations varying in concentrate to roughage ratios, Cowser and Montgomery (1969) reported increased apparent digestibility of dry matter, crude protein and energy. Dry matter intake decreased as the percent of concentrate in the ration increased.

Montgomery and Baumgardt (1965a) reported that the voluntary intake of sheep fed completely ground and pelleted mixtures of different proportions of lucerne and maize declined with increasing digestibility so that with each diet the voluntary intake of digestible energy was about the same. Therefore, they hypothesized that ruminants adjust voluntary food intake according to the physiological demand for energy if fill or rumen load does not limit their

consumption. Campling et al. (1962b) and Hemsley et al. (1963) observed increased cellulolytic activity of the rumen microflora, faster disappearance and a resulting increase in intake due to the addition of urea or protein to a low protein roughage ration.

Baile and Pfander (1964) suggested that with highly digestible diets distension of the gut was not important in controlling voluntary intake. Perhaps some products of digestion limited the intake of the more digestible diets by ruminants.

Mayer (1955), now refuted, postulated that the blood glucose concentration was a factor controlling intake of food in non-ruminants. The theory of the glucostatic control was based on chemoreceptors in the hypothalamus to monitor blood glucose levels. In ruminants, peripheral blood glucose concentration is low and infusions of glucose have not generally affected voluntary intake (Manning et al., 1959; Holder, 1963; Simkins et al., 1965b; and Baile and Mayer, 1968c).

Manning et al. (1959) suggested that blood acetate concentration in ruminants may act in a similar way to that proposed by Mayer for glucose in non-ruminants. Acetic acid is produced in large amounts in the reticulorumen and is absorbed; it is the only fatty acid normally found in significant amounts in peripheral blood and the concentration changes with time after feeding (Balch and Campling, 1962, and Campling, 1966b).

Intravenous infusion of sodium acetate, acetic acid



and propionic acid depressed intake in cattle (Dowden and Jacobson, 1960). Glucose, butyrate, valerate, hexanoic acid and lactate intravenous infusions did not alter intake. Holder (1963) reported that intravenous infusions of either glucose or acetate did not affect intake in sheep.

Intraruminal infusions of acetate have been shown to decrease intake (Simkins et al., 1965b; Weston, 1966; Rook et al., 1963; and Baile et al., 1965). Montgomery et al. (1963) observed a greater effect due to acetate than propionate or butyrate.

The regulation of intake by volatile fatty acids appears to be in the rumen since no depression was noted in duodenal infusions of propionate (Egan and Moir, 1965c) or abomasal infusions of acetate (Baile and Mayer, 1967c). Acetate receptors are more likely to be located on the lumen side of the reticulorumen than in an area where they respond to blood acetate since intraruminal infusions of acetate depress intake and intravenous infusions do not (Baile and Mayer, 1968b). These authors stated that the feed intake depression following an acetate infusion is related to satiety.

The rumen pH is influenced by the buffering action of saliva; however, no consistent effect upon voluntary feed intake has been shown due to dietary buffers (Bhattacharya and Warner, 1968; Kromann and Mayer, 1966; and Huber et al., 1969).

The extra heat realized during the assimilation of food designated as heat increment or specific dynamic effect

may be another controlling mechanism of food intake. Brobeck (1948) and (1955) proposed that animals may eat in response to a fall in heat production to keep warm and stop eating when heat production rises to prevent hyperthermia. Work with ruminants has given support to this theory (Balch and Campling, 1962; Simkins et al., 1965b; and Conrad, 1966). Other researchers have disputed the theory (Baile et al., 1967c and Baile and Mayer, 1968a).

There are many changes that occur within the body that directly or indirectly affect centers within the hypothalamus which in turn control eating behavior (Brobeck, 1955). Baile et al. (1967c and 1968d) reported that lesions in the ventromedial area of the hypothalamus produced sustained hyperphagia and subsequent rapid weight gain in ruminants.

This review has discussed some of the factors that may act as signals to the hypothalamic centers which include distension of the digestive tract, changes in the concentration of metabolites in the blood resulting from digestion, and a rise in heat production, etc. Balch and Campling (1962) reviewed these and other factors affecting intake of feed by ruminants. More research is needed in the area concerning the effect on intake of signals to the central nervous system.

### Voluntary Silage Intake

Conrad et al. (1964) concluded that factors affecting a ration low in digestibility (52% to 66%) were such things as body weight, reflecting roughage capacity, and undigested

residue per unit of body weight per day, reflecting rate of passage. Production of the animal, digestibility of the ration and metabolic body size are factors affecting intake of highly digestible rations (67% to 80%). Since corn silage falls in the high digestibility range, factors other than capacity for consumption must be explored.

Balch and Campling (1962) reported that intake of a wide range of dried forages was limited by bulk of ingesta within the rumen; however, the quantity of dry matter within the rumen was less for animals fed silage ad libitum than for those fed hay ad libitum (Thomas et al., 1961 and Waldo et al., 1965). Campling (1966c) found no relationship between the digestibility and the intakes of silages. It appears unlikely that bulk factors limit intake of all silages.

Ensiling has received widespread acceptance as an excellent method of forage preservation; however, the ensiling process was shown to reduce the feeding value of the corn plant primarily by decreasing the voluntary intake by growing dairy heifers (Noller et al., 1963). They reported average dry matter intakes of 2.39 and 1.71 kg per 100 kg body weight for green plant material and silage, respectively. Increases in consumption of dry matter as the plant matured were observed for both the green and the ensiled plant. Dinus et al. (1968) utilizing Holstein and Red Danish heifers, reported average dry matter intakes of 1.99 and 1.78 kg per 100 kg for green chop and the corn plant material after it had been ensiled.

It is well documented that voluntary intake of silage

1

E

(

S

V

A

S

S

a

l

r

r

r

r

r

r

r

r

r

r

r

r

r

r

r

r

r

r

Si

by ruminants is lower than that of hay made from the same crop (Moore et al., 1960; Hawkins et al., 1970; and Gordon et al., 1961). Mackenzie (1967) in a review article concluded that, when milk production or weight gains were compared, or both, in ruminants fed silage or hay made from the same plant material, slightly smaller dry matter intakes of silage were offset by higher production per unit of dry matter.

Huber et al. (1965) utilizing corn silage of 25, 30 and 33 percent dry matter reported dry matter intakes of 1.95, 2.13 and 2.31 kg per 100 kg of body weight.

Johnson and McClure (1968) and Klosterman et al. (1963a) reported increased dry matter intake with increasing dry matter of the silage. Feed efficiency was poorer on the more mature silage (Klosterman, 1963).

Henderson et al. (1971a) compared the performance of steers full fed all corn silage rations consisting of 35 and 46 percent dry matter corn silage and observed increased dry matter intake and poorer feed efficiency on the higher dry matter material.

Gordon et al. (1966) and Byers and Ormiston (1966) reported no significant difference in voluntary dry matter intakes of lactating cows fed corn silage between 27.6 percent and 55 percent dry matter. The effect of silage dry matter and consumption was confounded by grain additions.

Johnston and Cook (1970) reported a significant correlation of 0.65 between corn silage dry matter and dry matter intake. Hawkins (1969) using alfalfa reported a significant correlation of 0.45.

Moisture level of silage or forage does not appear to be responsible for the association between voluntary intake and silage dry matter. Water additions to the rumen (Camp-ling and Balch, 1961b) and increasing the moisture level of alfalfa hay to 65 percent (Mahapatro and Leffel, 1964) produced no consistent effect on dry matter intake. Others (Hillman et al., 1958; Hillman, 1959; and Thomas et al., 1961) have changed the dry matter of hay and silage and received no effect on dry matter consumption. Thomas et al. (1961) concluded that the differences in dry matter intake were due to products of fermentation.

The products of silage fermentation resulting from carbohydrate and protein degradation were reviewed earlier in this review. Possible relationships of some of these products and voluntary dry matter intake will be considered here.

Many researchers have attempted to relate volatile fatty acids present in the silage to a reduction in consumption. Dinius et al. (1968) added acetic acid at levels from 0 percent to 6 percent on a dry matter basis to green chop and corn silage. Acetic acid did reduce dry matter intake, but no significant effect on caloric intake was observed.

Wilkins et al. (1971) reported a negative correlation between the acetic acid content of 70 grass and legume silages and the voluntary consumption by sheep. McDonald and Whittenbury (1967) reported that acetic acid is formed in silage mainly by heterolactic fermentation of sugars and organic acids and later through the breakdown of amino acids during

secondary anaerobic fermentation as reported by Hutchinson et al. (1971). Wilkins et al. (1971) reported a positive correlation between acetic acid and ammonia content. It is possible that the low intake was due to low fermentation quality which was characterized by high levels of acetic acid resulting from degradation of amino acids.

Hutchinson et al. (1971) added acetic acid to ryegrass silage at two levels above that in the control silage. Silage pH and dry matter were held constant using potassium hydroxide and water. The percent acetate in the three silages was 2.0%, 5.0% and 8.8% of silage dry matter. When the three silages were fed to sheep, intake over a 24-hour period was unaffected. These researchers also reported that free acetic acid infused into the rumen reduced silage intake; however, the infused level of acetate was similar to the quantity of acetate consumed by the sheep fed the silage containing the high level of acetate.

Senel and Owen (1967) observed no reduction in voluntary intake when 2 percent acetate, 1 percent butyrate, or a combination of these acids were added to a hay-concentrate ration. However, a mixture of 4 percent acetate and 2 percent butyrate appeared to cause nasal irritation and reduced intake.

Later work by Senel and Owen (1966) using sorghum silage showed increased dry matter intake when acetate was added at levels up to 2.8% of the ration dry matter. Lactate addition to sorghum silage at a low level (5.90% DM) decreased intake but at the high level (9.03% DM) intake

higher than the control sorghum silage. Acetate and lactate improved feed efficiency, when compared to the control. They concluded that something other than acetate and lactate was depressing the consumption of silage.

Allen et al. (1971) observed no reduction in dry matter intake when acetic or lactic acid was added to a ration consisting of 75 percent concentrate and 25 percent corn silage. Feed efficiency values were similar to the control.

Emery et al. (1961) reported that lactic acid addition to corn silage reduced appetite in proportion to its concentration when fed to growing heifers. Corn silage and concentrate were fed to appetite. Feed efficiency increased in direct proportion to the lactic acid intake. This work indicates that the increased efficiency will in some cases more than compensate for the depressed feed intake.

Johnson et al. (1962) observed that steers fed a purification supplemented with lactic acid salivated abnormally; this appeared to be an attempt to neutralize the acid with the bicarbonate of the saliva.

There are conflicting opinions regarding the effect of lactic acid content of grass silage on dry matter consumption. McLeod et al. (1970) reported that sodium bicarbonate addition to grass silage increased the pH from about 4.0 to 5.4 and resulted in significant increases in dry matter intake ranging from 9.7 to 20.7 percent. The addition of equal levels of lactate (77.3% L(+) lactate and 22.7% D(-) lactate) to stepwise reduce the pH of a silage from 5.4 to



resulted in a maximum decrease in dry matter intake of percent. Reductions in dry matter intake were proportional to the amount of lactic acid added and the resulting decrease in pH. Other researchers (Harris et al., 1966; , 1943; and McCarrick et al., 1966) attribute the reduced intake of grass silages to the content of organic acids present.

Wilkins et al. (1971) examined seventy grass and legume silages and found a positive correlation between voluntary intake and lactic acid as a percentage of total acids.

Klosterman et al. (1960) calculated that one pound of lactic acid would replace 2.8 pounds of ration.

The type of lactic acid used to study the effect of lactate on dry matter consumption must be evaluated. Dunlop and Hammond (1965) reported the L(+) isomer is more readily metabolized in the rumen and liver than the D(-) isomer. Lactic acid in corn silage consists of both the D and L forms; however, the D(-) isomer is more abundant (Schaadt and Johnson, 1968).

Ternouth (1967) accounted for approximately 60 percent of the variation in voluntary feed intake due to changes in the osmolality of the rumen liquor. He altered the osmolality of the rumen contents prior to feeding by adding glucose or the sodium salts of the various volatile fatty acids.

He concluded that the reduction of voluntary feed consumption when silage rations are fed may be due to an increase in rumen osmolality resulting from the consumption of pre-fermented feed.

Others (Baile and Pfander, 1966 and Bergen, 1972) suggest that rumen osmolality per se is not an important factor in the control of feed intake by sheep. Bergen (1972) reported ruminal osmolality elevated above 400 m Osm/kg resulted in decreased feed intake in sheep. This study also indicated that ruminal osmolalities seldom reach this level on a high roughage or alfalfa silage ration.

The relationship of reduced dry matter consumption and osmolytic products will be considered now.

Neumark (1964) suggested that histamine may limit the intake of silage by ruminants since amines have been shown to have pharmacological effects in nonruminants (Neumark, 1964). In overfed sheep, Dain et al. (1955) identified histamine and tyramine as toxic constituents in the rumen. Neumark (1964) established a correlation between tryptamine content and silage palatability; however, none of the amines tested were found to directly affect appetite. Histamine enhanced the appetite depressing effect of formaldehyde. McDonald et al. (1963) found that the addition of tyramine to silage did not affect its level of consumption. Moto et al. (1964) and Wrenn et al. (1964) confirmed the findings of McDonald et al. (1963).

Amphetamine has received consideration since it is known to decrease food intake in monogastrics. Intraruminal administration of d-amphetamine sulfate had no effect on dry matter consumption of sheep and goats (Baumgardt, 1967). He observed a significant reduction in dry matter intake with

intravenous injection of dl-amphetamine phosphate. Bhattacharya and Warner (1967) observed similar effects with subcutaneous injections of an aqueous solution of amphetamine.

Attempts to link amines to the control of dry matter intake have been inconsistent. Further research in this area is needed to clarify the relationship of amines to the voluntary feed intake control by ruminants.

### Summary

The explanation of reduced voluntary dry matter consumption by ruminants fed silage compared to the same harvested in another manner continues to elude researchers. Perhaps a combination of products of fermentation is responsible for the depression in silage dry matter intake. Many silage additives have been utilized to improve the nutritive value of the silage and directly or indirectly alter the products of fermentation (mainly lactate and acetate); however, dry matter intake has not consistently been affected.

Most of the research completed has attempted to relate acid concentrations in silage to reduced dry matter intake; however, results have been variable.

Few attempts have been made to relate the form of the silage nitrogen or unidentified water soluble nitrogen compounds in corn silage to the depression in silage voluntary dry matter intake.

### III

#### MATERIALS AND METHODS

Six experiments -- two feeding trials; two metabolic studies; a silage fermentation study; and an intake, metabolic and in vitro study combined -- are included in this dissertation. All silages for all experiments, unless otherwise stated, were characterized by the methods described in Experiment I.

#### Experiment I - Steer Feeding Trial 1

##### Design

A 3 x 2 factorial design was utilized to compare the effects of maximizing and minimizing fermentation without the confounding effect of added nitrogen on silage proteolysis, dry matter consumption and performance of feedlot cattle.

The silage treatments shown in Table 1 were compared on an all corn silage ration and a 60% corn silage and 40% high moisture shelled corn ration on a dry matter basis.

##### Harvesting of Silages

All silages were harvested between August 26 and September 23, 1970, from a stand of hybrid corn averaging approximately 35 metric tons of 35% dry matter (DM) silage or 5 metric tons of shelled corn per hectare. All silage was stored in concrete silos fitted with metal roofs and top unloaders.

Control silage, which received no additive, was harvested over a two week period and stored in a 9.1 m x 18.3 m silo and averaged 36.7% DM at harvest.

Silage treated with 1.5% (of silage DM) formic acid was harvested over a two day period and stored in a 4.9 m x 15.2 m silo and averaged 34.1% DM.

Silage treated with 13.6 kg of limestone per metric ton of 35% dry matter silage was harvested over a two day period and stored in a 3.7 m x 15.2 m silo, and averaged 36.4% DM.

Limestone was applied by evenly spreading the required amount over the top of each self-unloading wagon load of silage prior to blowing into the silo.

Formic acid was applied by pumping the required amount of acid material directly into the blower housing as each load of silage was blown into the silo.

The average chemical composition of the corn plant material for each silo during harvest and feeding is shown in Table 1.

All silages were sampled every Monday, Wednesday and Friday during the feeding experiment for a dry matter determination. A composite sample of each silage was analyzed every two weeks during the feeding trial for nitrogen and organic acid fractions.

All silages were supplemented at feeding time with soy-mineral supplement (Table 2) (45% CPE on DM basis) were mixed prior to feeding at a ratio of 4.5 kg of supplement per 100 kg of silage on a 35% DM basis.

All rations were calculated to be isonitrogenous at 13% crude protein on a dry matter basis.

Table 1

## Silage Treatments Used in Experiment I - Feeding Trial

of Silage <sup>1</sup>	Additive Added at Ensiling kg/MT <sup>2</sup>	Supp. Added at Feeding kg/MT <sup>2</sup>
Control + soy-mineral <sup>3</sup> added at feeding		45.0
Formic acid treated + soy-mineral added at feeding	5.3	45.0
Limestone treated + soy-mineral added at feeding	13.6	45.0

three treatments were compared on all silage rations  
60% corn silage and 40% high moisture corn on a dry  
matter basis.

grams of supplement or additive added per metric ton  
) of 35% DM silage.

Table 2 for formulation of soy-mineral supplement.

Table 2

Formulation of Soy-Mineral Supplement  
(45% CPE on DM Basis)

Ingredient	Percent of Mixture
Calcium phosphate (20% Ca - 18.5% P)	3.45
Sulfur sulfate (22.5% S)	3.07
Trace mineral salt (high Zn)	3.45
Wheat meal (50% CPE)	90.04
TOTAL	100.00%

### Feeding Trial

Choice Angus steer calves, averaging 229 kg, when purchased in September, 1970 were used in this trial. They were acclimated on a full feed of regular corn silage, hay and one pound of 50% soybean meal per head daily until placed on experiment October 22, 1970. They were weighed on two consecutive days and the average of the two weights was used as the initial and final weight. The steers were assigned to blocks on the basis of the first-day weight and randomly assigned to the one of the six treatments following the second-day weight. Steers were terminated from the experiment when they reached Choice slaughter grade. Therefore, the number of days on feed varied as did the final weights. While on experiment, all lots of steers were fed ad libitum twice daily, and water was available at all times.

Immediately following the final individual weight, all cattle were trucked 100 miles for slaughter. They were killed upon arrival or early the next morning. After 48 hours in the cooler, carcasses were ribbed, graded by a federal grader, and carcass measurements taken. Kidney, heart and pelvic fat was estimated by the federal grader and fat and lean tracings were made of the 13th rib for accuracy in determining cutability grade, fat thickness and rib eye area.

### Silage Analysis

Composite samples of moist silage were analyzed for nitrogen and organic acid fractions and expressed as a per cent of dry matter.

Total nitrogen was determined by macro-Kjeldahl procedure and percent dry matter determined by oven drying for 24 hours at 55° C.

Silage extracts were prepared by homogenizing a 25 g portion of the sample in a Sorvall Omni-mixer with 100 ml of distilled water for two minutes and straining through two layers of cheesecloth. The pH of the homogenate was determined by using a Corning Model 12 pH meter.

A 27 ml sample of the extract was deproteinized using 1 ml of 50% sulfosalicylic acid (SSA) per nine ml of extract. The sample was then centrifuged at 18,000 rpm for 10 minutes and stored in a refrigerator for later analysis.

Water soluble nonprotein nitrogen was determined by macro-Kjeldahl procedures using the deproteinized homogenate.

Ammonia nitrogen in the water soluble nonprotein fraction was determined by the method of Conway (1950). Volatile

fatty acid content of the silage was determined by injecting 0.5 ml of the deproteinized silage homogenate into a

gas chromatograph. The column packing used was

Porosorb 101 with a 1.98 m x 0.05 cm teflon column. Carrier gas flow rate was 40 ml per minute N<sub>2</sub> and column oven temperature

was 188° C. The peak areas were converted to

micrograms per 100 ml by comparing with standard solutions

of volatile fatty acid analyzed at the same time. Color-

imetric procedures of Barker and Summerson (1941) were used

to determine lactic acid content of the deproteinized sample.



## Experiment II - Steer Feeding Trial 2

Design

A 5 x 2 factorial design was utilized to compare Pro-Sil and urea-mineral for stimulating fermentation and correcting protein and mineral deficiencies of corn silage when fed to feedlot cattle. These NPN treated corn silages were compared with corn silage not treated but supplemented with soybean meal at feeding time.

All five treatments shown in Table 3 were compared at the two levels of concentrate feeding used in Experiment I.

Harvesting of Silages

Silage yield per hectare, storage facilities and harvest dates were identical to those described in Experiment I.

Control silage, which received no additive, was harvested during a two week period and stored in a 9.1 m x 3.3 m silo and averaged 36.7% DM at harvest.

Silage treated with 22.5 kg of Pro-Sil (Table 4) per metric ton of 35% DM silage was harvested over a two day period, stored in three 4.9 m x 15.2 m silos, and averaged 36.5% DM.

Silage treated with 20.6 kg of urea-mineral (Table 5) per metric ton of 35% DM silage was harvested over a two day period, stored in a 4.9 m x 15.2 m silo, and averaged 36.1% DM during harvest.

Silage treated with urea-mineral and formic acid was harvested over a two day period, stored in a 3.7 m x 15.2 m silo,

Table 3

Silage Treatments Used in Experiment II - Feeding Trial

Type of Silage	Additive Added at Ensiling kg/MT <sup>2</sup>	Supp. Added at Feeding kg/MT <sup>2</sup>
Control + soy-mineral <sup>3</sup> added at feeding		45.0
Pro-Sil treated <sup>4</sup>	22.5	
Control + Pro-Sil added at feeding		22.5
Urea-mineral treated <sup>5</sup>	20.6	
Urea-mineral + formic acid treated	20.6 + 5.3	

Five treatments were compared on all silage rations  
of 60% corn silage and 40% high moisture corn on a dry  
matter basis.

Grams of supplement or additive added per metric ton  
(T) of 35% DM silage.

See Table 2 for formulation of soy-mineral supplement.

See Table 4 for formulation of Pro-Sil.

See Table 5 for formulation of urea-mineral supplement.

Table 4

Formulation of Pro-Sil<sup>1</sup>

Ingredient	Percent
Pro-Sil	16.54
Water and inert ingredients	61.15
Protein	13.60
Calcium	.7936
Phosphorus	.4850
Copper	2.0450
Strontine	3.8420
Fur	.9371
Magnesium	.4886
Carbon	.0597
Iron	.0088
Salt	.0002
Trace	.0530
TOTAL	100.00%

Pro-Sil applied for by Michigan State University.

Table 5

## Formulation of Urea-Mineral Silage Additive

Ingredient	Percent of Mixture
High grade urea (45% N)	30.35
Calcium phosphate (20% Ca - 18.5% P)	6.80
Copper sulfate (22.5% S)	6.05
Trace mineral salt (high Zn)	6.80
Ground shelled corn	50.00
TOTAL	100.00%

and averaged 36.8% DM. Both additives were added at levels described previously.

Control silage was supplemented at feeding time and mixed in a horizontal mixer with an equivalent of 22.5 kg of Pro-Sil per metric ton of 35% DM silage.

Urea-mineral was applied by evenly spreading the required amount over the top of each self-unloading wagon load of silage just prior to blowing into the silo.

The average chemical composition of the corn plant material for each silo during harvest and feeding is shown in Table 16. All silages were sampled for dry matter determination and lab analysis as described in Experiment I.

Control silage supplemented at feeding time with soy-mineral supplement (Table 2) (45% CPE on DM basis) was mixed prior to each feeding at a ratio of 4.5 kg of supplement per 100 kg of silage on a 35% DM basis.

All rations were calculated to be isonitrogenous at 13% crude protein on a dry matter basis.

### Feeding Trial

Procedures described for the feeding trial in Experiment I were followed in Experiment II.

### Experiment III - Metabolism Study with Corn Silage Varied in Extent of Fermentation

### Design

A 3 x 3 Latin Square design was utilized in this experiment. Three all silage rations were fed to three 18-month

Hereford steers fitted with permanent rumen cannulas  
 three 28-day periods. No supplemental nitrogen was fed.  
 Treatments were randomized by time and animals as shown in  
 Table 6. Out of each 28-day period, 21 days were allowed for  
 steers to adjust to the new ration before being placed in  
 collection stalls. After an adjustment period of 14 hours  
 (overnight) in the stalls, feed intake, fecal output, and  
 milk production were measured and sampled for chemical analy-  
 sis over a period of six days. During the day following col-  
 lection, jugular blood and rumen fluid samples were secured  
 immediately before feeding and at two hour intervals there-  
 after up to 10 hours, post-feeding. The experiment was ini-  
 tiated on January 17, 1971 and completed on April 10, 1971.

#### Feeding Regime

Steers in the collection stalls were fed twice daily at  
 8 a.m. and 5 p.m. The silage treatments utilized are shown  
 in Table 7. All rations were supplemented at feeding with a  
 mineral-vitamin mixture (Table 8) and thoroughly mixed. The steers  
 were fed ad libitum during the acclimation period and collec-  
 tion period. The respective silages were removed from the  
 stalls just prior to each feeding.

Representative samples of all rations were taken just  
 prior to feeding for laboratory analysis and dry matter deter-  
 mination. Feed not consumed was weighed, sampled and dis-  
 carded prior to the 8 a.m. feeding.

Table 6

Experiment III - Metabolic Study  
Design of Experiment

Period	Steer No.		
	1	2	3
	-----Ration-----		
1	A	B	C
2	B	C	A
3	C	A	B

Table 7

Experiment III - Metabolic Study  
Treatments Utilized

Ration	Silage Treatment	Additive Added kg/MT <sup>1</sup>
A	Formic acid	5.3
B	Control	-
C	Limestone	13.6

<sup>1</sup>Kilograms of additive added per metric ton (MT) of 35% DM silage.

Table 8

Experiment III - Metabolic Study  
Formulation of Mineral Supplement

Ingredient	Mineral Supplement <sup>1</sup>
Dicalcium - phosphate (20% Ca - 18.5% P)	3.89
Sodium sulfate (22.5% S)	3.46
Trace mineral salt (high Zn)	3.89
Ground shelled corn	88.76
TOTAL	100.00%

<sup>1</sup>Supplement added at rate of 10.4 kg per 100 kg of silage DM to all rations.

### Sample Collection

Total feces were allowed to pass through a wide-space steel grid in the floor immediately behind each steer and were collected in large plastic containers in a pit below the collection stalls. Feces were removed once daily and total output was weighed. A 5% aliquot was retained each day for nitrogen determination, a 100 g sample was analyzed daily for dry matter content and the remaining feces were discarded. At the end of the six-day collection period, all samples were thoroughly mixed and a composite 200 g sample was taken for immediate total nitrogen determination.

Total urine was collected in a plastic carboy (in the pit below the collection stalls) which contained 200 ml of 6 N sulfuric acid. The carboy was emptied daily and urine volume was measured, then diluted to 12 litres with water and an aliquot of 10% stored in a cooler. The remaining diluted urine was discarded.

After the six-day collection period, all urine samples were thoroughly mixed and a one-litre composite sample was taken for immediate nitrogen determination.

Samples of whole rumen contents were taken through the permanent rumen cannulas fitted to the steers. Rumen samples were strained through two layers of cheesecloth and 1 ml of mercuric chloride (saturated) was added to 19 ml of the strained rumen fluid in a test tube and retained for rumen ammonia determination. Five ml of the above 20 ml mixture were added to 1 ml of metaphosphoric acid and centrifuged at 10,000 rpm for 10 minutes. The supernatant was retained for volatile fatty acid analysis.



Jugular vein blood samples (10 ml) were taken with a 16 gauge needle into a heparinized test tube and retained for plasma urea analysis.

#### Laboratory Analysis

Dry matter of feed and feces samples was determined daily by oven-drying at 60° C for 24 hours.

Total nitrogen contents of feed, feces, and urine were analyzed by macro-Kjeldahl procedures on well-mixed wet samples.

Rumen volatile fatty acid concentrations were determined.

Blood samples were centrifuged at 6,000 rpm for 10 minutes, and the plasma recovered with a Pasteur pipette. Urea content of the plasma was determined by the micro-diffusion method of Conway (1950).

#### Experiment IV - Nitrogen Balance Study with NPN Silage Additives

##### Design

A 4 x 4 Latin Square design was utilized in this experiment. The silage treatments utilized are shown in Table 9. Treatments were randomized by time and animal as shown in Table 10. No supplemental nitrogen or minerals were fed. Four Angus steer calves without cannulas were used to study parameters of nitrogen metabolism on four all-silage rations. The experiment was initiated on January 24, 1970 and terminated on May 15, 1970. All procedures and analysis for this experiment were identical to those in Experiment III except blood and rumen samples were not taken.

Table 9

Experiment IV - Metabolic Study  
Treatments Utilized

Ration	Silage Treatment	Additive Added kg/MT <sup>1</sup>
A	Urea-mineral + Formic acid	20.6 + 5.3
B	Urea-Mineral	20.6
C	Pro-Sil Supplemented at Feeding <sup>3</sup>	22.5
D	Pro-Sil	22.5

<sup>1</sup>Kilograms of additive added per metric ton (MT) of 35% DM silage.

<sup>2</sup>See Table 5 for formulation of urea-mineral supplement.

<sup>3</sup>See Table 4 for formulation of Pro-Sil.

Table 10

Experiment IV - Metabolic Study  
Design of Experiment

Period	Steer No.			
	1	2	3	4
	-----Ration-----			
1	B	C	D	A
2	C	D	A	B
3	D	A	B	C
4	A	B	C	D

## Experiment V - Silage Fermentation Study

Design

A 16 x 2 factorial design was utilized to study the parameters of corn silage fermentation over time comparing untreated and Pro-Sil treated silages. The silage was harvested September 8, 1971 and a fresh sample was taken for laboratory analysis at the time of filling the experimental silos.

One gallon glass jars with rubber seal metal lids equipped with gas valves were used as experimental silos as shown in Figure 3. Fifteen jars were filled with 1.5 kg of untreated silage and another fifteen were filled with 1.5 kg of Pro-Sil treated silage. Pro-Sil was applied at a rate equivalent to 22.5 kg/MT of 35% dry matter silage and the silage was mixed in a small cement mixer. The silage was packed by hand in the jars, then a vacuum pump was used to exhaust all air ( $-.21 \text{ kg per cm}^2$ ). The jars were then filled with carbon dioxide ( $.21 \text{ kg per cm}^2$ ). The process was repeated and then the  $\text{CO}_2$  pressure was vented to zero  $\text{kg per cm}^2$ , thus creating an anaerobic atmosphere. The experimental silos were placed in an incubator at  $40^\circ \text{C}$  for the first three days, after which, they remained at room temperature ( $22^\circ \text{C}$ ) for the duration of the experiment. Pressure was vented to zero  $\text{kg per cm}^2$  daily during the fermentation.

Silage from one jar in each treatment was removed and frozen on each of the following days for laboratory analysis: 1 - 10, 15, 20, 30, 60 and 90.



Figure 3. Experimental Silo Unit. This one gallon glass jar with a rubber seal metal lid equipped with a gas valve was used as the experimental silo unit.

### Silage Analysis

Silage extracts were prepared by homogenizing a 25 g aliquot of the sample and 100 ml of distilled water with a Sorvall Omni-Mixer for two minutes and then straining the homogenate through two layers of cheesecloth. A portion of the unstrained homogenate was used to determine total nitrogen by micro-Kjeldahl procedures. After the first straining, the residue material was resuspended in distilled water at 60° C and then restrained. The two extracts were finally combined and a 30 ml aliquot of the combined extract was used to determine pH and soluble nitrogen. Another 27 ml aliquot of extract was deproteinized using one ml of 50% sulfosalicylic acid (SSA) per nine ml of extract. The sample was centrifuged at 18,000 rpm for 10 minutes and stored for nonprotein nitrogen, ammonia, volatile fatty acids and lactic acid determinations. Soluble nitrogen was determined by the micro-Kjeldahl method. Other silage parameters were determined as outlined in the procedures for Experiment I.

Determinations of neutral detergent fiber (cell wall constituents) and acid detergent fiber were made using the method of Goering and Van Soest (1970). Total nitrogen in the acid detergent fiber residue was determined by micro-Kjeldahl.

### Experiment VI - Intake, Metabolic and In Vitro Studies of Corn Silage Containing Varying Levels of Unidentified Soluble Nitrogen

#### Design

A 4 x 4 Latin Square design was utilized in this

1

experiment. Four silages were fed to four mature crossbred wethers fitted with permanent rumen cannulas over four 22-day periods. Weights were taken on days 5, 15 and 22 of each period. Animals were randomized by time and treatment as shown in Table 11. Out of each 22-day period, 14 days were allowed for the wethers to adjust to the new ration before being placed in collection crates. Intake was measured the last 10 days of the 14-day acclimation period. After the adjustment period, wethers were placed in collection crates and feed intake, fecal output, and urine production were measured and sampled for chemical analysis over a period of seven days. Water intake was also measured during the collection period. On the day following the collection period, jugular blood was sampled immediately before feeding and four hours after feeding. Rumen samples were secured immediately before feeding, one hour after feeding, two hours after feeding and at two hour intervals thereafter up to 8 hours post-feeding. The experiment was initiated on November 9, 1971 and terminated February 4, 1972.

#### Forage Preparation

Corn silage was harvested on August 30 and 31, 1971 at approximately 32% dry matter. All treatments with the exception of three barrels of autoclaved silage were made from one load of silage chopped on the 30th of August. The following four silage treatments will be discussed: 1) air dried, 2) sun dried 50% DM, 3) autoclaved, and 4) control silage.

The air dried silage treatment consisted of 29 burlap sacks filled with approximately 27.2 kg of fresh silage each. The sacks were placed in a gas heated crop drier at 118° F for four days. The DM was about 80% at the end of the drying process. Burlap sacks were then stored in steel barrels until fed.

The sun dried treatment was prepared by unloading the fresh silage in rows onto a paved parking lot that had been swept clean. The silage was spread into a layer about 5 to 10 cm deep and was mixed several times during the day using a garden rake. Eleven experimental silos were filled late in the afternoon with the 50% DM material.

Five barrels of autoclaved silage were prepared on August 30 and three were prepared on August 31. Each barrel contained about 70 kg of silage. Two metal barrels, 88.9 cm high and 57.2 cm in diameter with 1.27 cm holes drilled approximately 45 cm apart in the sides and bottom, were used to autoclave the silage. A metal pipe 5.1 cm in diameter and containing holes 10 cm apart was placed in the center of each barrel to facilitate autoclaving. The silage was autoclaved for one hour at 255° F and at a pressure of approximately 6.7 kg per square centimeter.

After autoclaving, the silage was dumped onto a clean cement slab and spread in a thin layer until it had cooled. It was then inoculated with 10% of a three day fermented silage, mixed, and placed in experimental silos.

The control silage was placed in experimental silos



without any treatment at approximately 84 kg per silo.

#### Experimental Silos

Metal barrels 88.9 cm high and 57.2 cm in diameter were used as containers. Two vinyl bags 12 mm thick, 1.37 m long, and 50.8 cm in diameter were placed in each barrel. The bags were filled with the various treatments and tramped several times to insure maximum compaction. After each bag was filled to the capacity of the barrel, a vacuum hose attached to an industrial floor sweeper was used to remove as much of the remaining air as possible. The bag was then sealed with tape. The above procedure does not apply to the air dried material which was stored in burlap sacks in the barrels.

#### Feeding Regime

The sheep were fed twice daily at 8:00 a.m. and 5:00 p.m. at ~ 15% excess of voluntary consumption, hence, insuring ad libitum intakes during the intake trial. While the wethers were in collection crates, they were fed at approximately 90% of voluntary intake. The ration was composed of the respective corn silage plus a mineral supplement (Table 12) added at 2% of the silage dry matter.

The silage was weighed prior to each feeding and the mineral supplement was thoroughly mixed with the silage. Unconsumed feed residue was removed and weighed each morning prior to feeding. Water was available at all times.

#### Sample Collection

Intake Period: During the 10-day intake study, daily

Table 11

Experiment VI - Metabolic Study  
Design of Experiment

Period	Treatment			
	Control Silage	Autoclaved Silage	Air Dried Silage	Sun Cured Silage
	Sheep No.			
1	2	4	3	1
2	4	3	1	2
3	3	1	2	4
4	1	2	4	3

Table 12

Experiment VI - Metabolic Study  
Formulation of Mineral and Vitamin Supplement

Ingredient	Percent
Dicalcium phosphate (26.5% Ca - 20.5% P)	47.3
Trace mineral salt (high Zn)	47.3
Sodium Sulfate (22.5% S)	4.7
Vitamin A (10,000 IU/g)	0.3
Vitamin D (9,000 IU/g)	0.1
TOTAL	100.00%

samples of the silage and unconsumed residue were taken and frozen. At the end of the intake trial, a composite sample of each silage treatment and unconsumed residue was oven dried at 60° C for 24 hours to determine dry matter consumption.

Collection Period: Daily samples of silages and unconsumed residue were frozen for laboratory analysis. Water intake was obtained by measuring the water presented to each sheep each morning and then measuring the unconsumed quantity 24 hours later. Evaporative loss was assumed to be negligible.

Total fecal collection was made by fitting each sheep with a canvas zipper bag collection harness. After weighing, the feces were placed in a cooler until the end of the collection period when they were thoroughly mixed and subsampled for laboratory analysis.

Total urine was collected in a two litre glass bottle which contained 25 ml of 20% sulfuric acid and 1 ml of 10% copper sulfate. The total urine volume was measured and then diluted with water to a volume of three litres. One-sixth of the diluted urine was saved from each of the seven days' collections and a composite sample was taken for later analysis.

The pH of the rumen samples was determined with a Corning Model 12 pH meter. A 50 gm aliquot was then dried at 60° C for 48 hours to determine rumen dry matter. The remainder of the rumen sample was strained through two layers of cheesecloth. One ml of saturated mercuric chloride was

added to 19 ml of the strained rumen fluid and restrained for ammonia analysis. Five ml of the above 20 ml mixture was added to 1 ml of metaphosphoric acid and centrifuged at 10,000 rpm for 10 minutes. The supernatant was retained for volatile fatty acid analysis.

The whole blood was centrifuged and a portion of the plasma was retained for plasma urea determinations. Two ml of the plasma was processed for plasma amino acid analysis by adding 0.2 ml of norleucine (1 $\mu$ m/ml) to 2 ml of plasma and then deproteinizing the sample with 0.2 ml of 50% sulfosalicylic acid. After one hour on ice, the sample was centrifuged at 18,000 rpm for 15 minutes. The supernatant was kept in the freezer for further analysis.

#### Laboratory Analysis

Dry matter of the fecal samples was determined daily by oven-drying at 60° C for 24 hours.

Total nitrogen contents of feed, feces and urine were analyzed by macro-Kjeldahl procedures on wet samples.

Rumen volatile fatty acid concentrations were determined by injecting samples into a Packard gas chromatograph, as described previously (page 51).

Rumen ammonia and blood urea levels were determined by the micro-diffusion method of Conway (1950).

Determination of amino acids was performed on a Technicon-TSM-1 amino acid analyzer, according to Bergen and Potter (1971) and Makdani, Huber and Bergen (1971).

Additional silage parameters were determined as in

## Experiment V.

### Procedures for In Vitro Fermentation

The basic system used in this series of trials was a modification of the Ohio System (Johnson, 1966). The substrate was prepared by taking each silage and lyophilizing it in a Stakes Model 21003F-2 Freeze Drying Unit. The freeze dried material was ground through a 40 mesh screen in a Wiley mill. The "Ohio" in vitro fermentation media (Johnson, 1966) was utilized. Rumen fluid inoculum was obtained from a donor sheep maintained on a Pro-Sil treated corn silage ration.

On the day of the initiation of the trial, rumen contents were collected from the donor sheep in the morning prior to feeding and strained through cheesecloth. Fifty ml quantities of the strained material were used to inoculate each fermentation bottle. Each bottle contained 4.0 g of ground freeze-dried silage, 150 ml of nutrient solution (Johnson, 1966) and 50 ml of the inoculum. During the course of the fermentation, carbon dioxide was continuously bubbled through the closed flask system. Duplicate samples of 10 ml were removed at 0 and 12 hours after the initiation of the trial and 20 ml duplicate samples were removed at 48 hours. These samples were centrifuged at 7,000 x g for 12 minutes and the resulting sediment was washed with distilled water and recentrifuged. The sediment was oven dried at 105° C for 24 hours and then subjected to a cellulose analysis by a modified Crampton and Maynard procedure (Dehority and Johnson,

1964). The 12 hour sample was used to estimate the rate of cellulose digestion while the 48 hour sample estimated the extent of digestion.

Total volatile base was determined on duplicate 5 ml samples taken at 0, 12, and 48 hours after the initiation of the trial. The micro-Kjeldahl distilling apparatus was utilized.

The micro-Kjeldahl procedure was used to determine total soluble nonprotein nitrogen. Duplicate 5 ml samples were treated with 5 ml of 10% trichloroacetic acid (TCA) and centrifuged. The precipitate was resuspended in distilled water and washed to ensure removal of all of the soluble nitrogen. Soluble nitrogen was determined in the TCA supernatants from samples removed at 0, 12, and 48 hours after the initiation of the in vitro digestion trial.

#### Statistical Analysis

All data from the feeding trials, metabolic trials and intake trial were analyzed on a CDC 3600 computer at Michigan State University Computer Laboratory. Least squares procedure was used to compare effects of the silage treatments (Harvey, 1960). Correlation coefficients were computed on all trials to more precisely define relationships among variables studied. Duncan's New Multiple Range Test was used to compare means for significant differences.

## IV

### RESULTS AND DISCUSSION

#### Experiment I - Feeding Trial 1

##### Chemical Analysis of Silage

Results of 18 different composite analyses of each silage and the high moisture corn used during the experiment are shown in Table 13.

Dry matter content of the silage ranged from 30.98% to 35.66%; the percent dry matter between fresh and ensiled silage varied little. Most published data indicate that corn silage dry matter yield per acre increases until dry matter content of the plant reaches approximately 35%. It then stabilizes for a few days and subsequently decreases at a rapid rate depending upon weather conditions (Johnson and McClure, 1968; Huber et al., 1968; and Gordon, 1966). Geasler (1970) reported that performance, average yield per acre, silo storage requirements, and fermentation parameters suggest that corn silage harvest should not be delayed beyond the 35% dry matter range.

##### Nitrogen Fractions

The apparent increase in total nitrogen content between fresh and ensiled values is attributable to sampling or laboratory errors since no nitrogen was added at silo filling time.

The insoluble nitrogen is the difference in total nitrogen and water soluble nitrogen. It contains true protein and small amounts of other organic forms of nitrogen such as nucleic acids, etc. The unidentified nitrogen is the portion of water soluble nitrogen not contained in the ammonia and urea fractions.

Water insoluble nitrogen content of control silage decreased approximately 41% during fermentation; whereas, decreases were 28% and 31%, respectively for formic acid and limestone treated silages. Water insoluble nitrogen destruction was calculated by comparing fresh values with corrected ensiled values. It was necessary to correct the ensiled values to remove the apparent sampling errors previously discussed. Less proteolysis in the formic acid and limestone treated silages resulted in slightly lower NPN levels in these two silages. The ammonia concentrations were lower in the formic acid treated (.06%) and limestone treated (.08%) silages when compared to the control silage (.11%), although differences were not significant for the three silage treatments. The unidentified water soluble nitrogen values followed the NPN concentrations. The reduction in NPN concentrations and ammoniacal nitrogen in formic acid treated silage is in agreement with results reported by Waldo et al. (1971b) and Huber (1970).

The unidentified nitrogen levels as a percent of total nitrogen in the ensiled material ranged from ~34% for the formic acid and limestone treated silages to ~41% for the





control silage. It can be concluded that maximizing fermentation with limestone or minimizing fermentation with formic acid did not greatly affect the percent of total nitrogen degraded to unidentified nitrogen compounds. This tends to indicate that plant enzymes rather than fermentation bacteria are responsible for the degradation of protein which is in agreement with results obtained by Russell (1908), Kirsch (1930), Hunter (1921) and Mabbitt (1951).

### Organic Acids

Total organic acid fractions (as percent of dry matter) were calculated by combining the total lactic, acetic and butyric acids. Other organic acids such as valeric and isovaleric were too low for accurate determination.

Formic acid treated silage contained only about 25% as much lactic acid as the control silage and the difference was highly significant (  $P < .01$  ). Acetic acid content of the formic acid treated silage was reduced 62% below the value obtained for control silage and the difference was highly significant (  $P < .01$  ). The depression in total organic acid content indicates decreased fermentation in the formic acid treated silage. These results are similar to those obtained by Huber (1970).

The limestone treated silage had 74% more lactate than the control silage and this increase was significant (  $P < .01$  ). The acetic acid content in the limestone treated silage was less than the level contained in the control silage. The increase in lactate was apparently due to the

Table 13

Average Chemical Analysis on Dry Matter Basis  
of Silages and High Moisture Corn Fed

Observation	Control		Formic Acid		Limestone		High Moisture Corn	
	No Treatment	Ensil <sup>1</sup>	Fresh <sup>1</sup>	Ensil <sup>1</sup>	Fresh <sup>1</sup>	Ensil <sup>1</sup>	No Treatment	Ensil <sup>1</sup>
Percent Dry Matter	36.69	34.91	34.13	30.98 <sup>A</sup>	36.38	35.66		75.44
<u>Nitrogen Fractions:</u>								
Total nitrogen	1.20	1.44 <sup>ab</sup>	1.25	1.49 <sup>a</sup>	1.20	1.32 <sup>b</sup>		1.49
Insoluble nitrogen	1.03	.75	1.00	.90 <sup>A</sup>	1.02	.78		1.26
Soluble nitrogen	.17	.69 <sup>A</sup>	.25	.59 <sup>AB</sup>	.18	.54 <sup>B</sup>		.23
Ammonium salts	.01	.11	.03	.06	.01	.08		.02
Urea	.00	.00	.01	.01	.00	.00		.00
Unidentified	.16	.58	.21	.52	.17	.46		.21
<u>Organic Acid Fractions:</u>								
Total organic acid	-----	9.44 <sup>B</sup>	-----	2.61	-----	14.94 <sup>A</sup>		.44
Lactic acid	-----	7.75 <sup>B</sup>	-----	1.97	-----	13.50 <sup>A</sup>		.42
Acetic acid	-----	1.67	-----	.64 <sup>A</sup>	-----	1.38		.02
Butyric acid	-----	.02	-----	-----	-----	.06		-----
pH	5.80	3.89 <sup>B</sup>	4.35	4.07 <sup>AB</sup>	5.49	4.21 <sup>A</sup>		5.53

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

<sup>1</sup>Statistical analysis not conducted.

Note: "Fresh" are the mean values of 8 composite samples taken during silo filling.

"Ensil<sup>1</sup>" are the mean values of 18 composite samples taken during feeding.

buffering effect of the limestone. Elevated lactic acid levels in the limestone treated silage are in agreement with results received by Klosterman et al. (1961b), Klosterman et al. (1963b) and Nicholson and Cunningham (1964). The limestone addition may have altered the activity of plant enzymes that degrade protein thus accounting for the decrease in proteolysis previously discussed. Although the pH of the three silages was significantly different ( $P < .01$ ), they were all within the range described by Barnett (1954) for maintaining desirable silage quality.

#### Effect of Maximizing and Minimizing Silage Fermentation on Performance of Feedlot Cattle

Complete performance of all lots of cattle is shown in Table 14. The means for all performance traits for cattle fed silage treated with additives not containing nitrogen are shown in Table 15. Average daily gain was not affected by silage treatment and there were only small differences in feed efficiency. Daily feed consumption values were slightly reduced for the two treated silages (control - 7.88 kg, formic acid treatment - 7.78 kg, and limestone treatment - 7.58 kg). The reduced intake of the limestone treated silage fed cattle may be due to significantly ( $P < .01$ ) higher lactic acid levels in the limestone treated silage; however, there are conflicting opinions concerning the effect of lactic acid on voluntary dry matter consumption (Emery et al., 1961; Senel and Owen, 1966; and Allen et al., 1971). If lactic acid content of the silage did depress the intake of

steers fed the limestone treated silage, then the dry matter intake of the steers fed the formic acid treated silage should have been the largest since it contained significantly ( $P < .01$ ) less lactate than either of the other treatments. Amines, which are possible products of proteolysis, have been reported to depress intake (Neumark, 1964 and Dain et al., 1955) and most proteolytic products of silage fermentation are contained in the unidentified nitrogen fraction. Unidentified nitrogen levels as a percent of total nitrogen were similar for all three treatments (34% to 41%), which may explain the resulting small differences in dry matter intake received on the three rations. Greater differences in the unidentified nitrogen levels fed are needed before definite conclusions can be made regarding their effect on voluntary dry matter consumption. The absence of an effect on average daily gain, feed consumption and feed efficiency by cattle fed the formic acid treated silage is in disagreement with results reported by Waldo et al. (1969) and Waldo et al. (1971). Improvement in animal performance has been shown for formic acid treatment of low dry matter grass and legume silage (~20% DM) (Saue and Breirem, 1969a; Waldo et al., 1970; and Waldo et al., 1969) or high dry matter corn silage (44% DM) (Huber, 1970). All silages in this experiment were in the dry matter range of 30.98% to 35.66% which was suggested by Geasler (1970) as the most effective stage of maturity to harvest corn silage to optimize all factors.

Feed cost per 100 kg gain was elevated for the formic

Table 14

Corn Silage Additives Compared  
(October 22, 1970 to June 17, 1971)

	Type of Silage Treatment and Supplement					
	Control		Formic Acid		Limestone	
	Soy-mineral Supplement		Soy-mineral Supplement		Soy-mineral Supplement	
	40% Sh.		40% Sh.		40% Sh.	
161 - 238 Day Test	All Silage	Corn-60% Silage	All Silage	Corn-60% Silage	All Silage	Corn-60% Silage
Lot No.	38	40	37	35	39	36
No. of steer calves	10	10	10	10	10	10
Av. initial wt., kg	245	245	244	244	243	247
Av. final wt., kg	403	420	397	414	405	414
Av. daily gain, kg	.71	.89	.70	.87	.73	.85
<u>Daily Feed, kg 85% DM:</u>						
Corn silage	6.64	4.30	6.71	4.11	6.77	3.91
Gr. sh. corn		3.42		3.32		3.10
Soy-mineral supplement	.84	.55	.88	.53	.85	.47
TOTAL	7.48	8.27	7.59	7.96	7.62	7.48
<u>Feed Efficiency:</u>						
Feed per kg gain, kg	10.58	9.26	10.86	9.15	10.36	8.78
Feed cost per 100 kg gain <sup>1</sup>	\$ 40.74	\$ 39.51	\$ 47.54	\$ 41.47	\$ 41.03	\$ 38.43
<u>Carcass Evaluation:</u>						
Carcass grade <sup>2</sup>	12.98	14.28	14.12	14.72	13.18	13.68
Marbling score <sup>3</sup>	15.51	19.01	18.09	20.89	15.19	17.11
Fat thickness, cm	1.54	1.77	1.62	1.51	1.64	1.67
Ribeye area, cm <sup>2</sup>	69.94	64.88	66.63	66.44	70.50	69.13
Percent K.H.P. fat <sup>4</sup>	3.62	3.77	3.38	3.58	3.33	3.47
Percent B.T.R. cuts <sup>5</sup>	49.36	47.87	49.09	48.88	49.27	48.88
Dressing percent <sup>6</sup>	60.52	61.78	59.30	60.98	60.95	61.31
Carcass price/100 kg	\$115.70	\$114.82	\$116.18	\$116.18	\$115.52	

<sup>1</sup>Feed costs based on 30% DM corn silage \$9.37/MT, urea-mineral \$88.18/MT, Pro-Sil \$71.65/MT, shelled corn \$49.60/MT, soy-mineral supplement \$110.23/MT.

<sup>2</sup>Good = 9, 10, 11; Choice = 12, 13, 14.

<sup>3</sup>Small = 10, 11, 12; Modest = 13, 14, 15; Moderate = 16, 17, 18.

<sup>4</sup>Percent of carcass weight in kidney, heart and pelvic fat.

<sup>5</sup>Percent of carcass weight in boneless, trimmed retail cuts.

<sup>6</sup>Cold carcass weight over off experiment weight.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

Table 15

Corn Silage Additives Compared  
(October 22, 1970 to June 17, 1971)

161 - 238 Day Test	Type of Silage Treatment and Supplement		
	Control Soy-mineral Supplement	Formic Acid Soy-mineral Supplement	Limestone Soy-mineral Supplement
No. of steer calves	20	20	20
Av. initial wt., kg	246	245	245
Av. final wt., kg	412	406	410
Av. daily gain, kg	.80	.79	.80
<u>Daily Feed, kg 85% DM:</u>			
Corn silage	5.47	5.41	5.34
Gr. sh. corn	1.71	1.66	1.55
Soy-mineral supplement	.70	.71	.69
TOTAL	7.88	7.78	7.58
<u>Feed Efficiency:</u>			
Feed per kg gain, kg	9.88	9.87	9.50
Feed cost per 100 kg gain <sup>1</sup>	\$ 40.08	\$ 44.20	\$ 39.82
<u>Carcass Evaluation:</u>			
Carcass grade <sup>2</sup>	13.63 <sup>ABb</sup>	14.42 <sup>Aa</sup>	13.40 <sup>Bb</sup>
Marbling score <sup>3</sup>	17.26 <sup>ab</sup>	19.49 <sup>a</sup>	16.15 <sup>b</sup>
Fat thickness, cm	1.67	1.56	1.64
Ribeye area, cm <sup>2</sup>	67.38	66.50	69.75
Percent K.H.P. fat <sup>4</sup>	3.70	3.48	3.40
Percent B. T. R. cuts <sup>5</sup>	48.61	48.99	49.07
Dressing percent <sup>6</sup>	61.15	60.14	61.13
Carcass price/100 kg	\$115.26	\$116.18	\$115.61

<sup>1</sup>Feed costs based on 30% DM corn silage \$9.37/MT, urea-mineral \$88.18/MT, Pro-Sil \$71.65/MT, shelled corn \$49.60/MT, soy-mineral supplement \$110.23/MT.

<sup>2</sup>Good = 9, 10, 11; Choice = 12, 13, 14.

<sup>3</sup>Small = 10, 11, 12; Modest = 13, 14, 15; Moderate = 16, 17, 18.

<sup>4</sup>Percent of carcass weight in kidney, heart and pelvic fat.

<sup>5</sup>Percent of carcass weight in boneless, trimmed retail cuts.

<sup>6</sup>Cold carcass weight over off experiment weight.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

acid treated silage fed group. This was due to the added cost of formic acid which resulted in no improvement in feed efficiency.

The slight improvement in feed efficiency for the limestone treated silage fed group offset the cost of treating. This improvement in feed efficiency is in agreement with previous work by Klosterman (1963b) and the summary by Essig (1968). One possible explanation for the increased feed efficiency of the limestone treated silage fed cattle may be a reflection of the significantly ( $P < .01$ ) higher lactic acid content of this silage. Increased feed efficiency when lactate was added to the ration was reported by Emery et al. (1961), Senel and Owen (1966), and Allen et al. (1971).

All groups had an average carcass grade of middle to high Choice; however, small differences were significant ( $P < .01$ ). Marbling scores ranged from moderate - to slightly abundant - and again the small differences were significant ( $P < .05$ ). Differences in all of the other carcass traits were small and insignificant.

## Experiment II - Feeding Trial 2

### Chemical Analyses of Silage Containing NPN Additions

Results of 18 different composite analyses of each silage and the high moisture corn used during the experiment are shown in Table 16.

Percent dry matter of the fresh and ensiled material varied little and all silages were within the dry matter range for excellent quality (Geasler, 1970).



### Nitrogen Fractions

Total nitrogen values of the three NPN treated silages compared to the control untreated silage were increased 44% by Pro-Sil treatment, 53% by urea-mineral treatment, and 57% by the urea-mineral plus formic acid treatment. In all cases, increases in total nitrogen accounted for essentially 100% of the Pro-Sil and urea applied. The apparent increase in total nitrogen of the control silage is attributed to sampling errors since no nitrogen was added at time of ensiling.

The water insoluble nitrogen content of the control silage decreased approximately 41% (fresh vs. adjusted ensiled). The control ensiled values were adjusted downward to compensate for the apparent increase in crude protein. The decrease in water insoluble nitrogen content of the silage during fermentation was 12% for the Pro-Sil treated silage, 24% for the urea-mineral treated silage and 18% for the urea-mineral plus formic acid treated silage.

These data indicate that Pro-Sil and urea-mineral additions had a sparing effect on water insoluble nitrogen and are in agreement with work by Beattie (1970), Huber and Hillman (1970) and Henderson, et al. (1971). This appears to be due to an increase in bacterial protein and/or a decrease in proteolysis during fermentation (according to Modyanov, et al., 1960 and Rayetskaya, et al., 1954)<sup>1</sup>.

Table 16 shows that approximately 36% of the nitrogen

---

<sup>1</sup>As reported by Owens (1968).

in Pro-Sil was recovered as insoluble protein in silage, 63% was recovered as ammonium salts (assuming all ammonia present was linked to organic acids to form ammonium salts)<sup>1</sup> and 1% as urea. The results agree with previous data of Henderson (1969) and (1970b).

When silage was treated with urea-mineral, 13% of the added nitrogen was recovered as water insoluble nitrogen, 63% as ammonium salts, 14% as urea and 10% in the unidentified fraction. The high ammonium salts value and low level of water insoluble nitrogen and urea in urea-mineral treated silage are not readily explainable. Apparently, a high level of urease was present in the fresh corn plant material at ensiling time (as suggested by Karr, et al., 1955) which reduced the added urea to ammonia. The ammonia was then combined with organic acids during fermentation. This is suggested by the high level of lactic acid produced in this silage.

When silage was treated with urea-minerals plus formic acid, 27% of the added nitrogen was recovered as water insoluble nitrogen, 7% as ammonium salts, 54% as urea and 12% remained in the unidentified fraction.

Actual levels of unidentified NPN in the four silages did not differ substantially; therefore, the elevated water insoluble nitrogen levels for the Pro-Sil, urea-mineral, and urea-mineral plus formic acid treated silages, as

---

<sup>1</sup>As suggested by Bentley, et al. (1955); Klosterman, et al. (1961); Johnson, et al. (1967); Huber and Hillman (1970); and Henderson, et al. (1970b).

compared to the control silage, are probably due to the production of microbial protein during fermentation rather than to a reduction in proteolysis.

It can be concluded that corn silage treated with either Pro-Sil or urea-mineral will have a higher water insoluble nitrogen content at feeding time than untreated silage.

### Organic Acids

Total organic acid (as percent of dry matter) was calculated by combining lactic, acetic and butyric acids. Other organic acids such as valeric and isovaleric were too low for accurate determination. Pro-Sil and urea-mineral treated silages had similar total organic acid contents which averaged 33% higher than the control. Urea-mineral plus formic acid treatment reduced the total acid content by 43%.

Lactic acid levels in the silage were significantly ( $P < .01$ ) increased by 39% for both the urea-mineral and Pro-Sil treated silages, when compared to the control silage. Acetic acid levels were unaffected by these two NPN treatments.

Treatment of silage with urea-mineral plus formic acid significantly ( $P < .05$ ) reduced both lactic and acetic acid levels by 43% and 46%, respectively, when compared to the control silage. This reduction is attributed to the formic acid addition discussed in Experiment I.

Thus, the neutralizing effects of Pro-Sil and urea mineral when added without formic acid resulted in an increased fermentation and bacterial activity, yielding significantly ( $P < .01$ ) higher amounts of lactic acid. This is in

Table 16

Average Chemical Analysis on Dry Matter Basis of  
Silages and High Moisture Corn Fed

Observation	Control		Pro-Sil		Urea-Mineral		Urea-Mineral-20.6		High Mois- ture Corn
	No Treatment		22.5 kg/MT		20.6 kg/MT		kg/MT, Formic Acid-5.3 kg/MT		
	Fresh <sup>1</sup>	Ensiled	Fresh <sup>1</sup>	Ensiled	Fresh <sup>1</sup>	Ensiled	Fresh <sup>1</sup>	Ensiled	No Treatment Ensiled <sup>1</sup>
Percent Dry Matter	36.69	34.91 <sup>a</sup>	34.52	34.57 <sup>ab</sup>	36.06	34.47 <sup>ab</sup>	36.75	33.26 <sup>b</sup>	75.44
<b>Nitrogen Fractions:</b>									
Total nitrogen	1.20	1.44 <sup>B</sup>	1.23	2.07 <sup>Ab</sup>	1.09	2.21 <sup>Aab</sup>	1.22	2.26 <sup>Aa</sup>	1.49
Insoluble nitrogen	1.03	.75	1.10	.97 <sup>A</sup>	.89	.85 <sup>B</sup>	1.03	.97 <sup>A</sup>	1.26
Soluble nitrogen	.17	.69 <sup>A</sup>	.13	1.10 <sup>B</sup>	.20	1.36 <sup>A</sup>	.19	1.29 <sup>A</sup>	.23
Ammonium salts	.01	.11	.01	.57 <sup>A</sup>	.01	.59 <sup>A</sup>	.01	.16	.02
Urea	.00	.00	.00	.01 <sup>c</sup>	.00	.11 <sup>B</sup>	.00	.44 <sup>A</sup>	.00
Unidentified	.16	.58	.12	.52	.19	.66	.18	.69	.21
<b>Organic Acid Fractions:</b>									
Total organic acid	-----	9.44 <sup>B</sup>	-----	12.31	-----	12.89	-----	5.36	.44
Lactic acid	-----	7.75 <sup>B</sup>	-----	10.78 <sup>A</sup>	-----	10.79 <sup>A</sup>	-----	4.45	.42
Acetic acid	-----	1.67	-----	1.47	-----	1.94	-----	.91 <sup>A</sup>	.02
Butyric acid	-----	.02	-----	.06	-----	.16	-----	-----	-----
pH	5.80	3.89 <sup>Ba</sup>	5.60	4.19 <sup>AB</sup>	5.72	4.28 <sup>A</sup>	5.65	4.12 <sup>AB</sup>	5.53

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

<sup>1</sup>Statistical analysis not conducted.

Note: "Fresh" values are the mean of 8 composite samples taken during silo filling.  
"Ensiled" values are the mean of 18 composite samples taken during feeding.



agreement with previous work by Huber, et al. (1968); Klosterman, et al. (1963); and Henderson, et al. (1970b, 1971).

The pH of the control silage (3.89) was significantly ( $P < .05$ ) lower than all other treatments; however, all silages were within the pH range suggested by Barnett (1954) as being required for maintaining excellent preservation of the silage during storage and feeding.

#### Effect of Additives on Feedlot Performance

Individual lot means for performance and carcass traits are shown in Table 17. The means for performance and carcass traits for cattle fed NPN treated silages are shown in Table 18. Steers receiving either Pro-Sil, urea-mineral or urea-mineral plus formic acid treated silage gained significantly faster than steers receiving the control silage supplemented at feeding time with soy-mineral ( $P < .05$ ) or Pro-Sil ( $P < .01$ ). The difference in gain between Pro-Sil supplemented and soy-mineral supplemented groups (.75 kg vs. .80 kg) was not significant.

Although Pro-Sil has a strong ammonia odor, and the nitrogen content is made up entirely of anhydrous ammonia; the ammonia readily combined with the organic acids contained in the silage. After approximately one minute of mixing in a horizontal mixer, the silage was free of ammonia odor and was quite similar to silage treated with Pro-Sil at time of ensiling with respect to physical characteristics and odor.



Previous work (Geasler and Henderson, 1970) showed a high correlation between feed efficiency and lactic acid content of the silage fed. The superiority of the NPN treated silages may be attributed to their higher lactic acid levels.

Differences in feed consumption were small; however, feed efficiency favored the NPN treated silages. Control silage supplemented with Pro-Sil at feeding time resulted in the poorest feed efficiency. Lower levels of organic acids in the control silage supplemented with Pro-Sil compared to Pro-Sil treated and urea-mineral treated silages may explain the reduced performance for this group. Another possible explanation of the lower daily gain and feed efficiency may be the formation of poorly utilizable NPN compounds other than ammonium salts and thus less available protein. Perhaps the latter explanation is more accurate since the urea-mineral plus formic acid treated silage had significantly ( $P < .01$ ) lower lactate and acetate than the control, but resulted in performance superior to the controls.

Feed cost per hundred kilograms of weight gain was lower for the NPN treated and supplemented silages. The increased feed cost for the urea-mineral plus formic acid treated silage fed group was due to the cost of the formic acid additon. Compared to the soybean supplemented silage, urea or Pro-Sil treament alone lowered feed cost approximately 25%. Feed cost for Pro-Sil supplementation at ensiling time was 17% less than when added at feeding time.

Carcass grade for all groups of cattle averaged between



Table 17

Corn Silage NPN Additives Compared  
(October 22, 1970 to June 17, 1971)

	Type of Silage Treatment and Supplement						
	Control		Pro-Sil		Pro-Sil		Pro-Sil Supplement
	Soy-mineral All Silage	40% Shelled Corn 60% Silage	All Silage	40% Shelled Corn 60% Silage	All Silage	40% Shelled Corn 60% Silage	
161 - 238 Day Test							
Lot No.	38	40	42	45	44	43	
No. of steer calves	10	10	9	9	10	10	
Av. initial wt., kg	245	245	244	246	247	244	
Av. final wt., kg	403	420	403	440	400	400	
Av. daily gain, kg	.71	.89	.73	1.01	.69	.80	
Daily Feed, kg 85% DM:							
Corn silage	6.64	4.30	6.94	4.47	7.39	4.16	
Gr. sh. corn		3.42		3.73		3.33	
Supplement	.84	.55			.23	.13	
TOTAL	7.48	8.27	6.94	8.20	7.62	7.62	
Feed Efficiency:							
Feed per kg gain, kg	10.58	9.26	9.56	8.14	11.05	9.49	
Feed cost per 100 kg gain <sup>1</sup>	\$ 40.74	\$ 39.51	\$ 28.82	\$ 31.70	\$ 33.31	\$ 36.96	
Carcass Evaluation:							
Carcass grade <sup>2</sup>	12.98	14.28	12.57	13.40	12.67	12.72	
Marbling score <sup>3</sup>	15.51	19.01	13.83	16.14	14.61	14.19	
Fat thickness, cm	1.54	1.77	1.67	1.54	1.33	1.21	
Ribeye area, cm <sup>2</sup>	69.94	64.88	64.50	69.31	63.31	65.19	
Percent K.H.P. fat <sup>4</sup>	3.62	3.77	3.83	3.00	3.37	2.93	
Percent B.T.R. cuts <sup>5</sup>	49.36	47.87	48.42	49.21	49.44	50.05	
Dressing percent <sup>6</sup>	60.52	61.78	59.59	59.93	57.71	59.05	
Carcass price/100 kg	\$115.70	\$114.82	\$115.48	\$114.69	\$115.04	\$115.30	

<sup>1</sup> Feed costs based on 30% DM corn silage \$9.37/MT, urea-mineral \$88.18/MT, Pro-Sil \$71.65/MT, shelled corn \$49.60/MT, soy-mineral supplement \$110.23/MT.

<sup>2</sup> Good = 9, 10, 11; Choice = 12, 13, 14.

<sup>3</sup> Small = 10, 11, 12; Modest = 13, 14, 15; Moderate = 16, 17, 18.

<sup>4</sup> Percent of carcass weight in kidney, heart and pelvic fat.

<sup>5</sup> Percent of carcass weight in boneless, trimmed retail cuts.

<sup>6</sup> Cold carcass weight over off experiment weight.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

Table 17  
Continued

Corn Silage NPN Additives Compared  
(October 22, 1970 to June 17, 1971)

161 - 238 Day Test	Type of Silage Treatment and Supplement				
	Urea-Mineral Treatment		Urea-Mineral Formic Acid Treatment		
	All Silage	40% Shelled Corn 60% Silage	All Silage	40% Shelled Corn 60% Silage	
Lot No.	41	48	47	46	
No. of steer calves	10	10	10	10	
Av. initial wt., kg	245	248	246	246	
Av. final wt., kg	413	444	414	443	
Av. daily gain, kg	.76	.99	.77	1.00	
Daily Feed, kg 85% DM:					
Corn silage	7.19	4.75	7.24	4.92	
Gr. sh. Corn Supplement		3.67		3.94	
TOTAL	7.19	8.42	7.24	8.86	
Feed Efficiency:					
Feed per kg gain, kg	9.43	8.48	9.44	8.83	
Feed cost per 100 kg gain <sup>1</sup>	\$ 28.20	\$ 32.82	\$ 32.80	\$ 36.52	
Carcass Evaluation:					
Carcass grade <sup>2</sup>	13.32	12.88	12.42	13.58	
Marbling score <sup>3</sup>	16.39	14.01	13.39	16.21	
Fat thickness, gm	1.36	1.74	1.10	1.56	
Ribeye area, cm <sup>2</sup>	69.25	68.56	71.63	68.69	
Percent K.H.P. fat <sup>4</sup>	3.43	3.47	2.63	3.02	
Percent B.T.R. cuts <sup>5</sup>	49.75	48.23	51.06	48.99	
Dressing percent <sup>6</sup>	59.41	61.05	58.60	60.24	
Carcass price/100 kg	\$115.85	\$114.60	\$116.95	\$114.93	

Table 18

Corn Silage NPN Additives Compared  
(October 22, 1970 to June 17, 1971)

161 - 238 Day Test	Type of Silage Treatment and Supplement			
	Control Soy-mineral Supplement	Pro-Sil Treated	No Treatment Pro-Sil Supplement	Urea- Mineral Treated
No. of steer calves	20	18	20	20
Av. initial wt., kg	246	246	245	246
Av. final wt., kg	412	422	400	430
Av. daily gain, kg	.80 <sup>ABb</sup>	.87 <sup>Aa</sup>	.75 <sup>Bb</sup>	.89 <sup>Aa</sup>
Daily Feed, kg 85% DM:				
Corn silage	5.47	5.71	5.78	5.97
Gr. sh. corn	1.71	1.86	1.66	1.84
Supplement	.70		.18	
TOTAL	7.88	7.57	7.62	7.81
Feed Efficiency:				
Feed per kg gain, kg	9.88	8.72	10.19	8.89
Feed cost per 100 kg gain <sup>1</sup>	\$ 40.08	\$ 30.18	\$ 35.40	\$ 30.60
Carcass Evaluation:				
Carcass grade <sup>2</sup>	13.63 <sup>a</sup>	12.99 <sup>ab</sup>	12.70 <sup>b</sup>	13.10 <sup>ab</sup>
Marbling score <sup>3</sup>	17.26 <sup>a</sup>	14.98 <sup>ab</sup>	14.40 <sup>b</sup>	15.20 <sup>ab</sup>
Fat thickness, cm	1.67 <sup>a</sup>	1.59 <sup>ab</sup>	1.26 <sup>c</sup>	1.56 <sup>abc</sup>
Ribeye area, cm <sup>2</sup>	67.38	66.88	64.31	68.88
Percent K.H.P. fat <sup>4</sup>	3.70 <sup>Aa</sup>	3.42 <sup>Aa</sup>	3.17 <sup>ABb</sup>	3.45 <sup>Aa</sup>
Percent B.T.R. cuts <sup>5</sup>	48.61	48.82	49.75	48.99
Dressing percent <sup>6</sup>	61.15	59.76	58.38	60.23
Carcass price/100 kg	\$115.26	\$115.08	\$115.19	\$115.24
				\$115.94

<sup>1</sup>Feed costs based on 30% DM corn silage \$9.37/MT, urea-mineral \$88.18/MT, Pro-Sil \$71.65/MT, shelled corn \$49.60/MT, soy-mineral supplement \$110.23/MT.

<sup>2</sup>Good = 9, 10, 11; Choice = 12, 13, 14.

<sup>3</sup>Small = 10, 11, 12; Modest = 13, 14, 15; Moderate = 16, 17, 18.

<sup>4</sup>Percent of carcass weight in kidney, heart and pelvic fat.

<sup>5</sup>Percent of carcass weight in boneless, trimmed retail cuts.

<sup>6</sup>Cold carcass weight over off experiment weight.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).



low and middle Choice; however, the group fed Pro-Sil supplemented silage was significantly ( $P < .05$ ) lower in carcass grade and marbling score than the control group. The Pro-Sil supplemented silage fed group had significantly ( $P < .05$ ) less fat thickness than the groups fed control and Pro-Sil treated silage. The steers fed urea-mineral plus formic acid treated silage also had significantly ( $P < .05$ ) less fat thickness than those fed control silage. Percent kidney, heart and pelvic fat significantly favored the Pro-Sil supplemented ( $P < .01$ ) and urea-mineral plus formic acid treated silage ( $P < .05$ ) fed groups. Other carcass traits were small and insignificant.

All Silage Ration vs. 40% Shelled Corn and 60% Silage Ration

Table 19 shows mean values for performance and carcass traits summarized across level of silage in the ration for Experiments I and II combined.

Average daily gain was significantly ( $P < .01$ ) higher for the cattle fed concentrate (.92 kg vs. .73 kg). Higher dry matter intake (8.13 kg vs. 7.40 kg), greater feed efficiency (8.84 vs. 10.14) and higher energy content of the ration containing added shelled corn accounted for the increase in daily gain. This is in agreement with many previous experiments conducted at this station. Cattle fed silage required a 12% longer feeding period to reach slaughter weight and Choice carcass grade (195 days vs. 220 days).

Feed cost per hundred kilograms was lower for the all

silage ration (\$36.06 vs. \$36.76). Using a standard yield of 35 metric tons of 35% DM silage or 5 metric tons of shelled corn per hectare, beef produced per hectare increased from 1149 kg for the 40% shelled corn group to 1730 kg for the all silage group. This represents a 51% increase in beef produced per hectare with an all silage ration. Using the actual selling price of the cattle, gross returns per hectare of corn fed was increased (48%) from \$803 for the 40% shelled corn group to \$1191 for the all silage group.

Cattle fed the all silage had significantly ( $P < .01$ ) greater percent of boneless, trimmed retail cuts and significantly ( $P < .05$ ) lower dressing percent. This was attributed to less fat thickness on the cattle fed all silage. The carcass grade of both groups of cattle averaged middle to high Choice; however, small differences significantly ( $P < .01$ ) favored the 40% shelled corn group. Therefore, Choice carcasses with a higher cutability can be produced on an all silage ration. No significant differences were found in other carcass traits.

#### Experiment III - Metabolism Study with Corn Silage Varied in Extent of Fermentation

##### Chemical Analysis of Silage

The three all silage rations used in Experiment I were compared in this metabolic study. The silages were not supplemented with additional nitrogen, but a mineral supplement was fed (Table 8). The chemical analysis of the silages is shown in Table 13 and discussed on pages 73 - 77.

Table 19

All Silage vs. 40% Shelled Corn and 60% Silage Ration  
(Dry Matter Basis)  
(October 22, 1970 to June 17, 1971)

161 - 238 Day Test	All Silage	40% Shelled Corn 60% Silage
No. of steer calves	69	69
Av. initial wt., kg	245	246
Av. final wt., kg	406	426
Av. daily gain	.73 <sup>A</sup>	.92
<u>Daily Feed, kg 85% DM:</u>		
Corn silage	7.00	4.38
Gr. sh. corn		3.51
Supplement	.40	.24
TOTAL	7.40	8.13
<u>Feed Efficiency:</u>		
Feed per kg gain, kg	10.14	8.84
Feed cost per 100 kg gain <sup>1</sup>	\$ 36.06	\$ 36.76
Kg beef produced per hectare corn fed <sup>7</sup>	1730	1149
Grass returns per hectare corn fed <sup>8</sup>	\$1191	\$803
<u>Carcass Evaluation:</u>		
Carcass grade <sup>2</sup>	13.03 <sup>A</sup>	13.61
Marbling score <sup>3</sup>	15.29 <sup>a</sup>	16.79
Fat thickness, cm	1.46	1.56
Ribeye area, cm <sup>2</sup>	67.94	67.44
Percent K.H.P. fat <sup>4</sup>	3.37	3.33
Percent B.T.R. cuts <sup>5</sup>	49.49 <sup>A</sup>	48.87
Dressing percent <sup>6</sup>	59.44 <sup>a</sup>	60.62
Carcass price/100 kg	\$115.83	\$115.17

<sup>1</sup>Feed costs based on 30% DM corn silage \$9.37/MT, urea-mineral \$88.18/MT, Pro-Sil \$71.65/MT, shelled corn \$49.60/MT, soy-mineral supplement \$110.23/MT.

<sup>2</sup>Good = 9, 10, 11; Choice = 12, 13, 14.

<sup>3</sup>Small = 10, 11, 12; Modest = 13, 14, 15; Moderate = 16, 17, 18.

<sup>4</sup>Percent of carcass weight in kidney, heart and pelvic fat.

<sup>5</sup>Percent of carcass weight in boneless, trimmed retail cuts.

<sup>6</sup>Cold carcass weight over off experiment weight.

<sup>7</sup>Based on corn yields of 35 MT of 35% DM silage or 5 MT of shelled corn per hectare.

<sup>8</sup>Based on selling price of cattle.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

### Rumen Ammonia and Blood Urea Concentrations

Mean values for rumen ammonia and blood urea are shown in Tables 20 and 21. The rumen ammonia concentration was highest at  $T_2$  (two hours post feeding) for the groups fed limestone treated and formic acid treated silages. The control silage produced a maximum concentration of ammonia at  $T_4$  and was higher at  $T_4$ ,  $T_6$  and  $T_8$  than the other two treatments. The limestone treated and formic acid treated silages had higher initial ( $T_0$ ) and final ( $T_{10}$ ) rumen ammonia concentrations than the control silage. Blood urea concentrations were highest during the period from four to eight hours post feeding for all three silages. The control silage produced blood urea levels that were higher at all times than the other two silage treatments and the limestone treated group had the lowest blood urea at all times. None of the rumen ammonia or blood urea values were significantly different.

### Rumen VFA Concentrations

Tables 22, 23 and 24 show mean values for acetate, propionate and butyrate concentrations expressed as mg of VFA per 100 ml of rumen fluid. There were no significant differences between treatments. However, the limestone treated silage had significantly ( $P < .01$ ) higher lactic acid concentration than either of the other two silage treatments and resulted in higher rumen acetate levels and more stable propionate levels than the control silage. The fate of lactate in the rumen is unclear. Baldwin et al. (1962) and



Table 20

Mean<sup>1</sup> Rumen Ammonia Values (mg/100 ml)

Time	Silage Treatment			SE <sup>2</sup>
	Control	Formic Acid Treated	Limestone Treated	
T <sub>0</sub>	1.17	2.44	4.10	1.76
T <sub>2</sub>	1.94	3.16	4.26	.77
T <sub>4</sub>	9.24	3.10	1.34	3.53
T <sub>6</sub>	5.44	1.50	2.03	2.69
T <sub>8</sub>	1.80	.73	.86	.64
T <sub>10</sub>	.58	1.20	2.30	.56

<sup>1</sup>Three observations per mean.<sup>2</sup>SE = Standard error of means.

No significant differences between means.

Table 21

Mean<sup>1</sup> Blood Urea Values (mg/100 ml)

Time	Silage Treatment			SE <sup>2</sup>
	Control	Formic Acid Treated	Limestone Treated	
T <sub>0</sub>	5.60	4.30	3.30	.90
T <sub>2</sub>	6.11	4.73	3.83	1.05
T <sub>4</sub>	7.01	5.43	4.33	1.22
T <sub>6</sub>	7.56	5.63	4.30	1.87
T <sub>8</sub>	7.76	5.00	4.16	1.78
T <sub>10</sub>	6.93	4.76	4.00	1.35

<sup>1</sup>Three observations per mean.<sup>2</sup>SE = Standard error of means.

No significant differences between means.

Bruno et al. (1962) used  $^{14}\text{C}$ -lactate in an in vitro fermentation and found acetate to be the main product of lactate fermentation. Others (Waldo et al., 1956; Waldo et al., 1960 and Wallnofer et al., 1966) have found an increase in the proportion of propionate in the rumen contents when rumen lactate concentration is elevated. Satter (1968) reported that both acetate and propionate are important metabolites of ruminal lactate, with propionate increasing in importance as the amount of dietary starch or lactate increases. Acetate is not a terminal end product of lactate metabolism but is used in the synthesis of butyrate.

#### Dry Matter Intake and Dry Matter Digestibility

As shown in Table 25, small differences in intake favored the control silage. These differences approached significance. The dry matter digestibility was not significantly different for the three silage treatments. Lower dry matter intake and digestibility were shown for the formic acid treated than the control silage. This is in disagreement with work done by Waldo et al. (1969) and Thomas et al. (1969) using formic acid treated grass and legume silages. Huber also (mimeo D-235) reported increased dry matter intake of 44% dry matter silage comparing formic acid and control treatments.

The nonsignificant differences in dry matter digestibility of the limestone treated and control silage fed groups was in agreement with previous results summarized by Essig (1968).

Table 22

Mean<sup>1</sup> Rumen Acetate Concentration (mg/100 ml)

Time	Silage Treatment			SE <sup>2</sup>
	Control	Formic Acid Treated	Limestone Treated	
T <sub>0</sub>	289.0	353.0	425.0	33.56
T <sub>2</sub>	246.3	371.0	401.3	39.08
T <sub>4</sub>	374.0	387.7	418.3	31.00
T <sub>6</sub>	391.0	446.3	419.0	14.34
T <sub>8</sub>	385.0	407.3	389.0	12.54
T <sub>10</sub>	400.0	389.7	431.7	49.44

<sup>1</sup>Three observations per mean.<sup>2</sup>SE = Standard error of means.

No significant differences between means.

Table 23

Mean<sup>1</sup> Rumen Propionate Concentrations (mg/100 ml)

Time	Silage Treatment			SE <sup>2</sup>
	Control	Formic Acid Treated	Limestone Treated	
T <sub>0</sub>	71.3	81.0	110.34	15.90
T <sub>2</sub>	67.0	102.0	109.0	8.35
T <sub>4</sub>	149.7	117.3	139.3	33.84
T <sub>6</sub>	147.7	149.3	138.7	49.97
T <sub>8</sub>	125.7	111.0	107.0	22.61
T <sub>10</sub>	102.7	139.3	126.7	8.04

<sup>1</sup>Three observations per mean.<sup>2</sup>SE = Standard error of means.

No significant differences between means.



Table 24

Mean<sup>1</sup> Rumen Butyrate Concentration (mg/100 ml)

Time	Silage Treatment			SE <sup>2</sup>
	Control	Formic Acid Treated	Limestone Treated	
T <sub>0</sub>	50.0	60.3	68.0	15.27
T <sub>2</sub>	44.3	60.3	59.0	16.73
T <sub>4</sub>	58.3	68.0	62.0	15.21
T <sub>6</sub>	58.3	81.0	65.7	13.48
T <sub>8</sub>	63.7	80.7	63.0	7.42
T <sub>10</sub>	60.0	83.3	71.0	11.79

<sup>1</sup>Three observations per mean.<sup>2</sup>SE = Standard error of means.

No significant differences between means.

### Nitrogen Balance

The mean values for all nitrogen balance parameters are shown in Table 25. The nitrogen intake varied considerably for the three silages (control - 141.0 g/day, formic acid treatment - 132.8 g/day and limestone treatment - 116.1 g/day), but differences were not significant. The increase in nitrogen intake for the control parallels the increase in dry matter intake and the difference in the latter two treatments was probably due to the elevated crude protein content of the formic acid treated silage (see Table 13).

The differences in nitrogen digestibility of the three silages (control - 70.6%, formic acid treatment - 47.3% and limestone treatment - 54.5%) were not significantly different.

Nitrogen digested (g/day), nitrogen retained (g/day) and nitrogen retained as a percent of nitrogen intake were significantly higher ( $P < .01$ ) for the control silage, compared to the two treated silages. Other nitrogen balance parameters were not significantly different.

#### Experiment IV - Nitrogen Balance Study with NPN Silage Additives

### Chemical Analysis of Silage

The four all silage rations utilized in this experiment were identical to the all silage rations utilized in Experiment II. The chemical analysis of the silages is shown in Table 16 and discussed on pages 81 - 86.



Table 25

## Effects of Silage Additives on Digestion Parameters

	Control	Silage Treatment		SE <sup>1</sup>
		Formic Acid Treatment	Limestone Treatment	
No. of steers	3	3	3	
DM intake, g/day	9675	8493	8510	185.4
DM digested, g /day	7379	5725	6244	215.7
Percent DM digested, %	75.3	64.9	73.0	3.24
Nitrogen intake, g/day	141.0	132.8	116.1	6.43
Fecal nitrogen, g/day	42.8	65.4	51.8	6.69
Nitrogen digested, g/day	98.2 <sup>A</sup>	67.3	64.4	3.64
Percent nitrogen digested, %	70.6	47.3	54.5	6.13
Urinary nitrogen, g/day	34.2	33.8	37.4	5.51
Nitrogen retained, g/day	64.0 <sup>A</sup>	33.6	27.0	2.48
N retained as % of N intake, %	44.1 <sup>A</sup>	21.0	22.1	1.25
N retained as % of N digested, %	65.2	49.9	41.9	

<sup>1</sup>SE = Standard error of means.

Note: Three observations per mean.

Values with no superscript or having the same superscript are not significantly different, A = (P &lt; .01), a = (P &lt; .05).



27

pa

di

W

h

z

S

ti

N

i

t

u

8

m

f

U

S

h

r

t

### Dry Matter Intake and Dry Matter Digestibility

Mean values for dry matter intake and digestibility parameters are shown in Table 26. Dry matter intake and dry matter digestibility were lowest for the silage treated with urea-mineral plus formic acid. The other three rations had similar dry matter intakes and higher dry matter digestibilities. The dry matter digestibility of the control, Pro-Sil treated and urea-mineral treated silages are similar to those obtained by Beattie (1970).

### Nitrogen Balance

Results of all nitrogen balance parameters are shown in Table 26. Daily nitrogen intake was similar for all three NPN treated silage rations (Pro-Sil - 87.0 g/day, urea-mineral - 93.3 g/day and urea-mineral plus formic acid - 86.5 g/day) and higher for the control soy-mineral supplemented silage ration (104.4 g/day).

Nitrogen digestibility was not significantly different for the four treatments (control - 70.7, Pro-Sil - 71.8, urea-mineral - 63.5 and urea-mineral plus formic acid - 55.3). All silage treatments produced a positive nitrogen balance. Although differences in nitrogen digestion parameters did exist, they were not significant.

### Experiment V - Silage Fermentation Study

Analysis of silage samples taken fresh and on days 1 through 10, 15, 20, 30, 60 and 90 after ensiling for the

Table 26  
Effects of Silage NPN Additives on Digestion Parameters

	Control Soy-mineral Supplement	Silage Treatment			SE <sup>1</sup>
		Pro-Sil Treated	Urea- Mineral Treated	Urea-mineral Formic Acid Treated	
No. of steers	4	4	4	4	
DM intake, g/day	4445	4410	4410	3766	222.7
DM digested, g/day	3237	3216	3342	2503	192.2
Percent DM digested, %	69.5	71.1	72.7	61.6	2.04
Nitrogen intake, g/day	104.4	87.0	93.3	86.5	7.41
Fecal nitrogen, g/day	26.7	24.1	28.0	30.3	1.03
Nitrogen digested, g/day	77.6	62.9	65.3	56.2	6.38
Percent nitrogen digested, %	70.7	71.8	63.5	55.3	4.10
Urinary nitrogen, g/day	47.0	43.5	50.8	43.4	3.88
Nitrogen retained, g/day	30.6	19.3	14.6	12.8	3.46
N retained as % of N intake, %	20.6	20.8	4.6	- 8.8	8.66
N retained as % of N digested, %	39.4	30.7	22.4	22.8	

<sup>1</sup>SE = Standard error of means.

Note: Four observations per mean.

No significant differences between means.

control and Pro-Sil treated silages are shown in Tables 27 and 28, respectively. Experimental silos were used and the results may not fully apply to farm size silos.

#### Control Silage Fermentation Parameters

As shown in Table 27, pH decreased rapidly from 5.72 in fresh material to 4.65 on the first day of fermentation. The reduction in pH was variable from day one through day twenty and then appeared to level off at approximately 4.00. This is in agreement with data reported by Geasler (1970).

There was a large increase in lactic acid production during the first day of fermentation (Figure 4). It appears that there was a trend for lactic acid production to continue to increase but at a much slower rate through day ten. Geasler (1970) reported similar trends. The rate of lactate production increased again between day ten and fifteen and remained at a level of approximately 4.00% of silage dry matter. Barnett (1954) concluded that lactic acid increased at a slow rate during phase one and two (day one to day two) but at an accelerated rate during phase three and four of fermentation.

The high level of acetic acid in the fresh material is not readily explainable. The fresh sample was taken approximately one hour after field chopping. The acetic acid production was variable up to about day nine and then appeared to remain steady at approximately 1.50% of silage dry matter rather than decrease as reported by Geasler (1970). Barnett (1954) reported acetic acid production was rapid

during phases one and two of fermentation and continued at a slower rate thereafter.

Soluble nitrogen and soluble NPN, expressed as a percent of dry matter, had a rapid initial increase (fresh - day one) and then leveled off until about day fifteen, when another increase occurred which held fairly constant through day ninety. The ammonia concentration followed the trend of soluble NPN except that it did not increase until day three. All of the initial increase in soluble NPN appears to be due to increases in the unidentified nitrogen compounds. Geasler (1970) reported rapid increases in water soluble nitrogen and water soluble NPN initially. A more accurate comparison of the nitrogen fractions is to express soluble nitrogen as a percent of total nitrogen as shown in Figure 4. It is apparent that the majority of the proteolytic action occurs early in the fermentation process (fresh - day two) and then tends to continue at a slow rate through day ninety. The variability of the results may be due to individual experimental silo variation.

Van Soest (1965) reported that when the cell wall constituents (CWC) made up 50 to 60% of the forage dry matter, they appeared to limit intake. The cell wall constituents in the control silage (Table 29) varied from 43.92 to 51.02% of the silage dry matter; therefore, CWC should not have limited dry matter intake. Van Soest (1962) showed that the nitrogen in the acid detergent fiber (ADF) is essentially indigestible; therefore, ADF was determined and was fairly

Table 27

Means<sup>1</sup> of Control Silage Fermentation Parameters by Days  
(All values expressed on a dry matter basis)

	Fresh	1	2	Day of Fermentation			5	6	7
				3	4				
pH	5.72	4.65	4.23	4.08	4.42		4.33	4.17	4.00
% Dry Matter, %	31.12	32.26	32.21	32.59	29.76		30.91	33.27	31.39
% Nitrogen, %	1.24	1.40	1.36	1.22	1.18		1.34	1.16	1.38
% Insoluble N, %	.85	.92	.87	.71	.67		.85	.65	.85
% H <sub>2</sub> O Soluble N, %	.39	.48	.49	.51	.51		.49	.51	.53
% H <sub>2</sub> O Soluble NPN, %	.31	.45	.46	.48	.49		.47	.49	.48
% NH <sub>3</sub> -N, %	.024	.023	.026	.039	.037		.030	.035	.036
% Unidentified N, %	.29	.43	.43	.44	.45		.44	.45	.44
% Lactic Acid, %	0	1.60	2.14	2.37	1.88		2.52	2.24	4.28
% Acetic Acid, %	.60	.86	1.01	1.00	.81		1.10	.59	.95

<sup>1</sup>Mean of 3 observations.

Table 27  
Continued

Means<sup>1</sup> of Control Silage Fermentation Parameters by Days  
(All values expressed on a dry matter basis)

	8	9	10	Day of Fermentation				30	60	90
				15	20					
pH	4.48	4.38	4.52	4.50	4.25	3.84	3.82	4.05		
% Dry Matter, %	32.68	32.45	32.06	31.06	30.96	32.98	29.98	30.24		
% Nitrogen, %	1.46	1.24	1.24	1.49	1.40	1.35	1.48	1.39		
% Insoluble N, %	.96	.72	.73	.90	.84	.81	.89	.80		
% H <sub>2</sub> O Soluble N, %	.50	.52	.51	.59	.56	.54	.59	.59		
% H <sub>2</sub> O Soluble NPN, %	.49	.49	.49	.58	.50	.51	.58	.57		
% NH <sub>3</sub> -N, %	.033	.034	.034	.040	.041	.041	.050	.053		
% Unidentified N, %	.46	.46	.46	.54	.46	.47	.53	.52		
% Lactic Acid	2.55	2.50	2.34	3.34	2.98	3.67	4.82	4.12		
% Acetic Acid	1.95	1.42	1.84	1.41	1.54	1.53	2.17	1.62		

<sup>1</sup>Mean of 3 observations.





constant at about 25% of DM. The nitrogen contained in the ADF residue decreased as the experiment progressed. Little significance can be ascribed to the reduction in ADF residue nitrogen since it comprises such a small percent of the total nitrogen (3.92 to 5.39%).

#### Pro-Sil Treated Silage Fermentation Parameters

A fresh Pro-Sil treated silage sample was not taken; therefore, the pH of the fresh sample was unavailable. Theoretical nitrogen parameters were calculated for the fresh sample based on the amount of Pro-Sil added. All values are shown in Table 28.

The pH for the Pro-Sil treated silage was about 3 units higher than the control silage on day one. The pH dropped over the ninety-day experiment to a low of 4.08 on day ninety. The pH was higher for Pro-Sil treated silage at all times as was expected due to the neutralizing action of the ammonia contained in the Pro-Sil.

The rate of lactic acid production was essentially equivalent to that of the control silage for the first two days and then it exceeded the control on all but one sampling time (Figure 4). The neutralizing effect of the ammonia seemed to have its primary effect on lactic acid production. The increased lactate is in agreement with other results reported by Henderson (1971e).

The acetic acid levels in the Pro-Sil treated silage are approximately the same as those of the control silage. Henderson (1971e) and Beattie et al. (1971) reported that acetic

acid content decreased with Pro-Sil addition.

Total nitrogen, water soluble nitrogen, water soluble NPN, water insoluble nitrogen and ammonia nitrogen were all higher in the Pro-Sil treated silage than in the control silage. This was in agreement with earlier work reported by Henderson (1971e). Most of the increase in soluble NPN was due to the increase in ammonia nitrogen; however, the unidentified nitrogen levels in the Pro-Sil treated silage were slightly higher than in the control silage and appeared to be fairly constant throughout the experiment. The water insoluble nitrogen level in the Pro-Sil treated silage increased to its maximum at day five when the soluble nitrogen (% of total nitrogen) was at a low level (Figure 4).

Since the unidentified nitrogen was slightly higher in the Pro-Sil treated silage than in the control silage, the elevated insoluble nitrogen level does not appear to be due to a reduction in proteolysis. Therefore, these data suggest that some soluble nitrogen may be incorporated into microbial protein, hence, lowering the soluble nitrogen fraction (% of total nitrogen) and increasing the insoluble nitrogen level. Everson et al. (1971) and Rayetskaya et al. (1964) used  $^{15}\text{N}$  urea and reported that microbial protein synthesis accounted for the increased insoluble nitrogen in NPN treated silages. The increase in water soluble nitrogen (% of total nitrogen) after day eight might be due to degradation of protein. The amount of insoluble nitrogen in the Pro-Sil treated silage, after the apparent degradation,

Table 28

Means<sup>1</sup> of Pro-Sil1 Treated Silage Fermentation Parameters by Days  
(All values expressed on a dry matter basis)

	Fresh	1	2	Day of Fermentation				5	6	7
				3	4					
pH	-----	7.80	7.35	5.10	4.72			4.60	4.53	5.03
% Dry Matter, %	31.12	31.69	33.72	33.03	34.85			31.80	33.23	33.87
% Nitrogen, %	2.00	1.90	2.14	1.84	1.92			2.21	1.97	1.91
% Insoluble N, %	.85	.75	.94	1.04	1.11			1.23	1.10	1.15
% H <sub>2</sub> O Soluble N, %	1.15	1.15	1.20	.80	.81			.98	.87	.76
% H <sub>2</sub> O Soluble NPN, %	1.07	1.15	1.20	.80	.80			.97	.86	.74
% Unidentified N, %	.29	.69	.71	.50	.50			.59	.54	.48
% NH <sub>3</sub> -N, %	.78	.457	.488	.298	.296			.384	.316	.263
% Lactic Acid, %	0	1.47	1.47	3.87	3.10			3.92	4.12	3.54
% Acetic Acid, %	.60	.57	.43	.96	.90			1.00	.96	.83

<sup>1</sup>Mean of 3 observations.



Table 28  
Continued  
Means<sup>1</sup> of Pro-Sil Treated Silage Fermentation Parameters by Days  
(All values expressed on a dry matter basis)

	Day of Fermentation						
	8	9	10	15	20	30	90
pH	4.60	4.40	4.62	5.20	5.10	4.40	4.08
% Dry Matter, %	34.91	33.01	32.05	34.36	33.21	32.97	32.26
% Nitrogen, %	1.80	1.66	1.76	2.09	2.06	1.70	1.69
% Insoluble N, %	1.00	.88	.91	1.00	.90	.89	.83
% H <sub>2</sub> O Soluble N, %	.80	.78	.85	1.09	1.16	.81	.86
% H <sub>2</sub> O Soluble NPN, %	.78	.81	.81	1.09	1.14	.78	.86
% Unidentified N, %	.50	.51	.49	.63	.70	.51	.59
% NH <sub>3</sub> -N, %	.279	.300	.321	.464	.436	.272	.267
% Lactic Acid, %	3.55	3.82	4.78	4.31	4.45	5.51	6.64
% Acetic Acid, %	1.25	1.18	.83	1.34	1.41	1.59	2.14

<sup>1</sup>Mean of 3 observations.



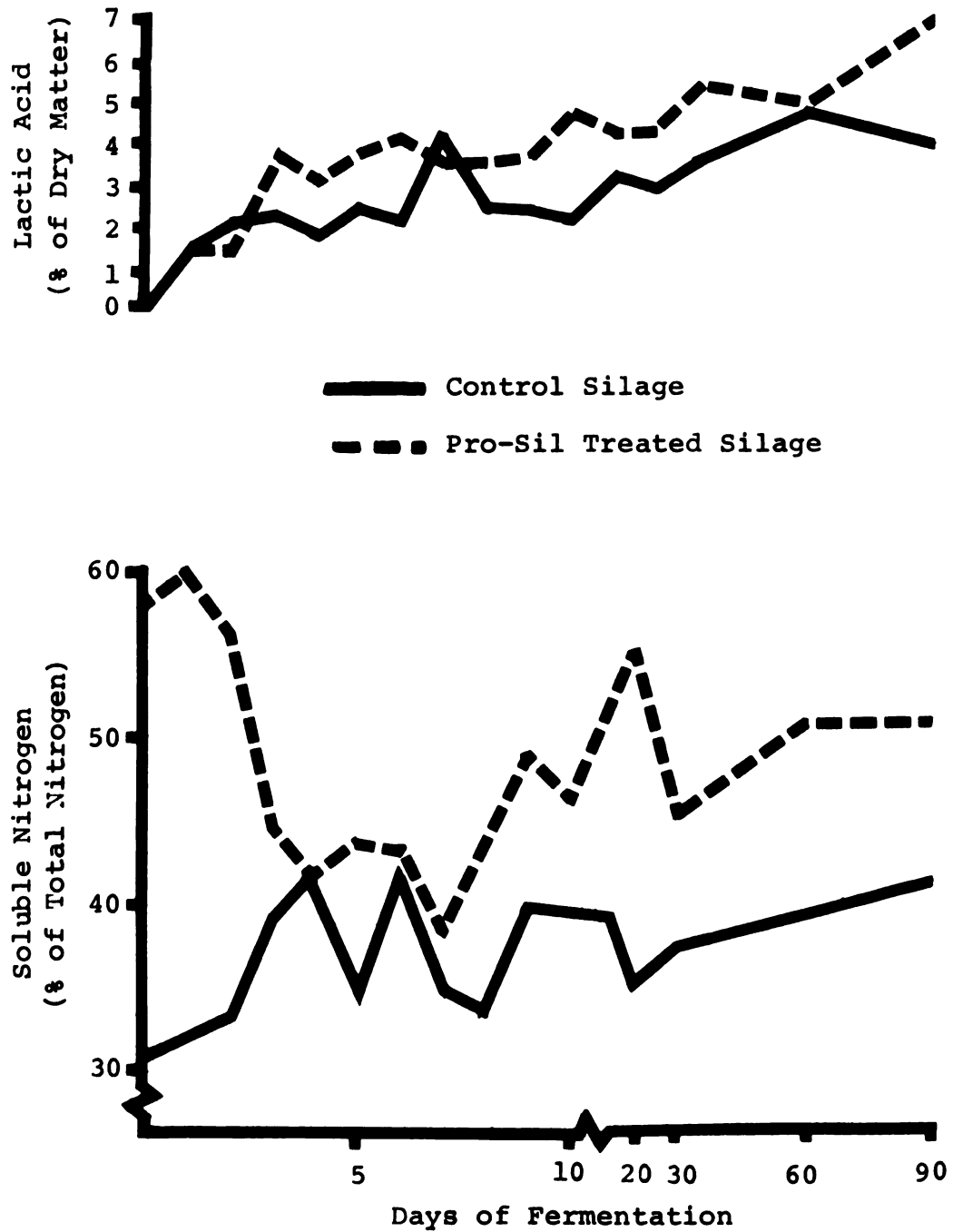


Figure 4. Changes in water soluble nitrogen and lactic acid concentration during fermentation.

wa

si

si

ma

va

me

on

sl

pi

to

sp

c.

in

de

s.

co

s.

Th

ra

is



was still greater than the insoluble nitrogen in the control silage. Increased insoluble nitrogen in Pro-Sil treated silage has been reported by Beattie (1970), Huber and Hillman (1970) and Henderson (1971e).

The cell wall constituent fraction and ADF nitrogen values are shown in Table 29. The discussion of these parameters in the control silage is applicable here, also. The one difference was that the nitrogen in the ADF residue was slightly higher than in the control silage.

#### Experiment VI - Intake, Metabolic and In Vitro Studies of Corn Silage Containing Varying Levels of Unidentified Soluble Nitrogen

##### Pilot Experiment

A pilot experiment was conducted in 1970 in an attempt to determine whether plant enzymes and/or bacteria were responsible for proteolysis (N solubilization) in corn silage.

Another purpose of this experiment was to evaluate autoclaving as a method of preventing proteolysis, thus altering the level of unidentified nitrogen compounds that may depress voluntary dry matter consumption.

Autoclaved silage (A) and autoclaved and inoculated silage (AI) were compared with fresh unfermented chopped corn (F) and control untreated silage (C).

The other two silage treatments studied were irradiated silage (I) and irradiated and inoculated silage (II). These two silages were irradiated with three megarads of radiation. This level of radiation was chosen because it is sufficient to kill all vegetative bacteria without

Table 29

## Cell Wall Constituents and ADF Nitrogen Values

Day of Fermentation	Cell Wall Constituents (% DM)	ADF (% DM)	% Nitrogen in ADF	Total Nitrogen (% DM)	% Total Nitrogen in ADF
Control Silage					
Fresh	48.57	25.82	.070	1.24	5.65
1	43.92	23.06	.071	1.40	5.10
5	47.02	24.24	.072	1.34	5.39
10	50.21	24.91	.068	1.74	3.92
30	49.56	24.49	.061	1.35	4.50
90	51.02	28.50	.058	1.39	4.19
Pro-Sil Treated Silage					
1	49.04	24.80	.099	1.90	5.23
5	42.90	22.58	.099	2.21	4.47
10	52.74	28.15	.083	1.76	4.71
30	45.18	23.98	.071	1.70	4.20
90	42.98	22.90	.066	1.69	3.91

harming plant enzymes (IAEA, 1970).

The AI and II silages were inoculated with 10% by weight of a silage in the third day of fermentation.

The A and I silage treatments that were not inoculated produced no bacterial growth in fluid thioglycollate medium. Bacterial growth was produced in fluid thioglycollate medium in a 24 hour incubation period with the following silage treatments: C, AI and II. The fermentation data (Table 30) and bacterial growth indicate that the attempted sterilization and reinoculation were successful.

The AI silage and the II silage did undergo fermentation as revealed by the lactic and acetic acid concentrations, which were essentially equal to the C silage. The A and I silages (not inoculated) contained no lactate and low levels of acetate indicating the absence of fermentation. This was expected since autoclaving and irradiating are bactericidal processes.

The inclusion of bacteria in the II silage resulted in no increase in water soluble NPN as a percent of total nitrogen compared to the I silage. The two irradiated silages and the C silage produced similar amounts of water soluble NPN as a percent of total nitrogen ( $\sim 27\%$ ). Therefore, it appears that plant enzymes were responsible for the proteolysis. Further evidence is suggested by the absence of proteolysis in the A and AI silages. The level of water soluble NPN as a percent of total nitrogen was essentially the same for the fresh and two autoclaved silages ( $\sim 11\%$ ).

Table 30

## Pilot Experiment Altering Unidentified Nitrogen Levels

	Silage Treatment				
	Fresh	Control	Autoclaved	Inoculated and Autoclaved	Irradiated and Inoculated
Dry matter, %	41.5	42.9	41.2	41.8	41.6
pH	5.63	3.98	5.10	3.72	3.84
<u>Nitrogen Fractions:</u>					
Total nitrogen, %	1.18	1.18	1.08	1.41	1.32
Insoluble nitrogen, %	.99	.82	.94	1.27	.94
Soluble nitrogen, %	.19	.36	.14	.14	.38
Soluble NPN, %	.13	.32	.14	.14	.34
Soluble NPN					
% of total nitrogen, %	11.0	27.0	13.0	9.9	25.8
NH <sub>3</sub> -N, %	.024	.038	.009	.014	.048
Unidentified nitrogen, %	.11	.28	.13	.13	.29
<u>Organic Acids:</u>					
Lactic acid	0	4.43	0	4.12	4.51
Acetic acid	0	.44	.12	.64	.82

Mean of 3 values.

Values are expressed as percent of dry matter unless stated otherwise.

Russell (1908) and Kirsch (1930) demonstrated that autoclaving silage, a process that denatures enzymes and sterilizes the silage, prevented proteolysis even if inoculated with bacteria. Hunter (1921); Kirsch (1930), and Mabbitt (1951) also reported that plant enzymes were responsible for proteolysis in silages.

Russell (1908) suggested that plant enzymes degrade protein to amino acids and that microorganisms may attack the nitrogenous products carrying them beyond the stage reached by plant enzymes. The amino acid content in the two irradiated silages was not evaluated; however, Bergen and Henderson (unpublished) have shown that amino acids compose less than .1% of corn silage dry matter. Hughes (1970a) reported that amino acids composed 63% of the NPN in grass silage ensiled for two months.

Since the AI silage resulted in fermentation essentially equal to the C silage in the absence of proteolysis, it was decided to use this silage treatment in Experiment VI. Other treatments altering the unidentified nitrogen content of silage were also compared in Experiment VI.

#### Chemical Analysis of Silage

Results of four composite analyses of the silages are shown in Table 31.

#### Dry Matter

The dry matter means were significantly different ( $P < .01$ ) and this difference was expected due to intended drying.

The dry matter levels for the four silage treatments were: control - 31.68%, autoclaved - 32.85%, sun-dried - 52.13% and air-dried - 84.51%.

### Nitrogen Fractions

The silages were chopped at the same time and allowed to dry on a paved surface (52% DM) or in a crop drier (85% DM); therefore, stage of maturity was not a factor and differences in nitrogen content of the silages were not anticipated. The means for total nitrogen did not differ significantly.

Geasler (1970) and Byers and Ormiston (1966) reported significant decreases in total nitrogen as stage of maturity and consequently dry matter increased. The total nitrogen content expressed as a percent of dry matter (% DM) ranged from 1.34% to 1.43% and is within the range reported by Geasler (1970) and Gorb and Lebedinski (1960) for corn silage.

Water insoluble nitrogen values (% DM) approached significance and when expressed as a percent of total nitrogen (% TN) the differences became significant ( $P < .01$ ). The correlation between silage dry matter and water insoluble nitrogen was significant ( $r = .50$ ,  $P < .05$ ). Hawkins (1969) using alfalfa silage and Geasler (1970) working with corn silage reported similar trends. The percent of water insoluble nitrogen (% TN) in the autoclaved silage was significantly ( $P < .01$ ) higher than the control silage and significantly ( $P < .05$ ) lower than the air dried silage.

an

an

co

co

wa

co

be

(.

an

(r

tr

of

n.

se

n.

t

c

l

H

d

s

B

m

The water soluble nitrogen levels (% DM) ranged from .28% to .55% and were significantly lower in the air dried and autoclaved silages than in the control silage ( $P < .01$ ) and the sun dried silage ( $P < .05$ ). The air dried silage contained only 50% of the water soluble nitrogen that was contained in the control silage. Water soluble nitrogen was reduced 33% in the autoclaved silage compared to the control silage. The difference in water soluble nitrogen between the autoclaved (.37%) and the air dried silage (.28%) was significant ( $P < .05$ ). Water soluble nitrogen and silage dry matter had a significant negative correlation ( $r = -.64$ ,  $P < .01$ ).

Since total nitrogen of all four silages was essentially the same, the water soluble nitrogen expressed as a percent of total nitrogen followed the changes of water soluble nitrogen expressed as a percent of dry matter. The water soluble nitrogen represented 27.61% and 19.58% of total nitrogen in the autoclaved and air dried silage, respectively; the difference was significant ( $P < .05$ ). Both the autoclaved and air dried silages had significantly ( $P < .01$ ) less water soluble nitrogen (% TN) than the control silage. Hawkins (1969) and Geasler (1970) reported that protein degradation during ensiling measured by increased water soluble nitrogen (% TN) decreased as dry matter increased. Brody (1965) also reported higher protein hydrolysis in moist silage than in drier silage.

Autoclaving reduced but did not inhibit proteolysis as



was the case in the pilot experiment. There are two possible explanations: either the silage was not autoclaved properly or proteolysis occurred prior to autoclaving. The later possibility seems most likely since data reported in Experiment V and by Geasler (1970) indicated that proteolysis is rapid during the first day of fermentation.

There were small but significant differences between silages in the ammonia levels (% DM); however, ammonia (% TN) was so small (range 1.50% to 4.00% of total nitrogen) that it appeared unimportant. The ammonia values observed for the control, autoclaved and sun dried silages are similar to those found by Henderson (1971c) and (1971e).

The unidentified nitrogen levels of the four treatments are shown in Table 31 and follow the trend of water soluble nitrogen levels. The air dried silage resulted in a significantly ( $P < .01$ ) lower level of unidentified nitrogen compounds than all other treatments. The autoclaved silage had significantly lower unidentified nitrogen content than the control silage ( $P < .01$ ) and the sun dried silage ( $P < .05$ ). The latter two treatments were not significantly different. The unidentified nitrogen expressed as a percent of soluble nitrogen did not vary greatly (80% to 85%) because changes in soluble nitrogen and unidentified nitrogen paralleled each other. A more meaningful expression is the percent of total nitrogen contained in the unidentified nitrogen fraction and these values were: control - 33.89, autoclaved - 22.81, sun dried - 29.55 and air dried - 15.95.

It is apparent that increasing the dry matter content of silage decreased the unidentified nitrogen (% TN) and these two silage parameters were significantly and negatively correlated ( $r = -.60$ ,  $P < .05$ ). The air dried silage had approximately 50% less unidentified nitrogen (% TN) than the control and sun dried silages. The unidentified nitrogen (% TN) in the control silage was approximately 34% and this is in agreement with values reported by Henderson et al. (1971c) of 30%.

Analyses of cell wall constituents and acid detergent fiber (ADF) in the four silages are shown in Table 32. The results did not vary greatly. The percent of the total nitrogen contained in the ADF fraction was reduced approximately 60% in the control and sun dried silages and 37% in the air dried silage compared to the autoclaved silage. The autoclaved silage contained 13.81% of the total nitrogen in ADF fraction and the air dried silage contained 8.53%. Nitrogen contained in the ADF is essentially indigestible as reported by Van Soest (1962).

### Organic Acids

Total organic acid content of the silage (% DM) and silage dry matter had a significant negative correlation ( $r = -.87$ ,  $P < .01$ ). The range in total acid content was from .03% to 12.16% of silage dry matter. It is apparent that the air dried silage did not undergo fermentation as revealed by virtually no organic acids and consequently a higher pH. The other three silages did undergo fermentation

wi

re

ac

by

te

ag

of

(.

s.

fe

to

to

w

s

t

v

z

v

o

with all three having higher lactic acid levels than reported by Barnett (1954) and Watson and Nash (1960). The acetic acid concentrations are near expected values reported by the above researchers. The pH paralleled the acid content of the silages. The acid concentrations illustrate again that there is less fermentation with increasing levels of dry matter as reported by Hawkins (1969) and Geasler (1970). In this experiment the acid content of the control, sun dried and air dried silages were all significantly different either at ( $P < .01$ ) or ( $P < .05$ ). It is important to note that the autoclaved silage did have less lactic and total acid content than the control; however, the difference was not significant. The acetic acid in the autoclaved silage in this experiment was significantly ( $P < .05$ ) lower than in the control silage.

### Studies In Vivo

#### Water Balance Trial

Water balance data are shown in Table 33. Free water intake by the sheep differed significantly ( $P < .01$ ) for the four silage treatments. Silage dry matter and free water intake were significantly correlated ( $r = .86$ ,  $P < .01$ ). The sheep fed the air dried silage drank 90% of their total water intake and the sheep on the control and autoclaved silages drank approximately 14% of their total water intake. The silage water intake of the sheep fed the control and autoclaved silages exceeded the total water intake of the sheep fed the air dried silage by an average of 14%.

Table 31  
Average<sup>1</sup> Chemical Analysis on Dry Matter Basis of Silages

Observation	Silage Treatment			
	Control	Autoclaved	Sun Dried	Air Dried
Percent dry matter	31.68	32.85	52.13 <sup>B</sup>	84.51 <sup>A</sup>
<u>Nitrogen Fractions:</u>				
Total nitrogen	1.42	1.34	1.37	1.43
Insoluble N	.87	.97	.89	1.15
Soluble N	.55 <sup>Aa</sup>	.37 <sup>Bb</sup>	.48 <sup>ABa</sup>	.28 <sup>Bc</sup>
NH <sub>3</sub> -N	.053 <sup>Aa</sup>	.043 <sup>ABa</sup>	.055 <sup>Aa</sup>	.020 <sup>Bb</sup>
Unidentified N	.47 <sup>Aa</sup>	.30 <sup>Bb</sup>	.40 <sup>ABa</sup>	.23
Insoluble N as % of total N	60.29 <sup>C</sup>	71.77 <sup>ABb</sup>	64.25 <sup>Bbc</sup>	80.53 <sup>Aa</sup>
Soluble N as % of total N	38.73 <sup>Aa</sup>	27.61 <sup>ABb</sup>	35.04 <sup>Aab</sup>	19.58 <sup>Bc</sup>
NH <sub>3</sub> -N as % of soluble N	9.55	11.77	11.32	7.20
NH <sub>3</sub> -N as % of total N	3.70	3.13	3.99	1.54 <sup>A</sup>
Unidentified N as % of soluble N	85.07	80.01 <sup>BC</sup>	82.42 <sup>AB</sup>	81.82
Unidentified N as % of total N	33.89 <sup>A</sup>	22.81 <sup>BC</sup>	29.55 <sup>AB</sup>	15.95 <sup>C</sup>
<u>Organic Acid Fractions:</u>				
Total organic acid	12.16 <sup>a</sup>	10.26 <sup>ab</sup>	8.31 <sup>b</sup>	0.03 <sup>A</sup>
Lactic acid	10.70 <sup>a</sup>	9.45 <sup>ab</sup>	7.45 <sup>b</sup>	0.00 <sup>A</sup>
Acetic acid	1.46 <sup>a</sup>	0.81 <sup>b</sup>	0.86 <sup>b</sup>	0.03 <sup>A</sup>
pH	3.68	3.71	4.36	5.57
				0.42

<sup>1</sup>Mean of 4 observations.

<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

Table 32

## Cell Wall Constituents and ADF Nitrogen Values

	Control	Silage Treatments		
		Autoclaved	Sun Dried	Air Dried
Cell wall constituents, %	49.73	51.57	55.80	46.77
Acid detergent fiber, %	25.70	27.18	29.09	23.87
ADF nitrogen, %	.069	.185	.072	.122
Total nitrogen, %	1.42	1.34	1.37	1.43
ADF N as % of total N, %	4.86	13.81	5.26	8.53

Sheep fed the 52% dry matter sun dried silage had a total water intake that was less than the silage water intake of the control silage fed sheep and equal to the silage water intake of the autoclaved silage fed sheep. Therefore, sheep fed the control and autoclaved silages were at the point of being forced to increase their water intake by eating.

Water intake (ml per g of dry matter intake) was significantly different ( $P < .01$ ) and decreased with increasing dry matter content of the silage fed. The values ranged between 2.51 for the control and 1.89 for the air dried silages. Hawkins using alfalfa reported values of 3.52 for 22% DM silage, 2.42 for 40% DM silage and 2.23 for 80% DM alfalfa. Calder et al. (1964) reported water to dry matter consumption ratio of 2.5 for hay and 3.3 for silage.

Fecal water did not differ significantly; however, total water output was significantly ( $P < .05$ ) higher for the control and autoclaved silages than the other two treatments. Total water balance varied between 788 ml and 458 ml but was not significantly different. Therefore, the primary method of regulating water balance was by urinary excretion which did differ significantly ( $P < .01$ ). Urinary water was significantly correlated to total water intake ( $r = .85$ ,  $P < .01$ ).

#### Dry Matter Intake

Silage dry matter intake measured when the sheep were fed ad libitum is shown in Table 34. Values are expressed

as dry matter intake g per kg body weight<sup>75</sup>. Differences in dry matter intake were not significant and were not correlated with silage acid content or water soluble nitrogen parameters. Geasler (1970) reported a negative correlation between water soluble nitrogen and dry matter intake ( $r = -.84$ ,  $P < .01$ ). Senel and Owen (1967) and Allen et al. (1971) observed no reduction in dry matter intake when acetic acid was added to the ration. Allen et al. (1971) reported no reduction in dry matter intake when lactic acid was added to the silage. However, King (1943), Emery et al. (1961), Harris et al. (1966) and McCarrick et al. (1966) attributed reduced dry matter intake to the content of organic acids.

Dry matter intake did not increase as silage dry matter increased. This is in disagreement with results reported by Klosterman et al. (1963a), Huber et al. (1965), Johnson and McClure (1968) and Henderson et al. (1971a). One explanation may be due to observed differences in eating habits of the sheep during the course of the experiment.

#### Dry Matter Digestibility

Dry matter digestibility differences (Table 34) were small and nonsignificant. The range in digestibility was from 66.93% to 71.60% and these values are within the range of those reported by Geasler (1970). The digestibility values were all above the 52% to 66% range where rumen fill may limit intake as suggested by Conrad et al. (1964). Campling (1966c) found no relationship between digestibility



---

Fre  
Sil  
Tot

Fec  
Ur:  
Tot

Wa

Wa  
dr

---

lv  
2S  
Va  
Fi

---

D

D

D  
i

D  
8

---

1  
2  
N

Table 33

Means<sup>1</sup> for Water Balance Study

	Treatments				SE <sup>2</sup>
	Control	Autoclaved	Sun Dried	Air Dried	
	ml	ml	ml	ml	
Free water	385	372	1304 <sup>B</sup>	1838 <sup>A</sup>	111.7
Silage water	2390 <sup>A</sup>	2239 <sup>A</sup>	938 <sup>B</sup>	196	99.0
Total intake	2775 <sup>a</sup>	2611 <sup>a</sup>	2242 <sup>b</sup>	2034 <sup>A</sup>	148.5
Fecal water	834	710	649	777	47.0
Urinary water	1154 <sup>a</sup>	1208 <sup>A</sup>	964 <sup>AB</sup>	800 <sup>B</sup>	76.4
Total output	1988 <sup>a</sup>	1918 <sup>a</sup>	1613	1577	95.2
Water balance	788	693	630	458	76.9
Water intake, ml/g dry matter intake	2.51 <sup>Aa</sup>	2.36 <sup>Aab</sup>	2.16 <sup>ABb</sup>	1.89 <sup>B</sup>	0.09

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P &lt; .01), a = (P &lt; .05).

Table 34

Means<sup>1</sup> for Dry Matter Intake and Digestibility

	Treatments				SE <sup>2</sup>
	Control	Autoclaved	Sun Dried	Air Dried	
Dry matter intake, g/day	1092	1085	1038	1073	30.67
Dry matter digested, g/day	738	724	735	778	41.37
Dry matter digestibility, %	67.65	66.93	70.58	71.60	2.03
Dry matter intake, g/kg body weight <sup>.75</sup>	65.81	68.84	63.47	68.48	4.10

<sup>1</sup>Means of 4 observations.<sup>2</sup>SE = Standard error of means.

No significant differences between means.

and intakes of silage which agrees with data in this experiment.

### Nitrogen Balance

There were no significant differences in the nitrogen balance values shown in Table 35. Since dry matter intake and silage nitrogen content were not significantly different for the four silage treatments, significant differences in nitrogen intake were not expected.

The percent nitrogen digested for the autoclaved silage was lower than the other three treatments and differences approached significance. Apparently, autoclaving denatured some protein rendering it indigestible and it contained more nitrogen in the ADF which is indigestible (Van Soest, 1962). It is impossible to determine the fate of the unidentified nitrogen compounds based on this trial due to lack of trends between nitrogen balance parameters and unidentified silage nitrogen compounds.

All sheep were in a positive nitrogen balance. If the unidentified nitrogen compounds were entirely unavailable (% of total nitrogen) then some of the sheep should have been in a negative nitrogen balance because the rations were just over the 8% crude protein level required for maintenance. None of the nitrogen balance parameters were significantly correlated to silage fermentation parameters.

### Rumen Ammonia and Blood Urea

Rumen ammonia values are shown in Table 36. Rumen

Table 35

Means<sup>1</sup> for Nitrogen Balance Study

	Treatments				SE <sup>2</sup>
	Control	Autoclaved	Sun Dried	Air Dried	
Nitrogen intake, g/day	15.62	14.68	14.11	15.23	0.88
Fecal nitrogen, g/day	6.61	7.68	6.13	6.06	0.49
Nitrogen digested, g/day	9.00	7.00	.798	9.18	0.83
Percent nitrogen digested, %	57.81	47.49	56.82	59.16	2.80
Urinary nitrogen, g/day	6.65	5.10	6.15	6.34	0.45
Nitrogen retained, g/day	2.36	1.90	1.83	2.83	0.97
N retained as % of N ingested, %	15.45	11.48	12.98	17.05	5.50
N retained as % of N digested, %	26.38	25.35	22.87	27.68	9.29
Fecal N as % of N intake, %	42.19	52.51	43.18	40.84	2.80
Urinary N as % of N intake, %	42.36	36.00	43.85	42.11	4.38

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

ammonia levels were significantly ( $P < .01$ ) higher at feeding time ( $T_0$ ) on the air dried and sun dried silage rations than on the control silage ration. This could be due to a longer or more extensive fermentation period in the rumen for the higher dry matter silages. Significantly ( $P < .05$ ) higher rumen ammonia levels were produced by the air dried silage ration than all other rations at  $T_0$ . Ammonia concentrations at other times were not significantly different for the four silage treatments. The autoclaved silage produced lower rumen ammonia levels than the other treatments at  $T_1 - T_6$ . This could be attributed to autoclaving since the digestibility of the nitrogen was lower than the other treatments.

The air dried silage had significantly ( $P < .01$ ) less unidentified nitrogen as a percent of total nitrogen and the rumen ammonia levels did not peak as high or drop as fast as they did in the control and sun dried silage. The unidentified compounds may have been (same for control and sun dried silage) converted to ammonia faster than the water insoluble protein which was higher in the air dried silage.

In vitro studies (discussed later) indicate that most of the unidentified nitrogen compounds are converted to ammonia. The relationship of low unidentified nitrogen compounds in the silage to more stable rumen ammonia levels did not hold true for the autoclaved silage. However, some of the protein was apparently altered in the autoclaved

Table 36

Mean<sup>1</sup> Rumen Ammonia Values (mg/100 ml)

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	5.67 <sup>C</sup>	6.95 <sup>BC</sup>	8.62 <sup>AB</sup>	10.68 <sup>Aa</sup>	0.59
T <sub>1</sub>	13.25	10.62	14.62	12.37	1.86
T <sub>2</sub>	12.18	8.23	12.15	10.48	2.14
T <sub>4</sub>	5.70	4.90	5.15	6.39	0.90
T <sub>6</sub>	3.35	3.55	4.07	5.66	0.79
T <sub>8</sub>	3.67	3.80	4.02	5.35	0.74

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

Table 37

Mean<sup>1</sup> Blood Urea Values (mg/100 ml)

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	8.10	8.77	9.07	9.78	0.54
T <sub>4</sub>	9.28	9.53	10.13	11.30	0.68

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

silage and thus responsible for the change in the relationship described above.

Although there were no significant differences in blood urea levels (Table 37), they followed rumen ammonia concentrations. There was an increase in blood urea with the higher dry matter silages. The autoclaved and control silages produced similar blood urea concentrations.

#### Rumen pH and VFA Concentrations

None of the silage treatments utilized in this experiment had a significant influence on rumen pH (Table 41). Rumen pH values exhibited a normal pattern (Fenner et al., 1967) of decrease in pH during active fermentation after feeding and then increased as fermentation declined. The drop in pH at  $T_8$  in the autoclaved and air dried silages can be ascribed to increased fermentation due to the consumption of silage over a longer period of time.

Mean rumen volatile fatty acid concentrations for the various silages fed are shown in Tables 38, 39 and 40. The small differences in VFA concentrations may be due to the eating habits of sheep on the different rations. The low level of propionate at all times after feeding (significant ( $P < .01$ ) at  $T_1$  and  $T_2$ ) is not readily explainable. The significantly low level of rumen butyrate in sheep fed the control silage compared to the sun dried silage ( $P < .05$ ) and the air dried silage ( $P < .01$ ) at  $T_0$  was not observed after feeding.

Table 38

Mean<sup>1</sup> Rumen Acetate Concentrations (mg/100 ml)

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	340.0	352.5	355.0	382.5	17.20
T <sub>1</sub>	385.0	417.5	385.0	397.5	17.14
T <sub>2</sub>	377.5	397.5	395.0	360.0	21.60
T <sub>4</sub>	360.0	402.5	362.5	380.0	14.58
T <sub>6</sub>	365.0	367.5	327.5	402.5	17.19
T <sub>8</sub>	332.5	382.5 <sup>a</sup>	330.0	372.5 <sup>a</sup>	11.70

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, a = (P < .05).

Table 39

Mean<sup>1</sup> Rumen Propionate Concentrations (mg/100 ml)

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	97.5	95.5	102.5	92.5	7.77
T <sub>1</sub>	174.6	165.0	162.5	105.0 <sup>A</sup>	11.06
T <sub>2</sub>	185.0 <sup>Aa</sup>	142.5 <sup>AB</sup>	162.5 <sup>Aa</sup>	107.5 <sup>B</sup>	11.79
T <sub>4</sub>	140.0	142.5	127.5	112.5	5.91
T <sub>6</sub>	125.0	120.0	105.0	97.5	7.60
T <sub>8</sub>	115.0	125.0	100.0	87.5	10.18

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).



Table 40

Mean<sup>1</sup> Rumen Butyrate Concentrations (mg/100 ml)

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	65.0 <sup>Bb</sup>	72.5 <sup>ABab</sup>	77.5 <sup>ABa</sup>	82.5 <sup>A</sup>	3.15
T <sub>1</sub>	70.0	65.0	90.0	80.0	9.47
T <sub>2</sub>	85.0	60.0	105.0	75.0	12.99
T <sub>4</sub>	70.0	65.0	87.5	90.0	6.42
T <sub>6</sub>	72.5	60.0	67.5	90.0	11.27
T <sub>8</sub>	62.5	57.5	65.0	77.5	6.58

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P < .01), a = (P < .05).

Table 41

Mean<sup>1</sup> Rumen pH

Time	Treatments				SE <sup>2</sup>
	Control	Auto-claved	Sun Dried	Air Dried	
T <sub>0</sub>	6.59	6.58	6.57	6.50	0.046
T <sub>1</sub>	6.44	6.49	6.39	6.48	0.056
T <sub>2</sub>	6.43	6.47	6.33	6.45	0.064
T <sub>4</sub>	6.51	6.44	6.42	6.43	0.027
T <sub>6</sub>	6.51	6.51	6.52	6.46	0.037
T <sub>8</sub>	6.51	6.47	6.54	6.44	0.022

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

No significant difference between means.

### Rumen Dry Matter

Rumen dry matter percentages are shown in Table 42. The significant differences at  $T_1$  may be a reflection of silage dry matter, since the control silage (31.68% DM) produced a rumen dry matter that was significantly lower than the sun dried silage ( $P < .05$ ) and the air dried silage ( $P < .01$ ). The rumen dry matters did not follow the expected pattern of decreasing with time after feeding and then increasing by eight hours post-feeding reported by Hawkins (1969). This may have been due to sheep eating throughout the day.

### Plasma Amino Acid Analyses

The average values for total essential, total non-essential, and sulfur containing amino acids are shown in Table 43. There were no significant differences in any of the values; this may have been due to the large variation as indicated by the standard error. The reasons for the variability are not readily explainable. Only the prefeeding values ( $T_0$ ) are shown because the values for four hours post-feeding ( $T_4$ ) were essentially the same. Fenderson and Bergen (1972) using four rations consisting of varying levels of roughage and/or grain (varied in protein from 6.9 to 20.2%) demonstrated that plasma amino acid concentrations were not affected by the diets used or time after feeding in sheep. Purser et al. (1966) administered a starch-glucose mixture intraruminally and observed a decline in plasma amino acid levels in sheep. However, the starch-glucose

Table 42

Means<sup>1</sup> for Rumen Dry Matter Determinations

Time	Treatments				SE <sup>2</sup>
	Control	Autoclaved	Sun Dried	Air Dried	
T <sub>0</sub>	8.25	9.00	9.13	9.50	0.34
T <sub>1</sub>	8.00 <sup>Bb</sup>	8.43 <sup>Bab</sup>	8.95 <sup>ABa</sup>	9.66 <sup>A</sup>	0.22
T <sub>2</sub>	7.80	8.42	9.00	9.02	0.28
T <sub>4</sub>	7.48	8.34 <sup>Ab</sup>	8.83 <sup>Aa</sup>	9.02 <sup>Aa</sup>	0.16
T <sub>6</sub>	8.00	8.42	8.37	9.53 <sup>A</sup>	0.19
T <sub>8</sub>	8.24	8.32	8.92	9.75	0.32

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

Values with no superscript or having the same superscript are not significantly different, A = (P &lt; .01), a = (P &lt; .05).

Table 43

Means<sup>1</sup> Plasma Amino Acid Concentrations (μm/100 ml)

	Treatments				SE <sup>2</sup>
	Control	Autoclaved	Sun Dried	Air Dried	
Total EAA T <sub>0</sub>	90.9	92.7	95.9	99.3	28.3
Total NEAA T <sub>0</sub>	198.6	192.8	235.0	246.1	65.2
NEAA/EAA T <sub>0</sub>	2.48	2.09	2.41	2.39	0.18
Lysine	9.75	9.63	9.2	9.2	3.9
Sulfur containing AA	7.58	7.78	8.1	7.6	2.2

<sup>1</sup>Mean of 4 observations.<sup>2</sup>SE = Standard error of means.

mixture was a more readily available source of energy than found in most conventional rations.

Salas (1971), using soybean supplemented corn silage rations fed to steers, reported NEAA/EAA ratios of 1.00. In this experiment, using unsupplemented corn silage, NEAA/EAA ratios of 2.09 to 2.48 were observed. The increased ratio was due to increased NEAA levels.

#### Studies In Vitro

The rate and extent of cellulose digestion, using silage alone and silage plus urea as the substrates, are shown in Table 44 for the four silage treatments. The digestibility of 12 hour samples estimated the rate of cellulose digestion while the 48 hour sample estimated the extent of digestion.

Cellulose digestibilities were about 50% less for the control and autoclaved silages without added nitrogen than the sun dried and air dried silages. This may suggest that nitrogen is limiting in the control and autoclaved silages; however, the water soluble nitrogen compounds in all silages were converted to volatile base ( $\text{NH}_3$ ) after 12 hours as illustrated in Figure 5.

At 48 hours, the two drier silages had greater cellulose digestibilities; however, the difference was not as great as at 12 hours.

When urea (108 mg ml) was added to the silage, cellulose digestibility was increased at 12 hours to the level attained at 48 hours with silage alone. Under these

conditions, nitrogen was not limiting. A higher cellulose digestibility was observed for the autoclaved silage (61.4%) than all other treatments which averaged approximately 54%. All of the water soluble non-ammonia nitrogen was converted to volatile base ( $\text{NH}_3$ ). The urea elevated the levels of water soluble nitrogen initially (Figure 6).

Based on these results, it appears that the water soluble unidentified nitrogen compounds are converted to ammonia and if they are converted to ammonia, they should not reduce voluntary dry matter intake. However, the unidentified nitrogen compounds may escape the rumen before being degraded to ammonia and thus could interfere with intake.

#### Correlation Coefficients

Correlation coefficients are shown in Appendix I.

Table 44

Means of Cellulose Digestibility for  
In Vitro Studies of Corn Silages<sup>1</sup>

Sampling Time	Silage Treatment			
	Control	Autoclaved	Sun Dried	Air Dried
	%	%	%	%
No N added in the <u>in vitro</u> system				
12 hr.	11.2	10.7	20.9	20.5
48 hr.	28.2	32.6	35.9	36.9
40 mg % Urea - N/100 ml in the <u>in vitro</u> system				
12 hr.	31.7	37.2	29.0	27.0
48 hr.	55.0	61.4	53.0	54.4

<sup>1</sup>Two observations per mean.

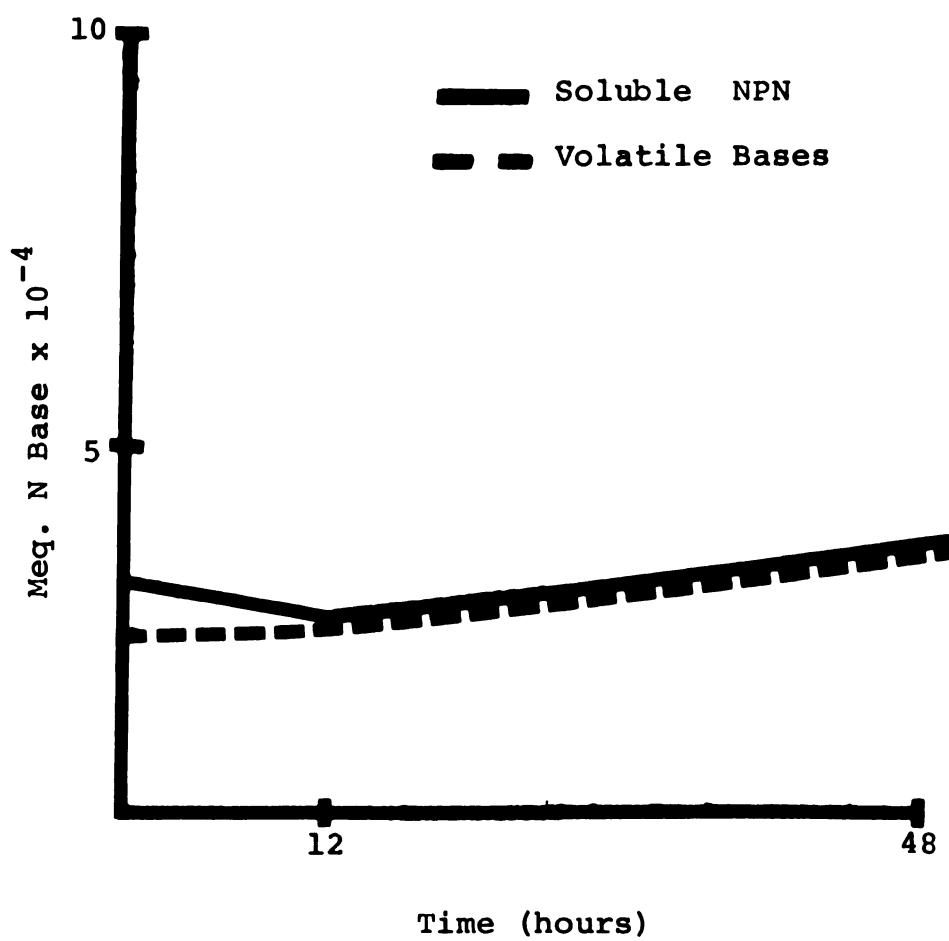


Figure 5. Changes in water soluble nitrogen and volatile bases during in vitro digestion.

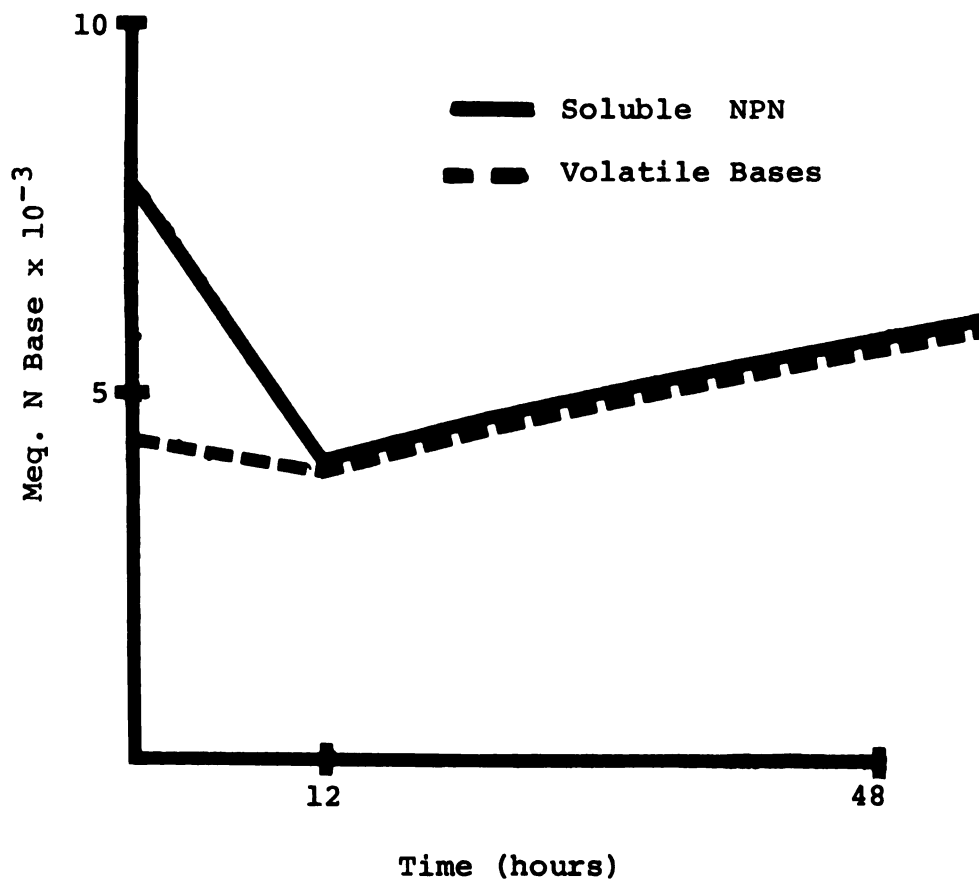


Figure 6. Changes in water soluble nitrogen and volatile bases during in vitro digestion (urea added).



## V

### SUMMARY

The results of six experiments are presented and discussed.

A common objective of all six experiments was to vary and evaluate the unidentified water soluble nitrogen fraction in corn silage, which represents 1/3 to 1/2 of the total silage nitrogen. The literature suggests that the unidentified nitrogen fraction may contain compounds responsible for depressed dry matter consumption by ruminants fed silage vs. hay or green chop. Perhaps the unidentified nitrogen fraction is unavailable for rumen microorganisms, thus reducing dry matter intake of silage as a result of less utilizable nitrogen. Attempts to link other products of silage fermentation (organic acids) to reduced silage dry matter intake have produced inconsistent results; therefore, other silage fermentation products such as the unidentified nitrogen fraction must be investigated.

Experiment I was designed to measure the effects of maximizing fermentation of corn silage with limestone treatment and minimizing fermentation with formic acid treatment without the confounding effects of added nitrogen on steer performance, silage nitrogen and acid fractions, and steer metabolic parameters, when compared to control silage

receiving no treatment. All three silages were fed to steer calves in a 161 - 238 day experiment and were compared on both an all silage ration and a 60% corn silage and 40% high moisture shelled corn ration on a dry matter basis. Results of the metabolic study, using the three all silage rations without protein supplementation, are presented in Experiment III. Organic acid levels were significantly ( $P < .01$ ) increased and decreased with limestone and formic acid treatments, respectively. The unidentified nitrogen compounds made up 41% of the total nitrogen in the control silage and 34% of the total nitrogen in the formic acid and limestone treated silages; therefore, the content of unidentified water soluble nitrogen compounds was not greatly affected by the extent of fermentation. Average daily gain was essentially identical for all treatments. Dry matter consumption was decreased slightly for the limestone treated silage; however, this was offset by an improvement in feed efficiency. Dry matter intake was not increased for the formic acid treated silage, which contained significantly ( $P < .01$ ) less lactate than the control or limestone treated silages. The nitrogen retained (g/day) was significantly ( $P < .01$ ) higher for the control silage and blood and rumen parameters were not significantly different.

Experiment II was designed to measure the effects of stimulating fermentation of corn silage with NPN additions on steer performance, nitrogen balance parameters and silage nitrogen and organic acid fractions. Five silage

treatments were studied: control untreated silage, Pro-Sil supplemented and treated silages, urea-mineral treated silage and urea-mineral plus formic acid treated silage. All silage treatments were compared on an all silage ration and a 60% corn silage and 40% high moisture shelled corn ration on a dry matter basis. The results of the nitrogen balance study, using the control soy-supplemented and the NPN treated all silage rations, are presented in Experiment IV. The neutralizing effect of Pro-Sil and urea-mineral addition without formic acid resulted in stimulated fermentation and bacterial activity, yielding significantly ( $P < .01$ ) greater lactate levels. Lactate production was reduced when formate was added to the urea-mineral treated silage. Total nitrogen was increased approximately 50% by NPN additions. Water insoluble nitrogen levels were significantly ( $P < .01$ ) elevated for all NPN treated silages compared to the control silage; however, unidentified nitrogen levels were not significantly altered by stimulating fermentation with NPN additions. The increase in water insoluble nitrogen content appears to be due to the production of microbial protein during fermentation rather than a reduction in proteolysis. Average daily gains were significantly higher for the NPN treated silages than for the control silage supplemented at feeding with soy-mineral ( $P < .05$ ) or Pro-Sil ( $P < .01$ ). Differences in feed consumption were small; however, feed efficiency and cost favored the NPN treated silages. Differences in nitrogen

balance parameters were not significantly different.

Experiment V was designed to monitor changes occurring during fermentation in untreated control and Pro-Sil treated corn silages. Samples of each silage treatment were taken when fresh and on days 1 through 10, 15, 20, 30, 60 and 90 after ensiling. Water insoluble nitrogen level in Pro-Sil treated silage was increased to a maximum at day five when the soluble nitrogen as a percent of total nitrogen had decreased to a low level (Figure 4). The unidentified nitrogen level was slightly higher in the Pro-Sil treated silage than in the control silage. These results indicate that water soluble nitrogen compounds are incorporated into microbial protein, consequently increasing the water insoluble nitrogen.

Experiment VI was designed to vary the resulting level of water soluble nitrogen compounds occurring in fermented silage and determine the effects of these compounds on silage dry matter intake and metabolic and blood parameters. In vitro studies were also conducted to examine the availability of the nitrogen contained in the unidentified compounds for rumen microorganisms. Dry matter intake was not affected by experimentally altered unidentified nitrogen levels in corn silage and was not significantly correlated with acid levels, nitrogen fractions or dry matter content of the silages. The unidentified nitrogen ranged from 15.95% to 33.89% of total nitrogen for the air dried and control silages, respectively. Silage dry matter

content and unidentified nitrogen levels had a significant negative correlation ( $r = -.60$ ,  $P < .05$ ). Results of the nitrogen balance study were not significantly different. The nitrogen balance data indicate that the unidentified nitrogen compounds are at least partially available for rumen microorganisms, if not entirely, otherwise some sheep would have been in a negative nitrogen balance.

In vitro studies showed that all of the unidentified nitrogen compounds were converted to volatile base ( $\text{NH}_3$ ) within a 12 hour period. Therefore, it is unlikely that the unidentified nitrogen compounds are responsible for reduced dry matter intake unless they escape the rumen before being degraded to ammonia.

It appears that plant enzymes are responsible for protein hydrolysis (increased levels of water soluble nitrogen) in silage, based on the literature and indirect evidence from the pilot experiment in Experiment VI. Plant enzyme levels were not measured in the pilot study, however. The plant enzymes may degrade protein to amino acids and either plant enzymes or microorganisms may carry the amino acids to other nitrogenous products as indicated in the literature. Perhaps bacterial activity is greater in corn silage than in grass and legume silages, since amino acid levels were never greater than .1% of the silage dry matter in Experiment V and levels of .56% of silage dry matter were reported by Hawkins (1969) using alfalfa silage.

The unidentified nitrogen compounds appear to be converted to ammonia, which is utilizable by rumen microorganisms. Therefore, unless they escape the rumen before being degraded they would not be expected to reduce silage dry matter intake. More research is needed before unidentified water soluble nitrogen compounds can be eliminated as depressors of silage dry matter intake. Once the compounds are identified in corn silage, it will be possible to follow their metabolism in vivo.

## BIBLIOGRAPHY

## BIBLIOGRAPHY

- Allen, C. K., H. E. Henderson and W. G. Bergen. 1971Aa. Ammonium salts as a source of crude protein for feedlot cattle. Research Report 143:16. Mich. Agr. Expt. Sta.
- Allen, C. K., H. E. Henderson and W. G. Bergen. 1972. Ammonium salts for feedlot cattle. J. Anim. Sci. 34: (in pub.) (Abstr.).
- Annett, H. E. and E. J. Russell. 1907. The composition of green maize and of the silage produced therefrom. J. Agr. Sci. 2:382.
- Archibald, J. G., J. W. Kuzmeski and S. Russell. 1960. Grass silage quality as affected by crop composition and by additives. J. Dairy Sci. 43:1648.
- Baile, C. A. and W. H. Pfander. 1964. Feed intake of sheep on various concentrate levels. J. Anim. Sci. 23:1205.
- Baile, C. A. and W. H. Pfander. 1965. Chemosensitive regulatory mechanism of ovine feed intake. Fed. Proc. 24:244.
- Baile, C. A., A. W. Mahoney and J. Mayer. 1967a. Preliminary report on feeding activity and hypothalamic temperature in goats. J. Dairy Sci. 50:1854.
- Baile, C. A., A. W. Mahoney and J. Mayer. 1967b. Preliminary report on hypothalamic hyperphagia in ruminants. J. Dairy Sci. 50:1851.
- Baile, C. A. and J. Mayer. 1967c. Intragastric injections of liquid diet, water, and acetate and meal patterns of goats. Am. J. Physiol. 213:387.
- Baile, C. A. and J. Mayer. 1968a. Hypothalamic temperature and the regulation of feed intake in goats. Am. J. Physiol. 214:677.
- Baile, C. A. and J. Mayer. 1968b. Effects of intravenous versus intraruminal injections of acetate on feed intake of goats. J. Dairy Sci. 51:1490.



- Baile, C. A. and J. Mayer. 1968c. Effects of insulin induced hypoglycemia and hypoacetoemia on eating behavior in goats. J. Dairy Sci. 51:1495.
- Baile, C. A., A. W. Mahoney and J. Mayer. 1968d. Induction of hypothalamic aphagia and adipsia in goats. J. Dairy Sci. 51:1474.
- Balch, C. C. and R. C. Campling. 1962. Regulation of voluntary food intake in ruminants. Nutr. Abstr. and Rev. 32:669.
- Baldwin, R. L., W. A. Wood and R. S. Emery. 1962. Conversion of lactate- $^{14}\text{C}$  to propionate by the rumen microflora. J. Bacteriol. 83:907.
- Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535.
- Barnett, A. J. G. 1954. Silage Fermentation. Academic Press, Inc., New York.
- Baumgardt, B. R. 1967. Regulation of food intake in ruminants. 5. Effect of amphetamine administered intraruminally and intravenously to sheep and goats. J. Dairy Sci. 50:1176.
- Baumgardt, B. R. 1970. Control of feed intake in the regulation of energy balance. In Proc. Third Internat. Symp. on the Physiology of Digestion and Metabolism in Ruminants. Oriel Press, England, pp. 235-253.
- Beardsley, D. W. 1964. Symposium on forage utilization: Nutritive value of forage as affected by physical form. II. Beef cattle and sheep studies. J. Anim. Sci. 23:239.
- Beattie, D. R. 1970. The effect of adding a liquid suspension of anhydrous ammonia, minerals and molasses to corn silage at ensiling, on fermentation parameters and beef cattle performance. M.S. Thesis. Mich. State Univ.
- Beattie, D. R., H. E. Henderson, M. R. Geasler and W. G. Bergen. 1971. Pro-Sil and urea addition to corn silage. Research Report 136:21. Mich. Agr. Expt. Sta.
- Belasco, I. J. 1954. New nitrogen feed compounds for ruminants - a laboratory evaluation. J. Anim. Sci. 13:601.
- Bentley, O. G., E. W. Klosterman and P. Engle. 1955. The use of urea to increase the crude protein content of corn silage for fattening steers. Ohio Agr. Expt. Sta. Res. Bul. 774.

- Bergen, W. G. and E. L. Potter. 1971. E - N - methyl lysine metabolism in sheep. *J. Anim. Sci.* 32:1324.
- Bergen, W. G. 1972. Rumen osmolality as a factor in feed intake control of sheep. *J. Anim. Sci.* 34 (In pub.).
- Bhattacharya, A. N. and R. G. Warner. 1967. Inhibitory effect of amphetamine of feed and water intake in sheep. *J. Anim. Sci.* 26:1445.
- Bhattacharya, A. N. and R. G. Warner. 1968. Voluntary feed intake of pelleted diets for cattle, sheep, and rabbits as affected by different alkali supplements. *J. Anim. Sci.* 27:1418.
- Blaxter, K. L. and F. W. Wainman. 1961. The utilization of food by sheep and cattle. *J. Agr. Sci.* 57:419.
- Blaxter, K. L., F. W. Wainman and R. S. Wilson. 1961. The regulation of food intake by sheep. *An. Prod.* 3:51.
- Bloom, S., N. L. Jacobson, R. S. Allen, L. D. McGilliard and P. G. Homeyer. 1957. Effects of various hay concentrate ratios on nutrient utilization and production responses of dairy cows. II. Observations on ration digestibility and on the excretion pattern of chromic oxide. *J. Dairy Sci.* 40:240.
- Brobeck, J. R. 1948. Food intake as a mechanism of temperature regulation. *Yale J. Biol. and Med.* 20:545.
- Brobeck, J. R. 1955. *Ann. N. Y. Acad. Sci.*, 63, 44.
- Brody, C. J. 1965. Nitrogen redistribution during ensilage at low moisture levels. *J. Sci. Food & Agr.* 16:508.
- Bruno, C. F. and W. E. C. Moore. 1962. Fate of lactic acid in rumen ingesta. *J. Dairy Sci.* 45:109.
- Byers, J. H., C. L. Davis, and C. E. Baylor. 1964. Feeding value of limestone-treated corn silage for lactating dairy cows. *J. Dairy Sci.* 47:1062.
- Byers, J. H. and E. E. Ormiston. 1966. Feeding value of corn silage made at three stages of development. *J. Dairy Sci.* 49:741. (Abstr.).
- Calder, F. W., J. W. G. Nicholson and H. M. Cunningham. 1964. Water restriction for sheep on pasture and rate of consumption with other feeds. *Can. J. Anim. Sci.* 44:266.
- Campling, R. C., M. Freer and C. C. Balch. 1961a. Factors affecting the voluntary intake of food by cows. 2. The relationship between the voluntary intake of roughages, the amount of digesta in the reticulo-rumen and the rate of disappearance of digesta from the alimentary tract. *Brit. J.*

Nutr. 15:531.

Campling, R. C. and C. C. Balch. 1961b. Factors affecting the voluntary intake of food by cows. I. Preliminary observations on the effect, on the voluntary intake of hay, of changes in the amount of the reticulo ruminant contents. Brit. J. Nutr. 15:523.

Campling, R. C. and M. Freer. 1962a. The effect of specific gravity and size on the mean time of retention of inert particles in the alimentary tract of the cow. Brit. J. Nutr. 16:507.

Campling, R. C., M. Freer, and C. C. Balch. 1962. Factors affecting the voluntary intake of food by cows. 3. The effect of urea on the voluntary intake of oat straw. Brit. J. Nutr. 16:115.

Campling, R. C., and M. Freer. 1966a. Factors affecting the voluntary intake of food by cows. 8. Experiments with ground, pelleted roughages. Brit. J. Nutr. 20:229.

Campling, R. C. 1966b. The control of voluntary intake of food in cattle. Outlook on Agr. 5:74.

Campling, R. C. 1966c. The intake of hay and silage by cows. J. Brit. Grassld. Soc. 21:41.

Campling, R. C. Physical regulation of voluntary intake. In Proc. Third Internat. Symp. on the Physiology of Digestion and Metabolism in Ruminants. Oriel Press, England, pp. 226-234. 1970.

Castle, E. J. 1956a. The rate of passage of foodstuffs through the alimentary tract of the goat. 1. Studies on adult animals fed on hay and concentrates. Brit. J. Nutr. 10:15.

Castle, E. J. 1956b. The rate of passage of foodstuffs through the alimentary tract of the goat. 3. The intestines. Brit. J. Nutr. 10:338.

Castle, M. E. and J. N. Watson. 1970a. Silage and milk production, a comparison between grass silage made with and without formic acid. J. Brit. Grassld. Soc. 25:65.

Castle, M. E. and J. N. Watson. 1970b. Silage and milk production, a comparison between wilted and unwilted grass silages made with and without formic acid. J. Brit. Grassld. Soc. 25:278.

- Conrad, H. R., J. W. Hilbs and A. D. Pratt. 1966. Regulation of feed intake in dairy cows. II. Association between digestible dry matter intake and cellulose digestibility in cows fed increasing levels of grain concentrate. J. Dairy Sci. 49:1038.
- Conway, E. I. 1950. Microdiffusion Analysis and Volumetric Error. Crosby Lockwood and Son Ltd., London.
- Coombe, J. B. and R. N. B. Kay. 1965. Passage of digesta through the intestines of the sheep. Retention times in the small and large intestines. Brit. J. Nutr. 19:325.
- Coppock, C. E. and J. B. Stone. 1968. Corn silage in the ration of dairy cattle: A review. New York State College of Agr.: Cornell. Misc. Bull. 89.
- Cowsert, R. L. and M. J. Montgomery. 1969. Effect of varying forage to concentrate ratio of isonitrogenous rations on feed intake by ruminants. J. Dairy Sci. 52:64.
- Dain, J. A., A. L. Neal and R. W. Dougherty. 1955. The occurrence of histamine and tyramine in rumen ingesta of experimentally overfed sheep. J. Anim. Sci. 14:930.
- Davis, G. K., R. B. Becker, P. T. Dix Arnold, C. L. Comar and S. P. Marshall. 1944. Urea in sorghum silage. J. Dairy Sci. 27:649. (Abstr.).
- Dehority, B. A. and R. R. Johnson. 1964. Estimation of the digestibility and nutritive value of forages by cellulose and dry matter solubility methods. J. Anim. Sci. 23:203.
- Derbyshire, J. C. and C. H. Gordon. 1969. Utilization of formic acid silages by milk cows. J. Dairy Sci. 52:936.
- Derbyshire, J. C. and C. H. Gordon. 1970. Additional observations of milk cow response on formic acid silages. J. Dairy Sci. 53:677.
- Derbyshire, J. C., D. R. Waldo and C. H. Gordon. 1971. Dairy cattle performance on formic acid silages. J. Dairy Sci. 54:805.
- Dexter, S. T., C. F. Huffman and E. J. Benne. 1959. Physical, chemical and biological principles in silage making. Proceedings of Mich. State Univ. Silage Conf. p. 31.
- Dinius, D. A., D. L. Hill and C. H. Noller. 1968. Influence of supplemental acetate feeding on the voluntary intake of cattle fed green corn and corn silage. J. Dairy Sci. 51:1505.

- Dowden, D. R. and D. R. Jacobson. 1969. Inhibition of appetite in dairy cattle by certain intermediates metabolites. *Nature* 188:148.
- Dox, A. W. and R. E. Neidig. 1912. The volatile aliphatic acids of corn silage. *Iowa Agr. Expt. Sta. Res. Bull.* 7.
- Dunlop, R. H. and P. B. Hammond. 1965. D-lactate acidosis of ruminants. *Ann. N. Y. Acad. Sci.* 119:1109.
- Egan, A. R. 1965a. The fate and effects of duodenally infused casein and urea nitrogen in sheep fed on a low protein roughage. *Australian J. Agr. Res.* 16:169.
- Egan, A. R. 1965b. Nutritional status and intake regulation in sheep. II. The influence of sustained duodenal infusions of casein or urea upon voluntary intake of low protein roughages by sheep. *Australian J. Agr. Res.* 16:451.
- Egan, A. R. and R. J. Moir. 1965. Nutritional status and intake regulation in sheep. 1. Effects of duodenally infused single doses of casein, urea and propionate upon voluntary intake of a low-protein roughage by sheep. *Australian J. Agr. Res.* 16:437.
- Emery, R. S., L. D. Brown, C. F. Huffman, T. R. Lewis, J. P. Everett and C. A. Lassiter. 1961. Comparative feeding value of lactic acid and grain for dairy cattle. 20:159.
- Eng, K. S., Jr., M. E. Riewe, J. H. Craig, Jr. and J. C. Smith. 1964. Rate of passage of concentrate and roughage through the digestive tract of sheep. *J. Anim. Sci.* 23: 1129.
- Essig, H. W. 1968. Urea-limestone-treated silage for beef cattle. *J. Anim. Sci.* 27:730.
- Everson, R. A., N. A. Jorgensen and G. P. Barrington. 1971. Effect of bentonite, nitrogen source and stage of maturity on nitrogen redistribution in corn silage. *J. Dairy Sci.* 54:1482.
- Fatianoff, N., M. Durand, J. L. Tisserand and S. Z. Zetter. 1966. The effect of wilting, mineral acidification and sodium metabisulfite on quality and nitrogen efficiency of alfalfa silages. Mimeo presented at European Conference on Silage. Rostok, East Germany.
- Fenderson, C. L. and W. G. Bergen. 1972. Effect of ration composition and protein level on plasma free tryptophan and ruminal microbial tryptophan content in sheep. *J. Anim. Sci.* 34: (In pub.).

- Fenner, H., F. N. Dickinson and H. D. Barnes. 1967. Relationship of digestibility and certain rumen fluid components to level of feed intake and time of sampling after feeding. *J. Dairy Sci.* 50:334.
- Fisher, L. J., J. R. Lessard and G. A. Lodge. 1970. Utilization of formic acid treated sorghum-sudan silage by dairy cows. *Can. J. Anim. Sci.* 51:371.
- Freer, M. and R. C. Campling. 1963. Factors affecting the voluntary intake of food by cows. 5. The relationship between voluntary intake of food, the amount of digesta in the reticulo-rumen and the rate of disappearance of digesta from the alimentary tract with diets of hay, dried grass or concentrates. *Brit. J. Nutr.* 17:79.
- Gale, E. F. 1941. Production of amines by bacteria. 4. The decarboxylation of amino acids by organisms of the group Clostridium and Proteus. *Biochem. J.* 35:66.
- Geasler, M. R. 1970. The effect of corn silage maturity, harvesting techniques and storage factors on fermentation parameters and cattle performance. Ph.D. Thesis. Mich. State Univ.
- Goering, H. K., C. H. Gordon, R. W. Hemken, P. J. Van Goest and L. W. Smith. 1970. Analytical measures of heat damaged forage and nitrogen digestibility. *J. Dairy Sci.* 53:676.
- Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis. USDA ARS Agr. Handbook 379.
- Goffart, M. A. 1877. The Ensilage of Maize and Other Green Fodder Crops. Trans. and Publ. by J. B. Brown. New York.
- Gorb, T. V. and S. S. Lebedinsky. 1960. Maize silage protein content enriched by addition of urea. (In Russian). *Vestn. Sel'skokhoz Nauki* 9:100.
- Gordon, C. H., J. C. Derbyshire, H. G. Wiseman, E. A. Kane and C. G. Melin. 1961. Preservation and feeding value of alfalfa stored as hay, haylage and direct-cut silage. *J. Dairy Sci.* 44:1299.
- Gordon, C. H., J. C. Derbyshire and J. L. Humphrey. 1966. The value of mature corn for silage. USDA Agr. Res. Ser. ARS 44-176.
- Graham, N. McC. and A. J. Williams. 1962. The effects of pregnancy on the passage of food through the digestive tract of sheep. *Australian J. Agr. Res.* 13:894.

- Greenhalgh, J. F. D. and G. W. Reid. 1967. Separating the effects of digestibility and palatability on food intake in ruminant animals. *Nature* 214:744.
- Hanis, C. E., W. F. Raymond and R. F. Wilson. 1966. The voluntary intake of silage. *Proc. 10th Int. Grassld. Congr.*, Helsinki 564.
- Harvey, W. R. 1960. Least squares analysis of data with unequal subclass numbers. *USDA Agr. Res. Ser. Publ. ARS* 20-8.
- Harvey, A. L., O. E. Kolari and J. C. Meiske. 1963. Feeding corn silage treated with limestone, urea or sodium metabisulfite at time of ensiling to calves. *Minn. Beef Cattle Grasslands Field Day*, p. 1.
- Hastings, W. H. 1944. The use of urea in commercial dairy feeds. *J. Dairy Sci.* 27:1015.
- Hatfield, E. E., D. L. Hixon, L. H. Paterson, W. Braman, A. P. Peter and U. S. Garrigus. 1971. Levels of protein for beef cattle finishing rations. *Illinois Beef Cattle Day Report*, 1971.
- Hawkins, D. R. 1969. The effect of dry matter levels of alfalfa silage on intake and metabolism in the ruminant. *Ph.D. Thesis. Mich. State Univ.*
- Hemsley, J. A. and R. J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea-supplemented low quality cereal hay by sheep. *Aust. J. Agr. Res.* 14:509.
- Henderson, H. E. and D. B. Purser. 1968. Urea additions to corn silage. *Mich. Beef Cattle Day Report* 1968, p. 29.
- Henderson, H. E. and M. R. Geasler. 1969. Pro-Sil addition (anhydrous ammonia, molasses, minerals) to corn silage. *Mich. State Univ. Exp. Sta. AH-BC 69D*.
- Henderson, H. E., D. B. Purser and M. R. Geasler. 1970a. Anhydrous ammonia, urea, and mineral additives to corn silage. *Research Report 108:3. Mich. Agr. Exp. Sta.*
- Henderson, H. E. and M. R. Geasler. 1970b. Anhydrous ammonia, molasses and mineral addition to rye silage. *Research Report 108:19. Mich. Agr. Exp. Sta.*
- Henderson, H. E., H. D. Ritchie, C. K. Allen and E. H. Cash. 1971Aa. Effect of housing systems and corn silage maturity on feedlot performance of Holstein steers. *Research Report 143:58. Mich. Agr. Exp. Sta., J. Anim. Sci.* 33:1140. (Abstr.).

- Henderson, H. E., D. R. Beattie, M. R. Geasler and W. G. Bergen. 1971b. Ammonia, mineral and molasses addition to corn silage. J. Anim. Sci. 33:285.
- Henderson, H. E., D. R. Beattie, M. R. Geasler and W. G. Bergen. 1971c. Pro-Sil treated vs. control corn silage with varying levels of concentrate addition for finishing yearling steers. Research Report 136:41. Mich. Agr. Expt. Sta., J. Anim. Sci. 33:285.
- Henderson, H. E., C. K. Allen, E. H. Cash and W. G. Bergen. 1971Ad. Corn silage-shelled corn rations and sources of crude protein. Research Report 143:5. Mich. Agr. Expt. Sta., J. Anim. Sci. 33:1167.
- Henderson, H. E., D. R. Beattie, M. R. Geasler and W. G. Bergen. 1971c. Pro-Sil, ammonia, urea-mineral and urea addition to corn silage for feedlot cattle. Research Report 136:33. Mich. Agr. Expt. Sta.
- Henderson, H. E., D. R. Beattie, M. R. Geasler and W. G. Bergen. 1970f. Effect of level of Pro-Sil addition to corn silage on performance of yearling steers. Research Report 136:15. Mich. Agr. Expt. Sta.
- Hillman, D., C. A. Lassiter, C. F. Huffman and C. W. Ducan. 1958. Effect of all-hay vs. all silage rations on dry matter intake of lactating dairy cows: moisture and pH as factors affecting appetite. J. Dairy Sci. 41:720. (Abstr.).
- Hillman, D. 1959. Appetite studies in dairy cattle grass silage vs. hay. Ph.D. Thesis. Mich. State Univ.
- Holder, J. M. 1963. Chemostatic regulation of appetite in sheep. Nature 200:1074.
- Huber, J. T., G. C. Graf and R. W. Engle. 1965. Effect of maturity on nutritive value of corn silage for lactating cows. J. Dairy Sci. 48:1121.
- Huber, J. T., R. A. Sandy, C. N. Miller and C. E. Polan. 1966. Recut corn silage for dairy cattle. J. Anim. Sci. 25:914. (Abstr.).
- Huber, J. T., C. E. Polan and R. A. Sandy. 1967. Urea-treated corn silage for lactating cows. J. Dairy Sci. 50:982.
- Huber, J. T., C. E. Polan and D. Hillman. 1968a. Urea in high corn silage rations for dairy cattle. J. Anim. Sci. 27:220.



- Huber, C. A., J. W. Thomas and R. S. Emery. 1968b. Response of lactating cows fed urea treated corn silage harvested at varying stages of maturity. J. Dairy Sci. 51:1806.
- Huber, J. T., R. S. Emery, J. W. Thomas and S. M. Yousef. 1969. Milk fat synthesis on restricted roughage rations containing whey, sodium bicarbonate, and magnesium oxide. J. Dairy Sci. 52:54.
- Huber, J. T. 1970. Formic acid treatment of urea corn silage harvested at different maturities. Mich. State Univ. Mimeo D-235. J. Anim. Sci. 31:244.
- Huber, J. T. and J. W. Thomas. 1971a. Comparison of ammonia and urea treated corn silages for lactating cows. J. Dairy Sci. 54:789. (Abstr.).
- Huber, J. T., R. E. Lichtenwalner and H. E. Henderson. 1971b. Pro-Sil addition to corn silage at feeding time. J. Anim. Sci. 33:287. (Abstr.).
- Hughes, A. D. 1970. The non-protein nitrogen composition of grass silages. II. The changes occurring during the storage of silage. J. Agr. Sci. 75:421.
- Hughes, A. D. 1971. The non-protein nitrogen composition of grass silage. III. The composition of spoilt silages. J. Agr. Sci. 76:329.
- Hutchinson, K. J. and R. J. Wilkins. 1971. The voluntary intake of silage by sheep. II. The effects of acetate on silage intake. J. Agr. Sci. 77:539.
- I.A.E.A. 1970. Training Manual on Food Irradiation Technology and Techniques. Tech. Report Series No. 114. Vienna.
- Jenkins, H. M. 1884. Report on the practice of ensilage, at home and abroad. Royal Agr. Soc. J. 20:126.
- Johnson, R. R., E. W. Klosterman and O. G. Bentley. 1962. Energy value of lactic acid and corn steep water and their effects on digestibility of ruminant rations. J. Anim. Sci. 21:887.
- Johnson, R. R., G. E. Ricketts, E. W. Klosterman and A. L. Moxon. 1964. Studies on the utilization and digestion of long, ground and pelleted alfalfa and mixed hay. J. Anim. Sci. 23:94.
- Johnson, R. R., E. W. Klosterman, K. E. McClure and L. J. Johnson. 1965. Studies on urea and diammonium phosphate additions to corn silage. Ohio Beef Cattle Res. Summary 7:14.

- Johnson, R. R. 1966. Techniques and procedures for in vitro and in vivo rumen studies. J. Anim. Sci. 25:855.
- Johnson, R. E., K. E. McClure, L. J. Johnson and E. W. Klosterman. 1967. Corn plant maturity. III. Distribution of nitrogen in corn silage treated with limestone, urea and diammonium phosphate. J. Anim. Sci. 26:394.
- Johnson, R. R. and K. E. McClure. 1968. Corn plant maturity. IV. Effects on digestibility of corn silage in sheep. J. Anim. Sci. 27:535.
- Johnston, J. F. W. 1843. On the feeding qualities of the natural and artificial grasses in different states of dryness. High. Agr. Soc. Scotland. Trans. 1:57.
- Johnston, W. E., G. W. Brandt, C. C. Brannon and W. C. Cook. 1970. Consumption of corn silage dry matter by bred heifers and its correlation with subsequent first-lactation production. J. Dairy Sci. 53:2:215.
- Karr, M. R., U. S. Garrigus, E. E. Hatfield, H. W. Norton, B. B. Doane. 1965. Nutritional and chemical evaluation of urea and of biuret in complete ensiled finishing diets by lambs. J. Anim. Sci. 24:469.
- Keith, J. M., W. A. Hardison, J. T. Huber and G. C. Graff. 1961. The effect of the physical form on the nutritive value of hay fed to lactating dairy cows. J. Dairy Sci. 44:1174.
- Kesler, E. M., J. M. Wilson and W. H. Cloninger. 1960. Corn silage versus mixed hay as roughage for Holstein calves. J. Dairy Sci. 43:298. (Abstr.).
- King, W. A. 1943. Comparison of limestone and sodium bicarbonate as neutralizers for phosphoric acid oat silage. J. Dairy Sci. 26:975.
- King, K. W. and W. E. C. Moore. 1957. Density and size as factors affecting passage rate of ingesta in the bovine and human digestive tracts. J. Dairy Sci. 40:528.
- Kirsh, W. 1930. Feed Conservation, Berlin. (In German). 2:127-133. (See Watson and Nash, 1960).
- Klosterman, E. W., R. R. Johnson, H. W. Scott, A. L. Moxon and J. Van Stavern. 1960. Whole plant and ground ear corn silages, their acid content, feeding value and digestibility. J. Anim. Sci. 19:522.

- Klosterman, E. W., A. L. Moxon, R. R. Johnson and A. P. Grifo, Sr. 1961a. Feeding value of limestone-urea treated whole plant corn silage with three levels of soybean oil meal. Beef Cattle Day Report Ser. No. 122, Ohio Agr. Expt. Sta.
- Klosterman, E. W., A. L. Moxon, R. R. Johnson, H. W. Scott and J. Van Stavern. 1961b. Feeding value for fattening cattle of corn silages treated to increase their content of organic acids. J. Anim. Sci. 20:493.
- Klosterman, E. W., R. R. Johnson, V. R. Cahill and P. Althouse. 1963a. Effect of stage of maturity upon the feeding value of corn silage and the effect upon carcass quality of feeding silage at different stages of growth and fattening cattle. Report of Beef Cattle Res., Ohio Agr. Expt. Sta., Wooster, Ohio. 131:14.
- Klosterman, E. W., R. R. Johnson, A. L. Moxon and H. W. Scott. 1963b. Feeding value of limestone-treated corn silages for fattening cattle. Ohio Agr. Expt. Sta. Res. Bull. 934.
- Kromann, R. P. and J. H. Meyer. 1966. Energy metabolism in sheep as influenced by interactions among the ration's energy content, physical form and buffers. J. Anim. Sci. 25:1096.
- Kroulik, J. T., L. A. Burkey and H. G. Wiseman. 1955. The microbial populations of the green plant and of the forage prior to ensiling. J. Dairy Sci. 38:256.
- Kroulik, J. T., L. A. Burkey, C. H. Gordon, H. G. Wiseman and C. G. Melin. 1955a. Microbial activities in alfalfa and orchardgrass ensiled under certain conditions in experimental silos. J. Dairy Sci. 38:263.
- Lessard, J. R., L. J. Fisher and G. A. Lodge. 1970. Formic acid as a preservative for high moisture silage. J. Dairy Sci. 53:142. (Abstr.).
- Lichtenwalner, R. E., J. T. Huber and W. G. Bergen. 1971. PAA and N-metabolism in dairy cows fed NPN-treated silages. J. Anim. Sci. 33:290. (Abstr.).
- Lopez, J., N. A. Jorgensen, R. P. Niedermeier and H. J. Larsen. 1970b. Redistribution of nitrogen in urea-treated and soybean meal-treated corn silage. J. Dairy Sci. 53:1215.
- Mabbitt, L. A. 1951. Proc. Soc. Appl. Bact. 14:147.

- Mackenzie, D. D. S. 1968. Production and utilization of lactic acid by the ruminant. A review. J. Dairy Sci. 50:1772.
- Macpherson, H. T. 1952a. Changes in nitrogen distribution in crop conservation. I. The rate and extent of protein breakdown in ensilage. J. Sci. of Food and Agr. 3:362.
- Macpherson, H. T. 1952b. Changes in nitrogen distribution in crop conservation. II. Protein breakdown during wilting. J. Sci. of Food and Agr. 3:365.
- Macpherson, H. T. 1962. Histamine tryptamine and tyramine in grass silage. J. Sci. of Food and Agr. 13:29.
- Mahapatro, B. B. and E. Cheffel. 1964. Effects of forage moisture content upon intake and VFA production in sheep. J. Anim. Sci. 23:883. (Abstr.).
- Makdani, D. D., J. T. Huber and W. G. Bergen. 1971. Effect of histidine and methionine supplementation on the nutritional quality of commercially prepared fish protein concentrate in rat diets. J. Nutr. 101:367.
- Manning, R., G. I. Alexander, H. M. Krueger and R. Bogart. 1959. The effect of intravenous glucose injections on appetite in adult ewes. Am. J. Vet. Res. 20:242.
- Mayer, J. 1955. Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. Ann. of the N.Y. Acad. of Sci. 63:15.
- McCarrick, R. B., K. D. Gardiner, D. B. Poole, T. A. Spillane and M. F. Maguire. 1966. The nutritive value of ammonium bisulfate and molasses silage. IV. Effect of mineral supplements with ammonium bisulfate silages on animal performance. Ir. J. Agr. Res. 5:1.
- McClure, W. H., J. P. Fontenot and R. C. Carter. 1972. Urea-treated corn silage and protein supplementation for fattening steer calves. J. Anim. Sci. 34:361. (Abstr.).
- McCullough, M. E. 1962. Some factors influencing intake of direct cut silage by dairy cows. J. Dairy Sci. 45:116.
- McCullough, M. E., L. R. Sisk and O. E. Sell. 1964. Influence of silage dry matter intake on efficiency of milk production. J. Dairy Sci. 47:650.
- McDonald, P., H. T. Macpherson and J. A. Watt. 1963. The effect of histamine on silage dry matter intake. J. Brit. Grassld. Soc. 18:280.

- McDonald, P., R. Whittenbury. 1967. Losses during ensilage. Brit. Grassld. Soc. Occ. Symp. 3:76.
- McLeod, D. S., R. J. Wilkins and W. F. Raymond. 1970. The voluntary intake by sheep and cattle of silages differing in free-acid content. J. Agr. Sci. 75:311.
- Miles, M. 1918. Silos, ensilage and silage. A practical treatise on the ensilage of fodder corn. Orange Judd Co. N.Y.
- Modyanov, A. V., S. P. Bezenko, M. I. Korotkov and E. V. Kiselev. 1960. The use of synthetic urea and ammonium sulphate for increasing the protein content of maize silage. (In Russian). Vestn. Sel'skokhoz Nauki (4):38.
- Montgomery, M. J., L. H. Schultz and B. R. Baumgardt. 1963. Effect of intraruminal infusion of volatile fatty acids and lactic acid on voluntary hay intake. J. Dairy Sci. 46:1380.
- Montgomery, M. J. and B. R. Baumgardt. 1965a. Regulation of food intake in ruminants. 1. Pelleted rations varying in energy concentrate. J. Dairy Sci. 48:569.
- Montgomery, M. J. and B. R. Baumgardt. 1965b. Regulation of food intake in ruminants. 2. Rations varying in energy concentration and physical form. J. Dairy Sci. 48:1623.
- Moore, L. A., J. W. Thomas and J. F. Sykes. 1960. The acceptability of grass/legume silage by dairy cattle. Proc. 8th Int. Grassld. Congr. p. 701.
- Morgan, M. E. and R. L. Pereira. 1962. Volatile constituents of grass and corn silage. I. Steam distillates. J. Dairy Sci. 45:457.
- Neumark, H. 1961. Amines in silage. Nature 190:839.
- Neumark, H. 1962. Tyramine and other amines in clover (Trifolium alerandrium) after incubation under sterile conditions. Nature 195:627.
- Neumark, H., A. Bondi and R. Volcani. 1964. Amines, aldehydes and keto-acids in silages and their effect on food intake by ruminants. J. Sci. of Food and Agr. 15:487.
- Nicholson, J. W. G. and H. M. Cunningham. 1964. Addition of limestone to immature corn and grass silages. J. Anim. Sci. 23:1072.

- Noller, C. W., J. E. Warner, T. S. Rumsey and D. L. Hill. 1963. Comparative digestibilities and intakes of green corn and corn silages with advancing maturity. J. Anim. Sci. 22:1135. (Abstr.).
- Okamoto, M., D. R. Waldo, R. W. Miller and L. A. Moore. 1964. Histamine levels in forages and dry matter intake of heifers. J. Dairy Sci. 47:1231.
- Owens, F. N., J. C. Meiske and R. D. Goodrich. 1968. The influence of urea or limestone additions in fermentation and feeding value of the ensiled corn plant (Zea mays). Proc. of Minnesota Nutrition Conference, p. 31.
- Owen, F. G. 1971. Silage additives and their influences on silage fermentation. Int. Silage and Res. Conf. p. 79.
- Pearse, G. R. and R. J. Moir. 1964. Rumination in sheep. 1. The influence of rumination and grinding upon the passage and digestion of food. Australian J. Agr. Res. 15:635.
- Peterson, W. H., E. G. Hastings and E. B. Fred. 1925. A study of the principal changes which take place in the making of silage. Univ. of Wisc. Agr. Expt. Sta. Bull. 61.
- Peterson, W. H., G. Bohstedt, H. R. Bird and W. M. Beeson. 1936. The preparation and nutritive value of A.I.V. silage for dairy cows. J. Dairy Sci. 18:63.
- Purser, D. B. and R. J. Moir. 1966. Rumen volume as a factor involved in individual sheep differences. J. Anim. Sci. 25:509.
- Purser, D. B., T. J. Klopfenstein and J. H. Cline. 1966a. Dietary and defaunation effects upon plasma amino acid concentrations in sheep. J. Nutr. 89:226.
- Rayetskaya, Yu, M. Rambidi, F. Kivutsan. 1964. Transformation of urea labeled with heavy nitrogen during ensilaging. (In Russian). Vestn. Sel'skokhoz. Nauki (2):16.
- Rodrigue, C. B. and N. N. Allen. 1960. The effect of fine grinding of hay on ration digestibility, rate of passage, and fat content of milk. Can. J. Anim. Sci. 40:23.
- Rook, J. A. F., C. C. Balch, R. C. Campling and L. R. Fisher. 1963. The utilization of acetic, propionic and butyric acids by growing heifers. Brit. J. Nutr. 17:399.
- Russell, E. J. 1907. The chemical changes taking place during the ensilage of maize. J. Agr. Sci. 2:392.

- Salas, F. J. 1971. Urea, ammonia and M-analog additions to corn silage rations for feedlot cattle. M.S. Thesis. Mich. State Univ.
- Satter, L. D. and W. J. Esdale. 1968. In vitro lactate metabolism by ruminal ingesta. Applied Microbiology 16: 680.
- Saue, O. and K. Breirem. 1969a. Formic acid as a silage additive. Proc. 3rd General Meeting of the European Grasslands Federation, Braunschweig, Germany. p. 161.
- Saue, O., and K. Breirem. 1969b. Comparison of formic acid silage with other silages and dried grassland products in feeding experiments. Proc. 3rd General Meeting of the European Grasslands Federation, Braunschweig, Germany. p. 282.
- Schaadt, H. and R. R. Johnson. 1968. Effects of maturity, fermentation time, and limestone and urea treatments on D(-) and L(+) lactic acid in corn silage. J. Dairy Sci. 51:5:802.
- Schmutz, W. G. 1966. The nutritive value of corn silages containing chemical additives as measured by growth and milk production of dairy animals. Ph.D. Thesis. Mich. State Univ.
- Senel, S. H. and F. G. Owen. 1966. Relation of dietary acetate and lactates to dry matter intake and volatile fatty acid metabolism. J. Dairy Sci. 49:1075.
- Senel, S. H. and F. G. Owen. 1967. Relation of dietary acetic and butyric acids to intake, digestibility, lactation performance, and ruminal and blood levels of certain metabolites. J. Dairy Sci. 50:327.
- Simkins, K. L., Jr., B. R. Baumgardt and R. P. Niedermeier. 1965a. Feeding value of calcium carbonate-treated corn silage for dairy cows. J. Dairy Sci. 48:1315.
- Simkins, K. L., Jr., J. W. Suttie and B. R. Baumgardt. 1965b. Regulation of food intake in ruminants. 4. Effect of acetate, propionate, butyrate and glucose on voluntary food intake in dairy cattle. J. Dairy Sci. 48: 1635.
- Snedecor, G. W. 1946. Statistical Methods. Iowa State College Press, Ames, Iowa.
- Taylor, J. C. 1959. A relationship between weight of internal fat, "fill", and the herbage intake of grazing cattle. Nature 184:2021.

- Ternouth, J. H. 1967. A factor limiting the ruminant's voluntary consumption of silage. J. Australian Inst. of Agr. Sci. 33:263.
- Thomas, J. W., L. A. Morre, M. Okamoto and J. F. Sykes. 1961. A study of factors affecting rate of intake of heifers fed silage. J. Dairy Sci. 44:1471.
- Thomas, J. W., L. D. Brown, R. S. Emery, E. J. Benne and J. T. Huber. 1969. Comparisons between alfalfa silage and hay. J. Dairy Sci. 52:195.
- Ulliyatt, M. J., K. L. Blaxter and I. McDonald. 1967. The relationship between the apparent digestibility of roughages in the rumen and lower gut of sheep, the volume of fluid in the rumen and voluntary feed intake. Anim. Prod. 9:463.
- Van Soest, P. J. 1962. Estimation of forage protein digestibility and determination of the effects of heat-drying upon forages by means of the nitrogen content of acid detergent fiber. J. Dairy Sci. 45:664.
- Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24:834.
- Varner, L., W. Woods and T. J. Klopfenstein. 1968. Urea or ammonium salts of volatile fatty acids as supplements to beef cattle rations. J. Anim. Sci. 27:1773. (Abstr.).
- Varner, L. and W. Woods. 1969. Urea or ammonium salts of volatile fatty acids as nitrogen sources for beef cattle. J. Anim. Sci. 29:174. (Abstr.).
- Varner, L. W. and W. Woods. 1971. Influence of ammonium salts of volatile fatty acids upon ration digestibility, rumen fermentation and nitrogen retention by steers. J. Anim. Sci. 33:1110.
- Virtanen, A. I. 1934. (From Watson and Nash, 1960). Contrib. Lab. Valio, Helsinki. No. 2.
- Voss, N. 1966. Amines and ammonia as products of protein decomposition in silage. Proc. 10th Int. Grassld. Congr. (Helsinki), p. 540.
- Waldo, D. R. and L. H. Schultz. 1956. Lactic acid production in the rumen. J. Dairy Sci. 39:1453.
- Waldo, D. R. and L. H. Schultz. 1960. Blood and rumen changes following the intraruminal administration of glycogenic materials. J. Dairy Sci. 43:496.



- Waldo, D. R., R. W. Miller, M. Okamoto and L. A. Moore. 1965. Ruminant utilization of silage in relation to hay, pellets and hay plus grain. II. Rumen content, dry matter passage, and water intake. J. Dairy Sci. 48:1473.
- Waldo, D. R., L. W. Smith, R. W. Miller and L. A. Moore. 1969. Growth, intake and digestibility from formic acid silage versus hay. J. Dairy Sci. 52:1609.
- Waldo, D. R., J. E. Keys, Jr. and C. H. Gordon. 1970. Direct-cut formic acid silage versus wilted silage for growth. J. Dairy Sci. 53:677. (Abstr.).
- Waldo, D. R., J. E. Keys, Jr., L. W. Smith and C. H. Gordon. 1971a. Effect of formic acid on recovery, intake, digestibility, and growth from unwilted silage. J. Dairy Sci. 54:77.
- Waldo, D. R. and J. C. Derbyshire. 1971b. The feeding value of hay crop silages. Int. Silage and Res. Conf. p. 141.
- Wallnofer, P., R. L. Baldwin and E. Stagno. 1966. Conversion of C<sup>14</sup> labeled substrates to volatile fatty acids by the rumen microbiota. Appl. Microbiol. 14:1004.
- Watson, S. J. and M. J. Nash. 1960. The Conservation of Grass and Forage Crops. Oliver and Boyd. London.
- Weston, R. H. 1966. Factors limiting the intake of feed by sheep. I. The significance of palatability, the capacity of the alimentary tract to handle digesta, and the supply of glucogenic substrate. Australian J. Agr. Res. 17:939.
- Weston, R. H. 1967a. Factors limiting the intake of feed by sheep. II. Studies with wheaten hay. Australian J. Agr. Res. 18:983.
- Weston, R. H. and J. P. Hogan. 1967b. The digestion of chopped and ground roughages by sheep. I. The movement of digesta through the stomach. Australian J. Agr. Res. 18:789.
- Wetterau, H. and W. Holyschuh. 1960-61. Die Erhöhung der Stickstoffgehaltes von Maisgärfutter Durch Zusatz Synthetischer Stickstoffverbindungen. Jahrb. Arbeitsgemeinschaft Fütterungsberatung 3:378.
- Wilkins, R. J. and R. F. Wilson. 1970. Experiments at Hurley with formic acid as a silage additive. Nat. Agr. Advisory Ser. Newsletter No. 1.

- Wilkins, R. J., K. J. Hutchinson, R. F. Wilson and C. E. Harris. 1971. The voluntary intake of silage by sheep. I. Interrelationships between silage composition and intake. J. Agr. Sci. 77:531.
- Wise, G. H., J. H. Mitchell, J. P. LaMasters and D. B. Roderick. 1944. Urea-treated corn silage vs. untreated corn silage as a feed for lactating dairy cows. J. Dairy Sci. 27:649. (Abstr.).
- Woodward, T. E. and J. B. Shephard. 1944. Corn silage made with the addition of urea and its feeding value. J. Dairy Sci. 27:648.
- Wrenn, T. R., J. Bitman, H. C. Cecil and M. L. Mench. 1964. Histamine excretion of cattle fed various rations. J. Dairy Sci. 47:882.

## APPENDIX

Table A1

## Simple Correlation Coefficients - Experiment 4 - Metabolic Study

Variable I	Variable II	r
Silage dry matter	Dry matter intake	0.043
Silage dry matter	% digestible dry matter	0.438
Silage dry matter	Silage unidentified water soluble N	-0.606*
Silage dry matter	Silage water soluble N	-0.645**
Silage dry matter	Silage NH <sub>3</sub> N	-0.659**
Silage dry matter	Silage water insoluble N	0.505*
Silage dry matter	Silage lactic acid	-0.849**
Silage dry matter	Silage acetic acid	-0.794**
Silage dry matter	Silage pH	0.757**
Silage dry matter	Free water intake	0.859**
Silage dry matter	Urinary water	-0.532*
Dry matter intake	Silage lactic acid	-0.021
Dry matter intake	Silage acetic acid	-0.175
Dry matter intake	Silage water soluble N	-0.089
Dry matter intake	Silage unidentified water soluble N	-0.087
Dry matter intake	Nitrogen intake	0.839**
Dry matter intake	Fecal nitrogen	0.579*
Dry matter intake	Urinary nitrogen	0.195
Dry matter intake	Nitrogen retained	0.679**
Dry matter intake	Urinary water	0.590*
Dry matter intake	Fecal water	0.794*
% digestible dry matter	Fecal nitrogen	-0.505*
% digestible dry matter	Nitrogen retained	0.445
% digestible dry matter	Dry matter intake	0.352
% digestible dry matter	Silage NH <sub>3</sub> N	-0.361

Table A1 (cont.)

Variable I	Variable II	r
% digestible dry matter	Silage water soluble N	-0.244
% digestible dry matter	Silage total N	-0.475
% digestible dry matter	Silage lactic acid	-0.447
Nitrogen intake	Fecal nitrogen	0.648**
Nitrogen intake	Urinary nitrogen	0.458
Nitrogen intake	Nitrogen retained	0.649**
Nitrogen intake	Silage total N	0.325
Fecal nitrogen	Nitrogen retained	0.184
Fecal nitrogen	Silage lactic acid	0.271
Urinary nitrogen	Nitrogen retained	-0.139
Urinary nitrogen	Silage unidentified water soluble N	0.305
Urinary nitrogen	Fecal water	0.484
Nitrogen retained	Silage water soluble N	-0.312
Nitrogen retained	Silage unidentified water soluble N	-0.306
Silage unidentified water soluble N	Silage lactic acid	0.546*
Silage unidentified water soluble N	Silage acetic acid	0.672**
Silage unidentified water soluble N	Silage water soluble N	0.994**
Silage unidentified water soluble N	Silage NH <sub>3</sub> N	0.580*
Silage water soluble N	Silage lactic acid	0.580*
Silage water soluble N	Silage acetic acid	0.717**
Urinary water	Total water intake	0.845**
Urinary water	Silage water intake	0.655**
Silage water intake	Total water intake	0.674**
Silage water intake	Free water intake	-0.806**

Critical Value<sup>1</sup>

\* (P &lt; .05) .497

\*\* (P &lt; .01) .623

<sup>1</sup>Snedecor, 1946



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 03061 6670