# UREA, AMMONIA AND M-ANALOG ADDITIONS TO CORN SILAGE RATIONS FOR FEEDLOT CATTLE

Thesis for the Degree of M. S. MICHIGAN STATE UNIVERSITY FERNANDO J. SALAS 1971

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#### ABSTRACT

# UREA, AMMONIA AND M-ANALOG ADDITIONS TO CORN SILAGE RATIONS FOR FEEDLOT CATTLE

by

#### Fernando J. Salas

Three experiments were conducted to investigate the effects of methionine hydroxy analog (M-analog) and different sources of crude protein supplementation to corn silage rations on steer performance, digestion parameters and levels of plasma amino acids.

In the feeding trial, a 4x2 factorial involving 80 steer calves was utilized in a 221-day feeding trial to study the effect of adding 3 g. of M-analog to the daily ration of steers receiving a full feed of corn silage (no grain) and four sources of supplemental crude protein. The analysis of the silages showed that Pro-Sil (ammoniated molasses, mineral mixture) and urea-mineral treated silages were higher in pH, lactic acid and organic protein (non-soluble nitrogen) than control silage which received additions of soy-mineral or Pro-Sil at feeding time. All silage rations were isonitrogenous and contained a S:N ratio of 1:10.

Steers receiving M-analog gained significantly faster (.82 vs. .73 kg. P<.01) and had significantly leaner carcasses with less fat as evidenced by a lower carcass grade (low Choice vs. middle Choice, P<.02). Daily DM consumption favored the M-analog group (7.0 vs. 6.3 kg.) and

feed efficiency values were idential.

For sources of supplemental crude protein, average daily gain was .77, .81, .79, and .72 kg. for control silage supplemented with soybean meal and minerals at feeding time, urea-mineral treated silage, Pro-Sil treated silage and control silage supplemented with Pro-Sil at feeding time respectively. Daily DM consumption was 6.7, 6.7, 6.7, and 6.5 kg. respectively and feed consumed per unit of gain was 8.7, 8.3, 8.5, and 9.1 respectively. Differences were small and insignificant between carcass traits. No significant interactions existed between type of silage and M-analog addition.

In the metabolic study, a 4x4 latin square was utilized to test the effect of daily supplementation of 3 g. of M-analog on steers fed Pro-Sil or urea-mineral treated silages. M-analog supplementation did not affect plasma urea or rumen ammonia levels. Regardless of M-analog additions, the average values of the five post-feeding sampling periods (2-hour intervals) indicated a faster ammonia release in the rumen and higher rumen ammonia levels for groups fed urea-mineral treated silage over Pro-Sil treated silage fed groups.

Rumen volatile fatty acids (VFA) concentrations at the various sampling periods were not significantly different for all groups. Overall mean values showed slightly depressed rumen VFA values for groups receiving M-analog. The nitrogen digestion parameters were not statistically different for the four treatments and all groups were in slightly negative nitrogen balance.

In the third experiment, the effect of feeding M-analog on plasma amino acid levels (PAA) in steers fed either corn silage and soybean meal or corn silage treated at ensiling time with Pro-Sil was investigated. The two silage rations were fed to two groups of 20 steers each; 1/2 of each group received 3 g. of M-analog per head daily. At the 180th day of the feeding trial, blood samples were secured from all steers before and 4 hours after the morning feeding. Plasma amino acid levels were then determined for the most rapid, median and slowest growing steer of each group. M-analog supplementation did not influence plasma total AA or Met and Cys levels. The average  $T_{A}/T_{0}$  for EAA and for NEAA were similar in all groups. No single EAA could be implicated by this procedure as limiting. The most consistent observation was that about 50% of the total PAA were EAA in steers fed silage rations.

# UREA, AMMONIA AND M-ANALOG ADDITIONS TO CORN SILAGE RATIONS FOR FEEDLOT CATTLE

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## A THESIS

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# TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION	1
LITERATURE REVIEW	4
Fermentation, Additives and Usage of Silage	4
Silage Fermentation	5
Losses Associated with the Ensiling Process	6
Nutritional Deficiencies of Corn Silage	10
Silage Additives	11
Non-protein Nitrogen Addition to Corn Plant	
Material at Silo Filling	13
Effects of Corn Silage Additives on Feedlot	
Performance	17
Non-Protein Nitrogen Utilization by Ruminants	19
Probable Causes of Limited Utilization of	
Non-Protein Nitrogen	23
Methionine Deficiency and Feeding M-Analog to	
Ruminants	27
Fate of Dietary Methionine in the Rumen	28
Bases for M-Analog Supplementation in Ruminants	29
Methionine Deficiency in NPN Supplemented	
Rations	30
Effect of Methionine in the Rumen	34
Effect of Methionine on Lipid Metabolism	35
Effect of Diet on Methionine Content in the	
Rumen	36
Effect of M-Analog on Milk Production	37
Effects of M-Analog Supplementation on Feedlot	
Cattle	37
MATERIALS AND METHODS	40
Experiment I - Steer Calf Feeding Trial	40
Harvesting of Silages	40
Feeding Trial	45
Silage Analysis	47
Experiment II - Metabolism Study	48
Feeding Regime	48
Sample Collection	50
Laboratory Analysis	51
Statistical Analysis	52

	Page
MATERIALS AND METHODS (Continued)	
Experiment III - Effect of M-Analog on Plasma	
Amino Acid Levels	52
Collection and Analysis Procedures	54
RESULTS AND DISCUSSION	55
Experiment I - Feeding Trial	55
Chemical Analysis of Silage	55
Effect of M-Analog Supplementation to All Corn	
Silage Rations	60
Sources of Supplemental Protein Compared	65
Experiment II - Metabolism Study	72
Chemical Analysis of Silage	72
Rumen and Blood Parameters	72
Rumen Volatile Fatty Acids	77
Nitrogen Digestion Parameters	81
Experiment III - Effect of M-Analog on Plasma	
Amino Acids	81
CONCLUSIONS	85
BIBLIOGRAPHY	88

# LIST OF TABLES

<u>Table</u>		Page
1	Average Analysis of Corn Silage and Nutrient Requirements of Feedlot Cattle	12
2	Experiment I - Feeding Trial	41
3	Formulation of Pro-Sil	42
4	Formulation of Soy-Mineral Supplement	42
5	Formulation of Urea-Mineral Silage Additive	44
6	Formulation of Methionine Hydroxy Analog Supplement	44
7	Experiment II - Metabolic Study Design of Experiment	49
7a	Experiment II - Metabolic Study Treatments Utilized	49
8	Experiment III - Effect of M-Analog Addition on Plasma Amino Acid Levels of Steers Fed Corn Silage	53
9	Average Chemical Analysis on Drymatter Basis of Silages Fed	56
10	Effect of Methionine Hydroxy Analog Addition	61
11	Sources of Crude Protein Compared	66
12	Effect of M-Analog Addition and Sources of Crude Protein	67
13	Ration Composition and Cost on 85% DM Basis	70
14	Experiment I - Capacity of Corn Silage to Produce Beef	71
15	Experiment II - Means of Rumen Ammonia Values	73
16	Experiment II - Means of Blood Urea Values	74

<u>Table</u>		Page
17	Experiment II - Mean Rumen Acetic Acid Concentrations	78
18	Experiment II - Mean Rumen Propionic Acid Concentrations	79
19	Experiment II - Mean Rumen Butyric Acid Concentrations	80
20	Experiment II - Digestion Parameters	82
21	Plasma Amino Acids Analysis	83

## LIST OF FIGURES

Figure		Page
1	Schematic illustration of equipment used in applying Pro-Sil to corn silage in a tower silo	43
2	Average daily gain during three periods of the feeding trial	62

#### INTRODUCTION

Recent studies have indicated that if the present relative rates of population growth and food production continue, it is probable that the excess of cereal grain and legume seeds, which are now used as animal feeds, will be critically diminished. Consequently, it will be of increasing concern for man to determine which animals most efficiently convert the surplus grain into more nutritionally complete foods. In this respect, beef cattle are the least efficient of all farm animals. However, they are able to survive and even be economically productive when full fed only materials non-edible by man; thus, they need not compete with man for food. Therefore, ruminant animals, in general, appear to be well equipped to insure their survival due to their peculiar digestive apparatus, which is anatomically and physiologically different from that of other mammalian species.

The ruminants' digestive system includes a symbiotic relationship with a massive microorganism population located in the fore stomach which is capable of digesting fibrous plant materials resistant to digestion by tissue elaborated enzymes. The final products of this process (volatile fatty acids) can provide most of the energy needed by the host

animal. In addition, rumen microorganisms synthesize protein from non-protein nitrogen compounds, vitamin K and all of the B-complex. The protein is transported to the small intestine where they are utilized in the same manner as in monogastric animals.

In the harvesting of grain and legume seeds, the stalks and leaves are left in the field in spite of the fact that, in some cases such as with corn, they contain up to 50% of the total plant gross energy. These residues can be converted by the ruminants into high quality foods which otherwise man would not obtain. The combination of the high yielding nature of corn plant and the ensiling process (which allows preservation of the plant at the stage of maturity where concentration of nutrients is maximum) results in a large amount of digestible energy produced for dairy and beef cattle. In areas where corn silage is well adapted, no other harvested crop surpasses corn silage in the amount of digestible energy produced per hectare.

Extensive research conducted at the Michigan station and other stations has clearly demonstrated that complete rations of high quality corn silage supplemented with non-protein nitrogen and minerals at silo filling time are capable of producing more kilograms of Choice grade beef cattle per hectare than any other crop. Nevertheless, there are inherent problems in feeding such rations. One of the major ones being the limited utilization of non-protein nitrogen by rumen microbes, which, in turn, do not provide

a sufficient quantity of amino acids for the optimum synthesis of host tissue protein.

There is considerable evidence that one of the deficient amino acids in growing ruminants is methionine, particularly when non-protein nitrogen supplemented silages are fed. Direct supplementation with methionine has not improved absorbable levels of methionine because it is degraded by rumen microbes before reaching absorption sites in the lower gut. Methionine hydroxy analog (M-analog) is the calcium salt of methionine hydroxy acid, and it has been suggested that 50% or more of the analog resists the rumen degrading action and is available in the lower gut for absorption.

The objectives of this study are:

- 1) To determine the effect of adding 3 g. of methionine hydroxy analog to the daily ration of steers full fed corn silage with no grain on steer performance, digestion parameters and plasma amino acid levels.
- 2) To assess the effect of two sources of non-protein nitrogen (Pro-Sil and urea-mineral) applied at silo filling time on the chemical and physical nature of the resulting corn silage.
- 3) To compare four methods of supplementing corn silage to correct its protein and mineral deficiencies: a) Pro-Sil applied at silo filling, b) urea-mineral applied at filling, c) Pro-Sil applied at feeding, and d) soymineral applied at feeding.

#### LITERATURE REVIEW

This review consists of three sections: (1) Fermentation, Additives and Usage of Silage; (2) Non-Protein Nitrogen Utilization by Ruminants; and (3) Methionine Deficiency and Feeding Methionine Hydroxy Analog to Ruminants.

## FERMENTATION, ADDITIVES AND USAGE OF SILAGE

It is well documented that corn silage contains from 65 to 70% total digestible nutrients when harvested from high yielding stands of hybrid corn at its proper state of maturity and when it has undergone a normal fermentation.

It is further well documented (Henderson, et al. 1968, 1970a, 1970b, 1971) that feedlot cattle can be fed to choice slaughter grade when the ration is made up entirely of well-fermented corn silage properly supplemented for protein, minerals, vitamins and growth stimulants. Henderson's data further show that the number of pounds of beef produced per hectare of corn fed is maximized when the entire corn crop is harvested as corn silage and that pounds of beef per acre of corn fed decrease in direct proportion to the percent of the corn crop harvested as shelled corn.

Data from a number of research stations (Newland, et al. 1965; Minish, et al. 1966; Peterson, et al. 1970; Hatfield, et al. 1971; and Henderson, et al. 1970b and 19 1971) show conclusively that daily drymatter consumption of

feedlot cattle decreases in direct proportion to the percent of the entire ration made up of corn silage. As a result of decreased drymatter consumption, average daily gain decreases with increasing levels of corn silage in the ration.

## Silage Fermentation

Watson and Nash (1960) have defined silage as succulent material produced by a process of controlled vital changes from a green crop or other material of high moisture content. Some of these changes were reported as early as 1907 by Annett and Russell, who noted a decrease in sugar content, an increase in non-protein nitrogen (NPN) and no change in crude fibre after fermentation had occurred.

Russell (1908) observed the appearance of volatile fatty acids (VFA) during fermentation. A decrease in pH during fermentation and its importance in preventing undesirable fermentation were reported by Esten and Mason (1912) and reviewed by Watson and Nash (1960).

During normal corn silage fermentation, organic acids are produced and specially lactic acid at the expense of plant carbohydrate (Coppock and Stone, 1968 and Barnett, 1954). Other research (Geasler and Henderson, 1969, and Cash, et al. 1971) has shown that corn silage drymatter (DM) consumption by steers is positively correlated with the lactic acid content of silage. Other workers have shown a negative correlation between intake and butyric acid levels. For these reasons and because of its higher caloric value, high lactic acid levels are characteristic of excellent-

quality corn silage.

## Losses Associated with the Ensiling Process

Annett and Russell (1907) compared green corn plant material with the resulting corn silage and found a reduction in nitrogen free extract and an increase in NPN content. Later studies showed these changes to be due to carbohydrate and protein degradation.

As quoted by Geasler and Henderson (1969), and based on the above concept, it is thought that the green corn plant is a more desirable feed for beef cattle than corn silage, which is inferior to the former in true protein, gross energy and, perhaps, in some minerals. The difference in the corn plant material before and after ensiling may be interpreted as silage losses.

Silage losses (generally referred to as a loss in drymatter) can be divided into two classes. These are: field or harvest and fermentation and storage losses. Relative to field losses, Geasler and Henderson (1968) have shown that drymatter yield per acre decreases rapidly after the corn plant reaches 40% DM. Drymatter losses will always occur after frost kills the plant cells since no further accumulation of DM can result from photosynthetic processes (Coppock and Stone, 1968). Geasler and Henderson reported a minimum loss when total corn plant DM was between 30% and 35% at harvesting time.

Regarding storage losses, these are commonly expressed as a percent of the silage drymatter and have been

categorized by Gordon (1967a) as gaseous, seepage and spoilage losses.

Gas losses are unavoidable and are mainly represented by CO<sub>2</sub> produced during the phase of respiration of the ensiling process (when the crop is placed in the silo, the cells of the plant still alive and respiring degrade simple sugars to CO<sub>2</sub> and water, according to Barnett (1954)).

Peterson (1925) reported that oxygen had disappeared from the silage mass at the end of five hours, whereas carbon dioxide concentration peaked at two days in his study. The amount of CO<sub>2</sub> evolved may be reduced markedly by a higher degree of mass compaction which in turn is influenced by particle size, silo structure and other factors.

Gases other than CO<sub>2</sub> may also be produced but they are quantitatively unimportant in the final silage. One is nitrogen dioxide, which has lethal effects on humans. A special report on this subject is available (Agricultural Research Services, USDA Pub. No. 810).

Seepage losses are avoidable and, according to Barnett (1954), are due to mechanical expression of excess water. Recent investigation by Geasler (1970) has shown that seepage equaled zero in corn silage when drymatter was near 35% and the pressure in the mass was 5 psi. Gay (1966) has shown that in silage containing more than 30% drymatter, the percent of drymatter loss seldom exceeds 1%, but these losses can be up to 15% of the drymatter if the silage drymatter is lower than 20%. Seepage losses can be avoided if

appropriate techniques are followed (Miller and Clifton, 1965) and adequate silo structures are utilized (Gordon, 1967a).

Spoilage losses are avoidable. Important factors relative to such losses are: a) adequate ensiling and handling procedures to prevent air from entering the mass in order to evade putrefying processes, and b) suitable levels of soluble carbohydrates in the crop utilized for ensiling. Work by Johnson, et al. (1966) indicated that the corn plant is well provided with soluble sugars after it reaches a 20% drymatter content; then, soluble sugar levels decline slowly with advancing maturity. He also states that corn silage can be successfully ensiled at almost all stages of maturity.

Fermentation losses are qualitative as well as quantitative. Qualitative losses involve mainly protein and carbohydrate degradation.

Plant protein breakdown and a resulting increase in water soluble NPN during the ensiling process is considered an unavoidable loss. Several factors may influence the level of proteolysis. Normally, proteolytic enzymes contained in the plant cell are entirely responsible for hydrolysis of protein according to Russell (1908), Hunter (1921) and Mabbit (1951).

Microorganisms may break down the plant protein under certain conditions. Watson and Nash (1960) stated that butyric acid producing bacteria may decompose plant

protein through the action of their enzymes, especially when crops low in soluble carbohydrates are undergoing fermentation. This is associated with the putrefying changes occurring when air is allowed in the silage mass. Furthermore, Barnett (1954) stated that under these conditions a further breakdown of the amino acids already formed by proteolysis will occur producing keto acids, amides, amines, carbon dioxide and ammonia. However, Rosenberg (1956) indicated that lactic acid-producing bacteria along with other bacteria capable of proteolysis can be of major importance in this respect.

The effect of heat on plant protein degradation is not well documented; however, McPherson (1952), working with corn plant cell extracts under anaerobic conditions showed that at  $30^{\circ}$ C considerably more protein breakdown occurred than at  $18^{\circ}$ C.

Gordon (1967b) reported a lower protein digestibility for overheated silage which was accompanied by lower DM consumption.

Brody (1960) analyzed numerous normal silages and showed that up to 25% of the total nitrogen was degraded to NPN, while research conducted at Michigan State University (Henderson, et al. 1970b and 1971) has demonstrated that nearly half of the true protein of the fresh material was degraded to ammonia and NPN during fermentation.

Carbohydrate degradation: During silage fermentation, the soluble carbohydrate content in the corn plant decreased from 83% to 39% (Johnson, et al. 1966). These sugars are converted mostly into lactic and acetic acids. Only the production of acetic acid involves a CO<sub>2</sub> release which escapes from the mass and is a quantitative loss. In a study by Peterson, et al.(1925), starch content from the corn plant material decreased from 10 to 30% during fermentation. No change in the crude fibre content during fermentation has been observed by Annett and Russell (1907) and Hunter (1921) and others.

It should be recognized that the decrease in carbohydrates as a result of bacterial action may not reflect a
net energy loss because ruminant animals utilize products of
bacterial fermentation; namely, lactic and acetic acid
(Coppock and Stone, 1968). Furthermore, lactic acid is the
most abundant acid in corn silage (Henderson, et al. 1970b
and 1971 and Owens, et al. 1968) and has a feeding value
almost equal to that of glucose (Kirch and Hidelbrand, 1930)
as quoted by Barnett (1954).

# Nutritional Deficiencies of Corn Silage

The relative nutritional value of corn silage is influenced by plant maturity, soil fertility, growing season, harvesting and storage methods, feeding practices and kind of supplementation utilized at ensiling or feeding. An excellent review of this topic is presented by Owen (1967).

Although good corn silage is high in energy, succulent, and highly acceptable by ruminants, it is not nutritionally complete for feedlot cattle when fed alone (Hatfield, et al. 1966). It is inherently deficient in protein and most of the essential minerals and vitamins.

The following table defines the deficiencies of corn silage relative to the needs of cattle, as established by Henderson and Geasler (1969).

## Silage Additives

A large number of substances have been tested for improving corn silage fermentation and improving its nutritional value and, particularly, to elevate its crude protein content.

Nitrogen fertilization of the growing corn plant generally has a minor effect on silage quality, although, in most instances, it increases the crude protein content (Owen, 1967). Alexander, et al. (1963) has shown increased protein content and higher yield in corn plant material when nitrogen fertilization was used.

Combining the corn plant with legumes at ensiling time may be feasible for areas where both kinds of plants can be grown simultaneously.

The addition of nitrogen compounds to the corn plant material either at ensiling or feeding time has been extensively tested and compared. Ammonia (or compounds that will yield ammonia in the silo) will combine with the organic acids and, thus, serve as a neutralizing agent which increases fermentation and the level of lactic acid produced.

Urea addition to corn silage at silo filling time is now a widespread practice in the United States (Klosterman,

TABLE 1

Average Analysis of Corn Silage and Nutrient Requirements of Feedlot Cattle

Element	Corn Plant 100% Dry Matter	Deficit 1/	Requirements2/
Nitrogen	1.30	0.78	2.08
Crude Protein $\frac{3}{2}$	8.13	4.78	13.00
Calcium	0.32	0.08	0.40
Phosphorus	0.25	0.05	0.30
Salt	0.089	0.411	0.500
Sulfur4/	0.140	0.084	0.224
Potassium	0.947	adequate	0.200
Magnesium	0.175	adequate	0.065
Manganese	0.007	adequate	0.0009
Iron	0.012	adequate	0.010
Copper	0.00005	0.00085	0.0009
Zinc	???	0.0038	0.0038
Iodine	???	0.00005	0.00005
Cobalt	???	0.000001	0.000001

<sup>1/</sup> Based on animal consuming 2.4% of body weight daily
in 85% DM feed.

<sup>2/</sup> Based on National Research Council (NRC) and recent research.

<sup>3/ 6.25</sup> times percent nitrogen.

<sup>4/</sup> Ratio of 1 part sulfur to 10 parts nitrogen.

1963; Huber, et al. 1967).

Non-protein Nitrogen Addition to Corn Plant Material at Silo Filling

When added to corn silage at filling time, the urea is distributed throughout the silage mass, affording the animals a gradual intake which may help avoid palatability and toxicity problems associated with high concentrations of urea (Hatfield, et al. 1959; Bloomfield, et al. 1961; and Campbell, et al. 1963).

Urease activity in urea treated silages was reported by Hastings (1944). He further noted that increased temperature caused an increase in urea hydrolysis. Karr, et al. (1965) found intense urease activity in the fresh chopped corn plant and noted a corresponding ammonia yield.

It is well documented that when corn plant material is (varying between 28% and 40% DM) treated with urea at ensiling time, approximately 50% of the applied urea remains as urea after fermentation. However, Bentley, et al.(1955), Hatfield, et al.(1966), and Huber, et al.(1968a) have found wide differences of urea recoveries ranging from 4% to 84%. Most of the hydrolised urea is recovered in the form of ammonia (Henderson, et al. 1970b; Lopez, et al. 1970b; and Huber, et al. 1968a).

In one of the earliest reports of urea addition to silage, Cullison (1944) observed a prolonged fermentation and improved palatability in chopped sorghum when treated with 0.5% of urea. The pH of the silage treated with urea

was found to be higher than the control silage. Davis (1944) and later Bentley, et al. (1955), among others, confirmed this observation. Klosterman, et al. (1963) postulated that higher pH and an elevated level of lactic acid were due to the buffering action of urea, which, after being reduced to ammonia, allows the fermentation to continue longer and bring about an increased concentration of bacterial end products. They concluded that elevated concentrations of lactic and acetic acid are usually associated with urea additions to silage. Klosterman's conclusion is supported by Polan, et al. (1967) and Huber, et al. (1968a).

McDonald and Henderson (1962) suggested that chemical changes, such as protein breakdown to amino acids, increase the buffering action during silage fermentation. However, Lopez, et al.(1970b) stated that amino acids are unlikely to make a contribution within the pH range of 4.0 to 6.0; but it appears more logical that organic acids formed may be neutralized by basic residues (ammonia and amines) produced from the amino acids within the stated pH range.

Ammonia and other forms of NPN are commonly found in higher concentrations in silage treated with NPN sources (Johnson, et al. 1967). Therefore, it appears most likely that ammonia is the neutralizing agent discussed by Klosterman (1963), Polan, et al.(1967) and Huber, et al.(1968a). Furthermore, its combination with the silage acids to form ammonium salts of the respective acids has been suggested by Bentley and co-workers (1955), Henderson, et al.(1969),

(1970b), (1971), and Johnson, et al. (1967).

Ammonium salts are comparatively better utilized for rumen microbial growth than urea (Wetterauh and Holzchuh, 1960, 1961) as reported by Coppock and Stone (1968). Moreover, Belasco (1954) and Hendrickx (1967) utilizing in vitro studies demonstrated higher nitrogen availability from organic ammonium salts, especially ammonium lactate (which appears to be formed in considerable quantities in NPN treated silages), and ammonium succinate than from urea. Belasco also attributed to these salts a stimulatory effect on nitrogen fixation by rumen micro flora.

Workers in Eastern Europe (Modyananou, et al. 1960, and Rayetskaya, et al. 1954, as quoted by Owens, et al. 1968) reported that urea additions to corn silage resulted in higher final levels of true protein, which agrees with results obtained by Johnson, et al. (1967) and Henderson, et al. (1971). The Russian workers suggested that increasing the length of the fermentation period resulted in the greater microbial growth and, hence, greater microbial protein synthesis. Lopez, et al. (1970b) did not observe an increase in true protein in a series of silage bag experiments when urea was added at ensiling time.

Biuret addition to corn silage is of interest because of its low toxicity (Meiske, et al. 1955; Reep, et al. 1955; Berry, et al. 1956; and Hatfield, et al. 1959), although it requires a longer adaptation period to reach maximum nitrogen utilization compared to urea (Farlin, et al. 1968, and Oljten,

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et al. 1968). Karr, et al. (1965) ensiled corn at filling time with biuret, which proved to be more stable than urea during corn silage fermentation. Biuret-treated silage showed higher DM consumption and nitrogen retention values than the urea-treated silage when fed to lambs. However, more data is needed to fully evaluate biuret as a silage additive.

Anhydrous ammonia as a source of nitrogen is 1/3 less expensive than urea. When it is combined with molasses and minerals (Pro-Sil) 1/, it has given excellent results (see page 18). Studies conducted at Michigan State University (Research Report 108 and 143) on numerous Pro-Sil treated silages show that about 2/3 of the applied ammonia combines with lactic and acetic acid during fermentation which resulted in an inexpensive way to produce ammonium lactate. The remaining 1/3 of the applied ammonia is recovered as organic nitrogen (apparently microbial protein). The high concentration of the ammonium salts are probably responsible for the good results obtained in the feeding trials.

Although Diammonium Phosphate (DAP) has been studied as a nitrogen source for ruminants (Cowman and Thomas, 1962; Oljten, 1962; and Noller, et al. 1967), no appreciable advantage over urea has been demonstrated. It is less palatable but provides extra phosphorous. Johnson, et al. (1967)

Commercial name patented by Michigan State University. See composition in Table 3, page 42.

reported lower levels of lactic acid on DAP treated silages than in urea-limestone or limestone treated silages.

Corn silage with soybean meal added at ensiling time exhibited lower concentration of lactic acid and an increased true protein destruction in the final silage as compared to urea treated silage (Lopez, et al. 1970a and 1970b).

Limestone (CaCO<sub>3</sub>) addition to corn silage at filling time is widely practiced. Ohio workers have shown a consistently higher organic acid content of silages treated either with 1% limestone, or 0.5% urea + 0.5% limestone (Klosterman, et al. 1963; Johnson and McClure, 1968).

Other additives to corn silage have been thoroughly reviewed by Barnett (1954), Watson and Nash (1960) and Coppock and Stone (1968).

# Effects of Corn Silage Additives on Feedlot Performance

1.- Comparison between ensiled NPN supplementation and no nitrogen supplementation (negative control) to corn silage.

Bentley, et al. (1965) with yearling steers, and Meiske and Goodrich (1968) with steer calves, showed conclusively that urea treated silage (at ensiling) produced significantly higher average daily gains (ADG) and had superior feed efficiency (FE) than control silage. Karr, et al. (1965) observed that additions of NPN in adequate amounts increased the growth rate 26%. This difference is obviously due to the lack of an adequate crude protein level in the control silage.

The recommended level of urea additions at ensiling

time is 10 to 15 lbs. per ton of fresh chopped corn plant material on a 35% DM basis, evenly distributed.

The following three comparisons were reported to have been made on isonitrogenous basis.

2.- NPN additions to corn silage at ensiling time versus NPN additions to corn silage at feeding time.

Henderson and Purser (1968) reported that addition of urea at ensiling was superior to urea addition at feeding, as measured by the rate of gain of beef heifers calves.

Similar results were presented by Klosterman, et al. (1955) on steers. In one experiment, Harvey, et al. (1963) showed similar daily gains on calves fed both kinds of rations; but slightly better feed efficiency was observed in favor of urea-treated silage.

When high-quality corn plant material is supplemented at ensiling time with suitable NPN sources, a better performance and a higher DM consumption from feedlot cattle may be anticipated than if supplementing the same NPN source at feeding time.

3.- Comparison between various NPN additives to corn silage at ensiling time.

Karr, et al. (1965) presented data showing that
Biuret treated silage had higher nitrogen retention in lambs
than urea treated silage. Henderson, Purser and Geasler
(1970b), compared Pro-Sil, a urea-mineral mixture, and urea
additions at ensiling time. Results showed that Pro-Sil and
urea-mineral supplementation improved performance of steers

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as compared to the urea addition. These data suggest that minerals may play a beneficial role during silage fermentation and that Pro-Sil was superior than urea alone as an additive to corn silage.

In general, NPN compounds modify silage fermentation, enhancing the nutritive value of the resulting silage.

4.- Ensiled NPN treated corn silage versus untreated corn silage supplemented with soybean meal at feeding time.

Stengerg, et al. (1968) reported that untreated corn silage supplemented with SBM produced higher daily gain on steers than urea treated silage, which is in agreement with reports by Bentley, et al. (1955), Klosterman, et al. (1963), Owens, et al. (1967), and Jordan, et al. (1965), among others. However, Henderson and Purser (1968) showed that urea treated silages produced lower feed costs per hundredweight of gain than untreated silage rations supplemented with soybean meal at feeding time.

### NON-PROTEIN NITROGEN UTILIZATION BY RUMINANTS

to be no significant discrepancy between ruminants and non-ruminant mammals in the essential chemical pathways of nitrogen metabolism in the various tissues of the body (McDonald, 1968, and Hogan and Weston, 1970). Perhaps the most remarkable discovery showing this similarity was by Black, et al. (1952), (1957) and Downes (1961). By using isotopical techniques, they conclusively determined that

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sheep and cattle have the inability to synthesize essential amino acids (EAA) at the tissue level. This metabolic feature has also been shown for man, rats and dogs. The work of Rose (1938) made it clear that EAA are indispensable for maintenance and optimum growth; thus, they must be provided in the diet or at least at absorption sites (as in the case of ruminants) in adequate amounts to meet the specie requirements.

Nevertheless, in Germany in the 1920's and later in the United States in the 1930's (see Church, et al. 1970, page 590; Beeson, 1969; Chalupa, 1968, and Loosli et al. 1949) there were reports showing that ruminant animals could survive and even grow on diets containing variable amounts of urea replacing natural protein. By 1949, Loosli, et al. obtained substantial growth, over a three-month period, in lambs and goats fed a purified diet devoid of amino acids, in which virtually all the nitrogen was in the form of urea but with adequate levels of energy. Simultaneously, in the same experiment, EAA analyzed by microbiologic techniques were 9 to 20 times greater in the rumen material and feces than in the feeds fed. This led to the conclusion that all EAA were synthesized by rumen microbes. These findings were later confirmed by Duncan, et al. (1953).

Therefore, ruminant animals are capable of using either preformed protein or NPN such as urea, biuret and ammonium salts to satisfy their protein requirements.

The following concepts are based on the combined descriptions of Bryant (1970) and Thomas (1966) on the fate of nitrogenous compounds in the rumen.

- 1.- Rumen microbial proteolytic activity breaks down dietary protein to peptides and amino acids.
- 2.- Amino acids and peptides are utilized to synthesize microbial cell components such as protein and nucleic acids.
- 3.- The vast majority of amino acids are catabolized to VFA,  $CO_2$  and  $NH_3$ .
- 4.- NPN compounds are reduced to  $NH_3$  and other products such as  $C0_2$  in the case of urea.
- 5.- NH<sub>3</sub>, as the major source of nitrogen, is utilized in the synthesis of microbial cell constituents such as protein, nucleic acids and cell wall molecules.

Excess ammonia not utilized by rumen microflora can be absorbed through the rumen wall into the blood stream, and a minute amount is metabolized in the rumen wall to form certain amino acids. The absorbed NH<sub>3</sub> may re-enter the rumen by diffusion from blood vessels in the rumen, by saliva, or by both routes, after being converted to urea in the liver. Diffusion of urea from the blood to the rumen is the major pathway of endogenous nitrogen reentering the rumen. Absorbed ammonia which does not return to the rumen is excreted as urinary urea, and a small amount is utilized in the tissues for synthesis of nitrogenous compounds such as amino acids, purine and pyrimidine bases.

Many factors modify the dynamics of the nitrogen cycle just described. When rumen and blood NH<sub>3</sub> are high, then nitrogen is lost through urine, resulting in an eventual loss of nitrogen available for microbial protein synthesis (Bloomfield, et al. 1960). The extent of NH<sub>3</sub> concentration in the rumen is related to the water solubility of the protein ingested and to the availability of rapidly fermentable carbohydrates (McDonald, 1952; McDonald and Hall, 1957; and Hungate, 1966). The most efficient use of nitrogen occurs when protein synthesis in the rumen exceeds nitrogen degradation rate (Thomas, 1966).

In beef cattle, usually, little dietary protein escapes from the catabolic-anabolic action of the rumen. From available data, under most dietary regimens, microbial protein would account for 70 to 90% of the protein passing to the omasum (Bergen, 1967). The digesta, including rumen microbial cells, passes to the abomasum and small intestine in a constant flow where it is digested to amino acids and absorbed in this form (Kay, 1969). The levels of EAA required by ruminants are yet unknown; however, from experimental data, microbial proteins are highly digestible and have a relatively high biological value (see Hungate, 1966, Table VII-10, page 308). Bergen, et al. (1968) give an excellent discussion on the latter subject. Thus, under normal conditions, ruminant animals depend largely upon microbial protein to meet their protein requirements.

# Probable Causes of Limited Utilization of Non-Protein Nitrogen

The inefficient utilization of high levels of NPN by ruminants is widely accepted. Oljten (1969) concluded from several studies, including his own, that when NPN completely replaces the protein in purified diets, growth and feed efficiency are about 65% as good as that of animals fed rations containing preformed protein. Furthermore, since the early work of Hart, et al. (1939) it has been observed that weight gains decrease as the level of NPN increases in diets for growing ruminants.

The efficiency of microbial protein synthesis from various sources of NPN has been measured in vitro by Belasco (1954) and Hendrickx (1967). These studies show that certain ammonium salts such as lactate, succinate and acetate are better utilized than urea. Certain amides resulted in almost no microbial protein synthesis (Belasco, 1954). Similar compounds may be present in the unidentified non-protein nitrogen fraction of silages.

a specific growth requirement for branched chain fatty acids such as isobutyric and isovaleric (Allison, et al. 1962). Such compounds provide essential carbon skeletons for certain amino acids such as valine and leucine. Cline, et al. (1966) reported low amounts of isovaleric and isobutyric acids in lambs fed a urea purified diet. These workers observed increased nitrogen retention and a higher digestibility when

isovalerate, isobutyrate and valerate were added to a 39% crude fibre ration, but no effect was observed on a higher crude fibre ration.

Thomas, et al. (1951) demonstrated that the use of NPN in ruminant rations increases the need for sulfur. The protein-rich feeds which are replaced by NPN are usually excellent sources of this mineral. Johnson, et al. (1970) showed that sulfur is required for microbial formation of cystine and methionine, which synthesis has been demonstrated by Emery, et al. (1957). Thus, sulfur may be a limiting nutrient in high NPN diets for ruminants. To overcome such deficiency, a nitrogen/sulfur ratio in the diet of 10-15 to 1 is recommended by Beeson (1969). A study by Huber, et al. (1970) showed that sulfur additions to dairy cows receiving silage treated with 0.75% urea as their only source of supplemental nitrogen, did not increase milk yields but there was a slight increase in efficiency of nitrogen utilization.

One of the major problems that prevents the efficient utilization of urea is the rapid rate of ammonia release in the rumen, as compared to the rate of ammonia uptake by rumen microbes. This results in a substantial loss of nitrogen escaping microbial synthesis in spite of the rumen nitrogen recycling mechanisms (Bloomfield, et al. 1960). In addition, an excess of urea may cause toxicity (Reep, et al. 1955). When the capacity of the liver to synthesize urea from excess portal ammonia is surpassed, ammonia concentration

rises in peripherial blood and may provoke symptoms as those of urea toxicity (Lewis, et al. 1957). However, when urea intake is gradual throughout the day (as in urea treated silage), it allows a slower rate of ammonia release in the rumen, and nitrogen utilization is improved (Campbell, et al. 1963, and Bloomfield, et al. 1961).

Other NPN sources with a slow ammonia release rate have been tested. Biuret has been shown to be much less toxic than urea due to its slower ammonia release rate, but no conclusive advantages have been observed (Meiske, et al. 1955; Berry, et al. 1956; Oljten, et al. 1968; and Farling, et al. 1968). A longer adaptation period for optimum utilization is required (Hatfield, et al. 1959; and Karr, et al. 1965).

Levels of nitrogen and energy fed have very important effects on NPN utilization. Campling, et al. (1962) continuously infused 75 or 150 g. of urea per day in aqueous solution into the rumen of cows fed 3% crude protein oat straw and observed increase in feed intake, fibre digestibility, and a faster particle passage. Likewise, Fontenot, et al. (1955) and Barth, et al. (1962) 1/2 showed increased nitrogen utilization of high urea rations when readily available carbohydrates were added to the diets. This was due to an increased microbial synthesis of amino acids (Conrad, et al. 1961). On the other hand, an excess of carbohydrates

<sup>1/</sup> As reported by McLaren, 1964.

relative to nitrogen present in the rumen will accumulate in the form of ATP, which retards fermentation (Hungate, 1966). In any case, nitrogen/energy imbalance may result in reduced feed intake according to Waldo (1968).

The above observations suggest that within the rumen, NPN diets which are inadequate in energy availability to rumen microbiota result in lower nitrogen utilization and diets low in energy but adequate in nitrogen levels result in a lower energy utilization. Thus, there must be a nitrogen available energy ratio which will allow maximum microbial growth and fermentation and thus provide greater quantities of nutrients to the host animal.

Data by Potter, et al. (1968) showed that the total quantities of nitrogen reaching the abomasum were approximately 80% as high for steers fed urea as for those fed all natural protein diets. Presumably, the extra 20% nitrogen was made up mostly from undegraded protein in the rumen passing to the abomasum in animals fed all vegetable protein rations.

The apparent limit in the animal productivity due to the limitations of rumen microorganisms to synthesize microbial protein can be overcome by administering high quality protein posterior to the rumen (through abomasal and duodenal fistulas) or by treating the protein-rich feeds in such a manner that protein will not be easily attacked by rumen microbes (heat, formaldehyde or tannic acid treatments). By these means, improvement in nitrogen utilization and

superior performance have been consistently reported (McDonald, 1968, page 147; Allison, 1970, pages 459-460; and Church, et al. 1970, pages 581-592). Bypassing the rumen with protein may not have practical application for feedlot cattle due to the competitive character of the bovine with man for protein in this case. Therefore, as forecasted by Armstrong (1968), NPN will be the only nitrogen supplement that ruminants can afford in the near future. Thus, improvement in NPN utilization by ruminants is imperative.

# METHIONINE DEFICIENCY AND FEEDING METHIONINE HYDROXY ANALOG TO RUMINANTS

Both EAA and NEAA must be available simultaneously and in sufficient quantities for efficient tissue protein synthesis. A limiting amino acid is defined as the one in a given diet which by quantity is least able to fulfill the animal's requirement. Practically speaking, it is the amino acid which will give the greatest growth response when supplemented to that diet (Potter, 1970).

There is well documented evidence that some amino acids are limiting when the protein fed ruminants is largely made up of NPN, although neither the amino acid requirements by ruminants (Purser, 1970) nor their actual availability to the host from microbial protein (Bergen, et al. 1967) are known. The likelihood that methionine may be a limiting amino acid in NPN supplemented diets for ruminants is also

supported by numerous experiments reviewed below. In addition, methionine requirements must be considered beyond protein needs in view of its special function in fat and vitamin metabolism, nucleic acids synthesis, and rumen activity (Chandler and Polan, 1970).

### Fate of Dietary Methionine in the Rumen

Only traces of free amino acids are found in the rumen. Methionine, along with other amino acids, is extensively deaminated by rumen bacteria and protozoa and later oxidized to the keto acid form which is then decarboxilated to fatty acids. However, dietary methionine in the rumen is degraded more slowly than other amino acids (Lewis and Emery, 1962).

Dethiometylation, incorporation to microbial cell components, and CO<sub>2</sub> production of methionine have been reported (Salsbury, et al. 1970, Belasco, 1971). Methionine has been suggested to be utilized for protozoa lipid synthesis (Patton, et al. 1968). Finally, some methionine may be absorbed directly through the rumen wall according to Cook, et al. (1965).

For these reasons, methionine in high NPN rations is not likely to reach the small intestine for absorption in significant amounts other than that contained in microbial protein. The same is true for cystine, which may substitute for about 30% of the methionine requirements, indicating that a fraction of methionine is transformed to cystine (Albanese and Orto, 1970).

### Bases for Methionine-Analog Supplementation in Ruminants

It has been demonstrated that deamination of amino acids occurs in the normal tissue metabolism of rats, and that those carbon skeletons of NEAA and EAA are re-aminated, usually being ammonia, aspartic and glutamic acids, the amino group donors (Frost, 1959). Furthermore, hydroxy and keto analogs of the EAA's (except lysine and threonine) have successfully been used to replace the corresponding natural occurring amino acids in man, rats, poultry and swine. For instance, Methionine hydroxy analog or M-analog (DL  $\alpha$  hydroxy  $\gamma$  methyl mercapto calcium-butyrate) is manufactured in large quantities at reasonable prices and has proven to be of value in practical supplementation of chick and swine feeds. Recently, Belasco (1971) has shown that bovine liver and kidney tissues are capable of converting the M-analog to functional methionine.

Nevertheless, the synthetic procedures currently used for amino acid production yield racemic mixtures (D and L stereoisomers) which represents a problem in view of the fact that intestinal absorption of D-amino acids is slower and metabolically unuseful. In spite of this, few of them such as D-methionine and D-phenylalanine can be utilized in the metabolism instead of L-forms (Jackson and Block, 1932-1933).

It seems that D-methionine is first deaminated to the corresponding keto acid by a specific D-amino oxidase present in some tissues and is then converted by transamination

to L-methionine (Albanese and Orto, 1970). Therefore, DL methionine might be expected to have similar effects to L-methionine after absorption through the intestine.

Belasco (1971) has shown with in vitro studies that L-methionine in rumen liquid disappeared rapidly after one hour and 95% had disappeared after four hours, while the M-analog suffered only 45% degradation four hours following inoculation. Belasco concluded that because the residence of methionine in the rumen is considerably less than four hours, degradation of M-analog is much less than that depicted in his experiment and, consequently, the efficiency of M-analog in ruminants is due, at least in part, to its resistance to microbial degradation.

Related to this, McCarthy (1970) postulated that:

1) M-analog escapes ruminal degradation and passes to the small intestine where it is absorbed, 2) M-analog, in free state, is absorbed directly from rumen, and 3) its presence also redirects microbial metabolism in the rumen to provide a better balance of nutrients, including a larger supply of methionine. All these factors may be involved, to some extent, in explaining the positive effects reported for M-analog.

### Methionine Deficiency in NPN Supplemented Rations

It has been shown that the supplementation of DL methionine to rations in which urea furnished 40% of the total nitrogen increased the rate of gain and nitrogen utilization in lambs (Loosli and Harris, 1945, and Lofgreen,

et al. 1947). Although Gallup, et al. (1952) found the same results, they were not statistically significant. Later McLaren, et al. (1965) tested retention of absorbed nitrogen in lambs fed high urea rations containing either DL methionine or L tryptophane, or both. All amino acid supplementations gave 15% greater response than controls. adaptation period to urea significantly affected nitrogen retention but did not influence the effect of amino acid This work, along with that of Loosli and supplementation. Harris (1945) and Lofgreen (1947) eliminated the possibility of methionine's effect as a sulfur source since the sulfur level in the control rations was equivalent to that of the methionine supplemented rations. This was later confirmed by other workers (see Chandler and Polan, 1970). In another experiment, when potassium ororate and methionine were fed either by capsule or in the milk, young calves gained 25% more weight than the controls (Wing, 1957). In contrast, Gosset, et al. (1962), Noble, et al. (1955), and Harbers, et al. (1961) did not detect an increase in growth rate of steers and lambs when methionine and lysine were added to the diets when sources of nitrogen were predominantly natural protein. This may indicate that methionine is less deficient in such diets. However, in various reports, DL methionine addition to high-urea rations fed to feedlot cattle did not improve performance (see Homer, 1970). One of the major reasons for these results is that this "unprotected" form of amino acid is rapidly destroyed in the

rumen (Oljten, 1969).

Other evidence which may reflect more clearly a need for supplemental methionine in ruminants are as follows:

Post-ruminal infusions in sheep of DL methionine mixed with other amino acids, intraperitoneal and intravenous injections of L methionine, and oral administration of M-analog, resulted in a significant improvement in nitrogen utilization, a better performance, and a superior wool growth (Wright, 1971; Reis and Schinckel, 1964; Reis, 1967; and Schelling and Hatfield, 1968).

Poley (1965) 1/ analyzed amino acid composition and levels of abomasal material and compared these results with amino acid requirements for swine, and suggested that methionine and cystine are generally limiting for the ruminant. Schelling, et al. (1967), upon expressing the amino acid composition of rumen contents (from sheep fed urea-purified diets) as a ratio of the amino acid composition for whole egg protein, suggested that histidine and methionine were in short supply to the host animal. Other studies (Purser, et al. 1966, and Salsbury, et al. 1970) suggested methionine and lysine, among others, as possible limiting amino acids in ruminants.

Virtanen (1966), Little, et al. (1966) and Schelling, et al. (1967) reported lowered plasma levels of EAA (in cows,

 $<sup>\</sup>frac{1}{}$  as reported by Bergen (1967).

steers, and lambs, respectively) when these ruminants were fed rations in which almost all the nitrogen was furnished by NPN sources. Furthermore, Virtanen (1966) found histidine and methionine to be relatively low compared to other plasma EAA in cows fed urea-purified diets. This was later confirmed by Jacobson (1967) with respect to plasma methionine in dairy cows fed corn silage rations. Finally, Schelling, et al. (1967) showed that increasing the protein level in diets for lambs resulted in a linear decrease of plasma methionine concentration but in none of the other EAA. Moreover, methionine supplementation did not affect methionine concentration in plasma. Schelling concluded that "the decreasing trend of methionine in plasma suggests something unique about this EAA. A limiting amino acid would not probably accumulate in the plasma as other amino acids would." However, Broderick, et al. (1970) administered encapsulated methionine to dairy cows fed a high concentrate ration and observed higher plasma levels of methionine. In this case, methionine requirements may have already been met by the high quantities of concentrates fed. Linton, et al. (1968) and Sibbald, et al. (1968) reported that encapsulated methionine fed to steers elevated the plasma methionine level, but they did not report the composition of the ration fed.

Most of the above literature supports the postulate of Jacobson, et al. (1967), who stated that "of all EAA's, sulfur-amino acids are found in the lowest concentrations in the rumen microorganisms and in bovine plasma. On this

basis alone, there exists the possibility that a sulfur amino acid could be the first limiting amino acid in meeting the physiological requirements of ruminants."

Effect of Methionine in the Rumen

Studies conducted in vitro and in vivo indicate that methionine and its hydroxy analog stimulate cellulose digestion in the rumen. Methionine was the only one among nineteen amino acids tested, to show this effect (Salsbury, et al. 1970, 1967 and 1964). Forty grams of M-analog per cow per day significantly increased drymatter and crude fibre digestibility as well as nitrogen retention (Polan, et al. 1970).

Williams and Moir (1951) showed that methionine supplemented sheep rations resulted in an increased number of rumen microbes. In tracer studies Patton, et al. (1968 and 1970) reported a significant improvement in the extent of lipid synthesis in in vitro systems supplied with L methionine and an increased synthesis of complex microbial lipids that were related to cell growth. The authors believe that most of the increase in lipids was associated with increased rumen protozoa. Chandler and Polan (1970) reported that inclusion of M-analog in the diet of a cow had a stimulatory effect on rumen protozoa since the total number of protozoa increased markedly. The slightly higher biological value of protozoa protein and its greater digestibility, as compared to rumen bacteria (Bergen, et al.

of amino acids available to cattle if proliferation of protozoa can be stimulated. Bergen, et al. (1967) also suggested the possibility that changes in the diet may also change the microbial population.

### Effect of Methionine on Lipid Metabolism

Chandler, et al. (1970) observed that adding M-analog to 10% fat rations for calves improved feed intake and performance. Rumen fill was reduced due to more efficient ruminal digestion. Associated with this, there was a significant reduction in the ruminal lipid pool size. This suggests a faster turnover of the lipids of the rumen and higher efficiency of rumen epithelium in the transport of lipids.

McCarthy, et al. (1969) postulated that a shortage of methionine may be important in the development of bovine ketosis because the significant decrease in serum protein (other than serum albumin) characteristic of ketotic cows returns to normal with injections of L methionine or M-analog oral dose. This could be related to a special function of methionine, reported by Trams, et al. (1966), in the binding of the lipid and protein moieties for the formation of the serum lipoproteins. Methionine, as a methyl donor in phospholipids formation, is required for the ruminant liver to synthesize and release serum lipoprotein. Davis and Sachan (1966) observed that the methyl-labeled group from methionine was rapidly incorporated into blood phospholipids.

The increased milk fat response to 40 g. of M-analog addition to the feed of cows (Griel, et al. 1968) is also

considered the result of a more efficient fat utilization.

Effect of Diet on Methionine Content in the Rumen

Forage proteins are low in lysine and methionine (Larson and Halverson, 1966). Further shortage of methionine in the feeds may occur during storage because of the susceptibility of the methyl-mercapto portion of methionine to oxidation (McCarthy, et al. 1969).

Conrad, et al. (1967) published estimates of ruminal methionine synthesis based upon the incorporation of labeled 35s into methionine. Ruminal synthesis of methionine per day, with dairy cows, was from 31 to 59 mg/kg of body weight. Apparent ruminal degradation of dietary methionine for high concentrate rations was 43% and for low concentrate rations These researchers observed that as the soluble NPN 63%. level in the diet increased, the synthesis of methionine in the rumen decreased. Complementarily, they determined that methionine formation in the rumen increases as the integrity of the plant protein fed is preserved. They further reported a 58%, or higher, degradation of dietary methionine in the rumen when haylage was fed, in contrast to a 44%, or higher, degradation for cows fed dried alfalfa. These data lead the conclusion that when feeding large amounts of silage (which has had about one half of its protein content reduced to soluble NPN compounds during fermentation) with no concentrates, the amount of methionine passing to the lower gut would be critically shortened and its supplementation is then indicated.

### Effect of M-Analog on Milk Production

In a study by Griel, et al. (1968), cows from four different dairy breeds responded to dietary supplementation of 40 g. of M-analog per head per day with an average of 4.4 pounds of milk increase daily. Higher milk production and milk fat percent, as a response to various levels of M-analog fed, were also reported by McCarthy, et al. (1969), while Polan, et al. (1970) obtained maximum response of milk production when 23 g. per head per day of the analog were fed. Milk fat test increased almost linearly with increasing levels of M-analog; but, when 80 g. were fed, milk production and feed intake were depressed, which may be attributable to an amino acid imbalance. This has also been reported by Griel, et al. (1968) and others.

In contrast to this, Williams, et al. (1970), using 12 g. of encapsulated DL methionine, did not observe effects on milk production. This was probably due to the low level of methionine fed. Grubaush and Olson (1971) reported no improvement in milk production at various levels of M-analog supplementation.

### Effects of M-Analog Supplementation on Feedlot Cattle

Gosset, et al. (1962), in an experiment with steers, fed 5 and 10 g. of the analog per head per day in a high-shelled corn ration and reported no improvement in average daily gain; but the 10 g. M-analog level depressed weight gain and feed intake. Beeson, et al. (1970) observed a slight depression in rate of gain with steers fed ear corn rations

when each animal was supplemented with 3 g. of M-analog per head per day. Feed intake was identical to the control groups. Hale, et al.(1970a) obtained slightly higher average daily gain values for control steers than for steers receiving 22 g. of M-analog per head per day. The M-analog addition caused a lower feed intake. The basal ration was 90% concentrates. After 52 days, the analog level was reduced to 11 g. per head per day, which resulted in identical feed consumption and average daily gain as compared to the control group.

Reports by Hale, et al. (1970b) and Lofgreen (1970) indicate no advantage from M-analog additions over control groups. Both experiments utilized rations composed of 90% concentrates and 10% roughage.

In contrast to this, work by Burroughs, et al. (1969) showed 13% faster gains and 10% better feed efficiency for heifer calves fed 3 g. of M-analog per head per day. The basal ration was composed of 80% rolled corn, 20% corn cobs, and a urea supplement. The initial weight and final weight for the heifer calves were 480 lbs. and 810 lbs. respectively. These workers postulated that M-analog resists rumen degradation, that it provides methionine for maximum tissue protein synthesis, and that too much of the analog is injurious to the protein synthesis in cattle. In view of this success, a second trial (Burroughs, et al. 1969b and 1970) was conducted; but this time heavier steers were utilized. Their initial and final weights were 745 and 1,175 lbs. The rations were

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similar to those in the first trial. During the first 72 days of the experiment, the 3 g. level of M-analog per day per head resulted in 7% better weight gains and feed efficiency than the control group, while the 4.5 g. level gave 6% and 3% higher average daily gain and feed efficiency, respectively, over the controls. The 9 g. level resulted in lower feed consumption and lower gains than the control group. For the total feeding period of 133 days, all levels of M-analog supplementation caused a reduction in daily feed consumption and average daily gain. Finally, Burroughs, et al. (1969b) reported that lambs fed 0.6 g. of M-analog per head daily gained 20% faster than controls. Higher levels of M-analog supplementation resulted in a corresponding decrease in feed consumption and a consequent depression in average daily gain.

#### MATERIALS AND METHODS

## EXPERIMENT I - STEER CALF FEEDING TRIAL DESIGN:

A 4x2 factorial design was utilized to study the treatments shown in Table 2.

### Harvesting of Silages

Corn for all silage treatments was harvested between August 26 and September 23, 1970, from a stand of hybrid corn averaging approximately 35 metric tons of 35% drymatter (DM) silage or 5 metric tons of shell corn per hectare. All silage was stored in concrete stave silos fitted with metal roofs and top unloaders.

Control silage (received no additive) was stored in a 9.1 m  $\times$  18.3 m silo and averaged 36.7% DM at harvest.

Silage treated with Pro-Sil (Table 3) was harvested over a two-week period and stored in three 4.9 m x 15.2 m silos and averaged 34.5% DM during harvest. Pro-Sil was applied by pumping the liquid material directly into the blower housing of the blower as each load of silage was blown into the silo, as shown in Figure 1. Pro-Sil additions were equivalent to 22.5 kg. per 1000 kg. of 35% DM silage.

Silage treated with urea-minerals (Table 5) was harvested over a two-week period, stored in a 4.9 m x 15.2 m silo and averaged 36.1% DM during harvest. The urea-minerals

TABLE 2

Experiment I - Feeding Trial

			· · · · · · · · · · · · · · · · · · ·
Lot No.	Type of Silage	Supplement Added kg./M.T.1/	M-Analog g./hd./day
11	Control + Pro-Sil 2/ added at feeding	22.5	3
12	Pro-Sil treated	22.5	3
13	Control + soy-mineral $\frac{3}{}$ added at feeding	45.0	3
14	Urea-mineral 4/ treated	20.6	3
44	Control + Pro-Sil added at feeding	22.5	0
42	Pro-Sil treated	22.5	0
38	<pre>Control + soy-mineral added at feeding</pre>	45.0	0
41	Urea-mineral treated	20.6	0

Each lot (treatment group) consisted of 10 Angus steer calves.

<sup>1/</sup> Kilograms of supplement added per metric ton (M.T.)
 of 35% DM silage.

<sup>2/</sup> See Table 3 for formulation of Pro-Sil.

<sup>3</sup>/ See Table 4 for formulation of soy-mineral supplement.

<sup>4/</sup> See Table 5 for formulation of urea-mineral supplement.

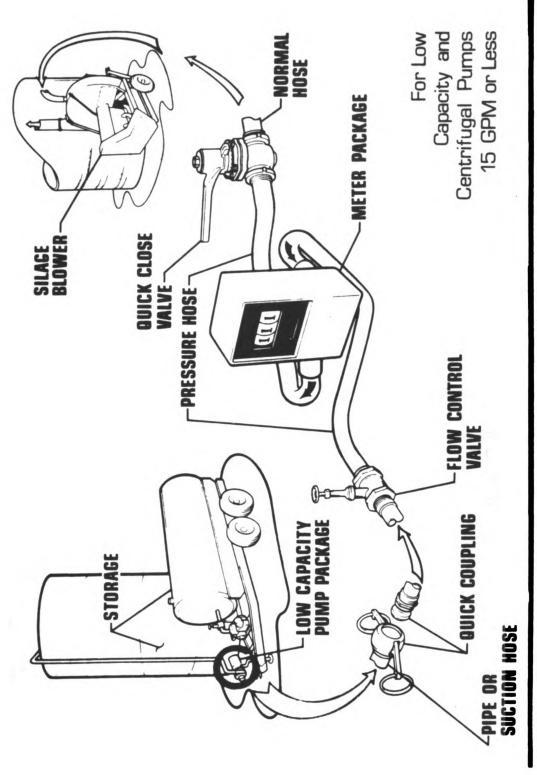
TABLE 3 Formulation of Pro-Sil  $\frac{1}{2}$ 

Element	Percent
Molasses Water and inert ingredients Nitrogen Calcium	16.54 61.15 13.60 .7936
Phosphorus Sodium Chlorine Sulfur	.4850 2.0450 3.8420 .9371
Magnesium Zinc Copper Cobalt Iodine	.4886 .0597 .0088 .0002
TOTAL	100.0%

1/ Patent applied for by Michigan State University

TABLE 4
Formulation of Soy-Mineral Supplement (45% CPE on DM Basis)

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



Schematic illustration of equipment used in applying Pro-Sil to corn silage in a tower silo. Fig. 1.

TABLE 5
Formulation of Urea-Mineral Silage Additive

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

TABLE 6
Formulation of Methionine Hydroxy Analog Supplement

Ingredient	Percent of Mixture
Methionine hydroxy analog (93%) $1/$	1.4211
Ground shelled corn	98.5789
TOTAL	100.000%

Note: Fed at the rate of 227 g. per head per day to provide 3 grams of M-analog per head per day.

Sold under the trade name of "Hydan" at \$1.87/kg. and provided by E.I. DuPont de Nemours & Co.

was applied by evenly spreading 20.6 kg. per metric ton of 35% DM silage over the top of each self-unloading wagon just prior to unloading and blowing into the silo.

The average chemical composition of the corn plant material for each silo during harvest and feeding is shown in Table 9, page 56. All silages were sampled every Monday, Wednesday, and Friday during the feeding experiment for ovendrying and drymatter consumption was computed daily. A composite sample of each silage was analyzed every two weeks during the feeding trial for nitrogen and organic acid fractions.

Control silage supplemented at feeding time with soy-minerals (Table 4) (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 35% DM basis.

Pro-Sil added to control silage (Table 3) at feeding time was applied at rates of 2.25 kg. per 100 kg. of 35% DM silage or its equivalent. Both supplements were combined thoroughly with silage in a horizontal mixer just prior to each feeding. All rations were calculated to be isonitrogenous at 13% crude protein (drymatter basis).

Steers of the treatment groups scheduled to receive M-analog (Table 6) received 227 g. of supplement per head per day containing 3 g. of M-analog. This was combined with the silage in a horizontal mixer just prior to each feeding.

### Feeding Trial

Choice Angus steer calves (80 heads) utilized in this

experiment were purchased in a series of Virginia feeder calf sales on September 22, 23, and 24, 1970, at average weight of 229 kg. All calves were vaccinated prior to or upon arrival for Infectious Bovine Rinotracheitis (IBR) and Parainfluenza Type 3 (PR3). Each calf was implanted with 24 mg. of stilbestrol and injected with two million units of Vitamin A. All animals were acclimated until the initiation of the experiment on October 22, 1970, on a full feed of corn silage and hay plus 1/4 kg. of 50% CPE soybean meal, containing minerals and vitamins, per head daily.

Steer calves were individually weighed on two successive days at the beginning and end of the experiment and the average of the two-successive-day weights in each case were used as initial and final weights. Steer calves were randomly assigned by initial weight blocks to the eight treatment groups. All lots of cattle were group weighed every 28 days during the course of the experiment. The experiment was designed to terminate individual animals when they reached Choice slaughter grade. Thus, the length of the feeding period varied from 192 to 238 days, and final weights varied from 358 to 470 kg.

All lots of steer calves were fed <u>ad libitum</u> twice daily, and water was supplied in automatic fountains at all times in all pens.

Immediately after the final weighing, all cattle were trucked 100 miles to a commercial slaughtering plant, allowed to stand overnight, and slaughtered the following morning.

Carcasses were allowed to hang 48 hours in the cooler, and then ribbed, graded by a federal grader, and carcass measurements taken. Kidney, heart and pelvic fat were 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

Daily drymatter consumption was determined by oven-drying the silage samples at 100°C for 24 hours. Composite samples of moist silage were analyzed for nitrogen and organic acid fractions and then expressed on a drymatter basis from analysis of a paired sample dried at 55°C for 48 hours.

Total nitrogen was determined by macro-Kjeldahl procedures.

Water extracts of silage were prepared by homogenizing 25 g. of fresh silage with 100 ml. of distilled water for one minute. The pH of the homogenate was determined by using a Corning Model 12 pH meter. The homogenate was strained through two layers of cheesecloth and total water soluble nitrogen was determined by the micro-Kjeldahl method.

A second sample of the strained homogenate was deproteinized with 1 ml. of 50% sulfosalicylic acid (SSA) per 9 ml. of homogenate, and a micro-Kjeldahl analysis of this fraction gave total water-soluble non-protein nitrogen fraction.

The remaining strained homogenate was deproteinized with 1 ml. of 50% SSA per 9 ml. of the strained homogenate as before, and then centrifuged for 10 minutes at 18,000 r.p.m. Volatile fatty acid content of the silage was determined by

injecting samples of this deproteinized silage extract into a Packard gas chromotograph. Lactic acid content of the silage was determined on the deproteinized extract by the colorimetric method of Barker and Summerson (1941).

## EXPERIMENT II - METABOLISM STUDY DESIGN:

A 4x4 Latin Square design was utilized in this experiment. Four silages were fed to four 18-month old Hereford steers fitted with permanent rumen cannulas over four 28-day periods. Treatments were randomize by time and animal as shown in Table 7. 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 urine production were measured and sampled for chemical analysis over a period of six days. During the day following collection, jugular blood and rumen fluid samples were secured immediately before feeding and at two-hour intervals thereafter up to 10 hours post-feeding. The experiment was initiated on March 6, 1971 and terminated on June 24, 1971.

### Feeding Regime

Steers in the collection stalls were fed twice daily at 8 a.m. and 5 p.m. The quantity of feed was calculated to supply 90% of maximum intake (as measured by previous ad libitum regime during the acclimation period). The respective

TABLE 7

Experiment II - Metabolic Study
Design of Experiment

		Stee	r No.	No.	
Period	1	2	3	4	
		Rat	ion		
1	D	Α	С	В	
2	Α	С	В	D	
3	С	В	D	A	
4	В	D	A	С	

TABLE 7a

Experiment II - Metabolic Study
Treatments Utilized

Ration	Silage treatment	Supplement Added kg./M.T.	M-Analog g./hd./day
A	Pro-Sil <sup>2</sup> /	22.5	3
В	Urea-mineral $\frac{3}{}$	20.6	3
С	Urea-mineral	20.6	0
D	Pro-Sil	22.5	0

<sup>1/</sup> Kilograms of supplement added per metric ton (M.T.)
 of 35% DM silage.

<sup>2/</sup> See Table 3 for formulation of Pro-Sil

<sup>3/</sup> See Table 5 for formulation of urea-mineral supplement

silages were removed from the silos just prior to each feeding.

The rations containing the M-analog supplement (Table 6) were thoroughly mixed with the supplement in a small cement mixer just prior to each feeding.

The treated silages scheduled to receive no M-analog supplement were fed similarly to the corresponding lots in the feeding trial.

Representative samples of all rations were taken just prior to feeding for laboratory analyses. Any feed which was not consumed was weighed, sampled and discarded prior to the 8 a.m. feeding.

### Sample Collection

Total feces were allowed to pass through a widespace 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 and frozen for nitrogen determination. A 100 kg.
sample was analyzed daily for drymatter content. Remaining
feces were discarded. At the end of the six-day collection
period, all frozen samples were thawed and thoroughly mixed.
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 6N sulfuric acid. The carboy was emptied daily prior to

morning feeding, and urine volume was measured and then diluted to 12 liters 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-liter 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 total volatile fatty acid and rumen ammonia determination.

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

### Laboratory Analysis

Drymatter of feed and feces samples was determined daily by oven-drying at 100°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. The column packing used was a Chromosorb 101 in a 6' column carrier, flow rate was 70 ml./min. N<sub>2</sub> and column oven temperature was 195°C. Samples were prepared by taking

5 ml. of strained rumen fluid and mixing it with 1 ml. of 25% metaphosphoric acid, centrifuging at 10,000 r.p.m. for 10 minutes. The peak areas were converted to milligrams per 100 ml. by comparing with standard solutions of volatile fatty acid analyzed at the same time.

Blood samples were centrifuged at 6,000 r.p.m for 10 minutes, and the plasma removed with a Pasteur pipette.

Urea and ammonia content of the plasma was determined by the micro-diffusion method of Conway (1960).

### Statistical Analysis

All data from the two experiments were analyzed on an CDC 3600 computer at the Michigan State University Computer Laboratory. Analysis of variance procedures were used in all experiments, and Duncan's New Multiple Range Test compared means for significant differences.

# EXPERIMENT III - EFFECT OF M-ANALOG ON PLASMA AMINO ACID LEVELS

Four lots of steer calves (10 head each) from Experiment I, at the 6th month of the feeding trial, were utilized in this experiment, which was initiated on March 29, 1971, and terminated on April 12, 1971. Treatments utilized in this experiment are shown in Table 8.

The silages used have been described in the pro-Cedures of Experiment I and their chemical composition is Shown in Table 9.

The steers were trained to consume all their feed in a two-hour period for two weeks. In the last day, blood

TABLE 8

Experiment III - Effect of M-Analog Addition on Plasma
Amino Acid Levels of Steers Fed Corn Silage

Lot No.	Type of Silage	Supplement Added kg./M.T.1	M-Analog g./hd./day
12	Pro-Sil <sup>2</sup> / treated	22.5	3
13	Control + soy-mineral $\frac{3}{}$ added at feeding	45.0	3
42	Pro-Sil treated	22.5	0
38	Control + soy-mineral added at feeding	45.0	0

<sup>1/</sup> Kilograms of supplement added per metric ton (M.T.)
 of 35% DM silage.

<sup>2/</sup> See Table 3 for formulation of Pro-Sil.

 $<sup>\</sup>underline{3}/$  See Table 4 for formulation of soy-mineral supplement

samples were taken from all steers before  $(\mathbf{T}_0)$  and 4 hours  $(\mathbf{T}_4)$  after the morning feeding. Plasma amino acid levels were then determined to compare values for the most rapid, median, and slowest growing steer of each group. The NEAA/EAA ratios at four-hour post-feeding  $(\mathbf{T}_4)$ , pre-feeding  $(\mathbf{T}_0)$  were computed as well as plasma amino acid levels at  $(\mathbf{T}_4)$  and  $(\mathbf{T}_0)$ .

### Collection and Analysis Procedures

Whole blood samples (10 ml.) were collected from the jugular vein through needle puncture in heparinized test tubes. Then samples were centrifuged at 5,000 r.p.m. for 10 minutes and plasma collected with Pasteur pipettes. To each milliliter of plasma, 0.1 ml. of 50% SSA solution and 0.1 ml. of 1.0 mM. norleucine were added, shaking softly after each addition. After one hour on ice within the cooler, the resulting precipitate was cleared by centrifugation at 18,000 r.p.m. for 15 minutes. The supernatant was kept in the freezer for further analysis. Determination of amino acids was performed in the Ruminant Nutrition Laboratory at Michigan State University on a Technicon - TSM - 1 amino acid analyzer, according to Bergen and Potter (1971) and Makdani, Huber and Bergen (1971).

#### RESULTS AND DISCUSSION

## EXPERIMENT I - FEEDING TRIAL

### Chemical Analysis of Silage

Results of 18 different composite analyses of each silage used during the experiment are shown in Table 9.

Percent drymatter between fresh and ensiled corn plant varied very little; all silages were considered to be excellent in quality.

Total crude protein (N x 6.25) values of the NPN treated silages (fresh vs. ensiled) was increased 5.24 percentage units by Pro-Sil treatment and 6.97 percentage units by urea-mineral treatment. In both cases, increases in crude protein recovered accounted for essentially 100% of the Pro-Sil and urea applied. The apparent increase in crude protein content between fresh and ensiled control silage is attributable to sampling errors since no nitrogen was added at silo filling time.

### Nitrogen Fractions:

All nitrogen fraction values shown in Table 9 are expressed as crude protein (N x 6.25). The term "organic Protein" (arrived at by difference between total nitrogen and NPN-nitrogen) may include in addition to true protein other forms of organic nitrogen such as small amounts of nucleic acids, polypepto amino sugars, etc.

(All nitrogen values are expressed as Crude Protein, N  $\times$  6.25) Average Chemical Analysis on Drymatter Basis of Silages Fed TABLE 9

	Control-	Control-no treat.	Pro-Sil-2	Pro-Sil-22.5 kg./M.T.	Urea-Mineral	ineral
Observation	Fresh	Ensiled	Fresh	Ensiled	Fresh Ensil	Ensiled
Percent Drymatter	36.69	34.91	34.52	34.57	36.06	34.47
Crude Protein Fractions: Total crude protein	7.47	8.99	7.73	12.97	6.82	13.79
Organic protein NPN protein	6.38 1.09	4.68 4.31	6.89	68.9	5.56 1.26	5.32
Ammonium salts Urea Unidentified	.08	. 65 . 02 3.64	.06 .01 .77.	3.57 .08 3.24	.00	3.65 .70 4.12
Organic Acid Fractions: Total organic acid	!!!	9.44		12.31		12.89
Lactic acid Acetic acid Butyric acid		7.75		10.78 1.47 .06		10.79 1.94 .16
Нd	5.80	3.89	5.60	4.19	5.72	4.28

"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. Note:

Percent organic protein was reduced 42% during fermentation for the control silage when compared to fresh material, which resulted in a substantial organic protein loss. In contrast, decreases in organic protein for Pro-Sil and urea-mineral treated silages were approximately 12% and 24% respectively. Organic protein destruction values were calculated by comparing the "fresh" and the "adjusted ensiled" values for Pro-Sil and urea-mineral treated silages. In this fashion, ensiled values for control, Pro-Sil and urea-mineral were compared. This was necessary because of sampling errors involved between "fresh" and "ensiled" as previously discussed.

These data indicate that Pro-Sil or urea-mineral additives had a significant organic protein sparing effect on silage, which has been shown recently by Beattie (1970), Huber and Hillman (1970) and Henderson, et al. (1971). The probable factors accounting for this sparing effect are increased bacterial protein and/or decreased proteolysis during fermentation (according to Modyanov, et al. 1960, and Rayetskaya, et al. 1954) 1/

Values presented in Table 9 show that approximately 36% of the nitrogen added through Pro-Sil was recovered as organic protein in the silage, 63% was recovered as ammonium salts (considering that all the ammonia present was linked)

 $<sup>\</sup>perp$  As reported by Owens (1968)

to organic acids to form ammonium salts) $\frac{1}{}$  and 1% as urea. These results are in agreement with data by Henderson (1969) and (1970b).

For silage treated with urea-minerals, 13% of the added nitrogen was recovered as organic protein, 63% as ammonium nitrogen, 14% as urea, and 10% as unidentified NPN.

The high ammonium salts value and low level of organic protein 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 most of the added urea to ammonia, which later combined with organic acids during fermentation. This is substantiated by the high level of lactic acid produced in this silage.

Actual levels of unidentified NPN in the three silages did not differ substantially; therefore, the elevated organic protein levels in the Pro-Sil and urea-minerals treated silages, as compared to the control silage, are probably due to production of microbial protein during fermentation rather than to a reduction in proteolysis. The unidentified NPN appear to have a low value for rumen microbial protein synthesis (Hawkins, 1970) and may be

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

partially responsible for the reduction in DM intake on high silage rations (Geasler, 1970).

It can be concluded that corn silage treated with either Pro-Sil or urea will have a higher organic protein content at feeding time than silage not treated.

Organic Acids:

Total organic acid fractions (as percent of drymatter) were calculated by combining total acetic, lactic, and butyric acids. The amounts of other organic acids such as valeric and isovaleric were too low for accurate determination. Pro-Sil and urea-mineral treated silage showed similar total organic acid contents which averaged 33% higher than the control silage, as was expected. Both NPN treated silages exhibited similar lactic acids concentrations, which averaged 39% higher than the control silage. Acetic and butyric acid values were similar for the three silages. Thus, the neutralizing effects of Pro-Sil and urea additions resulted in an increased fermentation and bacterial activity, yielding significantly higher amounts of total organic acids, particularly lactic acid, which is in agreement with previous work by Polan, et al. (1967), Huber, et al. (1968), Klosterman, et al. (1963), and Henderson, et al. (1970b, 1971).

The pH of the control silage (3.89) was lower than the Pro-Sil (4.19) and urea-mineral (4.28) treated silages. However, the pH level of all silages was in the range required for maintaining excellent preservation of the silage during storage and feeding.

# Effect of M-Analog Supplementation to All Corn Silage Rations

M-analog and their controls appear in Table 10. This comparison shows a highly significant (P <.01) increase in average daily gain in favor of the steers receiving 3 g. of M-analog per head per day (.87 kg. vs .73 kg.). These results are in agreement with results reported for heifers by Burroughs, et al. (1969a) using the same level of the analog in the ration. Other experimental feeding trials have shown no improvement in weight gains when higher levels of M-analog were fed to steers (Burroughs, et al. 1969a and 1970; Lofgreen, 1970; and Hale, et al. 1970a) or when M-analog was added to high concentrate rations (Gosset, et al. 1962; Hale, et al. 1970a and 1970b; and Lofgreen, 1970).

The pattern of average daily gain during the feeding trial for steers fed M-analog supplemented diets and their controls is compared in Figure 2. The difference between the two groups favored M-analog fed steers during the first 83 days of the feeding trial (approximately 1.0 kg. vs .90 kg. daily). During the following 78 days average daily gain was similar for the two groups. This pattern agrees with that reported by Burroughs, et al. (1970b) for steers. However, in the present experiment, the last third (76 days) of the feeding trial favored cattle receiving M-analog supplement again (approximately .56 kg. vs .40 kg. daily), resulting in an overall 13% faster daily gain for M-analog fed cattle

TABLE 10

Effect of Methionine Hydroxy Analog Addition (October 22, 1970 to June 17, 1971)

192-238 Day Test	3 grams M-Analog Daily	No M-Analog
No. of yearling steers	39	39
Av. initial weight, kg.	247	246
Av. final weight, kg.	427	405
Av. daily gain, kg.	.817	.726
Daily Feed, kg. 85% DM:		
Corn silage	7.770	7.050
Pro-Sil	.059	.059
M-analog supplement	.227	
Soy-mineral supplement	.227	.227
TOTAL	8.283	7.336
Feed Efficiency:		
Feed per kg. gain, kg.	10.13	10.09
Feed cost per 100 kg. gain $1/$	\$33.13	\$32.51
Carcass Evaluation:		
Carcass grade 2/	11.91	12,89 <sup>a</sup>
Marbling score 3/	12.03	15.09 <sup>A</sup>
Fat thickness, cm.	1.40	1.50
Ribeye area, sq. cm.	72.3 <sup>a</sup>	68.9
Percent K.H.P. fat 4/	2.94	3.56 <sup>A</sup>
Percent B.T.R. cuts 5/	50.03 <sup>a</sup>	49.25
Dressing percent $\underline{6}/\overline{}$	57.72	59.28
Carcass price/100 kg.	\$115.60	\$115.80

<sup>1/</sup> Feed costs based on 30% DM corn silage \$9.37/M.T., ureamineral \$8.20/M.T., Pro-Sil \$71.66/M.T., shelled corn \$51.4/M.T, soy-mineral supplement \$132.30/M.T.

Significance: Values having different superscript differ significantly: A (P<.01), a (P<.05).

<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.

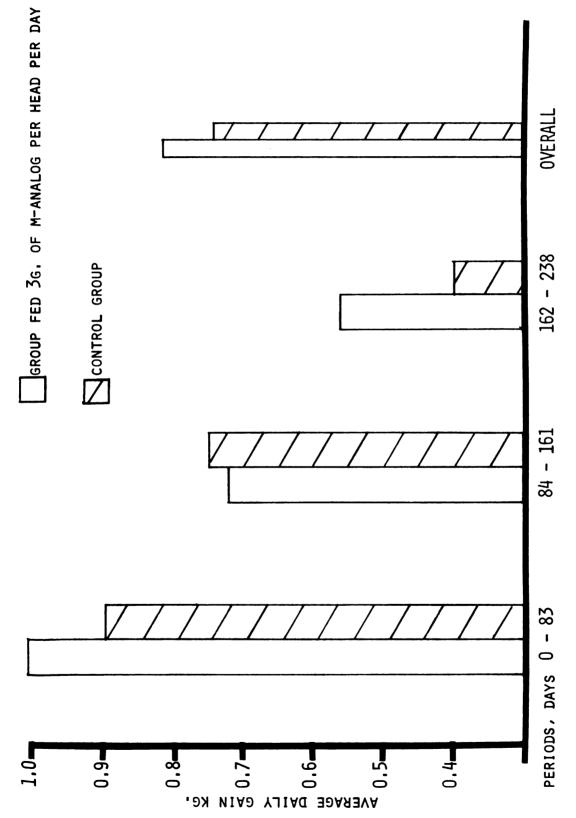


Figure 2. -- Average daily gain during three periods of the feeding trial.

over the total 192- to 238-day test.

Average daily drymatter consumption also favored the group receiving M-analog (8.23 kg. vs 7.33 kg. daily).

However, no difference was found in kilograms of feed required to produce a kilogram of weight gain (10.13 kg. vs 10.09 kg.). This is in contradiction to Burroughs, et al. (1969a) who reported a 10% improvement in feed efficiency for heifers receiving 3 g. of M-analog daily.

The addition of M-analog to the ration increased overall ration cost; and, since there was no difference in feed efficiency, feed costs per 100 kg. of weight gain were slightly elevated for the M-analog fed group (\$36.16 vs \$32.54), as shown in Table 10.

After the cattle had been in experiment 80 days, it became visually evident that the steers receiving M-analog were not fattening as rapidly as the control group, although they were gaining more rapidly. According to Drill (1954), methionine can donate methyl groups for choline synthesis, which is a very active lipotropic substance. As a constituent of phospholipid molecules, choline is essential for the transport of fat and cholesterol and it accelerates the metabolism of lipids in general. A fatty infiltration condition in some tissues can be alleviated by administering methionine.

It was also observed that the hair coats of the M-analog fed group were more glossy and they also shed several weeks earlier than the control group. These

phenomena can be explained on the basis of the high content of cystine reported in hair proteins and the fact that methionine can be effectively converted into cystine during metabolism. Therefore, M-analog could stimulate hair growth and vigor. This stimulatory effect of M-analog was unequivocally proven in wool growth of sheep (Wright, 1970, and Downes, et al. 1970).

Values for carcass quality traits in Table 10 support the visual observations. All lots receiving M-analog exhibited less fat and more lean tissue in the carcass than cattle fed control rations. Level of marbling was significantly (P <.01) depressed from "modest +" for the control group to "small +" for the M-analog fed group. This resulted in a significant (P <.02) depression in carcass grade from middle choice for the control animals to low choice for the M-analog fed group.

Percent kidney, heart and pelvic fat was significantly (P <.01) reduced from 3.56% of the total carcass weight for the control group to 2.94% for the M-analog fed group, and rib eye area was significantly (P <.03) increased from 69.8 cm<sup>2</sup> for the control group to 72.3 cm<sup>2</sup> for the M-analog group. Cutability values of 50.03% vs 49.25% of carcass weight in boneless, trimmed, retail cuts significantly (P <.02) favored the M-analog fed group.

The decreased carcass fat in cattle fed M-analog is attributable to the lipotropic action of methionine already discussed.

All data presented yield sufficient evidence to state that M-analog reached tissue levels in physiologically tangible amounts, especially because of differences in hair coats and body fat, but its role as supplier of limiting amino acid is somewhat obscured by the fact that increased average daily gain was at least partially due to the higher feed intake observed in cattle fed M-analog supplement, and feed efficiency was essentially equal for control and M-analog supplemented cattle.

### Sources of Supplemental Protein Compared

Complete results of this comparison are presented in Table 11. Performance of all treatment groups was considered good and no interaction between type of supplement and M-analog existed (see Table 12).

Average daily gain of steers between treatments was not significantly different. However, steers fed silages treated at filling time with either Pro-Sil or urea-minerals gained faster than steers fed control silage supplemented with soy-mineral or Pro-Sil at feeding time (approximately .80 kg. vs .74 kg.). The superiority of both NPN treated silages in this experiment may be attributed to their higher organic acid levels than in control silage.

The slightly faster rate of gain for steers fed

Pro-Sil or urea-minerals treated silages (.80 kg.), as

compared to control silage supplemented with soy-minerals

(.77 kg.) at feeding, is in agreement with a recent study

conducted at the Michigan station by Cash and Henderson (1971).

TABLE 11

Sources of Crude Protein Compared (October 22, 1970 to June 17, 1971)

	Control Soy-mineral Supplement	Urea- Mineral Treated	Pro-Sil Treated	Control Pro-Sil Supplemen
No. of yearling steers	20	20	18	20
Av. initial weight, kg.	247	245	247	247
Av. final weight, kg.	416	424	419	405
Av. daily gain, kg.	.767	.813	.790	.717
Daily Feed, kg. 85% DM:				
Corn silage	6.864	7.445	7.777	7.251
Pro-Sil				.227
M-Analog supplement	.114	.114	.114	.114
Soy-mineral supplement	.872			
TOTAL	7.850	7,559	7.891	7.592
Feed Efficiency:				
Feed per kg. gain, kg.	10.23	9.67	9.98	10.58
Feed cost per 100 kg. gain $1/$	\$39.76	\$29.60	\$30.66	\$31.34
Carcass Evaluation:				
Carcass grade 2/	12.40	12.70	12.39	12.11
Marbling score 3/	14.55	14.30	12.89	12.50
Fat thickness, cm.	1.57	1.35	1.57	1.30
Ribeye area, sq. cm.	72.4	74.5	69.2	66.3
Percent K.H.P. fat 4/	3.23	3.23	3.39	3.15
Percent B.T.R. cuts 5/	49.75	50.10	49.00	49.71
Dressing percent 6/	57.11	59.85	59.22	57.82
Carcass price	\$115.90	\$116.10	\$116.10	\$114.60

<sup>1/</sup> Feed costs based on 30% DM corn silage \$9.37/M.T., urea-mineral \$88.20/M.T., Pro-Sil \$71.66/M.T., shelled corn \$51.40/M.T., soy-mineral supplement \$132.30/M.T.

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

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

<sup>4/</sup> Percent of carcass weight in kidney, heart and pelvic fat.
5/ Percent of carcass weight in boneless, trimmed retail cuts.

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

TABLE 12

Effect of M-Analog Addition and Sources of Crude Protein (October 22, 1970 to June 17, 1971)

		No M-Analog	alog		3	g. M-Analog Daily	og Daily	
192-238 Day Test	Control Soy-Supp.	Urea- Treated	Pro-Sil Treated	Control Pro-Sil Supp.	Control Soy Supp.	Urea- Treated	Pro-Sil Treated	Control Pro-Sil Supp.
Lot No.		42	41	44	13	12	14	11
No. of yearling steers		10	6	10	10	10	6	10
Av. initial weight, kg.		246	245	247	248	244	249	248
Av. final weight, kg.		414	403	400	429	435	435	410
Av. daily gain, kg.	•	.758	.726	969.	.822	.863	.849	.735
Daily Feed, kg. 85% DM:	••							
Corn silage	7.55	6.95	7.20	7.40	7.09	8.61	8.29	7.10
M-Analog supplement				67.	.23	. 23	.23	. 23
Soy-mineral supplement TOTAL	. 84 8.39	6.95	7.20	7.63	8.22	8.84	8.51	7.63
Feed Efficiency:	;	,	,	,	,	•	,	
Feed per kg. gain, kg.	10.51	9.16	9.91	10.98	66.6	10.24	10.03	10.27
gain $1/$	\$40.51	\$27.64	\$29.69	\$33.13	\$39.16	\$32.03	\$31.37	\$29.87
Carcass Evaluation:	13.00	13,30	12.56	12.70	11.80	12.10	12.22	11.50
Marbling score 3/	15.60	16.30	13.78	14.70	13.50	12.30	12.00	10.30
Fat thickness, cm.	1.52	1.35	1.65	1.32	1.52	1.27	1.45	1.22
Ribeye area, sq. cm.	72.2	71.4	66.4	65.4	72.5	77.3	72.0	67.1
Percent K.H.P. fat 4/	3.60	3.45	3.83	3,35	α	3.00	2.94	2.95
Percent B.T.R. cuts 5/	49.38	49.73	48.43	49.46	50.13	50.47	49.56	49.95
Dressing percent 6/	60.47	59.47	•	57.66	۲.	60.23	٥.	57.98
Carcass price/100 kg.	\$116.00	\$116.10	\$115.80	\$115.30	\$115.90	\$116.20	\$116.40	\$113.90

Feed costs based on 30% DM corn silage \$9.37/M.T., urea-mineral \$88.20/M.T., Pro-Sil \$71.66/M.T., shelled corn \$51.40/M.T., soy-mineral supplement \$132.30/M.T. Good=9,10,11; Choice=12,13,14. Small=10,11,12; Modest=13,14,15; Moderate=16,17,18. Percent of carcass weight in kidney, heart and pelvic fat.

Percent of carcass weight in boneless, trimmed retail cuts. Cold carcass weight over off experiment weight. 9151413161 However, a number of previous reports released by the Michigan station show similar growth rates and feed efficiency in steers fed the three types of rations.

Steers fed silage supplemented with Pro-Sil at feeding time produced the lowest average daily gain (.72 kg.), but this result was still considered in the range of good performance with a substantial reduction in feed cost over the soy-minerals supplemented group.

Daily feed consumption was nearly identical for all groups with the exception of the steers receiving control silage with Pro-Sil addition at feeding time. The latter group consumed approximately 4% less feed, which was obviously the reason for the diminished rate of growth for this group.

The nitrogen content of Pro-Sil is made up entirely of anhydrous ammonia and it readily combined with the organic acids contained in the silage mass. Although Pro-Sil has a strong ammonia odor when released from the hermetic system where it is maintained, after approximately one minute of mixing with the silage in a horizontal mixer, the silage was free of the ammonia odor and was quite similar to silage treated with Pro-Sil with respect to physical and odor characteristics.

Feed efficiency was similar for all four treatments.

Nevertheless, Pro-Sil and urea-minerals treated silages were

more efficient than control silage supplemented with either

soy-minerals or Pro-Sil. Work by Geasler and Henderson (1970)

indicates a high association between feed efficiency and lactic acid content of the silage fed. In this experiment, two NPN treated silages had a combined average of 39% higher lactic acid content than the control silage, which may account for the superior drymatter intake and feed efficiency obtained with the treated silages.

Feed cost per hundred kilograms of weight gain was about \$10.00 higher for cattle fed control silage supplemented with soy-mineral than the other three treatment groups. The later three feeding treatments differed less than \$2.00 in feed cost to produce 100 kg. of beef gain. The method of Pro-Sil supplementation favored "ensiling time" addition by 68¢ over at "feeding time" addition.

Costs of crude protein sources are shown at the bottom of Table 13.

Carcass grade for all groups of cattle averaged between low and middle choice with no significant differences between the four groups. For the remaining carcass quality traits, differences were small and insignificant. All groups yielded between 49% to 50% of the carcass weight in boneless, trimmed, retail cuts.

Finally, a brief analysis of corn silage potential to produce beef is shown in Table 14. Based on the comparison of protein supplements just discussed, kilograms of beef produced by hectare averaged 1427 kg., thus corresponding to 8.4 head per hectare of corn silage crop during a fattening period of approximately 220 days. If shelled corn had been

Ration Composition and Cost on 85% TABLE 13

		Percent Ration Composition	Composition		+ 000
Lot No.	Corn Silage	$\frac{\text{Soy}}{\text{Supp.}\underline{1}/}$	Pro-Sil Supp. $\underline{2}/$	M-Analog Supp.3/	100 kg. of 85% DM 4/
38 13	88.4 86.2	11.6 11.0		2.8	3.85 3.92
42 12	$\frac{100}{97.4} \frac{5}{5}$		1   1   1   1	2.6	3.01
41 14	$\frac{100}{97.3} \frac{6}{6}$		       	2.7	3.00
44 11	96.8 94.1	  	$\frac{3.2}{2.9} \frac{7}{7}$	3.0	3.01 2.91

See Table 4 for formulation of soy-mineral supplement. See Table 3 for formulation of Pro-Sil. See Table 6 for formulation of M-analog supplement. S 1/2/WI

the Supplement was fed at

41

rate of 227 g. per head per day. Feed cost based on \$9.37/M.T. for 30% DM corn silage; \$71.66/M.T. for Pro-Sil; \$132.30 for soy-mineral supplement; \$88.20/M.T. for urea-mineral supplement;

and \$75.63/M.T. for M-analog supplement. Treated with 22.52 kg. Pro-Sil per metric ton of 35% DM silage at ensiling. Treated with 20.62 kg. of urea-mineral per metric ton of 35% DM silage 16/5

at ensiling.

Silage was treated with 22.52 kg. Pro-Sil per metric ton of 35% DM silage at feeding

Experiment I - Capacity of Corn Silage to Produce Beef TABLE 14

	Non-Prot treat Pro-Sil	Non-Protein Nitrogen treated silage ro-Sil Urea-mineral	Control Silage supplemented at feeding time Sov-mineral Pro-Sil	supplemented ng time Pro-Sil	Mean
Weight gain in 220 days, kg.	172	179	169	158	170
Kg. of feed required per kg. of gain	24.23	23.48	24.84	25.69	24.56
Beef kg. produced per hectare $1/$	1444	1491	1409	1362	1427
Heads maintained/ha. during fattening period	8.4	8.3	8.3	9.8	8.4
Feed cost per 100 kg. gain	\$30.66	\$29.60	\$39.76	\$31.34	\$32.84
Approximate feed cost per animal per 220 days	\$52.74	\$52.98	\$67.19	\$49.51	\$55.61

Based on 35 M.T. yield/ha. of 35% drymatter corn silage.

exclusively used, approximately 720 kg. of beef per hectare would have been produced. For whole plant corn silage yield and shell corn yield equivalents, see page 40.

## EXPERIMENT II - METABOLISM STUDY

## Chemical Analysis of Silage

The four silage rations utilized in this experiment were the same as four of the rations utilized in Experiment I. The chemical analysis of the silages utilized is discussed in page  $^{55}$  and shown in Table 9.

### Rumen and Blood Parameters

Mean values for rumen ammonia and plasma urea levels are shown in Tables 15 and 16 ( $T_0$  = pre-feeding,  $T_2$  = 2 hours post feeding, etc.) Rumen ammonia level at pre-feeding time ( $T_0$ ) was significantly (P >.05) higher for the group receiving silage treated with urea-minerals and no M-analog addition (10.3 mg./100 ml.) than for those of Pro-Sil treated silage plus M-analog, Pro-Sil without M-analog, and urea-mineral treated silage plus M-analog fed groups (3.6, 1.9, and 3.3 mg./100 ml. respectively). Since the two M-analog containing rations (combined with urea-minerals or Pro-Sil) gave lower rumen ammonia than their respective controls, M-analog may account for the lower ammonia concentration at  $T_0$  in the urea-minerals plus M-analog fed group.

Two hours after feeding  $(T_2)$ , rumen ammonia concentrations were at a peak for all treatment groups (except the Pro-Sil plus M-analog treatment group, which was highest

TABLE 15

Experiment II - Means of Rumen Ammonia Values (mg./100 ml. rumen fluid)

	Pro-Si	Pro-Sil Treated Silage	ilage	Urea-min	Urea-mineral Treated Silage	Silage
Time	Control	M-Analog	Overall Mean	Control	M-Analog	Overall Mean
$^{\mathtt{T}_{0}}$	3.6	1.9	2.8	10.3ª	3.3	8.9
${\tt T}_2$	14.5	16.7	15.6	20.6	21.9	21.3
Т4	6.6	18.4	14.2	17.6	13.1	15.4
$^{\mathrm{T}}6$	3.4	8.9	5.1	11.4	9.9	0.6
${f T}_8$	7.0	6.4	6.7	8.9	7.9	8.4
$^{\mathrm{T}_{10}}$	3.3	4.1	3.7	3.1	5.3	4.2
OVERALL MEAN	7.0	0.6	8 .0	12.0	7.6	10.9

Values having different superscript differ significantly,  $^{\rm a}$  (P<.05). Significance:

TABLE 16

Experiment II - Means of Blood Urea Values (mg./100 ml. plasma)

	Pro-Si	Pro-Sil Treated Silage	ilage	Urea-min	Urea-mineral Treated Silage	Silage
Time	Control	M-Analog	Overall Mean	Control	M-Analog	Overall Mean
${\tt T}_0$	7.2	8.4	7.8	6.3	7.6	8.5
$^{\mathrm{T}_2}$	8.2	8.8	8.5	10.5	0.6	8.6
$^{\mathrm{T}}_{4}$	10.9	10.1	10.5	12.5	9.5	10.9
T	0.6	10.4	7.6	12.3	10.4	11.4
T <sub>8</sub>	7.7	8.6	8.8	10.9	9.5	10.2
$^{\mathrm{T}_{10}}$	7.6	0.6	8.3	10.00	8.5	9.3
OVERALL MEAN	8.4	9.4	6.8	10.9	0.6	10.0

Significance: No significant differences were found between means.

at  $T_4$ ) and the urea-minerals fed groups had higher levels of rumen ammonia than the Pro-Sil fed group (21.3 mg./100 ml. vs 15.6 mg./100 ml.) However, no significant differences were detected among treatments at  $T_2$ , which was also true for the other periods ( $T_4$  through  $T_{10}$ ). At  $T_4$ , a sharper drop in ammonia concentration was observed in the ureamineral groups as compared to the Pro-Sil groups. At  $T_6$ , rumen ammonia levels for the urea-minerals fed groups were still higher (15.4 mg./100 ml. vs 14.2 mg./100 ml.), which was evident throughout the whole sampling period (from  $T_0$  to  $T_{10}$ ).

From  $T_6$  to  $T_{10}$ , Pro-Sil treated silages produced lower rumen ammonia concentrations for  $T_6$ ,  $T_8$ , and  $T_{10}$  (5.1, 6.7, 3.7 mg./100 ml.) respectively, than the groups fed urea-mineral silage, (with corresponding values of 9.0, 8.4, and 4.2 mg./100 ml.) Treatment groups receiving M-analog combined with either Pro-Sil or urea-minerals did not differ from the group fed without the analog, except for the depression shown at  $T_0$ . Average concentration of rumen ammonia for all periods ( $T_0$  to  $T_{10}$ ) were identical for groups fed rations with 3 g. of M-analog per day per head (9.4 mg./ 100 ml.) and groups fed no M-analog (9.5 mg./100 ml.), while the groups receiving urea-minerals averaged approximately 36% higher levels in rumen ammonia concentrations than the Pro-Sil groups; however, the overall mean values for rumen ammonia were not statistically analyzed.

Analysis of plasma urea from all groups did not show statistically significant differences in any period. The highest values for all treatment groups were observed at  $\mathbf{T}_4$  and  $\mathbf{T}_6$ . In all treatments, no noticeable deviations from the general pattern were recorded. Averaged values for plasma urea for all periods in each treatment showed no effect of M-analog or NPN additives. However, values for the Pro-Sil groups averaged 1.1 mg./100 ml. lower than for those receiving urea-mineral treated silage.

Combining rumen ammonia and plasma urea data, it can be concluded that, in general, no significant alteration on the two parameters was obtained when 3 g. of M-analog per head daily was added to NPN treated silages.

However, when NPN sources were compared, ureamineral treated silages gave a faster and higher release of ammonia in rumen after feeding than Pro-Sil treated silage.

Urea is hydrolysed rapidly in the rumen, whereas ammonia from ammonium salts tends to be released at a slower rate and is probably more readily fixed by rumen microbes. Pro-Sil treated silage had an equal ammonium salts level and much less urea than urea-minerals treated silage (see Table 9).

The ammonia concentration in the rumen was reflected in the pattern of plasma urea values. Therefore, in this experiment, nitrogen from Pro-Sil treated silage apparently was lost from the rumen to a lesser extent than that from silages treated with urea-mineral additive.

### Rumen Volatile Fatty Acids

The mean concentration for rumen acetic, propionic, and butyric acid are shown in Tables 17, 18, and 19. Overall means show that the highest values for all acids were observed at the periods  $\mathbf{T}_2$  and  $\mathbf{T}_4$  and decreased gradually to  $\mathbf{T}_{10}$ , where all acids values were similar to those at  $\mathbf{T}_0$ .

At none of the sampling times were any of the concentrations of the fatty acids significantly different for the four treatments utilized. Averaged acetic acid values were higher for the urea-minerals groups as compared to the Pro-Sil groups (488.6 mg./100 ml. vs 436.2 mg./100 ml.)

This was also true for average propionic values (173 mg./100 ml. vs 161.8 mg./100 ml.) and butyric acid values (130.5 mg./100 ml. vs 97 mg./100 ml.)

Both the Pro-Sil and the M-analog treatments had consistently slightly depressed mean levels of individual and total volatile fatty acids.

M-Analog decreased slightly all rumen volatile fatty acid values. This may be due to a faster fatty acid turnover in the rumen, including enhanced rumen absorption of the fatty acids when M-analog is fed (as suggested by Chandler, et al. 1970). In addition, methionine seems to increase lipid synthesis in rumen microbial cells (Patton, et al. 1968 and 1970), which could also account for lowered fatty acid values when M-analog was included in the feeds. The possibility that fiber digestibility was depressed by the M-analog addition in this experiment is unlikely. On the contrary, it has been

TABLE 17

Experiment II - Mean Rumen Acetic Acid Concentrations (mg./100 ml.)

	Pro-Si	Pro-Sil Treated Silage	ilage	Urea-min	Urea-mineral Treated Silage	Silage
Time	Control	M-Analog	Overall Mean	Control	M-Analog	Overall Mean
T <sub>0</sub>	417	410	414	464	456	460
${\tt T}_2$	208	460	484	511	469	490
$\mathtt{T}_{4}$	487	478	483	547	542	545
$^{ m T}_6$	444	449	446	514	456	485
T8	449	390	420	517	475	496
Tlo	383	366	375	446	441	444
OVERALL MEAN	448	426	437	200	473	487

Significance: No significant differences were found between the means.

TABLE 18

Experiment II - Mean Rumen Propionic Acid Concentrations (mg./100 ml.)

	Pro-Si	Pro-Sil Treated Silage	ilage	Urea-min	Urea-mineral Treated Silage	Silage
Time	Control	M-Analog	Overall Mean	Control	M-Analog	Overall Mean
т <sub>о</sub>	132	112	122	144	126	135
$^{\mathrm{T}_2}$	199	239	219	215	206	211
$^{\mathrm{T}}4$	206	209	208	214	200	207
$^{\mathrm{T}}6$	177	165	171	186	166	176
т 8	167	128	148	168	158	163
$^{\mathrm{T}_{10}}$	145	116	131	150	143	147
OVERALL MEAN	171	162	167	180	167	174

Significance: No significant differences were found between the means.

TABLE 19

Experiment II - Mean Rumen Butyric Acid Concentrations (mg./100 ml.)

And the second s						
	Pro-Si	Pro-Sil Treated Silage	ilage	Urea-min	Urea-mineral Treated Silage	Silage
Time	Control	M-Analog	Overall Mean	Control	M-Analog	Overall Mean
H <sub>0</sub>	45	91	70	119	105	112
${\mathtt T}_2$	124	104	114	132	109	121
$\mathbf{T}_{oldsymbol{4}}$	129	93	111	166	138	152
$^{ m T}_{ m 6}$	122	88	105	147	141	144
Т8	113	85	66	174	113	144
$^{T_{10}}$	66	71	82	123	101	112
OVERALL MEAN	105	68	9.7	144	118	131

Significance: No significant differences were found between the means.

consistently shown that M-analog supplementation stimulates cellulose digestion (Salsbury, et al. 1967 and 1970; and Polan, et al. 1970).

### Nitrogen Digestion Parameters

Table 20 shows mean values for nitrogen digestibility and no significant differences were detected among the four treatment groups. However, drymatter intake slightly favored the Pro-Sil treated silage. Drymatter digestibility was also similar in all four groups. The percent of nitrogen digested favored the group receiving urea-minerals treated silage with no M-analog over the other three groups. This treatment also produced the highest urinary nitrogen output.

M-analog showed no significant effects in any of the nitrogen digestion parameters tested.

All treatment groups were in negative nitrogen balance (approximately -5 to -10 g. of nitrogen per head daily).

This can be explained on the basis of the age of the steers utilized for this metabolic trial. At the end of the experiment, the animals were approximately 2 years old. Under these conditions, the need for limiting amino acids for growth, if any, could not be shown by nitrogen retention values.

# EXPERIMENT III - EFFECT OF METHIONINE HYDROXY ANALOG ON PLASMA AMINO ACIDS

The average values for total essential amino acids (EAA), total non-essential amino acids (NEAA), and sulfur amino acids are shown in Table 21. The NEAA/EAA ratio was

TABLE 20
Experiment II - Digestion Parameters

	Ureated Control	Silage		Sil Silage M-Analog
Number of steers	4	4	4	4
DM intake g./day	7205	7398	7900	7411
DM digested g./day	4817	4468	5231	4688
Percent DM digested	66.7	60.1	65.2	63.6
Nitrogen intake g./day	161.0	156.0	162.6	158.3
Nitrogen excreted in feces g./day	94.8	106.6	105.3	106.2
Nitrogen digested g./day	66.2	49.3	57.3	52.1
Percent nitrogen digested	40.2	27.7	29.0	33.4
Nitrogen excreted in urine g./day	n 71.6	65.3	66.4	57.4
Nitrogen retained g./day	-5.4	-4.2	-9.4	-5.3

Plasma Amino Acids Analysis ( $\mu$ -moles/100 ml. of plasma) TABLE 21

	Control Silage + Soy Supplement Control M-Analog	upplement M-Analog	Pro-Sil Treated Silage Control M-Analog	ed Silage M-Analog
Total EAA T $_{ m 0}$	91.8	95.7	74.6	76.5
Total NEAA $ exttt{T}_0$	97.8	96.2	92.4	0.86
NEAA/EAA T $_0$	1.07	1.00	1.24	1.28
$ ext{T}_4/ ext{T}_0$ EAA x 100	78.8	85.5	104.3	93.7
$\mathtt{T_4/T_0}$ NEAA x 100	80.4	80.2	94.2	93.2
Methionine $\mathtt{T}_0$	2.7±0.1	2.9+0.2	2.2±0.1	2.4+0.2
Methionine $\mathtt{T}_4$	1.9+0.0	2.5±0.2	2.3±0.2	2.5±0.2
Cystine $\mathtt{T}_0$	2.0+0.0	1.8+0.2	1.8+0.2	2.0+0.2
Cystine $\mathtt{T}_4$	2.2+0.2	1.9+0.1	1.7±0.3	2.0+0.1

3 Steers per Value.

similar in steers fed control silage plus soy-minerals supplement (1.07). Control silage plus soy-minerals plus M-analog (1.00), Pro-Sil treated silage (1.24), or Pro-Sil treated silage plus M-analog (1.28). Average values for the two M-analog treatments (1.03) and control groups (1.26) were unaffected by the analog. Three grams of M-analog added to the daily ration of steers did not influence the total plasma amino acid levels nor the concentration of methionine or cystine. Ratios of amino acids levels at To and  $T_{\Delta}$  can be indicators of amino acid deficiencies. If an amino acid is available in lower amount than required for protein synthesis at  $T_A$ , when presumably substantially more energy is available for protein synthesis, the particular amino acid should be found in a markedly low concentration in plasma with respect to its level at  $T_0$ , in which case it can be considered as being deficient.

The  $T_4/T_0$  ratios for NEAA and EAA for all treatment groups did not differ substantially. Finally, no single essential amino acid was found sufficiently lower when individually computed by this procedure to indicate whether it was deficient. Incidentally, a consistent observation was that for all the steers in this experiment, approximately 50% of total plasma amino acids were essential amino acids, whereas this value is about 40% or less in steers fed diets other than silage.

### CONCLUSIONS

### EFFECTS OF M-ANALOG ON FEEDLOT CATTLE

When steer calves are full fed on all corn silage rations, the supplementation of 3 grams of M-analog per head per day increases the rate of growth and drymatter consumption. However, previous research, as was the case in this experiment, seem to indicate that as feedlot cattle mature, they become less responsive to M-analog supplementation. Furthermore, all silage rations with M-analog addition appear to produce better results than high concentrate rations.

The supplementation of three grams of M-analog per head per day is absorbable in tangible amounts which produce visual changes in the hair coat of young cattle and diminish deposition of subcutaneous fat. M-analog has a lipotropic effect that is reflected in low carcass fat content as compared to control animals.

M-analog may stimulate the transport of rumen volatile fatty acids and synthesis of rumen microbial lipids, both of which would represent a reduction in the level of volatile fatty acids in the rumen.

The daily supplementation of 3 grams of M-analog per head to mature steers fed NPN treated silage does not affect nitrogen digestibility or nitrogen retention.

Finally, research is needed to precisely determine the effect of M-analog at different ages of cattle.

# EFFECTS OF PRO-SIL AND UREA-MINERAL ADDITIONS TO CORN SILAGE AT ENSILING

The main advantages of treating corn silage with Pro-Sil or urea-mineral supplements at filling time as compared to a control silage are: higher levels of crude protein and minerals sufficient to satisfy the requirements of cattle, which also facilitate the feeding processes; the substantial increase of lactic acid and true protein content in the final silage; and, in addition, high amounts of ammonium salts are formed particularly in Pro-Sil treated silage. All these factors enhance silage utilization by ruminants and explain its superiority over non-treated silages.

### SOURCES OF SUPPLEMENTAL PROTEIN COMPARED

Pro-Sil or urea-mineral treated silages produce equal or slightly higher average daily gain in steer calves than soy-mineral or Pro-Sil supplemented to corn silage at feeding time. As method of supplementation, Pro-Sil at ensiling is definitely superior to Pro-Sil at feeding.

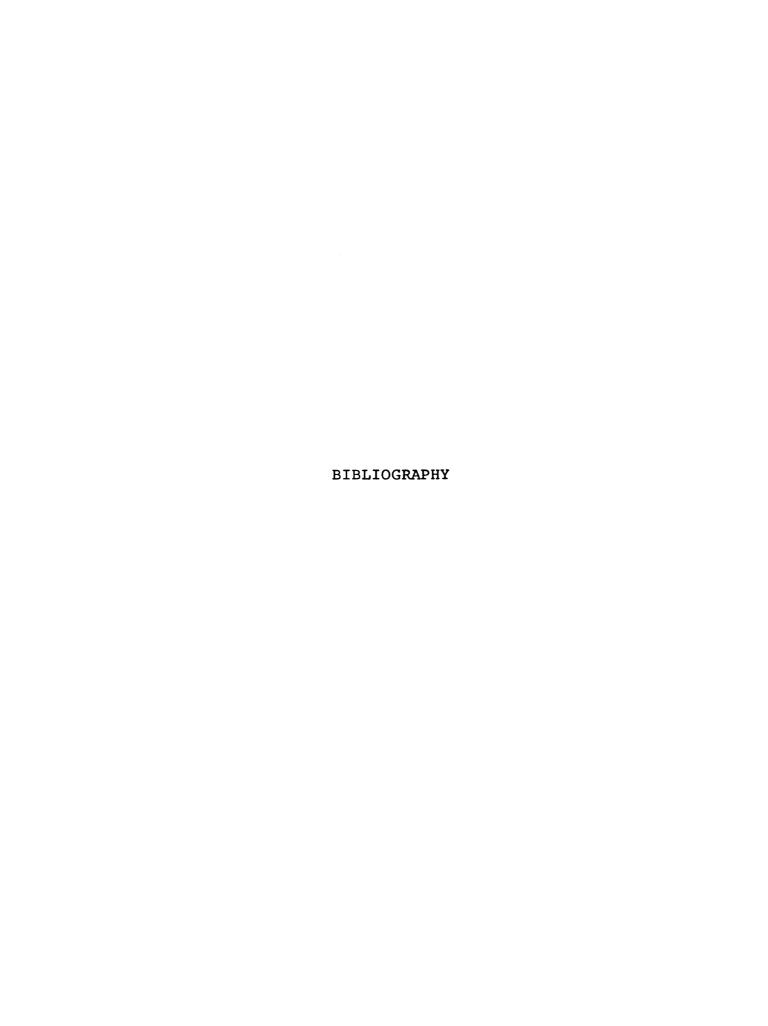
Feeding Pro-Sil or urea-mineral treated silage to steer calves reduces the feed cost of 100 kg. of gain about \$10.00 relative to the feed cost of soy-mineral supplemented at feeding time.

Urea-mineral treated silages produce a faster rumen ammonia release after feeding and a higher rumen ammonia

level during the following ten-hour period than Pro-Sil treated silage. This pattern is reflected in plasma urea levels.

Pro-Sil silage appears to depress slightly the levels of rumen volatile fatty acids. Urea-mineral treated silage fed to mature steers may present a higher nitrogen digestibility but also a higher nitrogen urinary excretion when compared to Pro-Sil treated silage. Varying the supplemental source of nitrogen in all-silage rations affects very little the nitrogen balance in mature steers.

Choice quality cattle can be produced when fed a ration made up entirely of high-quality corn silage properly supplemented.



#### **BIBLIOGRAPHY**

- Albanese, A. A., and Orto, L. A. 1970. "Protein and Amino Acids", Modern Nutrition in Health and Disease.
  4th ed. Edited by M. G. Wohl and R. S. Goodhart Philadelphia: Lea and Febiger.
- Alexander, R. A.; Hentges, J. F.; Robertson, W. K.; Borden, G. A.; and McCall, J. T. 1963. "Composition and Digestibility of Corn Silage as Affected by Fertilizer Rate and Plant Population", J. Animal Sci. 22:55.
- Allison, M. J.; Bryant, M. P.; and Doetsch, R. M. 1962.
  "Studies of Metabolic Functions of Branched-Chain
  Volatile Fatty Acids, Growth Factors for Ruminococci",
  J. Bact. 83:523
- Allison, M. J. 1970. "Nitrogen Metabolism of Ruminal Microorganisms", Physiology of Digestion and Metabolism in the Ruminant. Edited by A. F. Phyllipson. Newcastle, England: Oriel Press.
- Annett, H. E., and Russel, E. J. 1907. "The Composition of Green Maize and of the Silage Produced Therefrom", J. Agr. Sci. 2:382.
- Armstrong, D. G. 1968. "Use of Non-Protein Nitrogen in Animal Feeding", Chemistry and Industry, p. 894-898.
- Barnett, A. J. G., Silage Fermentation. New York: Academic Press, Inc., 1954
- Beeson, W. M. 1969. "Urea in the Ration: How Much can be Safely Added?", J.A.V.M.A. 154:1220.
- Beeson, W. M.; Perry, T. W.; and Huber, D. A. 1970. "Methionine Hydroxy Analogue and Payzone as Additives to High Urea Supplemented Finishing Rations for Beef Cattle", Indiana Cattle Feeders Day, p. 11.
- Belasco, I. J. 1954. "New Nitrogen Feed Compounds for Ruminants A Laboratory Evaluation", J. Animal Sci. 13:601.

- Belasco, I. J. 1971. "Stability of Methionine Hydroxy Analog in the Rumen Fluid and its Conversion in vitro to Methionine by Calf, Liver and Kidney", submitted for publication to the Journal of Dairy Science, 1971.
- Bentley, O. G.; Klosterman, E. W.; and Engle, P. 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. 1967. "Studies of the Effect of Dietary and Physiological Factors on the Nutritive ...."

  Thesis for Degree of Ph. D., Ohio State University.
- Bergen, W. G.; Purser, D. B.; and Cline, J. H. 1967.
  "Enzimatic Determination of the Protein Quality of Individual Rumen Bacteria", J. Nutr. 92:357-64.
- Bergen, W. G.; Purser, D. B.; and Cline, J. H. 1968. "Effect of Ration on the Nutritive Quality of Rumen Microbial Protein", J. Animal Sci. 27:1497-1501.
- Berry, W. T.; Riggs, J. K.; and Kunkel, H. O. 1956. "The Lack of Toxicity of Biuret to Animals", J. Animal Sci. 15:225.
- Black, A. L.; Kleiber, M.; and Smith, A. H. 1952. "Carbonate and Fatty Acids as Precursors of Amino Acids in Casein", J. Bio. Chem. 197:365-70.
- Black, A. L.; Kleiber, M.; Smith, H. H.; and Stwart, D. N. 1957. "Acetate as a Precursor of Amino Acid of Casein in the Intact Dairy Cow", Biochem. Biophys. Acta. 23:54.
- Bloomfield, R. A.; Garner, G. B.; and Muhrer, M. E. 1960.

  "Kinetics of Urea Metabolism in Sheep", J. Animal
  Sci. 19:1248.
- Bloomfield, R. A.; Welsch, G. B.; Garner, J.; and Muhrer, M. E. 1961. "Effect of Sixteen Times a Day Feeding an Urea Utilization", J. Animal Sci. 20:926 (Abstr.)
- Brody, C. J. 1960. "Redistribution of Nitrogen in Grass and Leguminous Fodder Plants During Wilting and Ensilage", J. Sci. Food and Agr. 11:276.
- Brooderick, G. A.; Kowalczyk, T.; and Satter, L. D. 1970.

  "Milk Production Response to Supplementation with
  Encapsulated MET per Os or Casein per Abomasum",
  J. Dairy Sci. 53:1714-21

- Bryant, M. P. 1970. "Microbiology of the Rumen", <u>Duke's</u>
  Physiology of Domestic Animals. 8th ed. <u>Edited</u>
  by E. Duke. Ithaca, New York: Cornell University
  Press.
- Burroughs, W.; Trenkle, A.; Ternus, C.; and Cooper, C. 1969a.

  "Initial Experiment of Methionine-Hydroxy-Analogue-Calcium Added to an All-Urea Supplement for Finishing Calves", Iowa State Univ. A. S. Leaflet. R-122.
- Burroughs, W.; Trenkle, A.; Ternus, C.; and Cooper, C. 1969b.

  "Different Levels of Methionine-Hydroxy-AnalogueCalcium Added to All-Urea Versus All-Plant Protein
  Supplements for Finishing Lambs and Yearling Steers",
  Iowa State Univ. A. S. Leaflet. R-128.
- Burroughs, W.; Trenkle, A.; Ternus, C.; and Cooper, C. 1970.
  "Different Levels of Methionine-Hydroxy-Analogue-Calcium Added to All-Urea Versus All-Plant Protein Supplements for Finishing Yearling Steers." Iowa State Univ. A. S. Leaflet. R-134.
- Campbell, J. R.; Howe, W. N.; Martz, F. A.; and Merilan, C. P. 1963. "Effects on Frequency of Feeding an Urea Utilization and Growth Characteristics in Dairy Heifers", J. Dairy Sci. 46:131.
- Campling, R. C.; Freer, M.; and Balch, C. C. 1962. "Factors
  Affecting the Voluntary Intake of Food by Cows". III.
  "The Effect of Urea on Voluntary Intake of Oat Straw",
  Brt. J. Nutrition 16:115.
- Cash, E. H.; and Henderson, H. E. 1971. "Corn Silage Additives and Concentrate Levels Compared", Report of Beef Cattle Research, 1971. Michigan State Univ., p. 28-44.
- Chalupa, W. 1968. "Problems in Feeding Urea to Ruminants", J. Animal Sci. 27:207.
- Chalupa, W.; Evans, J. L.; and Stillions, M. C. 1964.
  "Metabolic Aspects of Urea Utilization by Ruminant Animals", J. Nutr. 83:77.
- Chandler, P. T.; Patton, R. A.; and Polan, C. E. 1970.

  "Methionine Analog and Ruminant Metabolism", J. Animal
  Sci. 31:238 (Abstr.)
- Chandler, P. T., and Polan, C. E. 1970. "Consideration for the Need of Supplemental Methionine in Ruminant Nutrition", Feedstuffs, Vol. 42, No. 32, p. 50.

- Church, D. C., et al. 1970. Digestive Physiology and Nutrition of Ruminants, Vol. 2: Nutrition. Published by D. C. Church. Corvalis, Oregon.
- Cline, T. R.; Garrius, U. S.; and Hatfield, E. E. 1966.

  "Additions of Branched and Straight-Chain Volatile
  Fatty Acids to Purified Lamb Diets and Effects on
  Utilization of Certain Dietary Components", J. Animal
  Sci. 25:734.
- Conrad, H. R.; Hibbs, J. W.; and Pratt, A. D. 1967. "Effect of Plane of Nutrition and Source of Nitrogen on Methionine Synthesis in Cows", Jour. Nutr. 976:343-47.
- Conrad, H. R.; Hibbs, J. W.; Pratt, A. D.; and Dovin, R. R. 1961. "Nitrogen Metabolism on Dairy Cattle", J. Dairy Sci. 44:85.
- Cook, R. M.; Brown, R. E.; and Davis, C. L. 1965. "Protein Metabolism in the Rumen". I. "Absorption of Glycine and Other Amino Acids", J. Dairy Sci. 48:475.
- Coppock, C. E., and Stone, J. B. 1968. "Corn Silage in the Ration of Dairy Cattle"; review of New York State College of Agriculture, Bul. 89.
- Cowman, G. L., and Thomas, O. O. 1962 "Diammonium Phosphate as a Source of Nitrogen and Phosphorus for Beef Cattle", J. Animal Sci. 21:992 (Abstr.)
- Cullison, A. E. 1944. "The Use of Urea in Making Silage from Sweet Sorghum", J. Animal Sci. 3:59.
- Davis, G. K.; Becker, R. B.; Dix Arnold, P. T.; Comar, C. L.; and Marshall, S. P. 1944. "Urea in Sorghum Silage", J. Dairy Sci. 27:649 (Abstr.)
- Davis, C. L., and Sachan, D. S. 1966. "Effect of Feeding a Milk Fat Depressing Ration on Fatty Acids Composition of Blood Lipids", J. Dairy Sci. 49:1567.
- Downes, A. M. 1961. "On the Amino Acids Essential for the Tissues of Sheep," Aust. J. Biol. Sci. 14:254.
- Duncan, C. W.; Aramala, I. P.; Huffman, C. F.; and Lucke, R. W. 1953. "A Quantitative Study of Rumen Synthesis on the Bovine on Natural and Purified Rations". II. "Amino Acids Content of Mixed Rumen Protein", J. Nutr. 49:41.
- Emery, R. S.; Smith, C. K.; and Fai To, L. 1957. "Utilization of Inorganic Sulfate by Rumen Microorganisms." II. Appl. Microbial 5:363.

- Farlin, S. D.; Garrigus, U. S.; Hatfield, E. E. 1968.
  "Changes in Metabolism of Biuret During Adjustment to a Biuret-Supplemented Diet", J. Animal Sci. 27:785.
- Fontenot, J. P.; Gallup, W. D.; and Nelson, A. B. 1955.
  "Effects of Added Carbohydrate on the Utilization of Nitrogen in Wintering Rations", J. Animal Sci. 14:807.
- Gallup, W. D.; Pope, L. S.; and Whitehair, C. K. 1952.
  "Value of Added Methionine in Low Protein and Urea for Lambs", J. Animal Sci. 11:572.
- Gay, N. 1966. "Making High-Quality Whole-Plant Corn Silage for Finishing Beef Cattle", Iowa State Univ. Agr. Exp. Sta. Leaflet. R-86.
- Geasler, M. R. 1970. "The Effect of Corn Silage Maturity, Harvesting Techniques and Storage Factors on Fermentation Parameters and Cattle Performance". Thesis for Degree of Ph. D., Michigan State Univ.
- Geasler, M. R.; and Henderson, H. E. 1968. "Effect of Stage of Maturity and Fineness of Chop on Yield and Feeding Value of Corn Silage." Michigan Beef Cattle Day Report 1968, p. 47-58.
- Geasler, M. R., and Henderson, H. E. 1969. "What goes in your Silo?", Michigan State Univ. Mimeo. AH-BC-50.
- Gordon, C. H. 1967a. "Storage Lossed in Silage as Affected by Moisture Content and Structure", J. Dairy Sci. 50:397.
- Gordon, C. H. 1967b. "Effects of Heat and Silage Composition", J. Dairy Sci. 50:6:983 (Abstr.)
- Gosset, W. H.; Perry, T. W.; Mohler, M. T.; Plumlee, M. P.; and Beeson, W. M. 1962. "Value of Supplemental Lysine, Methionine, Methionine Hydroxy Analog and Trace Mineral Salts on High Urea Fattening Rations for Beef Steers", J. Animal Sci. 21:248.
- Griel, L. C.; Patton, R. A.; McCarthy, R. D.; and Chandler, P. T. 1968. "Milk Production Response to Feeding Methionine Hydroxy Analog to Lactating Dairy Cows", J. Dairy Sci. 51:1866.
- Grubaugh, W. R.; and Olson, H. H. 1971. "Supplementation of Lactating Cows with Methionine Hydroxy Analog", J. of Dairy Sci. 54:790.

- Hale, W. H.; Theurer, B.; Marchello, J. A.; Taylor, B.; and Essig, H. 1970a. "Effect of Alfalfa and Cotton Seed Hulls Fed at Two Levels and Length of Feeding Period for a 90% Concentrate Ration Fed to Fattening Steers", Arizona Cattle Feeders Day, Series P-12. Report 3.
- Hale, W. H.; Theurer, B.; Marchello, J. A.; and Taylor, B. 1970b. "MHA and Various Levels of Wheat in 90% Concentrate Fattening Rations", Arizona Cattle Feeders Day, Series P-12. Report 7.
- Harbes, L. H.; Oljten, R. R.; and Tillman, A. D. 1861.
  "Lysine Supplementation for Sheep", J. Animal Sci. 20:880.
- Hart, E. B.; Bohstedt, C.; Deoblad, H. J.; and Wegner, M. L. 1939. "The Utilization of Simple Nitrogenous Compounds Such as Urea and Ammonium Bicarbonate by Growing Calves", J. Dairy Sci. 22:785.
- Harvey, A. L.; Kolari, O. E.; and Meiske, J. C. 1963. "Feeding Corn Silage Treated with Limestone, Urea or Sodium Bisulfite at Time of Ensiling to Calves", Beef Cattle Field Day. Minnesota Agr. Exp. Sta. B-40a, p. 1.
- Hastings, W. H. 1944. "The Use of Urea in Commercial Dairy Feeds", J. Dairy Sci. 27:1015.
- Hatfield, E. E.; and Garrigus, U. S. 1967. "Recommended Levels of Additives for 'Balancing' Corn Silage and High-Moisture Corn", Illinois Beef Cattle Day Report AS-634e., p. 27.
- Hatfield, E. E.; Garrigus, U. S.; Forbes, R. M.; Newman, A. L.; and Gaither, W. 1959. "Biuret A Source of NPN for Ruminants," J. Animal Sci. 18:1208.
- Hatfield, E. E.; Hixon, D. L.; Paterson, L. H.; Braman, W.; Peter, A. P.; and Garrigus, U. S. 1971. "Levels of Protein for Beef Cattle Finishing Rations", Illinois Beef Cattle Day Report, 1971.
- Hatfield, E. E.; Melgi, E. F.; Albert, W. W.; Teuscher, J. S.; and Garrigus, U. S. 1966. "Fortifying Corn Silage with Non-Protein Nitrogen." Illinois Cattle Feeder Day, p. 18.
- Hawkins, D.R.; Henderson, H. E.; and Geasler, M. R. 1967.
  "Corn Silage, Concentrate Levels and Hormones for Finishing Steer and Heifer Calves", Michigan Beef Cattle Day Report, 1967, p. 33-48.

- Hawkins, D. R.; Henderson, H. E.; and Purser, D. B. 1970.
  "Effect of Drymatter Levels of Alfalfa Silage on
  Intake and Metabolism in the Ruminant", J. Animal
  Sci. 31:617-625.
- Henderickx, H. K. 1967. "The Effectiveness of Urea and Other Non-Protein Nitrogen Compounds in Ruminant Feeding", Urea as a Protein Supplement. Edited by M. H. Briggs. New York: Pergamon Press.
- Henderson, H. E.; Beattie, D. R.; Geasler, M. R.; and Bergen, W. G. 1970a. "Pro-Sil, Ammonia, Urea-Mineral and Urea Addition to Corn Silage for Feedlot Cattle", Michigan Beef Cattle Res. 1971, P. 33.
- Henderson, H. E.; Allen, C. K.; Cash, E.; Bergen, W. G. 1971.

  "Pro-Sil Vs. Soybean Meal for Supplementing 0%, 1/2%,
  1%, and 2% Concentrate Rations", Michigan Beef
  Cattle Res., 1971, P. 5.
- Henderson, H. E., and Geasler, M. R. 1969. "Pro-Sil Addition (Anhydrous Ammonia, Molasses, Minerals) to Corn Silage", Michigan State University Exp. Sta. AH-BC 69D.
- Henderson, H. E., Geasler, M. R. 1970. "Regular Corn Silage Full Fed Varying Levels of Protein, Sources of Protein and Concentrate Levels", Michigan Beef Cattle Res. 1970, P. 27.
- Henderson, H. E., and Purser, D. B. 1968. "Urea Additions to Corn Silage", Michigan Beef Cattle Day Report 1968, p. 29.
- Henderson, H. E.; Purser, D. B.; and Geasler, M. R. 1970b.
  "Anhydrous Ammonia, Urea and Mineral Additives to
  Corn Silage", Report of Beef Cattle Res. 1970.
  Michigan State Univ., p. 3.
- Hogan, J. P.; and Weston, R. H. 1970. "Quantitative Aspects of Microbial Protein Synthesis in the Rumen."

  Physiology of Digestion in the Ruminant. Edited by A. T. Pillipson. England: Ovel Press Limited.
- Huber, J. T., and Hillman, D. 1970. "Protein Additives for Corn Silage for Dairy Cattle", Michigan State Univer. Mimeo. D-236.
- Huber, J. T.; Polan, C. E.; and Hillman, D. 1968b. "Urea in High Corn Silage Rations for Dairy Cattle", J. Animal Sci. 27:220.
- Huber, J. T., and Thomas, J. W. 1971. "Urea-Treated Corn Silage in Low Protein Rations for Lactating Cows", J. of Dairy Sci. 54:224-230

- Huber, C. A.; Thomas, J. W.; and Emery, R. S. 1968a. "Response of Lactating Cows Fed Urea-Treated Corn Silage Harvested at Varying Stages of Maturity." J. Dairy Sci. 51:11:1806.
- Huber, J. T.; Sandy, R. A.; Polan, C. E.; Bryant, H. T.; and Blaser, R. E. 1967. "Varying Levels of Urea for Dairy Cows Fed Corn Silage as the Only Forrage."

  J. of Dairy Sci. 50:8:1241-47.
- Hungate, R. E. 1965. The Rumen and its Microbes. New York: Academic Press, Inc.
- Hunter, C. A. 1921. "Bacteriological and Chemical Studies of Different Kinds of Silage", J. Agr. Res. 21:767.
- Jackson, R. W., and Block, R. J. 1932-1933. "Metabolism of d and I-Methionine", Proc. Soc. Exp. Biol.and Med. 30:587.
- Jacobson, D. R.; Barnett, J. W.; Carr, S. B.; and Hatton, R. H. 1967. "Voluntary Feed Intake, Milk Production, Rumen Content and Plasma Free Amino Acid Levels of Lactation Cows on Low Sulfur and Sulfur Supplemented Diets", J. Dairy Sci. 50:1248.
- Jacobson, D. R.; Soewardi, B.; Barnett, J. W.; Hatton, R. H.; and Carr, S. B. 1969. "Sulfur, Nitrogen and Amino Acid Balance and Digestibility of Low Sulfur and Sulfur Supplemented Diets Fed to Lactating Cows", J. Dairy Sci. 52:472.
- Johnson, R. R.; Balwani, T. L.; Johnson, L. J.; McClure, K. E. and Dehority, B. A. 1966. "Corn Plant Maturity." II. "Effect on in vitro Cellulose Digestibility and Soluble Carbohydrate Content", J. Animal Sci. 25:612.
- Johnson, W. H.; Goodrich, R. D.; and Meiske, J. C. 1970.

  "The Role of Sulfur in Ruminant Nutrition", Technical Release 70-11a. Int. Min. & Chemical Corp. Skokie, Ill.
- Johnson, R. E., and McClure, R. E. 1968. "Corn Plant Maturity".

  IV. "Effects on Digestibility of Corn Silage in Sheep",
  J. Animal Sci. 27:535.
- Johnson, R. E.; McClure, K. E.; Johnson, L. J.; and Klosterman, E. W. 1967. "Corn Plant Maturity". III. "Distribution of Nitrogen in Corn Silage Treated with Limestone, Urea and Diammonium Phosphate", J. Animal Sci. 26:394.
- Jordan, R. R.; Meiske, J. C.; Goodrich, R. D.; Kolari, O. E.; and Harvey, A. L. 1965. "All-in-one for Wintering Calves", Minnesota Agr. Sta. Beef Cattle Field Day. B-61, p. 17.

- Karr, M. R.; Garrigus, U. S.; Hatfield, E. E.; Norton, H. W.; and Doane, B. B. 1965. "Nutritional and Chemical Evaluation of Urea and of Biuret in Complete Ensiled Finishing Diets by Lambs", J. Animal Sci. 24:469.
- Kay, R. N. 1969. "Digestion of Protein on the Intestines of Adult Ruminants", Proc. Nutr. Soc. 28:140.
- Klosterman, E. W.; Johnson, R. R.; McClure, K. E.; and Johnson, L. G. 1966. "Full Utilization of the Corn Plant as Complete Silages for Fattening Cattle and Wintering Beef Cows", Ohio Beef Cattle Res. Summary 6:7.
- Klosterman, E. W.; Johnson, R. R.; Moxon, A. L.; and Scott, H. W. 1963. "Feeding Value of Limestone-Treated Corn Silage for Fattening Cattle", Ohio Agr. Exp. Sta. Bul. 934.
- Klosterman, E. W.; Moxon, A. L.; Johnson, R. R.; and Grifo, A. P. 1961. "Feeding Value of Limestone Urea Treated Whole Plant Corn Silage with Three Levels of Soy Oil Meal", Beef Cattle Day Report. Ohio Agr. Exp. Sta. Serial No. 122.
- Larson, R. L.; and Halverson, A. W. 1962. "Protein Quality of Alfalfa Concentrate", Agr. Food. Chem. 10:422.
- Lewis, T. R.; and Emery, R. S. 1962. "Relative Deamination Rates of Amino Acids by Rumen Microorganisms", J. Dairy Sci. 45:1363.
- Lewis, D.; Hill, K. J.; and Annison, E. F. 1957. "Studies on the Portal Blood of Sheep". I. "Absorption of Ammonia from the Rumen of the Sheep", Biochem. Jour. 66:587.
- Linton, J. H.; Loughheed, T. C.; and Sibbald, L. R. 1968.
  "Elevation of Free Methionine in Bovine Plasma",
  J. Animal Sci. 28:1179.
- Little, C. O.; Bradley, N. W.; and Mitchell, G. E. 1966.
  "Plasma Amino Acids in Steers Feed Urea Supplements",
  J. Animal Sci. 25:260 (Abstr.)
- Lofgreen, G. P. 1970. "Methionine Hydroxy Analog and/or Sulfur in Rations Containing Urea", California Feeders Day. 10:64.
- Lofgreen, G. P.; Loosli, L. K.; and Maynard, L. A. 1947.
  "The Influence of Protein Source Upon Nitrogen
  Retention by Sheep." J. Animal Sci. 6:343.
- Loosli, J. K.; Harris, L. E. 1945. "Methionine Increases the Value of Urea in Lambs", J. Animal Sci. 4:435-437.

- Loosli, J. K.; Williams, H. H.; Thomas, W. E.; Harris, E. H.; and Maynard, L. A. 1949. "Synthesis of Amino Acids in the Rumen", Science 11:144.
- Lopez, J.; Jorgensen, N. A.; Larsen, H. J.; and Niedermaier, R. P. 1970a. "Effect of Nitrogen Source, Silage of Maturity, and Fermentation Time on pH, and Organic Acid Production in Corn Silage", J. Dairy Sci. 53:1225.
- Lopez, J.; Jorgensen, N. A.; Niedermeier, R. P.; and Larsen, H. J. 1970b. "Redistribution of Nitrogen in Urea-Treated and Soybean Meal-Treated Corn Silage", J. Dairy Sci. 53:1215.
- McCarthy, R. D. 1970. "The Role of Methionine Hydroxy Analog in Ruminant Nutrition", Feedstuffs, Jan. 24.
- McCarthy, R. D., Porter, G. A.; and Griel, L. C. 1969.

  "Bovine Ketosis and Depressed Fat Test in Milk,
  a Problem of Methionine Metabolism and Serum Lipoprotein Aberration", J. Dairy Sci. 51:459.
- McDonald, I. W. 1952. "The Role of Ammonia in Ruminal Digestion of Protein", Biochem. Jour. 51:86-90.
- McDonald, I. W. 1968. "Nutritional Aspects of Protein Metabolism in Ruminants", Austr. Vet. Journal 44:145.
- McDonald, I. W.; Hall, R. J. 1957. "The Conversion of Casein into Microbial Protein in the Rumen", Biochem. J. 67:400.
- McDonald, P.; Stirling, A. G.; and Henderson, A. R. 1962.
  "Buffering Capacity of Herbage Samples as Factor
  in Ensilage", J. Dairy Sci. 53:1215.
- McLaren, G. A.; Anderson, G. C.; and Barth, K. M. 1965.
  "Influence of Methionine and Tryptophan on Nitrogen
  Utilization by Lambs Fed High Levels of Non-Protein
  Nitrogen", J. Animal Sci. 24:231.
- McLaren, G. A. 1964. Symposium: Microbial Digestion in Ruminants Nitrogen Metabolism in the Rumen. J. Animal Sci. 23:577.
- MacPherson, H. T. 1952. "Protein Breakdown in Silage Making", F. Sci. Fd. Agric. 3:362-65.
- Mabbit, L. A. 1951. Proc. Soc. Appl. Bact. 14:147-150.
- Meiske, J. C.; and Goodrich, R. D. "Effect of Urea Addition to Corn Silage in Steers", Minn. Beef Cattle Feeders Day. 1969.

- Meiske, J. C.; Van Ardsell, W. J.; Luecke, R. W.; and Hoefer, J. A. 1955. "The Utilization of Urea and Biuret as Sources of N. for Gaining Fattening Lambs", J. Animal Sci. 14:941.
- Miller, W. J.; and Clifton, C. M. 1965. "Relation of Drymatter Content in Ensiled Material and Other Factors to Nutrient Lower by Seepage", J. Dairy Sci. 48:917.
- Minish, G. L.; Newland, H. W.; and Henderson, H. E. 1966.
  "The Relationship of Concentrate Level and Feeder
  Grade to Production Factors and Carcass Characteristics", Mich. Beef Cattle Day Rep., p. 54-56.
- Munro, H. N. 1964. "General Aspects of the Regulation of Protein Metabolism by Diet and Hormones", Mammalian Protein Metabolism. Edited by H. N. Munro and J. B. Allison. Vol. I. New York: Academic Press.
- Newland, H. W.; Henderson, H. E.; and Minish, G. L. 1965.
  "Energy Levels for Different Grades of Cattle Fed
  Two Kinds of Corn Silage", Mich. Beef Cattle Day.
  1965, p. 3.
- Noble, R. L.; Pope, S. P.; and Gallup, W. D. 1955. "Urea and Methionine in Fattening Rations for Lambs", J. Animal Sci. 14:132.
- Noller, C. H.; Colenbrander, V. F.; Jone, G. T.; Cummings, K. R.; and Muller, L. D. 1967. "Feeding Value of Ammoniated Polyphosphate Treated Corn Silage."
  J. Animal Sci. 26:927 (Abstr.)
- Oljten, R. R. 1969. "Effects of Feeding Ruminants Non-Protein Nitrogen, as the Only Nitrogen Source", J. Animal Sci. 28:673.
- Oljten, R. R.; Styler, L. L.; Kozak, A. A.; and Williams, E. E. 1962. "Evaluation of Urea, Biuret, Urea Phosphate and Uric Acid as NPN Sources for Cattle", J. Nutr. 94:193.
- Oljten, R. R.; Waller, G. R.; Nelson, A. B.; and Tillman, A. D. 1963. "Ruminant Studies with DAP and Urea", J. Animal Sci. 22:30.
- Oljten, R. R.; Williams, B., Jr.; and Richardson, G. V. 1968.
  "Urea vs. Biuret in a Roughage Diet for Steers",
  J. Animal Sci. 27:1173.
- Owen, F. G. 1967. "Factors Affecting Nutritive Value of Corn and Sorghum Silage", J. Dairy Sci. 50:404.

- Owens, F. N.; Meiske, J. C.; and Goodrich, R. D. 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.
- Patton, R. A.; McCarthy, R. D.; and Griel, L. C. 1968.
  "Lipid Synthesis by Rumen Microorganisms". I.
  "Stimulation by Methionine in vitro", J. Dairy
  Sci. 51:1310.
- Patton, R. A.; McCarthy, R. D.; and Griel, L. C. 1970.

  "Lipid Synthesis of Rumen Microorganisms". II.

  "Further Characterization of the Effects of
  Methionine", J. Dairy Sci. 53:460.
- Peterson, L. A.; and Hatfield, E. E. 1970. "Effect of Protein and Energy Levels for Finishing Steer Calves on Corn Silage and High-Moisture Corn Diets", Illinois Beef Day Report, 1970, R-93, p. 3-8.
- Peterson, W. H.; Hostings, E. G.; and Fred, E. B. 1925.

  "A Study of the Principle Changes which Take Place in the Making of Silage", Wisconsin Agr. Exp. Sta.

  Res. Bul. 61.
- Polan, C. E.; Chandler, P. T.; and Miller, C. N. 1970.
  "Methionine Hydroxy Analog: Varying Levels for Lactating Cows", J. Dairy Sci. 53:607.
- Polan, C. E.; Huber, J. T.; and Miller, C. N. 1967. "Recovery of Nitrogen from Urea-Treated Silage", J. Dairy Sci. 50:616 (Abstr.)
- Potter, E. L. 1970. "Amino Acid Studies in Ruminants: Investigation of a Method for Determining the Limiting Amino Acids", Thesis for Degree of Ph. D. Michigan State University.
- Potter, G. D.; Little, C. O.; and Mitchell, G. E. 1968.
  Abomasal Nitrogen in Steers Fed Soybean Meal or
  Urea", J. Animal Sci. 28:711
- Potter, E. L.; Purser, D. B.; and Bergen, W. G. 1970.
  "Duodenal Protein Infusion and Plasma Amino Acids",
  J. Animal Sci. 31:251.
- Purser, D. G. 1970. "Nitrogen Metabolism in the Rumen: Microorganisms as a Source of Protein for the Ruminant Animal", J. Animal Sci. 30:988-1001.
- Purser, D. G., Klotenstein, T. J.; and Cline, J. H. 1966.
  "Dietary and Defaunation Effects Upon Plasma Amino Acids Concentrations in Sheep", J. Nutr. 89:226.

- Reep, W. W.; Hale, W. H.; and Burroughs, W. 1955. "The Influence of Oral Administration of NPN Compounds as Protein Substitutes in Lamb Fattening Rations", J. Animal Sci. 14:901.
- Reep, W. W.; Hale, W.; Cheng, E. W.; and Burroughs, W.
  1955. "The Influence of Oral Administration of NonProtein Nitrogen Compounds Upon Blood Ammonia and
  Urea Levels in Lambs", J. Animal Sci. 14:118.
- Reis, P. J. 1967. "The Growth and Composition of Wool", IV. Austr. J. Biol. Sci. 20:809-825.
- Reis, P. J.; and Schinckel, P. G. 1964. "Some Effects of Sulfur Containing Amino Acids on the Growth and Composition of Wool", Austr. J. Biol. Sci. 16:218.
- Rose, W. C. 1938. "The Nutritive Significance of the Amino Acids", Physiol. Rev. 18:109.
- Rosenberg, R. F. 1950. The Isolation and Cultivation of Obligate Anaerobes from Silage", J. Appl. Bact. 19:173.
- Russell, E. J. 1908. "The Chemical Changes Taking Place During the Ensilage of Maize", J. Agr. Sci. 2:392-410.
- Salsbury, R. L.; and Haenlein, G. E. 1964. "Effect of Amino Acids on Cellulose Digestion in vitro", J. Animal Sci. 23:891.
- Salsbury, R. L.; Maruil, D. K.; Woodmansee, C. W.; and Haenlein, G. E. 1970. "Utilization of Methionine and Methionine Hydroxy Analog by Rumen Microorganisms in vitro", J. Dairy Sci. 54:390-396.
- Salsbury, R. L.; and Zika Kis, J. P. 1967. "Sulfur Metabolism of Rumen Microorganisms", J. Animal Sci. 26:929.
- Schelling, G. T.; and Hatfield, E. E. 1968. "Effect of Abomasally Infused Nitrogen Sources on Nitrogen Retention of Growing Lambs", J. Nutr. 96:319.
- Schelling, G. T.; Hinds, F. C.; and Hatfield, E. E. 1967.

  "Effect of Dietary Protein Levels, Amino Acids
  Supplementation on a Nitrogen Source Upon the Plasma
  Free Amino Acid Concentration in Growing Lambs",
  J. Nutr. 92:339.
- Sibbald, R.; Lougheed, T. C.; and Linton, J. H. 1968.

  "A Methionine Supplement for Ruminants", Proc. of the 2nd World Conference of Animal Production.

- Stanberg, D.; Tolman, W.; Harris, L.; and Woods, W. 1968.
  "Urea Additions to Corn Silage at Ensiling and at Feeding Time", J. Animal Sci. 27:1177 (Abstr.)
- Thomas, J. W. 1966. "Protein, Kinds and Amount to Feed to Dairy Cattle", Feedstuffs, Vol. 38, No. 40, p. 58.
- Thomas, W. E.; Loosli, J. K.; Williams, H. H.; and Maynard, L. A. 1951. "The Utilization of Inorganic Sulfates and Urea Nitrogen by Lambs", J. Nutr. 43:515-523.
- Trams, E. G.; Brown, E. A.; Lauter, C. J. 1966. "Lipoprotein Synthesis". I. "Rat Plasma Lipoprotein Composition and Synthesis from Radioactive Precursors", Lipids. 1:309.
- Virtanen, A. I. 1966. "Milk Production of Cows on Protein-Free Feed", Science. 153:1603.
- Waldo, D. R. 1968. Symposium: Nitrogen Utilization by the Ruminant Nitrogen Metabolism in the Ruminant.
  J. Dairy Sci. 51:265.
- Watson, S. J.; and Nash, M. J. 1960. The Conservation of Grass and Forage Crops. London: Oliver and Boyd.
- Williams, L. R., Martz, F. A.; and Hilderbrand, E. S. 1970.

  "Feeding Encapsulated Methionine Supplement to
  Dairy Cows", J. Dairy Sci. 53:1709-13.
- Williams, V. J., and Moir, R. J. 1951. "Ruminal Flora Studies in Sheep", III. Austr. J. Biol. Sci. Res. 84:377.
- Wing, J. M. 1957. "Effects of Method of Feeding Methionine and Potassium Ororate to Young Calves", J. Dairy Sci. 40:1617.
- Wright, P. L. 1971. "Body Weight Gain and Wool Growth Response to Formaldehyde Treated Casein and Sulfur Amino Acids", J. Animal Sci. 33:137-141.

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