EFFECT OF TREATING CORN STOVER WITH AQUEOUS AMMONIA AND UREA ON NUTRITIONAL VALUE.

by

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DEDICATION.

This manuscript is dedicated to the memory of my sister whose untimely death occurred on

Chapter I

INTRODUCTION

One of the major crop residues produced worldwide is corn-residue. Over 162 million metric tons of corn by-product is produced in the U. S. each year (Key and Smith, 1981). Hence, it is of paramount importance to expand the utilization of this crop by-product for economic and environmental reasons.

Recently, the use of cereal grains for animal feeding has been criticized on the grounds that this puts pressure on human food supplies and that more emphasis should, therefore, be put on beef and dairy production from crop by-products. Crop by-product is a poor quality roughage and the extent of its utilization depends on the daily energy requirements of the animals in question and their acceptance and effective utilization of the by-product. For roughage to be widely utilized it must serve as a useful source of energy and must be economical.

The available nutrient content of low quality roughage indicates that crop by-products are deficient in protein, P, soluble carbohydrate, and are high in lignin and waxy undegradable components, which tend to limit their nutritive value. Crop by-products are noted for their bulkiness, which limits intake.

Numerous attempts have been made to improve the nutritional value of corn by-product and other low-quality roughages. Methods tested include chemical treatment with alkali solutions, NH_3, and urea; physical treatment involving grinding, pelleting and chopping; supplementation; and biological treatment with enzymes capable of weakening or breaking lignocellulosic bonds.

There has been some success with the use of alkali. However, animals fed alkali-treated roughages, and the soil on which wastes are applied may be exposed to hazardous mineral residues. Scientists have focused on the use of NH_3 and urea which not only increase digestibility but also enhance the N content of the crop by-product.

Chemical treatment of corn stover with ammonia and urea solutions has not been extensively investigated . As the utilization of crop by-products become more effective, and as grains become relatively more expensive or unavailable, crop by-products seem likely to have a high potential in practical ruminant feeding programs of the future.

This study addressed the influence of treating poor quality roughages with ammonia and urea solutions on the utilization of poor quality roughages, as measured by changes in chemical composition, digestibility and utilization of the roughage.

Chapter II

LITERATURE REVIEW

Over 162 million metric tons of corn residues (dry basis) are produced annually in United States (Keys and Smith, 1981). This figure represents over one half the total available crop residue supply. It has been established that this crop residue is underutilized due to its low nutritional value (Klopfenstein and Owens, 1981).

Nutritive Value of Corn Stover.

Colenbrander et al. (1971) reported that corn stover supplies some energy for ruminants, is high in neutral detergent fiber (NDF), and low in crude protein (CP). The nutritive value of corn stover decreases, whereas, the lignin content increases as the plant matures (McDonnell and Klopfenstein, 1980). As the plant cell walls mature, the lignin content increases, with a disproportionate decrease in fermentability. Previous studies have established that lignin physically encapsulates cellulose myofibrils in a hydrophobic matrix, thus preventing wetting and access of cellulase enzyme complexes (Morrison, 1974). However, recent studies have shown that lignin is a complex polymer of phenyl-propane units which is covalently bound to both cellulose and hemicellulose fractions (Evans, 1979).

Digestibility may be influenced by moisture, composition of straw, type and variety of plant, maturity of plant and temperature (McDonnell and Klopfenstein, 1980). Different havesting systems will also produce corn crop residues with varying feeding value. Koers et al. (1970) obtained gains of .3 kg/d in calves fed supplemented corncobs, and Berger et al. (1979) reported .65 kg/d gains with growing calves fed supplemented stalklage. However, Paterson et al. (1979) reported digestibilities as low as 37%, which may be insufficient for maintenance.

Treating Crop Residues.

For many years, nutritionists have attempted to improve the nutritive value and preservation of livestock feeds, especially crop by-products. The low quality of crop residues may be improved by either manipulating the harvest time and procedure, generating plant varieties capable of producing higher quality residues (Sundstol et al. 1978), or treating the residue.

Physical and Mechanical Processing. The major principle of physical or mechanical processing is to diminute the particle sizes of the diets. This could be achieved in various ways such as chopping or shredding, grinding (Greenhalgh and

Wainman, 1972) pressure cooking (Jackson 1978) and production of briquettes, pellets and cubes (Sundstol and Coxworth, 1984). Owens (1978) reported that grinding or pelleting may increase the rate of passage of crop residue through the gastro-intestinal tract (GIT) of ruminants, reduce rumination time and decrease the frequency and intensity of rumen contraction.

The study conducted by Owens (1978) revealed that grinding and pelleting may decrease digestibility by 9 to 15% percentage units, while increasing voluntary consumption. However, it has been pointed out that the increase in efficiency of utilization of digestible energy due to grinding compensated for the decrease in digestibility (Arnarson, 1980). Norgaard (1981) indicated that the increased feed intake and utilization of digestible energy due to grinding could be associated with various factors such as lowered energy costs of masticating, ruminating and digesting. Futhermore, he stated that the energy loss due to methane formation and fermentation in the rumen may be less, and the change in fermentation patterns may increase the digestible energy.

Grinding may increase the voluntary intake and digestible energy intake of crop residues up to 30% (Greenhalgh and Waiman, 1972). Moreover, the increase in energy intake may be higher than 30% because grinding will improve the net energy value of the straw. Carmona and Greenhalgh (1972) reported the possibility of an additive effect of grinding and alkali treatment . The digestible energy intake by sheep on a ground and alkali-treated straw diet was 15% higher than that of sheep fed a chopped alkali-treated straw diet (Carmona and Greenhalgh, 1972).

Donefer (1976) suggested that the digestibility of sugarcane bagasse and wood chips may be improved by pressure cooking. He treated samples of bagasse with 4% NaOH under high pressure steam (8 kg/cm^2 at 170 C) and observed an increased in vitro cellulose digestibility of 15 to 17 percentage units (initial digestibility was 25%). Temperature and pressure above those used by these authors may give higher rate of digestibility (Jackson 1978). Guggolz et al.(1971) subjected straw to 30 kg/cm^2 pressure and obtained over 20% increase in in vitro digestibility. The authors reported over 40% increase in digestibility when 3 g NaOH/100g straw were added before pressure cooking. Conversely, Garrett et al. (1974) treated rice straw with pressure cooking only and observed decreased intake and digestibility when fed to sheep. However, the intake and digestibility were increased when NaOH was added prior to pressure cooking.

Biological Treatment. Biological treatment of roughages is based on the use of fungi or enzymes which can disintegrate lignocellulosic bonds. Organisms that are capable of degrading cellulose may not be ideal, since they can adversely deplete the straw of the available nutrients that are degradable by ruminants (Jackson, 1978; Ibrahim and Pearce, 1983). They claimed that white-rot fungi have the capacity to degrade lignin to a minimum level. In vitro experiments with this fungi showed a significant increase in digestibility (Jackson, 1977).

Chemical Treatment of Crop Residues. It has been established that as plants mature, the lignin content increases (Kamstra et al., 1958) and lignin has very low digestibility. Digestibility may be improved by treating with chemicals. Four chemicals which have been routinely used in experimentation with the various straws are NaOH, $Ca(OH)_2$, KOH and NH_3 (Rounds et al., 1976; Klopfeinstein, 1978).

The modes of action for chemical treatment of crop residues have been demonstrated by Waller (1976). In general, chemical treatment solubilizes some of the hemicellulose without changing the cellulose content. The reaction breaks the ether linkages between lignin and cellulose or hemicellulose (Lau and Van Soest, 1981). There have also been suggestions of a swelling effect of chemical treatment on cellulose and hemicellulose (Waller, 1976).

Chemical treatment of crop by-product may be done by various methods, such as thermoammoniation (Oji et al., 1977; Oji and Mowat, 1979 and Oji et al., 1979), NH_3 freeze procedure (Dale et al., 1982) and the Norwegian Stack method (Sundstol et al., 1978).

Thermoammoniation is a method which involves a very short time of treatment. According to Oji et al. (1977) this procedure entails the circulation of hot ammoniated air in an oven chamber packed with crop residue. These researchers found that this procedure improved in vitro dry matter digestibility (IVDMD). In vitro dry matter digestibility of treated stover was increased by 20 percentage units over that of the untreated stover. However, prolonged ammoniation, particularly at temperatures above 100 C, may be detrimental in decreasing nitrogen and energy digestibilities (Chomyszyn and Ziolecka, 1972). Thermoammoniation reaction may be completed within 24 h (Mowat, 1979).

Ammonia freeze procedure has been widely utilized on an industrial basis since it was developed at Colorado State University (Dale et al., 1982). The procedure involves the mixture of crop by-product with liquid NH_3 (1:1; w/w). The mixing of NH_3 and straw should be done inside a highly pressurized reactor, which should be opened later to allow sudden release of the accumulated pressure through a rotating ball valve. The evacuation of the pressure would drop the temperature of the mixture to freezing temperature. At this point Dale et al. (1982) stressed that the liquid NH_3 would be trapped to allow a rapid expansion of the cell structures into vapor and collapse of cell walls. There was a substantial improvement in cellulase activity and digestibility of the straw after this treatment. According to these authors the NH_3 may be recompressed and recycled for economic reasons.

Sundstol et al. (1978) developed the "stack" method of ammoniation. This method involves the injection of anhydrous NH_3 three-quarters of the way into the stack of bales of straw. The bales should be squarely arranged and properly sealed with polyethylene sheets. The Norwegian workers reported that the treated straw changed in color from yellow to a brownish color after ammoniation and also the structure of the material was slightly softened. Similar observations were reported previously by Martynov (1972). The color change was related to the oxidation and condensation of the aldehydic fractions in sugars with nitrogenous bases, via the Maillard reactions. According to Sundstol et al. (1978) the "stack" method increased the CP content of the straw by

6.6 percentage units and organic matter digestibility was increased by 10 to 15 percentage units.

Ammonia appears to react similarly to NaOH, but the reaction time is much longer, up to 20 d (Waiss et al., 1972), compared to 24 h for NaOH treatment. In ammonia treatment the N content of the treated materials could be increased by the NH_3 residue, which is a source of non-protein nitrogen (NPN), and eliminate the detrimental effects of mineral residues observed in other chemical treatment such as NaOH.

Treatment of crop residues has been shown by many researchers to improve digestibility and animal performance, if the mineral imbalance created by alkali treatment is corrected (Itoh et al., 1979).

Ammonia Treatment.

There is a need for practical methods of treating low quality roughages in order to increase their feeding value. One of these is the ammoniation method (Klopfeinstein and Owen, 1981). Ammoniation of low quality roughage may be done by using aqueous (liquid) or anhydrous (gas) NH_3.

Aqueous Ammonia. Aqueous NH_3 is prepared by dissolving NH_3 in water. Sundstol and Coxworth (1984) suggested that the purity may range from 25 to 30% NH_3. Liquid NH_3 may be preferred to anhydrous NH_3 when the material to be treated is very dry. Furthermore, in arid and tropical regions better results are obtained with liquid NH_3 than anhydrous NH_3 (Sundstol and Coxworth, 1984).

The solution may be injected into stacked bales of straw (Borhami et al., 1982), or watering cans may be used to spray the solution on hay, forages and bales of straw (Johri et al., 1982). Another method involves the use of an open container containing NH_3 solution, which may be placed on top of stacks of straw (Saadulaah et al., 1981). The stacks must be properly sealed to allow even distribution of the chemical (Berger, 1981).

Anhydrous Ammonia. Anhydrous NH_3 is about 100% pure (Sundstol and Coxworth, 1984), and application of it results in rapid reaction and fast penetration into stacks (Sundstol et al., 1978). The Norwegian workers suggested that very small amounts of anhydrous NH_3 are required for an effective reaction to occur (Sundstol et al., 1978).

Anhydrous NH_3 may be toxic and dangerous to handle. Therefore, special equipment and experienced personnel are required (Sundstol and Coxworth, 1984).

Ammoniation of poor quality roughage may serve two major purposes which are: 1) increase the N content, which serves as NPN source in the diet and 2) the absence of mineral residue remaining which might be detrimental to the animals or the soil on which the manure is applied. It should be noted that animals will refuse to eat ammoniated diet unless it is aerated or mixed with fermented feed (Klopfenstein and Owens, 1981).

Ammoniated roughages provide substrate for fiber digestion and microbial protein synthesis. However, good sources of energy are required for efficient utilization of ammoniated roughage (Klopfenstein and Owens, 1981). Previous workers have showed that ammoniation may improve feeding quality and digestibility of crop residue (Oji et al., 1977; Sundstol et al., 1978; Oji and Mowat, 1979; Oji et al., 1979; Peterson et al., 1981 and Cochran et al., 1984). Klopfenstein and Owens (1981) observed that dry matter digestibility increased from 37% for sheep fed stalks with 2 g NH_3/100g dry matter (DM), whereas, addition of 3 or 4 g NH_3/100g DM did not improve digestibility further. Generally, these researchers reported about 10 percentage units increase in DM digestibility of treated corn stalk over untreated stalks.

Oji et al. (1977) treated corn stover with 2% and 3% NH_3 and reported an increased total nitrogen digestibility of the roughage. No fermentation was observed during storage and no mold was formed. These workers suggested that ammonia may be an effective fungicide and preservative for moist hay. This observation agrees with previous work by Knapp et al. (1974) with hay and Bothast et al. (1973) with grains .

High dry matter intake was reported by Oji et al. (1977) and Garrett et al. (1979) when ammoniated corn stover was fed to sheep. Sundstol et al. (1978) reported reduced palatability in NH_3-treated forages at high moisture levels. This was due to incomplete aeration, which may be related to the high-moisture content.

Fernandes et al. (1986) reported improved nutritive value in terms of digestibility, feed intake, daily gain, feed efficiency and N content when ammoniated corn stover was fed to sheep.

Paterson et al. (1981) observed that NH_3 treatment of corn stalks or cobs consistently improved dry matter intake (DMI). They suggested that this was due to the action of NH_3 in solubililizing the hemicellulose fraction of the cell wall. This observation agrees with those reported by previous workers (Tarkow and Feist, 1969; Waller, 1976; Klopfenstein, 1978).

One of the disadvantages of ammoniation is associated with drastic loss of NH_3 during aeration, which may eventually cause reduced nitrogen content of the treated materials

(Suleiman et al., 1979). However, Borhami et al. (1982) reported that NH_3 loss was reduced and N content was increased when ammoniated straw was treated with organic acid. Another purpose of organic acid treatment of ammoniated straw is the prevention of NH_3 loss.

Quantity of Ammonia Used. The effect of amount of NH_3 used on the quality of treated straw was first investigated by Nikolaeva (1938). He treated straw with NH_3 solution to arrive at 5% NH_3 which resulted in improved nutritive value of the straw over the control. Another study conducted by Waiss et al. (1972) showed that larger amount (5.2%) of NH_3 resulted in higher enzymatic solubility of rice straw, compared to smaller amount (2.6%). Waagepetersen and Vestergaard-Thomsen (1977) treated barley straw with three levels of NH_3 (3.4, 4.4, 5.9%) for 14 d at temperatures ranging from 15 to 55 C. They reported a significant increase in nutritive value of the straw when the NH_3 level was increased from 3.4 to 5.9%. Sundstol et al. (1978) reported a higher dry matter digestibility of oat straw treated with 2.5% NH_3 over 1.0% NH_3. However, only slight increase of dry matter digestibility was observed at 4.0% level.

According to Kernans et al. (1979) the optimum level of dosage should be between 3.0 and 4.0% NH_3 on roughage dry matter basis, and the economical level is between 2.5 to 3.5% NH_3.

Temperature. Although NH_3 is a slow reacting chemical, its reaction frequency may be stimulated by high temperature (Borhami et al., 1982; Sundstol and Coxworth, 1984). Waage-petersen and Vestergaard-Thomsen (1977) pointed out that temperature could exert a positive effect on NH_3-treated roughages by stimulating the reaction and reducing the treatment period. They stressed that temperature up to 45 C would only require 3 to 7 d to complete the chemical reaction, while freezing temperature would slow down the reaction, thus, prolonging the duration of treatment.

Sundstol et al. (1978) commented that immediate treatment of crop by-product after harvest may be more ideal, especially when the temperature is high and stable. In arid and tropical regions chemical treatment of crop by-product may be all year round activity (Mbatya et al., 1983).

Duration of Treatment. It has been established that NH_3 is a slow reacting chemical, hence , it is expected to have a long reaction time (Sundstol and Coxworth, 1984). Sundstol et al. (1978) referred to the fact that longer period of treatment at very low temperature and shorter treatment period at high temperature are required to achieve complete reaction toward improved nutritional value of crop by-product. They recommended that straw should be treated for 4 to 8 wks at 5 to 15 C, 1 to 4 wks at 15 to 30 C and for temperature above 30 C , 1 wk should be enough. The study conducted by Kernan et al. (1978) agrees closely with the previous authors. They stressed that temperature and duration of treatment are indirectly proportional to each other.

Moisture Content. The moisture content of the treated material has a major effect on the extent of ammonia and urea reaction. It has been established that moisture up to 30% was enough to initiate chemical reaction (Waiss et al. 1972). More recent work has showed that moisture content up to 50% was ideal when a large quantity (5 to 7%) of NH_3 is used to treat poor quality roughage (Borhami et al., 1982).

According to Said et al. (1982) very dry straws in the arid and tropical regions should be wetted prior to NH_3 or urea treatment. They stressed the preference of aqueous NH_3 over anhydrous NH_3 in these dry regions. However, if the only alternative is anhydrous NH_3, the straw could be wetted prior to treatment to ensure even distribution of chemicals. It should be noted that high moisture is not without its problems. High moisture may predispose the treated material to mold, which may result in handling difficulties (Sundstol et al., 1978).

Pressure. The stacks should be properly sealed immediately after treatment. Sundstol et al. (1978) reported that over 50% of the chemical may be lost if enough pressure is not exerted on the stacks. Oji et al. (1978) suggested that puncturing of the polyethylene sheets should be avoided throughout the period of treatment.

Sundstol and Coxworth, (1984) reported that appropriate pressure would help to maintain the temperature inside the stacks and prevent NH_3 loss during treatment. They further stated that stacks should remain sealed pending the feeding period.

Initial Composition and Quality of the Crop Residue. The initial chemical composition of the crop by-product may influence the degree of chemical reaction that occurs during treatment. Kernan et al. (1979) investigated the effect of NH_3 treatment on wheat, oat and barley straws. Samples were randomly taken from four different locations. Their analyses revealed that wheat straw has the largest amount of CP

(8.1%), followed by barley and oat straws with 5.3 and 4.7% CP, respectively. In similar studies conducted by Horton and Steacy (1979) and Horton (1981), wheat straw showed the highest improvement, compared to barley and oat straws.

The effect of NH_3 treatment on the in vitro digestibility of corn stover, wheat and rice straws was studied by Kiangi et al. (1981). They observed that the largest effect of NH_3 was attributed to rice straw.

Said (1981) conducted a digestion trial with ammoniated corn cobs, corn stover and untreated corn cobs. He reported the higher daily gain with lambs fed ammoniated corn cobs, compared to those fed ammoniated corn stover and untreated corn cobs. A performance study done by Patersen et al. (1981) showed no positive effect on steers fed ammoniated corn stover, whereas, there was an increased average daily gain from 390g/d (untreated) to 720g/d in steers fed ammoniated corn cobs.

Kernan et al. (1981) treated various cereal by-products with NH_3 and established that chemical treatment was most ideal for poor quality roughage characterized by low digestibility. However, highly digestible materials may be treated with ammonia or urea for preservative purposes (Winther, 1978).

Urea Treatment.

Urea is an economical source of N and it may serve as good substitute for NH_3. It has been established that urea is easier and safer to handle than NH_3 (Ibrahim and Pearce, 1983; Ibrahim et al. 1983a; Oji and Mowat, 1977). William and Innes (1983) commented that the rate of urea hydrolysis may be influenced by the initial moisture content of the straw, temperature and urease activity.

Urea has been used in various ways to improve the nutritive value of poor quality roughages. Numerous methods of urea application have been researched, such as supplementation at feeding time (Saadullah et al., 1982; Ibbotson, 1983; Dias-Da-Silva, 1986), spraying on baled or unbaled straw (Saadullah et al., 1981), ensiling with straw, stack treatment and soaking of bales (Dias-Da-Silva, 1986).

It has been established that crop by-product such as corn stover has enough urease for complete urea hydrolysis, whereas, wheat straw is deficient in urease (Oji and Mowat, 1977; Jayasuriya and Perera, 1982 and Ibrahim et al., 1983b). These authors stressed the need to supplement crop residue which are lacking in urease with a readily-available source of urease such as jackbean (canavalia saueri) meal. Jayasuriya and Pearce (1983) reported a speedy reaction rate and a decreased treatment time when urease was added to treated straw during chemical treatment.

The studies conducted by Coombe (1981) showed that urea has a dual purpose of increasing intake and digestibility. He stressed that these effects enable ruminants to increase their digestible organic matter intake, especially, when fed only cereal straw. It was shown by Coombe (1981) that urea-treated straw was superior to urea-supplemented straw. He reported about 34% increase in digestible organic matter intake of sheep fed urea-treated wheat straw, compared to those fed urea-supplemented straw.

Oji and Mowat (1977) treated corn stover with urea solution. They observed that over 70% of the urea applied was decomposed within 2 d and the urea was totally hydrolyzed at d 20. However, Coxworth and Kullman (1978) reported contrasting results with wheat straw treated with urea and aqueous NH_3. These researchers suggested that the reason for decreased rate of urea hydrolysis was related to insufficiency of urease enzyme in wheat straw.

The use of urea as NPN feed supplement has been researched extensively. Tillman and Swift (1953) investigated the utilization of ammoniated crop by-products, urea and natural plant nitrogen source. These reseachers found that urea-supplemented diets were digested as well as soybean oil meal supplemented diets. Furthermore, they observed that the sheep receiving the urea diet retained slightly more N than the sheep receiving the soybean meal diet. However, both diets were superior to ammoniated feedstuffs.

According to Donefer et al. (1969) the digestibility and palatability of straw may be improved by treatment with 2.5% NH_3 from urea. Hershberger et al. (1959) studied the effect of different levels of urea. They reported that both cellulose digestion and protein synthesis increased with increased levels of urea. These workers suggested that the availability of the N added via NH_3 may not be as high as that from urea source. A similar suggestion was made by Sundstol et al. (1978).

Arnason (1979) reported that neutralization of NaOH-treated straw with H_3PO_4 and urea increased the CP content and digestibility of the treated straw. Ulloa et al. (1984) applied different levels of urea to forage crops. They reported an increased CP content and a decreased hemicellulose fraction in the urea-treated forage. The CP content of sorghum stover was increased from 4 to 6% when the roughage was treated with urea solution (Rekib et al., 1982). These researchers further studied the effect of urea treatment on voluntary intake. They found that the fortified sorghum stover was highly palatable to the animals. It was suggested that the high intake and increased digestibility was a direct effect of urea treatment. This observation agrees with previous results reported by Campling et al. (1962) and Colovos et al. (1967), but in contrast to that of Minson et al. (1961), who did not find any significant improvement in the digestibility of straw treated or supplemented with urea. The results of the experiment conducted by Johri et al. (1982) showed that mean voluntary dry matter intake increased progressively with increased level of urea impregnation of wheat straw up to 3%, then declined at higher levels of impregnation. Saadullah (1978) reported that although 2% of N, dry basis, increased digestibility, higher levels (up to 3%) showed no further rise in digestibility.

Doulberg et al. (1981) has shown that urea treatment of straw may not only improve digestibility but also the N balance of the animals. This agrees with results of the work by Moller and Hvelplund (1978).

The studies conducted by Waagepetersen and Vestergaard-Thomsen (1977) involved the treatment of straw with urea at 30 C for 7 d and 45 C for 3 d. The results showed an improvement in the nutritional value of the straw with both treatments, with maximum effect at 45 C for 3 d.

Doulberg et al. (1981) treated wheat straw with urea and they reported an increased intake, in vivo and in vitro dry matter digestibilities and N fixation from urea treatment. It was established that high moisture could enhance urea hydrolysis. Rashiq (1980) stressed that 65% moisture was better than 40% when treating straw with urea.

Anderson (1978) reported that bales treated with liquid urea were more durable, compact and easier to handle, compared to untreated bales. He also stressed that the weights of the treated bales increased from 20.5 to 34.1 kg, which could improve the hauling characteristics. Furthermore, he observed an improved intake and reduced wastage of the treated straw.

Colenbrander et al. (1983) compared the performance of dairy cows fed complete mixed diets containing NH_3 or urea as partial substitutes for soy protein. They reported an increased CP from 13.6 to 16% when urea or NH_3 was added to corn silage and stressed that without the use of NPN , the corn silage diet would have been deficient in CP. Furthermore, these researchers stated that substantial savings in feed costs were realized by providing some supplemental CP as NH_3 instead of soybean protein.

Chapter III

OBJECTIVES

The overall objective was to study the utilization by sheep of crop by-product treated with aqueous NH_3 or urea. The specific objectives were to:

1. Investigate the effect of NH_3 and urea treatment on chemical composition and nutritional value of corn stover.

2. Compare the utilization of N in NH_3- or urea-treated corn stover, and N in urea supplement.

Chapter IV

MATERIALS AND METHODS

Chemical Treatment of Corn Stover.

Twenty seven rectangular bales of corn stover were used for each of three treatments. One half of the bales were not treated (control) one fourth were treated with NH_3 and one fourth were treated with urea. The bales to be treated were stacked on black polyethylene sheets as described by Sundstol et al. (1978). Each bale was sprayed with garden watering cans to arrive at 3% NH_3 (w/w) for NH_3 treatment and 5.86% urea for urea treatment. The average weight of the bale was 40 kg. The bales were sprayed with known quantity of liquid chemicals on both sides to raise moisture to 40%. The bales were stacked in three layers designated as top, middle and bottom, each layer made up of nine bales (figure 1).

Black polyethylene sheet was laid down in an open ground. The size of the sheet was determined by the surface area of the stacks. All the bales were thoroughly sprayed as fast as possible to minimize ammonia loss. The stacks were tightly closed with the extra length of the plastic sheets. Heavy weights were placed on sides and top of the stacks. The bases of the stacks were covered with soil.

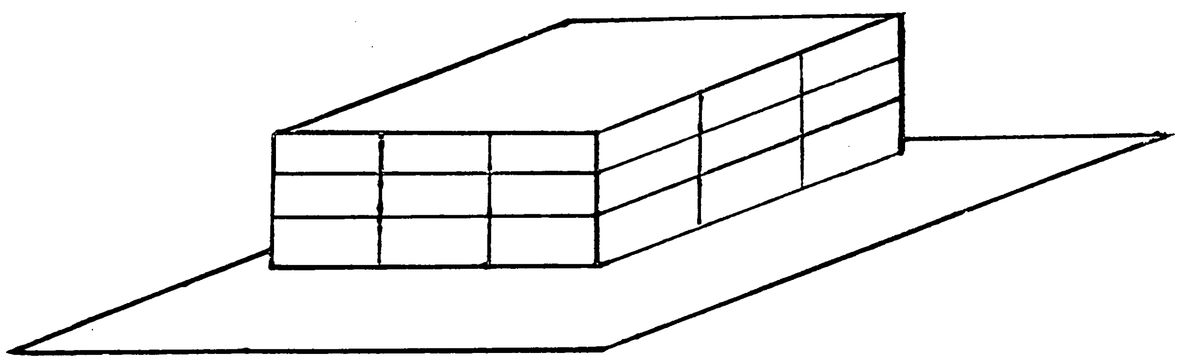

FIGURE 1. THE STACK OF BALES OF CORN STOVER.

The control bales were stacked inside the barn following similar stacking arrangement as the treated bales (figure 1) The treated stacks were left outside for 129 d, to allow adequate urea hydrolysis since the experiment was conducted during the winter season. The mean maximum temperature was 8.0 C and the mean minimum temperature was -4.0 C during the duration of chemical treatment. However, the minimum temperature was -23.3 C, and the maximum temperature was 27.8 C. According to Sundstol et al. (1978) low temperature could be compensated by extended time of treatment.

The stacks were opened and excess NH_3 was allowed to dissipate for 3 d, samples were randomly taken from different cores from representative bales of both treated and control bales.

Samples were dried at 60 C, ground through 1 mm screen and analyzed for CP by Kjeldahl procedure (AOAC, 1980). The remainder of the ground samples were placed in sample jars and used for in vitro dry matter digestibility as described by Tilley and Terry (1963).

Data from the experiment were statistically analyzed by using the GLM procedure of Statistical Analyses System (SAS, 1982). The experiment was designed as a 3 x 3 Factorial design, using the heights (top, middle & bottom) and treatments as main effects. The interaction between the two main effects was also tested. Differences among treatments were tested by using the following contrast: 1) Untreated (control) vs NH_3 and urea, 2) NH_3 vs urea. The means of all the data were analyzed by using the Least Squares Means procedure.

In Vitro Digestibility

One ruminally cannulated Angus steer was fed corn stover ad-libitum for 14 d. The corn stover was supplemented with .91 kg of soybean meal at each feeding period (morning and evening). Water was supplied at all times except for 2 h prior to collection of rumen ingesta for inoculum.

Rumen ingesta was collected from the steer 2 h after the withdrawal of feed and water, strained and filtered through an eight-layered cheesecloth into a thermos. Reasonable speed was employed to transport the rumen fluid to the laboratory for the in vitro dry matter digestibility (IVDMD).

The IVDMD procedure used in this experiment was similar to the two-stage procedure of Tilley and Terry (1963). The stover was ground on a Wiley mill through a 1 mm screen.

Metabolism Trial.

Twenty four crossbred wethers averaging 35 kg body weight were used. The initial weights of the sheep were taken prior to commencement of the experiment. Sheep were blocked by initial weights and randomly allotted to four diets; 1) Corn stover alone, 2) Urea-supplemented corn stover, 3) Ammonia-treated stover and 4) Urea-treated stover (table 1). Sheep were offered 600 g dry matter from corn stover per day. All diets were supplemented with 40 g molasses at each feeding, diluted to 80 g with water before being mixed. Composition of the diets is given in table 1.

Feed grade urea (10 g) was added to 50 ml of water and fed to sheep on diet 2, at each meal to make it isonitrogenous with the treated stover diets.

The mineral supplements consisted of 16 g/d of dicalcium-phosphate, and 10 g/d of both iodized salt and limestone. All diets were supplemented with equal amounts of minerals. The minerals were supplemented to meet the NRC recommendations of 6.6 g Ca and 3.2 g P for growing sheep weighing 34 kg and to maintain a desirable Ca:P ratio (NRC, 1985). The stover was chopped through a 3.75cm screen. The control diet was mixed with 200 g water just before feeding to reduce dustiness, wastage and improve acceptability by the animals.

Table 1. COMPOSITION OF DIETS FED TO THE SHEEP

	Treatment			
Item	Untreated	Urea Supplement	Ammonia Treated	Urea Treated
Ingredient composition[a], g/d				
Corn stover	601.33	601.33	596.31	595.37
Dicalcium phosphate	15.15	15.15	15.15	15.15
Limestone	9.99	9.99	9.99	9.99
Molasses	57.60	57.60	57.60	57.60
Salt	9.97	9.97	9.97	9.97
Urea	-	19.76	-	-
Chemical composition				
Dry matter[c,d],%	87.85	88.12	75.50	76.07
Acid detergent fiber[a,f],%	46.22	43.68	48.47	44.89
Crude protein[a,b],%	4.57	12.67	11.88	11.91
Cellulose[a],%	36.79	35.49	38.68	36.44
Hemicellulose[a,d,e],%	29.09	29.53	19.20	26.71
Neutral detergent fiber[a,d,f],%	75.32	73.21	67.68	71.60
Lignin[a],%	7.69	7.08	7.94	7.04
Gross energy[a], k cal/g	3.86	3.68	3.83	3.83

[a]Dry basis

[b]Untreated differs from other treatments ($P < .01$)

[c]Untreated differs from other treatments ($P < .05$)

[d]Urea supplement differs from ammonia and urea treatment ($P < .05$)

[e]Ammonia treatment differs from urea treatment ($P < .01$)

[f]Ammonia treatment differs from urea treatment ($P < .05$)

All sheep were treated for internal parasites with ivermectin. They were kept in false bottom metabolism crates similar to those described by Briggs and Gallup (1949), permitting separate collection of urine and feces. Sheep were fed during 2 h feeding periods at 12 h intervals. Water was provided at all times except during the feeding periods.

The trial started with 2-d adaptation to stalls during which the sheep were fed corn stover supplemented with soybean meal. This was followed by 5-d transition period to the experimental diets. A preliminary period (10 d), after animals were placed on test diets was followed by a 7-d total collection of feces and urine.

Feed samples and refusals were collected from 2 d before the start until 2 d prior to the end of the collection period and frozen immediately. At the end of the trial these samples were composited by animals, subsampled, dried in a forced air oven and ground through 1-mm screen in a Wiley mill before analysis.

Feces were collected once daily and dried in a forced air oven at a maximum of 60 C for 24 h. Dried feces were composited by animals, in plastic lined metal cans with loose lids. Composited feces were weighed and subsampled at the end of collection period.

Urine were collected in polyethylene containers containing 15 ml of 1:1 (v/v) water and H_2SO_4 solution plus 300 ml of water. Total urine was collected once daily, diluted to a constant weight (4500 g) and a 2% aliquot (by volume) was taken each day. Urine samples were composited by sheep and stored pending analysis.

On the last day of collection, ruminal fluid samples were taken 2 h postfeeding, using a stomach tube. The samples were strained through four layers of cheesecloth and used for determination of pH (electrometrically), and volatile fatty acids (VFA) (Erwin et al., 1961) and NH_3-N (Beecher and Whitten, 1970). Blood samples were taken by jugular puncture 6 h post feeding and analyzed for urea-N (Coulombe and Favrean, 1963).

Dried feed, feed refusals and fecal samples were ground through 1-mm screen in a Wiley mill and analyzed for Kjeldahl N (AOAC., 1980), acid detergent fiber (ADF) (Goering and Van Soest, 1970), neutral detergent fiber (NDF) (Van soest and Wine, 1967), lignin, and cellulose (Van Soest and Wine, 1968). Hemicellulose was determined by difference, and energy was determined by Parr Adiabatic bomb calorimeter (table 2). Urine samples were analyzed for N (AOAC, 1980).

Data from metabolism trial were statistically analyzed by using the GLM procedure of Statistical Analyses System (SAS, 1982). Differences among treatments were tested by using the following contrasts: 1) Untreated stover vs supplemented, ammonia-treated and urea-treated stovers. 2) Supplemented vs ammonia-treated and urea-treated stovers. 3) Ammonia-treated stover vs urea-treated stover.

Chapter V

RESULTS AND DISCUSSION.

Changes in Appearance and Chemical Composition.

The main characteristics of the treated corn stover were the changes in color and texture. The NH_3-treated bales of stover turned dark brown, and had a slight ammonia smell. The urea-treated bales of stover were light brown in color, and had a pungent smell of ammonia. The treated stovers were softer, more pliable and less coarse, compared to the untreated stover. Similar observations have been reported by Martynov (1972) and Sundstol et al. (1978) when they treated wheat straw with anhydrous ammonia. Moreover, Schuerch and Davidson (1971) reported similar results with NH_3 treatment of wood chips.

Buettner (1978) showed that the browning of ammoniated wheat straw occurred at room temperature and could be more intense with increasing rate of ammonia, time of exposure and temperature. The color changes could be associated with the various chemical reactions that occurred during the treatment such as oxidation and condensation of the aldehydic fractions in sugars with the nitrogenous bases via the Maillard reactions (Schuerch and Davidson, 1971).

The softening of the treated stover could be due to the reactions of aqueous NH_3 and urea described by Klopfenstein (1978). He described these reactions as solubilization of hemicellulose, disintegration of bonds between cell wall constituents and crosslinks between the cell wall. Other reactions associated with NH_3 and urea treatment include ammonolysis and breaking of bonds between lignin and structural carbohydrates with the formation of amides (Frape, 1984). These reactions may result in fiber softening and swelling of corn stover observed in our experiment.

The DM values were similar between top and middle layers of the treated corn stover (67.1 to 67.7%), whereas, the bales on the bottom layers were the highest in moisture (59.7% DM) compared to others with 67% DM. It could be theorized that the high moisture content of the bales on the bottom layer was due to excess liquid that escaped from both top and middle layers following the application of the chemical solution. However, these differences were negligible after the aeration period. The differences in CP among the layers were not significant ($P>.05$; table 2). Layers by treatment interaction was not statistically different ($P>.05$).

TABLE 2. CRUDE PROTEIN IN THE STACKS[a,b]

	Treatment of stover			
Location[c]	Untreated	Ammonia-treated	Urea-treated	SE[d]
Top	3.70	12.88	11.62	.37
Middle	4.18	11.92	13.12	.37
Bottom	4.32	12.68	12.78	.37
Average[e]	4.07	12.50	12.54	.37

[a]Dry basis.

[b]Each value represents the average of four measurements.

[c]Crude protein did not differ between locations ($P<.05$).

[d]Standard error of means.

[e]Means for ammonia and urea-treated stover were higher ($P<.01$).

The ADF was higher (P<.05) in ammonia-treated stover and was similar in control and urea-treated stover (table 3). Neutral detergent fiber and hemicellulose were higher (P<.05) in control than the treated stover. Similar results were reported by Fernandez et al. (1986) when they treated corn stover with 3% anhydrous NH_3. Ammonia-treated stover had lower (P<.05) values than the urea-treated stover. Lignin content was slightly higher in ammonia- than urea-treated stovers.

Crude protein was increased (P<.01) over 200% by both ammonia and urea treatments. The initial CP of the stover prior to chemical treatment averaged 4.07%. After treatment the CP increased (P<.01) to 12.54% for urea treatment and 12.50% for ammonia treatment (table 3). However, there was no significant difference between NH_3 and urea treatment. The increase in CP by 8.5 percentage units agrees closely with results reported by Klopfenstein and Owen (1981). Generally, the total CP from the treated stover was below the projected amount of about 13%.

The smell of NH_3 noticed when the stacks were uncovered was an indication of NH_3 loss. Various researchers have reported about 50 to 75% loss in NH_3 during and after chemical treatment of low quality roughages (Oji et al., 1977; Sundstol et al., 1978 and Suleiman et al., 1979).

TABLE 3. EFFECT OF CHEMICAL TREATMENT ON THE CRUDE PROTEIN, FIBER COMPONENTS AND IN VITRO DRY MATTER DIGESTIBILITY OF CORN STOVER[a]

Item	Treatment			
	Untreated	Ammonia-treated	Urea-treated	SE[b]
	-------------- % --------------			
Dry matter[c]	88.68	65.55	64.00	1.53
Acid detergent fiber[d,e]	51.54	56.50	51.94	.96
Crude protein[c,d]	4.07	12.50	12.54	.37
Neutral detergent fiber[c,d,e]	86.92	78.20	82.75	1.41
Cellulose[d,e]	42.30	44.70	42.12	.65
Hemicellulose[d,e]	35.3	21.70	30.81	1.20
Lignin[d,e]	8.64	9.18	8.14	.96
In vitro dry matter digestibility[c,e]	42.62	55.00	50.00	.99

[a]Each value represents an average of 12 measurements.

[b]Standard error of means.

[c]Untreated stover differs ($P<.01$) from ammoniated and urea-treated stovers.

[d]Dry basis.

[e]Ammoniated stover differs ($P<.05$) from urea-treated stovers.

No mold or fermentation characteristic was observed before and after uncovering the stacks, indicating good preservative effects of ammonia and urea. This feature makes the use of NH_3 and urea attractive as moist hay and wet forage preservatives.

The results of our experiment support the fiber solubilization reaction described by Klopfenstein (1978). The decrease in hemicellulose content obtained in NH_3- and urea-treated stover could be explained as the chemical reaction which broke down the bonds between digestible fibers and lignin as described by Tarkow and Feist (1969). According to Klopfenstein et al. (1972) lignin content is generally not reduced by chemical treatment. However, treatment could disintegrate the bonds between lignin and other fibers, thus, causing an improved intake and digestibility (Coombe, 1981).

The results of the in vitro dry matter digestibility study are shown in table 3. In vitro dry matter digestibility was higher (P<.01) for treated than untreated stover. The value was higher (P<.01) for NH_3- than urea-treated stover. Ammonia treatment increased the in vitro dry matter digestibility by 12 percentage units while, the values for urea-treated stover increased by 7 percentage units over the untreated stover.

The improved in vitro dry matter digestibility from aqueous NH_3 and urea apparently resulted from the action of NH_3 on breaking the lignocellulosic bonds by solubilizing some hemicellulose (Waller et al., 1976). Furthermore, this reaction could break the ether linkages between lignin and cellulose or hemicellulose (Lau and Van Soest, 1980). Tarkow and Feist (1969) suggested that the swelling of cellulose and hemicellulose due to chemical treatment could lead to greater flexibility of the lignocellulosic materials, and therefore, increase digestibility. According to Saenger et al. (1982) this increased digestibility could be due to availability of increased substrate to the appropriate enzymes.

The responses of NH_3 and urea treatments in this study agree with those reported by Coxworth et al. (1977), Oji et al. (1977) and Kiangi and Kategile (1981). The reduced effectiveness of urea treatment may be related to incomplete hydrolysis of urea to ammonia, especially, since the experiment was conducted during cool weather as was the case in this study. It has been established that high stable ambient temperature is required for efficient hydrolysis of urea and ammonia reactions (Sundstol et al., 1978). Researchers have suggested that the most appropriate level for ammonia treatment is 3 %, above which there is no economic advantage

(Sundstol et al., 1978; Borhami et al., 1982 and Coxworth and Owen, 1984).

Apparent In Vivo Digestibility

Apparent digestibilities of dry matter and fiber components were improved ($P<.05$) by both NH_3 and urea treatment of stover (table 4). The DM digestibility was increased by over 10 percentage units by ammonia treatment and by over 5 percentage units by urea treatment. Dry matter digestibility in sheep fed ammonia-treated stover was higher ($P<.01$) than for urea treatment. Dry matter digestibility tended to be higher in the sheep fed urea-supplemented stover, compared to those fed unsupplemented stover. However, dry matter digestibility was higher ($P<.01$) in sheep fed the treated stover than in those fed the urea-supplemented stover. Digestibility of ADF, NDF and cellulose followed a similar trend as dry matter digestibility.

Lignin digestibility was higher ($P<.01$) in the sheep receiving NH_3- and urea-treated stover than those fed urea-supplemented stover. However, the lignin digestibility was higher ($P<.01$) in sheep fed NH_3-treated stover than the sheep fed urea-treated stover.

TABLE 4. EFFECT OF CHEMICAL TREATMENT ON APPARENT DIGESTIBILITY OF CORN STOVER[a]

Component	Treatment				
	Untreated	Untreated plus urea supplement	Ammonia-treated	Urea-treated	SE[b]
	%				
Dry Matter[c,d]	55.08	56.77	61.47	57.19	1.20
Acid detergent fiber[c,e,f]	56.76	57.52	66.37	58.84	1.47
Lignin[f,g]	32.90	22.07	38.83	25.00	1.96
Cellulose[c,e,f]	60.58	64.19	73.22	65.11	1.65
Neutral-detergent fiber[c,d,e]	59.08	61.75	69.34	62.94	1.57
Hemicellulose[c,f]	62.80	67.74	76.14	66.05	1.55
Gross energy[c,d,e]	48.11	52.72	59.87	54.64	1.47
Crude protein[c,d,e]	26.38	73.68	61.13	65.20	1.50

[a]Each value represents mean of six measurements.

[b]Standard error of means.

[c]Untreated stover differs from other treatment ($P<.01$).

[c]Untreated stover differs from other treatment ($P<.05$).

[d]Ammoniated stover differs from urea-treated stovers ($P<.05$).

[e]Urea supplemented differs from ammoniated and urea-treated stovers ($P<.05$).

[f]Ammoniated stover differs from urea-treated stover ($P<.01$).

[g]Urea supplemented differs from ammoniated and urea-treated stovers ($P<.01$).

The digestibility of gross energy was higher ($P<.05$; table 4) in the sheep fed supplemented or treated stover, compared to those fed the untreated stover. Aqueous NH_3 and urea treatment improved the apparent digestibility of gross energy in corn stover. The energy digestibility was higher ($P<.05$) in sheep consuming NH_3-treated stover, than those fed urea-treated stover. The results demonstrate that aqueous NH_3 and urea treatment improved the nutritional value of corn stover, in agreement with results reported by Orskov et al. (1983) when anhydrous and aqueous ammonia-treated barley straw were fed. The superior nutritional value of aqueous NH_3-treated stover agrees with results reported by Saenger et al. (1980) and Oji et al. (1977). Arnason and Mo (1977) reported an increased fiber digestion in vivo when barley straw treated with 3.5% anhydrous NH_3 for 3 wk was fed to sheep. The improved fiber digestibility may be due to the various chemical reactions that occurred during the treatment of stover. Tarkow and Feist (1969) illustrated that increased fiber digestibility could be stimulated by the ammonolysis reaction which was suspected to occur whenever alkali and ammonia or urea were used to treat poor quality roughages:

$$RCOOR' + NH_3 \longrightarrow RCONH_2 + HOR'$$

where, R' is carbohydrate and R is a more complex carbohydrate. The mode of this reaction involved the hydrogen atom of a carboxylic acid or a phenylpropane unit of lignin. This reaction could result in increased fiber saturation point causing swelling and flexibility and ammonolysis of ester cross-links between lignin and digestible carbohydrates such as cellulose and hemicellulose. The outcome of this chemical reaction may improve the fiber utilization by ruminants, and result in an increased rate and extent of cellulose and hemicellulose digestion (Rexen and Thomsen, 1976).

According to Orskov et al. (1983) the use of urea as a method of supplying NH_3 relies on its immediate and complete hydrolysis to NH_3, but some time is required for the straw and urea to interact together. Urea hydrolysis may not be imminent when supplementing urea at feeding period, therefore, insufficient chemical reaction between the stover and urea may occur. This may be a valid explanation for the low digestibility from urea-supplemented, compared to the chemically-treated diets.

The lower fiber digestibilities for urea-treated stover than ammonia-treated stover, may be due to the fact that urease was not added to the urea-treated stover and that the experiment was conducted during cold weather. Researchers have established that high temperature is required to speed up the activity of urease, an enzyme responsible for urea hydrolysis to NH_3 (Itoh et al., 1979; Orskov et al., 1981; Saenger et al., 1982; Orskov et al., 1983 and Fernandez et al., 1986). Conversely, low temperature may inhibit this reaction.

Another possible reason for lower digestibility of urea-treated stover may be the extent of adherence of chemicals to the corn stover. It seems that the NH_3 resulting from urea treatment attaches loosely to the corn stover, compared to that of NH_3-treated stover (Tarkow and Feist, 1969). Other reactions that occurred during NH_3 and urea treatment may have resulted in some N being tied up with indigestible fiber particles. A similar suggestion in support of the effect of Maillard reaction that occurred during the roughage treatment has been reported by Oji et al. (1977). Goering et al. (1972) have described the N resulting from this reaction as acid detergent insoluble N (ADIN), which is a common characteristic of heat-damaged protein. Goering and Waldo (1974) described the browning reaction as a non-enzymatic reaction, which could lead to the formation of indigestible C-N bonds between proteins and sugars, hemicellulose or other compounds. Reduced performance of milk cows recieving this type of brownish diet has been reported (Goering et al., 1972). Solaimon et al. (1979) treated wheat straw with

3.3% NH_3. They reported an increased crude protein content from 4.6 to 11%. However, of the N remaining after 24 h of aeration, 43.4% was identified with NH_3 and 12.6% was fiber bound.

Sheep fed the treated stover and urea-supplemented stover showed higher ($P<.01$) apparent digestibility of crude protein than the sheep fed the untreated stover. Sheep fed the untreated stover showed the lowest crude protein digestibility. The crude protein digestibility was higher ($P<.05$) in the sheep receiving urea-supplemented stover than for those fed NH_3- and urea-treated stover. Even though NH_3 treatment was superior to urea treatment in all respects, both treatments were superior to the untreated stover.

The increasing digestibility in vivo followed a similar trend as the in vitro dry matter digestibility (IVDMD) (table 5). The results of in vitro dry matter digestibility could be positively related to the values obtained for apparent dry matter digestibility, since both indicate that aqueous NH_3 and urea improve the utilization of corn stover. The results of our studies gave slightly higher in vivo DM digestibility than IVDMD. This results contradict those reported by Paterson et al. (1981) where the IVDMD slightly overestimated the apparent in vivo DM digestibility of anhydrous NH_3-treated corn stalks.

TABLE 5. IN VITRO VS. IN VIVO DRY MATTER DIGESTIBILITY OF CORN STOVER

Dry matter digestibility	Treatment				
	Untreated	Urea supplement	Ammonia-treated	Urea-treated	SE[a]
In vitro[b,c,d]	42.62	-	55.00	50.00	.99
In vivo[c,e,f]	55.08	56.77	61.47	57.19	1.20

[a]Standard error of means.

[b]Each value represents an average of 12 measurements.

[c]Ammonia-treated stover differs from urea-treated stover ($P<.05$).

[d]Untreated-stover differs from ammonia-treated stover ($P<.01$).

[e]Each value represents an average of six measurements.

[f]Untreated-stover differs from ammonia-treated and urea-treated stover ($P<.01$).

Nitrogen Retention

Fecal N excretion was similar for the sheep fed untreated corn stover alone or supplemented with urea, and was lower than for sheep fed treated stover. Excretion in feces was higher ($P<.05$) for sheep fed NH_3- than those fed urea-treated stover (table 6). The urinary excretion was higher ($P<.01$) in sheep fed urea-supplemented stover compared to those fed treated stover. It seems that greater portion of the nitrogen supplemented was excreted in feces and urine less utilized. The urinary N excretion was higher ($P<.01$) in sheep fed urea-treated stover, compared to those receiving ammonia-treated stover.

Negative N retention was obtained for sheep fed untreated stover without urea supplement. Nitrogen retention was improved by supplementing N and treating with aqueous NH_3 and urea. Nitrogen retention was low but positive for the sheep fed urea-supplemented stover. Nitrogen retention was higher ($P<.01$) in sheep consuming both NH_3- and urea-treated stover, compared to those fed untreated stover supplemented with urea. Sheep fed NH_3-treated corn stover showed a higher ($P<.01$) N retention than those fed urea-treated stover.

TABLE 6. NITROGEN UTILIZATION BY SHEEP FED THE UNTREATED, UREA SUPPLEMENTED AND TREATED STOVER[a]

	Treatment				
Item	Untreated	Untreated plus urea supplement	Ammonia-treated	Urea-treated	SE[b]
Intake[c], g/d	4.78	12.61	12.81	12.62	.28
Excretion					
Fecal[d,f], g/d	3.77	3.76	5.41	4.72	.18
Urinary[c,d,f], g/d	2.22	8.61	4.93	6.40	.21
Total[c,d], g/d	5.99	12.37	10.33	11.12	.28
Retention[c,d,f]					
g/d	-1.21	.24	2.48	1.50	.13
% of intake[c,d,g]	-25.22	1.93	19.24	11.86	1.84
% of absorbed[c]	-128.18	2.69	33.45	18.88	12.47

[a]Each value represents an average of six measurements.

[b]Standard error of means.

[c]Untreated stover differs from other treatments ($P<.01$).

[d]Urea supplemented differs from ammonia and urea-treated stovers ($P<.01$).

[e]Urea supplemented differs from ammonia and urea-treated stovers ($P<.05$).

[f]Ammonia-treated differs from urea-treated stovers ($P<.01$).

[g]Ammonia-treated differs from urea-treated stovers ($P<.05$).

The negative N balance obtained in the sheep fed untreated stover could be related to low N intake, which was lower than the NRC recommendation (NRC, 1985). Similar results were reported by Ortigues (1983) when she supplemented poor quality hay with different N and carbohydrate sources. She obtained a negative N retention for lambs fed the diets.

Ruminal Fluid Parameters.

Ruminal pH was high for lambs fed all diets. However, the pH for animals fed the untreated stover was lower ($P<.05$) than for those fed other diets (table 7). Sheep on the control diet had an average pH of 7.0, whereas, sheep fed other diets had pH in the range of 7.40 to 7.48 (table 7). The sheep fed control diet had the lowest level of ruminal NH_3-N. The average ruminal fluid NH_3-N was higher ($P<.01$) in sheep fed urea-supplemented stover than sheep fed ammonia-and urea-treated stover. The large concentration of ruminal NH_3 N for the sheep fed urea-supplemented diet may be due to the rapid hydrolysis of urea to NH_3.

Although the ruminal NH_3 concentration in sheep fed untreated diet was the lowest value, it was higher than the 5mg/100ml optimal value of ruminal NH_3-N suggested by Satter and Slyter (1974) for maximum rate of microbial protein synthesis.

TABLE 7. RUMINAL FLUID pH, AMMONIA NITROGEN AND BLOOD UREA NITROGEN OF SHEEP FED TREATED AND UNTREATED CORN STOVER[a].

	Treatment				
Item	Untreated	Untreated plus urea supplement	Ammonia-treated	Urea-treated	SE[b]
Ruminal fluid					
pH[c]	7.10	7.48	7.40	7.42	.06
Ammonia-N[c,d],mg/dl	15.47	20.66	18.40	18.81	.66
Blood urea-N[c,e,f], mg/dl	9.82	31.64	16.13	21.11	1.17

[a]Each value represents the mean of six measurements.

[b]Standard error of means.

[c]Untreated stover differs from other treatment ($P<.01$).

[d]Urea supplemented differs from ammonia and urea-treated stovers ($P<.05$).

[e]Urea supplemented differs from ammonia and urea-treated stovers ($P<.01$).

[f]Ammonia-treated differs from urea-treated stovers ($P<.01$).

The efficiency of nitrogen use could be related to availability of energy. The energy supply from molasses supplemented in our studies might be inadequate for maximum N metabolism, especially in the case of the urea-supplemented diet. According to Roffler and Satter (1974), the ruminal NH_3 concentration could be positively related to dietary crude protein and negatively related to total digestible nutrient concentration. Hence, mean ruminal NH_3-N may be used to predict the utilization of nonprotein N supplementation.

Ruminal NH_3-N is a major precursor of microbial protein (Nolan and Leng, 1972). However, the amount of ruminal NH_3 required for optimal microbial growth is not clear. Various researchers have suggested values from 5.0 to 10mg/100ml (Satter and Slyter, 1974; Leng and Nolan, 1984) and 23.5mg/100ml (Mehrez et al., 1977). Ruminal NH_3 obtained in our experiment ranged from 15 to 20mg/100ml. The results agree with values reported by Mehrez et al. (1977).

Wallace et al. (1979) has demonstrated that the urease activity of the rumen microbes could be affected by rumen NH_3 concentration. High ruminal NH_3 will reduce the urease activity and vice versa.

Blood Urea Nitrogen.

The lowest ($P<.01$) blood urea concentration was obtained in sheep fed the untreated stover (table 7). Blood urea was higher ($P<.01$) in sheep fed urea-supplemented stover, compared to those fed treated stover. Blood urea N, is a pool with an input from urea synthesis by liver and kidney tissue and various exits including cycling to the digestive tract and urinary output. Concentration of urea in plasma has been used as an index of N status of cattle and sheep (Preston et al., 1965). In the present study it is not a good index, since the level for the urea-supplemented sheep was higher than for those fed treated stover, but N retention was lower for the lambs fed urea-supplemented stover.

Ruminal Fluid Volatile Fatty Acids.

The total VFA concentration was generally low, but similar among sheep fed all diets. (table 8). The concentration of total VFA varied between 53.7 and 55.8 µ mole/ml. Molar proportions of VFA were similar among sheep fed all diets.

The branched VFA were not different ($P>.05$; table 8) with treatment. However, valeric acid was detected in the ruminal fluid of some sheep while isovaleric acid was not detected by the gas chromatography in some sheep.

TABLE 8. RUMINAL FLUID VOLATILE FATTY ACIDS OF SHEEP FED TREATED AND UNTREATED CORN STOVER[a]

	Treatment				
Item	Untreated	Untreated plus urea supplement	Ammonia-treated	Urea-treated	SE[b]
Total, u mole/ml	55.84	53.74	55.34	55.85	3.71
Moles/100 moles					
Acetic	70.52	71.33	69.17	70.64	2.87
Propionic	21.31	21.78	23.24	22.41	.91
Isobutyric	0.28	0.25	0.24	0.36	.02
Butyric	7.47	5.98	6.85	5.92	.36
Isovaleric	0.15	0.13	0.11	0.28	.03
Valeric	0.29	0.34	0.39	0.40	.03

[a]Each value represents the average of six measurements.

[b]Standard error of means.

The low VFA concentration could be partly responsible for the high ruminal pH of all the sheep used for this experiment. Also, the low VFA is a direct indication of low ruminal fermentation.

According to Leng (1973) high proportions of the branched chain VFA indicate that proteolysis and/or deamination is actively high and vice versa. Therefore, the low proportions of both isovalerate and isobutyrate obtained in our studies were evidences of low deamination rate. No significant difference was found among the individual VFA. However the acetate was high in all sheep used for this experiment.

Chapter VI

SUMMARY

Experiments were conducted to study the effect of treatment of rectangular bales of corn stover with aqueous ammonia and urea solutions. The stacks were left covered for 129 d, after which they were uncovered, aerated and sampled for laboratory analyses.

Treated stover was higher in crude protein content. The distribution of N inside the stack was uniform and similar among treated stovers. Other effects of the chemical treatment included color changes of the stover from yellow to brown. Treated materials were softened, and more pliable than the untreated stover. It was evident that NH_3 was lost during the chemical treatment, confirmed by the smell of ammonia during and after chemical treatment.

In vitro digestibility was improved by both aqueous ammonia and urea treatments. However, the effect was greater for ammonia-treated stover than for urea-treated stover.

A metabolism trial was conducted with 24 cross-bred lambs with an average body weight of 34 kg, randomly allotted to four diets; 1) corn stover alone, or 2) supplemented with urea, 3) ammonia-treated corn stover and 4) urea-treated corn stover. All diets were supplemented with molasses and minerals.

Aqueous NH_3 and urea treatment improved dry matter and fiber digestibility in sheep. The improvement was greater in sheep fed NH_3-treated stover, than in those fed urea-treated stover. The in vivo dry matter digestibility followed a similar trend with the in vitro dry matter digestibility.

Certainly, there is need for more research to study the problems confronting the utilization of corn stover from the point of view of effective harvesting, preservation and upgrading with ammonia, urea or other additives.

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EFFECT OF TREATING CORN STOVER WITH AQUEOUS AMMONIA AND UREA ON NUTRITIONAL VALUE.

by

Johnson Obamehinti

Committee Chairman: Joseph P. Fontenot

Animal Science

(ABSTRACT)

Experiments were conducted to study the effect of treatment of rectangular bales of corn stover with aqueous ammonia and urea solutions. The stacks were covered with polyethylene sheets for 129 d, after which they were uncovered, aerated and sampled for laboratory analyses.

Treated stover was higher ($P<.01$) in crude protein content. The N was similar among treated stovers. There was no significant difference among the layers of stacked bales. Other effects of the chemical treatment included color changes of the stover from yellow to brown. Treated materials were more pliable than the untreated stover. It was evident that NH_3 was lost during the chemical treatment, confirmed by the smell of ammonia during and after chemical treatment.

In vitro digestibility was improved by both aqueous NH_3 and urea treatment. However, the effect was greater ($P<.01$) for NH_3-treated stover than for urea-treated stover.

A metabolism trial was conducted with 24 cross-bred lambs with an average body weight of 34 kg, randomly alloted to four diets; 1) corn stover alone, or 2) supplemented with urea, 3) ammonia-treated corn stover and 4) urea-treated corn stover. All diets were supplemented with molasses and minerals. Aqueous NH_3 and urea treatment improved ($P<.01$) dry matter and fiber digestibility in sheep. The improvement was greater (P<01) in sheep fed ammoniated stover, than those fed urea-treated stover. The in vivo dry matter digestibility followed a similar trend with the in vitro dry matter digestibility.