THE EFFECT OF EXTRUSION COOKING ON NUTRITIONAL COMPONENTS OF SORGHUM STOVER TREATED WITH SODIUM HYDROXIDE AND TRACE MINERALS

by

GARY PAUL CROSSLIN

A.A., Independence Community Junior College, 1971
B.S., Kansas State University, 1974

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Approved by:

Charles Deyoe
Major Professor

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
Factors Effecting the Feeding Value of High-Fiber, Low-Quality Roughages	3
The Use of Sodium Hydroxide for Digestibility Improvement of Low-Quality Roughages	5
The Effect of Sodium Hydroxide Addition on Chemically Analyzed Fiber Components	6
In Vitro and *In Vivo* Studies	7
Trace Minerals	11
Effects of Copper and Manganese Additions on Metabolism of Ruminant Animals	11
METHODS AND MATERIALS	17
RESULTS AND DISCUSSION	23
Reducing Sugar	23
In Vitro Dry Matter Disappearance	26
In Vitro Gas Production	30
Nylon Bag Dry Matter Digestibilities	35
Van Soest Chemical Analyses	35
SUMMARY	65
LITERATURE CITED	67
APPENDIX	74
ACKNOWLEDGMENTS	77

INTRODUCTION

One of the most demanding challenges facing the scientist today is to supply the food and feed processor with knowledge to meet the nutritional needs of the ever-expanding population.

Today's world can be divided into two groups when classified on the basis of their food supply. The first group is the technically developed, food abundant countries which include the United States, Canada, Europe, Argentina and Uruguay in South America. Approximately one-third of the world population falls within this group receiving 3,000 calories per person daily (73).

The second group is the technically underdeveloped countries which comprise two-thirds of the population and exist on 2,000 calories per person per day. Asia, Africa and tropical America make up this group (73).

The differences between the diets of these two groups are not only quantitative, but also qualitative.

As of today, in many parts of the world, protein is deficient. Fortunately for man, the ruminant animal is available to convert low-quality roughages to a high-quality protein.

Cellulose is one of the most widely distributed, naturally occurring organic compounds in the world, comprising nearly one-third of the world's plant material.

Pollution problems arise in disposal of low-quality cellulosic by-products such as paper pulp and crop residues. A

devised plan for improved utilization of these wastes in animal feeding would not only aid in the food scarcity problem, but also help solve the pollution problem.

Calorically, these materials are potentially rich. Nutritionally, however, the energy present does not avail itself to the rumen microorganisms to any great extent. This is due to a complexity of factors which will be further discussed.

The object of this research was to improve the utilization of a low-quality, high-fiber crop residue. Processing techniques, such as extrusion cooking and addition of chemicals and trace minerals were employed to upgrade the nutritional value of sorghum stover (NRC 1-07-961). Evaluations were made via *in vitro*, *in vivo* and chemical techniques.

REVIEW OF LITERATURE

Factors Effecting the Feeding Value of High-Fiber, Low-Quality Roughages

The feeding value of forages is a function of how much can be consumed and to what extent the energy can be digested. These factors are interrelated and depend mainly on the chemical composition of the forage. The digestibility is influenced by the relative amount of solubles, cellulose, hemicellulose, silica and lignin. Feed intake is related to these composition factors as well as the rate of digestion and rate of removal of the fiber mass through the rumen.

The inability of ruminant animals to avail themselves to most of the energy in cellulosic materials is explained by one or more of three factors: 1. lignin acts as an inert barrier between the carbohydrate and the energy system (4,34,35,56,57, 76), 2. the cellulose is too highly crystalline to quickly avail itself to enzyme action (5,17,80) or 3. that silica inhibits the cellulose digestibility (76).

Lignin. Lignified fiber is of two distinguishable types, potentially digestible or indigestible. The indigestible portion disappears from the rumen only by removal. The removal is related to that amount present and is a linear-semilog function of time (78). The digestible portion disappears by both removal and digestion. These two rates total to one rate, which is proportional to the amount present and again may be expressed as a linear semilog function of time (78).

Lignin contains a considerable portion of alcoholic, phenolic and other oxygen containing groups (56). The groups as mentioned are frequently involved in hydrogen bonds. Hydrogen bonds are generally very weak, unless arranged in a highly organized manner, such as in cellulose. This organization of hydrogen bonds is nonexistent in lignin (56).

The presence of the ligno-carbohydrate complexes is firmly established. These complexes exhibit an implication of chemical linkage, their exact nature merits further study.

Quinone methides derived from coniferyl alcohol are believed to be the intermediates in lignin formation and readily combine with sugars and form ether linkages (56). These compounds are believed to be the models for the lignin-carbohydrate bond in wood.

Straw treated with β-glycosidase resulted in an increase in phenolic-hydroxyl groups (56). These results differ from that of the ether bond theory.

The chemical bond theory cannot explain all of the resistance to cellulolytic breakdown. The incrustation theory is an important factor in enzyme attack. The three-dimensional lignin complex inhibits the large enzyme molecules from entering the structure (4,34,35,56,57,76).

Silica. Silica may act as a soluble cellulolytic inhibitor (30,64,76). The addition of soluble silica (sodium metasilicic acid) or aqueous extracts into *in vitro* systems reduced the degradation of forage fiber (78).

The Use of Sodium Hydroxide for Digestibility Improvement of Low-Quality Roughages

Early workers attempted to increase the digestibility of roughages by sodium hydroxide addition in hopes of freeing digestible nutrients from the lignin and silica (3). This process included washing the residues with water after alkali treatment to eliminate the effects that the alkali might have on *in vitro* or *in vivo* systems. This technique rinsed away the soluble nutrients and, in many cases, lowered the nutritional value.

The action of sodium hydroxide on high fiber material may be explained in a three-fold manner (3). First, separation and dissolving of the silicic acid, which may be involved in the incrustment theory. Secondly, splitting off the methoxy and acetyl groups from lignin. This results in acetic acid formation, which will neutralize some of the sodium hydroxide present (54). The delignification removes the complex "shell" while swelling opens up the complex and allows entrance of enzymes for attack (80). Electromicrographs of lignin dispersed in the cell walls treated with sodium hydroxide support this theory (54). Thirdly, forcing or springing the bonds which link lignin and cellulose together (3). The swelling of fiber in water in presence of sodium hydroxide is a result of cleavage of ester bonds on xylene chains acting as cross-links that maintain swelling (54). At room temperatures sodium hydroxide dissolves part of the hemicellulose in wood and converts a portion of lignin to a soluble alkali-lignin complex (54).

The Effect of Sodium Hydroxide Addition on Chemically Analyzed Fiber Components

Acid Detergent Fiber. Varying results have been obtained depending on concentration of sodium hydroxide and substrates used. Graded levels of 2, 4, 6 and 8% NaOH significantly decreased acid detergent fiber with each increasing level of alkali on rye-grass straw (2). Similar data were obtained by other workers on oat straw, poplar bark and reconstituted whole barley (25,53,62); while others could show an increase or no response on corn stover and wheat straw (36,54,80). The possible reason for disagreement was the procedures used in treating materials. The researchers that indicated no difference, rinsed the treated material with water to remove excess NaOH and, while doing so, probably washed away soluble nutrients and sclubilized silica. The same reasoning applies for neutral detergent fiber, where increases were reported by some workers (62); while others reported a decrease (24,25,53).

Cellulose, Hemicellulose. Steam and NaOH treated whole plant barley yielded significantly higher cellulose digestibility than the untreated control (53). However, with NaOH treated poplar bark, no net change in cellulose disappearance was observed (25).

A 42% decrease in hemicellulose content was observed in NaOH treated wheat straw (80). A significant disappearance was also found in NaOH treated poplar bark (25).

Lignin. Research has shown that a 10% NaOH treatment on ground corn cobs reduced lignin content by 26% (14). Similar

reports of decreased lignin content have been reported on treated straw, hardwood sawdust, poplar bark and corn cobs (2,25,33,35, 58). Contradictory results were reported by other researchers who found no net change in lignin content (29,36,54,62,80).

Gross Energy. Gross energy, as measured by a bomb calorimeter, was significantly increased in reconstituted whole plant barley treated with NaOH (52); while no change was observed in NaOH treated hardwood sawdust (35).

In Vitro and In Vivo Studies

Chemical treatment responses for improved in vitro dry matter digestibility (IVDMD) is species specific (54). Washing the substrate prior to IVDMD affects the nutrients remaining and the results obtained (35). Washing partially removes the cell solubles, silica and lignin dissolved by NaOH. Therefore, fiber and ash content could increase in the remaining material (35). The organic acids produced by the in vitro fermentation would lower the pH of the system (54). As the pH decreases, the dissociated silicate would revert to undissociated silicic acids, which could repolymerize to silicic acids and form a gel (30). These gels may form a barrier within the cell walls and inhibit in vitro fermentation (30). These conclusions agree with other workers who suggest that silica may be a soluble cellulolytic inhibitor (64,76).

Method of processing, whether spraying, soaking and/or degree of heat treatment has a significant effect on in vitro and in vivo systems. A simple spraying method yielded no increase in IVDMD, while soaking for 24 hours yielded a highly

significant difference (2). Heat treatment (pressure cooking) increased the IVDMD of NaOH treated sawdust over two-fold (58). The reducing sugar content was reported significantly increased via the combined effects of pressure cooking and chemical treatment (58). Temperatures of 100 C for 90 minutes resulted in IVDMD increases of 10 percentage units over that treated at room temperature and atmospheric pressure (54). The digestibility of straw via the enzymatic method was more than doubled when a 3% NaOH treatment was used in conjunction with steam treatment at 28 kg/cm^2 for three minutes (79).

Particle size had little or no effect on IVDMD (2,24). However, small particle size will yield a slightly higher fiber digestibility in vitro as a result of the increased surface area exposed to microbial attack in a closed system. In vivo fiber digestion is inversely correlated to particle size. Smaller particles spend less time in the rumen due to a faster rate of removal, thus, they are exposed for less time to microbial attack.

A search of the literature reveals that a range of 4-12% sodium hydroxide treatment (depending on substrate and conditions) increased IVDMD from 20 to over 100% (25,33,35,36,38,53, 54,58,62,74,80).

Neutralizing unreacted NaOH with hydrochloric or acetic acid does not increase microbial degradation (30). Unreacted NaOH present does not appear to alter in vitro systems.

The in vivo dry matter digestibility tended to be lower than the in vitro tests; however, a marked improvement in utilization still existed.

With sheep there was a significant improvement in feed consumption, weight gain, feed efficiency and carcass quality when steam alkali-treated straw (compared to control) was incorporated in the diet at approximately 65%. These animals performed similar to those fed a 60% concentrate ration (36).

Similar performance occurred when lambs were fed treated wheat straw and corn cobs (37). A 17% improvement in organic matter digestibility was noted when feeding wheat straw treated with 5% NaOH (37). Treating corn cobs more than doubled the average daily gains of lambs when fed at 80% of the ration (37).

Chemical treatments appear to improve in vivo dry matter digestibility without a significant reduction in lignin content or lignin to fiber ratio. Some treatments may reduce lignin content, but yield a significantly lower dry matter digestibility (80). These findings leave the side-effects unclear.

Chemical additions tend to shift rumen volatile fatty acid concentrations. Propionic concentration increased linearly with increased levels of NaOH, while isovaleric significantly declined (52). The acetic:propionic ratio tended to decrease linearly with increased additions of NaOH.

Rumen fluid lactic acid increased and resulted in lower rumen pH with increased NaOH additions (52). This indicates a more fermentable substrate was available to the microorganisms.

Several hours after feeding a linear decrease in rumen fluid ammonia and plasma urea was observed (52). These observations may be hypothesized as increased microbial protein

synthesis; thus leaving less free ammonia present in the rumen at any given time.

NaOH treated feedstuffs do not appear to exhibit detrimental effects on the physiology of the rumen. Young calves fed a ration containing 10% NaOH for six months demonstrated no adverse effects (43). Frequent urination and increased water intake was the only behavior change observed. Sheep fed NaOH treated reconstituted whole plant barley for a long period of time did indicate tendencies of alkaline urine, osmotic diuresis and hemoglobinuria (53).

Sodium has been reported as being slightly stimulatory to rumen bacteria (9,10). It was also found to inhance volatile fatty acid production and to be a required element for rumen bacteria (11).

Sodium is very important in maintance of buffer systems and regulations of osmotic pressures and body fluid volume (15). Excess sodium intakes are excreted via the urine, resulting in a greater water intake to maintain water balance. Extreme excesses of sodium result in hypertonicity of extracellular fluid, resulting into intracellular dehydration and eventually, death simply because the kidney is overridden (15).

Very high levels of sodium chloride (up to 12.8% of the ration) have been fed to growing lambs with no adverse effects on digestibility or growth (49). Upon postmortem examination, the high salt fed lambs had significantly larger kidneys. Steers also withstood a 9.3% NaCl diet without adverse effects. High

salt additions had no effect on either *in vitro* cellulose digestion (32) or on *in vivo* digestion of the steers (12).

Trace Minerals

Maximum cellulose utilization is very important to ruminant animals that depend on roughages as their primary energy source. In order to utilize the vast amounts of low-quality, fibrous feedstuffs, the maximum efficiency of utilization must be obtained. To achieve maximum cellulose digestion, the rumen microorganisms must obtain a balanced supply of all nutrients. The energy and nitrogen requirements have been studied with little attention given to the trace elements.

In the paper pulping industry, the presence of trace minerals has been shown to significantly influence the delignification rate and paper pulp yield (39). In the presence of oxygen and alkali, manganese increased the pulp yield, pulp viscosity and nearly doubled the delignification rate of fiberized wood. Cooper increased the delignification rate, but reduced both the pulp yield and viscosity. Yield losses were contributed mainly to the disappearance of hemicellulose. Viscosity decreases were due to the formation of free radicals created by the oxidative attack on the carbohydrate chains.

Effects of Copper and Manganese Additions on Metabolism of Ruminant Animals

Copper. Copper is a catalyst oxidant and is a constituent of many enzymes and red blood cells (15).

Low level additions of copper have been found in limited cases to stimulate cellulose digestion and microbial protein

synthesis (22,81). Other researchers have not demonstrated improvement of microbial activity with copper additions (28,41, 45). Low levels of copper addition have been found to be inhibitory to rumen microorganisms (48).

The liver contains the highest concentrations of copper in animal tissue. When feeding sheep, the amount of liver copper storage was proportional to their intake, within the range of 3-20 mg/day (19). Over a six-month period copper was stored to the extent of 5% of the intake.

No information was found on the site of copper absorption in ruminants, but rats readily absorb copper from the stomach (75).

Urinary excretion of copper is known to be low in most species of animals. When cattle were fed copper-containing grass and alfalfa, urinary copper excretion amounted to 1.2-1.9% of the total copper excretion (40). Young bulls fed labeled copper excreted 75% of the dose in the feces and 3% via the urine within a five-day period (16).

Copper requirements and toxicity levels for ruminants can not be clearly defined when interfering factors are present in the diet.

Copper complexes with molybdenum sulfate and the complex is biologically unavailable (15,21.72).

Chelating agents, such as EDTA and penicillamine, can significantly decrease copper absorption and increase urinary copper excretion (42).

The chemical form of copper changes its biological value. Copper acetate is absorbed to a greater extent than that of

copper sulfate (19,68). Toxic levels of copper sulfate and copper acetate were fed to sheep and those consuming copper in acetate form died sooner than did the copper sulfate animals (68).

Dietary calcium carbonate supplementation to sheep has been shown to reduce copper availability where dicalcium phosphate did not (19).

There is no regulatory mechanism for controlling copper intake in ruminants; therefore, the liver may accumulate high concentrations. The extent of copper toxicity depends on the animal species (55,70,77) and the breed within a species (44). Sheep are less tolerant to copper toxicity than are cattle (72). Toxic symptoms occurred in Border-Leicester sheep when fed 100 mg copper per day, but when the same amount was fed to Merino sheep, no toxic symptoms were observed (44).

Chronic copper toxicity in ruminants has three distinct phases (15). 1. Gradual accumulation of copper in the liver where blood copper content is normal with no observed clinical signs of toxicity. 2. Blood copper concentration doubles, billirubin concentration increases and liver function decreases, resulting in reduced hematocrit. Sorbitol dehydrogenase, arginase and glutamate-oxaloacelate transaminase in the blood serum increases in activity (31). Stage three is known as the hemolytic crisis (15). Dullness, anorexia and acute thirst are observed. Bellering animals reveal that abdominal pain is occurring. The mucous membranes indicate presence of jaundice. Total hemoglobin level drops dramatically to low levels, while methemoglobin increases (47). Blood hemoglobin has been shown

to drop as much as 10 g % within 48 hours. Blood glutathione concentration falls and whole blood copper increases five to eight times the normal concentration (69). These sudden changes that occur in the final stage are a result of degeneration of the liver cells, accompanied by a sudden release of copper into the blood stream. Animals generally die within two to four days after the onset of the hemolytic crisis (15).

Copper toxicity in cattle is ill-defined. Estimates of safe levels of 70-100 ppm copper for periods of 112 days have been made (55). Chronic cases of toxicity have been reported when feeding young calves milk replacer containing 115 ppm (63). However, in contrast, young calves were fed as much as 500 ppm daily and did not develop clinical symptoms until 20 weeks of age (70).

Adult cattle are more resistant to copper toxicity than young cattle (67). Yearling heifers and cows have been shown to tolerate repeated doses of 20-40 g of copper sulfate and repeated single doses up to 100 g (63). The single lethal dosage occurred at between 200-300 g. But yearling cattle receiving 200-300 mg of copper sulfate per head per day for 16 months demonstrated no ill-effects (63).

The recommended safe level for sheep is between six to seven ppm calculated copper in dry feed; whereas, 19 ppm was observed to be toxic (50). Hemolytic crises were observed in lambs when fed 40 ppm copper in 20 weeks (60). Feeding lambs 80 ppm copper for 6-12 weeks resulted in very high liver copper values and eventually death (20). Contradictory results have

been reported that when one gram of copper sulfate was fed 13-15 weeks, no symptoms occurred (8).

As revealed by this literature review, copper requirements and toxicity levels are dependent upon many interactions. It is very difficult to access a set toxic amount for any species.

Manganese. Manganese is involved in enzyme activation, many of which can be activated by other divalent ions (15). Manganese is more specific for activating the enzymes that are involved in oxidative phosphorylation, fatty acid and cholesterol synthesis (15).

As in other trace minerals, manganese is found in relatively high concentrations in the liver, kidney and pancreas (15). The normal liver manganese concentration in sheep and cattle is 6-8, 9-12 ppm on a dry basis, respectively (71).

Manganese requirements for cattle have been suggested to be near 20 ppm (7,59). The maximum safe level appears to be about 1000 ppm for both cattle and sheep.

Manganese deficient heifers have been shown to cycle slower and breed later than control animals (59). The newborn from these animals exhibited weak pasterns. Common symptoms in calves born from manganese deficient mothers were bone deformities, enlarged joints, twisted legs, stiffness and general weakness.

Manganese additions of 7.5 ppm increased cellulose digestion after chelating agents were added (41). Low level additions to washed rumen suspensions demonstrated a stimulus to cellulose digestion (22,45). Deletion of manganese resulted

in a marked reduction in cellulose digestion (13). Other researchers could not demonstrate a stimulatory effect on cellulose digestion by manganese additions (28).

Excess manganese has been found to interfere with iron metabolism by antagonizing the enzyme system at the absorption site, thus affecting iron availability (23,46). Calves fed up to 4900 ppm manganese produced variable growth performances and had decreases in hemoglobin and serum iron values (18).

High level intakes of manganese have been shown to increase the fecal excretion of both calcium and phosphorous, while plasma levels remained unchanged (23).

Calves fed 2460 ppm of manganese as manganese sulfate for a period of 84 days exhibited a depressed feed intake and decreased weight gains (18). Lower levels of 820 ppm supplemental manganese caused no adverse effects on either growth or appetite. This indicates the decrease in performance from the higher manganese levels, is a reflection on the decreased feed intake.

METHODS AND MATERIALS

Raw Material Preparation

One metric ton of dehydrated, .953 cm sorghum stover (NRC 1-07-961) pellets were procured from CK Processing, Manhattan, Kansas. The material was cleaned using an oscillating pellet scalper to remove fine particles and grain that was present due to handling of the pellets after processing.

Following cleaning, the material was ground through a hammermill equipped with a .318 cm hole diameter screen. The ground material was sacked and stored for further processing.

Pressure Cooking

Sorghum stover samples were processed in an experimental pressure chamber at the Kansas State University Physical Plant.

The assigned trace mineral mixtures (appendix A) were dissolved in half the amount of water required to bring the moisture content of the 41 g samples to 55%. This mixture was well mixed in a 1000 ml beaker by a stirring rod before the other half of the water and appropriate amounts of sodium hydroxide were added (appendix A). This was done to prevent precipitation of the trace minerals before being uniformly distributed. Samples were then placed in rectangular trays and spread in a layer approximately 1.27 cm in depth.

Pressure cooking of the samples was accomplished with saturated steam at 8.79 kg/cm^2 for two minutes.

Following cooking, samples were mixed and spread in a thin, uniform layer and dried under ambient conditions.

Extrusion Cooking

Sorghum stover samples were processed using a Wenger[a], model X-25 continuous cooker extruder at the Kansas State University Feed Mill.

The specified trace mineral mixtures (appendix A) were dissolved in half the amount of water required to raise the moisture content of the 18.61 kg samples to 55%. This material was well mixed using a 45 kg capacity double ribbon mixer before adding the other half of the water containing the assigned amounts of sodium hydroxide (appendix A). After the dissolved NaOH was added, the samples were allowed to mix for 5 minutes. This procedure was followed to allow the trace minerals to be well distributed before the addition of the strong alkaline solution.

Following mixing, samples were extruded through a .476 cm die hole with the extruder head temperature reaching 115.6 C. The material was dried, following extrusion cooking, in a forced air, heated horizontal drier.

Representative samples of each batch were taken and ground through a Wiley mill equipped with a #40 mesh screen before any laboratory tests were conducted.

Reducing Sugar Analysis

The standard AACC sodium thiosulfate, alkaline ferricyanide reducing sugar analysis (1) was used with modifications.

---

[a]Wenger Manufacturing Company, Inc., Sabetha, Kansas.

Ten gram samples of finely ground, extruded sorghum stover were weighed to the nearest .1 mg. The weighed samples were placed in a high speed Lourdes blender, along with 80 ml of distilled water, for 2 minutes. Samples were then vacuum filtered using Whatman's #2 filter paper. Twenty ml of distilled water were poured through the vacuum filter as a wash. Supernatant volumes were adjusted to 100 ml and pH recorded (appendix B). The pH was adjusted to 4.6 by the addition of concentrated HCl. Duplicate 5 ml aliquots were volumetrically pipeted to large test tubes to which 10 ml of .1 N alkaline ferricyanide solution had previously been added. The solutions were well mixed and placed in boiling water for exactly 20 minutes. Following boiling, the tubes were rapidly cooled in tap water. This supernatant-alkaline ferricyanide mixture was transferred to clean 125 ml Erlenmeyer flasks with three washings of an acetic acid salt solution of 25 ml total volume. One ml of starch-potassium iodide indicator was added to the mixture before titrating with .1 N sodium thiosulfate. A magnetic stirrer was used during titration.

Reducing power was obtained by extrapolating from a standard curve prepared from known, graded concentrations of maltose. Values were expressed as mg of reducing sugar contained per ml of solution.

In Vitro Techniques

Inoculum. Rumen fluid was collected from an Angus X Holstein steer receiving a high-roughage, low-concentrate diet. Rumen fluid was withdrawn with a suction syringe and transferred

to a previously-warmed insulated jug. The rumen fluid was quickly taken to the laboratory where, after vigorous shaking, it was strained through four layers of cheese cloth into a clean 1000 ml graduated cylinder. All rumen fluid samples were collected at least 12 hours after feeding.

In Vitro Dry Matter Digestibility. Dry matter digestibility was determined by the method of Tilley and Terry (66) with slight modifications. A .4 g sample, 8 ml rumen fluid and 32 ml of McDougall's artifical saliva were used to facilitate the use of 50 ml tubes, instead of 100 ml tubes used in the original reference.

In Vitro Gas Production. A gas production measuring device was set up similar to that described by Behnke (6). The procedures were modified as explained below.

Four gram samples, in duplicates, were mixed with 100 ml of phosphate buffer and 50 ml of strained rumen fluid. The described mixture was placed in a water bath with a constant temperature of 39 C. Readings of water displaced (gas produced) were taken at 30 minute intervals, followed by gentle swirling to insure good mixing. This procedure was continued for a four-hour period.

Nylon Bag Technique

Samples were placed in nylon bags and suspended in an Angus X Shorthorn steer at the Kansas State University Beef Animal Research Unit. The steer was maintained on a corn silage, prairie hay, low-concentrate ration.

All samples had been ground through a Wiley mill equipped with a #40 mesh screen and were placed in 72 thread count nylon bags. The bags used, measured approximately 10 cm X 15 cm. Before weighing samples, the nylon bags and strings were dried to a constant weight in a drying oven. Five gram samples were weighed and transferred to the nylon bags, tied with nylon cord and reweighed. Sample sacks were identified by knot coding in the cord.

Weights were placed on the top of each sample bag to minimize mobility during ruminal contractions. Sample cords were secured to the cap of the fistula.

Ten sample bags (five samples in duplicate) were placed in the fistulated steer for a period of 48 hours. Placement of samples was just below the strata mat to insure optimum microbial activity.

Following the digestion period, the samples were washed in water to remove the ruminal material on the outside of the bags. Samples were then oven dried at 102 C for 16 hours and reweighed. Dry matter disappearance was found by difference between the initial and final weights. All samples were adjusted according to the sodium hydroxide content.

A duplicated control was included in each trial to account for microbial and environmental variations.

For each trial, the control's dry matter disappearance was assigned a value of 100%. Each treated sample value was divided by the control value for each trial to obtain the percent of change in dry matter disappearance over that of the control.

Chemical Analyses

Van Soest procedures (26) for forage fiber analyses of the samples were conducted by the Department of Animal Science and Industry Service Laboratory, Kansas State University.

Statistical Analyses

The effects of sodium hydroxide and trace mineral additions were analyzed by the least squares analysis of variance (65) in a 3 X 6 factoral design. Duncan's multiple range test was used to determine the significance of differences between means (65).

RESULTS AND DISCUSSION

Reducing Sugar Values

Reducing sugar values are summarized in table 1. Highly significant differences ($P<.01$) were found between all treatments, as well as interactions between treatments (table 2).

Extrusion cooking alone increased ($P<.05$) the reducing sugar content when compared to that of uncooked, untreated samples (table 1). These findings agree with other work (58) that demonstrated a significant increase in reducing sugar content when pressure cooking sawdust.

The 8% NaOH level increased ($P<.05$) the reducing sugar content when compared to all other treatments combining the effects of NaOH additions and extrusion cooking (table 1). This may be explained in part by a partial dissolving of cellulose and hemicellulose (54) thus creating more reducing ends (tables 13,15).

The 4% NaOH treatments significantly ($P<.05$) decreased the reducing sugar content when compared to the 8% NaOH addition (table 1). An increase ($P<.05$) still remained, however, when comparing the 4% NaOH level to that of the uncooked, untreated stover. The decrease in reducing sugar values caused by 4% NaOH treatments are not clearly understood, but may be a result of carbohydrate complexing.

The addition of trace minerals demonstrated no definite distinguishable trends. All trace mineral treatments in the

TABLE 1. REDUCING SUGAR ANALYSIS[a] OF EXTRUDED AND CHEMICALLY TREATED SORGHUM STOVER

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	13.16 13.10	14.42^{g} 14.56	14.27^{h} 13.14	14.68^{efg} 14.84	15.18^{f} 15.26	14.27^{fg} 15.11	14.33
8	20.90^{c} 20.79	24.32^{b} 24.48	24.43^{b} 24.46	20.64^{cd} 20.47	20.16^{d} 20.32	23.65 23.71	22.36
0	17.85 17.87	20.90^{c} 21.00	19.15^{e} 19.23	18.85^{e} 18.85	20.05^{d} 20.20	14.80^{fg} 14.78	18.63
$\bar{X}$	17.28	19.95	19.10	18.05	18.53	17.72	
Uncooked, Untreated	13.92 13.60						

[a](Mg maltose/ml) reducing power expressed as maltose equivalent.

bcdefgh Cell averages not having a superscript in common differ significantly ($P<.05$).

TABLE 2. ANALYSIS OF VARIANCE ON DATA FOR THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON REDUCING SUGAR CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	387.931	2	193.965**
Trace minerals	28.390	5	5.678**
NaOH X Trace minerals	64.858	10	6.486**
Error	1.010	18	.056
Total	482.189	35	

absence of NaOH resulted in different ($P<.05$) reducing sugar values (table 1). The interaction between NaOH and trace mineral additions were highly significant ($P<.01$) (table 2).

One can deduce from these data that addition of copper yielded superior ($P<.05$) reducing sugar values when compared to manganese (table 1). All trace mineral treatments in the absence of NaOH resulted in increased ($P<.05$) reducing sugar values (table 1). Trace minerals did not significantly increase ($P<.05$) the reducing sugar values when in the presence of NaOH (table 1).

In Vitro Dry Matter Disappearance

Special sample treatment was employed before In Vitro Dry Matter Disappearance (IVDMD) determinations were performed. Samples were boiled in ethanol for five minutes, rinsed and filtered to remove the soluble material. This procedure was followed to remove potentially toxic amounts of trace minerals and silica and lignin dissolved by the NaOH treatment (35). The dissolved silica has been suggested as impairing in vitro fermentation (30). It was hoped this procedure would bring out the effect the treatments had on the remaining dry matter and, most importantly, fiber digestibility.

Values for IVDMD of treated sorghum stover samples are shown in table 3. Both sodium hydroxide levels and trace mineral additions had highly significant ($P<.01$) effects on IVDMD (table 4).

The samples treated with 8% NaOH demonstrated over a two-fold increase in IVDMD when compared to treatments with no

TABLE 3. EXTRUDED SORGHUM STOVER IN VITRO DRY MATTER DISAPPEARANCE (%) AS EFFECTED BY TRACE MINERAL AND SODIUM HYDROXIDE ADDITIONS

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	34.106 26.671	36.896 47.745	36.216 25.698	18.056 28.490	3.069 7.345	24.113 19.106	26.454
8	45.648 38.822	48.341 51.186	48.653 35.314	31.801 48.549	14.438 12.215	52.145 49.471	39.711
0	25.751 29.843	15.425 20.086	20.193 17.971	20.024 17.838	0.000 0.000	20.770 18.757	17.302[c]
$\bar{X}$	33.470[ab]	36.775[a]	30.670[ab]	27.455[b]	6.173	32.390[ab]	
Uncooked, Untreated	17.664[c] 20.759						

[abc]Column and row averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 4. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON *IN VITRO* DRY MATTER DISAPPEARANCE OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	3046.712	2	1523.356**
Trace minerals	3658.992	5	731.798**
NaOH X Trace minerals	548.156	10	54.816
Error	502.281	18	27.905
Total	7756.141	35	

additions of NaOH (table 3). The 4% treatment also resulted in a significant ($P<.05$) increase in IVDMD (table 3). These data are in agreement with results found by other workers (54,58,79), who found heat treatments and NaOH additions to be advantageous to IVDMD. The increase in IVDMD may be explained by partially dissolving the silica and lignin, thus, removing the complex shell and allowing the material to swell for an improved microbial attack (80).

Heat and steam addition (extrusion cooking) in the absence of NaOH resulted in no differences ($P<.05$) in IVDMD when compared to the uncooked, untreated sorghum stover (table 3). This finding was not in agreement with other work where an increase in IVDMD was observed when processing sawdust in a pressure chamber (58). The possible difference may be due to differences in retention time spent in the steam heated environment. The retention time in the pressure chamber was 15 minutes. Maximum temperature and pressure conditions are found only during a very short period in the cooker extruder; therefore, the extruded samples were exposed to maximum conditions only briefly.

Trace mineral additions appear to be more beneficial in aiding IVDMD at the 0 and 4% NaOH level (table 3). This improvement vanishes with the 8% NaOH treatment. The depressing effect of 1000 ppm of copper and manganese on IVDMD may be explained by the presence of toxic residues remaining, which inhibited microbial activity. Other researchers have found that low levels of copper limit microbial activity (41,48).

In Vitro Gas Production

The amount of gas produced by microorganisms has been correlated (.95) with dry matter disappearance (61). The more fermentable a substrate is, the larger the amount of gas produced. Relative amounts of gases produced (CO_2, H_2, CH_3) are coupled with the extent of microbial protein synthesis and volatile fatty acid formation.

Pressure cooking and extrusion cooking were compared, and the effect of neutralizing or not neutralizing 8% NaOH with hydrochloric acid were evaluated based on gas production (table 5). All treatments were statistically different ($P<.01$) (table 6). Extrusion cooking resulted in greater gas produced ($P<.05$) than pressure cooking (table 5). Neutralizing the 8% NaOH was found to improve ($P<.01$) gas production (table 6). These findings are not in agreement with those of other researchers who found the presence of unreacted NaOH bore no harmful effects on in vitro systems (30,45). This difference may be due to the NaOH to innoculum ratio or to the in vitro technique used.

Gas production values of extruded sorghum stover, as effected by sodium hydroxide levels and neutralization, are summarized in table 7. All treatments were found to have a significant ($P<.05$) effect on gas production (table 8). The interaction between treatments was due to the microbial inhibition of the non-neutralized 8% NaOH treatment (table 7). All treatments, with the exception of the non-neutralized 8% NaOH treatment, resulted in greater ($P<.05$) gas production than the untreated, uncooked sorghum stover sample (table 7). The

TABLE 5. GAS PRODUCTION AS EFFECTED BY PRESSURE COOKING OR EXTRUSION COOKING VERSUS NEUTRALIZED 8% NaOH AND NON-NEUTRALIZED 8% NaOH TREATED SORGHUM STOVER (Mls of Water Displaced During Four Hours)

	Non-Neutralized 8% NaOH	Neutralized (HCl) 8% NaOH	$\bar{X}$
Pressure Cooked	43.5 44.0	71.5 70.5	57.4
Extrusion Cooked	53.0 52.0	78.5 77.5	65.3
$\bar{X}$	48.1	74.5	
Uncooked, Untreated	51.5		

TABLE 6. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF PRESSURE COOKING OR EXTRUSION COOKING VERSUS NEUTRALIZED 8% NaOH AND NON-NEUTRALIZED 8% NaOH ON GAS PRODUCTION OF SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Cooking method	124.031	1	124.031**
Neutralizing	1391.281	1	1391.281**
Cooking X Neutralizing	1.531	1	1.531
Error	1.625	4	0.406
Total	1518.468	7	

TABLE 7. GAS PRODUCTION OF EXTRUDED SORGHUM STOVER AS EFFECTED BY SODIUM HYDROXIDE LEVELS AND NEUTRALIZATION (Mls of Water Displaced During Four Hours)

	Neutralized NaOH (HCl)	Non-Neutralized NaOH	$\bar{X}$
4% NaOH	72.0 73.5	62.5 61.0	67.3
8% NaOH	78.5 77.5	53.0[a] 52.0	65.3
$\bar{X}$	75.4	57.1	
Uncooked, Untreated	51.5[a]		

[a]Non-significant cell averages connected by superscripts.

TABLE 8. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECTS OF SODIUM HYDROXIDE LEVELS VERSUS NEUTRALIZED NaOH AND NON-NEUTRALIZED NaOH ON GAS PRODUCTION OF SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
NaOH level	8.000	1	8.000*
Neutralizing	666.125	1	666.125**
Level X Neutralizing	105.125	1	105.125**
Error	3.250	4	.813
Total	782.500	7	

neutralized 8% NaOH treated, extruded, sorghum stover sample resulted in greater ($P<.05$) gas production than any of the treatments (table 7).

Nylon Bag Dry Matter Digestibilities

Nylon bag dry matter digestibilities are summarized in table 9. Digestibility values were calculated by assigning the control (uncooked, untreated sorghum stover) a value of 100%. All treatments were divided by the control value to obtain the percent improvement over the control.

Sodium hydroxide additions resulted in significant ($P<.01$) digestibility effects (table 10). Neither trace mineral source, nor amount effected the nylon bag digestibility values (table 10).

Eight percent and 4% NaOH additions resulted in 250 and 163% dry matter digestibility increases, respectively, when compared to the extruded sorghum stover without NaOH treatment (table 10).

Due to the high variation among samples, trace mineral source or level was not found to have a significant effect on nylon bag dry matter digestibilities (table 9).

Trace mineral levels were assumed to be nontoxic in the nylon bag technique due to the low concentrations in relation to the large rumen volumes, but could have given localized effects.

Cell Wall Content

Cell wall content values are shown in table 11. The analysis of cell wall content represents the holocellulose and lignin

TABLE 9. NYLON BAG DIGESTIBILITIES OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS
(Percent Improvement Above Control)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	17.00 24.30 24.95 42.01	20.81 7.45 7.56 18.82	23.38 16.95 24.67 30.82	20.75 21.91 23.71 16.49	8.07 10.42 29.30 15.10	21.92 6.71 16.65 21.50	19.64
8	11.94 17.04 52.23 36.71	38.23 23.34 31.69 31.69	24.21 20.29 33.53 29.88	39.94 43.73 22.74 14.11	23.54 32.22 32.02 36.79	32.60 31.13 38.35 26.65	30.19
0	18.39 13.10 15.17 11.47	18.34 5.96 14.40 13.05	15.42 10.86 14.13 11.25	5.63 9.54 18.60 9.93	18.60 9.93 12.58 13.83	2.07 6.23 3.75 17.74	12.08
$\bar{X}$	23.69	19.28	21.28	20.59	20.20	18.77	

TABLE 10. ANALYSIS OF VARIANCE ON THE DATA, NYLON BAG DIGESTIBILITIES OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide levels	3972.125	2	1986.063**
Trace mineral levels	183.104	5	36.621
NaOH X Trace minerals	554.763	10	55.476
Error	3439.137	54	63.688
Total	8149.129	71	

TABLE 11. CELL WALL CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS
(Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	62.163^{bc} 61.890	61.934^{bc} 65.593	65.265^{a} 61.755	61.800^{bc} 62.276	62.051^{bc} 60.955	60.958^{c} 60.955	62.325
8	53.720^{de} 55.428	52.854^{ef} 51.057	47.788^{f} 54.109	53.293^{def} 53.766	52.777^{ef} 52.524	55.471^{d} 55.449	53.181
0	60.758^{c} 60.533	61.242^{c} 59.245	61.884^{bc} 61.173	61.884^{bc} 61.171	60.350^{c} 60.740	63.966^{ab} 64.814	61.450
$\bar{X}$	59.078	57.935	59.297	58.942	58.397	60.263	
Uncooked, Untreated	70.409 68.766						

^{abcdef}Cell averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 12. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON THE CELL WALL CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	611.042	2	305.521**
Trace minerals	19.143	5	3.829
NaOH X Trace minerals	57.552	10	5.755**
Error	26.386	18	1.466
Total	714.123	35	

content as a sum. Cell wall analyses appear to partition the nutritively available and soluble components from those that are unavailable and dependent on microbial fermentation (26).

Sodium hydroxide treatment significantly ($P<.01$) effected the cell wall content values of extruded sorghum stover (table 12). Eight percent NaOH treatments reduced ($P<.05$) the cell wall values when compared to all other treatments (table 11). This decrease in cell wall content is due to the partial solubilization of cellulose and hemicellulose (tables 13,15). Four percent NaOH treatments, when compared to extruded sorghum stover, failed to statistically reduce ($P<.05$) cell wall content (table 11). However, both the 4% NaOH treatment and the extruded, untreated stover resulted in lower ($P<.05$) cell wall contents than the uncooked, untreated material (table 11).

Trace mineral additions did not effect overall cell wall contents significantly ($P<.05$) (table 12). There were values within the data that did suggest differences. Trace mineral additions at the 8% NaOH treatment level had a more pronounced effect on decreasing the cell wall content (table 11). A significant ($P<.01$) interaction was present (table 12). The interaction present was apparently due to sample variance, rather than the trace mineral, NaOH associative relationship.

Cellulose

Values for cellulose contents are summarized in table 13. Sodium hydroxide additions significantly effected ($P<.01$) the cellulose contents of sorghum stover (table 14). The 8% NaOH treatment reduced ($P<.05$) the cellulose content in extruded

TABLE 13. CELLULOSE CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	31.470	29.723	26.759	27.518	30.378	28.683	29.083[a]
8	24.613	26.903	25.294	26.892	26.730	23.965	25.730
0	29.870	29.380	29.370	30.140	28.760	29.790	29.612[a]
$\bar{X}$	28.650	28.667	27.257	28.180	28.620	27.477	
Uncooked, Untreated	33.475						

[a]Row averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 14. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITION ON CELLULOSE CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	53.183	2	26.591**
Trace minerals	5.969	5	1.194
Error	19.336	10	1.934
Total	78.488	17	

sorghum stover from 29.79% to 23.97% (table 13). These findings agree with those of researchers (52,53) who found significant decreases in cellulose content in steam and NaOH treated whole plant barley.

Treatment with 4% NaOH did not result in differences in cellulose content from that of extruded only, sorghum stover (table 13). These results suggest that a 4% NaOH treatment was not severe enough for cellulose breakdown.

Extrusion cooking alone, with no additives, reduced ($P<.05$) the cellulose content when compared to the unprocessed sample (table 13).

Trace mineral additions had no effect on lowering the cellulose content of the extruded sorghum stover (table 14).

As a result of decreased cellulose, more reducing ends should be formed. The reducing sugar data (table 1), agrees with this concept.

Hemicellulose

Data on hemicellulose content are shown in table 15. Hemicellulose content is estimated by the difference between cell walls and acid detergent fiber. Highly significant differences ($P<.01$) were found due to all treatments, as well as significant interactions between treatments (table 16).

Eight percent NaOH additions lowered ($P<.05$) the hemicellulose content in extruded sorghum stover (table 15). These results are in agreement with other workers that have reported significant hemicellulose decreases following treatment of wheat straw (80) and poplar bark (25) with NaOH. The 4% NaOH treatment

TABLE 15. HEMICELLULOSE CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	18.949^{cde} 21.503	19.573^{cde} 20.613	17.919 17.914	20.790^{abc} 20.792	21.642^{a} 21.643	21.528^{ab} 21.527	20.362
8	12.258^{fg} 12.257	9.493^{h} 9.491	10.152^{h} 10.155	11.588^{g} 11.587	13.014^{f} 13.009	15.757 15.755	12.037
0	19.600^{de} 19.547	19.930^{cde} 19.926	20.610^{abcd} 20.609	19.210^{e} 19.210	20.522^{bcd} 20.521	23.053 23.050	20.484
$\bar{X}$	17.355	16.502	16.222	17.193	18.388	20.107	
Uncooked, Untreated	25.455 25.460						

^{abcdefgh}Cell average not bearing superscripts in common differ significantly ($P<.05$).

TABLE 16. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON HEMICELLULOSE CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	562.610	2	281.305**
Trace minerals	61.341	5	12.278**
NaOH X Trace minerals	25.841	10	2.584**
Error	3.818	18	.212
Total	653.660	35	

did not statistically effect the hemicellulose content; however, a decreasing trend is present (table 15).

Trace mineral treatments resulted in lower ($P<.05$) hemicellulose values when compared to the extruded only, sorghum stover (table 15). With the interactions present, it is difficult to determine which trace mineral and concentration was responsible for maximizing hemicellulose degradation.

Extrusion cooking with no additives provided sufficient energy to reduce the ($P<.05$) hemicellulose content more than that of the uncooked, untreated sample (table 15). The combination of high temperature, high pressure and shear in the extruder plus chemical treatments increases these effects.

Acid Detergent Fiber

The acid detergent fiber fraction contains lignin, cellulose and silica (26). This fraction is used as a preparatory step in the determination of lignin. Values for this determination are summarized in table 17.

Highly significant differences ($P<.01$) were found for all treatments, including the interactions between treatments (table 18). The interactions present are due to the marked decreases ($P<.05$) in cellulose (table 13), silica (table 21) and increases found in lignin (table 22). The complexity of these data makes it difficult to extract conclusions. The lignin and silica components of the acid detergent fiber fraction will individually be discussed later. However, upon examination of table 17, a decreasing trend is present when comparing the extruded materials to the untreated, uncooked samples.

TABLE 17. ACID DETERGENT FIBER CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS
(Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	39.758^{efgh} 40.075	40.696^{cd} 41.318	38.920^{h} 39.114	41.294^{cd} 40.676	40.441^{def} 40.547	38.801^{gh} 40.057	40.141
8	42.218^{a} 42.421	42.242^{a} 42.687	40.355^{de} 41.299	41.443^{abc} 42.440	34.380^{fgh} 39.905	39.641^{fgh} 39.770	41.147
0	40.672^{cd} 41.424	40.141^{defg} 40.493	40.653^{d} 41.185	41.887^{ab} 42.450	39.501^{fgh} 39.992	41.608^{bcd} 41.065	40.919
$\bar{X}$	41.090	41.258	40.250	41.695	39.967	40.153	
Uncooked, Untreated	44.095 44.170						

^{abcdefgh}Cell averages not bearing superscripts in common differ signficantly ($P<.05$).

TABLE 18. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON THE ACID DETERGENT FIBER CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	6.677	2	3.339**
Trace minerals	14.912	5	2.982**
NaOH X Trace minerals	15.228	10	1.523**
Error	3.361	18	.187
Total	40.180	35	

Other research that has been reported on acid detergent fiber values of alkali treated material, show mixed results. Decreases in acid detergent fiber have been reported by some researchers (2,25,53,62) on alkali treated substrates, while other work found an increase or no response (25,53,62).

Lignin

Sodium hydroxide treatments increased ($P<.01$) (table 20) the lignin values (table 19) in the extruded sorghum stover samples. These data are in disagreement with those of other researchers, who found lignin content to decrease in alkali treated straw, sawdust, poplar bark and corn cobs (2,25,33,35, 58). The increase in lignin content may be a result of losses in other materials which results in an apparent increase in lignin or the effect may be the formation of artifacts due to heat and NaOH treatment, thus, altering the true lignin values.

Trace mineral additions indicated no effect ($P<.05$) on lignin content (table 20).

Silica

Silica values for extruded sorghum stover samples were significantly ($P<.01$) lowered by NaOH treatments (tables 21,22). Dissolved silica, however, has been shown to be a cellulolytic inhibitor (78). Consequently, the lowering of silica content should be noted only in respect to other data. Neither trace mineral treatments, nor extrusion cooking alone lowered the values for silica content in processed sorghum stover.

Cell Solubles

Soluble cell components (table 23) are comprised of lipids, soluble carbohydrates, most proteins and other water soluble

TABLE 19. LIGNIN CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	5.812 7.675	6.060 8.351	7.009 7.462	8.657 7.456	5.113 5.909	5.272 9.214	6.927
8	13.474 10.563	9.491 11.088	11.094 11.467	10.487 11.177	8.364 10.455	11.753 10.276	10.802
0	5.420 6.061	5.900 5.870	5.522 5.949	6.321 6.885	5.086 5.534	6.152 5.955	5.887
$\bar{X}$	8.165	7.792	8.083	3.492	6.603	8.100	
Uncooked, Untreated	4.755 5.764						

TABLE 20. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON LIGNIN CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	616.018	2	80.509**
Trace minerals	13.095	5	2.619
NaOH X Trace minerals	6.454	10	.645
Error	22.583	18	1.255
Total	203.150	35	

TABLE 21. SILICA CONTENT OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	3.70	4.49	4.89	4.67	4.75	4.39	4.482
8	3.76	3.90	3.61	3.79	4.05	3.57	3.780
0	5.24	5.18	5.19	5.15	5.56	5.37	5.282
$\bar{X}$	4.233	4.523	4.563	4.537	4.787	4.443	
Uncooked, Untreated	5.71						

TABLE 22. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON SILICA CONTENT OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	6.775	2	3.387**
Trace minerals	.483	5	.097
Error	.694	10	.069
Total	7.952	17	

TABLE 23. SOLUBLE CELL CONTENTS OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	41.836^{def} 42.109	42.060^{de} 42.691	46.378 47.758	42.199^{def} 42.244	41.948^{def} 41.723	43.041^{d} 43.044	43.081
8	54.279^{bc} 52.571	55.145^{ab} 56.942	60.211^{a} 53.890	54.706^{abc} 54.234	55.221^{abc} 55.475	52.528^{c} 52.550	54.810
0	39.241^{fg} 39.466	38.757^{efg} 40.754	38.115^{g} 38.826	38.625^{g} 38.218	36.634^{g} 38.234	36.033 35.185	38.419
$\bar{X}$	44.912	46.055	47.525	45.032	45.368	43.727	
Uncooked, Untreated	29.590 31.233						

^{abcdefg}Cell averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 24. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON THE CELL SOLUBLES OF EXTRUDED SORGHUM STOVER

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	1711.658	2	855.829**
Trace mineral	48.671	5	9.734**
NaOH X Trace minerals	40.842	10	4.084*
Error	28.102	18	1.561
Total	1829.273	35	

matter (26). This fraction is assumed to be completely digested. Therefore, changes in this measurement should reflect the availability of nutrients.

All treatments, including interactions between treatments were statistically different ($P<.01$) (table 24). The 8% NaOH treatment created over 43% more soluble material than did the extruded non-alkaline treated material (table 23). When comparing the 8% alkali treatment with that of the 4% treatment, a 27% increase in cell solubles was found (table 23). The increase in cell solubles indicate solubilization and, therefore, decreases in cellulose, hemicellulose and silica (tables 13,15,21).

Trace mineral additions failed to establish consistent effects on cell solubles values (table 23).

Extrusion cooking resulted in increased ($P<.05$) cell soluble yields when compared to the untreated, uncooked samples (table 23). The alkaline treatment effects were additive to those of the extrusion cooking process.

Hot Water Insoluble Matter

Hot water insoluble matter (table 25) contains the cell wall material not readily soluble in hot water.

Alkali treatment significantly reduced ($P<.01$) the hot water insoluble material remaining in extruded sorghum stover (tables 25,25). A 26% reduction in hot water insoluble matter was observed when comparing the extruded 8% NaOH treated stover to that of extruded nonalkaline treated stover (table 25). The extruded 8% alkaline treatment resulted in an additional 19%

TABLE 25. HOT WATER INSOLUBLE MATTER OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	69.333 70.546	68.477 64.567	65.307 66.551	66.118 67.260	67.174 66.870	66.603 66.264	67.085
8	56.660 55.929	56.802 56.723	55.587 56.143	56.484 55.651	55.064 54.835	56.961 59.968	56.397
0	69.547 70.129	70.559 70.891	72.936 72.744	73.993 74.399	72.285 71.942	73.212 63.739	71.362
$\bar{X}$	65.352	64.672	64.873	65.647	64.642	64.453	
Uncooked, Untreated	77.561 77.287						

TABLE 26. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON THE HOT WATER INSOLUBLE MATTER OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	1426.044	2	713.022**
Trace minerals	6.261	5	1.252
NaOH X Trace minerals	71.973	10	7.197
Error	60.541	18	3.363
Total	1564.819	35	

decrease in insoluble matter when compared to the extruded 4% NaOH treated stover (table 25).

Extrusion cooking alone produced lower ($P<.05$) hot water insoluble values than the untreated, uncooked material (table 25). These findings are in close agreement with the treatment effects on cell wall content (table 11).

Trace minerals failed to produce any effect on the hot water insoluble material remaining in the extruded sorghum stover.

Nitrogen Content in Hot Water Insoluble Matter

Nitrogen content of the hot water insoluble matter in extruded sorghum stover is summarized in table 27. This determination is an estimate of nitrogen availability. True protein nitrogen in forages may be estimated by multiplying the hot water insoluble nitrogen content by a factor of 6.25 (26) (appendix C). The hot water coagulates the protein, which is then insoluble. It, therefore, remains in the residue and can be used for nitrogen determination and estimates of true protein content.

Sodium hydroxide significantly ($P<.01$) effected the nitrogen content remaining in the hot water insoluble fraction (table 28). The 8% alkaline treatment lowered ($P<.05$) the nitrogen content of the hot water insoluble material (table 27). These results agree with that of other work which found a decrease in protein availability due to alkaline treatments (2,54,62). These data indicate that the more severe the alkaline treatment, the greater the decrease in coagulatable protein. The sodium

TABLE 27. NITROGEN CONTENT OF THE HOT WATER INSOLUBLE MATTER IN EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS
(Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	.550^{ab} .553	.512^{bcd} .462	.396^{cd} .544	.513^{abcd} .515	.479^{cd} .463	.513^{abcd} .492	.496
8	.415^{ef} .378	.372^{f} .374	.406^{ef} .383	.340^{f} .333	.352^{f} .346	.451de .441	.379
0	.553^{ab} .556	.536^{abc} .518	.545^{ab} .538	.567^{a} .566	.562^{a} .554	.505^{abcd} .494	.536
$\bar{X}$	.497	.458	.463	.468	.455	.480	
Uncooked, Untreated	.433^{ef} .430						

^{abcdef}Cell averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 28. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON THE NITROGEN CONTENT OF THE HOT WATER INSOLUBLE MATTER IN EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	.1590	2	.0795**
Trace minerals	.0073	5	.0015
NaOH X Trace minerals	.0247	10	.0025*
Error	.0143	18	.0008
Total	.2053	35	

hydroxide treatments may have broken down some of the protein to free ammino acids, and thus, increased hot water soluble nitrogen and decreased the coagulatable protein.

Trace mineral addition failed to demonstrate any effect ($P<.05$) on the nitrogen availability (table 28).

Alternate Pepsin Insoluble Nitrogen

Alternate pepsin insoluble nitrogen (table 29) gives an estimate of the nitrogen availability to the enzyme pepsin. This procedure simulates the available nitrogen metabolism in the nonruminant animal, or nitrogen digestion in the abomasum of the ruminant.

Sodium hydroxide additions significantly ($P<.01$) effect the pepsin insoluble nitrogen values (table 30). Both alkaline treatments reduced ($P<.05$) the availability of nitrogen to the enzyme pepsin (table 29). This agrees with the results on the nitrogen content in hot water insoluble matter (table 27). Both analyses indiate that NaOH additions have altered the nitrogen components in the treated material.

Trace mineral additions failed to effect these nitrogen values (table 30).

TABLE 29. ALTERNATE PEPSIN INSOLUBLE NITROGEN VALUES OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS (Percent of Total Nitrogen on 100% Dry Matter Basis)

NaOH (%)	Trace Minerals (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	23.64	26.97	18.92	9.90	10.69	23.32	18.91[a]
8	37.12	32.86	25.89	35.61	35.33	28.57	32.56
0	11.84	21.67	12.94	12.97	10.75	11.34	13.59[a]
$\bar{X}$	24.22	27.17	19.25	19.49	18.92	21.08	
Uncooked, Untreated	12.36						

[a]Row averages not bearing superscripts in common differ significantly ($P<.05$).

TABLE 30. ANALYSIS OF VARIANCE ON THE DATA, THE EFFECT OF SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS ON ALTERNATE PEPSIN INSOLUBLE NITROGEN VALUES OF EXTRUDED SORGHUM STOVER

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square
Sodium hydroxide	1149.194	2	574.597**
Trace minerals	165.562	5	33.112
Error	270.349	10	27.035
Total	1585.105	17	

SUMMARY

In vitro, *in vivo* and chemical studies indicate that processing methods, alkali and trace mineral additions can result in increasing the nutrient availability of dehydrated sorghum stover.

Extrusion cooking in the presence of sodium hydroxide significantly increased ($P<.01$) the reducing sugar content of dehydrated sorghum stover. The addition of 8% NaOH (as is basis) resulted in more reducing sugar content than did any other treatment. The treatments containing copper produced higher reducing sugar values than did the manganese treated samples.

A two-fold increase in *in vitro* dry matter digestibility (IVDMD) was observed in the 8% NaOH treated extruded stover compared to extruded stover in the absence of alkali. Trace mineral additions decreased IVDMD apparently by microbial inhibition.

Extrusion cooked alkali-treated stover gave superior ($P<.05$) *in vitro* gas production results when compared to alkali treated, pressure cooked material. Neutralizing the unreacted NaOH with HCl was found to increase ($P<.05$) the microbial activity on both the extruded and pressure cooked material.

Nylon bag dry matter disappearance was enhanced by NaOH treatments. The 8% NaOH treatment gave a 250% dry matter digestibility increase when compared to extruded samples in the absence of NaOH. Trace mineral additions displayed no apparent effects on the nylon bag dry matter digestibilities.

Forage fiber analyses indicated a significant ($P<.05$) decrease in cell walls, cellulose, hemicellulose, silica, hot water insoluble matter and the nitrogen content in hot water insoluble matter in the 8% NaOH treated, extruded sorghum stover when compared to the extruded stover in the absence of NaOH. The 8% NaOH treatment solubilized significantly ($P<.05$) more of the cell components than the 4% alkali treatment.

Trace minerals used in addition to the alkali treatments gave nonconsistent results in the chemical components. Some additional responses may have been achieved, indicating that further study is needed.

LITERATURE CITED

1. A.A.C.C. 1962. Cereal Laboratory Methods (7th Ed.). American Association of Cereal Chemists. St. Paul, Minnesota.

2. Anderson, D. C. and A. T. Ralston. 1973. Chemical treatment of ryegrass straw: *in vitro* dry matter digestibility and compositional changes. J. Anim. Sci. 37:148.

3. Archibald, J. C. 1924. The effect of sodium hydroxide of the composition, digestibility, and feeding value of grain hulls and other fibrous materials. J of Agric. Res. 27:245.

4. Baker, F. and S. T. Harris. 1947. Microbial digestion in the rumen (and ceacum), with special reference to the decomposition of structural cellulose. Nut. Abstr. Rev. 17:3.

5. Baker, T. I., G. V. Quicke, O. G. Bentley, R. R. Johnson and A. L. Moxon. 1959. The influence of certain physical properties of purified celluloses and forage celluloses on their digestibility by rumen microorganisms *in vitro*. J. Anim. Sci. 18:655.

6. Behnke, K. C. 1973. Factors affecting starch damage and its measurement in extrusion cooked cereals. Master's Thesis Kansas State University.

7. Bentley, O. G. and P. H. Phillips. 1951. The effect of low manganese rations upon dairy cattle. J. Dairy Sci. 34:396.

8. Bordon, P. J. and A. Robertson. 1962. Experimental copper poisoning in sheep. Vet. Rec. 74:252.

9. Bryant, M. P., I. M. Robinson and H. Chu. 1959. Observations on the nutrition of bacteroides succinogenes - A ruminal cellulolytic bacterium. J. Dairy Sci. 42:1831.

10. Burroughs, W., A. Latona, P. DePaul, P. Gerlaugh and R. M. Bethke. 1951. Mineral influences upon urea utilization and cellulose digestion by rumen microorganisms using the artificial rumen technique. J. Anim. Sci. 10:693.

11. Caldwell, D. R. and R. F. Hudson. 1974. Sodium, an obligate growth requirement for predominant rumen bacteria. Appl. Microbiol. 27:549.

12. Cardon, B. P. 1953. Influence of a high salt intake on cellulose digestion. J. Anim. Sci. 12:536.

13. Chamberlain, C. C. and W. Burroughs. 1962. Effect of floride, magnesium and manganese ions on in vitro cellulose digestion by rumen microorganisms. J. Anim. Sci. 21:428.

14. Chandra, S. and M. G. Jackson. 1971. A study of various chemical treatments to remove lignin from course roughages and increase their digestibility. J. Agric. Sci. 77:11.

15. Church, D. C. 1974. Digestive Physiology and Nutrition of Ruminants. D. C. Church publisher, distributed by O and B Books, Corvallis, Oregon 2:453.

16. Comar, C. L., G. K. Davis and L. Singer. 1948. The fate of radioactive copper administered to the bovine. J. Biol. Chem. 174:905.

17. Cowling, E. B. and W. Brown. 1969. Structural features of cellulostic materials in relation to enzymatic hydrolysis. Am. Chem. Soc. Adv. in Chem. Ser. No. 95:152.

18. Cunningham, G. N., M. B. Wise and E. R. Barrick. 1966. Effect of high dietary levels of manganese on the performance and blood constituents of calves. J. Anim. Sci. 25:532.

19. Dick, A. T. 1954. Studies on the assimulation and storage of copper in crossbred sheep. Austral. J. Agric. Res. 5:511.

20. Doherty, P. C., R. M. Barlow and K. W. Angus. 1969. Spongy changes in the brains of sheep poisoned by excess dietary copper. Res. Vet. Sci. 10:303.

21. Dowdy, R. P. and G. Matrone. 1968. Copper-molybdenum interaction in sheep and chicks. J. Nutr. 95:191.

22. Ellis, W. C., W. H. Pfander, M. E. Muhrer and E. E. Pickett. 1958. Molybdenum as a dietary essential for lambs. J. Anim. Sci. 17:180.

23. Gallup, W. D., A. B. Nelson and H. E. Darlow. 1952. Forage manganese as a possible factor affecting calcium and phosphorous metabolism of range beef cattle. J. Anim. Sci. 11:783. (Abstr.).

24. Gharib, F. H., R. D. Goodrich, J. C. Meiske and A. M. Elserafy. 1975. Effects of grinding and sodium hydroxide treatment on poplar bark. J. Anim. Sci. 40:727.

25. Gharib, F. H., J. C. Meiske, R. D. Goodrich and A. M. Elserafy. 1975. In vitro evaluation of chemically-treated poplar bark. J. Anim. Sci. 40:734.

26. Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses. United States Department of Agriculture Handbook No. 379.

27. Hartman, R. H., G. Matrone and G. H. Wise. 1955. Effect of dietary manganese on hemoglobin formation. J. Nutr. 57:429.

28. Hubbert, F., E. Cheng and W. Burroughs. 1958. Mineral requirements of rumen microorganisms for cellulose digestion in vitro. J. Anim. Sci. 17:559.

29. Huffman, J. G., Kitts and C. R. Krishramurti. 1971. Effects of alkali treatment and gamma irradiation on the chemical composition and in vitro rumen digestibility of certain species of wood. Can. J. Anim. Sci. 51:457.

30. Hutanuwatr, N., F. C. Hinds and C. L. Davis. 1974. An evaluation of methods for improving the in vitro digestbility of rice hulls. J. Anim. Sci. 38:140.

31. Ishmael, J., Gopinath and J. MacHowell. 1972. Experimental chronic copper toxicity in sheep-biochemical and hematological studies during development of lesions in liver. Res. Vet. Sci. 13:22.

32. Johnson, C. E., L. H. Harbors and J. M. Prescott. 1959. Effect of alkaline drinking water on the pH and microbial activity of the rumen. J. Anim. Sci. 18:599.

33. Jones, M. J. and T. J. Klopferstein. 1967. Chemical treatments of poor quality roughages. J. Anim. Sci. 26:1492. (Abstr.).

34. Kamstra, L. D., A. L. Moxon and O. G. Bentley. 1958. The effects of stage of maturity and lignification on the digestion of cellulose in forage plants by rumen microorganisms in vitro. J. Anim. Sci. 17:199.

35. Keith, E. A. and L. B. Daniels. 1976. Acid or alkali-treated hardwood sawdust as a feed for cattle. J. Anim. Sci. 42:888.

36. Klopfenstein, T. J., V. E. Krause, M. J. Jones and W. Woods. 1972. Chemical treatment of low quality roughages. J. Anim. Sci. 35:418.

37. Klopfenstein, T. and W. Woods. 1970. Sodium and potassium hydroxide treatment of wheat straw and corn cobs. J. Anim. Sci. 31:246. (Abstr.).

38. Koers, W., M. Prokop and T. Klopfenstein. 1972. Sodium hydroxide treatment of crop residues. J. Anim. Sci. 35:1131. (Abstr.).

39. Landucci, L. L. and N. Sanyer. 1973. Influence of transition metals in oxygen pulping. Paper presented 166th National Meeting of A.C.S., Chicago, Illinois, August 26-31.

40. Lesperance, A. L. and V. R. Bohman. 1963. Effect of inorganic molybdenum and type of roughage on the bovine. J. Anim. Sci. 22:686.

41. Little, O., E. Cheng and W. Burroughs. 1958. Effects of chelating agents on cellulose digestion in vitro by rumen microorganisms. J. Anim. Sci. 17:1190. (Abstr.).

42. MacPherson, A. and R. G. Hemingway. 1967. The effect of intravenous injection of chelating agents on urinary copper excretion by sheep. Br. Vet. J. 123: 410.

43. Mahendra, S. and M. G. Jackson. 1971. The effect of different levels of sodium hydroxide spray treatment of wheat straw on composition and digestibility by cattle. J. Agric. Sci. 77:5.

44. Marston, H. R. and H. J. Lee. 1948. The effects of copper deficiency and chronic overdosage with copper on Border-Leicester and Merino sheep. J. Agric. Sci. 38:229.

45. Martinez, A. and D. C. Church. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. J. Anim. Sci. 31:982.

46. Matrone, G., R. H. Hartman and A. J. Clawson. 1959. Studies of a manganese-iron antagonism in the nutrition of rabbits and baby pigs. J. Nutr. 67: 309.

47. McClosker, P. J. 1968. Observations on blood copper in the sheep II.-chronic copper poisoning. Res. Vet. Sci. 9:103.

48. McNaught, M. L., E. C. Owen and J. B. Smith. 1950. Net exchange of certain inorganic ions and water in the alimentary tract of the milk-fed calf. Biochem. J. 46:36.

49. Meyer, J. H., W. C. Weir, N. R. Ittner and J. D. Smith. 1955. The influence of high sodium chloride intakes by fattening sheep and cattle. J. Anim. Sci. 14:412.

50. National Research Council. 1975. Nutrient requirements of sheep. Natl. Acad. Sci. Washington, D. C. 5th Ed.

51. Neathery, M. W. and W. J. Miller. 1976. Maximum safe levels and toxicity of essential trace elements for livestock and poultry. Proc. Georgia Nutr. Conf.

52. Oloade, B. G. and D. N. Mowat. 1975. Influence of whole-plant barley reconstituted with sodium hydroxide on digestibility, rumen fluid and plasma metabolism of sheep. J. Anim. Sci. 40:351.

53. Oloade, B. G., D. N. Mowat and G. C. Smith. 1973. Digestibility and nitrogen retention of NaOH treated diet. J. Anim. Sci. 37:352. (Abstr.).

54. Oloade, B. G., D. N. Mowat and J. E. Wineh. 1970. Effect of processing methods on the in vitro digestibility of sodium hydroxide treated roughages. Can. J. Anim. Sci. 50:657.

55. O'Mary, C. C., M. C. Bell, M. N. Sneed and W. T. Butts, Jr. 1970. Influence of ration copper on minerals in hair of hereford and holstein calves. J. Anim. Sci. 31:626.

56. Pew, J. C. and P. Weyna. 1962. Fine grinding, enzyme digestion, and the lignin-cellulose bond in wood. Tappi 45:247.

57. Pigden, W. J. and D. P. Heaney. 1969. Lignocellulose in ruminant nutrition. Amer. Chem. Soc. Adv. in Chem. Ser., No. 95:245.

58. Reddy, M. R. 1970. Improved utilization of cellulosic materials using different processing techniques. PhD. Dissertation. Kansas State University.

59. Rojas, M. A., I. A. Dyer and W. A. Cassatt. 1965. Manganese deficiency in the bovine. J. Anim. Sci. 24:664.

60. Ross, D. B. 1964. Chronic copper poisoning in lambs. Vet. Rec. 76:875.

61. Roth, M. 1972. Factors affecting starch damage during the steam flaking of sorghum grain. Master's thesis. Kansas State University.

62. Sacena, S. K., D. E. Otlerby, J. D. Donker and A. L. Good. 1971. Effects of feeding alkali-treated oat straw supplemented with soybean meal or non-protein nitrogen on growth of lambs and on certain blood and rumen liquid parameters. J. Anim. Sci. 33:485.

63. Shand, H. and Lavis. 1957. Chronic copper poisoning in young calves. Vet. Rec. 69:618.

64. Smith, G. S., E. C. Smith, Raul de la Torre and A. L. Neumann. 1971. Soluble silica in rumen cultures. J. Anim. Sci. 33:245. (Abstr.).

65. Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods (6th Ed.). Iowa State College Press, Ames, Iowa.

66. Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassland Soc. 18:104.

67. Todd, J. R. 1969. Chronic copper toxicity of ruminants. Proc. Nutr. Soc. 28:189.

68. Todd, J. R., J. F. Gracey and R. H. Thompson. 1962. Studies on chronic copper poisoning. I. Toxicity of copper sulfate and copper acetate in sheep. Br. Vet. J. 118:482.

69. Todd, J. R. and R. H. Thompson. 1963 Studies on chronic copper poisoning. II. Biochemical studies on the blood of sheep during the hemolytic crises. Br. Vet. J. 119:161.

70. Todd, J. R. and R. H. Thompson. 1965. Studies on chronic copper poisoning. IV. Biochemistry of the toxic syndrome in the calf. Brit Vet. J. 121:90.

71. Underwood, E. J. 1962. Trace Elements. Academic Press, New York, New York.

72. Underwood, E. J. 1971. Trace Elements in Human and Animal Nutrition (3th Ed.). Academic Press, New York, New York.

73. United States Department of Agriculture. U.S.D.A. 1974. Foreign agricultural economics report #19.

74. University of Arkansas. 1976. Treated, untreated sawdust compared in dairy rations. Feedstuffs 48(8):14.

75. Van Campen, D. R. and E. A. Mitchell. 1965. Absorption of Cu^{64}, Zn^{65}, Mo^{99} and Fe^{59} from ligated segments of the rat gastrointestinal tract. J. Nutr. 86:120.

76. Van Soest, P. J. and L. H. P. Jones. 1968. Effect of silica in forages upon digestibility. J. Dairy Sci. 51:1644.

77. Vohra, P. and F. H. Kratzer. 1968. Zinc, copper and manganese toxicities in turkey poults and their alleviation by EDTA. Poultry Sci. 47:699.

78. Waldo, D. R., L. W. Smith and E. L. Cox. 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55:125.

79. Wilson, R. K. and W. J. Pigden. 1964. Effect of sodium hydroxide treatment on the utilization of wheat straw and poplar wood by rumen microorganisms. Can. J. Anim. Sci. 44:122.

80. Yu, Yu, J. W. Thomas and R. S. Emery. 1975. Estimated nutritive value of treated forages for ruminants. J. Anim. Sci. 41:1742.

81. Zerebocov, P. I., V. F. Vrakin and N. S. Sevelev. 1971. Effect of trace elements on processes of rumen metabolism and N-metabolism in ruminants. Nutr. Abst. Rev. 41:937.

APPENDIX A

SODIUM HYDROXIDE AND TRACE MINERAL ADDITIONS TO SORGHUM STOVER SAMPLES

NaOH[b] (%)	Trace Minerals[a] (ppm)					
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----
4	1[c]	2	3	4	5	6
8	7	8	9	10	11	12
0	13	14	15	16	17	18
Uncooked, Untreated	19					

[a]Calculated trace mineral content (ppm, as is basis).

[b]Sodium hydroxide content (%, as is basis).

[c]Denotes sample number.

APPENDIX B

pH OF SUPERNATANT USED IN REDUCING SUGAR ANALYSES

Sample Number	pH
1	9.00
2	8.90
3	9.15
4	9.05
5	9.00
6	9.30
7	10.10
8	10.00
9	10.10
10	10.05
11	10.05
12	9.90
13	5.25
14	6.35
15	6.80
16	6.30
17	6.15
18	6.95

APPENDIX C

TRUE CRUDE PROTEIN[a] OF EXTRUDED SORGHUM STOVER AS EFFECTED BY NaOH AND TRACE MINERAL LEVELS
(Percent on 100% Dry Matter Basis)

NaOH (%)	Trace Mineral (ppm)						
	500 Mn ----	500 Cu ----	250 Mn 250 Cu	500 Mn 500 Cu	1000 Mn 1000 Cu	---- ----	Combined $\bar{X}$
4	3.439 3.461	3.203 2.892	4.335 3.825	3.204 3.218	2.996 2.897	3.210 3.075	3.308
8	2.592 2.366	2.325 2.338	2.539 2.397	2.132 2.083	2.204 2.165	2.822 2.756	2.388
0	3.459 3.478	3.354 3.243	3.411 3.366	3.548 3.542	3.517 3.464	3.158 3.091	3.381
$\bar{X}$	3.127	2.889	3.307	2.950	2.868	3.015	

[a]Calculated by multiplying nitrogen content in hot water insoluble matter by 6.25.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to his major professor, Dr. Charles Deyoe, for his guidance and assistance in the research and preparation of this manuscript.

Appreciation is extended to Dr. William J. Hoover, former head of the Department of Grain Science and Industry, for the use of the facilities and equipment to conduct this work.

Thanks are also given to Dr. L. H. Harbers and Dr. P. A. Seib for serving on the advisory committee and reviewing this manuscript.

Finally, the author wishes to express gratitude to his loving wife, Karen, for her sacrifices, encouragement and support.

THE EFFECT OF EXTRUSION COOKING ON NUTRITIONAL COMPONENTS OF SORGHUM STOVER TREATED WITH SODIUM HYDROXIDE AND TRACE MINERALS

by

GARY PAUL CROSSLIN

A.A., Independence Community Junior College, 1971
B.S., Kansas State University, 1974

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Maximum cellulose utilization is of great importance to ruminants that depend on roughages as their primary caloric intake. With vast amounts of low-quality fibrous feedstuffs available, this efficiency of utilization is important. The object of this research was to improve the nutrient availability of low-quality crop residue by processing techniques, trace mineral additions and the incorporation of sodium hydroxide. The processed materials were evaluated by *in vitro*, *in vivo* and chemical techniques.

Dehydrated sorghum stover pellets were ground through a hammer mill equipped with a .318 cm hole diameter screen. This material was subjected to various levels of trace minerals, sodium hydroxide and processing techniques. Pressure cooked samples were obtained by applying saturated steam at 8.79 kg/cm^2 for two minutes. Extruded samples were accomplished by passing the material through a cooker extruder equipped with a .476 cm die hole. The concentrations of sodium hydroxide used were 4 and 8% of the dehydrated sorghum stover on an as is basis. Calculated copper and manganese were added at 250,000 and 1000 ppm with different combinations of each.

Extrusion cooking in the presence of NaOH increased the reducing sugar content of sorghum stover significantly ($P<.01$). The 8% NaOH addition was found to maximize reducing sugar content. Trace mineral additions increased ($P<.05$) reducing sugar contents. Copper additions yielded superior reducing sugar values when compared to manganese.

Extruded sorghum stover in the presence of 8% NaOH resulted in over a two-fold increase in *in vitro* dry matter digestibility (IVDMD). Trace mineral additions decreased IVDMD apparently by microbial inhibition.

Extrusion cooked alkali-treated stover gave superior *in vitro* gas production results when compared to that of the alkali-treated, pressure cooked material. Neutralizing the unreacted NaOH with HCl was found to enhance the microbial activity significantly ($P<.05$) of both the extruded and pressure cooked material.

Sodium hydroxide additions produced dramatic increases in the nylon bag dry matter disappearance. The 8% NaOH treatment gave a 250% dry matter digestibility increase when compared to extruded samples in absence of NaOH. Trace mineral additions displayed no apparent effects on the nylon bag dry matter digestibilities.

Forage fiber analyses indicated a significant ($P<.05$) decrease in cell walls, cellulose, hemicellulose, silica, hot water insoluble matter and the nitrogen content in hot water insoluble matter in the 8% NaOH treated, extruded sorghum stover when compared to the extruded, untreated samples. Cell solubles were drastically increased ($P<.05$) in the alkali-treated stover compared to extruded stover in the absence of NaOH. The 8% NaOH treatment solubilized significantly ($P<.05$) more of the cell components than did the 4% alkali treatment.

Trace minerals used in addition to the alkali treatments gave nonconsistant results in the chamical components. Some

additional responses may have been achieved, but further study is needed before recommendations can be made.