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THE UNIVERSITY OF ALBERTA

BY

JOYCE PHUMZILE SHONGWE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE INFLUENCE OF ANTHRAQUINONE DERIVATIVES WITH ALKALI IN IMPROVING THE NUTRITIVE VALUE OF CEREAL STRAWS submitted by JOYCE PHUMZILE SHONGWE IN partial fulfilment of the requiments for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

Supervisor

Date: May 29, 1987

ABSTRACT

Seven in vitro digestibility studies and two in vivo studies with sheep were conducted to determine the effect of inclusion of sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) in the treatment of wheat and barley straw with alkali.

In the first study the effect of AQ-SA on microbial activity was examined. The addition of AQ-SA to the fermentation medium at levels 0, 0.02, 0.05 or 0.08% of the straw dry matter (DM) had no significant effect on in vitro organic matter digestibility (IVOMD) or gas production. When straw treated with 3% NaOH was used AQ-SA levels of 0.2 or 0.5% in the fermentation medium reduced ($P<0.05$) IVOMD by 16 and 43%, respectively. No decrease in IVOMD occured with the 0.2% or 0.5% AQ-SA levels with NH_4OH treated straw. The lignin content in straw treated with NH_4OH at 60°C for 24 h was reduced ($P<0.05$) from 10.0 to 7.9% when AQ-SA was added during the treatment. Further decreases in lignin occured when glucose was added and AQ-SA was present in amounts greater than 0.05% of straw DM.

In the other studies the relationships of temperature, moisture, alkali concentration and duration of treatment on the effectiveness of AQ-SA treatment were investigated. Treatment of wheat straw with 4% NH_4OH plus 0.05% AQ-SA at 100°C for 2, 4, 8 and 24 h resulted in IVOMD of 55, 58, 64 and 68%, respectively, which were higher ($P<0.05$) than the values of 51, 55, 59 and 60% obtained without AQ-SA treatment. IVOMD ranged ($P<0.05$) from 66% in barley straw treated with 2%NH_4OH plus AQ-SA to 75% with a 6%NH_4OH plus AQ-SA treatment compared with values of 62% and 73% obtained in straw treated with 2%NH_4OH and

6% NH_4OH alone.

In in vivo studies the effect of treatment with NH_3 or NH_3 plus AQ-SA on the nutritive value of straw for sheep was determined at 0°C or 17°C. The Estimated digestible energy content of the barley straw treated at 0°C and 17°C was increased by 14% and 11%, respectively, while voluntary intake was increased by 15% and 19%.

Evidence obtained from these studies indicated that when anthraquinone compounds were included in the alkali treatment of straw the lignin content was reduced, which resulted in increased IVOMD, voluntary intake and in vivo digestibility of the treated material

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I. INTRODUCTION

Straw and other fibrous materials are by products of cereal grain production (Sundstol and Owen, 1984). On a weight to weight basis, more straw is produced than grain (National Research Council, 1983). It has been estimated that 1.5 billion tons of crop residues are produced annually on a world wide basis (National Research Council, 1983).

Traditionally straw and other fibrous by-products are used for many purposes, such as a feed for ruminant animals, bedding, paper making and as farmyard manure (Waiss et al., 1972; Said et al., 1982; Berhâne, 1983). In some places where cereal straw is produced in excessive amounts, it is customary to burn it in the field (Anderson and Ralston, 1978). Increased interest in controlling environmental pollution makes it imperative to seek alternative ways of disposing or utilizing straw rather than burning (Waiss et al., 1972; Grossbard, 1979; Sundstol and Owen, 1984).

In less developed countries straw and other fibrous by-products serve as the major dietary source of energy for ruminant animals (Jayasuriya, 1979; Kategile, 1982). In Africa for example, it is estimated that 70% of the livestock depend on crop residues for part of their nutrition during the dry season and this dependency is expected to increase (Mosi and Lambourne, 1982). Although crop residues are low in nutritional value, they are the only feed resource available during the dry season and at this time the limited natural vegetation is of even lower quality (Kategile, 1982; Mosi and Lambourne, 1982).

Presently, about one third of the world's production of cereal grain is fed to farm animals (Sundstol and Owen, 1984). In man's search

for future sources of feed for the expanding human population the time may come when we cannot afford the loss of calories associated with the feeding of cereal grain to livestock (Davis, 1973; Leatherwood, 1973; Carter, 1974). The herbivores animals, especially ruminants, need not be competitive with man for cereal grains. In fact if the unique features of these animals are fully utilized, they should complement man's needs for energy and high quality protein (Davis, 1973). The ruminant can serve as a vital link between man and organic cellulose (Van Soest, 1973, Hespell, 1979, Akin and Barton, 1983). However, the cellulose in straw is inefficiently utilized by ruminants because of the lignin which encrusts the cellulose and hemicellulose as the plant matures.

It has been shown that treatment of cereal straw with anhydrous ammonia (NH_3) breaks the covalent bonds between lignin and cellulose or hemicellulose resulting in increases in digestibility (Lin et al., 1986). Bryce (1980) reported that lignin in wood and other fibrous by-products is located in the middle lamella between fibers and within the cell walls. During alkali treatment of the fibrous materials dissolution of the hemicellulose and the lignin located within the cell walls occurs whereas the lignin located in the middle lamella remains unchanged (Bryce, 1980; Glasser, 1980).

Increases in the rate of delignification of wood and straw occurs when they are treated with alkali and anthraquinone compounds (Holton, 1977; Dimmel and Palasz, 1984). The effectiveness of anthraquinone on fibrous by-products is based on its redox properties combined with its stability to alkali. Glasser (1980) reported that the redox potential

seemed to facilitate lignin solubilization, resulting in higher delignification rates and less cellulose and hemicellulose breakdown to monosaccharides by the peeling reaction. Therefore it appears that anthraquinone compounds may have potential in delignifying fibrous by-products used as feed for ruminants. This would be of significant importance, since increased amounts of straw can be used in rations for ruminants.

Several in vitro studies were conducted to investigate the effect of the sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) on wheat and barley straw. The objective of the first study was to investigate the effect of alkali with different levels of AQ-SA on wheat straw and microbial activity. The objective of the second study was to investigate the effect of various factors which might influence the effectiveness of AQ-SA on wheat straw; also the effect of different derivatives of AQ on barley straw were compared. The objective of the third study was to investigate the effect of treatment with AQ-SA and ammonia (NH_3) on the nutritive value of barley straw for sheep.

LITERATURE REVIEW

STRAWS, CORN STOVER AND WOOD PRODUCTS USED IN ANIMAL FEEDS

Among the common cereal straws, it is generally accepted that oat straw is more digestible than barley straw, and barley straw is more digestible than wheat straw (Kernan et al., 1980; Raininko et al., 1981; Sundstol and Owen, 1984). Oat straw does not have awns, whereas some cultivars of wheat and barley straw do (Sundstol and Owen, 1984). Also the higher nutritive value of oat straw is attributed to the fact that oats is harvested before the plant reaches full maturity, hence the lignin content of the plant material has not reached its maximum.

It is estimated that about 15 million tones of barley straw are produced annually in Western Canada (Canada Grains Council, 1985). Barley straw is second to oats straw in crude protein content, and is relatively higher in cellulose and hemicellulose content than the other cereal straws (Stoskopf, 1985). Jackson (1977) reported a lignin content of 7% in barley straw compared with 11%, 7% and 12% for oats, rice and wheat straw respectively. The silica content of barley straw varies between 2.5 to 3% compared with 13% and 6% for rice and wheat straw. Coxworth et al. (1980) reported that in vitro organic matter digestibility (IVOMD) for barley straw containing 3-4% crude protein varied between 45 and 52%, depending on the variety.

Wheat straw is available in North America in greater quantities than any other straw, but it is less desirable as a feed for livestock (Nicholson, 1979; Klopfenstein, 1978). White et al. (1981) reported IVOMD of 37.0% for wheat straw as compared to 42% for barley straw.

Spring wheat straw has a higher crude protein and dry matter digestibility than winter wheat straw (Knipfel and Townley-Smith, 1982). White et al. (1981) found that semidwarf varieties of spring wheat had higher IVOMD (40.8%) than did standard height varieties (33.1%) of spring wheat and concluded that the quality of straw used in this study was inversely related to the height of the straw.

Rye straw is rated as equal to wheat and less desirable than barley or oat straw by livestock feeders (Nicholson, 1979). Erikson (1981) determined the IVOMD of 11 samples of one cultivar of rye and found an average of 42% with a range of 30-50%. This was a lower average than found for oats (54%), barley (48%) or wheat (46%) straw in the same study.

Rice straw is produced in large quantities in many countries. Most of the rice straw is either ploughed in or burnt directly in the field (Waiss et al., 1972; Jayasuriya, 1979). Jackson (1978) found that the stem of rice straw was more digestible than the leaves. This is the opposite of what was found in other straws (Coxworth et al., 1980). Rice straw contains more silica (12-15%) and less lignin (6-7%) than other straws which normally contain 3-5% silica and 10-12% lignin (Jackson, 1978; Van Soest, 1982).

Corn stover is generally used as a major part of the diet for dry pregnant beef cows (Berger et al., 1979; Klopfenstein et al., 1979). The cows may be turned to the field after harvest of the grain to clean up the crop residue, or the stover may be chopped ensiled, and fed similar to silage (Jayasuriya, 1979; Mosi and Lambourne, 1982). Berger et al. (1979) in two feeding trials with steers compared maize stover with normal maize silage, and reported average daily gains of

0.65 kg for early harvested stover, 0.48 kg for late stover and 0.88 kg for normal maize silage.

Other crop residues available in large amounts in Western Canada which could be utilized for livestock feeding include sunflower crop residue, faba bean straw and pea straw (Coxworth et al., 1980; Horton and Steacy, 1979). Kernan et al. (1980) reported an IVOMD of 68% for sunflower crop residue and 66% for matured Jerusalem-artichoke forage. These by-products were not improved by ammonia treatment. In the same study IVOMD of 61% and 50% were reported for faba bean and pea straw, respectively.

Many species of wild ruminants and domestic goats browse trees rather than graze forages (Sundstol and Owen, 1984). The use of wood wastes as feed has been studied and reviewed (Baker et al., 1975; Nicholson, 1981). The leaves and twigs represent the most digestible parts of these wastes but they are also the most difficult to collect for use as feed since they are usually discarded in the forest (Nicholson, 1981). The availability of nutrients from wood processing wastes, such as bark and sawdust, is too low to be useful as feed for livestock (Nicholson, 1981). The fines from chemical pulping mills are nearly pure cellulose and are well digested by ruminants but chemical pulping to produce feed as a primary product has proven to be too expensive for animals (Baker et al., 1975; Nicholson, 1981).

CHEMICAL CHARACTERISTIC OF CEREAL STRAW

Cereal straw consists of highly lignified cell wall material, which often constitutes up to 80% (Table I.1; Jackson, 1977;

Theander and Aman, 1984) of the dry matter (DM).

Cellulose, the most common organic compound in the world, amounts to about 20-40% of the dry matter of all higher plants (Dehority, 1973; Lehninger, 1982). Cellulose as isolated from forage, tends to contain pentoses, mainly xylose and some arabinose, in addition to β 1-4 glucan (Van Soest, 1982). The nonglucan substances of the plant are commonly regarded as contaminants and are impossible to remove without destructive degradation of the cellulose (Bailey, 1973; Akin, 1976; Van Soest, 1982). It has been proposed that cellulose molecules possess crystalline and amorphous regions (Evans, 1979). Cellulose enzymes can readily degrade the more accessible amorphous portion but are less able to attack the poorly accessible crystalline material (Bailey, 1973; Van Soest, 1982). Cellulose crystallinity is diminished by alkali treatment and ball milling (Van Soest, 1982; Akin and Barton, 1983). These treatment procedures renders the cellulose susceptible to enzymes that would not otherwise attack it (Evans, 1979; Van Soest, 1982).

Hemicellulose is the noncellulose polysaccharide fraction that is readily hydrolyzed with acid and extractable with alkali (Bailey, 1973). In general it is poly β 1-4 D-xylopyranose with branches of arabino-gluco and galactopyranosides (Dehority, 1973; Van Soest, 1982; Akin and Barton, 1983). The ester linkages of hemicellulose and 1-3 glucosides are susceptible to alkaline attack (Theander and Aman, 1978; Van Soest, 1982). Jackson (1977) and Van Soest (1982) have reported that alkali treatment of cereal straw hydrolysis hemicellulose to five carbon sugars which can be utilized by the noncellulolytic enzymes in the rumen.

Pectin substances are also present in cell walls and are comprised

of chains of galacturonic acid, galactans and arabinans (Aspinall, 1973). The pectins are not pure but are mixed, branched, and form complicated polysaccharide structures (Van Soest, 1982). Like starch, pectin is linked α 1-4 leading to the nonlinearity and coiling of the polygalacturonic acid chain (Van Soest, 1982; Esau, 1965).

Lignin is a plant polymer containing phenylpropanoid units which are thought to arise from precursor alcohols (p-coumaryl, coniferyl and sinapyl alcohols) (Hartley and Jones, 1977; Jung and Fahey, 1984). Theander and Aman (1984) reported that the p-coumaryl, coniferyl and sinapyl alcohols are important in the biosynthesis of lignin via a complex dehydrogenation process. The binding of lignin to cell wall carbohydrates results in a rigid cell wall known to resist digestion by rumen microorganisms (Van Soest, 1973). Using ultraviolet fluorescence microscopy, Harris and Hartley (1976) reported that p-coumaric and ferulic acids are bound to unlignified as well as lignified cell walls in grasses. Yields of 1.57 mg p-coumaric acid and 6.39 mg ferulic acid per g of cell wall have been found in rye grass (Hartley et al., 1976). The quantity of phenolic compounds present in grasses increases with advancing maturity of the plant material (Theander et al., 1981).

The average content of minerals in cereal straws is presented in Table I.2. It is known that the mineral content varies widely depending on agronomical factors and also with the amount of contaminating soil. Anderson (1978) reported that cereal straws are low in phosphorus and marginal in calcium in terms of animal requirements. Preston and Leng (1984) agree with this finding and also reported that cobalt, copper, sulphur and sodium may also be limiting. Mathison et al. (1981)

reported that magnesium and selenium were limiting for beef cows fed barley straw.

PLANT FACTORS AFFECTING DIGESTIBILITY

Most plants have developed protective measures to defend themselves against predation and biodegradation (Van Soest, 1982). These protective measures affect the utilization of the forage by ruminants by limiting DM intake and the extent of digestion (Kernan et al., 1980; Van Soest, 1982).

Lignin and phenolic compounds

Van Soest (1969) reported that the amount of carbohydrate and protein constituents in plant varies with and is related to, among other things, the degree of lignification. Covalent bonds between lignin and the plant polysaccharides do form as the plant matures and these bonds reduce feed intake and digestibility more than lignin per se (Barton and Akin, 1977). Evans (1979) and Van Soest (1982) have reported that the cell wall polysaccharides in fibrous materials are only available to ruminant animals that have a symbiotic relationship with microorganisms capable of hydrolysing the polymers to simple endproducts which may then be utilized by the host animal. However, the availability and fermentability of the cell wall polysaccharides (i.e. cellulose and hemicellulose) is negatively correlated with the stage of maturity of the plant material (Evans, 1979; Van Soest, 1982).

The lowering of the digestibility of the plant cell wall organic

matter by lignin is believed to be caused by the physical inaccessibility of cellulose to the microbial enzymes due to lignin encrustation of the cellulose microfibrils (Akin and Barton, 1983), the inability of microbial enzymes to degrade the lignin polymer (Van Soest, 1982) and the inhibition of enzymes by phenolic constituents of lignin (Nadia et al., 1972). P-coumaryl alcohol yields p-coumaric acid in the rumen which inhibits both the microbial growth and degradation of cellulose by rumen microorganisms (Akin, 1982). An increased lag time and reduced growth rate was observed when a level of 0.1% p-coumaric acid was added to the broth media, and the addition of 0.15% p-coumaric acid totally prevented degradation of cellulose by the rumen microorganism (Akin, 1976; Hartley et al., 1976). In another study, Akin (1982) found that the broth medium containing 0.1% p-coumaric acid inhibited xylan utilizing microorganisms. Hungate (1966) observed a decline in motility with time for both entodiniomorphs and holotrichs protozoa when they were incubated with 0.1% p-coumaric acid. Akin (1982) and Jung and Fahey (1984) agree that among the three acids, p-coumaric acid is more toxic to the rumen microorganisms than is ferulic or sinapic acid.

Silica

The content of silica in the plant material varies between 3-5% except for rice straw and tropical grasses which contain more silica (Van Soest, 1970). Silica is associated with lignin in maintaining the structural rigidity of the plant cell wall and protection against microbial invasion (Chesson, 1980). Removal of silica by chemical

treatment improves straw digestibility (Hartley, 1981; Chesson, 1980). The mechanism of action of silica in depressing DM digestibility has not yet been determined (Van Soest, 1982), however it has been postulated that silica might reduce digestion rate by lowering the availability of essential trace minerals (Smith and Nelson, 1975).

Cutin

Cutin is found in the cuticle of the plants, in hulls from rice, and in cotton seeds. Cutin is indigestible and it lowers the plant cell wall digestibility (Sundstol and Owen, 1984). Goering and Van Soest (1970) reported that cutin may be estimated using the detergent system of analysis as the organic acid detergent fiber (ADF) residue resistant to 72% sulfuric acid and permanganate oxidation.

Tannins

Tannins are polyphenolic compounds of high molecular weight which interact with, and precipitate proteins. They are classified into two groups according to their reaction with hydrolytic agents: hydrolysable and condensed tannins (Cabrera and Martin, 1985). The hydrolysable tannins are easily eliminated under the action of hydrolytic agents such as gastric juice and do not represent a problem in animal feeding (Cabrera and Martin, 1985). Tannins play a positive role in plant life such as increasing the resistance to root diseases, reducing the attractiveness of seeds to birds and in preventing fungal attack during germination (Jones et al., 1973). Van Soest (1981) found that tannins

in straw decreased fiber digestion, and that the rumen bacteria adapted to high tannins were more efficient in digesting the fiber than unadapted microoganisms.

FACTORS INFLUENCING MICROBIAL ACTIVITY

Ruminant animals are a distinct group of mammals in which pregastric digestion of plant feedstuffs by microorganisms occurs in the rumen (Hespell, 1979). The rumen microbial population consists of diverse species of bacteria and protozoa of varying and overlapping metabolic versatilities (Leatherwood, 1973; Hespell, 1979). This is reflected in the ruminants ability to adapt to numerous feedstuff ranging from grasses and legumes to agricultural byproducts.

Although the availability of forage nutrients influences the extent and rate of fiber digestion, rumen conditions must be optimal for maximum fiber digestion (Williams, 1983). Hespell (1979) reported that the physical-chemical and nutritional factors affects the growth and efficiency of ruminal bacteria. The major physical-chemical factors include temperature, pH, oxidation-reduction potential, osmotic pressure, and to a lesser extent, surface tension and viscosity (Van Soest, 1973; Hespell, 1979; Akin and Barton, 1983). However, the nutritional factors would appear to be more important than physiochemical factors as determinants of rumen bacterial growth (Hungate, 1966; Hespell, 1979).

Digestion of the plant cell wall and roughage intake decreases when ruminant diets contain 30 to 45% concentrates (Fahmy et al., 1984a; Williams, 1983). Starch increases lag time for in vitro dry matter

digestion of grass and legume fiber (Mould et al., 1983). However, the rate of degradation is not affected after the onset of digestion (Mould et al., 1983). Cellobiose and glucose inhibits the activity of R. albus and cellobiose inhibites the attachment of B. succinogenes to cellulose (Minato and Suto 1978).

MICROBIAL DIGESTION OF CELLULOSE AND HEMICELLULOSE

Cellulose Digestion

Fermentation of cellulose by the rumen microbial community begins with the hydrolysis of cellulose to soluble products (Hungate, 1966). The bacteria are the major microbial degraders of the cell wall (Hungate, 1966; Cheng et al., 1983). The major cellulolytic species in the rumen include Ruminococcus albus, R. flavefaciens and Bacterioides succinogenes (Bryant and Wolin, 1973). Bacterioides fibrisolvens degrades cellulose but not to a great extent (Bryant and Wolin, 1973, Hungate, 1966). Bacteroides succinogenes is the most active cellulose digester according to studies using cellulose powder (Bryant et al., 1963), grass and alfalfa cellulose isolated from plant cell walls (Dehority and Scott, 1967) or intact hay, straw and cotton fiber (Cheng et al., 1983). Maximum digestion of barley straw is attained from in vitro incubation with B. succinogenes (Stewart et al., 1979) R. flavefaciens (Lathan et al., 1979) or R. albus (Graham et al., 1985).

Many noncellulolytic rumen bacteria degrade xylans and pectins, and synergistic interactions between cellulolytic and noncellulolytic species have been shown to enhance cellulose degradation (Dehority and

Scott, 1967). Bacteroides succinogenes, R. flavefaciens, R. albus and B. fibrisolvens do not ferment fatty acids, lactate, glycerol and amino acids, however, it has been found that some strains of Butyrivibrio fibrisolvens ferment some amino acids (Dehority, 1973; Bryant, 1973). Strains of cellulolytic species usually require some carbon sources other than those used as the energy source (Bryant and Wolin, 1973). Of interest in this respect is the requirement for carbon dioxide or bicarbonate (Dehority, 1971). Ammonia has been found to be essential as the main nitrogen source for Ruminococcus albus and B. succinogenes and is utilized as a sole source of nitrogen by most of these strains (Hungate, 1966, Dehority, 1971). Lack of sulphur reduces the growth and efficiency of the rumen bacteria (Hobson, 1979).

Hungate (1966); Coleman et al (1976) showed that entodiniomorph protozoa were cellulolytic. As these protozoa constitute only a small part of the protozoan population, the amount of cellulose digested by rumen protozoa is considered to be small (Hungate, 1975; Clarke, 1977). Russell (1984) reported that protozoa are involved in maintaining optimal rumen conditions for bacterial fiber digestion such as consuming large quantities of starch, sequestering starch from rumen bacteria and preventing rapid bacterial fermentation and subsequent depressions in rumen pH.

The most common anaerobic fungi found in the rumen of sheep and cattle fed fibrous diets are the flagellated zoospores (Sphaeromonas communis) and the mycelium (Neocallimasti frontatis) type of fungi (Gordon and Ashes, 1984). Bauchop (1981) reported that the rumen fungi are closely associated with the more slowly digested fractions of plant tissues. Soetanto et al. (1985) showed a large population of fungi in

the rumen of sheep fed chaffed lucerne and meadow hay. Mycelial type fungi were isolated in the rumen of animals fed wheat straw supplemented with methionine but the type producing sporangium from spherical bodies were not (Gordon and Ashes, 1984). It was also reported that the mycelial type fungi digested organic matter, cellulose, acid detergent and neutral detergent fibers better than the nonmycelial type when methionine was added in the diet although neither type of fungi digested the lignin component of the straw (Gordon and Ashes, 1984)

A number of factors which influences the fungal population densities in the rumen have been established. The most important factors include diurnal fluctuations, influence of diet, rumen pH, presence of toxic substances and microbial interactions (Soetanto et al., 1985). Although all of these factors are important, Soetanto et al. (1985) suggested that changes in the rumen pH was probably the most important factor governing the fungal population.

Hemicellulose Digestion

A number of cellulolytic bacteria can degrade, but do not utilize hemicellulose of intact bromegrass (Coen and Dehority, 1970). Hemicellulose degradation by non-utilizing strains of cellulolytic bacteria is a nonspecific function closely associated with cellulose activity (Dehority, 1973). Therefore symbiotic relationship exist between hemicellulose degrading, non-utilizing bacteria and non-hemicellulose degrading and utilizing bacteria (Dehority, 1973). Variability in the ability to attack hemicellulose depends on the

bacterial strain, plant species and plant maturity (Van Soest, 1961). Bacteroides succinogenes and B. fibrisolvens are the major bacteria capable of degrading and utilizing the hemicellulose in intact bromegrass (Dehority, 1973).

METHODS OF IMPROVING THE FEED VALUE OF LOW QUALITY ROUGHAGES

Different methods of improving the nutritive value of low protein fibrous by-products for livestock have been investigated. These include physical, chemical and biological processing methods as well as the provision of supplemental nutrients (Latham, 1979; Robles et al., 1980; Mathison, 1981; Walker, 1984).

PHYSICAL PROCESSING

Particle size reduction

Physical processing by milling, grinding, pelleting and chopping enhance DM intake of fibrous by-products by the animals (Jackson, 1977; Walker, 1984). Milling treatments disrupts the fiber structure of polymers at the molecular level and increases the digestibility of carbohydrates (Stone et al., 1969). Ball milling was demonstrated by Walker (1984) to be the most effective procedure to increase the digestibility of wood. In vitro dry matter digestibility of matured forages, especially timothy hay is increased by wet ball milling (Dehority and Johnson, 1961).

Mechanical comminution of coarse agricultural residues increases

daily feed intake by the animal (Walker, 1984). This is because the density of the feed may be increased and also the chewing time required to reduce ingested material to a particle size sufficient for passage from the rumen is decreased (Walker, 1984; Owen et al., 1984).

Fine grinding of forages increases in vitro digestibility (Dehority, 1973; Robles et al., 1980) but does not increase in vivo digestibility (Donefer, 1973; Walker, 1984). In vitro, both large and small particles may be digested because passage rate is not a factor, therefore this may result in a greater extent of digestion than would occur in vivo (Sundstol and Owen, 1984). Cowling and Kirk (1976) found that grinding increased the surface available on forages but only moderately, because of the length-width relationships of fibers. Mathison (1981) reported that grinding barley straw through a hammer mill (4.75 mm) had no measurable influence on straw consumption or average daily gain compared to chopped straw when fed to mature pregnant beef cows.

Owen (1978) noted that the advantage of grinding straw is that it can be easily incorporated into complete feeds. Ground roughages are frequently subjected to a compaction procedure such as cubing or pelleting before being fed to animals (Moore, 1963).

Stevens (1981) summarized a number of benefits of pelleting low quality roughages. These included a more uniform and desirable appearance, increased density, less dust, ease of handling, reduced segregation and waste. Muller and Bergner (1976) found that pelleting of untreated straw decreased crystallinity of cellulose and increased the content of degradable cellulose.

Levy et al. (1972) pelleted rations containing 15 and 30% wheat

straw and found that feed intake and organic matter digestibility were greatest with the low level of straw. Weisenburger and Mathison (1976) confirmed this finding, and reported that the steers fed the least barley straw produced the heaviest and fattest carcasses, indicating that these animals retained more energy and thus utilized their diet more efficiently than the steers fed a diet containing higher levels of straw. Burts (1966) showed that when 2.5 kg of pelleted straw was substituted for long straw, gain was increased due to reduced time required to consume the food and to reduced energy expenditure in chewing and ruminating.

STEAM TREATMENT

High-pressure steam treatment has also been examined as a means of improving the digestibility of various forages (Kernan et al., 1980). Coxworth et al. (1980) found that wheat straw, sedge hay and brome hay had the greatest response to steam treatment as measured by enzyme solubility. However, this was not as evident when the response was evaluated by the in vitro rumen fluid technique (Coxworth et al., 1980). In the same study, it was found that sunflower tops and Jerusalem artichoke forage had an initial IVOMD of 65-73% which remained unchanged following high-pressure steam treatment. Knipfel et al. (1981) showed that rations consisting of 65% straw steamed by Stake Technology in a continuous-flow steamer resulted in higher DM digestibility and intake than when rations containing 75% of the same straw treated with anhydrous ammonia were fed. Oji and Mowat (1979) reported that steam treated corn stover added at a level of 87% of the

diet for lambs resulted in significantly increased DM intake and apparent digestibility of organic matter, gross energy, cellulose, neutral detergent fiber (NDF) and acid detergent fiber (ADF) in comparison with untreated corn stover. It was also reported that acetate to propionate ratio in rumen fluid was decreased with steam treatment. Rates of passage of particulate matter was reduced by more than 20% with steam treatment (Oji and Mowat, 1979).

Campbell and Freer (1966) reported that steam treatment of fibrous forages increased the level of phenolic compounds in the material. The phenolic content in rice straw was increased from 0.43%, prior to steam treatment to 0.73% after steam treatment at 7 kg cm^{-1} pressure for 10 min and to 2.28% after treatment at 42.2 kg cm^{-1} pressure for 30 sec. Garret et al. (1979) reported no significant differences in feed intake and feed efficiency between straw steamed for 30 sec and unsteamed straw when fed to wethers. It was, however, noted that rice straw steamed for 90 sec depressed OMD and animals fed this material actually suffered significant daily weight losses during the trial. Since the phenolic compounds are bacteriostatic their presence in the feed could have an inhibitory effect on the rumen function (Campbell and Freer, 1966). Walker (1984) suggested that the main effect of steam treatment seemed to be an increase in the water solubility of some straw components and not to an increase in carbohydrate digestibility.

CHEMICAL TREATMENT

Bases

Many chemicals have been screened in laboratory experiments for potential to enhance digestibility of low quality crop residues. However, only five chemicals are being routinely used in experimentation with animals. These are sodium hydroxide (Jackson, 1977; Klopfenstein et al., 1979; Coombe et al., 1979; Chesson, 1980), ammonium hydroxide (Coxworth et al., 1980; Sundstol and Owen, 1984), anhydrous ammonia (Horton and Steacy, 1979; Knipfel et al., 1981), calcium hydroxide (Klopfenstein and Rounds, 1974; Walter and Klopfenstein, 1975) and potassium hydroxide (Woods and Klopfenstein, 1970). The first three of these compounds are used more frequently than the latter two.

The actions of alkali treatment on low quality roughages are well documented (Klopfenstein, 1978; Theander and Aman, 1984). Klopfenstein (1978) reported that chemical treatment of fibrous by-products solubilized hemicellulose but not cellulose, and increased the extent and rate of cellulose and hemicellulose digestion in vitro. Evans (1979) and Van Soest (1982) suggested that sodium hydroxide treatment of fibrous by-products may also decrease cellulose crystallinity due to swelling of cellulose microfibrils. This hypothesis is substantiated by increases in extent of digestion of alkali treated material (Van Soest, 1982).

It has been shown that the lignin content of the plant varies with the stage of maturity. Different materials respond differently to

alkali treatment. Ololade et al. (1970) found no changes in the lignin content of alfalfa stem and corn stover treated with 2, 4 and 8% NaOH. Bryce (1980) reported that the rate of hemicellulose dissolution in straw and wood with alkali was related to the removal of lignin within the secondary cell walls. It has also been shown that breaking of the ester bonds between lignin and hemicellulose or cellulose and the β-aryl ether bonds of lignin occurs with alkali treatment resulting in improvement in digestibility (Lin et al., 1986). Phenolic compounds (ferulic, p-coumaric and trans-ferulic acid) are released from the cell wall structure by alkali treatment (Chesson, 1980; Hartley, 1981).

Rexen (1977) showed that increases in digestibility of barley straw occured both in vivo and in vitro when 4% sodium hydroxide was used. Higher levels of sodium hydroxide increased the in vitro digestibility but did not increase the in vivo digestibility in the study of Fahmy and Orskov (1984b). Singh and Jackson (1977) demonstrated that IVOMD of wheat straw increased from 0 to 4% sodium hydroxide and did not increase thereafter. Klopfenstein et al. (1979) showed that IVOMD of corn stover increased with 3% sodium hydroxide, and a further increase was obtained with 5% sodium hydroxide treatment. However, when the corn stover treated with 5% NaOH was fed as the only roughage in the diet in vivo digestibility decreased in comparison to the 3% NaOH treatment. Coombe et al. (1979) reported that steers fed wheat straw treated with 6% NAOH had a high water intake and therefore DM digestibility was decreased due to a faster rumen turnover. Fahmy and Orskov (1984b) found that treatment of low quality roughages with sodium hydroxide decreased the rate of cellulose digestibility. This was due to rapid rates of passage from the reticulo-rumen caused by changes in the rumen

osmolality and increased water consumption (Kristensen, 1981; Fahmy and Orskov, 1984b). In contrast to sodium hydroxide treated straw, ammoniated straw does not induce increases in water intake and thus does not enhance the rate of passage of material from the rumen (Kristensen, 1981).

Calcium hydroxide $Ca(OH)_2$ is not very soluble in water and is less effective on the fibrous materials than NaOH (Doyle et al., 1986). Thus to be effective, $Ca(OH)_2$ needs to be used at higher concentrations and the treatment time must be longer than with NaOH for reactions to occur. Winugroho et al. (1984) found that when $Ca(OH)_2$ was applied in a soaking process, increased IVOMD of rice straw resulted. However, this method of treatment suffered from increased losses of organic matter (Winugroho et al., 1984). Verma et al. (1982) found no improvement in feed intake and digestibility in sheep fed wheat straw treated with $Ca(OH)_2$ and suggested that the high Ca content in the straw might have upset the ratio between calcium and the other nutrients such as phosphorus.

Treatment of straw with potassium hydroxide (KOH) produces a material with a similar feed value to straw treated with NaOH (Rounds and Klopfenstein, 1974). Increased feed intake and digestibility of crop residues treated with KOH have been measured in vivo. However, KOH is costly and is therefore not commonly used for treating fibrous by-products.

The potential improvement from alkali treatment of low quality roughages is not achieved in vivo if large amounts of concentrate is included in the diet (Horton, 1978; Raininko et al., 1981). Knipfel et al. (1981) and Streeter et al. (1983) attributed the poor response of

alkali treated roughages in mixed diets to the inhibitory effects of readily fermentable carbohydrate on the synthesis and/or activities of ruminal cellulose and hemicellulose.

AMMONIATION OF STRAW

Factors influencing ammoniation process

In Western Canada, the use of anhydrous ammonia for treating fibrous by-products is justified compared to using more feed grain with untreated straw, or feeding hay, if the grain or hay is relatively expensive (Coxwoth et al., 1980). Ammoniation of crop residues might also become more attractive during a drought when hay and feed grain supplies are severely restricted (Horton and Steacy, 1979). Sundstol and Owen (1984) reported that the ammoniation of low quality roughages has no negative effects on the animals, is economical and simple for on farm processing, and produces a palatable product of good nutritive value.

Sundstol et al. (1978) reported increased IVOMD of straw with increasing levels from 1 to 4% NH_3 and, that even though increasing the level of ammonia to 5% resulted in a higher crude protein content, IVOMD was decreased.

Ammonia is a weak base and reacts very slowly, as with other chemical reactions, its effectiveness is accelerated by increasing temperature (Kernan et al., 1981; Sundstol and Owen 1984). Ammoniation at 20° or 30°C increased DM intake and crude protein content of wheat straw in comparison to straw ammoniated at 3°C (Laytimi et al., 1983).

Horton (1978) and Coxworth et al. (1980) observed poor treatment responses by ammoniation at temperatures below 5°C. However, there is a significant interaction between treatment temperature and time (Waagerpetesen, 1977). This means that low treatment temperatures can be compensated for to a large extent by increasing the time of treatment (Waagerpetesen and Thomsen, 1977; Sundstol and Owen, 1984).

Improvement in straw digestibility by ammoniation is directly proportional to straw moisture content (Coxworth and Kullman, 1978). Higher moisture levels facilitate the introduction and equilibration of gaseous ammonia into straw (Kernan et al., 1981). Higher crude protein concentrations and IVOMD values occur in straw ammoniated with a moisture content ranging between 20 and 30% (Sundstol et al., 1978). Kiangi and Kategile (1981) found an increase in the crude protein content in straw ammoniated with a moisture content of 40%, but IVOMD was not improved compared to straw containing 30% moisture. Although high moisture content in straw gives better treatment effects, this advantage is outweighed by the greater risk of mouldiness (Sundstol and Coxworth, 1984). In this regard however, ammonia treatment has a preservative effect on the treated material if applied in sufficient amounts (Thorlacius and Robertson, 1984).

It is well documented that different materials respond differently to ammonia treatment. Waiss (1972) indicated that ammonia was more effective on plant materials with an initial low digestibility. Horton and Steacy (1979) and Horton (1981) found greater improvement in the digestibility of wheat straw than barley and oat straw, though the digestibility after treatment was still higher for both barley and oat straw. The in vitro and in vivo digestibility of corn stover and rice

straw is improved by ammonia treatment under varying conditions (Kiangi and Kategile, 1981). Treating corn cobs with 3% ammonia resulted in improved daily gain of steers from 0.39 kg day^{-1} to 0.72 kg day^{-1} (Sundstol and Coxworth, 1984).

Increase of nitrogen in straw by ammoniation

It has been shown that ammoniation of low quality roughages provides a source of nonprotein nitrogen to the rumen microoganisms (Lawlor et al., 1981). The increase in nitrogen content and DM digestibility value by ammoniation of low quality roughages depends on the amount of ammonia applied, the moisture content of the substrate, treatment temperature, storage period prior to feeding and substrate particle size (Sundstol and Owen, 1984). Kategile (1982) reported that the protein content of barley straw increased from 2.8 to 9.9% after treatment with 3% ammonia. Increasing the level of ammonia to 5.5% increased the protein content of fibrous by-products, but did not increase in IVOMD (Coombe et al., 1979; Coxworth and Kernan, 1980). Nitrogen is incorporated into ammonia treated feedstuff in three distinct forms: 1) water soluble ammonia salts, 2) water soluble non-ammonia nitrogen, and 3) water insoluble, non-ammonia nitrogen (Gordon and Chesson, 1983). Water soluble ammonia N is well utilized by rumen microoganisms. The water soluble non-ammonia N is theoretically bound to soluble organic compounds and is also available to the microbes. The availability of the water insoluble N could be important during active periods of fiber digestion (Gordon and Chesson, 1983).

Effect of ammoniation on the physical structure of straw

Ammoniation increases the fragility of wheat straw, indicating greater susceptibility to mechanical fractures (Zorrilla-Rois et al., 1985). Ammoniated straw undergoes a more rapid reduction in particle size during chewing and rumination as compared to the control straw (Zorrilla-Rios et al., 1985). Van Soest (1982) reported that changes in the physical structure due to alkali treatment may not be advantageous to the ruminant because of reduced time of microbial attachment on the treated straw. Small particle size is associated with increased rates of passage and decreased rate and extent of digestion (Van Soest, 1980). However, small particle size also increases feed intake.

BIOLOGICAL PROCESSING

Straw has been treated with fungi to improve its nutritive value. According to Zadrazil (1984) straws, wood and other plant residues are mainly mineralized by fungi. Groups of rot fungi capable of hydrolysing different polymers of the plant include sweet rot, brown rot and white rot fungi. Sweet rot fungi can metabolize soluble sugars or other easily digestible cell constituents of the plant (Zadrazil, 1984). The more resistent plant polymers, such as cellulose and hemicellulose are primarily decomposed by brown rot fungi (Zadrazil, 1984; Chang and Steinkraus, 1972). Zadrazil (1984) reported that the white rot fungi can be divided into three categories. Firstly, fungi that decompose hemicellulose and cellulose initially and then decompose lignin. Secondly, fungi that metabolize more lignin initially than cellulose

and hemicellulose. Thirdly, fungi that degrade all kinds of plant cell polymers simultaneously. Ibrahim and Pearce (1980) found that each species of white rot fungi would only be beneficial for a specific fibrous material. For example, the best white rot fungi for wheat straw may not be the best for rice straw. Soeyono et al. (1984) reported that IVOMD of rice straw increased from 32 to 45% when incubated with Pleurotus species for 21 days.

SUPPLEMENTATION OF LOW QUALITY ROUGHAGES

The ability of ruminants to utilize roughages depends on the rumen microbial activity, which depends on an adequate nutrient supply (Anderson, 1978). Maximum digestibility and intake depends upon supplementation. Cereal residues have inadequate nitrogen to satisfy microbial growth requirements (Coombe et al., 1979; Weisenburger et al., 1976). The addition of branched chain VFA, peptides and amino acids to cereal straw diets low in true protein increases microbial protein production and also improves the efficiency of the rumen microorganism in digesting the forage (Dixon et al., 1981). Alfalfa hay, high protein feeds and cereal grains have favorable influences on the digestibility of low quality roughages (Anderson, 1978). Wiedmeier et al. (1983) supplemented wheat straw with soybean meal to formulate dietary crude protein concentration of 4.5, 8.0, 9.5 and 11% and found that the digestibility for DM, cellulose and hemicellulose increased linearly with increasing crude protein content. Weisenburger et al. (1976) reported no differences in feed intake of cows fed diets of either chopped or pelleted barley straw containing 5.7, 6.6 or 9.7%

crude protein. However, weight gains increased from 10 to 29 kg as the protein level increased from 5.7 to 9.7%.

Campling and Freer (1966) found that a daily intraruminal urea infusion increased voluntary intake of ground and pelleted oat straw. The addition of molasses to the straw diet supplemented with urea reduced protein and fiber digestibility, passage rate and straw intake (Anderson, 1978). Spraying a 0.5% urea solution on long straw did not improve intake and daily gain of growing bullocks (O'Donovan, 1968). While urea supplementation does not improve growth performance in cattle fed diets containing barley straw (Smith et al., 1980) or wheat straw, fishmeal supplementation of barley straw diets improved steer growth performance in studies of Smith et al. (1980).

ANTHRAQUINONE

The effect of anthraquinone and its derivatives in improving the rate of delignification, and stabilization of the resulting polysaccharides, in wood was first demonstrated by Bach and Fiehn in 1972. Holton of C.I.L. in Canada picked up this information and patents were applied for by 1975. Anthraquinones were approved for use in the pulping industry by the Food and Drug Administration in the United States, and are now being used commercially.

MECHANISM OF ACTION OF ANTHRAQUINONE ON WOOD AND FIBROUS BYPRODUCTS

Holton (1977) demonstrated that treatment of fibrous by-products such as wood, straw and baggase in an alkali solution with

anthraquinone monosulphonate sodium salt (AQ SA) solubilized lignin and other noncellulose components, such as gums, and produced cellulose which was suitable for the manufacture of paper products. Fleming et al. (1978); Kubes et al. (1980) confirmed this finding and reported substantial increases in pulp yield after treatment of wood chips with anthraquinones which function as redox catalyst (Figure I.1).

Fleming et al. (1980) and Dimmel and Palasz (1984) found that the anthrahydroquinone (AHQ, a reduced form of AQ) played an important role during pulping. The compound increased delignification by a combination of at least two effects: promotion of lignin framentation reactions and retardation of lignin condensation reactions. Two theories, the adduct and single electron transfer, have been proposed to explain the mechanism of action of anthraquinone (AQ) compounds on wood and other fibrous by-products. Haggin (1984) proposed that adducts were formed during alkaline pulping of fibrous materials, and that they were involved in the delignification process. According to the adduct theory AHQ dianions add electrons to the β-aryl bonds of the solubilized lignin and fragment it to phenolic compounds which are alkali or water soluble (Fig I.2). Presence of the correct functional groups from wood in the reaction reduced the oxidized AQ to AHQ which allows the process to repeat again. There is some doubt about this mechanism however, since Dimmel and Palasz (1984) reported that adduct formation was not necessary for delignification reactions, and that the adduct might be detrimental in that irreversible AQ bound products may be formed. The formation of the bound AQ products might reduce the amount of AQ present in solution for the reaction.

The electron transfer theory (Fig I.3) proposes that

anthrahydroquinone dianions and radical anions function as electron transfer catalysts, which mediate the transfer of electrons from the insoluble carbohydrate polymers to lignin quinonemethides, fragmenting them to phenolate radicals. In such a mechanism, no bonding or adducts are involved.

AMOUNT OF ANTHRAQUINONE AND REDUCING AGENTS USED IN WOOD PULPING

It has been shown that AQ compounds are effective in very small quantities. Holton (1977) reported that a level of 0.06% AQ was required for the soda process (NaOH) to match the Kraft process (sulphate salts) in pulp yield. Increasing the level of AQ to 0.13, 0.25, and 2% provides progressively greater yield gains over the Kraft process (Holton, 1977). However, the response to AQ was not linearly dependent with the concentration, especially in terms of cooking rate. Virkola et al., (1981) suggested that a balance between the alkali to wood ratio would give the AQ the advantage during the delignification process over the Kraft method.

Several reducing agents have been tried to increase the effectiveness of the AQ compounds on fibrous materials. Hocking et al. (1980) reported that glucose reduced AQ rapidly at room temperature in 1 M aqueous sodium hydroxide but not as rapidly as sodium dithionite. It was also reported that, although cellobiose reduced AQ slowly at room temperature in 1 M aqueous sodium hydroxide, it reduced AQ much more rapidly at 50°C. Cotton linters reduced AQ rapidly at 130°C (Hocking et al., 1980). The order of the reduction efficiency of the reducing agents is glucose> cellobiose> hydrocellulose> cotton linters

(Fleming et al., 1978; Hocking et al., 1980).

SAFETY OF ANTHRAQUINONE COMPOUNDS

Anthraquinone and its derivatives have shown no hazardous effect on the personnel handling the chemical in the pulping paper industry (Holton 1977) and no special techniques or equipment were required for their application. However at this time there is very little information available on the toxicity and metabolic behavior of anthraquinone and its derivatives in mammals. In addition no feeding trials with farm animals have been conducted using fibrous by-products treated with anthraquinones.

Martin et al. (1983) reported that dosing a sheep with 50 mg Kg^{-1} body weight of 1-methylaminoanthraquinone (MAAQ) resulted in 27% of the total dose being accounted for as colored products excreted in the urine and 16% as coloured products excreted in the feces. Approximately 50% of the total dose was metabolized to metabolites which could not be detected by their color. Less than 0.005% of the 2-and 4-hydroxy glucuronide conjugates of MAAQ were found in the milk of the lactating ewe. A concentration of approximately 2 ppm anthraquinone was found in the bile of the sheep. Martin et al. (1983) concluded that MAAQ was likely to be metabolized by mammals and that retention of MAAQ and its metabolites by body tissues and fluids was minimal.

Zanella et al. (1979) tested the liquor obtained when 0.1% AQ was added in kraft processing for acute toxicity and observed that the liquor was non-toxic to fathead minnows and Daphnia magna fish.

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TABLE 1.1

Chemical composition (%) of straw dry matter[1]

Roughage	Cell walls	Hemicellulose	Cellulose	Lignin
Barley straw	81	27	44	7
Oat straw	73	16	41	11
Paddy straw	79	26	33	7
Wheat straw	80	36	39	10
Sorghum stover	74	30	31	11
Chickpea straw	62	20	30	10
Lucerne straw	69	19	38	11
Sugarcane bagasse	82	29	40	13
Sugarcane trash	80	26	36	10
Paddy hulls	86	14	39	11
Cottonseed hulls	91	15	59	13

[1]Adapted from (Theander and Aman, 1984)

TABLE I.2

Content of minerals in cereal straws dry matter[1]

Mineral	Unit	Barley	Oats	Rice	Rye	Spring wheat	Winter wheat
Ash	g/kg	60	59	189	39	61	50
Silica	g/kg	15	11	130	34	31	32
Ca	g/kg	2.9	3.9	2.4	2.8	3.2	2.1
P	g/kg	0.8	0.9	0.9	1.0	0.8	0.8
Mg	g/kg	1.0	1.5	1.2	0.9	0.9	1.1
K	g/kg	14.0	21.9	13.2	9.8	11.8	10.0
Na	g/kg	-	-	-	0.5	0.5	0.5
Cl	g/kg	7.7	8.1	-	2.5	6.1	3.5
S	g/kg	1.4	2.5	1.3	1.2	1.4	1.6
Fe	mg/kg	305	214	347	300	420	230
Mn	mg/kg	27	89	-	44	-	36
Zn	mg/kg	60	138	-	25	-	54
Cu	mg/kg	3.9	6.5	-	3.0	-	3.1
Mo	mg/kg	0.4	0.6	-	0.1	-	0.4
I	mg/kg	0.4	0.3	-	0.5	0.8	0.6
Co	mg/kg	0.3	0.09	-	0.05	-	0.08
F	mg/kg	-	-	-	-	-	4.5

[1]Adapted from (Theander and Aman, 1984).

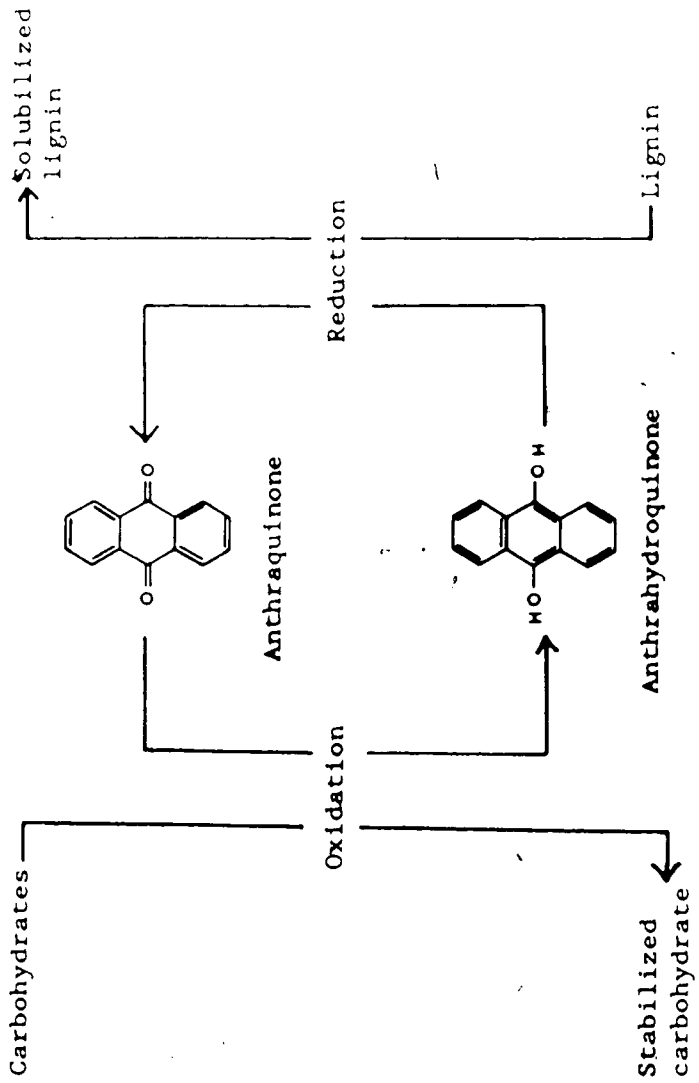

Figure I.1 Redox cycle involving anthraquinone (adapted from Haggin, 1984).

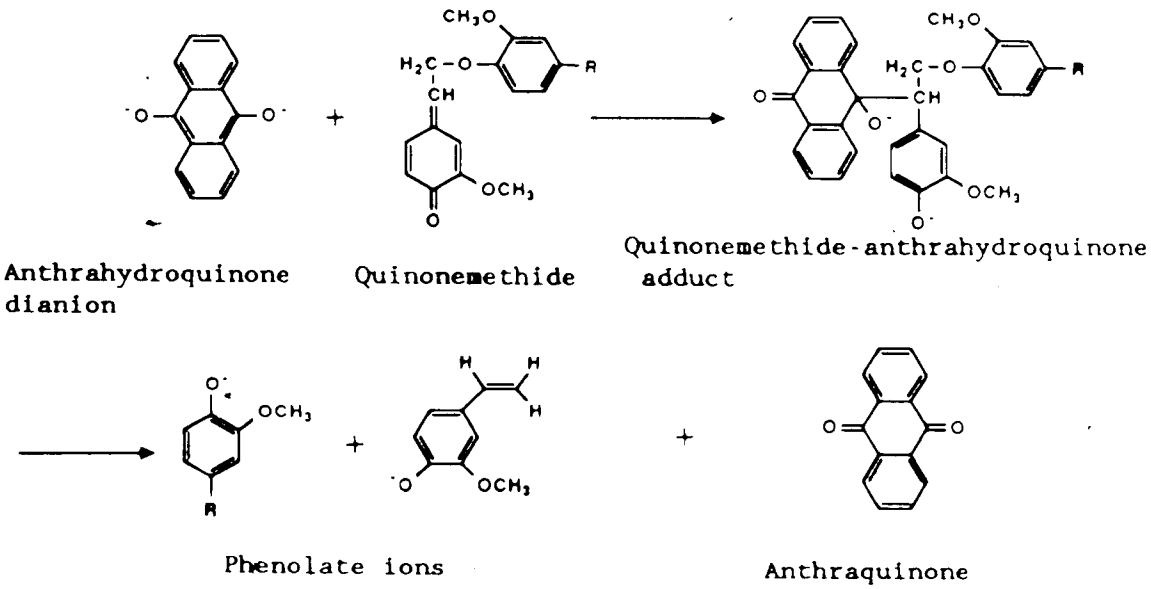

Figure I.2 Double electron theory of lignin fragmentation with anthraquinone (adapted from Haggin, 1984).

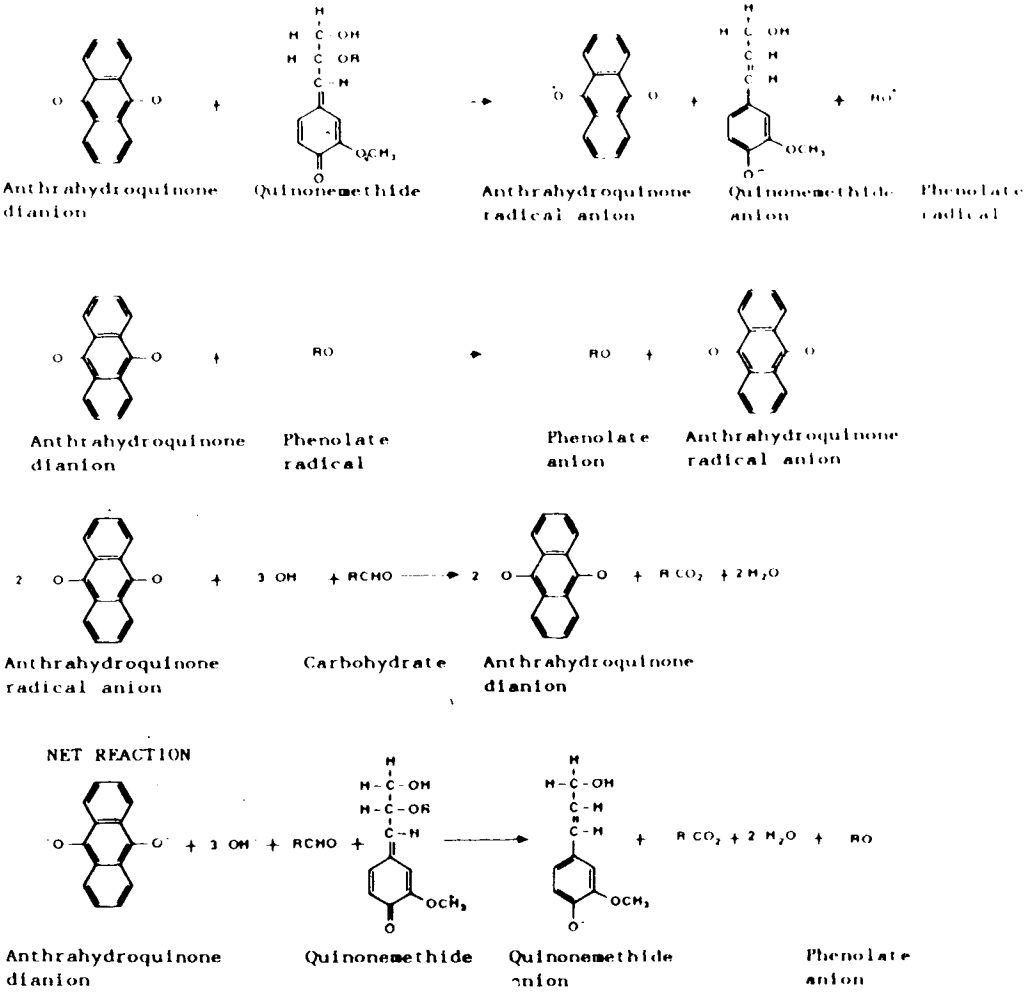

Figure 1.3 Single electron transfer theory of lignin fragmentation with anthraquinone (adapted from Dimmel and Palasz, 1984)

II. EFFECT OF ANTHRAQUINONE 2-SULFONIC ACID TREATMENT ON MICROBIAL ACTIVITY AND ON IN VITRO DEGRADABILITY OF WHEAT STRAW

INTRODUCTION

The demand for increased crop production has placed greater pressure on agricultural land use. Research has effectively demonstrated that beef cows can be successfully maintained on rations consisting primarily of crop residues (Annonymous, 1987; Grings and Males, 1987). However, because of the resistance of fiber to digestion by ruminal microbes, untreated crop residues generally do not provide sufficient energy to support maintenance functions let alone optimum levels of growth and lactation in ruminant animals (National Research Council, 1983; Lin et al., 1986). The resistance to digestion of the plant fiber by ruminal microbes is attributed to the crystalline structure of cellulose and its close physical association with lignin (Van Soest, 1982; Lin et al., 1986).

Different methods of processing cereal straws to improve the feed value and utilization by the ruminant animal have been examined. The alkali treatment procedure is the most commonly used and has been researched extensively, as evidenced in reviews by Jackson (1977); Klopfenstein (1978) and Chesson (1980). Improvement in animal performance has been measured when treated crop residues have been fed. It has been shown that treatment of crop residues with NaOH or NH_4OH breaks the β-aryl ether bonds of lignin and the ester linkages between lignin and cellulose or hemicellulose and causes a swelling effect on the cellulose microfibrils (Evans, 1979; Harbers et al.,

1982, Bryce; 1980). Jackson (1977) reported that alkali treatment decreased the cellulose crystallinity and increased the surface area. Therefore increases in the extent of in vitro bacterial digestion of cellulose and hemicellulose occur (Evans, 1979).

Anthraquinone (AQ) derivatives enhance the rate of delignification in fibrous materials during alkali treatment by transfering electrons to the lignin molecule and preventing any reversal reactions from occuring (Dimmel et al., 1981). Holton (1977) and Virkola et al. (1981), in wood pulping, reported increased pulp yields due to increased delignification when a level of 0.06% AQ was used with NaOH. Increasing the concentration of AQ to 0.13, 0.25 and 2% of the weight of wood gave higher pulp yield than with the conventional kraft pulping process but the effect of AQ on the wood was not linearly dependant on its concentration (Holton, 1977). Hermadi (1981) reported that AQ improved the pulp yield from straw by 6% above that obtained in the conventional sulphate pulping process.

Fleming et al. (1978) reported that the addition of glucose as a reducing sugar enhanced the effectiveness of the AQ, resulting in more lignin being solubilized. There are, however, some contradictory opinions about the need for a reducing sugar when anthraquinone is used since the dissolved carbohydrates in fibrous materials are a likely source of reducing power (Kubes et al., 1980).

The objectives of this study were to determine if AQ-SA had any detrimental effects on rumen microoganisms, to examine the need for a reducing sugar for increasing the effectiveness of the AQ-SA treatment in solubilizing the lignin in the straw, and to investigate the effect of treating wheat straw with NaOH or NH_4OH with various

concentrations of the sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) on the in vitro organic matter digestibility (IVOMD) and rate of gas production from microbial fermentation.

MATERIALS AND METHODS

EXPERIMENT 1 Effect of anthraquinone 2-sulfonic acid on microbial activity

Approximately 10 g samples of wheat (Triticum vulgare, variety Katepwa) straw, ground through a 1 mm screen were treated with 3% NaOH (wt/wt, dry matter (DM) basis) or 4% NH_4OH in duplicate. The chemicals were dissolved in approximately 80 mL water and the solutions were then mixed with the straw samples in 1 L pyrex jars which were then sealed and held in an oven at 100°C for 4 h. Straw treated with water alone was also prepared. At the end of the treatment period duplicate 1.0 g samples of the treated straw materials were added to 250 mL Erlenmeyer flasks for in vitro studies. A stock solution of AQ-SA was prepared by dissolving 200 mg AQ-SA in 20 mL water. This solution was added to the flasks using a microliter pipette to provide AQ-SA in amounts equivalent to 0, 0.02, 0.05, 0.08, 0.2 and 0.5% of the straw (wt/wt, DM basis). Samples of straw treated with water alone were also prepared for the in vitro assay.

A 409 kg steer fed 9 kg of timothy bromegrass hay served as the source of the rumen fluid for the in vitro experiments, which were conducted according to the procedure of Tilley and Terry (1963). Glucose and urea were added in the incubation medium according to the

method used at the research station , Lethbridge, for in vitro forage digestion. Total gas produced during the digestion of the straw materials in vitro was measured between 2 and 26 h of the 48 h incubation period using simplified manometer equipment. After 48 h of incubation 2 mL of a 6N HCL was added to each sample to lower the pH to 2 and then 5 mL (25 g L^{-1}) pepsin solution was added and contents of the flasks were incubated for another 48 h for digestion of the microbial protein. Organic matter digestibility was determined as the loss in weight of DM of the material ashed at 600°C in a muffle furnance oven for 3 h. Analysis for DM was measured by standard procedures (Association of Official Analytical Chemists, 1980).

EXPERIMENT 2 Effect of glucose on the effectiveness of anthraquinone in delignifying straw

Ten g samples of wheat straw which had been ground through a 1 mm screen were treated with 4% NH_4OH (wt/wt, DM basis) and with one of six different levels of AQ-SA (0.0, 0.03, 0.05, 0.08, 0.2 and 0.5% of straw, in combination with three levels of glucose (0, 3, and 5% of straw DM). Each AQ-SA and glucose treatment had two replications. The treatment procedure followed was the same as that used in experiment 1 with the exception that the pyrex jars were held at 60°C for 24 h. At the end of the treatment period samples were analysed for lignin using the 72% sulfuric acid method as described by Goering and Van Soest (1970).

EXPERIMENT 3 Effect of anthraquinone 2-sulfonic acid and alkali on wheat straw

Wheat straw ground through a 1 mm screen was used for this experiment. Ten g samples of straw were placed in pyrex jars and 80 ml of solutions containing AQ-SA at levels of 0.02, 0.05, 0.08, 0.2, and 0.5% of straw DM with 3% NaOH or 4% NH_4OH (wt/wt, DM basis) were added. Straw treated with water alone was included as the control treatment. Each treatment was prepared in duplicate. The samples were held at 100°C for 4 h and at the end of the treatment period samples were removed and frozen until in vitro measurements and microbial gas production were conducted as outlined in experiment 1.

STATISTICAL ANALYSIS

Data for microbial gas production and lignin were analysed using a two way analysis of variance procedure in experiments 1 and 3 with time and level of anthraquinone considered in the analysis. In experiment 3 the NaOH treatment was considered separately from the NH_4OH treated straw since the in vitro procedure was not done at the same time. Data for IVOMD obtained in these experiments were analysed using a one way analysis of variance. Experiment 2 was set up as a 3x6 factorial design with three glucose and six anthraquinone 2-sulfonic acid treatments. If significant F ratios were obtained, differences among treatment means were further tested using the Student-Newman-Keuls' test (Steel and Torrie, 1980).

RESULTS

EXPERIMENT 1 Effect of anthraquinone 2-sulfonic acid on microbial activity

The addition of levels of 0, 0.02, 0.05 or 0.08% AQ-SA to the treated straw had no significant ($P>0.05$) effect on the IVOMD of NaOH treated wheat straw (Table II.1). However, the addition of 0.2 and 0.5% AQ-SA reduced IVOMD by 16 and 43%, respectively, below that which occured in straw treated with NaOH with no addition of AQ-SA.

There were no significant differences ($P>0.05$) in gas production as a result of microbial activity among the straw treated with NaOH with the addition of 0, 0.02, 0.05 or 0.08% AQ-SA. However, the addition of 0.2 and 0.5% AQ-SA to NaOH treated straw reduced mean ($P<0.05$) gas production over the 6 h periods from approximately 28 mL to 19 mL and 11 mL g^{-1}, respectively, which were lower ($P<0.05$) than that obtained with untreated straw (Table II.1).

Straw treated with NH_4OH alone had an IVOMD of 21% above that observed in the untreated straw. The addition of 0, 0.02, 0.05, or 0.08 AQ-SA to NH_4OH treated straw resulted in nonsignificant ($P>0.05$) increases in IVOMD. In contrast to the situation with NaOH treated straw, there was no decrease in IVOMD when the 0.2, or 0.5% levels of AQ-SA were added.

Mean gas production measured over the 6 h periods with straw treated with NH_4OH was not influenced ($P>0.05$) by the anthraquinone (0, 0.02, 0.05 or 0.08% AQ-SA) concentration. However, the addition of 0.2 or 0.5% AQ-SA reduced ($P<0.05$) gas production to 25 mL,

respectively, in a 6 h period from the 29 ml. obtained when no AQ SA was present (Table II.1).

The two way interactions for time by treatment for gas production were significant (P<0.001) for straw treated with NaOH and NH_4OH. The interaction in NaOH treated straw occured because no change in gas production with increasing time of incubation occured with the 0.5% AQ SA treatment. In straw treated with NH_4OH with 0.5% AQ-SA, gas production decreased in the 14-20 h period as compared to the 8-14 h period which was different than the trend in gas production observed with the other levels of AQ-SA.

EXPERIMENT 2 Effect of glucose on the effectiveness of anthraquinone 2-sulfonic acid

The lignin content in the DM treated with NH_4OH plus 0.03% AQ-SA and NH_4OH alone did not differ (P>0.05) but the lignin content was higher (P<0.05) than the lignin content of material treated with higher levels of AQ-SA (Tables II.2). There were no differences (P>0.05) in the lignin content in straw treated with NH_4OH plus 0.05, 0.08 or 0.2% AQ-SA (Table II.2). Straw treated with NH_4OH plus 0.5% AQ-SA gave a mean concentration of 7.5% lignin in the DM which was lower (P<0.05) than that obtained in the other treatments.

In evaluating the effect of glucose on the lignin content of the treated material (Table II.2), it is important to consider the dilution effect that the added glucose would have on the lignin. In this regard if the untreated straw contained 10.4% lignin, it would have been expected that 3 and 5% added glucose would have decreased the lignin

content of DM to 10.1 and 9.9%, respectively. Glucose had no effect on lignin content of the DM when AQ-SA levels were 0.05% or less. At 0.08 and 0.2% AQ-SA lignin content was reduced (P<0.05) when 5% glucose was added. The lignin contents of DM of material treated with 0.5% AQ-SA were 9.0, 8.3, and 5.2% when 0, 3, or 5% glucose was added, respectively (Table II.2), indicating that with the high AQ-SA level even the low level of glucose was effective in promoting delignification.

The two way interaction for anthraquinone by glucose factors was significant (P<0.001) and resulted from a larger effect of glucose on the lignin content of straw with increasing concentrations of AQ-SA.

EXPERIMENT 3 Effect of anthraquinone 2-sulfonic acid and alkali on wheat straw

There were no differences (P>0.05) in IVOMD between straw treated with NaOH plus 0, 0.02, 0.08 or 0.2% AQ-SA (Table II.3). However, treatment of straw with 3% NaOH plus 0.05% AQ-SA resulted in an IVOMD of 70% which was higher (P<0.05) than that obtained in the other treatments. IVOMD decreased (P<0.05) to 64% in straw treated with NaOH plus 0.5% AQ-SA.

Treatment of the straw with 3% NaOH gave an overall mean of 29 mL g^{-1} gas production during each 6 h period which was higher (P<0.05) than the 23 mL g^{-1} obtained with the untreated straw. There were, however, no significant (P>0.05) differences in the overall means of gas production obtained in straw treated with NaOH plus the different levels of AQ-SA (Table II.3). Furthermore, the two way interaction of

treatment by time for the straw treated with NaOH plus the different levels of AQ-SA was not significant ($P>0.05$).

The IVOMD of straw was improved ($P<0.05$) from 52 to 70% when it was treated with 4% NH_4OH. Level of AQ-SA had no signficant ($P>0.05$) effect on the IVOMD with the exception ($P<0.05$) of the 0.05% level which resulted in an IVOMD of 78% (Table II.3).

Gas production increased ($P<0.05$) from 15 mL to 25 mL g^{-1} in a 6 h period when straw was treated with NH_4OH (Table II.3). Straw treated with NH_4OH and 0.05% AQ SA resulted in a mean of 35 mL g^{-1} gas production, which was higher ($P<0.05$) than the values obtained in the other treatments. The second highest (31 mL) amount of gas production was obtained in straw treated with NH_4OH plus 0.08% AQ SA. There were no significant ($P>0.05$) differences among the overall means of gas production from straw treated with NH_4OH plus 0.02, 0.2% AQ-SA, or and straw treated with NH_4OH alone however the amount of gas production obtained in these treatments was higher ($P<0.05$) than the 20 mL g^{-1} obtained in straw treated with NH_4OH plus 0.5% AQ-SA (Table II.3).

DISCUSSION

The results obtained in experiment 1 demonstrated that the addition of AQ-SA at levels of less than 0.08% of straw DM had no effect on microbial activity. However, the 0.2 and 0.5% levels of AQ-SA resulted in lower gas production from rumen microorganisms when incubated with NaOH or NH_4OH treated straw. Therefore, it is probable that AQ-SA at concentrations in excess of 0.08% of straw DM was inhibitory to the

rumen microorganisms. No evidence in the literature was found to confirm this negative effect on microoganisms at these concentrations.

In contrast to the apparent negative effect of high levels of AQ-SA on microbial activity when the AQ-SA was added at the time of the in vitro procedure, when straw was treated with AQ-SA and the base at 100°C for 4 h (Table II.3) no decrease in microbial gas production was observed with the 0.2 and 0.5% AQ-SA treatments. Since recoveries of AQ compounds ranging from 99 to 100% have been found in the pulping liquor (Nilsson and Samuelson, 1982) these results suggest that the reduction in microbial activity observed when higher levels of AQ-SA are present were compensated for by the increased delignification achieved.

It is well established that AQ and its derivatives are useful in delignifying wood (Holton, 1977). Anthrahydroquinone (AHQ), the reduced form of AQ, plays an important role during the pulping process (Bryce, 1980; Dimmel and Palasz, 1984). The compound increases delignification by a combination of at least two effects: promotion of lignin fragmentation reactions and retardation of lignin condensation reactions (Bryce, 1980; Dimmel et al., 1981). The proposed mechanism of AQ-SA action in pulping with wood, straw and baggase, outlined in Figures II.1 and II.2 may help explain the reactions which may have occured during these experiments which resulted in increased delignification and increased susceptibility of the lignocellulose material to microbial attachment.

In experiment 2 evidence was obtained which suggested that the amount of lignin in straw was reduced with increasing level of glucose and AQ-SA (Table II.2). This agrees with the results of Fleming et al. (1978) and Hocking et al. (1981) with wood. Dimmel et al. (1981)

reported that the presence of glucose in the pulping liquor reduced anthraquinone (AQ) to anthrahydroquinone (AHQ) at a more rapid rate than when glucose was not present. This prevented any reversal reactions of the cleaved ester linkages and B-aryl ether bonds of lignin in bases from occuring. It was also suggested that the glucose captured quinonemethides species thereby lowering their concentration and preventing condensation reactions. It appears that it is desirable to have the AQ in the reduced form during the initial phase of the bases treatment (Bryce, 1980). Glucose may be especially useful in reducing the AQ faster during the initial phases of treatment (Hocking et al., 1981). At the later phases of the bases treatment, bases will be neutralized by the acid products formed during the reaction (Bryce, 1980; Dimmel and Palasz, 1984).

The improvement in the IVOMD obtained in straw treated with NaOH or NH_4OH as compared with the control treatment agrees with numerous results (Klopfenstein, 1978; Jackson, 1977; Ololade et al., 1970). The IVOMD results obtained in experiment 3 demonstrated that the 0.05% level of AQ-SA improved the effectiveness of either NaOH or NH_4OH in improving the IVOMD from in vitro incubation of the treated straw. The 0.05% level which gave the highest IVOMD results was similar to the 0.06% level of AQ-SA reported by Holton (1977) and Dimmel et al. (1984). Holton (1977) reported that the increased pulp yield from wood treated with the 0.06% AQ-SA occured because of increased delignification and stabilization of cellulose and hemicellulose from the alkali peeling reactions. These authors found no further increase in pulp yield when the AQ-SA was used at levels greater than the 0.06% of the wood weight.

The results reported herein clearly demonstrated that AQ-SA, when used with alkali and glucose, caused delignification in cereal straw. However, higher levels of AQ-SA may be toxic to rumen microoganisms and this problem may limit the use of this compound in treating fibrous materials for feeding ruminant animals. Further work is needed to determine the effect of concentration of alkali, time and temperature of straw treatment, effectiveness of different AQ derivatives, and the effect of AQ treated straw on animals.

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Table II.1

Effect of adding the sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) to the incubation medium on microbial gas production (mL g^{-1} straw) measured over 6 (h) periods and on in vitro organic matter digestibility (IVOMD) of alkali treated wheat straw (experiment 1)

Type of Straw	AQ-SA (% of straw)	IVOMD (%)	Gas Production Time (h)					SEM[1]
			Mean	2-8	8-14	14-20	20-24	Treatment x Time
Sodium hydroxide								
Untreated	-	53.2b	23.3b	14.1bc	26.2c	29.8b	23.2b	1.3***
Straw+3%NaOH	-	62.9c	28.4c	19.3c	31.3c	34.9c	28.1c	
Straw+3%NaOH	0.02	62.5c	27.9c	18.6c	30.0c	34.6c	28.3c	
Straw+3%NaOH	0.05	62.1c	28.4c	17.6c	29.6c	37.7c	29.8c	
Straw+3%NaOH	0.08	60.7c	27.6c	15.4bc	27.8c	37.8c	29.4c	
Straw+3%NaOH	0.2	52.9b	19.0b	11.4ab	14.4b	27.0b	23.0b	
Straw+3%NaOH	0.5	36.0a	10.9a	8.5a	9.9a	11.8a	13.4a	
SEM[1]		1.1***	0.8***					
Ammonium hydroxide								
Untreated	-	46.6a	23.0a	13.7a	27.7a	31.4b	19.2a	0.9***
Straw+4%NH_4OH	-	56.6b	29.0c	21.8bc	34.8c	38.5d	21.0ab	
Straw+4%NH_4OH	0.02	59.9b	27.4bc	21.5bc	32.9bc	34.4c	20.4ab	
Straw+4%NH_4OH	0.05	60.3b	28.5c	21.3bc	32.3bc	38.0d	22.4ab	
Straw+4%NH_4OH	0.08	59.6b	28.5c	19.3bc	33.8bc	36.8cd	24.0b	
Straw+4%NH_4OH	0.2	56.4b	25.8b	18.7b	31.2bc	34.1bc	19.3a	
Straw+4%NH_4OH	0.5	56.7b	25.2b	22.7c	30.7b	27.8a	19.6a	
SEM[1]		2.6***	0.5***					

[1] n=8 for overall gas production, n=2 for IVOMD and treatment by time interaction of gas production means.

a-d Means within the same column not followed by the same letter are significantly different (P<0.05).

*** significant at P<0.001.

TABLE II.2

Effect of anthraquinone 2-sulfonic acid (AQ-SA) and glucose levels on lignin (% of treated material) when used in the ammonium hydroxide treatment of straw.

Glucose added (%)	AQ-SA level (% of straw dry matter) 0	0.03	0.05	0.08	0.2	0.5	Overall mean	SEM[1] glucose	SEM[1] anthraquinone	SEM[1] x glucose
0%	10.4a	9.9a	9.5a	9.4a	9.4a	9.0a	9.6a	0.11***		0.27***
3%	10.5a	10.3a	9.4a	9,0a	8.6ab	8.3b	9.3a			
5%	9.8a	9.5a	9.4a	8.0b	7.9b	5.2c	8.3b			
Overall mean	10.0x	10.3x	8.9y	8.6y	8.9y	7.9z	9.3		0.15***	

[1] n=6 for overall mean of anthraquinone levels, n=12 for glucose levels and n=2 for anthraquinone by glucose interaction.

abc Means within the same column not followed by the same letter are significantly different ($P<0.05$).

xyz Means within the same row not followed by the same letter are significantly different ($P<0.05$).

*** significant at $P<0.001$.

Table II.3

Effect of treating wheat straw with base and various concentrations of the sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) on in vitro organic matter digestibility (IVOMD) and on microbial gas production (mL g^{-1} straw) measured over 6 (h) periods (experiment 3)

Type of Straw	IVOMD (%)	Gas Production Time (h) Mean	2-8	8-14	14-20	20-24	SEM[1] Treatment x Time
Sodium hydroxide							
Untreated	50.0a	22.9a	17.8	26.4	27.7	19.9	1.8[ns]
Straw+3%NaOH	67.4c	29.1b	20.5	33.0	36.2	27.5	
Straw+3%NaOH+0.02AQ-SA	65.3bc	30.6b	23.2	35.1	37.4	26.7	
Straw+3%NaOH+0.05AQ-SA	70.1d	29.3b	22.1	32.2	35.6	27.3	
Straw+3%NaOH+0.08AQ-SA	66.5c	29.8b	24.6	35.8	34.9	25.5	
Straw+3%NaOH+0.2AQ-SA	64.8bc	30.5b	22.0	34.3	38.0	27.6	
Straw+3%NaOH+0.5AQ-SAQ	63.6b	29.5b	23.2	34.7	34.7	25.7	
SEM[1]	0.2***	0.8***					
Ammonium hydroxide							
Untreated	51.6a	15.4a	8.2a	13.2a	19.7a	20.5a	1.1***
Straw+4%NH_4OH	69.8b	25.1c	15.7bc	19.1b	32.6d	33.2c	
Straw+4%NH_4OH+0.02AQ-SA	66.4b	24.1c	18.1c	22.9bc	28.6bc	27.8b	
Straw+4%NH_4OH+0.05AQ-SA	78.4c	35.0e	25.7d	34.6e	42.4e	37.5d	
Straw+4%NH_4OH+0.08AQ-SA	71.8b	30.5d	17.1c	31.1d	39.7e	34.2c	
Straw+4%NH_4OH+0.2AQ-SA	70.6b	23.6c	15.5bc	20.9bc	30.1cd	27.9b	
Straw+4%NH_4OH+0.5AQ-SA	67.9b	20.3b	12.2b	17.8b	25.9b	25.5b	
SEM[1]	1.4***	0.5***					

[1]n=8 for overall gas production, n=2 for IVOMD and treatment by time interaction of gas production means.

a-d Means within the same column not followed by the same letter are significantly different (P<0.05).

ns; not significant at P>0.05; *** significant at P<0.001.

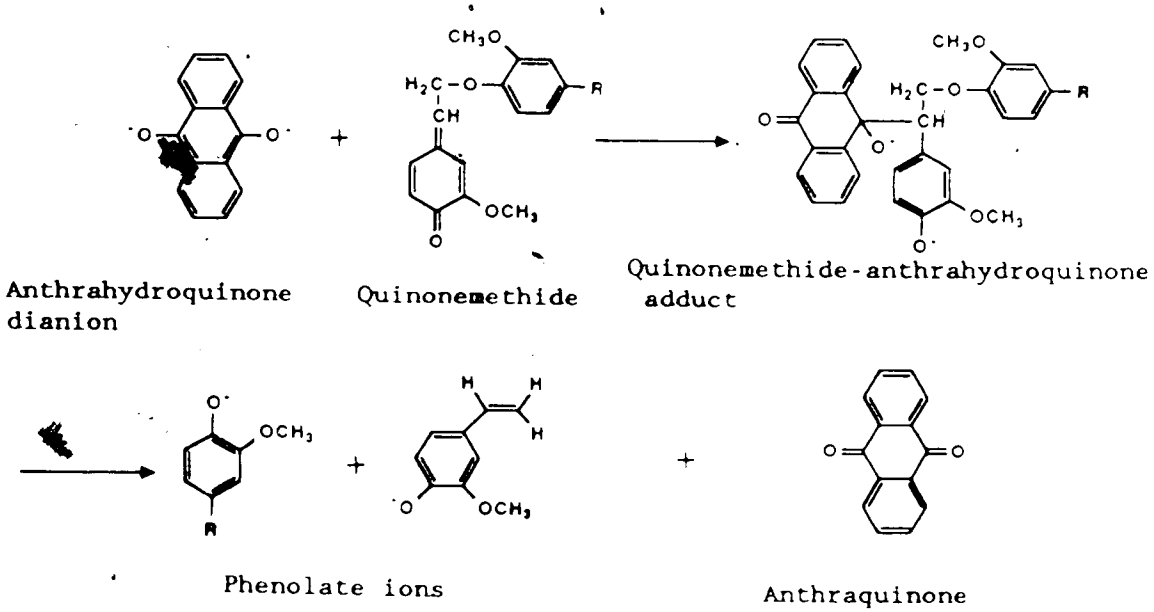

Figure II.1 Double electron theory of lignin fragmentation with anthraquinone (adapted from Haggin, 1984).

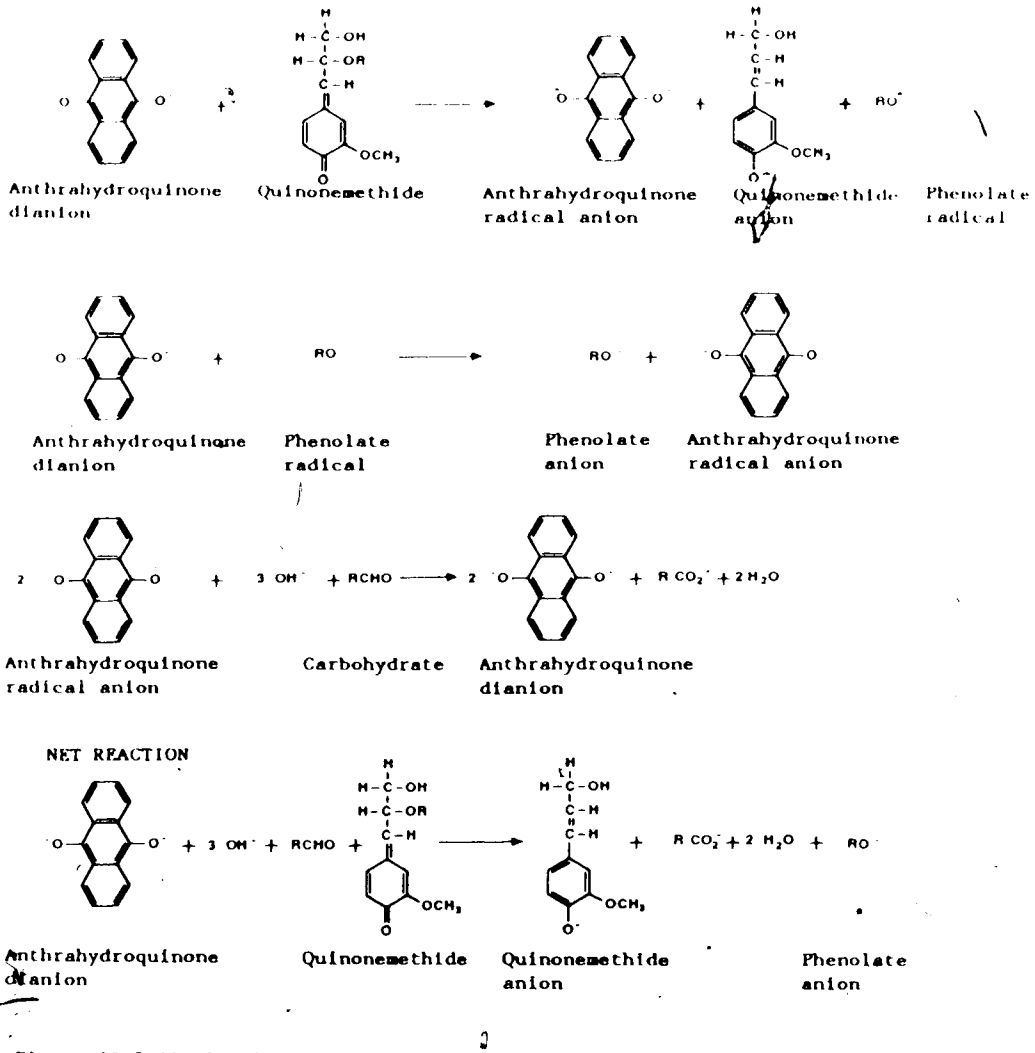

Figure II.2 Single electron transfer theory of lignin fragmentation with anthraquinone (adapted from Dimmel and Palasz, 1984).

III. EFFECTS OF ANTHRAQUINONE 2-SULFONIC ACID, TEMPERATURE, MOISTURE CONTENT AND AMMONIUM HYDROXIDE LEVELS ON IN VITRO ORGANIC MATTER DIGESTIBILITY OF WHEAT STRAW

INTRODUCTION

Cereal straw is an abundant by-product of the grain industry and is high in energy yielding polysaccharides such as cellulose and hemicellulose. However, it is inefficiently utilized by ruminants because it is high in lignin and low in other essential nutrients. The high lignin content decreases the rate and extent of dry matter (DM) digestibility (Darcy and Belyca, 1980). Treatment of straw with NaOH or NH_4OH solubilizes the ester linkages between lignin and cellulose or hemicellulose and the β-aryl ether bonds of lignin (Harbers et al., 1982). In addition treatment of straw with alkali causes the cellulose microfibrils to swell thus enabling the rumen microoganisms to readily degrade the available cellulose or hemicellulose (Evans, 1979).

Various factors influence the effect of bases on the nutritive value of straw. It is well documented that the in vitro organic matter digestibility (IVOMD) of straw treated with bases increases with temperature. Ololade et al. (1970) reported an IVOMD of 59% for barley straw treated with 4% sodium hydroxide (NaOH) at 60°C for 1 h compared with 49% for straw held at 23°C.

Length of treatment could also influence IVOMD. For example, Kiangi and Kategile (1981) demonstrated that straw treated with bases at 100°C for 5 min had an IVOMD of 59% whereas straw treated for 90 min had an IVOMD of 65%.

It has been shown that the moisture content of the material influences the effectiveness of the ammoniation procedure. The IVOMD of oat straw containing 10% moisture and treated at -20°C with 3% NH_3 was 52% which was lower than the IVOMD of 56% obtained when the straw contained 50% moisture (Sundstol et al., 1979).

Treatment of straw with different concentrations of alkali can also influence IVOMD. Ololade et al. (1970) reported that IVOMD for barley straw increased from 38% with 0% NaOH to 81% with 12% NaOH. However, no significant improvement in the IVOMD occured between the 8 and 12% NaOH concentrations.

The recognition of a role for anthraquinone (AQ) and other related compounds in alkaline pulping has created interest in the chemistry of its reactions with lignocellulose materials. These compounds increase the delignification rate of fibrous materials during alkaline pulping (Holton, 1977; Dimmel and Palasz, 1984). Straw has also been treated with AQ for pulping purposes and increases in pulp yield due to increased delignification have been reported (Sandor, 1981).

The objectives of this study were to investigate the effect of the sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) and other anthraquinone compounds with bases on the IVOMD of straw and to examine the relationship of temperature, moisture, bases concentration and length of treatment on the effectiveness of the anthraquinone treatment.

MATERIALS AND METHODS

EXPERIMENT 1 Effect of temperature

Wheat (Triticum vulgare, variety Katepwa) straw was ground through a 1 mm screen and treated with 3% (wt/wt, dry matter (DM) basis) NaOH, 4% ammonium hydroxide (NH_4OH) or with these bases plus 5 mg AQ SA per 10 g straw DM. Straw with no added chemical served as the control. In the treatment procedure 10 g samples of the straw were treated with 80 mL of the solutions containing the chemicals. Samples were held at 4, 22 and 40°C for 5, 9, 17, 28 and 42 days in plastic bags which were tied with rubber bands. In addition, straw samples were also treated in a similar manner in pyrex jars and heated at 100°C for 2, 4, 8 and 24 h. Two samples of each treatment were incubated at each of the different temperatures and time periods. After treatment samples were frozen until analyzed.

DM and IVOMD determinations were carried out after samples had been thawed and any free water evaporated off with a fan to facilitate representative sampling. Since so many samples were involved, only straw treated at one temperature could be included in any single in vitro determination.

EXPERIMENT 2 Effect of moisture level

Samples of ground wheat straw (approximately 100 g DM) were placed in a Rotary mixer (Model C-100, Hobart Manufacturing Company, Ltd., Toronto, Ontario) and water, or a solution containing 50 mg AQ-SA per

100 g straw DM, was sprayed on the straw as it mixed. The amount of water added was calculated to yield a final mixture which contained 15, 25 and 35% water. The straw samples were then transfered to 3.5 liter plastic bags, a 0.3 cm i.d. plastic tube was inserted in the mouth of the bag, held in place with an elastic band and anhydrous ammonia (NH_3) from a cylinder was added to the bag. The amount of NH_3 added to the bag was determined by weighing the bag as the gas was added. Samples of straw treated with only the three levels of water were included as the control treatments. Each treatment was prepared in duplicate and the samples were held at 4 and 22°C for 5, 10, 15 and 20 days. At the end of each treatment period samples were frozen until analysed.

EXPERIMENT 3 Effect of ammonium hydroxide concentration

Levels of 0, 2, 4, or 6% (wt/wt of straw, DM basis) ammonium hydroxide (NH_4OH) were mixed with 25 g water per 100 g straw DM. The solutions containing different amounts of NH_4OH were then mixed with or without 50 mg AQ-SA per 100 g straw and used to treat 100 g ground wheat straw samples. Each treatment was prepared in duplicate and the samples were kept in 3.5 liter plastic bags and held at 22°C for 5, 10, 15 and 20 days. At the end of each treatment period samples were removed and frozen until analysed.

EXPERIMENT 4 Effect of different anthraquinone derivatives

Four percent NH_4OH (wt/wt of straw, DM basis), with or without 50

mg per 100 g straw DM of the sodium salt of anthraquinone 2-sulfonic acid or anthraquinone 2-carboxylic acid, was mixed with water (25% wt/wt of straw, DM basis) and used to treat 100 g barley (Hordeum vulgare, variety Leduc) straw which was ground through a 1 mm screen. The water insoluble anthraquinone and 2-methylanthraquinone derivatives were dissolved in ethanol and then sprayed on the straw while mixing before treatment with 4% NH_4OH. A sample of barley straw with no added chemical was also included as a control. Samples were held at 40°C for 10 days, after which IVOMD was determined.

A 409 kg steer fed 9 kg of timothy brome grass hay served as the source of the rumen fluid for the in vitro experiments which were conducted according to the procedure of Tilley and Terry (1963). After 48 h of incubation with rumen fluid, 2 mL of 6N HCL was added to each sample to lower the pH to 2 and then 5 mL (25 g L^{-1}) pepsin solution was added for digestion of the microbial protein and contents of the flasks were further incubated for 48 h. Organic matter was measured as the loss in weight of DM at 600°C in a muffle furnace oven for 3 h. All samples for DM and IVOMD determinations were done in duplicate. Dry matter analysis was done by the standard methodology (Association of Official Analytical Chemists, 1980).

STATISTICAL ANALYSIS

Experiment 1 conformed to a factorial design with the following model within each temperature:

$$Y_{ijk}=u+T_i+D_j+TD_{ij}+e_{k(ij)}$$

where:

Y_{ijk}-IVOMD;

u-overall mean;

T_i-the effect of the ith treatment;

D_j-the effect of the jth duration;

TD_{ij}-the ith treatment by the jth duration interaction and

$e_{k(ij)}$-the random error term .

Data in this experiment were analyzed using analysis of variance. If significant F-ratios were obtained, means of significant factors and their interations were further tested using the Students-Newman-Keuls' test (Steel and Torrie, 1980).

A similar design was used for experiment 2 with moisture levels, treatment by temperature, treatment by moisture levels and treatment by duration interactions being considered. In experiment 3, amount of NH_4OH added, time and their interaction were the factors used in the analyses. In experiment 4 treatment was the only factor considered in the analyses.

RESULTS

EXPERIMENT 1 Effect of temperature

On the 9th day of treatment mold growth was observed on the surface of straw treated with 3% NaOH and 3% NaOH plus AQ-SA which was held at 22 and 40°C. As the treatment period was extended beyond the 9th day the mold growth increased and became more visible. The pH of the samples was found to have dropped from 10.0 to 5.6 after 9 days of treatment. No signs of mold growth, however, were observed on the straw

treated with NH_4OH or NH_4OH plus AQ-SA and held at these temperatures.

Significant ($P<0.05$) improvements on the overall IVOMD occured in straw treated with NaOH or NH_4OH under all treatment temperatures (Table III.1). Improvements ($P<0.05$) of 6, 3, 9 and 7 digestibility units were obtained in straw treated with NaOH at 4, 22, 40 and 100°C over that achieved with NH_4OH alone. The addition of AQ-SA to NaOH showed no significant ($P>0.05$) differences in IVOMD to that obtained in straw treated with NaOH alone at 22 and 40°C. However, at 4 and 100°C AQ-SA plus NaOH improved ($P<0.05$) the IVOMD by 2 and 6 digestibility units, respectively.

Significant ($P<0.05$) improvements in the IVOMD were obtained when AQ-SA was used with NH_4OH to treat the straw at all temperatures, with improvements ranging from 3 to 9 digestibility units above that obtained with NH_4OH alone (Table III.1). There were no overall differences ($P>0.05$) in IVOMD when AQ-SA was used with NaOH or NH_4OH to treat straw at 4, 22 and 40°C, however, at 100°C NaOH plus AQ-SA was superior ($P<0.05$) to NH_4OH plus AQ-SA (68.6 vs 61.1%).

For straw treated at 4°C and 22°C IVOMD of the NaOH treated straw progressively increased with time up to 17-28 days and declined thereafter (Table III.1 and Appendix III.1). With NH_4OH, maximum IVOMD was attained at 9 to 17 days at these temperatures. At 40°C maximum IVOMD was reached at 5 days for both alkali treatments, while at 100°C the highest IVOMD values were obtained at day 1 which was the longest treatment time. When AQ-SA was used with NaOH or NH_4OH to treat the straw the highest IVOMD were obtained after 9 days of treatment for straw treated at 40°C with both bases. At 100°C the

highest IVOMD obtained with AQ-SA present were not reached until 1 day.

EXPERIMENT 2 Effect of moisture level

Treatment of straw with NH_3 improved ($P<0.05$) the overall IVOMD by 8 digestibility units. When AQ-SA was used with NH_3 to treat the straw IVOMD was improved ($P<0.05$) by 3 units over that attained with NH_3 alone (Table III.2).

There was no significant ($P>0.05$) overall effect of temperature on IVOMD (Table III.2). Treatment of straw with AQ-SA plus NH_3 improved ($P<0.05$) IVOMD by 2 and 3 digestibility units at 4 and 22°C, respectively, above that obtained with NH_3 alone. The two way interaction between treatment and temperature was significant ($P<0.001$), this interaction largely resulted from the smaller effect of temperature on IVOMD of straw treated with NH_3 than on the control or straw treated with NH_3 plus AQ-SA (Table III.2).

The addition of moisture to yield the 25% level in the straw improved ($P<0.05$) IVOMD by 4 digestibility units over that obtained in straw with moisture levels of 15 and 35%, respectively. There were, however, no differences ($P>0.05$) in the mean IVOMD of straw with the 15 or 35% moisture levels. The two way interaction of treatment by moisture was also significant ($P<0.001$). This interaction resulted from smaller differences between the IVOMD of straw containing the 15, 25 or 35% moisture levels with NH_3 treatment alone than in the control or straw treated with NH_3 plus AQ-SA (Table III.2).

An increase ($P<0.05$) in the overall mean IVOMD occured with increasing time of treatment up to 15 days. The IVOMD of straw treated

for 5 and 20 days was not significantly ($P>0.05$) different. The two way interaction of treatment by duration factors was significant ($P<0.001$), and resulted from the decrease in IVOMD of the control straw between 15 and 20 days of treatment in contrast with the increase in IVOMD observed in this time period with treated straw.

EXPERIMENT 3 Effect of ammonium hydroxide concentration

The overall mean IVOMD increased ($P<0.05$) from 51.9 to 73% as the amount of NH_4OH solution added to the straw was increased from 0 to 6% (Table III.3). There was, however, no significant ($P>0.05$) difference in IVOMD between the 4% and 6% concentrations of NH_4OH. When AQ-SA was present, an increase in the overall mean IVOMD above that obtained with NH_4OH alone was only obtained with the 2% NH_4OH treatment. Significant improvements in IVOMD were, however, obtained when AQ-SA was included with the 4% NH_4OH treatment after 15 days and with the 6% NH_4OH treatment at 10 and 15 days (Table III.3).

Treating the straw for 10 and 15 days gave similar ($P>0.05$) IVOMD but the mean IVOMD for these days were higher ($P<0.05$) than the IVOMD obtained in straw treated for 5 and 20 days. The two way interaction of treatment by time factors was significant ($P<0.04$), and occured as a result of the different levels of NH_4OH with, or without AQ-SA, peaking at different time periods (Appendix III.2, Table III.3). The IVOMD of the 2% NH_4OH and 2% NH_4OH plus AQ-SA progressively increased with time up to 20 days. In 4% NH_4OH and 4% NH_4OH plus AQ-SA treatments maximum IVOMD was reached at 10 days of treatment and with 6% NH_4OH plus AQ-SA the maximum IVOMD was not reached until 15

days of treatment.

EXPERIMENT 4 Effect of different anthraquinone derivatives

An improvement ($P<0.05$) of 11 digestibility units was obtained by treating barley straw with NH_4OH (Table III.4). Treatment of the straw with anthraquinone 2-sulfonic acid, anthraquinone 2-carboxylic acid, 2-methylanthraquinone and anthraquinone improved ($P<0.05$) the IVOMD by 7, 9, 12 and 13 digestibility units, respectively, over that achieved in straw treated with 4% NH_4OH alone. There was no significant ($P>0.05$) differences in IVOMD among the anthraquinone derivatives even though anthraquinone and 2-methylanthraquinone gave slightly higher IVOMD than the other compounds (Table III.4).

DISCUSSION

During the treatment of straw a rapid rise in temperature occured after application of NH_3 or NH_4OH into the plastic bags. Waagepetersen and Thomsen (1977) and Sundstol and Owen (1984) also reported a similar observation and suggested that the immediate rise in temperature is of limited significance and that ambient temperature has the greatest effect on the speed of reaction between the chemical and the straw.

The improvement in IVOMD obtained with alkali treatment in these experiments agrees with numerous other reports (Klopfenstein, 1978; Jackson, 1977; Evans, 1979). It has been suggested that alkali treatment results in the breaking of bonds between lignin and

hemicellulose or cellulose, and causes a swelling effect on the cellulose. This increases the digestibility of the straw by the rumen microoganisms (Evans, 1979; Lin et al., 1986). Also, increased IVOMD could occur because alkali treatment separates the contiguous parenchymal cells of the straw and ruptures the inner cuticle from the stem of the plant, as indicated by Harbers et al. (1982).

The increase of 21 units in IVOMD values obtained in experiment 3 after treatment of the straw with increasing concentrations of NH_4OH from 0 to 4% of the straw weight were similar to those obtained by Sundstol et al. (1979) and Sundstol and Owen (1984). These authors also observed no further increase in IVOMD of wheat straw at concentration of more than 5% NH_4OH even though increases in crude protein were observed.

The IVOMD of straw treated with 25% moisture levels was improved by 4 digestibility units above that obtained in straw treated with 15 or 35% moisture levels (Table III.2). The poor IVOMD obtained in untreated straw treated with 35% moisture was attributed to increased mold growth in the material. This observation is in agreement with the results reported by Thorlacius and Robertson (1984) who observed 100% mold growth on high moisture alfalfa hay stored in plastic bags for 21 days. Similar IVOMD values were obtained in straw treated with or without AQ-SA and treated with the 15 and 35% moisture levels. The lower IVOMD obtained in straw treated with these levels of moisture content than with the 25% moisture level did not appear to be caused by mold growth. It is possible that the 15 and 35% added moisture content may not have been well distributed in the straw which may have reduced the effectiveness of the chemicals on the material.

Treatment of the straw at 40°C for more than 17 days resulted in increased mold growth which may have resulted from the drop in pH due to the formation of acidic products. The rather similar overall means of IVOMD obtained in straw treated at 4 and 22°C in experiment 2 may have occured because of the treatment time of 20 days being long enough to cancel any differences due to temperature.

AQ-SA improved mean IVOMD of straw by -1 to 5 digestibility units with NaOH and 1 to 9 digestibility units with NH_4OH in the various experiments. Holton (1977), Fleming et al. (1978) and Bryce (1980) reported that when 0.06% AQ-SA was added to a solution of NaOH and heated with wood, increased pulp yield due to increased delignification occured. Fleming et al. (1980) and Dimmel and Palasz, (1984) reported that the AQ compounds during the reaction cycled through an oxidation-reduction process, and transfered electrons to active sites of the solubilized lignin β-ether bonds thus preventing any reversal reactions. It is also reported that the increased amount of alkali consumed during pulping of wood, resulting in the formation of glucuronic acid, was prevented by the presence of AQ compounds (Bryce, 1980).

It was found that treatment of barley straw with NH_4OH plus the 2-methylanthraquinone or anthraquinone resulted in slightly higher IVOMD values than when anthraquinone 2-sulfonic acid or anthraquinone 2-carboxylic acid were used (Table III.4). These results agree with the results of Holton (1977), Virkola et al. (1981) and Werthemann (1981) who found increased effectiveness of these compounds on the fibrous by-products, and suggested that the non-polar electron donating groups of these compounds appear to enhance their activity. The sulfonate and

carboxylic group of the AQ-SA and anthraquinone 2- carboxylic acids in contrast, are electron withdrawing groups which, although they enhance the compounds solubility in alkaline solution, reduces the effectiveness of the additives.

The interactions for length of treatment and alkali with or without AQ-SA obtained in experiment 1 agrees with reports by Waagepetersen and Thomsen (1977) and Sundstol et al. (1979). It appears that higher IVOMD may be obtained in a shorter time in straw treated with AQ-SA plus alkali or base than when the alkali is used alone. Bryce (1980) and Clayton (1970) reported that the extended long treatment time of wood with alkali resulted in acidic degradation products which reduced the effectiveness of the alkali. It was also found that this problem was prevented by the addition of AQ compounds (Bryce, 1980).

The results obtained in these experiments confirmed that temperature, moisture, amount of alkali and treatment time influenced the effectiveness of the alkali on the straw. The addition of AQ-SA significantly improved the IVOMD of straw treated with NH_4OH and of straw treated with NaOH at 4°C and 100°C. Slightly greater increases in IVOMD may have been obtained if anthraquinone or 2- methylanthraquinone had been used rather than anthraquinone 2-sulfonic acid because of their increased affinity to the non-polar electron-donating groups of these compounds. On the basis of these results, it would be predicted that the nutritive value of crop residues used as feed for the ruminant animals would be improved by using anthraquinone and its derivatives. In vivo trials are necessary to confirm this possibility.

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TABLE III.1

Effect of base and 0.05% (wt/wt) anthraquinone 2-sulfonic acid (AQ SA) on the in vitro organic matter digestibility (%) of wheat straw treated at different temperatures

Time (Days)	Control	3%NaOH	3%NaOH +AQ-SA	4%NH_4OH	4%NH_4OH +AQ-SA	SEM[1] Overall means	SEM[1] Time*Treatment
Temperature of 4°C							
5	43.0a	54.6b	60.7c	53.6b	57.8bc		1.1***
9	42.0a	56.1b	60.9c	55.7b	62.9c		
17	44.1a	57.1bc	63.6d	54.6b	60.8cd		
28	38.1a	61.5c	57.7c	51.4b	57.8c		
42	34.3a	59.8d	57.0cd	42.5b	55.0c		
Overall means	40.3a	57.8c	60.0d	51.6b	58.9cd	0.5***	
Temperature of 22°C							
5	50.2a	74.2bc	76.4c	72.0b	76.4c		1.4***
9	49.6a	72.5b	77.4c	72.0b	78.3c		
17	50.4a	76.3b	77.2b	75.7b	79.7c		
28	46.8a	72.5c	74.5c	68.0b	72.8c		
42	41.6a	74.9d	61.1b	68.4c	62.7b		
Overall means	47.7a	74.1c	73.3c	71.2b	74.0c	0.6***	
Temperature of 40°C							
5	48.8a	73.5b	69.2b	70.6b	76.7c		1.6***
9	44.2a	72.7c	74.0d	62.3b	80.0e		
17	49.1a	70.3d	69.2cd	56.7b	65.4c		
28.	50.7a	68.3bc	73.3c	54.5a	65.0b		
42	43.3a	57.8b	66.5c	53.5b	57.6b		
Overall means	47.2a	68.5c	70.4c	59.5b	68.9c	0.7***	
Temperature of 100°C							
0.08	44.9a	58.1c	60.0c	51.3b	54.9bc		0.3***
0.17	47.5a	60.1b	64.6c	55.1b	57.7b		
0.33	48.3a	66.2c	72.6d	59.0b	63.9c		
1.0	50.2a	69.2c	77.1d	59.8b	67.7c		
Overall means	47.7a	63.4d	68.6e	56.3b	61.1c	0.2***	

[1]n=10 for overall means and n=2 for duration by treatment interaction.
a-e Means within the same row not followed by the same letter are significantly different (P<0.05).
***significant at (P<0.001).

TABLE III.2

Effect of 0.05% (wt/wt of straw) anthraquinone 2-sulfonic acid (AQ-SA) and NH_3 on in vitro organic matter digestibility (%) of wheat straw treated at different moisture levels, temperature and times

Item	Overall mean	Treatment Interactions: Control	4%NH_3	4%NH_3+AQ-SA	SEM[1]
Temperature (°C)					
4	52.9x	46.4a	55.2b	57.2c	0.38***
22	54.0x	47.6a	55.7b	58.7c	0.38***
Overall mean	53.5	47.0a	55.4b	58.0c	0.27***
SEM[1]	0.22[ns]				
Moisture (%)					
15	52.3x	45.2a	55.2b	56.6b	0.47**
25	56.5y	51.0a	57.0b	61.4c	0.47**
35	51.6x	44.8a	54.1b	56.0b	0.47**
Overall mean	53.5	47.0a	55.4b	58.0c	0.27**
SEM[1]	0.27***				
Duration of treatment (days)					
5	50.9x	45.7a	52.3b	54.7c	0.54***
10	54.9y	52.1a	54.4b	58.3c	0.54***
15	57.3z	47.4a	61.1b	63.4c	0.54***
20	50.7x	42.7a	53.9b	55.4c	0.54***
Overall mean	50.7	42.7a	53.9b	55.4c	0.27***
SEM[1]	0.31***				

[1] n=72 for temperature, n=48 for moisture, n=36 for duration, n=24 for temperature by treatment interaction, n=16 for treatment by moisture interaction and n=12 for duration by treatment interaction.

abc Means within the same row among treatment interactions not followed by the same letter are significantly different (P<0.05).

xyz Means within the overall means not followed by the same letter are significantly different (P<0.05).

ns, not significant at (P<0.05); **,*** significant at P<0.01, P<0.001.

Table III.3

Effect of concentration of ammonium hydroxide (NH_4OH) with 0.05% (wt/wt of straw) anthraquinone 2-sulfonic acid on in vitro organic matter digestibility (%) of wheat straw treated at 22^0C

Time (days)	Treatment								SEM[1]
	Control	2%NH_4OH	2%NH_4OH +AQ-SA	4%NH_4OH	4%NH_4OH +AQ-SA	6%NH_4OH	6%NH_4OH +AQ-SA	Overall mean	Treatment x Time
5	49.0a	55.3b	62.2c	68.9e	66.2d	69.6e	68.3e	62.8w	0.32***
10	52.1a	63.0b	66.1c	75.9e	78.4f	72.3d	76.9e	69.2y	
15	52.1a	64.2b	66.4c	70.1d	75.4e	76.1e	79.7f	69.1y	
20	54.4a	66.0b	69.1c	69.3c	68.9c	74.1d	73.0d	67.8x	
Treatment means	51.9a	62.1b	65.9c	71.0d	72.2d	73.0de	74.5e	67.2	0.27***
SEM[1]								0.05***	

[1]n=8 for treatment, n=14 for time and n=2 for time by treatment interaction.
a-f Means within the same row not followed by the same letter are significantly different (P<0.05).
wxy Means within the same column not followed by the same letter are significantly different (P<0.05).
*** significant at P<0.001.

TABLE III.4

Effect of 4% ammonium hydroxide (NH_4OH) and anthraquinone derivatives (0.05% wt/wt of straw) on the in vitro organic matter digestibility (IVOMD) of barley straw*

Treatment	IVOMD (%)
Control	57.0a
NH_4OH	68.0b
NH_4OH+anthraquinone 2-sulfonic acid	75.1c
NH_4OH+anthraquinone 2-caboxylic acid	76.7c
NH_4OH+anthraquinone	80.3c
NH_4OH+2-methylanthraquinone	80.6c
SEM[1]	1.6***

[1] n=2

abc Means within the same column not followed by the same letter are significantly different (P<0.05)

*** significant at P<0.001

IV. EFFECT OF ANTHRAQUINONE 2-SULFONIC ACID ON THE NUTRITIVE VALUE OF BARLEY STRAW FOR EWES

INTRODUCTION

It is estimated that about 1/8 million tonnes of barley straw are produced annually on a world wide basis (FAO, 1985). Most of the barley straw is either burnt or ploughed back into the soil to improve the soil structure and to act as a potentially important source of plant nutrients (Horton, 1978).

As a feed for livestock, cereal straws have always been a second choice to grass and legumes (Anderson, 1978; Horton, 1978; Chesson, 1980) since straw is low in most nutrients required by the ruminant animal. Most cereal straws are characterized by low protein, high lignin and low digestible and metabolizable energy content (Van Soest, 1982; Lin et al., 1986). Chesson (1980) and Stoskopf (1986) have reported that barley straw contains a high content of polysaccharides (44% cellulose and 28% hemicellulose) which can serve as a potential source of dietary energy for ruminants. Theander and Aman (1984) stated that the structural polysaccharides which comprise the carbohydrate fraction are, however, only partially degraded by rumen microoganisms because of the cross linkages which form between lignin and the carbohydrates as the plant matures.

Processing of cereal straw by physical or chemical means to improve digestibility and nutritive value has received considerable attention in recent years. Knipfel et al. (1981) reported that improving the nutritive value of straw, by using chemicals such as anhydrous ammonia

(NH_3) or by alteration of the fertility level of the soil, has the benefits of increasing the quality of the roughage and providing the opportunity to include a larger proportion of the straw in the diet of the ruminant animal. This can be of considerable importance in times of conventional hay shortage or when supplemental feed sources such as grains are expensive. It has been reported that intake and digestibility of cereal straw by the ruminant animal increases significantly after treatment with NH_3. Knipfel et al. (1981) and Sundstol and Owen (1984) suggested that the increased intake of cereal straw after ammoniation was attributed to increases in the crude protein content which made the material more palatable.

Studies conducted in the pulping and paper industries have shown that anthraquinone and its derivatives accelerate the rate of delignification of fibrous by-products during alkaline pulping (Holton, 1977; Dimmel and Palasz, 1984). Results presented in chapters 2 and 3 demonstrated that anthraquinone 2-sulfonic acid (AQ-SA) also causes delignification of straw which supports the results of (Holton, 1977; Fleming et al., 1980 and Dimmel and Palasz, 1984). In addition, when straw was treated with either NH_4OH or NaOH plus a level of 0.05% AQ-SA (wt/wt) an improvement in the in vitro organic matter digestibility (IVOMD) was obtained. Anthraquinone has to date not been used to treat fibrous by-products such as cereal straws for feeding farm animals.

The objective of this study was to investigate the effect of anthraquinone 2-sulfonic acid treatment on the nutritive value of barley straw for ewes.

MATERIALS AND METHODS

The barley (Hordeum vulgare, variety Leduc) straw used in these studies was chopped through a 2.5 cm size screen using a tub grinder. In experiment 1 three piles of barley straw of approximately 172 kg each were formed on polyethylene sheets. The first and second piles of straw were sprinkled with 10% water (wt/wt), with the straw and water being thoroughly mixed with a rake as the water was added. The third pile was sprayed with a 10%, of a solution providing 0.05% (wt/wt of straw, dry matter basis) of the sodium salt of anthraquinone 2 sulfonic acid (AQ-SA) and mixed in a similar manner. Each of the piles of straw were then covered with the plastic and sealed. Anhydrous ammonia (4% wt/wt of straw, air dry basis) was added to the pile sprayed with AQ-SA solution and to one pile sprayed with water, over a time period of approximately 20 min, through a 10 m length of plastic tubing (2.5 cm diameter). The three piles were left outside for a period of 20 days before being used for feeding. During this time the mean, mean maximum and mean minimum temperatures were -3, 0 and -5°C respectively. In experiment 2, two piles of the same weight were treated with NH_3 or NH_3 plus AQ-SA in a similar manner and were kept in a barn heated to a temperature of 17°C for a period of 10 days before opening.

Two feeding trials were conducted at different time periods to evaluate the straw treated at 0° and at 17°C. In the first experiment, three groups of four Suffolk ewes with an average weight of 43 kg were randomly assigned to the control, NH_3 or NH_3 plus AQ-SA straw treatments. The experimental regimen consisted of an ad libitum intake period of 18 days, followed by a 5 day adjustment period to a feeding

level of 600 g straw and then by a 7 day fecal and urine collection period. In addition to the straw each animal received 254 g of concentrate DM (Table IV 1) at 0800h. The sheep were held in individual feeding pens for the voluntary intake period and were transfered to metabolic crates for the digestibility study. Animals were weighed at the start and end of the voluntary intake and digestibility periods.

Samples of rumen fluid were taken using a stomach tube from each animal on the last day of the digestibility study and processed according to the procedure of Kellogg (1969) for subsequent analysis of volatile fatty acids (VFA). The rumen fluid was analysed for individual VFA by Varian 3700 gas chromatography, using an OV-351 (30 m x .25 mm ID) fused silica capillary column. The oven temperature was programmed to increase from 130°C to 180°C at 10°C min^{-1} and held at 180°C for 3 min. Caproic acid was used as internal standard for quantitation. Peak areas were obtained using a Hewlett Packard 3500 computer system with the chromatograph for integration. Analysis for anthraquinone in the urine samples was done using a high performance liquid chromatograph (Varian 5500) by the procedure of Nilsson and Samuelson (1982).

Experiment 2 was conducted in a similar manner except that two groups of five Suffolk ewes weighing 50 kg were assigned to NH_3 and NH_3 plus AQ-SA straw treatments. The animals were held in metabolic crates throughout the experiment and straw intake was set at 700 g of (as-fed basis) during the digestibility period.

Weights and samples of feed offered and refused were taken daily during the last 7 days of the voluntary intake and during the digestibility study in both experiments. Daily urine and fecal collection were also made for each animal during the 7 day

digestibility study.

Samples of each of the straws fed during the digestibility studies were further ground through a 1 mm screen and used for in vitro organic matter digestibility (IVOMD) determinations. The rumen fluid used in the in vitro procedure was collected from a 409 kg steer which was fed 9 kg timothy bromegrass hay daily. The procedure of Tilley and Terry (1963) was followed for the incubation of the straw with rumen fluid. After 48 h, 2 mL 6N HCL was added to each flask to lower the pH to 2 and then 5 mL (25 $g \cdot L^{-1}$) pepsin solution was also added to each flask, and the flasks were further incubated for a period of 48 h. Organic matter digestibility was measured as the loss in weight of DM at 600°C in a muffle furnace oven for 3 h. All samples for IVOMD determinations were run in duplicate.

Feeds, refused feed, feces and urine samples were analysed for dry matter (DM) and nitrogen (N) by standard methodology (Association of Official Analytical Chemists, 1985). Gross energy was determined by using an adiabatic bomb calorimeter (Model no. 1241 Parr Instrument Company, Inc. Moline, Illinois). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the method described by Goering and Van Soest (1970). Analysis for lignin was conducted using the 72% sulfuric acid method as described by Goering and Van Soest (1970). The digestible energy of straw was calculated by estimating the amount of fecal energy attributable to soybean meal and barley grain using digestible energy values from the National Research Council (1985) sheep bulletin.

STATISTICAL ANALYSIS

Data for apparent digestibility, nitrogen retention, voluntary intake, estimated voluntary digestible energy intake for straw and the total diet, and the ratio of acetic to propionic acid were subjected to a one way analysis of variance, with three or two treatment levels in experiment 1 and 2, respectively. If significance F ratios were obtained in experiment 1 differences among treatment means were further tested using the Student Newman Keuls' test (Steel and Torrie, 1980). The chemical composition and the IVOMD of the straws were also compared using a one way analysis of variance procedure.

RESULTS

EXPERIMENT 1

The crude protein content of the straw was increased ($P<0.05$) from 3.7 to 7.6% when 4 %NH_3 (wt/wt, air dry basis) was added to the straw. When NH_3 plus AQ-SA was used to treat the straw the crude protein was 7.9% (Table IV.2). The NDF content of the straw was decreased ($P<0.05$) by treatment with NH_3 or NH_3 plus AQ-SA compared with the control. There was a slight, though nonsignificant reduction in the ADF and lignin when straw was treated with NH_3 and NH_3 plus AQ-SA. Treatment of straw with NH_3 and NH_3 plus AQ-SA resulted in similar ($P>0.05$) values of IVOMD, however, both values were higher ($P<0.05$) than the IVOMD value obtained in the untreated straw.

Sheep fed straw treated with ammonia increased their intake

slightly, but the intake was not significantly ($P>0.05$) different from that obtained from the sheep fed the untreated straw (Table IV.3). Treatment of straw with NH_3 plus AQ-SA resulted in slightly higher ($P>0.05$) intakes of straw DM by sheep than that achieved when NH_3 treated straw was fed.

The digestibility of all straw components examined were increased ($P<0.05$) by NH_3 treatment as compared with untreated straw (Table IV.3). Treatment with NH_3 plus AQ-SA increased ($P<0.05$) the digestibility of the gross energy by a further 6 digestibility units but had no significant ($P>0.05$) influences on the other items even though in general digestibilities were slightly higher when AQ-SA was present. The estimated straw digestible energy content of 5.9, 9.3 and 10.6 MJ Kg^{-1}DM untreated straw, straw treated with NH_3, and straw treated with NH_3 plus AQ-SA, respectively, were all significantly different ($P<0.05$).

The estimated voluntary digestible energy intake of digestible energy from straw by sheep were 189, 313 and 436 KJ $Kg^{-.75}$ d^{-1} for the untreated straw, straw treated with NH_3, and straw treated with NH_3 plus AQ-SA, respectively ($P<0.05$). The estimated total voluntary digestible energy intake of 592 KJ $Kg^{-.75}$ obtained in the group fed straw treated with NH_3 and the concentrate was not different ($P>0.05$) from the 714 KJ $Kg^{-.75}$ obtained in the group fed straw treated with NH_3 plus AQ-SA and the concentrate (Table IV.3). However, these mean values were higher ($P<0.05$) than 470 KJ $Kg^{-.75}$ obtained in the group fed untreated straw.

The calculated N intake by the group of animals fed untreated straw and for those fed NH_3 or NH_3 plus AQ-SA straw treatments were

different ($P<0.05$). Also, the urinary N excretions from the group fed straw treated with NH_3 and NH_3 plus AQ-SA were similar and higher ($P<0.05$) than the urinary N obtained in the group fed untreated straw (Table IV.3).

The concentrations of acetic acid in the rumen fluid of animals fed untreated, NH_3, and NH_3 plus AQ-SA straw treatments were not ($P>0.05$) different (Table IV.4). The differences in the concentrations of propionic acid obtained among the groups fed the different straw diets were significant ($P<0.05$). There was no major ($P>0.05$) differences in the ratio of acetic to propionic acid between the groups of animals fed the different straw diets.

EXPERIMENT 2

During the application of the NH_3 in the piles of straw kept in the barn, a rapid increase in heat was produced in the piles. The piles of straw remained very warm for approximately 1 day then cooled slowly to ambient temperature by 3 days after treatment. At the time of feeding, it was noted that the texture of the straw treated at 17°C was softer than straw treated with NH_3 at 0°C.

The straws treated with NH_3 and NH_3 plus AQ-SA did not differ in crude protein and NDF content (Table IV.2). The ADF and lignin contents were reduced ($P<0.05$) by 0.7 and 2.4 percentages in straw treated with NH_3 plus AQ-SA. The IVOMD was improved ($P<0.05$) by 6 percentage units in straw treated with NH_3 plus AQ-SA above that obtained in straw treated with NH_3 alone (Table IV.2).

Voluntary DM intake of straw and of the total diet by the sheep

were increased ($P<0.05$) by 19 and 15%, respectively, in straw treated with NH_3 plus AQ-SA (Table IV.5). The digestibility of DM, gross energy, crude protein and NDF were each increased ($P<0.05$) by 4 percentage units in straw treated with NH_3 plus AQ-SA. There were no differences ($P>0.05$) in the digestibility of the ADF and lignin fractions of the diets (Table IV.5). The estimated digestible energy content of the diet and the treated straw were 12 and 10 MJ Kg^{-1}DM, respectively, when AQ-SA treated straw was used. These values were higher ($P<0.05$) than 11 and 9 MJ Kg^{-1}DM obtained in the group fed straw treated with NH_3 alone. Furthermore the estimated voluntary digestible energy intake of straw treated with NH_3 plus AQ-SA and of the total diet were increased ($P<0.05$) by 33 and 21%, respectively, above that obtained with the NH_3 treatment alone.

There were significant ($P<0.05$) differences in the calculated N intake and the N excreted in the fecal matter of sheep fed straw treated with NH_3 and straw treated with NH_3 plus AQ-SA (Table IV.5). Nitrogen retained by both groups of sheep fed the two diets did not differ ($P>0.05$).

Feeding the animals straw treated with NH_3 and NH_3 plus AQ-SA did not result in significant ($P>0.05$) differences in the concentration of acetic, or propionic acid in the rumen or in the ratio of acetic to propionic acid (Table IV.4).

DISCUSSION

The apparant rapid rise in temperature which occured as the NH_3 was injected into the piles of barley straw agrees with the observation

reported by Sundstol and Owen (1984). According to Waagepetersen and Thomsen (1977) the increase in temperature varies between 40 and 60°C, depending on the temperature at the start, dosage of ammonia, moisture content of the material, and other factors.

Ammoniation increased the crude protein content of the straw by 10% in straw treated at 0°C. This increase is in agreement with the results of Horton (1978) and that reported in (Annonymous, 1987) and confirm that the ammoniation procedure has an added advantage over other treatment procedures in that it improves the crude protein of crop residues.

The effect of NH_3 in not reducing the lignin component of straw agrees with the results of Lin et al. (1986). The lower lignin content obtained in straw treated with NH_3 plus AQ-SA than with NH_3 alone at 17°C agrees with the results obtained with wood by Holton (1977), Fleming et al. (1980) and Dimmel and Palasz, 1984). It has been suggested that the β-aryl ether bonds of lignin accept electrons from anthrahydroquinone which rapidly fragments the β-linkages to form phenolic products which are alkali or water soluble. The higher ($P<0.05$) IVOMD obtained in the straw treated with NH_3 plus AQ-SA than in the straw treated with NH_3 at 17°C was correlated to this decrease in lignin in the straw, which agrees with the suggestion that lignin in the straw reduces the digestibility of the plant dry matter by the rumen microoganisms (Chesson, 1980; Theander and Aman, 1984). In addition, it has been speculated that NH_3 may have caused a swelling effect on the plant cellulose which enabled the rumen microoganisms to penetrate and readily degrade the straw (Evans, 1979; Chesson, 1980).

The significant improvement in feed intake obtained in straw

treated with NH_3 agrees with numerous other results (Horton, 1978, Knipfel et al., 1981; Saenger et al., 1983). The increased nitrogen content of the ammoniated straw may have caused the straw to be consumed more readily by the sheep. In addition the increased feed intake may have occured due to a more rapid reduction in particle size during chewing and rumination of the straw treated with NH_3 (Zorrilla-Rios et al., 1985). The increased feed intake obtained in straw treated with AQ-SA plus NH_3 was correlated with the decreased lignin content of the straw.

The increase in digestibility of DM, crude protein and gross energy obtained in both experiment 1 and 2 when straw was treated with NH_3 are in agreement with the results of Horton (1978), Saenger et al. (1983) and Knipfel et al. (1981). The significantly higher digestibility of DM, crude protein and NDF obtained in straw treated with NH_3 plus AQ-SA in comparison with NH_3 at 17°C but not at 0°C suggests that AQ-SA may not have been effective at 0°C. However, improvements in gross energy digestibility were obtained with straw treated at 0°C (Table IV.3) and improvements in IVOMD were obtained at 4°C in wheat straw treated with AQ-SA (chapter 3) which suggests that AQ-SA may be effective even at these low temperatures. It is well documented that ammoniation of straw at low temperatures does not improve the feed value of the material as much as when ammoniation is carried out at a higher temperature.

The high values of N retention obtained from animals fed the control, NH_3 and NH_3 plus AQ-SA straw diets treated at 0°C was related to the relatively large amount of concentrate being fed. Lawlor et al. (1981) also reported increased N retention in steers fed a high

concentrate supplement with untreated or ammoniated straw.

The higher levels of propionic acid obtained in the group of animals fed NH_3 or NH_3 plus AQ SA treated straw than animals fed untreated straw in experiment 1 is consistent with other results (Horton, 1978; Knipfel et al., 1981). Greater amounts of propionic acid are produced with diets of increased digestibility and increased ruminal passage rates (Horton, 1978; Saenger et al., 1983).

No deleterious effect on animal health were observed in the group fed straw treated with NH_3 plus AQ-SA. Urine samples of two ewes fed the straw treated with NH_3 plus AQ-SA were analysed for anthraquinone and it appeared that only approximately 1% of the daily dose was excreted in the urine. It is still not completely clear how anthraquinones are metabolized by mammals, although Martin et al. (1983) established that 1-methylaminoanthraquinone (MAAQ) was metabolized through three major metabolic mechanisms by a lactating sheep: N-demethylation, 2-and 4-Hydroxylation of the amino sustituted ring, and conjugation of MAAQ and its metabolites with glucuronic acid. Zanella et al. (1979) tested the toxicity of AQ on Daphna magnia fish and found that the chemical was nontoxic to the animals. More work is needed to be able to make any conclusive statements about the toxicology and metabolic behavior of the AQ compounds by ruminant animals.

It appears that the procedure of using AQ-SA with NH_3 offers a new potential method which may improve the feed value of fibrous materials used as feed for ruminant animals. According to the National Research Council (1985) sheep bulletin, it can be calculated that a digestible energy intake of 504 KJ $kg^{-.75}$ would be required for

maintenance of a 50 kg sheep fed a diet containing 10 MJ DE kg^{-1}DM. The sheep used in these experiments ate enough straw treated with NH_3 plus AQ-SA to provide approximately 440 KJ $kg^{-.75}$ of digestible energy which means that sheep fed the AQ-SA treated straw would need to be supplemented with an additional source of energy to achieve a maintenance feeding level. However, with beef cattle it might not be necessary to provide supplemental energy in NH_3 plus AQ-SA treated diets. Grings and Males (1987) found no significant difference in weight gains of cows receiving NH_3 treated wheat straw alone or a mixture of untreated straw and bromegrass haylage before calving and that positive weight gains were observed with both treatments. Since AQ-SA treatment appears to improve the effectiveness of the ammoniation procedure, AQ-SA treated straw may thus provide an adequate energy intake for the maintenance of cattle with the possibility of some productive function being supported as well.

The results obtained in these experiments demonstrated that the ammoniation procedure improved the nutritive value of straw. Sheep increased straw voluntary intake by 15-19% when AQ-SA was included in [redacted]mmoniation procedure and the digestibility of energy in straw was improved by 11-14%. The intake of digestible energy from the straw by the sheep was thus increased by 32-39%. Further studies with AQ compounds are warranted.

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TABLE IV.1

Ingredients and composition of concentrate supplement.

Item	Amount
Ingredient (% air dry basis)	
Barley, dry rolled	65.0
Soybean meal	32.1
Calcium carbonate[1]	1.40
Salt	1.36
Selenium premix[2]	.10
Vitamin A premix[3]	.04
Vitamin E premix[4]	.01
Analysis of dry matter	
Dry matter (%)	87.40
Crude protein (%)	21.60
Acid detergent fiber (%)	2.62
Neutral detergent fiber (%)	7.57
Lignin (%)	0.25
Gross energy MJ Kg^{-1}	4.42

[1]Calcium carbonate contained 38.5% Ca.
[2]Selenium premix contained 200mg Se kg^{-1}.
[3]Vitamin A premix contained 10,000,000 IU vitamin A kg^{-1}.
[4]Vitamin E premix contained 500,000 IU vitamin E kg^{-1}.

TABLE IV.2

Crude protein, cell wall constituents and in vitro organic matter digestibility of control and treated barley straw in experiments 1 and 2

Item	Experiment 1[1]				Experiment 2[2]		
	Control	NH_3	NH_3+AQ-SA	SEM	NH_3	NH_3+AQ-SA	SEM
Crude protein (%)	3.7a	7.5b	7.9c	0.1***	9.5	9.4	0.1^{ns}
Neutral detergent fiber	83.2c	81.1b	80.0a	0.2***	76.7	76.7	2.4^{ns}
Acid detergent fiber	55.3a	55.3a	55.0a	0.5^{ns}	53.1	51.6	0.2*
Lignin (%)	7.6a	7.2a	6.8a	0.2^{ns}	6.4	5.7	0.1**
IVOMD (%)	55.0a	60.0b	60.9b	0.3***	63.2	67.4	0.5**

[1]Treatments were control straw, straw treated with 4% (wt/wt) NH_3 and straw treated with 4% NH_3 plus 0.05% anthraquinone 2-sulfonic acid (AQ-SA) at 0^0C, n=2.

[2]Treatments were straw treated with 4% (wt/wt) NH_3 and straw treated with 4% NH_3 plus 0.05% anthraquinone 2-sulfonic acid (AQ-SA) at 17^0C, n=2.

abc Means within the same row not followed by the same letter are significantly different (P<0.05).

ns, not significant, (P>0.05).

*,**,*** significant at P<0.05;P<0.01; P<0.001.

TABLE IV.3

Voluntary feed intake, digestibility and estimated voluntary digestible energy intake by sheep when fed control, ammoniated (NH_3) and ammoniated (NH_3) plus anthraquinone 2-sulfonic acid (AQ-SA) barley straw treated at 0°C (experiment 1)

Item	Treatment			SEM
	Control	NH_3	NH_3+AQ-SA	
No. of animals	4	4	4	
Animal wt. (kg)	42.8	43.3	43.5	1.6^{ns}
	Voluntary DM intake			
DM intake (g $Kg^{-.75}$)				
Straw	31.9a	35.6ab	41.1b	2.2*
Total diet	47.2a	50.7ab	56.1b	2.2*
Estimated digestible energy intake (KJ $Kg^{-.75}$)				
Straw	189a	313b	436c	26.4***
Total diet	470a	592b	714b	26.0***
	Digestibility period			
DM intake (g $Kg^{-.75}$)				
Total diet	40.8	43.6	42.8	1.1^{ns}
Straw intake in diet (%)	62.7	65.4	64.8	
Apparent digestibility (%)				
Dry matter	52.9a	62.5b	64.6b	0.7***
Gross energy	51.5a	61.4b	64.5c	0.7***
Crude protein	55.8a	62.3b	64.2b	1.2***
Neutral detergent fiber	36.8a	57.8b	58.6b	1.4***
Acid detergent fiber	37.3a	59.7b	59.9b	1.8***
Lignin	11.1a	23.0b	20.4b	2.5***
Digestible energy(MJ kg^{-1}DM)				
Diet	9.3a	11.3b	12.2c	4.3***
Estimated straw	5.9a	9.3b	10.6c	0.2***
Urinary energy (KJ day^{-1})	310	384	486	43.8^{ns}
Nitrogen balance (g day^{-1})				
Intake	11.2a	14.5b	14.7c	0.02***
Fecal	4.8	5.5	5.2	0.2^{ns}
Urinary	4.3a	7.0b	8.1b	0.5***
Retention	2.2	2.2	1.3	0.5^{ns}

abc Means within the same row not followed by the same letter are significantly different (P<0.05).
ns, not significant at P>0.05.
*,**,*** significant at P<0.05; P<0.01; P<0.001.

TABLE IV.4

Effect of feeding ammonia (NH_3) treated and ammonia (NH_3) plus anthraquinone 2-sulfonic acid (AQ-SA) treated barley straw on acetic and propionic acid concentrations (m mol L^{-1}) in rumen liquor in experiments 1 and 2

Item	Experiment 1[1]				Experiment 2[2]		
	Control	NH_3	NH_3+AQ-SA	SEM	NH_3	NH_3+AQ-SA	SEM
Acetic acid	38.4	46.8	44.2	2.2^{ns}	42.4	38.6	3.2^{ns}
Propionic acid	8.6a	12.3c	10.3b	0.4***	10.1	9.6	1.0^{ns}
Acetic/ propionic	4.5	3.9	4.4	0.2^{ns}	4.2	4.1	0.2^{ns}

[1]Treatments were control straw, straw treated with 4% (wt/wt) NH_3 and straw treated with 4% NH_3 plus 0.05% anthraquinone 2-sulfonic acid (AQ-SA) at 0°C, n=4.

[2]Treatments were straw treated with 4% (wt/wt) NH_3 and straw treated with 4% NH_3 plus 0.05% anthraquinone 2-sulfonic acid (AQ-SA) at 17°C, n=5.

ab Means within the same row not followed by the letter are significantly different ($P<0.05$).

ns, not significant at ($P>0.05$); *** significant at $P<0.001$

TABLE IV.5

Voluntary feed intake, digestibility and estimated voluntary digestible energy intake by sheep when fed ammoniated (NH_3) and ammonia (NH_3) plus anthraquinone 2-sulfonic acid (AQ-SA) barley straw treated at 17°C (experiment 2)

Item	Treatment		SEM
	NH_3	NH_3+AQ-SA	
No. of animals	5	5	-
Animal wt. (kg)	51	49	0.8^{ns}
	Voluntary intake period		
DM intake (g $Kg^{-.75}$)			
Straw	38.0	45.1	1.3**
Total diet	51.4	59.2	1.3***
Estimated digestible energy intake (KJ $Kg^{-.75}$)			
Straw	332	440	13.7***
Total diet	579	702	13.6***
	Digestibility period		
DM intake (g $Kg^{-.75}$)			
Total diet	41.3	42.5	0.5^{ns}
Straw intake in diet (%)	67.6	67.5	-
Apparent digestibility (%)			
Dry matter	61.3	64.9	0.5***
Gross energy	59.6	63.2	0.7**
Crude protein	62.1	65.9	0.7**
Neutral detergent fiber	59.4	63.0	0.8**
Acid detergent fiber	58.7	61.3	0.9^{ns}
Lignin	25.2	25.4	2.8^{ns}
Digestible energy(MJ kg^{-1}DM)			
Diet	10.9	11.5	0.1**
Estimated for straw	8.8	9.8	0.2**
Urinary energy (KJ day^{-1})	574	555	8.8^{ns}
Nitrogen balance (g day^{-1})			
Intake	16.8	16.7	0.0***
Fecal	6.4	5.7	0.1***
Urinary	9.8	10.5	0.4^{ns}
Retention	0.6	0.5	0.4^{ns}

ns, not significant at ($P>0.05$).
,* significant at $P<0.01$, $P<0.001$.

V GENERAL DISCUSSION

Cellulose is the most abundant organic molecule grown in the world (Lehninger, 1982). Rumen bacteria are capable of degrading this material to simple endproducts which may then be utilized by the host animal. In this thesis chemical treatment of crop residues to improve their feed value for the ruminant animal has been examined since these by-products are a major potential source of low quality material for ruminants. Because of time limitations the results were not extended to wood products although these are also major potential sources of energy for ruminants as discussed in chapter 1.

Limitations to the use of cereal straw is the crystallinity of cellulose and its close physical association with lignin (Evans, 1979; Harbers, 1982). Therefore methods for improving the nutritive value of straw should concentrate on these two facets.

Alkali treatment diminishes cellulose crystallinity by causing the cellulose microfibrils to swell (Van Soest, 1982), and also breaks the ester bonds between lignin and cellulose or hemicellulose and the β-aryl ether bonds of lignin (Evans, 1979; Lin et al., 1986). Treatment of cereal straws with anhydrous ammonia (NH_3) improves the crude protein content of the treated material, and thus provides one of the deficient nutrients in straw. It has been shown that different materials respond differently to alkali treatment because of the stage of maturity of the plant, as well as the location of the lignin within the plant (Bryce, 1980; Horton, 1981). This suggests that the effectiveness of alkali or other treatments on the material may be influenced by other factors such as treatment temperature, moisture

content and alkali concentration.

The sodium salt of anthraquinone 2-sulfonic acid (AQ-SA) has been shown to improve the efficiency of alkali delignification in lignocellulotic materials used for pulping purposes. Therefore the use of AQ-SA with alkali and bases to improve the feed value of low quality roughages for ruminants was investigated. Studies reported in chapter II confirmed that AQ-SA could aid in the delignification of cereal straws since the lignin content was reduced from 10.4 to 9.0% when no glucose was present and to 5% when glucose was present. This established that the AQ-SA would be beneficial in delignification of straw under mild conditions as well as under high temperatures in which it has been found to be effective by other workers (Fleming et al., 1978) and this provided justification for pursuing this project.

At this time it is not known how AQ-SA results in delignification or improves the in vitro and in vivo digestibility of straw under these mild conditions. It is apparent from experiments in chapter II, however, that a reducing sugar such as glucose greatly enhanced the effectiveness of high levels of AQ-SA in reducing the lignin content in the straw when NH_4OH was used as a base. Furthermore, experiments in chapters II and III demonstrated that AQ-SA resulted in a greater increase in microbial degradation of straw when NH_4OH was used in the treatment procedure than when NaOH was used. The results also demonstrated that optimum conditions for AQ-SA treatment were high pH, moisture content of about 25%, and treatment temperature ranging from 4°C to 100°C. Although lower IVOMD were obtained in straw treated at lower temperature, extending the time improved IVOMD, thus suggesting that the length of treatment influenced the effectiveness of the

chemicals on the straw.

Sheep fed barley straw treated with NH_3 plus AQ-SA increased intake by 15-19% and intake of digestible energy (DE) from straw by 32-39% (chapter IV). These results suggested that changes on the plant structure could have occured, thus causing the sheep to readily consume and degrade the straw. The crude protein was increased by 50-60% by NH_3, and this may have improved the palatability of the straw. A change of 32-39% in DE intake has tremendous implications concerning the nutritive value of cereal straw.

Since straw treated with NH_3 alone has been successfully used in maintenance rations for cows (Annonymous, 1987; Grings and Males, 1987), it would appear that treatment of straw with alkali plus AQ-SA would provide all of the energy required for maintenance by the animal and that AQ treated straw may be useful in production diets as well. This would be of significant importance especially for developing countries where cattle primarily depend on straw during the dry season, when weight losses in cattle are common.

Further work is needed before AQ-SA and or other AQ derivatives can be used in processing feed for animals. Potential toxicity problems are the major concern. Evidence in chapter II indicated that the material could inhibit microbial metabolism when present in amounts greater than 0.08% of straw DM. Animal results with anthraquinone compounds are limited to studies of Zanella et al. (1979) with fish and Martin et al. (1983) with sheep. However, the measurement of approximately 1% of the AQ-SA ingested in the urine of animals in our experiment and appearance of 1-methylaminoanthraquinone (MAAQ) products in urine of sheep in the trial of Martin et al. (1983) suggests that the product is absorbed.

This suggests that residues of AQ will occur in meat and that the product may never be cleared for animal feeding.

Even though this may be true, this research demonstrated that AQ compounds have a potential for improving the feed value of straw and may have a similar effect on other lignocellulotic materials such as wood which could be used for feeding ruminants. A search for other compounds which delignify fibrous materials without toxic problems is warranted. In this regard it is of interest to determine the toxicity of the insoluble derivatives of AQ tested on straw which resulted in higher IVOMD than that achieved from the soluble AQ-SA.

The effect of high levels of the AQ derivatives on straw and on the rumen microoganisms needs to be looked at. Furthermore, research on the effect of AQ and its derivatives on the environment, soils, plants and to the animals is needed before the product can be used on a widespread basis in animal feeds

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APPENDIX 1

Effect of base and 0.05% (wt/wt) anthraquinone 2-sulfonic acid (AQ-SA) on the in vitro organic matter digestibility (%) of wheat straw treated at different temperatures.

Time (days)	Control	3%NaOH	3%NaOH +AQ-SA	4%NH_4OH	4%NH_4OH +AQ-SA	Overall means
			Temperature of 4°C			
5	43.0y	54.6w	60.7x	53.6y	57.8x	53.9x
9	42.0y	56.1wx	60.9x	55.7y	62.9z	55.5y
17	44.1y	57.1x	63.6y	54.6y	60.8y	56.1z
28	38.1x	61.5y	57.7w	51.4x	57.8x	53.3w
42	34.3w	59.8y	57.0w	42.5w	55.0w	49.7v
SEM[1]	0.62***	0.62***	0.62***	0.62***	0.62***	0.18***
			Temperature of 22°C			
5	50.2y	74.2wx	76.4x	72.0x	76.4y	69.9y
9	49.6y	72.5w	77.4x	72.0x	78.3yz	70.0y
17	50.4y	76.3x	77.2x	75.7y	79.7z	71.9z
28	46.8x	72.5w	74.5x	68.0w	72.8x	66.9x
42	41.6w	74.9wx	61.1w	68.4w	62.7w	61.7w
SEM[1]	0.83***	0.83***	0.83***	0.83***	0.83***	0.25***
			Temperature of 40°C			
5	48.8x	73.5y	69.2x	70.6z	76.7y	67.8y
9	44.2w	72.7y	74.0y	62.3y	80.0z	66.6y
17	49.1x	70.3x	69.2x	56.7x	65.4x	62.1x
28	50.7y	68.3x	73.3y	54.5w	65.0x	62.4x
42	43.3w	57.8w	66.5w	53.5w	57.6w	55.7w
SEM[1]	0.73***	0.73***	0.73***	0.73***	0.73***	0.22***
			Temperature of 100°C			
0.08	44.9w	58.1w	60.0w	51.3w	54.9w	53.8w
0.17	47.5x	60.1x	64.6x	55.1x	57.7x	57.0x
0.33	48.3x	66.2y	72.6y	59.0y	63.9y	62.0y
1.0	50.2y	69.2z	77.1z	59.8y	67.7z	64.8z
SEM[1]	0.41***	0.41***	0.41***	0.41***	0.41***	0.12***

[1]n=10 for overallmeans and n=2 for duration by treatment interaction.
v-z Means within the same column not followed by the same letter are significantly different (P<0.05).
***significant at (P<0.001).

APPENDIX II

Effect of ammonium hydroxide concentration with 0.05% (wt/wt) anthraquinone 2-sulfonic acid (AQ-SA) on in vitro organic matter digestibility (%) of wheat straw

Time (days)	Control	2%NH4OH	2%NH4OH +AQ-SA	4%NH4OH	4%NH4OH +AQ-SA	6%NH4OH	6%NH4OH +AQ-SA	Overall Mean
5	49.0w	55.3w	62.2w	68.9w	66.2w	69.6w	68.3w	62.8w
10	52.1x	63.0x	66.1x	75.9y	78.4z	72.3x	76.9y	69.2y
15	52.1x	64.2y	66.4x	70.1x	75.4y	76.1z	79.7z	69.1y
20	54.4y	66.0z	69.1y	69.3wx	68.9x	74.1y	73.0x	67.8x
SEM[1]	0.32***	0.32***	0.32***	0.32***	0.32***	0.32***	0.32***	0.05***

[1]n=8 for treatment, n=14 for time and n=2 time by treatment interaction.
w-z Means within the same column not followed by the same letter are significantly different (P<0.05).
*** significant at (P<0.001).