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Chemical Composition And Nutritional Benefits Of Acid Resistant Hemicellulose.

James Edward Williams

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Chemical Composition and Nutritional Benefits of Acid Resistant Hemicellulose

DISSERTATION

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Of
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In Partial Fulfillment Of The Requirements For
The Degree Of Doctor Of Philosophy

by
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INTRODUCTION

The future of animal production depends upon the supply of additional feedstuffs as well as improving the efficiency of utilization of roughages. One of the components of feedstuffs which is present in industrial wastes and comprises approximately 25 percent of the dry matter of most roughages is the cell wall constituent-hemicellulose.

As a result of rumen microbial fermentation, hemicellulose serves as a major energy source for ruminants. The ruminant utilizes approximately 60 to 70 percent of the hemicellulose by rumen microbial enzymatic degradation to oligosaccharides and monosaccharides. However, the availability of hemicellulose, as determined by enzymatic hydrolysis and chemical extraction, is adversely affected by the physiochemical relationship with lignin and other cell wall constituents. As a result, partially degraded lignin-hemicellulose fractions pass from the rumen to other segments of the intestinal tract where further degradation may occur.

Unidentified factors capable of stimulating growth of guinea pigs have been reported in alfalfa and other plants. Recently, a degraded cell wall constituent from corncobs was identified as a lignin-hemicellulose fraction and termed acid resistant hemicellulose (ARH) because of its resistance to prolonged refluxing in 1 N H_2SO_4. ARH was found to stimulate growth and N retention of rats and improve *in vitro* protein synthesis by rumen microorganisms. This lignin-hemicellulose fraction may be prepared from a wide variety of low quality roughages and industrial wastes. Masonex, a waste product in the wood industry and a good source

of lignin-hemicellulose, improved N retention, apparent N digestibility, weight gain, and feed efficiency of cattle fed soybean meal rations. Thus lignin-hemicellulose fractions from low quality roughages and industrial cellulosic wastes should not be overlooked for their significance in animal nutrition.

Research concerning hemicellulolytic degradation products and their influence on rumen microbial protein synthesis is quite limited and is necessary for a better understanding of their nutritional significance. It was for this purpose that work was conducted to examine the influence of various hemicellulolytic degradation products (ARH) on in vitro protein synthesis by rumen microorganisms. Since lignin is intimately associated with hemicellulose, it was of interest to examine the chemical composition (carbohydrate and free phenolics) of various ARH fractions. Since plant cell wall constituents are enzymatically degradable, research was performed to compare chemically-produced and enzymatically-produced hemicellulolytic degradation fractions.

LITERATURE REVIEW

I. Hemicelluloses

Present day conditions strongly emphasize the use of low quality as energy sources for the future of animal production. One way of meeting the needs would be the utilization of agricultural and industrial waste products that are presently being disposed. Most of these waste products as well as other roughages contain the cell wall constituent hemicellulose which comprises approximately 25% of the dry matter.

Hemicelluloses are a complex group of heterogeneous polysaccharides which are water insoluble and extractable by mild alkaline treatment. The major constituents of plant hemicelluloses are the xylans joined by B 1-4 linkages with branching residues of L-arabinose, D-glucose, D-glucuronic acid, D-galacturonic, and lesser amounts of L-rhamnose, L-fucose, and methylated neutral sugars (Aspinall, 1959). Most pasture plants contain three types of polymers making up the complicated hemicellulose molecule. Hemicellulose A may be isolated from the 10% alkaline-treated plant source by neutralizing the solution to pH 4.5 with glacial acetic acid (O'Dwyer, 1926). The maximum extraction of hemicellulose A from the solution occurred upon addition of a slight excess of glacial acetic acid. The hemicellulose A fraction is a water insoluble linear heteroxylan which contains uronic acids and a small amount of arabinose. The hemicellulose B polymer has been fractionated by addition of half a volume of acetone to the filtrate from the hemicellulose A extraction. This fraction is a

water-soluble, highly branched polymer which in addition to pentoses is rich in galactose and uronic acids. A hemicellulose C fraction may be isolated by addition of a further volume of acetone to the filtrate of hemicellulose B extract. This linear polymer is more soluble than hemicellulose A but contains more arabinose and less uronic acid than linear A polymer (O'Dwyer, 1926). The branched hemicellulose B polymer and linear hemicellulose C polymer have been termed hemicellulose B and isolated as a single component in 2 volumes of ethanol (Gaillard, et al. 1965). Most grasses, legumes and cereal grains contain representatives of these 3 polymers differing slightly in monosaccharide content (Gaillard, et al. 1965). The action of rumen microbial enzymes on these fractions showed that branched B polymer from grass and clover was the most resistant to enzymatic degradation (Gaillard, 1966). However the linear B polymer was hydrolyzed most rapidly possibly because of its solubility, shorter chain length, and lower uronic acid content as compared to the linear A hemicellulose fraction (Gaillard, et al. 1965). Furthermore, hemicelluloses from roughages would be better digested when they contain more of the linear B polymer (Gaillard, 1966). It should be pointed out that differences in composition of individual sugars of these 3 polymers may account for clover hemicellulose being less digestible than grass hemicellulose in the ruminant (Gaillard, 1962). However, Beveridge and Richards (1973) reported no differences in the rate of hydrolysis by rumen enzymes of linear hemicellulose B and branched hemicellulose B polymers from tropical spear grass. It was suggested

that the ryegrass linear B fractions contained major quantities of glucose which is rapidly released upon digestion and may have accounted for the rapid hydrolysis of linear B hemicelluloses.

It was found that during growth of the plants the uronic portion of hemicellulose became progressively less digestible (Waite, et al. 1964). In vitro digestion studies which were based on acid hydrolysis of hemicellulose, have also shown that xylans are the least digestible of all the hemicellulose constituents (Burdick and Sullivan, 1963). Also, Gaillard (1966) found from testing 29 roughages for digestibility of cell wall constituents and anhydruronic acid, that uronic acid residues had the lowest digestibility of all fractions, while xylose was shown to be less digestible than arabinose (Gaillard, 1962). The more digestible residues hemicellulose, such as arabinose, were fermented in the silo leading to a lower digestibility of silages as compared to fresh forage or hay. (Van Soest, 1969).

Gaillard et al. (1965) demonstrated that galactose and uronic acid were released less readily than pentoses when branched hemicellulose B polymers from clover were tested with rumen microbial enzyme extracts. It was suggested that these hexose sugars and their derivatives may block access of the pentosanases to the pentose linkage. However, in the ruminant these blocking groups could be removed by other enzyme systems present in other rumen microorgansims (Gaillard, et al. 1965). Dehority (1973) showed that all strains of cellulolytic rumen bacteria were capable of degrading isolated hemicelluloses from a form insoluble in 80% ethanol to a soluble form. However, only these

strains which could utilize xylans were able to use isolated hemicelluloses for growth. In attempts to explain the mechanism of enzyme action, results seem to indicate that cellulolytic rumen bacteria produce constitutive extracellular enzymes capable of non-specific cleavage of B 1-4 xylosidic linkages of isolated hemicellulose or xylan. It was further suggested that possibly the nature of this enzyme system may be similar to the mode of action of cellulase.

Other studies have indicated that chemical barriers existing between cell wall constituents would interfere with hemicellulose utilization (Alexander, 1971). As a result of no improvement in nitrogen utilization in male weanling rats fed holocellulose as compared to those fed ARH and isolated hemicellulose, it was postulated that a strong bond exists between cellulose and hemicellulose accounting for the barrier to utilization (Alexander, 1971). It was further suggested that bounding may possibly be between the cellulose and the carboxyl group of uronic acid moieties of hemicellulose since uronic acid residues have been found the most difficult to hydrolyze in graded acid hydrolysis of hemicellulose (Alexander, 1971). In measuring non fermentable reducing sugars and converting xylan, the hemicellulose digestibility was higher for those rats fed isolated hemicellulose as compared to holocellulose (Alexander, 1971). Apparently the bonding between hemicellulose and cellulose contributes to the poor digestibility of these cell wall constituents. Once these bonds between cell wall constituents are hydrolyzed by mild acid treatment, as in the case of ARH, the animal microflora can utilize the isolated hemicell-

ulose. With respect to the nonruminant, Van Soest (1969) found that the rat on an alfalfa diet had a cellulose digestibility of 21% and a hemicellulose digestibility of 47%. It was shown that the arabinofuranoside fraction, which is present in hemicellulose may be easily hydrolyzed by a weak acid solution such as 1 N sulfuric acid, while the pyranosidic polysaccharides, existing in cellulose, require a much higher strength of acid to achieve the same hydrolysis (Van Soest, 1969).

Packett et al. (1965) showed with in vitro studies with rumen microorganisms that digestibility of cellulose in roughages was adversely affected by the addition of isolated hemicellulose B. Hemicellulose B incorporation into the diet of the animal resulted in an increased VFA production and an increased molar ratio of propionate with corresponding reduction in acetate. It was felt that apparently the rumen microflora utilize the pentosans of hemicellulose in preference to cellulose. However, the possibility of there being a change in microflora upon hemicellulose addition was not ignored.

II. Phenolic Compounds

Geissman 1962, has defined phenols as organic compounds formed as secondary metabolites in plants which help protect the living plant from attack by microorganisms capable of causing disease. In some plant phenols tie up nutrients and prevent oxidation and digestion by animals (Anonymous, 1976). However, under aerobic and alkaline conditions, the hydrogen bond joining phenols to carbohydrates or proteins may be reversed enabling the complex to be utilized by the animal (Anonymous, 1976).

Phenolic compounds have a variety of metabolic functions and structures. Phenolic compounds have been categorized into 3 groups according to genetic origin: (1) acetogenins which are derived from combination of acetate fragments and include anthroquinone pigments and naphthoquinone; (2) compounds derived from shikimic-prephenic pathway and characterized by the presence of a C_6-C_3 structural unit (phynylpropanoid); (3) compounds formed by combination of acetate-derived fragments with those derived from phenylpropanoids and included flavonoids and isoflavonoids (Geissman, 1962). Of interest are the polyphenolic compounds because of their growth inhibitory role (Tamir and Alumot, 1970) and the flavonoids because of their stimulatory role of basal metabolic rate (BMR) (McLaren et al. 1966).

The ability of polyphenols to retard growth is not a new concept. The growth retardation of grain sorghums have been directly related to the level of tannin present in these samples (Chang and Fuller, 1964). Vohra et al. (1966) found tannic acid to be the most growth depressing of several plant tannins. Furthermore the metabolites of tannic acid, pyrogallol and pyrocatechol, are much more growth depressing than the parent compound (Potter and Fuller, 1968, Rayudu et al. 1970). Tamir and Alumot (1970) fed polyphenol fractions to rats and noticed a growth depression. It was felt that the growth depression resulted from binding of proteins by tannins, yielding insoluble proteins in the feces. Joslyn and Glick (1969) found that condensed tannins were less growth depressing than gallolyl glucose. However, Kratzer et al. (1975) found that tannins were about equally growth inhibitory in the chick when dosages were computed on an equal

total phenol content, regardless of the nature of the hydrolyzable or condensed tannic compound.

Booth et al. (1959) reported that gallic acid depressed growth of rats and caused fatty livers. Fuller et al. (1967) observed that arginine and ornithine reduced the toxicity of 1% tannic acid more than either one alone and completely alleviated the adverse effects of .5% tannic acid.

Potter and Fuller (1968) using thin layer chromatography (TLC) demonstrated that gallic acid, 4-O-methyl gallic acid, and pyrogallol was present in the urine of hens fed either tannic acid or gallic acid. Tannic acid was apparently hydrolyzed to gallic acid and a large part of this material was O-methylated and excreted in the urine as 4-O-methyl acid. Methionine and choline serve as methyl donors for detoxifying gallic acid.

The role of flavonoids in the stimulation of basal metabolic rate (BMR) has been demonstrated by research conducted at the WVU Agricultural Experiment Station. McLaren et al. (1964) found that dried grass silage (DGS) stimulated BMR and increased thyroid secretory activity of male rats. This DGS was obtained from equal amounts of wheat, vetch, orchardgrass and alfalfa. The stimulation of BMR of rats was attributed to the 80% ethanol extract of DGS (McLaren et al., 1966). The 80% ethanol extract of spinach, lettuce, sweet potatoes, lemons, oranges, and cane molasses also stimulated BRM over rats fed a basal diet (McLaren et al., 1973[b]). From paper chromtographic analysis, it was suggested that flavonoids were responsible

for the BMR-stimulatory activity. McLaren and Michaelis (1974) also determined that the influence of extractives of dried grass silage (EDGS) on BMR was linear when the diet contained a higher level of iodine. However, supplemental concentrated EDGS did not influence rat BMR when iodine intake was reduced from 0.123 to 0.062 mg/100 gm. of diet. Kahle and McLaren (1975) showed that inclusion of 20% unextracted DGS in the diet slightly but not significantly, decreased endogenous urinary nitrogen (EUN) below that of rats fed alpha-cellulose or ground wheat straw as the source of roughage. However, EUN was greater and N retention was decreased over basal-fed rats by the inclusion of 80% ethanol extract of DGS in a 4% egg albumin basal diet. It appeared that flavonoids in DGS contributed in a catabolic effect on protein metabolism, as shown for BMR. Stelzig and Quasim (1973) fractionated the DGS into 8 fractions. Two of these fractions were found to significantly elevate BMR. Using ultraviolet spectral-analysis and paper chromatography, these pure flavonoids were identified as tricin and 5,7-dihydroxy-3',4',5',-trimethoxyflavone. It was postulated that these flavonoids stimulated BMR more than other flavonoids because of their resistance to degradation by the intestinal flora.

III. Phenolic-Carbohydrate Complexes

The importance of phenolic-carbohydrate complexes has been realized within the last few years. McLaren (1950) reported that the non-sugar colored components of cane molasses could be fractionated with 80% ethanol into a insoluble portion containing lignin and hemi-

cellulose and a soluble portion which was made up of a mixture of phenolics. Using column chromatography and organic solvent fractionation, it was shown that the 80% ethanol extract contained phenolic compounds and a mixture of flavonoids. Carbohydrate-bound phenols were isolated and one group was shown to be flavonoid in nature while others remained as unidentified phenolic compounds. A simpler procedure became available and a spectrophotometric method of quantitatively determining these compounds were developed by McLaren (1954).

Certain fractions of these carbohydrate-bound phenolic compounds were shown to be flavonoid in nature while others remained as unidentified phenolics. Markham (1972) found that flavonoid-like natural products behave in a manner suggesting the existence of a bond between the polyphenolic compound and the cell wall. A flavonoid-polysaccharide from liverwort was isolated and it was found that the polysaccharide was a hemicellulose containing approximately 18 sugar residues. The bond was glycosidic and involved the galacturonic residue in the polysaccharide, and the 7' and 4' hydroxyl groups on the flavone.

Hartley (1973) has revealed that carbohydrate esters of ferulic acid were released from grass cell-walls by cellulase action. The molecular weight of these esters varied from a few hundred to over 50,000. Hydrolysis of the carbohydrate moiety showed it to contain xylose, arabinose, and glucose units. It was felt that these carbohydrate esters of ferulic acid obtained by cellulase treatment form part of the phenolic monomer-carbohydrate fraction present in lignin-carbohydrate complexes (Hartley, 1973).

There has been considerable evidence that lignin is esterified to hemicellulosic polysaccharides. Using cellulolytic enzymes, Pew and Weyna (1962) could not remove all other carbohydrates from wood without degrading the lignin. The lignin-xylan and lignin carbohydrate complexes isolated by Bolker and Wang (1969) could not be separated into individual components by electrophoresis. All evidence seems to suggest that complexing occurs only between lignin and hemicellulosic polysaccharides (Morrison, 1972). Furthermore, lignin-protein complexes are unlikely to occur as it is only in the primary cell wall that a protein (extension) has an integral role while lignin is only laid down when secondary thickening of the wall takes place (Lamport, 1965; Albersheim, 1965).

Morrison (1973) investigated the capabilities of various solvents in dissolving lignin-carbohydrate complexes from crude cell wall preparations. He found that 1 N alkali dissolved the highest percentage of the cell wall but the composition of the isolated lignin-carbohydrate complex was very different from that of the other complexes, dissolved by the other solvents but similar to that of grass hemicellulose (70% xylose, 20% arabinose, and 5% galactose and 5% glucose). A dimethyl sulphoxide extraction gave by far the highest yield of dry matter. A fractional precipitation of the extracts (dimethyl sulphoxide) revealed that 50%-75% ethanol precipitated 84% of the carbohydrate as estimated by the phenol-sulfuric acid method and this precipitate was designated Component A. Furthermore, additional precipitation of 11% carbohydrate from the residual solution resulted by concentration with ethanol (1:1)

and this precipitate was designated Component B. Molecular sieve chromatography revealed that both components contained carbohydrates with MW 150,000 which were eluted in the void volume and contained both lignin and carbohydrate. The ratio of carbohydrate to lignin revealed that carbohydrate was the dominant component of A, whereas the B component had less carbohydrate. The alkaline extract on neutralization to pH 5 with acetic acid remained soluble. However, upon addition of ethanol it was precipitated and thus classified as Hemicellulose B. The lignin-hemicellulose complex extracted with alkali was shown to have 5 components varying in MW from 10,000 to greater than 100,000 and each component had lignin and carbohydrate association (Morrison, 1973). Blake and Richards (1971) have reported that MW ranges of hemicelluloses are difficult to determine because hemicellulose aggregates in aqueous solutions making fractionation by gel chromatography difficult.

If these lignin carbohydrate complexes exist then they should be produced by the action of rumen microorganisms and present in the rumen liquor. Gaillard and Richards (1975) demonstrated that soluble lignin-carbohydrate complexes are formed by the action of rumen microorganisms on grass. The cell free rumen liquor which was isolated and fractionated by gel permeation chromatography contained carbohydrates and proteins of MW greater than 10,000. All components of one peak were eluted beyond the volume of monosaccharides, which indicated an interaction of the dextran with the solutes. According to Gaillard and Richards (1975) this would be typical of aromatic molecules of lignin and it was suggested that carbohydrates are associated with lignin. In confirmation of these

findings, the U.V. spectra and I.R. spectra of this peak were comparable to that of lignin.

Various reports have mentioned a chemical bond between carbohydrate and lignin in the cell walls of plants (Sarkanen, 1971). As previously mentioned, Morrison (1973) pointed out the presence of 5 complexes in grasses whose solubility was ascribed to the carbohydrate. When hydrolyzed the complex became insoluble in water. Furthermore, Morrison reported that hydrolysis with alkali destroyed the ester linkages as determined by absorbance at 325 nm. Thus different types of linkages between carbohydrate and lignin have been reported (Gaillard and Richards, 1975). Furthermore, some are alkali-labile while others are alkali-stable but susceptible to acid hydrolysis. However, Gaillard and Richards (1975) reported that there was no production of lignin-free polysaccharides by subjecting these complexes to acid and alkaline treatment.

The fact that soluble lignin-carbohydrate complexes are produced from fodder in the rumen has important implications in the study of ruminant digestion and nutrition. Bondi and Meyer (1943) demonstrated that a change occurs in the lignin component of plants as it passes through the gut. Although there was no change in solubility, MW, methoxyl and N content during digestion, the fecal lignin had a lower aldehyde content. It was postulated that the side chain had undergone hydrogenation preventing oxidative fission and formation of aldehyde groups. Along with the disappearance of nonphenolic OH groups from fecal lignin there was evidence that a change occurred in the lignin molecule as it passed through the gut. It has been reported that lignin is partially digested as a result of the dissolution of lignin-carbohydrate complexes

which pass from the rumen as a polymer in solution (Dekker et al. 1972). Hartley and Jones (1976) found that cellulase treatment of leaf blades of Italian ryegrass (Lolium multiflorum) resulted in a release of 41% of the cell walls. Allobiose was the main sugar along with glucose and higher oligosaccharides. Considerable amounts of carbohydrate esters of ferulic and p-coumaric acid were released. The cellulolytio enzyme (carboxymethyl cellulase) released 51% of the cell walls containing glucose, xylobiose, xylose and arabinose along with higher oligosaccharides and carbohydrate esters of ferulic and p-coumaric acid. However, a commercial hemicellulase removed only tracted of the esters. It was postulated that the esters are attached to cellulose chains and the function of the phenolic esters may be to act as intermediates in the process of lignification.

IV. In Vitro Studies

As early as 1891, Zuntz as cited by Martson (1948), suggested the possibility of using non-protein nitrogen as an alternative nitrogen precursor for rumen microbes in the synthesis of protein. Hart et al. (1939) conducted long term growth studies with calves in which the replacement of 43% of the ration's nitrogen with urea was slightly less than that of 66% casein. Furthermore, there was no adverse affect on the host's tissue protein due to the replacement with urea. In 1940, Wegner et al. confirmed in vitro that urea was utilized but not as effectively as casein as a nitrogen source for ruminal protein synthesis. In 1943, Pearson and Smith confirmed that urea is hydrolyzed to ammonia which is utilized to form microbial protein. In order to more accurately

simulate *in vivo* conditions and minimize changes in microbial population, urea was incubated in a rumen liquor media for four hours.

It became necessary to develop a medium which closely simulated the actual rumen environment. McDougall (1949) determined the mineral composition of sheep's saliva and developed "artificial saliva". Many modifications in the composition of artificial saliva were made in order to study *in vitro* rumen function. In measuring *in vitro* digestion of cellulose, Burroughs *et al.* (1950) noted that trace minerals in rumen liquor must be considered for normal rumen function and growth. Bentley *et al.* (1954 and 1955) and Bryant and Doetsch (1955) discovered that certain short chain fatty acids, notably valeric acid, and biotin were growth factors for cellulolytic rumen microorganisms.

As a means of improving the *in vitro* system, Louw *et al.* (1949) placed the reaction media in a dialysis sack to remove inhibitory end-products formed during fermentation. However Johnson *et al.* (1958) and El-Shazly *et al.* (1960) showed that removal of the end products did not affect the activity of cellulolytic microorganisms. This system was termed the "continuous flow", "Chemostat" or "open system" and was used during the 1940's and early 1950's as an attempt to better simulate *in vivo* conditions.

The use of the Warburg respirometer as a modification of the all-glass *in vitro* system developed from work by Quin (1943), who measured gas production from carbohydrate fermentation by rumen liquor in air-tight vessels. McBee (1953) used the Warburg respirometer to determine fermentation rates of various carbohydrates by rumen microorganisms of

sheep fed different diets. Hungate (1966) developed a continuous fermentation system with the Warburg respirometer from which they related the rate of digestion of various substrates to the gas pressure increases during fermentation.

The most active in vitro utilization of urea by ruminant microorganisms was observed to occur between 30°C and 40°C (Pearson and Smith, 1943). With a constant temperature, there was no correlation between pH and the amount of protein synthesized as long as the pH ranged between 6.3 and 7.4. A continuous culture maintained at 6.7 was found to contain the types of microorganisms observed to be present in high concentrations in the rumen. Slyter et al. (1966) noted that at a pH of 5.0, the variety of microorganisms cultured was typical of that observed in vivo. Broberg (1957) found that with the change in pH from 6.8 to 5 there occurs a tremendous change in redox potential with a concomitant alteration in bacterial activity. A redox potential of -150 mV or lower was noted by Bryant (1959) to be necessary to initiate in vitro fermentation.

Among the many factors studied in in vitro rumen fermentations, the effect of various types and levels of carbohydrates on urea utilization has received the most attention to date (Wegner et al. 1940; Pearson and Smith, 1943; Arias et al., 1951; McNaught, 1951; Hudman and Kunkel, 1953; Hoover et al., 1963; Bloomfield et al., 1964; Smith, 1965). At the West Virginia Experiment Station this area of interest has received considerable attention. McLaren et al. (1965) observed that over a length of time increased levels of readily fermentable

carbohydrates fed to sheep positively affected retention of absorbed nitrogen. Concurrently, Smith (1965) demonstrated in *in vitro* studies that there was a positive linear relationship between the amount of readily available carbohydrate and the amount of protein synthesized by rumen microorganisms. He also showed that xylose utilization was affected by the level of glucose and the source of the inoculum.

The importance of the studies on utilization of low quality roughages in animal nutrition was demonstrated with nitrogen metabolism trials with sheep fed corn cobs or wheat straw as the sole source of roughages. Smith *et al.* (1960) showed that nitrogen utilization was increased when corn cobs replaced wheat straw as the sole roughage. Later studies showed that an improvement in nitrogen retention occurred in lambs fed semi-purified diets containing corn cobs by substitution of 8% of the all-urea supplemented ration with an isonitrogenous amount of enzymatically hydrolyzed casein (McLaren *et al.*, 1962). However, no improvement in nitrogen retention occurred by substituting wheat straw for corn cobs when enzymatically hydrolyzed casein was included in the ration (Peters, 1963).

Incorporation of the solids contained in each of successive extractions of ground corn cob with diethyl ether, hot 95% ethanol, 80% ethanol and hot water did not influence *in vitro* rumen microbial protein synthesis. However, supplementation of the medium with the filtrate resulting from .1N H_2SO_4 hydrolysis of the organic solvent and aqueous extracted corn cob did increase *in vitro* rumen microbial protein synthesis (Mc Laren *et al.*, 1968). Previous procedures for isolation of this corn cob factor involved refluxing corn cobs in 1N H_2SO_4 for 6 hours and

neutralizing the filtrate to pH 6 with calcium carbonate. The corn cob factor was recovered from the neutralized filtrate by ethanol precipitation. The fraction solubilized by refluxing in 1N H_2SO_4 for 6 hours and insoluble in 67% ethanol was termed acid resistant hemicellulose since it was not completely hydrolyzed to simple sugars (McLaren et al., 1968). This characteristic of certain hemicellulose fractions has been attributed to the acid resistant glycosidic bonds linking uronic acids to pentoses (Whistler and Smart, 1953).

Woolf (1969) demonstrated that either the non-dialyzable fraction of corn cob ARH or molasses ARH stimulated in vitro protein synthesis by rumen microorganisms. The ash portion of while molasses did not improve in vitro protein synthesis. Replacement of 10% of urea nitrogen with enzymatically hydrolyzed casein (EHC) resulted in an additional improvement in in vitro protein synthesis over that obtained in the media with all nitrogen furnished by urea. The stimulation in protein synthesis due to 10% EHC replacement of urea nitrogen tended to exhibit its influence independently of that of the ARH factor.

Until recently, little research has been conducted to ascertain the nutritional benefits of hemicellulose other than as an energy source. Fung et al. (1950) attempted to measure the amount of liver glycogen formed by feeding hemicellulose to phlorhizinized rats. The results indicated that hemicellulose was not a source of glycogen for the rat.

Reid and Briggs (1953) found that a semi-synthetic diet containing variable amounts of alfalfa and kale produced a growth response with guinea pigs comparable to a good commercial pelleted diet. This indicated that alfalfa and kale may contain a growth factor although it was

also shown that animals could thrive as well without supplements of fresh kale.

Other workers also recognized the ability of 10% dehydrated alfalfa along with other roughages, to improve the purified diet of guinea pigs (Lakhanpahl et al. 1966). The unidentified factor appeared to be organic in nature since the ash of alfalfa gave no response. When tested individually the alcohol extract and alcohol residue of alfalfa resulted in no growth response.

Work by Singh et al. (1968) attempted to determine the constituents of raw cabbage and alfalfa which improved the growth of guinea pigs. The growth stimulatory effect of cabbage was attributed to the supply of ascorbic acid to the animal. However it was suspected that alfalfa contained a growth stimulant other than ascorbic acid. Fractionation of alfalfa with water, alcohol and petroleum ether proved futile in removing the active constituent. Furthermore, the water soluble and 95% ethanol extract of alfalfa produced no growth response in guinea pigs.

In attempts to determine the influence of ARH from hemicellulose on nonruminants, the West Virginia group conducted nitrogen metabolism trials and growth trials with rats. Cuppett (1969) showed that ARH significantly improved nitrogen retention by adult male rats. The improvement in nitrogen retention occurred as the result of the incorporation of ARH from holocellulose extracted from spinach, alfalfa, and corn cobs into semi-purified diets at the 0.1% level (Cuppett, 1969). The addition of .1% ARH semi-purified soybean basal diet significantly improved weight gains and feed conversions of male weanling rats (Cuppett,

1969; Yang, 1971; Alexander, 1971). Yang (1971) found that addition of .1% ARH and .3% ARH to diets containing 11.6% and 14.5% soybean protein improved growth rate and feed conversion of male, weanling rats. However, the improvement was significant only at the 14.5% protein level with both concentrations. With the 11.6% protein level only the 0.3% dietary level of ARH gave significant improvement in weight gain and feed conversion.

Studies of Alexander (1971) indicated that degree of extraction influenced the effects of cell wall constituents upon nitrogen utilization of rats. There was no improvement in nitrogen utilization by male, weanling rats fed a semi-purified diet containing holocellulose, ethanol-extracted corn cobs, or acid hydrolyzed corn cobs. However, the addition of hemicellulose of ARH to semi-purified diets significantly improved nitrogen utilization.

McLaren et al. (1974) showed that fractions of corn cob ARH responsible for improvement in growth and feed conversion were organic in nature since the incorporation of the inorganic portion of corn cob ARH into a casein basal diet did not improve growth or feed conversion of weanling male rats. Furthermore, McLaren et al. (1968) and Williams (1974) demonstrated that the non-dialyzable portion of corncob ARH was responsible for the growth activity and increased in vitro rumen microbial protein synthesis. In addition it was shown that inclusion of corn cob ARH into a casein basal diet at the 0.05% level was optimal for growth rate and feed conversion of weanling male rats.

It was thought that carbohydrate source may influence growth-promoting ability of ARH. Williams (1975) showed that inclusions of corn-

starch or dextrose, and the interaction between the two and ARH accounted for most of the improvement in weight gains. The effect of alpha-cellulose or acid hydrolyzed alpha-cellulose on the growth promoting ability of ARH was significant at ($P < .10$).

A striking growth response occurred when rats were fed cornstarch and acid-hydrolyzed alpha-cellulose. The effect of acid hydrolysis of alpha-cellulose was only apparent in those rats fed cornstarch rather than dextrose as the carbohydrate source of the ARH diet. It would appear that cornstarch may exert some influence on the ability of ARH to promote growth of rats (Williams et al. 1975).

Growth-promoters which are similar to corncob ARH have been found in the non-dialyzable fraction of 80% ethanol extracts of cane molasses and refiner's syrup (McLaren et al., 1973a). The incorporation of 0.03% carbohydrate-bound phenolics into the basal diet significantly increased growth over the basal fed rats (McLaren et al., 1973a). However another carbohydrate-bound phenolic fraction with phenolics of the flavonoid group increased BMR, but did not influence growth when added to the basal diet at 0.03% level (McLaren et al., 1973a). Alkaline cleavage of the black phenol-carbohydrate complex yielded a mixture of phenols and a non-dialyzable free hemicellulose fraction which stimulated growth of rats when fed at the 0.03% level. The cleaved phenolic fraction had either negative effect or no effect at all on rat growth (Fahey et al. 1976).

Acid hydrolysis of the black phenol-carbohydrate complex yielded an insoluble lignin residue which made up approximately 18% of the fraction (Fahey et al., 1976). Hartley (1973) has characterized, in

several grasses, a soluble complex with a hemicellulosic side chain bonded to ferulic acid and para-coumaric acid through ester linkages. These phenols are probably monomers which enter into enzyme catalyzed condensation to form core lignin in grasses.

Other lignin-hemicellulosic degradation complexes, which may be similar to ARH from corn cobs, have been tested for their growth-promoting action. Hatfield (1972) demonstrated that Masonex supplementation of soybean meal significantly improved N retention in the rumen, and resulted in higher N digestibility as well as a general improvement in feed intake, weight gain and feed efficiency. In vitro studies by Hatfield found that as levels of Masonex increased, there was a significant reduction in ammonia production. In vivo studies were conducted with lambs receiving a basal ration of 62% corn, 10% cellulose plus mineral-vitamin-antibiotic supplementation. The diet was altered with 12% soybean meal plus various levels of cane molasses (12%-0) and Masonex (0-12%). The average daily gain and feed efficiency increased as percentage of Masonex increased. Nitrogen retention was progressively higher as the level of Masonex increased. Hatfield (1972) has postulated that the beneficial biological activity of Masonex may result from intimately mixing Masonex with the protein source. It has been suggested that the intimate association may enable the animal to more efficiently utilize the protein protected by Masonex. Overfield and Hatfield (1976) compared the supplementation of fresh and aged phenolic-hemicellulose extracts (tradename Masonex) to a basal ration of cracked corn, soybean meal, urea, and 6% corn silage for cattle. They noted that a fresh hemicellulose extract increased daily feed intake, daily gain, and feed

efficiency over the basal-fed animals while the aged product decreased performance. These investigators also reported that inclusion of hemicellulose extracts in cattle rations, as compared to a basal, decreased the incidence of abscessed livers.

Bartley (1976) reported on ammonia toxicity tests and observations of rumen protein synthesis with "Masonex" and suggested some possible urease inhibition by phenolic hemicellulose extracts. In confirmation of this, Fernando and Roberts (1976) presented evidence that naturally occurring phenolic compounds may be used to effectively inhibit urease activity in the soil, thereby preventing loss of nitrogen. Feather (1976) also conducted research on the inhibition of urease by phenolic-hemicellulose extracts. He tested aged and fresh samples of phenolic-hemicellulose extracts and concluded that non-sugar fractions of the products appeared to be contributing to the urease inhibition. The fresh products showed higher inhibition than aged products and whatever causes the inhibition is obviously less effective after aging and was highly dialyzable with some of it being polymeric in nature. Lang (1976) used young guinea pigs to compare a basal diet to a basal diet containing phenolic-hemicellulose extracts as the substitute for all of the carbohydrates, basal diet containing freeze-dried alfalfa solubles and a guinea pig chow as the sole source of intake. He found that the phenolic-hemicellulose extracts and alfalfa solubles stimulated feed intake and weight gains equally and significantly more than the basal diet but not equivalent to the chow. These components increased feed efficiency significantly over the basal diet and almost twice as much as the chow. In conclusion, it was felt that the chemical activity

of carbohydrate and phenolics is complex and more work must be conducted before the nutritional effects of phenolic hemicellulose extracts may be determined.

The metabolic role of ARH on N utilization in the ruminant may be similar to its function in the monogastric animal and the function of Masonex in the ruminant animal. McLaren et al. (1976) found an improvement in in vitro microbial protein synthesis as well as N retention and cellulose digestion of lambs fed all urea-supplemented, semipurified rations containing ARH. Furthermore, the improvement in N utilization and growth of rats as the result of ARH supplementation might have been due to the influence of ARH on intestinal microbial metabolism (McLaren et al., 1974).

Materials and Methods

Fractionation procedures for ARH preparations in Growth Trials

ARH from ground corncobs was subjected to various chemical treatments to ascertain the influence of chemical composition of these fractions on biological activity. The procedures involved cation resin purification, carbon treatment, water extraction and dialysis. The purification of ARH by removing excess calcium salts, phenolic compounds, and low molecular weight sugars would elucidate the chemical composition of ARH responsible for stimulating the growth of rats.

The crude ARH used in Trials 1 and 2 was prepared from ground corncob which had not been extracted with organic solvents. Ground corncobs were refluxed in 1N H_2SO_4 for 6 hours and filtered to remove the unhydrolyzed residue and the ARH recovered, according to the procedure of McLaren et al. (1974a).

The water extracted ARH fraction was prepared from ground corncobs, as described by McLaren et al. (1974a) and shown in Figure 1. The precipitated ARH was resuspended in water (10:1 volume by weight) to remove low molecular weight sugars and then reprecipitated in 2 volumes of 95% ethanol. The ARH was recovered by centrifugation at 1000xG for 30 minutes and designated the high molecular weight ARH fraction.

The preparation of cation resin-treated ARH, as shown in Figure 1, was conducted by neutralization of the filtrate to pH 5.8 with barium hydroxide followed by filtration to remove the barium salts. The filtrate was treated with Dowex 50W-X8 cation resin to remove the excess barium salts and the effluent dialyzed against water for three days

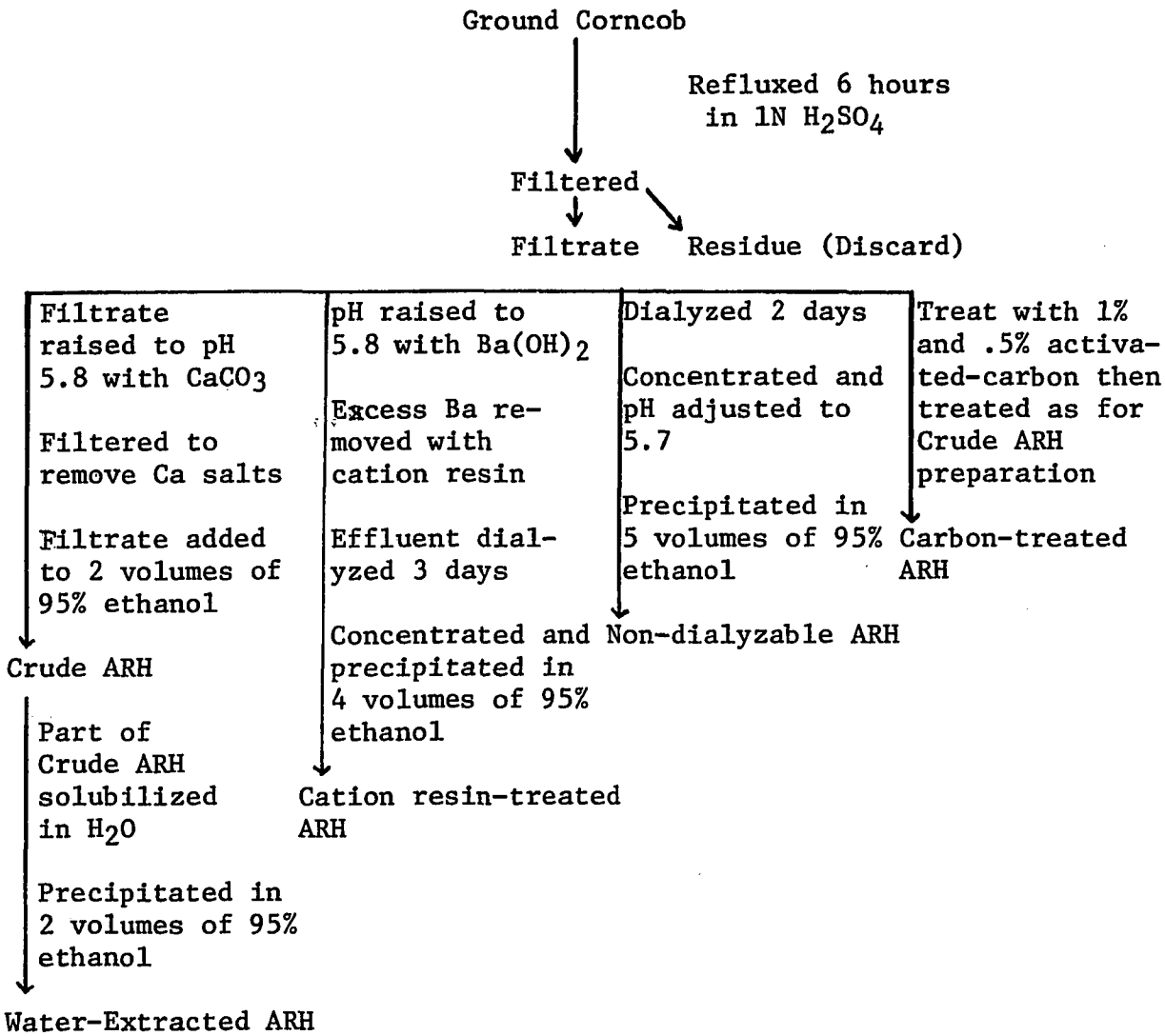


Figure 1 Flow diagram of procedures used in preparing various ARH preparations from corncob.

with frequent changes of the water to remove low molecular weight compounds. The non-dialyzable hydrolysate was concentrated in a forced-air oven at 50°C to one-sixth of its original volume. The concentrated non-dialyzable hydrolysate was placed in 4 volumes of 95% ethanol and the precipitate recovered by centrifugation at 1000xG for 30 minutes.

The non-dialyzable ARH was prepared by dialyzing the corncob hydrolysate for several days to remove small molecular weight sugars. The non-dialyzable fraction was concentrated to one-sixth of the original volume and neutralized to pH 5.7 with calcium carbonate and placed in 5 volumes of 95% ethanol. The residue was recovered by centrifugation at 1000xG for 30 minutes and washed with acetone to obtain a dry ARH material.

The activated carbon-treated ARH was obtained by treating the corncob hydrolysate successively with 1% and .5% activated carbon at 4°C prior to neutralization to pH 5.8 with calcium carbonate. After removal of the calcium salts by filtration, the filtrate was added to 2 volumes of 95% ethanol to precipitate the ARH.

Preparation of Alkaline-Cleaved ARH Fractions

The non-dialyzable ARH fraction was subjected to 10% NaOH in order to cleave the lignin-hemicellulose bonds and obtain a carbohydrate-free fraction. These experiments were undertaken to confirm work by Fahey et al. (1976) who demonstrated that alkaline cleavage of a black-phenol carbohydrate complex in cane molasses resulted in a biologically active hemicellulose fraction. The biological activity of the alkaline-cleaved ARH fractions was determined by testing their

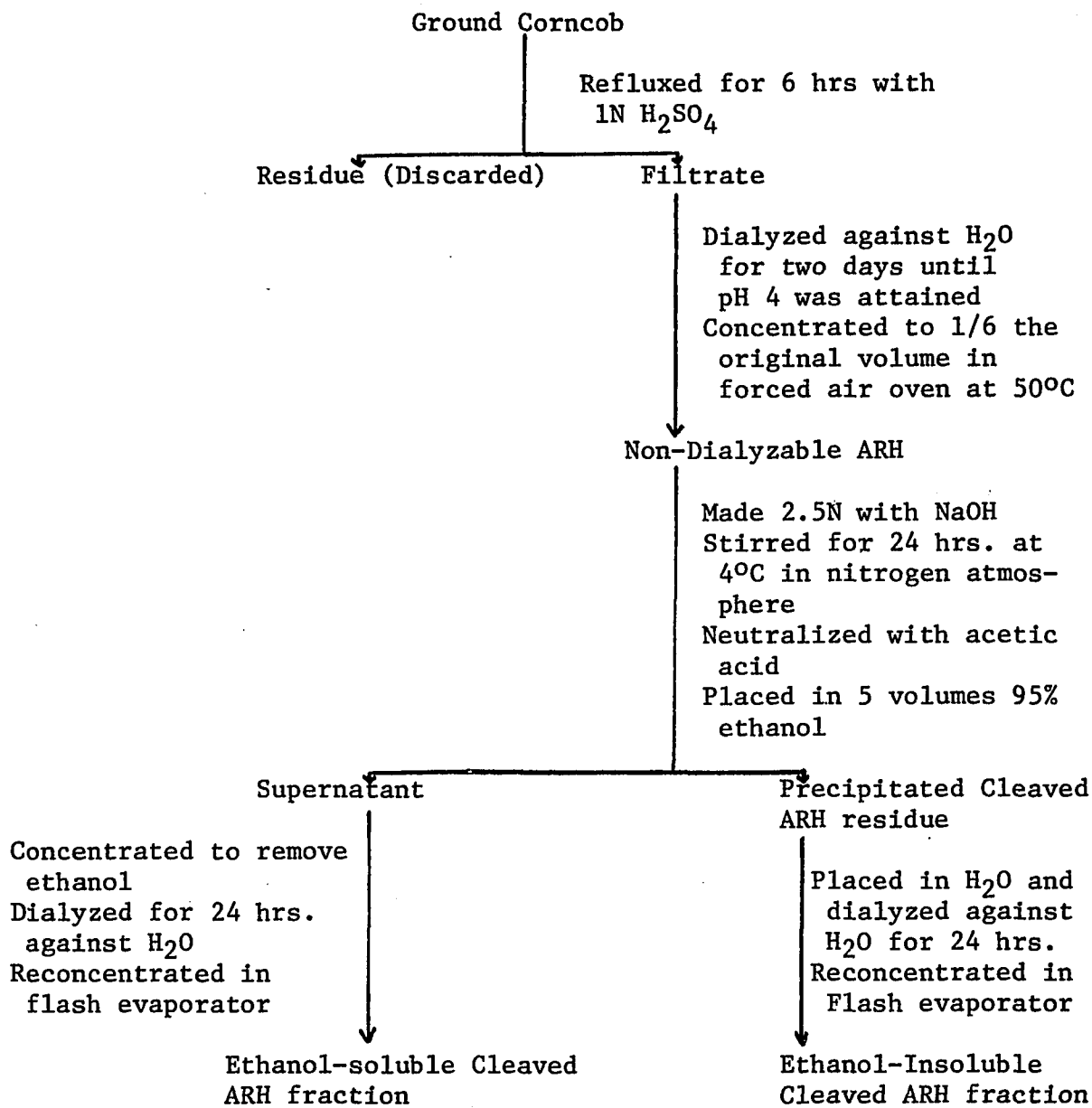


Figure 2 Flow diagram of the procedure used for the alkaline cleavage of acid-resistant hemicellulose (ARH) from ground corn cob.

influence on growth and feed utilization of rats.

ARH fractions were prepared as shown in Figure 2 and used in Trial 3. Ground corncobs (225g) were refluxed in 1 N H_2SO_4 for 6 hours. The filtrate was dialyzed against tap water for 2 days until pH_4 was attained. The filtrate was concentrated to 1/6 of its original volume. The solution was termed the non-dialyzable ARH fraction. It was made 2.5N with sodium hydroxide and stirred for 24 hours in a nitrogen atmosphere at 4°C. The alkaline solution was neutralized with acetic acid and placed in 5 volumes of 95% ethanol. The ethanol-insoluble fraction was centrifuged at 1000xG for 30 minutes. The insoluble residue was placed in water and dialyzed against water to remove excess salts. Finally, it was lyophilized and termed the ethanol-insoluble alkaline-cleaved ARH fraction. The supernatant of the ethanol-precipitated alkaline-cleaved ARH was freed of its alcohol content by flash evaporation. Any excess salts were removed by dialysis against tap water and the fraction was dried by lyophilization in a Virtis lyophilizer and termed the ethanol-soluble alkaline-cleaved ARH fraction.

Preparation of Diets

The composition of the basal diet fed to rats is shown in Table 1.

Experimental Animals

1. Rat Trials

Male albino rats of Wistar strain and weighing approximately 60 grams were used in each study. The rats were housed individually in galvinized cages and fed a commercial stock diet for 2 days before they were fed the basal and experimental diets. The animals were

TABLE 1

Composition of the Basal Diet Fed to Rats

Diet Constituent	Amount
	g
Cornstarch	67.3
Casein	14.4
DL-methionine	0.3
USP XIV salt mixture[1]	4.0
Corn Oil[2]	6.0
Fish liver oil[3]	1.0
Vitamin mixture[4]	5.0
Acid hydrolyzed Alpha-cellulose[5]	2.0
Total	100.0

[1]Salt mixture USP XIV, General Biochemicals, Cleveland, Ohio: supplemented with 2 mg Cu/Kg diet (as $CuSO_4$) and 4 mg Zn/Kg diet or 8 mg Zn/Kg diet (as $ZnCO_3$).

[2]Supplemented with 5 mg of alpha tocopherol per kg.

[3]Contained not less than 2500 IU vitamin A and 600 IU vitamin D per gram.

[4]According to Rao et al. (1959).

[5]Nutritional Biochemical Corp., Cleveland, Ohio.

provided with 10 grams of feed daily for the first 5 days and 15 grams of feed daily for the remainder of the trial. The animals were provided with tap water ad libitum.

Experimental Design of Animal Trials

Trial 1

Two growth trials, including 10 rats per treatment in each experiment, were conducted to compare the growth effects of regular corn cob ARH to that of a cation resin purified ARH. Ten rats were randomly assigned to one of three treatments - a basal, basal + .05% regular corn cob ARH; and basal + 0.05% cation resin treated ARH. The trials were conducted for 17 days and the animals were weighed weekly.

Trial 2

Two growth trials, involving 10 rats per treatment in each experiment, were carried out to determine the influence of ARH preparation on the growth of rats. Ten rats were randomly assigned to one of four treatments - a basal, a basal + 0.05% carbon-treated ARH, basal + 0.05% non-dialyzable ARH and a basal + 0.05% water-extracted corn cob ARH. The growth trials were conducted for 21 days and the rats were weighed weekly.

Trial 3

Two growth trials, involving 10 rats per treatment in each experiment, were conducted to compare the growth response of rats fed an ethanol-insoluble and ethanol-soluble alkaline-cleaved ARH fraction. Ten rats were randomly assigned to one of three treatments - a basal, a basal + .05% ethanol-insoluble alkaline-cleaved ARH, and a basal +

.05% ethanol-soluble alkaline-cleaved ARH fraction. The trials were conducted for 21 days and the rats were weighed weekly.

Fractionation Procedures for ARH preparations used in the in vitro Experiments

ARH was prepared from hemicellulose isolated from the crude plant source and from the delignified plant source in order to determine if the lignin moiety was a necessary component for the fraction to exert a biological response. ARH was fractionated from corncob hemicellulose, delignified corncob hemicellulose, white oak woodchip hemicellulose, delignified white oak woodchip hemicellulose and delignified alfalfa hemicellulose. Ground corncobs and delignified ground corncobs also served as sources for hemicellulose A and hemicellulose B. ARH was extracted from these hemicellulose components to determine if the chemical composition of the hemicellulose moiety influenced the biological response exerted by these fractions. The biological activity of these ARH fractions was determined by testing their ability to improve in vitro microbial protein synthesis.

Preparation of Holocellulose

Holocellulose was prepared by heating (60°C) ethanol extracted crude plant material (60g and 600 ml of water) in a water bath according to the modified procedure of Jayne-Wise (Whistler and Smart, 1953) as shown in Figure 3. Sodium chlorite (60g) and acetic acid (24 ml) were added to the solution in two equal portions at zero time and at 45 minutes for a total of 1.5 hours. The residue was washed free of chlorine with water and then air dried. Holocellulose contains hemicellulose, cellulose and residual lignin.

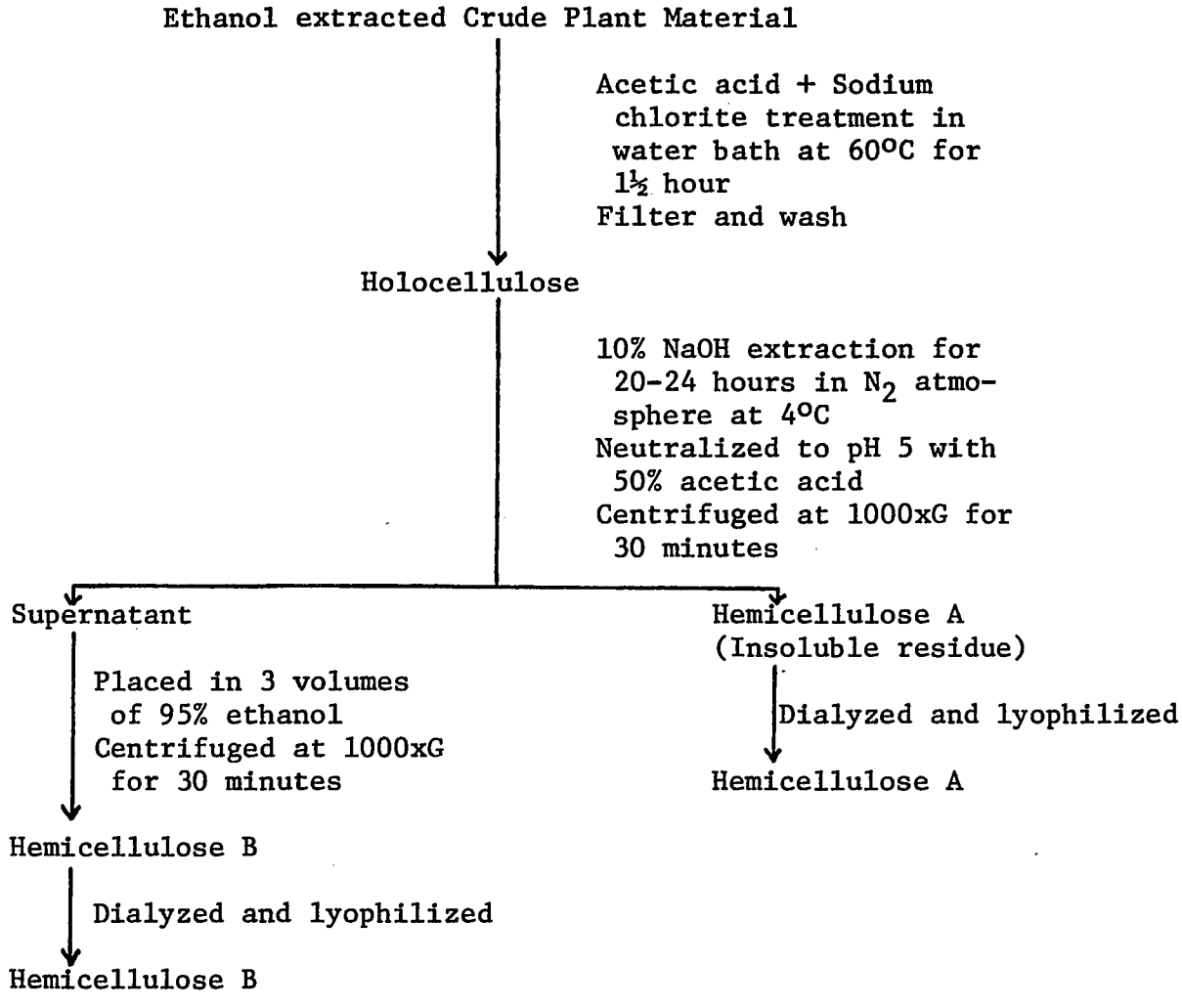


Figure 3 Preparation of holocellulose and hemicellulose A and B from 80% ethanol extracted crude plant material.

Preparation of Hemicellulose

Hemicellulose was prepared by mixing 200 grams of holocellulose (as shown in Figure 3) or the ethanol extracted crude plant material as shown in Figure 4, with 2 liters of 10% sodium hydroxide. The mixture was sealed under nitrogen gas for 20-24 hours at 4°C and then neutralized with acetic acid and centrifuged at 1000xG for 30 minutes. The residue recovered was termed hemicellulose A. The filtrate was placed in 3 volumes of 95% ethanol and the precipitate was recovered by centrifugation at 1000xG for 30 minutes. The residue was termed hemicellulose B. Both hemicellulose fractions were dialyzed to remove excess salts and lyophilized to obtain dry hemicellulose fractions.

The hemicellulose fractions, prepared from the ground plant source and from holocellulose, were refluxed in 1 N H_2SO_4 for 6 hours as described by McLaren et al. (1974a) for the preparation of regular corncob ARH.

Experimental procedure for in vitro experiments

Rumen liquor for these experiments was obtained from two sheep, one a wether and the other a ewe. Both of the sheep, of the Suffolk breed, were maintained on an all-hay diet of mostly orchard grass and alfalfa.

The rumen liquor was sampled from the animals prior to the morning feeding by drawing the rumen contents by vacuum into a flask. The rumen contents were strained through 4 layers of cheese cloth into a pre-warmed, CO_2 filled flask. The flask containing the rumen liquor was maintained in a 39°C water bath to keep the cells at their physiological temperature.

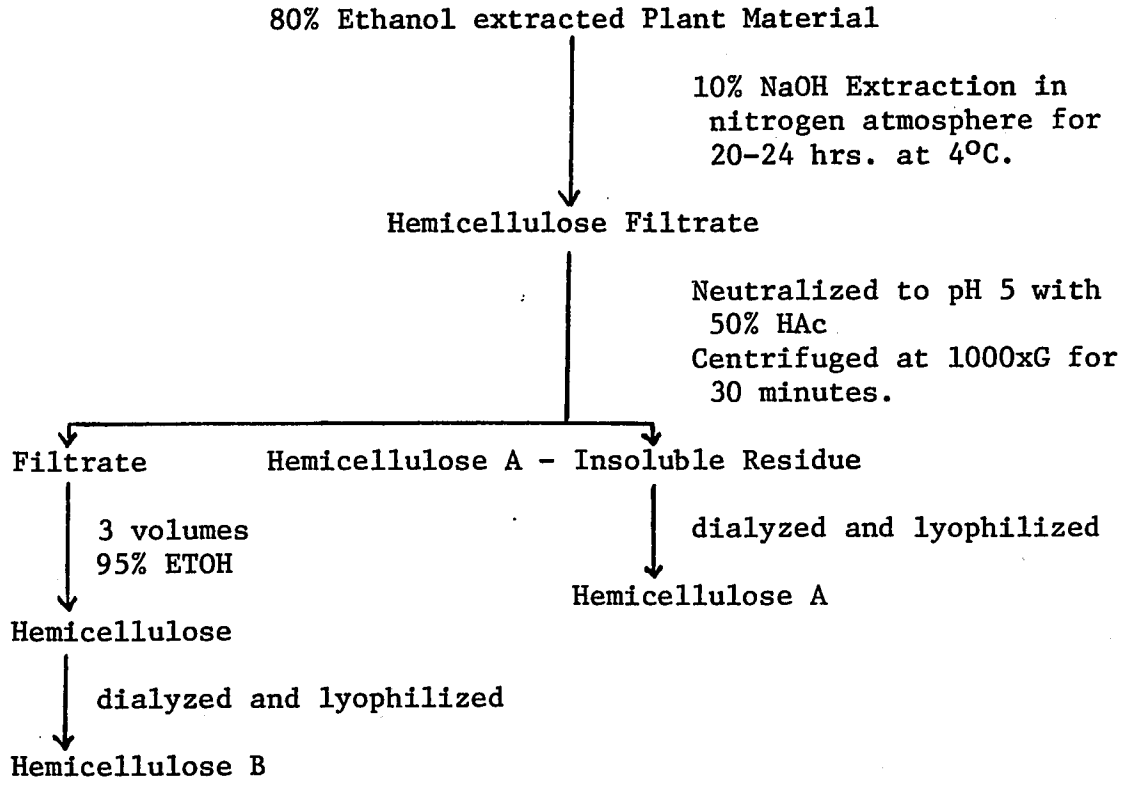


Figure 4 Flow diagram for the preparation of hemicellulose A and B from 80% ethanol extracted plant source.

The 50 ml Warburg flask which contained the basal reaction medium, as shown in Table 2, or the basal reaction medium plus the constituent to be tested for enhancing in vitro protein synthesis, were pre-warmed to 38°C and the atmosphere evacuated with CO_2 prior to inoculation with rumen liquor. Five ml aliquots of strained rumen liquor were added to the Warburg flasks. The contents of the Warburg vessel were flushed with CO_2 for 30 minutes to ensure anaerobiosis. The fermentation medium was allowed to incubate for 6 hours with slight mechanical agitation provided by the Warburg respirometer since it was found that maximum protein synthesis had occurred by this time. This agitation simulated the mixing conditions occurring in the rumen during fermentation.

After the incubation period, the rumen microbial protein was precipitated in 160 ml of 95% ethanol followed by washing the flasks with 5 ml of 95% ethanol. Zero hour protein precipitates were prepared in the same fashion. After allowing the samples to stand for at least 4 hours, the protein precipitates were recovered by centrifugation at 1000xG for 30 minutes. The supernatant was discarded and the protein residue was taken up in water and dialyzed against water for 2 days to remove any nonprotein nitrogen compounds. The samples were lyophilized in order to obtain the dry material.

Protein N was determined after dialysis of the ethanol precipitated proteins by means of the Kjeldahl method (A.O.A.C., 1965) utilizing the Technicon 40 block digestor and autoanalyzer as described by Isaac and Johnson (1976). The net protein N synthesis was obtained by subtracting the protein N content of the reaction vessel at zero time

TABLE 2

Composition of the Basal Reaction Medium

Ingredient	Quantity
Strained rumen liquor	5 ml
McDougall's artificial saliva	20 ml
Glucose	500 mg
Urea	75 mg

[a]McDougall (1949) and modified by the addition of the following trace minerals/liter: Ferrous sulfate 9.9 mg, Manganous sulfate 4.0 mg, cobaltous chloride 2 mg, and cupric sulfate 2.0 mg.

from protein N content of the reaction vessel after the 6 hour incubation period.

The nitrogen content of a standard sample of orchard grass leaves was determined by runming a Kjeldahl nitrogen using the boric acid method. The nitrogen content of the orchard grass leaves was 2.6± 0.05%. This was in agreement with the nitrogen values recorded by National Bureau of Standards which was 2.5±.1%. During each trial using the block digestor and the Technicon Autoanalyzer, nitrogen content was determined on the orchard grass leaves and found to be 3.0±.3% for all trials.

Experimental Design of in vitro experiments

One replicated in vitro experiment, involving an average of 4 reaction vessels per treatment, was conducted to test the influence of ARH isolated from corncob hemicellulose, woodchip hemicellulose, delignified corncob hemicellulose, delignified woodchip hemicellulose, and delignified alfalfa hemicellulose on rumen microbial protein synthesis. Net protein N synthesis was determined by subtracting the N present in 5 ml of rumen fluid precipitated at 0 time from the protein nitrogen in the reaction vessels after the 6 hour incubation period. The improvement in protein N synthesis was determined by comparing the 6 hour controls and the 6 hour controls + 0.02% ARH fractions.

Experimental Procedure for Rumen Liquor Trial

An experiment was conducted in order to isolate a lignin-hemicellulose fraction from the rumen fluid of a fistulated cow. The lignin hemicellulose fraction would be compared as far as biological activity and chemical composition to ARH from ground corncobs. In order to

isolate a lignin hemicellulose fraction which was low in protein N, ground corn cobs were fed as the sole roughage to the animal.

A fistulated Holstein nonlactating cow weighing approximately 430 kg was fed a semi-purified urea-corncob ration for 28 days. The corncobs were the roughage source and the readily fermentable carbohydrates were provided by corn starch and dried cane molasses. The ration had a TDN value of 11.7 lbs. The composition and average daily intake of the ration are presented in Table 3. The percentage nitrogen in the urea ration was 2.0%, or a 12.5% protein equivalent. The composition and average daily intake of the mineral mixture is shown in Table 4.

The animal was gradually adjusted to the all urea semi-purified ration by feeding 1/4 increments of the semi-purified ration with ground orchard grass hay for a 2 day period. After a 6 day adjustment period the animal was placed on the 100% corncob-all urea ration. The animal consumed the entire ration throughout the 28 day trial.

Trial 2 was conducted in which the fistulated cow was fed 37.6% ground sawdust as the sole roughage source. The remaining constituents were the same as trial 1, as shown in table 3. The animal was adjusted to the urea-semi-purified ration over a 6 day period. The Holstein cow consumed the entire ration each day of the 11 days.

Trial 3 was conducted by feeding timothy hay as the sole roughage to 2 fistulated sheep during 28 days. The sheep were also allowed water and salt ad libitum during the trial.

Rumen liquor and fecal samples were obtained from the fistulated dairy cow previously fed a semi-purified diet containing 47% corncobs.

TABLE 3

Average Daily Intake of Rations Fed to Holstein Dairy Cow for Trial 1 and Trial 2[a]

Constituent	Trial 1 Quantity	Trial 2 Quantity
Ground Corn Cobs	4457.4	-
Ground Sawdust	-	3343.1
Dried Molasses	2222.4	2222.4
Mineral Mix[a]	252.7	252.7
Concentrate Mixture	3067.5	3067.5
Urea	376.1	376.1
Corn Starch	2398.3	2398.3
Corn Oil	266.6	266.6
Fish Oil	26.6	26.6
Total	10000.0	8885.7

[a]Thomas et al. (1951)

TABLE 4

Average Daily Intake of Mineral Supplement[a]

Ingredient	Quantity
NaCl	33.6
K_2HPO_4	59.4
$CaHPO_4$	52.4
MgO	18.1
$CaSO_4 \cdot 2H_2O$	77.8
$CaCO_3$	5.6
$Fe(C_6H_5O_7) \cdot H_2O$	5.2
Trace minerals[b]	.7
Total	252.8

[a]Thomas et al. (1951)

[b]The trace mineral mixture supplied the following as percent of the total mineral mixture: KI .016%, $MnSO_4 \cdot 4H_2O$ .130%, $ZnCl_2$ .022%, $CuSO_4 \cdot 5H_2O$ .028%, $CoCl_2 \cdot 6H_2O$ .067%, and CaF_2 .004%.

The rumen fluid was taken in the morning prior to feeding and strained through 4 layers of cheese cloth. The strained rumen liquor was centrifuged at 39,000xG for 30 minutes to remove bacteria and food debris. The cell-free rumen fluid was either dialyzed against water for 5 days with frequent changes of the water to remove small molecular weight compounds and salts or precipitated in ethanol without prior dialysis. It was found that dialysis removed none of the high molecular weight lignin-hemicellulosic fraction. The sample was then placed in 4 volumes of 95% ethanol in order to precipitate the lignin-hemicellulose fraction. The fraction was recovered by centrifugation at 1000xG for 30 minutes. The residue was taken up in water and lyophilized to obtain a dry fraction. This lignin-hemicellulose fraction was tested for its biological activity by measuring its influence on in vitro microbial protein synthesis and compared to ARH from ground corn cobs. The fecal samples, which were taken in conjunction with the rumen fluid, were immediately dried at 50°C and stored for analysis. Dried fecal samples were extracted with alcohol:benzene, (2:1 v/v) for 40 hours to remove lipid material, pigments and chlorophyll. The extracted fecal material was refluxed in 1 N H_2SO_4 for 6 hours, as previously described for corncob and other plant material. The residue was filtered and the filtrate neutralized with calcium carbonate. The calcium salts were removed by filtration and the neutralized hydrolysate place in 2 volumes of 95% ethanol to precipitate the ARH fraction. The precipitate was recovered by centrifugation at 1000xG for 30 minutes.

Chemical Analysis

Paper Chromatography

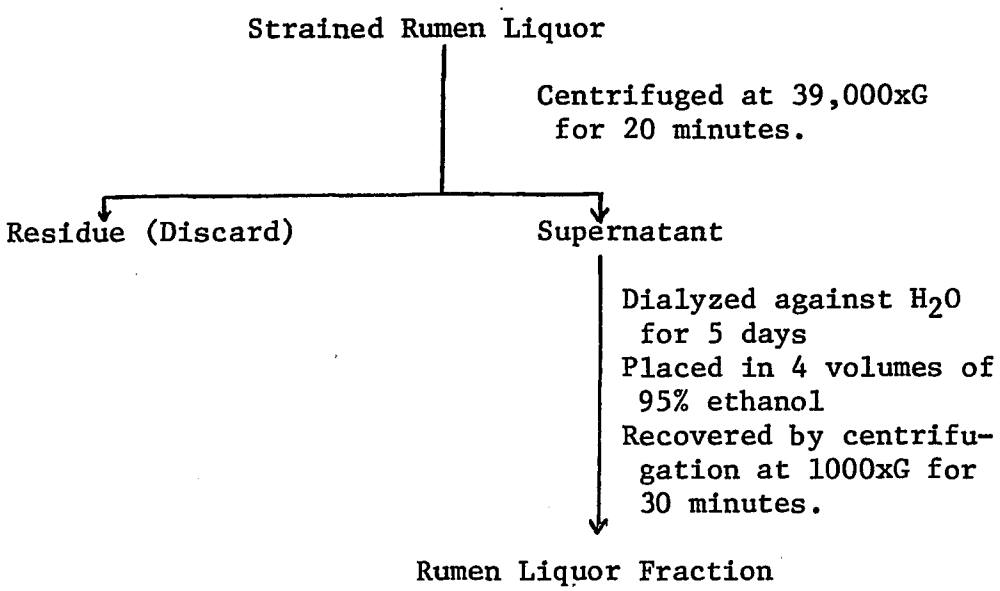


Figure 5. Flow diagram for the preparation of a rumen liquor fraction from strained rumen liquor.

Detection of carbohydrate derivatives and phenolic compounds in the ARH fractions was achieved by paper chromatography. These ARH fractions were prepared by taking the neutralized hydrolysate prior to alcohol precipitation and treating with cation resin to remove excess calcium salts. The samples were concentrated to 1 ml by lyophilization and spotted on paper in quantities of 100 ul. The solvent system used for carbohydrate derivatives was butanol:acetic acid:water (2:1:1 v/v) (Myre and Smith, 1960) and ethylacetate:acetic acid:formic acid:water (18:3:1:4 v/v) (Whistler and Gaillard, 1961). Phenolic compounds were also detected with these solvent systems by observation under U.V. light. The carbohydrate derivatives were detected with 5% aniline hydrogen phthalate in acetic acid (Whistler and Gaillard, 1961) and 3% anisidine hydrogen chloride in butanol. The spots were developed by drying the chromatograph in a forced-air oven at 100°C. The R_f value or Rx value (R_f value for the individual spot divided by the R_f value for xylose) along with the characteristic color was recorded for each spot.

Phenol Assay

The various ARH samples (2.5 mg) were added to 1 ml of 1 N H_2SO_4 and thoroughly mixed in a tissue homogenizer. This solution was brought to a volume of 25 ml with distilled water. The phenol concentration was determined on 1 ml of the sample by the Folin-Denis method according to Swain and Hillis (1959). The absorbance of the ARH samples was compared to a standard curve for reagent grade (ACS) phenol.

Smith-Phenol Assay

The various ARH samples (2.5 mg) were added to 1 ml of 1 N H_2SO_4

and mixed thoroughly in a tissue homogenizer. This solution was brought up to a volume of 25 ml with distilled water. The carbohydrate concentration was determined by the Smith-Phenol assay procedure of Dubois et al. (1956). This procedure was modified in that 1% reagent grade phenol was used rather than 5% phenol (Smith, 1976). The absorbance of the various ARH samples was compared to a standard curve for xylose.

Gel Chromatography

The ARH fractions were separated by gel chromatography in order to determine the molecular weight of these fractions. G-100 Sephadex resin of medium porosity was used because it was speculated that the molecular size would fall between 10,000 and 100,000. Dextran standards with molecular weights of 10,000 and 70,000 were used to compare to the MW of the lignin-hemicellulose complexes. A column with dimensions of 60 cm by 2.5 cm was used and the fractions were eluted with 0.025 M Ammonium acetate at a flow rate of .6 ml/minute. The carbohydrate fraction was assayed by the Smith-Phenol method of Dubois et al. (1956) while the lignin portion was determined by the U.V. absorbance at 280 nm since the protein content of the alkaline-cleaved fractions was very low.

Miscellaneous Chemical Analysis

Moisture and ash determinations were carried out on all ARH fractions according to the AOAC method (1965). Kjeldahl N determinations were conducted using the block digestor and technicon autoanalyzer according to the procedure of Isaac and Johnson (1976). Atomic absorption analysis of the minerals present in the inorganic portion of ARH

samples was performed on a Jarrell-Ash Series 82-360 Atomic Absorption/ Flame Spectrometer. An estimation of lignin in the rumen liquor fractions was obtained on the residue of the acid hydrolyzed lignin-hemicellulose fraction from rumen liquor by the AOAC method, (1965), as outlined by Ellis et al. (1946).

Statistical Analysis

The analysis of variance was used to test the overall significance of the various treatments. Dunnett's and Duncan's multiple range tests were conducted to test significance among the control and treatment means and among means of treatments (Snedecor and Cochran, 1967). Standard deviations and standard errors of the mean (SEM) were calculated according to methods outlined in Steele and Torrie, 1960.

RESULTS AND DISCUSSION

Data on growth of rats fed different ARH preparations are presented in table 5 and 6. In trial 1, the inclusion of cation resin-treated ARH into the diets of male weanling rats at 0.05% improved growth and feed efficiency over the basal fed rats by 12.9 and 11.7% ($P < .05$), respectively. An increase in the organic matter of cation resin-treated ARH as compared to regular corncob ARH resulted in a slight improvement in growth response over the basal fed rats.

In trial 2, the incorporation of each of the ARH preparations into the basal diet (0.05%) resulted in a significant increase ($P < .05$) in growth and feed conversion of male weanling rats (table 6). It would appear that removal of low molecular weight sugars by dialysis and phenolic products by carbon treatment did not alter the growth response due to ARH. However, water extraction of the precipitated ARH seemed to remove a portion of the active fraction of ARH, since the feeding of the ARH preparation yielded the smallest growth response.

The cation resin-treated ARH had the largest percentage of total carbohydrates of the five preparations as shown in table 9. In trial 1, there was not an appreciable improvement in growth of rats receiving cation resin-treated ARH as compared to corncob ARH. However, in trial 2 water extraction of ARH caused a 31% reduction in total carbohydrates as compared to corncob ARH. In the other ARH preparations, the total carbohydrate content was not significantly different. The similarities of the total carbohydrates of these ARH preparations were in agreement with the growth response of rats receiving these fractions in the semi-purified diet.

TABLE 5

Influence of Acid-resistant Hemicellulose Preparations on the Growth Rate and Feed Conversion of Weanling Male Rats[1]

Diet	No. of Rats	Average Daily Gain	Improvement Over Basal	Feed Conversion	Improvement in Feed Conversion
		g	%	g feed/g gain	%
Basal	18	6.0±.2	--	2.1±.1	--
Basal + 0.05% ARH	18	6.6±.2[2]	10.0	1.9±.1[3]	9.5
Basal + 0.05% cation resin-treated ARH	18	6.7±.2[2]	11.7	1.9±.1[3]	9.5

[1]Trial was conducted for 17 days.

[2]Greater ($P < .05$) than that by rats fed the basal diet within this trial.

[3]Less ($P < .05$) than that by rats fed the basal diet within this trial.

TABLE 6

Influence of Acid-resistant Hemicellulose Preparations on the Growth Rate and Feed Conversion of Weanling Male Rats[2]

Diet	No. of Rats	Average Daily Gain	Improvement Over Basal	Feed Conversion	Improvement in Feed Conversion
		g	%	g feed/g gain	%
Basal	20	6.2±.1	--	2.5±.1	--
Basal + 0.05% H_2O ext. ARH	20	6.7±.1[2]	8.1	2.3±.1[3]	8.0
Basal + 0.05% non-dialyzable ARH	20	7.0±.1[2]	12.9	2.3±.1[3]	12.0
Basal + 0.05% carbon-treated ARH	20	6.9±.2[2]	11.3	2.2±.1[3]	8.0

[1]Trial was conducted for 21 days.

[2]Greater ($P < .05$) than that by rats fed the basal diet within this trial.

[3]Less ($P < .05$) than that by rats fed the basal diet within this trial.

TABLE 7

Analysis of Variance of Growth Data Comparing Carbon-Treated ARH, Water-Ext. ARH, Non-dialyzable ARH.

Source	df	SS	MS	F
Total	77	29.85		
Treatment	3	5.96	1.99	15.3*
Trial	1	14.69		
Error	73	9.20	.13	

*Significant at $P < .01$

Analysis of Variance Comparing Feed Conversion Data of Carbon-treated ARH, Water Extr. ARH and Non-dialyzable ARH Fractions

Source	df	SS	MS	F
Total	77	4.33		
Treatment	3	.89	.297	6.46*
Trial	1	.09		
Error	73	3.35	.046	

*Significant at $P < .01$

TABLE 8

Analysis of Variance of Growth Data Comparing the Purified and Regular Corn Cob ARH

Source	df	SS	MS	F
Total	53	32.94		
Treatment	2	6.68	3.34	9.97*
Trial	1	9.52	9.52	
Error	50	16.74	.34	

*Significant at $P < .01$

Analysis of Variance of Feed Conversion of Rats fed Purified and Regular Corncob ARH Fractions

Source	df	SS	MS	F
Total	53	3.10		
Treatment	2	.69	.35	11.67*
Trial	1	.93	.93	
Error	50	1.48	.03	

*Significant at $P < .01$

The free phenolic content was reduced by 77% upon carbon and cation resin treatment of the ARH preparations. The phenolic content of the water-extracted ARH was slightly higher than corncob ARH and significantly higher than other ARH preparations. Fahey, et al. (1976) demonstrated that the phenolic portion of the molasses lignin-hemicellulose fraction caused a growth depression when fed to rats. Perhaps the high free phenolic content and lower total carbohydrate content of the water-extracted ARH preparation may have resulted in the lower growth response.

A portion of the organic matter of the ARH fractions was not accounted for by the Smith-phenol and Folin-Denis assays. This residual organic matter maybe attributed to nitrogen, phenolic groups failing to react because they were bound to carbohydrate, and carbohydrate since various sugars in ARH respond differently to the phenol-sulphuric acid method. It may be inferred that the values for carbohydrates and free phenols are only approximate estimations. The nitrogen content, may contribute a small percentage of the residual organic matter. Since corncobs contain approximately .5% nitrogen as protein the amount in ARH would be extremely low.

The presence of hemicellulose degradation products among the organic portion of ARH has been confirmed by chromatographic analysis. With solvent system 1, five R_f values were obtained for three ARH preparations. These R_f values for cation resin-treated ARH, carbon-treated ARH and water-extracted ARH were similar with $R_f$values for oligouronic acid (.18), mono-o-methyl aldobiuronic acid (.41), galacturonic acid (.34) and glucuronic acid (.51). The R_f value .074, which existed as a component of all ARH preparations, may possibly be a high molecular weight

TABLE 9

Total Carbohydrate and Total Free Phenol Content of Acid Resistant Hemicellulose (ARH) Fractions

Fractions	Organic Matter	Total Carbohydrate[1]	Free Phenol[2]
	%	%	%
Cation resin-treated ARH	56.0	54.3	1.3
Non-dialyzable ARH	27.0	15.8	5.5
Carbon-treated ARH	22.0	12.5	1.5
Water-extracted ARH	21.0	10.7	6.8
Corncob ARH	22.0	15.6	6.1

[1]Total carbohydrate determined using xylose as the standard in the phenol-surfuric acid method.

[2]Total free phenol determined by Folin-Denis Method.

hemicellulose degradation residue. Similar hydrolytic products have been identified in alfalfa hemicellulose (Myre and Smith, 1960).

Four R_x values existed for three of the ARH preparations with solvent system 2. According to the report of Whistler and Gaillard (1961), aldobiuronic acid (.75) and galacturonic acid (.54) appeared to be present in all three ARH preparations. Aldotriuronic acid (.30) appeared to be present in the cation resin-treated ARH and the carbon-treated ARH preparations. The poor resolution of the non-dialyzable ARH preparation in both solvent systems suggests that it contained a high molecular weight phenolic-hemicellulose degradation product.

The ARH preparations had a similar R_f value in the range of .26-.35 for solvent system 2. The R_f value .28 agrees with the xylose standard (.29). However, the range would indicate the presence of other components. In the cation resin-treated ARH and the carbon-treated ARH preparation, phenolics were detected in the same range (.33-.35) with ultraviolet light. This suggests that the carbohydrate components migrated at the same rate as the phenolic portion, and could infer chemical bonding between the two classes of components.

The phenolic-hemicellulose ARH fraction from acid hydrolyzed corncobs appeared beneficial to the rat as evidenced by a growth response. Other work has demonstrated the occurrence of phenolic-hemicellulose complexes in cane sugar products and their growth stimulus to the rat (Fahey, 1976). These phenolic-hemicellulosic degradation products (ARH) which may be obtained from forages and cellulosic wastes may prove to be of nutritional significance to the animal.

Mineral analysis by atomic absorption spectroscopy was carried out

TABLE 10

Paper Chromatographic Separation of Carbohydrate Components of Corn-Cob ARH Factors

Fraction	Butanol-acetic Acid-water 2:1:1	Ethylacetate-acetic Acid-formic Acid-water 18:3:1:4	
	R_f	R_f	R_x
Cation resin-treated ARH	.07	.07	.29
	.18	.14	.57
	.42	.22	.74
	.51	.28-.36	.96
	.60		
Non-dialyzable ARH	.05	No separation	
	.34		
Carbon-treated ARH	.07	.06	.21
	.15	.15	.52
	.24	.21	.71
	.34	.26-.34	.92-1.18
	.40		
Water-extracted ARH	.07	.15	.53
	.15	.21	.73
	.24	.27	.94
	.34	.35	1.20

on the ARH fractions after ashing since the inorganic portion made up approximately 70% of the actual materail (table 11). However, the cation resin-treated ARH fraction was an exception to this since the organic portion of this fraction accounted for 56% of the actual material. The macro minerals present in highest quantities were calcium and potassium. Of the trace minerals, zinc and manganese accounted for the largest amount. Since the USPXIV salt mixture was lacking in zinc and marginal in copper, the mineral mixture was supplemented with either 4 mg or 8 mg of zinc per kilogram of diet and 2 mg of copper per kilogram of diet. However, the ARH preparations supplied a minimal amount of zinc and copper to the diet (1.4 ug of zinc and .3 ug of copper per day). These findings were in agreement with McLaren et al. (1974a) who indicated that contribution of trace minerals from the inorganic portion of ARH was minimal. Furthermore, it substantiates the report of McLaren et al. (1974a) that the inorganic portion of ARH was not responsible for the growth response in weanling, male rats.

From these studies, phenolic-hemicellulose ARH fractions from acid hydrolyzed corncobs were beneficial to rats as evidenced by growth response and improved feed conversions. Furthermore, this study confirms reports by McLaren et al. (1974a) and Fahey et al. (1976) who have demonstrated the occurrence of phenolic hemicellulosic complexes in cane sugar products and a wide variety of plant sources and their growth stimulus to the rat. Since these phenolic hemicellulosic degradation products (ARH) may be obtained from a variety of low quality forages and cellulosic wastes, these may prove to be of nutritional significance to the animal.

TABLE 11

Mineral Composition of Acid-Resistant Hemicellulose Fractions after Ashing

Element	Purified ARH	Nondialyzable ARH	Carbon-Tr ARH	Washed ARH	Corncob ARH
Copper ppm	38	36	19	17	16
Nickel ppm	34	26	--	19	--
Manganese ppm	64	118	68	29	73
Zinc ppm	186	612	165	271	364
Chromium ppm	32	10	6	13	8
Iron mg/gr	11.4	5.6	7.1	5.3	123
Potassium mg/gr	.4	7.2	11.4	17.2	17.4
Magnesium mg/gr	.6	7.0	.3	.2	.6
Calcium me/gr	12.6	148.8	191.0	196	162.5

Reports have indicated that phenolic-hemicellulosic complexes are beneficial to the nutrition of ruminant and nonruminant animals (McLaren et al., 1974; McLaren et al., 1976; Fahey et al., 1976; and Hatfield et al., 1972). Furthermore, these phenolic-hemicellulose complexes occur in a variety of natural sources (McLaren, 1950; Fahey et al., 1976; Markham, 1972; Morrison, 1973; and Hartley, 1973). Fahey et al. (1976) has reported that alkaline cleavage of a black phenol-hemicellulosic complex from cane molasses resulted in a carbohydrate moiety and phenolic moiety. When included in a basal diet at 0.03%, only the carbohydrate moiety yielded a significant growth response in weanling male rats. However this growth response was only 68% of that produced with the phenolic-bound carbohydrate fraction. Perhaps, subjection of the carbohydrate-phenol complex to alkaline treatment proved to be too severe for cleavage of the ester bonds. This work demonstrated that certain carbohydrate moieties, appearing free of phenols by qualitative tests, are nutritionally beneficial. However, since the biological activity was less than that obtained with the original lignin-hemicellulosic complex perhaps a portion of the phenolic moiety is necessary for optimal activity.

With this in mind, non-dialyzable ARH was cleaved by alkaline treatment in attempts to obtain a pure carbohydrate moiety which yielded a significant growth response in rats. The alkaline cleavage of non-dialyzable ARH resulted in an ethanol-insoluble fraction and an ethanol-soluble fraction as shown in figure 2. The incorporation of the ethanol-insoluble fraction into a casein basal diet at 0.05% level significantly

TABLE 12

Influence of Alkaline-Cleaved Corncob ARH On The Growth of Rats[a]

Treatment	No. of Animals	Average Daily Gain	Per Cent Improvement	Feed Conversion	Per Cent Improvement
		G	%		%
Basal	20	5.95±.11		2.44±.06	
Basal + Ethanol-Insoluble Fraction	20	6.38±.07[b]	7.2	2.27±.03[b]	7.0
Basal + Ethanol-Soluble Fraction	20	6.04±.08[c]	1.7	2.34±.03[c]	3.3

[a]Trial was conducted for 20 days.

[b]Significantly different with Dunnett's test at $P < .05$.

[c]Not significantly different with Dunnett's test at $P < .05$.

TABLE 13

Influence of Alkaline-Cleaved Corn Cob ARH on *in vitro*[b] Protein Synthesis with Rumen Microorganisms

6 Hour Control N	Ethanol-Insoluble Fraction N	Ethanol-Soluble Fraction N
mg	mg	mg
3.9±.2	5.4±.5[a]	4.3±.3

[a]Significantly different from 6 hour control with Dunnett's test at $P < .05$.

[b]Eight tubes per treatment.

TABLE 14

Analysis of Variance of Growth Data for the Alkaline Cleaved Fractions

Source	df	SS	MS	F
Total	59	10.56		
Treatment	2	2.05	1.03	17.4*
Replication	1	5.19		
Error	56	3.32	.059	

*Significant at $P < .01$

Analysis of Variance of Feed Conversions for the animals fed the Alkaline-Cleaved ARH fractions

Source	df	SS	MS	F
Total	59	2.18		
Treatment	2	.29	.145	7.3*
Replication	1	.78		
Error	56	1.11	.0198	

*Significant at $P < .01$

increased the average daily weight gain and feed conversion of weanling male rats over basal fed rats during a 20 day trial. The ethanol-soluble fraction actually inhibited growth up to two weeks but this inhibition leveled off during the third week.

The biological activity of these two fractions was also compared by testing their ability to improve in vitro protein synthesis of rumen microorganisms. Only the incorporation of the ethanol-insoluble fraction at the .02% level significantly increased protein N over 6 hour controls. Unlike the growth depression observed in the rat, the inclusion of the ethanol-soluble fraction did not inhibit protein N synthesis. Apparently the microflora in the rumen are not susceptible to the toxic effect of free phenolics as the monogastric animal. A similar non-inhibitory effect on in vitro microbial protein synthesis of lignin-hemicellulose complexes from cane molasses, which are high in phenolics, was demonstrated by Woolf (1969).

The examination of the total carbohydrate and free phenol content of these two fractions revealed that an incomplete separation of carbohydrate and phenol moiety occurred. In each fraction the carbohydrate and free phenols made up approximately 60% and 20% of the organic matter, respectively. However, the organic matter of the ethanol-soluble fraction was 77.3% higher than that of the ethanol-insoluble fraction. Although Fahey et al. (1976) reported an alkaline-cleaved carbohydrate moiety from cane molasses, the present finding agrees with Morrison (1973). By fractionation with gel chromatography, Morrison reported that the 5 components extracted by alkaline treatment had carbohydrate and lignin associated with it.

TABLE 15

Total Carbohydrate and Phenol Content of the Ethanol-Insoluble and Ethanol-Soluble Fractions from ARH

Fraction	Total Carbohydrate	Total Phenol[2]	Org Matter
	%	%	%
Ethanol-insoluble Fraction	18.8	6.7	32.1
Ethanol-soluble Fraction	34.2	11.5	56.9

[1]Total carbohydrate was determined using xylose as the standard by the phenol-sulfuric acid method.

[2]Total phenol was determined by Folin-Denis method.

The carbohydrate components of alkaline-cleaved fractions separated with two solvent systems by paper chromatography is shown in Table 16. The ethanol-insoluble fraction did not move in either solvent system and was hydrolyzed in order to identify individual carbohydrate components. The carbohydrate components of the alkaline-cleaved fractions were similar to aldobiuronic acids (R_f .41 and R_x .75 with solvent system 1 and 2, respectively) and galacturonic acid (R_f .34 and R_x .52 with solvent system 1 and 2, respectively). Aldotriuronic acids (R_x .30) were identified as a component of the ethanol-soluble fraction. The corresponding R_f value .13 of the ethanol-soluble and ethanol-insoluble fraction may be a derivative of uronic acid since glucuronic acid and galacturonic acid had R_f values of .16 and .15, respectively. All of these components of alkaline-cleaved fractions are indicative of hydrolyzed components of hemicellulose and have been identified by Myre and Smith (1960) and found by Fahey (1976) to be present in the alkaline-cleaved black phenol carbohydrate complex.

Fractionation studies on Sephadex G-100 were undertaken to obtain an estimation of molecular size of these alkaline-cleaved fractions. The fractions were passed through G-100 Sephadex (60 cm by 2.5 cm) column at a flow rate of .6 ml/min and eluted with 0.025 M ammonium acetate. The ethanol-insoluble fraction came off around 170-220 ml and had peaks for carbohydrate and lignin as shown in figure 6. Although the carbohydrate peak was eluted slightly ahead of the lignin peak, there was considerable overlapping of the two moieties to indicate a bond between the fractions.

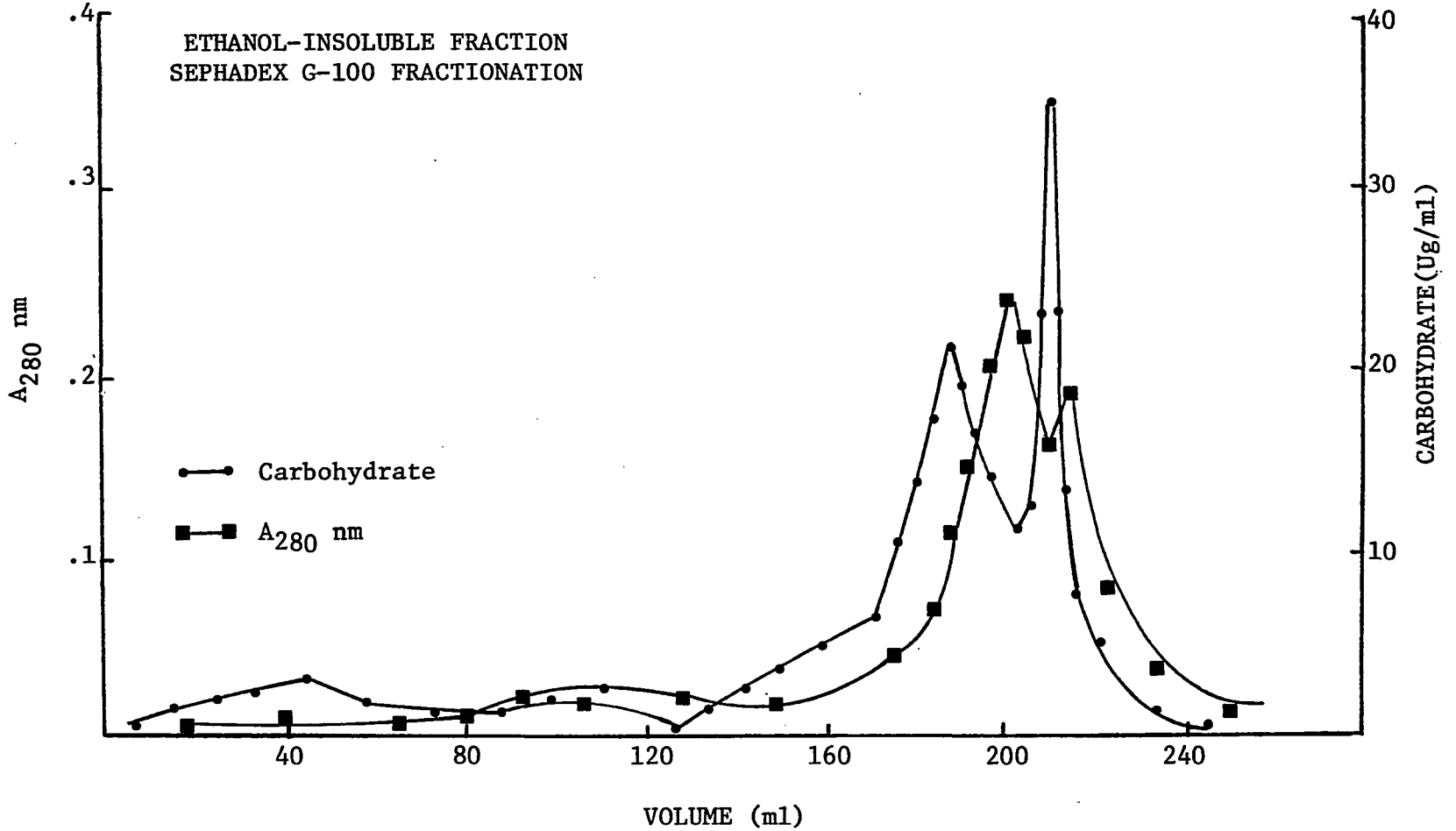
TABLE 16

Paper Chromatography of the Carbohydrate Components of the ETOH-Soluble and ETOH-Insoluble Cleaved ARH Fractions

Fraction	1 BAW 2:1:1	2 EA:HAC:FA:H_2O 18:3:1:4	
	R_f	R_f	R_x[1]
Ethanol-Soluble	.34	.22	.75
	.42	.16	.54
		.13	.46
		.08	.28
Ethanol-insoluble	No Movement	No Movement	
Hydrolyzed	.30	.21	.73
Ethanol-Insoluble	.34	.12	.45
	.43	.04	.13

[1] R_x value obtained by dividing R_f value for carbohydrate components by the R_f value for xylose.

FIGURE 6


ETHANOL-INSOLUBLE FRACTION
SEPHADEX G-100 FRACTIONATION
.4
.3
.2
.1
A_{280} nm
40
30
20
10
CARBOHYDRATE (Ug/ml)
Carbohydrate
A_{280} nm
40
80
120
160
200
240
VOLUME (ml)

The ethanol-soluble fraction came off between 195-240 ml. In both the ethanol-soluble and ethanol-insoluble fractions this was well beyond the elution volume for the 70,000 and 10,000 molecular weight dextran standards and would indicate an interaction of aromatics, such as a lignin degradation fraction with the dextran gel. Gaillard and Richards (1975) and Morrison (1973) also presented evidence that such an interaction between lignin degradation fractions and the dextran gel may occur, precluding any possibility of molecular weight determination of lignin-hemicelluloses with dextran gels, such as Sephadex.

The ethanol-soluble fraction had one peak for carbohydrates and three peaks for lignin (figure 7). The spectrum of the peaks for both moieties indicated that the carbohydrate peak of the ethanol-insoluble fraction was degraded and yielded the large peak of the ethanol-soluble fraction. The lignin peak of the ethanol-soluble fraction also shifted slightly to the right indicating the degradation of the carbohydrate-bound phenolics.

It has been determined that lignin displays a typical U.V. spectral analysis of maximum absorption at 280 nm and a minimum at 258 nm (Sarkanen and Ludwig, 1971). Gaillard and Richards (1975) demonstrated this typical U.V. spectrum for lignin-hemicellulose complexes fractionated from rumen fluid. The ethanol-soluble fraction revealed this typical U.V. spectra as shown in figure 8. However, the ethanol-insoluble complex had a leveling off in this region which is an indication that there is more of the carbohydrate than the phenolic moiety in the ethanol-insoluble complex.

FIGURE 7

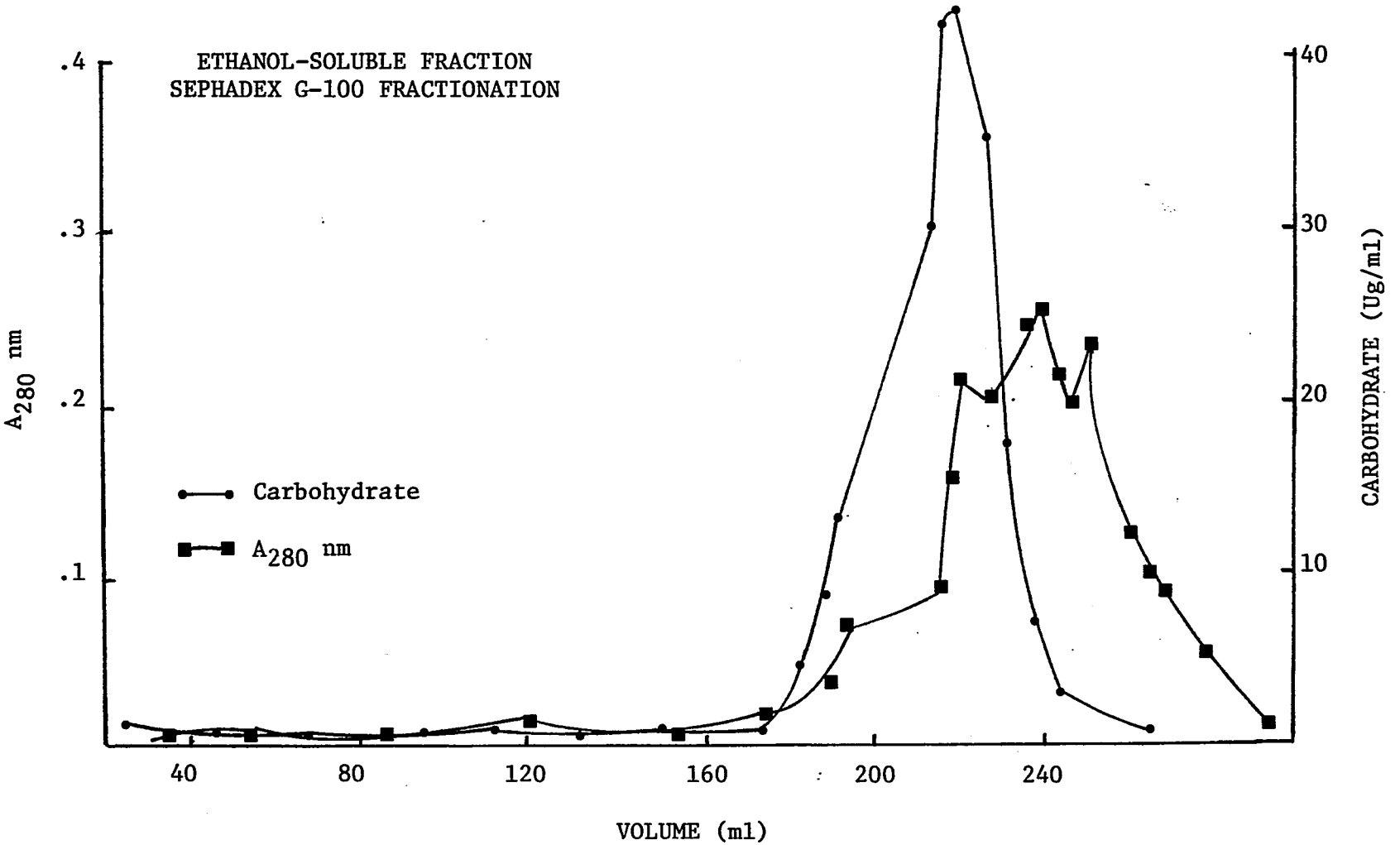
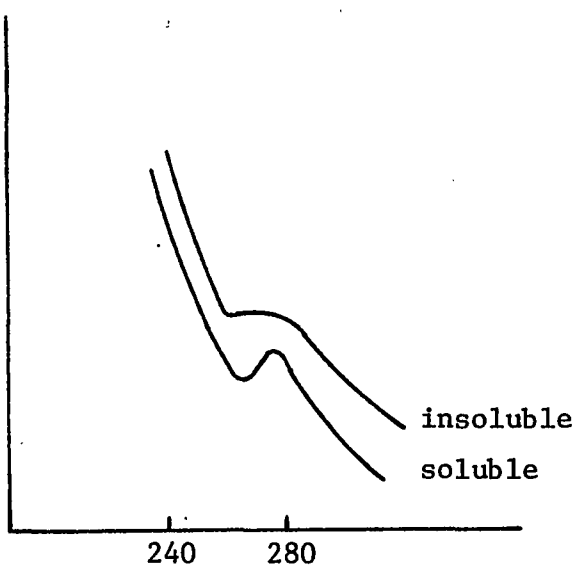

ETHANOL-SOLUBLE FRACTION
SEPHADEX G-100 FRACTIONATION
.4
.3
.2
.1
A_{280} nm
40
30
20
10
CARBOHYDRATE (Ug/ml)
Carbohydrate
A_{280} nm
40
80
120
160
200
240
VOLUME (ml)

FIGURE 8

UV SPECTRA OF ALKALINE
CLEAVED ARH FRACTIONS



This work confirmed reports by Fahey et al. (1976) and demonstrated that alkaline cleavage of acid-resistant hemicellulose produced an ethanol-insoluble lignin-hemicellulosic degradation complex. As far as biological activity in rats and microorganisms, this complex was different from the ethanol-soluble lignin hemicellulosic degradation component. According to gel chromatography and the U.V. spectrum the presence of lignin degradation products appeared to interfere with the dextran gel and prevent molecular weight determination of these lignin hemicelluloses. However, there appeared to be slightly lower molecular weight carbohydrate-bound lignin in the ethanol-soluble component, which may have accounted for the growth inhibition during the first two weeks.

Lignin-hemicellulose degradation fractions termed ARH has been shown to improve in vitro microbial protein synthesis (Woolf, 1969 and McLaren et al., 1976). Cuppett (1969), Alexander (1971) and McLaren et al. (1974) demonstrated that ARH fractions from holocellulose had biological activity since they enhanced rat growth. The exact chemical composition of lignin hemicellulose degradation fractions responsible for the stimulation of in vitro microbial protein synthesis remains unknown. With this in mind, 0.02% ARH from hemicellulose extracted from delignified and untreated corncob, white oak woodchips, and alfalfa were added to reaction vessels to compare their ability to improve in vitro microbial protein synthesis.

As shown in table 17, the incorporation of 0.02% ARH from hemicellulose extracted from the delignified plant and the untreated plant

TABLE 17

Influence of Hemicellulose Degradation Fractions on In Vitro Microbial Protein Synthesis

	No. of Tubes	6 Hour Control[c] N	Delignified Plant N	Untreated Plant N
		mg	mg	mg
Corncob Hemicellulose (ARH)	6	9.1±.2	10.3±.5 (13%)[ad]	10.3±.6 (13%)[ad]
Corncob Hemicellulose A (ARH)	8	4.3±.2	6.6±.7 (54%)[bd]	6.1±.7 (42%)[ad]
Corncob Hemicellulose B (ARH)	8	8.0±.6	9.5±.9 (19%)[bd]	8.9±.8 (11%)[ad]
Woodchip Hemicellulose (ARH)	10	6.0±.1	7.3±.2 (22%)[ad]	7.0±.2 (17%)[ad]
Alfalfa Hemicellulose (ARH)	7	4.3±.3	5.4±.1 (26%)[ad]	-

[a]Treatments were significantly different from control at $P < .05$ with Dunnett's test.
[b]Treatments were significantly different from control at $P < .01$ with Dunnett's test.
[c]Expressed as Kjeldahl N.
[d]Values in parenthesis represents the percent net protein synthesis over the 6 hour control.

TABLE 18

Analysis of Variance of *In Vitro* Protein Synthesis Data of Corncob Hemicellulose ARH

Source	df	SS	MS	F
Total	23	70.4		
Treatment	2	23.9	11.95	20.1*
Trial	1	34.6	34.6	58.2*
Effor	20	11.9	.595	

*Significant at P< .01.

Analysis of Variance for the Inlfuence of Corncob Hemicellulose B ARH on *In Vitro* Protein Synthesis

Source	df	SS	MS	F
Total	23	117.62		
Trial	1	102.92	102.9	366.3*
Treatment	2	10.24	5.12	18.3*
Error	20	5.61	.28	

*Significant at P< 01.

TABLE 19

Analysis of Variance of the Influence of Corncob Hemicellulose ARH on *In Vitro* Protein Synthesis

Source	df	SS	MS	F
Total	17	193.6		
Trail	1	183.8	183.8	680.7*
Treatment	2	6.1	3.05	11.35*
Error	14	3.7	.27	

*Significant at $P < .05$

Analysis of Variance of the Influence of Woodchip Hemicellulose ARH on *In Vitro* Protein Synthesis

Source	df	SS	MS	F
Total	29	21.0		
Trial	1	8.8	8.8	88.0*
Treatment	2	9.7	4.83	48.3*
Error	26	2.6	.10	

*Significant at $P < .01$

Analysis of Variance of the Influence of Alfalfa Hemicellulose ARH from Holocellulose on *In Vitro* Protein Synthesis

Source	df	SS	MS	F
Total	13	7.32		
Trial	1	.82	.82	3.7
Treatment	1	4.14	4.14	19.26*
Error	11	2.37	.22	

*Significant at $P < .01$

to the reaction medium significantly improved *in vitro* protein synthesis over the 6 hour control. In most plant sources tested, ARH from delignified plant hemicellulose had a more beneficial effect on *in vitro* protein synthesis than ARH from hemicellulose extracted from the plant which had not been delignified with sodium chlorite. However, there were no differences between ARH from corncob hemicellulose and ARH from delignified hemicellulose. When the individual hemicellulose components were compared there was more improvement in *in vitro* protein synthesis with ARH from corncob hemicellulose A and B isolated from holocellulose as compared to hemicellulose from the plant source. The differences in biological activity as measured by *in vitro* protein synthesis may be attributed to the yield of ARH produced as a result of acid hydrolysis. Perhaps delignification of the plant source prior to isolation of hemicellulose may result in a more complete hydrolysis and a larger yield of ARH.

As shown in table 20, ARH fractions prepared from delignified hemicellulose components (hemicellulose, hemicellulose A and hemicellulose B) were tested to compare their biological activity. The incorporation of all ARH fractions at the 0.02% level significantly improved *in vitro* microbial protein synthesis. ARH from delignified hemicellulose produced the largest net protein N synthesis over the 6 hour control in comparison to ARH from delignified hemicellulose A and B. The addition of 0.02% ARH from delignified hemicellulose A or delignified hemicellulose B to the reaction medium resulted in no difference in *in vitro* protein synthesis over the 6 hour control. As a result, the chemical

TABLE 20

Influence of Corncob Hemicellulose ARH Fractions from Holocellulose on *In Vitro* Protein Synthesis

No. of Tubes	6 Hour Control N	Hemicellulose A ARH N	Hemicellulose B ARH N	Hemicellulose ARH N
	mg	mg	mg	mg
8	7.5±.3	8.3±.10[a]	8.2±.07[c]	8.6±.08[b]
Net protein synthesis above control (%)		11	9	15

Significant differences determined with Dunnett's test
[a] $P < .05$
[b] $P < .01$
[c] $P < .10$

Analysis of Variance of the Influence of Hemicellulose Degradation from Holocellulose on *In Vitro* protein Synthesis

Source	df	SS	MS	F
Total	31	12.59		
Trial	1	.30	.30	1.05
Treatment	3	4.53	1.51	5.26*
Error	27	7.76	.287	

*Significant at $P < .01$

composition of hemicellulose A and hemicellulose B components (ARH), did not influence the biological activity of these two fractions. Since there was less net protein N synthesis with ARH from hemicellulose A and B as compared to ARH from total hemicellulose perhaps the acid hydrolysis of delignified hemicellulose components influenced the yield of the lignin-hemicellulosic degradation fraction (ARH).

The chemical composition of the hemicellulose degradation fractions (ARH) is shown in table 21. The percentage total carbohydrate content of ARH fractions from hemicellulose and delignified hemicellulose did not appear to follow a general trend. The total carbohydrate content of ARH fractions from hemicellulose and delignified hemicellulose was not significantly different ($P < .05$). This was in agreement with the biological activity of ARH from hemicellulose and delignified hemicellulose in that there was no difference in improvement of net protein N synthesis produced by these 2 fractions.

In comparing the hemicellulose A and B components, the total carbohydrate content of the hemicellulose fraction (ARH) was significantly greater ($P < .05$) than that of the delignified hemicellulose fraction (ARH). However the incorporation of ARH from delignified hemicellulose A and B as compared to ARH from hemicellulose A and B resulted in a greater improvement in net protein N synthesis over the 6 hour control. As a result the quantity of total carbohydrate in ARH fractions from hemicellulose A and B did not influence net protein N synthesis. Perhaps, the delignification and fractionation procedures for the preparation of hemicellulose A and B proved to be too severe and the final

TABLE 21

Organic Matter, Percentage Total Carbohydrate and Free Phenol Content of ARH Fractions from Hemicellulose and Holocellulose

	Constituent	Organic Matter	Total Carbohydrate	Total Free Phenol	N
		%	%	%	%
Corn Cob	Hemicellulose ARH	18.3	12.3[b]	1.6[c]	2.0
	Delignified Hemicellulose ARH	24.0	19.1[bc]	3.1[f]	1.5
Corn Cob	Hemicellulose A ARH	20.5	28.1[f]	3.1[h]	2.2
	Delignified Hemicellulose A ARH	23.5	18.4[cd]	3.7[i]	1.7
Corn Cob	Hemicellulose B ARH	25.2	23.8[e]	1.6[b]	1.8
	Delignified Hemicellulose B ARH	29.8	24.7[d]	3.5[e]	1.5
Woodchip	Hemicellulose ARH	37.4	17.5[a]	5.0[fg]	.7
	Delignified Hemicellulose ARH	36.3	22.8[b]	3.4[cd]	1.4
Alfalfa	Delignified Hemicellulose ARH	42.2	28.6[b]	2.4[a]	6.6

When computed as percent of organic matter values with different subscripts are significantly different at $P < .05$ according to Duncan's Multiple Range Test.

TABLE 22

Analysis of Variance of the Carbohydrate Content of the Hemicellulose ARH Fractions

Source	df	SS	MS	F
Total	43	1,233.2		
Treatment	8	1,114.3	139.3	40.9*
Error	35	118.9	3.4	

*Significant at $P < .01$.

Analysis of Variance of Free Phenol Content of the Hemicellulose ARH Fractions

Source	df	SS	MS	F
Total	17	18.45		
Treatment	8	18.40	2.3	383.3*
Error	9	.05	.006	

*Significant at $P < .01$.

hydrolysis of hemicellulose yielded an ARH fraction whose chemical nature rendered it less active.

The carbohydrate content of woodchip hemicellulose and delignified woodchip hemicellulose (ARH) fractions were significantly different ($P < .05$). The carbohydrate content of hemicellulose and delignified hemicellulose (ARH) fractions varied directly with the biological activity of these fractions. This trend was in agreement with the biological activity and carbohydrate content for the corncob hemicellulose (ARH) fractions.

The free phenol content of hemicellulose and delignified hemicellulose (ARH) fractions is shown in table 21. ARH from delignified corncob hemicellulose as compared to ARH from corncob hemicellulose had a higher free phenol content. This indicates that there is a considerable residual lignin moiety in the delignified corncob hemicellulose fraction. The free phenol content for ARH from delignified corncob hemicellulose as compared to ARH from corncob hemicellulose was actually 47% greater for ARH from delignified hemicellulose B. Although all fractions were biologically active, the delignified plant hemicellulose fractions as compared to the plant hemicellulose fractions were more active. As a result, in the case of corncob hemicellulose fractions, the higher free phenol content was associated with biological activity.

Delignification of woodchips by sodium chlorite treatment reduced the residual lignin moiety while the percentage total carbohydrate was significantly increased. The biological activity of ARH prepared from hemicellulose from delignified woodchips, as measured by *in vitro* microbial protein synthesis was slightly greater than that of ARH prepared from untreated woodchip hemicellulose. With respect to corncob

and woodchip hemicellulose, the total carbohydrate content and biological activity had the same general trend. Apparently, in corncobs as opposed to woodchip, the lignin moiety is more strongly bound to the hemicellulose fraction which accounts for the greater content of free phenol in delignified hemicellulose. Since ARH from delignified woodchip hemicellulose showed a lower free phenol content and higher biological activity than that of ARH from woodchip hemicellulose, and since previous studies (Williams, 1977) have shown that reduction in phenol content did not influence growth response with rats, it was suggested that phenol content is not necessary for biological activity.

The nitrogen content of hemicellulose fractions is also shown in table 21. The nitrogen content of ARH prepared from delignified corncob hemicellulose was less than the N content of ARH prepared from untreated corncob hemicellulose. This suggests that delignification of hemicellulose removes a portion of the nitrogen associated with the lignin moiety. Morrison (1972) indicated that lignin-protein complexes were unlikely to occur in the plant, since protein has an integral role in the primary cell wall and lignin is laid down during thickening of the secondary cell wall. Since corncobs contain low levels of protein nitrogen and the ARH fractions from delignified corncob hemicellulose contained a residual lignin moiety, it is suggested that the nitrogen in these ARH fractions is bound to lignin.

The nitrogen content of ARH from woodchip hemicellulose is much less than that of ARH from corncob hemicellulose. The higher amount of

nitrogen in ARH from delignified woodchip hemicellulose as compared to ARH from woodchip hemicellulose would suggest that the nitrogen is not bound to lignin. In support of this, Bondi and Meyer (1943) stated that forage and wood lignins may be distinguished from each other in that wood lignins do not contain nitrogen.

Again, in some of the ARH fractions a portion of the organic matter could not be attributed to total carbohydrate, free phenols or nitrogen. Since the protein nitrogen content of corncobs and woodchips are low, the residual organic matter content of these fractions may be due to the variation in the reaction response of sugars to the phenol-sulphuric acid method or the inability of carbohydrate-bound phenols in lignin to react with the Folin-Denis reagents.

Using solvent system 1 and 2, various carbohydrate fractions were identified as components of the hemicellulose fractions from corncob, woodchips and alfalfa. For most of the hemicellulose fractions, two carbohydrate components were identified as aldobiuronic acids (R_f value .41 with solvent system 1 and R_f value .21 with solvent system 2) and aldotriuronic acids (R_f value .24 with solvent system 1 and .09 with solvent system 2). An additional component was identified as galacturonic acid (R_f value .30 with solvent system 1) for hemicellulose A and delignified hemicellulose A fractions. These components of hemicellulose were similar to hydrolytic products found in alfalfa hemicellulose (Myre and Smith, 1960).

Hemicellulose fractions (ARH) from woodchip and alfalfa were found to contain aldobiuronic acid (R_f .41), oligouronic acid (R_f .16) and aldotriuronic acid (R_f .24) with solvent system 1. Using solvent system

TABLE 23

Paper Chromatographic Separation of Plant Hemicellulose Degradation Fractions

Fractions	B:A:W[1] 2:1:1	EA:A:FA:W[2] 18:3:1:4
	R_f	R_f
Delignified Corncob	.40	0.21
Hemicellulose ARH	.25	0.09
Delignified Corncob	.42	0.22
Hemicellulose A ARH	.30	0.08
Delignified Corncob	.36	0.21
Hemicellulose B ARH	.23	0.09
Corncob	.42	0.22
Hemicellulose ARH	.24	0.09
Corncob	.41	0.22
Hemicellulose A ARH	.30	0.09
Corncob	.36	0.22
Hemicellulose B ARH	.22	0.08

[1]Represents the solvents butanol:acetic:water in a ratio of 2:1:1 according to volume.

[2]Represents the solvents ethylacetate:acetic acid:formic acid: water in a ratio of 18:3:1:4 according to volume.

TABLE 24

Paper Chromatographic Separations of ARH Fractions from various plant sources

Fractions	B:A:W[1] 2:1:1	EA:A:FA:W[2] 18:3:1:4
Alfalfa ARH	.37	.31
	.26	.21
	.16	.08
Delignified Alfalfa Hemicellulose ARH	.36	.36
	.26	.25
	.16	.09
Woodchip ARH	.37	.31
	.24	.21
	.15	.08
Delignified Woodchip Hemicellulose ARH	.34	.35
	.24	.23
	.15	.09

[1]Represents the solvents butanol:acetic acid:water in a ratio of 2:1:1 according to volume.

[2]Represents the solvents ethylacetate:acetic acid:formic acid: water in a ratio of 18:3:1:4 according to volume.

2, aldobiuronic acid (R_f .21), aldotriuronic acid (R_f .09) and xylose (Rf .30) were identified as components of these same hemicellulose fractions. These components were similar to those present in corncob hemicellulose ARH fractions and identified by Myre and Smith (1960) to be present in alfalfa hemicellulose hydrolytic products. As a result, corncob acid-resistant hemicellulose is similar as far as carbohydrate components and biological activity to ARH from hemicellulose isolated from wood, corncob, and alfalfa.

It has been shown that in ruminants available carbohydrate components of foods are mainly fermented in the rumen (Gaillard and Van't Klooster, 1969). According to McLeod and Minson (1974), the herbage cell wall carbohydrates including hemicellulose should be divided into a lignin-free fraction which is totally digested and a lignified fraction which is undigested. Gaillard and Richards (1975) demonstrated that soluble lignin carbohydrate complexes in rumen fluid released by enzymatic attack, pass from the rumen undigested. Research at WVU has demonstrated that a lignin hemicellulose fraction isolated from can molasses and degradation products of acid hydrolysis of several roughages improved in vitro protein synthesis and stimulated growth of rats (Fahey et al., 1976; McLaren et al., 1976). It was the purpose of this research to demonstrate that enzymatically hydrolyzed lignin hemicellulose fractions in rumen liquor possessed biological activity as measured by in vitro protein synthesis of rumen microorganisms.

The cell-free rumen liquor isolated by centrifugation and alcohol precipitation was analyzed to determine its chemical composition. The chemical composition of the rumen liquor fractions is shown in table 25.

The total carbohydrate, free phenol, and nitrogen content of the rumen liquor fractions accounted for approximately 30% of the organic matter. As a result, the rumen liquor fractions were acid hydrolyzed for 6 hours with 1 N H_2SO_4 in order to obtain a more accurate estimation of the total carbohydrate and free phenol content. In addition the lignin content was determined on the residual rumen liquor fraction which remained after hydrolysis.

The total carbohydrate content and free phenol content of the rumen liquor fraction is shown in table 25. The total carbohydrate content of the rumen liquor fractions accounted for approximately 58% of the soluble fraction after acid hydrolysis. The corncob rumen liquor fractions showed an increase in total carbohydrate content for the third and fourth week collection over the first and second week collection. This would suggest that the corncob rumen liquor fractions were different in chemical nature as far as their ease of hydrolysis. The rumen liquor fraction collected from the sawdust-fed cow had the lowest carbohydrate content.

The free phenol content of the rumen liquor fractions accounted for approximately 27% of the amount solubilized by acid hydrolysis. The free phenol content of the acid hydrolyzed rumen fluid sample probably represents a portion of the lignin moiety which was acid hydrolyzed. The lower amounts of free phenol were found in the acid hydrolysates of rumen liquor fractions from timothy hay fed-sheep and sawdust fed-cow. The total carbohydrate and free phenol content accounted for all the acid hydrolyzed portion of timothy hay rumen liquor fraction. However, only 61% of the acid-hydrolyzed rumen liquor

TABLE 25

Chemical Composition of Rumen Liquor Fractions

Fraction	ORG Matter	N	Total Carbohydrate	Total Free Phenol	Amount Solubilized By Acid Hydrolysis[a]	Residual Lignin
	%	%	%	%	Mg	%
Corncob 1st wk rumen Liquor Fraction	79.4	3.8	40.7	24.0	70	13.3
2nd wk rumen Liquor Fraction	75.9	3.7	36.4	20.2	77.0	11.6
3rd wk rumen Liquor Fraction	80.4	4.1	52.4	25.0	73.0	12.3
4th wk rumen Liquor Fraction	76.6	3.9	58.3	21.0	86.0	6.0
Timothy Hay Rumen Liquor Fraction	84.2	2.5	47.9	13.7	57.0	27.8
Wood Rumen Liquor Fraction	75.1	3.2	30.1	16.5	77.0	11.1

[a]100 mg was acid hydrolyzed

fraction collected from the sawdust-fed cow could be accounted for by total carbohydrate and free phenol determination. This suggests that the lignin fractions from sawdust rumen liquor was more resistant to acid hydrolysis than that of the corncob rumen liquor fraction.

The nitrogen content of the corncob rumen liquor fractions did not vary from the first to the fourth week. In addition, the nitrogen content of timothy hay and sawdust rumen liquor fraction was lower than that of corncob rumen liquor fraction. A portion of this N in the cell-free rumen liquor fraction may arise from glycoproteins added to the digesta and not associated with the cell wall polysaccharides. Gaillard and Richards (1975) demonstrated that upon acidification, 83% of the protein in the cell-free rumen liquor fraction remained in solution while most of the cell wall carbohydrate material was precipitated. An analysis of the lignin content of the residual rumen liquor fraction indicated that the lignin content accounted for approximately 12% of the dry matter of corncob and sawdust rumen liquor fractions and 28% of the timothy hay rumen liquor fraction. The low lignin content of the fourth week corncob rumen liquor fraction could not be explained. Perhaps, an alteration in the microflora may result in more complete degradation of cell wall constituents.

As a result, a majority of the cell-free rumen liquor fraction is a soluble lignin-carbohydrate complex. The rumen liquor fraction was acid hydrolyzed in order to identify carbohydrate components by paper chromatography. The carbohydrate components, xylose (R_f value .47 with solvent system 1 and R_f .31 with solvent system 2) aldobiuronic acid (R_f value .41 with solvent system 1 and R_f .22 with solvent system

TABLE 26

Paper Chromatography of Carbohydrate Components of Hydrolyzed Rumen Liquor Fraction

B:A:W[1] 2:1:1	EA:A:FA:W[2] 18:3:1:4
R_f	R_f
.33 (RD)	.22 (GR)
.41 (RD)	.31 (RD)
.48 (RD BN)	.35 (RD BN)
.51 (RD BN)	.41 (RD BN)
.65 (RD BN)	.57 (RD BN)

[1]The solvents B:A:W represents butanol:acetic acid:water at a ratio of 2:1:1 according to volume.
[2]The solvents EA:A:FA:W represents ethylacetate:acetic acid:formic acid:water at a ratio of 18:3:1:4 according to volume.

2) and galacturonic acid (R_f .33 with solvent system 1) were identified as hydrolyzed components of the rumen liquor fraction. The component (R_f .35 with solvent system 2) has been found in corncob ARH (table 26) but was not identified. The other two carbohydrate components could not be identified but were found to give ninhydrin positive tests for amino acids indicating that they were protein-bound carbohydrates (glycoproteins). These carbohydrate components were similar to hydrolyzed hemicellulose components identified by Myre and Smith (1960) and found to be present in the acid-resistant hemicellulose fractions previously mentioned in the discussion. These results confirm reports by Gaillard and Richards (1975) that soluble lignin-hemicellulose complexes are preduced in the rumen.

These lignin-hemicellulose fractions from cell-free rumen liquor were similar to corncob acid-resistant hemicellulose in terms of carbohydrate components and contained a degraded lignin moiety. As a result these degraded lignin-hemicellulose fractions were tested for biological activity as measured by in vitro microbial protein synthesis. As shown in table 27, the incorporation or 0.04% lignin-hemicellulose fraction from cell-free rumen liquor to the reaction medium significantly improved in vitro protein synthesis. The lignin-hemicellulose fraction was dialyzed extensively in order to determine whether its biological activity was influenced. There was a significant improvement in in vitro protein synthesis as a result of dialyzing the lignin-hemicellulose fraction. Apparently, the low molecular weight dialyzable material present in the isolated rumen liquor fraction was either toxic or not biologically active. Also, the supernatant of the

TABLE 27

Influence of Lignin-Hemicellulose Fractions from Cell-Free Rumen Liquor on *In Vitro* protein synthesis Expressed as Nitrogen (N)

No. of Tubes	6 Hr. Control	.04% dial Rumen Liquor Fraction	.04% undial Rumen Liquor Fraction	.04% Supernatant of Alcohol ppt Rumen Liquor Fraction
	N	N	N	N
	Mg	Mg	Mg	Mg
8	8.2±.1	10.8±.5*	9.8±.4*	8.6±.1
Net protein synthesis above control (%)		32	20	5

*Values are significantly different at $P < .01$ according to Dunnett's Test.

Analysis of Variance on the Influence of Lignin Hemicellulose RumenLiquor on *in vitro* protein synthesis

Source	df	SS	MS	F
Total	31	58.9		
Trial	1	9.98	9.98	19.6*
Treatment	3	35.3	11.7	23.1*
Error	27	13.7	.51	

*Significant at $P < .01$

alcohol precipitated rumen liquor fraction was tested for biological activity. The incorporation of this fraction, into the reaction vessel resulted in no significant improvement in vitro protein synthesis.

Rumen liquor samples were collected weekly during the four week corncob trial and the eleven day sawdust trial, and tested for biological activity as measured by in vitro protein synthesis. As shown in table 28, the incorporation of isolated rumen liquor lignin-hemicellulose fractions at 0.02% level to the reaction vessel revealed that only the first and second week rumen liquor fractions significantly improved in vitro microbial protein synthesis over the 6 hour control. In addition, the lignin hemicellulose fraction from the sawdust-fed cow significantly improved in vitro protein synthesis over the 6 hour control. The results indicate a change in the chemical nature of the rumen liquor lignin-hemicellulose fraction collected during the third and fourth week of the trial which may have altered the biological activity. In comparing chemical conposition of these fractions, it suggests that ease of acid hydrolysis and low lignin content of the third and fourth week rumen liquor lignin-hemicellulose fractions may be associated with poor biological activity.

Table 29 shows the biological activity of isolated rumen liquor lignin-hemicellulose fractions collected from a cow fed sawdust and a sheep fed timothy hay as the primary roughage source. The incorporation of both fractions at 0.02% level to the reaction medium significantly ($P < .01$) improved in vitro microbial protein synthesis over the 6 hour control. However, the rumen liquor lignin-hemicellulose fraction from the sawdust-fed cow had significantly greater biological activity.

TABLE 28

Influence of Weekly Rumen Liquor Lignin-Hemicellulose (LH) Fractions on *in vitro* microbial protein synthesis expressed as nitrogen (N)

No. of Tubes	6 hour Control	1st week L.H. Fraction	2nd week L.H. Fraction	3rd week L.H. Fraction	4th week L.H. Fraction	Sawdust L.H. Fraction
	N	N	N	N	N	N
	mg	mg	mg	mg	mg	mg
6	10.7±.8	11.9±.9*	11.8±.8*	11.3±.9	11.2±.9	11.9±1.0*
Net protein synthesis above control (%)		11	10	6	5	11

*Values significantly different from control at $P < .05$ according to Dunnett's test.

Analysis of Variance on the influence of Lignin-Hemicellulose on *in vitro* protein synthesis.

Source	df	SS	MS	F
Total	35	146.48		
Trial	1	133.56	133.56	702.9*
Treatment	5	7.42	1.48	7.82*
Error	29	5.50	.190	

*Significant at $P < .01$.

TABLE 29

Influence of Isolated Rumen Liquor Lignin-Hemicellulose (LH) fractions from sawdust-fed and timothy hay-fed ruminants on *in vitro* protein synthesis expressed as nitrogen (N)

No. of Tubes	6 hr. Control	Sawdust Rumen Liquor Fraction	Timothy Hay R.L. Fraction
	N	N	N
	mg	mg	mg
8	7.2±.3	8.9±.6*	7.9±.5*
Net protein synthesis above control (%)		24	10

*Values were significantly different at $P < .01$.

Analysis of variance on the influence of Isolated Rumen Liquor Lignin Hemicellulose Fractions on *in vitro* protein synthesis

Source	df	SS	MS	F
Total	23	51.38		
Trial	1	34.44	34.44	215.3*
Treatment	2	13.82	6.91	44.3*
Error	20	3.12		

*Significant at $P < .01$.

Perhaps, the similarity of the chemical composition of the biologically active-sawdust and corncob rumen liquor lignin-hemicellulose fractions may be an explanation for this.

In conclusion, these soluble lignin-hemicellulose complexes isolated from the rumen liquor are biologically active when tested by adding them to an in vitro system and measuring their influence on microbial protein synthesis. The lignin-hemicellulose complexes did vary in lignin content and the ease of acid hydrolisis which may have contributed to the variation in biological activity. Among the fractions chromatographic analysis indicated that carbohydrate components of the lignin-hemicellulose complexes were similar as far as R_f values to those existing in acid-resistant hemicellulose. This would indicate that soluble lignin-hemicellulose complexes enzymatically produced in the rumen are chemically and biologically similar to those produced by acid hydrolysis. As a result, the essentiality of these soluble lignin-hemicellulose complexes to the growth and well-being of the microbial population and ultimately the ruminant animal must be realized.

Various fecal samples from ruminants fed corncobs, sawdust, or bromegrass as the sole roughage served as sources for acid-resistant hemicellulose (ARH). Studies have demonstrated that there is a change in fecal hemicellulose components in comparison to feed hemicellulose (Van Soest, 1975). It has been postulated that lignification, cell wall organization, and other criteria affect hemicellulose digestion. It was the purpose of this research to determine whether fecal hemicellulose fractions (ARH) have biological activity. The incorporation of 0.02% ARH from feces from animals fed corncob, sawdust (table 30)

and bromegrass (table 31) to the reaction medium significantly improved ($P < .01$) *in vitro* microbial protein synthesis over the 6 hour control. Although all ARH fractions from weekly fecal samples had significant biological activity, there was a linear trend toward increased biological activity for ARH fractions taken during the third and fourth week. The fecal ARH fraction from the Holstein cow fed sawdust as the sole roughage produced the lowest biological activity of all samples tested in this experiment.

The chemical composition of these fecal ARH samples, as shown in table 32 was presented in attempts to explain the variation in biological activity. There was an increasing trend in percentage total carbohydrate content among the fecal ARH fractions for the four week collection period. The percentage free phenol content of the fecal ARH fractions also followed this linear trend from the first week to the fourth week fecal ARH sample. It is suggested that the increasing total carbohydrate content and free phenol content of the fecal ARH fractions from the first to the fourth week collection may account for the linear increase in biological activity of these same fractions. However, the poor biological activity of the fecal ARH fraction from the sawdust-fed cow could not be explained by the total carbohydrate and free phenol content, since it was higher than that of the other ARH fractions. Perhaps, a continuation of the trial, in which the Holstein was fed sawdust, may have resulted in an increase in biological activity of the fecal ARH fraction. If this may be the case, it would appear that the lignin-hemicellulose fraction would be extensively degraded and increase the yield of the biologically active lignin-hemicellulose fraction upon acid hydrolysis.

TABLE 30

Influence of ARH fraction from weekly fecal samples on *in vitro* protein synthesis

No. of Tubes	6 hr. Control	1st week Fecal ARH	2nd week Fecal ARH	3rd week Fecal ARH	4th week Fecal ARH	Sawdust Fecal ARH
6	10.42±.54	11.79±.95*	12.34±.82*	12.49±.79*	12.56±.75*	11.64±88*
Net protein synthesis above control (%)		13	18	20	21	12

*Values were significantly different at $P < .01$ according to Dunnett's test.

Analysis of Variance of the Influence of ARH fractions from fecal samples on *in vitro* protein synthesis

Source	df	SS	MS	F
Total	35	133.9		
Trial	1	101.5	101.5	230.7*
Treatment	5	19.6	3.95	8.9*
Error	29	12.9	.44	

*Significant at $P < .01$

TABLE 31

Influence of ARH from bromegrass feces on *in vitro* microbial Protein Synthesis

No. of Tubes	6 hr. Control	Bromegrass fecal ARH
	MgN	MgN
11	7.6±.6	9.0±.5*
Net protein synthesis above control (%)		18

*Significantly different from 6 hour control ($P < .01$) according to Dunnett's Test.

Analysis of Variance on the influence of Fecal Bromegrass ARH on *in vitro* protein synthesis

Source	df	SS	MS	F
Total	21	80.9		
Trial	1	6.8	6.8	57*
Treatment	1	11.0	11.0	92*
Error	19	2.3	.12	

*Significantly different at $P < .01$.

TABLE 32

Total carbohydrate and free phenol content of ARH fractions from fecal samples

Roughage Source	Fecal ARH Samples	Organic Matter	Total Carbohydrate	Free Phenol	N
		%	%	%	%
Corn cob	1st week	29.2	6.0	.7	6.2
	2nd week	29.6	10.6	.8	6.2
	3rd week	32.3	12.4	1.6	5.9
	4th week	33.9	13.2	1.1	5.9
Sawdust		31.0	13.6	1.0	6.2

SUMMARY

The present work was conducted to determine the influence of methods of preparation of corncob acid-resistant hemicellulose on its chemical composition and rat growth stimulation. ARH preparations involved either treatment with cation resin, solubilization of crude ARH and reprecipitation in ethanol, treatment of the hydrolysate with carbon or recovery of the non-dialyzable portion of corncob hydrolysate. All ARH preparations significantly improved growth and feed utilization of rats when fed at the 0.05% level of the diet. The reduction of the phenolic content of the ARH preparation did not influence rat growth. The carbohydrate components of the ARH preparation were similar to carbohydrate components of hemicellulose reported by other workers.

Alkaline cleavage of corncob ARH with 10% NaOH resulted in an ethanol-insoluble fraction which significantly improved growth and feed utilization of rats and in vitro protein synthesis of rumen microorganisms. According to chemical analysis, U.V. spectral analysis and Sephadex fractionation, the ethanol-insoluble fraction contained carbohydrate-lignin complexes, whereas the ethanol-soluble fraction contained carbohydrate-bound lignin complexes with less carbohydrate. Paper chromatographic analysis indicated that both fractions contained typical carbohydrate components of hemicellulose.

ARH was prepared from hemicellulose isolated from untreated and delignified corncobs, woodchips and alfalfa. ARH was also prepared from the hemicellulose A and B components which had been isolated from delignified and untreated corncobs. All ARH preparations from hemicellulose isolated from the untreated and delignified plant sources

significantly improved *in vitro* microbial protein synthesis as measured by Kjeldahl N. The biological activity of ARH preparations appeared to be associated with carbohydrate content. Again, the carbohydrate components of these ARH preparations from hemicellulose were similar to those for corncob acid-resistant hemicellulose.

The purpose of another segment of this research was to isolate soluble lignin-hemicellulose complexes from cell-free rumen liquor and compare them to ARH in terms of biological activity and chemical composition. Cell-free rumen liquor fractions were isolated from rumen fluid collected from a Holstein cow fed corncobs for four weeks and sawdust for eleven days as the sole roughage. After acid hydrolysis, total carbohydrate and lignin represented major constituents of the rumen liquor fraction. Paper chromatographic analysis of the acid hydrolysate revealed that the carbohydrate components of the rumen liquor fraction were similar to those of hydrolyzed hemicellulose and corncob ARH. Since nitrogen represented a minor component, the lignin-hemicellulose complex was the major constituent of the rumen liquor fraction. The addition of the lignin-hemicellulose complexes isolated from rumen liquor to the reaction medium significantly improved *in vitro* microbial protein synthesis. As a result, this research demonstrated that soluble lignin-hemicellulose complexes enzymatically produced in the rumen were similar in chemical composition and biological activity to acid-resistant hemicellulose.

ARH was recovered from fecal fractions collected from a Holstein cow fed corncob and sawdust as the sole roughage. Fecal ARH from the corncob and sawdust-fed cow improved *in vitro* microbial protein synthesis. The

total carbohydrate content of fecal ARH appeared to be associated with the biological activity since a greater improvement in *in vitro* protein synthesis occured with those fractions higher in total carbohydrate content.

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ABSTRACT

This research has shown that corncob lignin-hemicellulose fractions (ARH) stimulate growth and improve feed conversions of rats when fed at 0.05% of the semi-purified casein ration. The reduction of the phenolic content of corncob ARH by carbon treatment and cation resin treatment did not affect the growth response. In addition, the growth response was attributed to the non-dialyzable portion of corncob ARH. All corncob ARH fractions contained aldobiuronic acid, oligouronic acid and galacturonic acid as carbohydrate components.

Alkaline cleavage of corncob ARH produced two distinct fractions differing in biological activity and chemical nature. Only the ethanol-insoluble fraction significantly improved growth and feed conversion of rats when added to the ration at 0.05% level and stimulated in vitro microbial protein synthesis. Both the ethanol-soluble and ethanol-insoluble fraction had similar percentages of total carbohydrate and free phenol and contained aldobiuronic acid and uronic acid as carbohydrate components. According to U.V. spectral analysis and Sephadex fractionation, the ethanol-insoluble fraction contained carbohydrate-lignin complexes, whereas the ethanol-soluble fraction contained carbohydrate-bound lignin complexes with less carbohydrate.

Degraded lignin-hemicellulose fractions (ARH) from hemicellulose were isolated from untreated and delignified corncob, woodchips, and delignified alfalfa. The addition of 0.02% ARH from hemicellulose extracted from the untreated plant and the delignified plant to reaction vessels resulted in a significant improvement in in vitro protein synthesis. In corncobs and woodchips this increase in biological activity

appeared to be associated with total carbohydrate content. These ARH fractions from hemicellulose contained aldobiuronic acid, xylose, and uronic acid.

Cell-free rumen liquor fractions were isolated from rumen fluid from a Holstein cow fed corncobs for four weeks and sawdust for eleven days and from two Suffolk sheep fed timothy hay for four weeks as the sole roughage. The total carbohydrates and lignin represented major constituents of the cell-free rumen liquor fraction. Similar carbohydrate components were found in the cell-free rumen liquor fraction as existed in hydrolyzed hemicellulose and in corncob ARH. The addition of 0.02% rumen liquor lignin-hemicellulose fraction to the reaction vessel significantly improved in vitro microbial protein synthesis.

ARH was recovered from fecal fractions collected from a Holstein cow fed corncob and sawdust as the sole roughage. Fecal ARH from the corncob and sawdust-fed cow improved in vitro microbial protein synthesis. The improvement in in vitro protein synthesis appeared to be associated with the total carbohydrate content of fecal ARH fractions.

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