CHARACTERISTICS OF RUMINAL FIBER DIGESTION WITH CATTLE FED HIGH CONCENTRATE DIETS

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CHAPTER I

INTRODUCTION

In commercial feedlots, large amounts of grain are fed to ruminants. High grain diets reduce the ability of ruminal microbes to digest roughage sources (legumes, grass forages and byproduct) for energy (VFA) and microbial protein production. But precisely which portion of the fiber within a roughage is most affected and what specific ruminal factors are responsible have not been fully elucidated.

Fiber is included in ruminant diets to maintain proper ruminal function and to obtain maximum feed and energy intake. Typical feedlot diets include from 5 to 15 % of dry matter as roughage or forage (Galyean and Goetsch, 1993). With forage-based diets, the quantity of forage consumed is the main factor limiting animal production (Minson, 1990). To maximize intake with high forage diets, forages of high quality (rich in protein; low in NDF) are preferred. With lower quality forages, processing (particle size reduction) often will increase feed intake. However, fiber particles need to be longer than 10 mm in order to stimulate rumination (Welch and Hooper, 1988). With high concentrate diets, any benefit from enhancing the quality of forage are not as evident because forage bulk does not limit energy intake. Nevertheless, forage or roughage fulfills several roles shown in Table 1.

Table 1. Roles of roughage in feedlot diets

Objective	Functional roughage component(s)
Provide nutrients for animal/microbes	Protein, cell contents, minerals, vitamins
Reduce rate of grain consumption.	Long fiber
Increase chewing of diet during eating	Water-absorbing components
Increase saliva flow	Dry, coarse NDF, ADF
Provide inert ruminal bulk	ADF, lignin, NDF
Ruminal scratch factor-rumination	Large particle floating ADF, NDF
Buffer ruminal contents	Protein, minerals
Slowly released energy/protein	NDF, protein
Refuge for protozoa	Large floating particles

Compared to diets for finishing beef cattle, diets for dairy cattle typically contain much more forage. In recent years, neutral detergent fiber (NDF; cell wall constituents) has largely replaced net energy as the basis for formulation of dairy diets. The National Research Council report for Dairy Cattle (1989) indicates that diet dry matter should contain a minimum of 25% NDF with at least 75% of the NDF being supplied by coarse forage. To maintain normal rumen function and to avoid depressions in forage fiber digestion, the ratio of ruminally degradable starch to forage NDF should not exceed 1:1 (Poore et al., 1991).

In addition to directly affecting diet digestibility, adding roughage to concentrate diets or vice versa can result in interactions between diet constituent or associative

effects. Associative effects usually are defined as synergistic or antagonistic effects of two or more feed components on digestibility of the diet. Negative associative effects with higher concentrate diets usually are attributed to depression of starch digestion in rumen, perhaps associated with decreased ruminal residence time, due to the presence of fiber. In contrast, with forage-rich diets, rapidly fermented non-structural carbohydrate, such as starch can decrease digestibility of cell wall constituents by mechanisms that are not fully understood. Chappell and Fontenot (1968) demonstrated that adding purified starch to the rumen depressed the extent of fiber digestion in the rumen. Mould and Orskov (1983) also discussed the idea of "starch effect" and Miron et al. (1996) demonstrated that adding wheat and sorghum to forage diets, even though pH was not depressed, decreased rate and extended the lag time for NDF digestion.

Neither ruminants nor non-ruminants produce enzymes that degrade cell walls; instead, microbes in the digestive tract (the ruminal ecosystem and the cecum and large intestine of ruminants and non-ruminants) are responsible for degrading and utilizing complex carbohydrates (Akin, 1993). Although ruminal bacteria, protozoa and fungi all can digest most structural carbohydrates found in forage, the fiber-digesting microbes are much more sensitive to depressions in pH than are the microbes that digest starch and other stored polysaccharides (Dehority, 1993). Most microorganisms in the rumen utilize readily fermented carbohydrate as their main energy source.

A number of factors can alter rate or extent of fiber digestion in the rumen (Hoover, 1986). Those include 1) carbohydrate preferences by rumen microorganisms, 2) rumen pH, 3) number of cellulolytic organisms, 4) rumen ammonia nitrogen level, 5) production and the activity of cellulases and hemicellulases and 6) enzyme binding as

well as 7) inhibition either through catabolite repression (Miron et al.1996) or through proteinaceous inhibitors that are bacteriocidal for fibrolytic organisms (Piwonka and Firkins, 1996), and 8) toxicity of volatile fatty acids at low pH (Russell and Wilson, 1996)

Using in vitro procedures, Grant and Mertens (1992a, 1992b) and Grant and Weidner (1992) indicated that the depression in fiber digestion from added starch could be attributed largely to a pH decline below 6.0; in addition, an additional but small portion of the depression was attributed directly to a "carbohydrate effect" above pH of 6.5.

The goal of the research in this thesis was to examine the degree to which rate and extent of disappearance of fiber and fiber components is altered by pH and(or) deficiencies of other nutrients. Although most past research has examined only Neutral Detergent Fiber (NDF) disappearance, we attempted to examine disappearance of Acid Detergent Fiber (ADF) and hemicellulose fractions, as well. Both in vitro and in situ research methods were employed in order to investigate fiber disappearance. Because direct extrapolation of in vitro results to in vivo conditions is complicated by diurnal fluctuations of pH in vivo, we employed in situ methods to simulate in vivo conditions more precisely. In addition, effects of cell content extraction and of ammonia nitrogen concentration in the rumen were investigated for their potential to limit fiber disappearance.

CHAPTER II

LITERATURE REVIEW

Fiber in Ruminant Diets

Increased nutrient competition from humankind makes livestock production more dependent on efficient utilization of fiber, forage, and indigestible byproducts. Beef cow-calf production and beef cattle growing programs depend heavily on grazed and harvested forages. Forage research generally has been focused on increasing the ability of the forage plant to store more potential energy and to increase the availability of nutrients from forages to ruminants (Hatfield, 1993). Structural polysaccharides, i.e., cellulose, hemicellulose, and pectin, account for 400 to 700 g/kg of dry matter (350-800 g/kg of the organic matter (OM); Jung and Allen, 1995) in most forages and carry most of the gross energy in forages (Dehority, 1993, and Goetsch and Galyean, 1993). NRC (1989) indicated that diets for dairy cattle should consist of a minimum of 28% NDF with 75% of this NDF coming from forage. Varieties and forms of dietary feedstuffs used in diets for ruminants depend heavily on cost, local availability (to avoid high transportation cost), type of livestock system and management, and maintenance of animal and ruminal health (Marshall et al., 1992).

As noted in Table 1, fiber plays several roles in diets for ruminants. In general, fiber sources with larger particle size, through enhancing ruminal mixing and stimulating rumination, will help to maintain ruminal function. In addition, ruminally digested fiber from forage can provide a substantial proportion of dietary energy for lactating dairy and beef cattle (Stensig and Robinson, 1997). Dietary fiber level also can impact feed intake,

ruminal fill, particle retention time, passage rate and microbial activity in rumen. Owens et al. (1997) indicated that diets in most feedlots of the southern Great Plains contain 8% or less roughage. Swingle (1995) suggested that roughage level in feedlot diets range 0 to 15%. Galyean and Goetsch (1993) indicated that 140 to 200 g/kg total dietary NDF are provided by 7 to 15% of DM as forage in finishing diets. One widely fed source of roughage, corn silage, typically contains about 50% of its dry matter as corn grain. In contrast with other roughage sources, it provides both concentrate and forage in the diet. According to the review by Owens et al. (1997), the optimal roughage to include in grain-rich feedlot diets should have 1) large enough particle size to aid ruminal mixing, dilute ruminal acids, and stimulate rumination to increase flow of saliva containing bicarbonate to buffer of ruminal acids; 2) low enough density both to float in rumen for long residence time and to stimulate rumination by scratching the cardia, and 3) a low digestibility so that it is retained in the rumen so that less needs to be fed. Note that for these functions, roughage is merely considered a diluent and not a source of nutrients for either microbes or the animal. Hence, plastic particles might be considered ideal as a source of roughage. Indeed, plastic particles and pot scrubbers (Loerch et al., 1991) have been tested and found useful as a source of roughage for cattle fed feedlot diets.

Physical characteristics of fiber including particle size and density can influence diet utilization, ruminal fermentation, and post-absorptive metabolism, as well as animal health and performance (Mertens, 1997). He postulated that fiber or NDF must have a large particle size if it is to serve as floating mat in the liquid pool of rumen; that, in turn, will help maintain adequate concentrations of fat in milk and to avoid acidosis. He introduced the term effective NDF (eNDF). Defined as the percent of NDF remaining on

a 1.18 mm screen after dry seiving (NRC, 1996), eNDF is the only portion of NDF that supposedly is effective in stimulating rumination. Earlier, Sudweeks et al. (1979) devised a roughage value index (RVI) to represent a similar index for roughages. RVI was calculated as the number of minutes of rumination that was achieved from addition of 1 kg of a specific roughage to the diet. Compared with eNDF, RVI is a more direct index of the impact of a specific roughage on rumination. However, RVI can vary with forage moisture and processing and cannot be predicted directly from feed analysis.

Conventional Feedlot Diets and Digestibility

Although ruminants can grow and reproduce successfully when fed diets that range from 100% roughage to 100% concentrate, dietary energy density or total digestible nutrients (TDN) needs to exceed about 65% of DM for ruminants to achieve maximum energy intake and thereby consume enough energy to reach a maximum rate of gain or level of milk production. This latter factor is the main economic factor that drives ruminant production. In order to achieve maximum rate of gain with an optimal feed efficiency, the percentage of forage in feedlot diet must be minimized, particularly in forage-deficient regions of the world where forage or roughage availability is limited and cost is high. In addition, cost of net energy for gain often is greater for forage than grain components of the diet (Calderon-Cortes and Zinn, 1996). Yet forage typically is included in feedlot diets in an attempt to reduce the incidence of health (acidosis) problems and to achive high and steady feed intakes. Though counter to the concept that long forages are preferable for maximum benefits on ruminal health, dry forages usually are chopped or ground and may even be pelleted before being added to feedlot diets in order to simplify handling, mixing, and feeding with grain-rich diets.

Besides adding nutrients (protein, minerals, vitamins) to a diet, forages in a feedlot diet help neutralize ruminal acids through 1) dilution of ruminal contents and 2) increasing salivary secretion that in turn elevates ruminal pH (Gill et al., 1981). However, including extra forage to a feedlot ration can decrease daily gain and feed efficiency by reducing intake of energy and dilution of dietary calories. Diets containing moderate amounts of roughage (20 to 60%) also may reduce ruminal digestion of fiber (as compared to diets higher in roughage) and of starch (as compared to diets lower in roughage and richer in starch). However, type and form of forage may be as important as or more important than level of forages in the diet, because particle size, density and bulk of forages all can affect chewing, rumination, and ruminal retention. Swingle (1995) indicated that typical feedlot diets contain from 0% to 15% roughage. He proposed that roughage has two distinct impacts, altering productivity and animal health. Of these two, he considered that maintaining rumen function to avoid metabolic diseases is much more important than supplying nutrients for microbes or the animald. However, associative effects between the roughage and grain constituents of the diet and adverse effects on rate of gain must be considered when determining the optimum fiber level in the diet (Swingle, 1995).

Gill et al. (1981) conducted a feeding trial with 5 different levels (8 to 24% of diet DM) of a single roughage mixture (50% ground alfalfa hay plus 50% corn silage). Two types of grain were fed - steam-flaked corn and high moisture corn. As roughage level was increased, dry matter intake increased and feed efficiency was adversely affected, but average daily gain remained unchanged across this range of roughage levels.

Bartle et al. (1994) fed finishing steers diets containing 10, 20, or 30% cottonseed hulls or alfalfa hay. Only with 30% cottonseed hulls was gain depressed. Gains were similar at a level of 10% roughage whether the roughage came from cottonseed hulls or alfalfa hay. But when the roughage content of the diet was increased, steers fed alfalfa gained faster than those fed cottonseed hulls. They attributed this to the higher NDF content of the cottonseed hulls. In a study by Kreikemeier et al. (1990), steers fed diets containing no roughage had much lower feed intakes than steers fed 5, 10 or 15% roughage. They attributed the depressed intake with the 0% roughage diet to very rapid fermentation of steam rolled wheat in the diet.

Diet have been formulated based upon NDF content by the dairy industry for many years (Mertens, 1983). When diets are formulated to provide the same amount of NDF, milk production will be similar regardless of source of fiber according to his initial studies. Subsequently, concerns about effects of particles size and ruminal passage rate led him to formulate diets based on eNDF rather than total NDF. Roughages typically are processed before being included in feedlot diets for both beef and dairy cattle primarily to improve mixing and handling characteristics of the totally mixed ration (Panichnantakul et al., 1990). When finely ground, forages may not prevent acidosis. Physical characteristics of forages also may interact with physical form of the concentrate in the diet (Goetsch et al., 1986). With whole shelled corn, addition of long chopped hay increased the extent of ruminal digestion, but with ground corn, addition of hay decreased the extent of ruminal digestion. They noted further that including cottonseed hulls and prairie hay resulted in greater total tract starch digestion than including alfalfa hay as a source of roughage. Panichnantakul et al. (1991) noted that rate of gain was faster and

cost of gain was lower when wheat straw was substituted for alfalfa as a source of roughage in whole shelled corn finishing diets for steers. However, in a subsequent trial, these same authors (1992) found that performance of steers fed the wheat straw diet was inconsistent. One problem with this roughage source is its low bulk density after grinding; this may lead to poor diet mixing and to separation and selection by animals. In contrast to results by Goetsch et al. (1987), Panichnantakul et al. (1992) found that feeding long hay (4 inches vs 2 inches) increased the extent of ruminal digestion of organic matter, starch and fiber digestion.

Fiber Digestion in Rumen

Although digestion is relatively slow, the ability to digest cellulose and other cell wall components in the rumen gives ruminants an advantage over other mammals (Breazile and Houghton, 2000). Fiber is an important source of energy for ruminal microbes and, in turn, the ruminant (Tamminga, 1993). Fiber also aids in maintaining proper ruminal stratification, in stimulating rumination, and providing a site for microbes to attach and avoid being flushed out of the rumen.

Digestion is the process of degradation of food molecules to simple compounds that can be absorbed across the gastrointestinal tract wall (Merchen and Bourquin, 1994). This process occurs during acid hydrolysis in stomach and with enzymatic cleavage in intestine. Although digestion in abomasum and small intestine is important for both ruminants and non-ruminants, the major site of fermentation for ruminants is the rumino-reticulum although additional fermentation will occur in the cecum and large intestine. Ecology is defined as interrelationship between living organisms and their environment

(Russell, 1988). The rumen provides an ideal, aqueous and well-buffered ecological system for growth of anaerobic microorganisms.

Structural polysaccharides or cell wall constituents are the primary fraction of plant cells that are incompletely degraded; the fraction of food that is indigestible by enzymes produced by the animal itself is commonly called "fiber" (Chesson and Forsberg, 1994; Tamminga, 1993). The primary plant polysaccharides are pectic substances, hemicellulose and cellulose and some additional polysaccharides that are rare and specific to certain roles such as recovery from plant wounds, i.e., callose, mannans, xylan, galactan, galacturonan are homoplymers and arabinans, beta-D-glucans, arabinogalactrans, rhamnogalacturonans, xylans, and xyloglucans (Moore and Hatfield, 1994). Pectic subtances usually comprise about 1 to 4 % of the dry weight of grasses (monocots) and 5 to 10 % of the dry weight of legumes (dicots) (Dehority, 1993); pectin concentrations decrease as plants mature, being replaced by lignin, cellulose, and hemicellulose (Fahey and Berger, 1988). Consequently, the structural polysaccharide composition of forages varies with plant class, tissue type and maturity, morphological location, and environmental conditions (Himmelsbach, 1993). Cellulose, the most abundant organic molecule in nature (Moore and Hatfield, 1994), consists of glucose molecules linearly linked with beta 1-4 covalent bonds. Beside producing glucose molecules, hydrolysis of cellulose produces the intermediate disaccharide cellobiose (4-O-beta- glucopyranosyl-beta-D-glucopyronase) (Fahey and Berger, 1988) that in turn is degraded by cellobiase.

Numerous digestion studies have been conducted using isolated polysaccharides and(or) specific isolated strains of rumen microorganisms to examine details of

degradation of cell wall constituents in rumen. Fractionation of structural carbohydrates of the cell wall usually involves treatment with chemicals to remove cytoplasmic organelles (Moore and Hatfield, 1994). The Van Soest (1963) detergent system, the most widely used method for extraction of cell contents, leaves structural polysaccharides of plant cell walls behind. Although it does not partition cell wall carbohydrates into specific chemical entities, this method is an attempt to subfraction fiber into classes that differ in their availability for microbial digestion in the rumen. Other procedures have been used to isolate plant cell walls. Sodium dodecyl sulfate can be used to removes cytoplasmic proteins and dimethyl sulfoxide to remove starch (Moore and Hatfield, 1994). The Uppsala method was developed to prepare alcohol insoluble residues by Theander et al (1991). KOH extraction, treatment with acidic Na_2ClO_2, treatment with hydrolases, alcohol precipitation, anion exchange, and gel filtration chromotography are additional methods for isolation and fractionation of polysaccharides (Moore and Hatfield, 1994).

Efects of Lignin on Fiber Digestion

Although not a structural polysaccharide but a complex polyphenol, lignin is closely related with plant cell walls. An indigestible biopolymer of plant cell walls, lignin provides strength and rigidity to cell walls, and reduces water loss by limiting permeability. Since most cell wall polysaccharides are bound to lignin in plants, lignin often is one of the major factors limiting digestion of cell wall constituents.

To understand, fiber digestion, one must consider the various sub-components of plant cell walls. Certain fiber fractions (pectin, hemicellulose) are more digestible than others (cellulose, cutin) whereas others (lignin, ADF bound N) are virtually indigestible.

Dynamic models can be used to help describe fiber digestion (Tamminga, 1993). Unfortunately, study of digestion of isolated cell wall fractions in situ may prove misleading because interactions among the fiber fractions are ignored (Moore and Hatfield, 1994). In addition, crystallinity of cellulose and extent of polysaccharide linkage with lignin can influence accessibility of constituents to microbial attack and thereby reduce rate and extent of ruminal degradation.

Fiber digestion by herbivores is affected by many factors; these include 1) characteristics of the fiber (chemical and physical), 2) time for fermentation, which depends on rate of passage of forage from the rumen, and 3) activity of microflora of gastrointestinal tract (Dehority, 1993). Cell wall digestion in rumino-reticulum consists of two distinct activities or period: 1) hydrolysis of polysaccharides by microbial enzymes and 2) conversion of released monosaccharides to pyruvate and subsequently to volatile fatty acids (acetate, propionate and butyrate), fermentation gases (CO_2 and CH_4) and heat. Hydrolysis is the rate-limiting step.

Lignin concentration and forage digestibility within a forage type (legume or grass) are inversely related. Covalent bonds between lignin and cell wall polysaccharides limit accesibility of fiber to microbial enzymes (Moore and Hatfield, 1994). Phenolics and their esters also can inhibit growth of ruminal microbes and thereby reduce fiber digestion (Weimer, 1993).

Microbes involved in Fiber Digestion

The primary ruminal microbes involved with fiber digestion are bacteria. Although their contribution to the cellulose digestion is small, protozoa and fungi also

possess limited cellulolytic activity (Moore and Hatfield, 1994). Only a limited number of the thousands of species of bacteria in the rumen can digest cellulose. The four most prevalent cellulase producing ruminal bacteria are: Bacteroides succinogenes, Ruminococcus albus, Ruminococcus flavefaciens and Butyrivibrio fibrisolvens (Yokoyama and Johnson, 1988, Fahey and Berger, 1988, Chesson and Forsberg, 1988, and Moore and Hatfield, 1994). Most cellulolytic microorganisms also can ferment hemicellulose, but some additional species, including Eubacterium ruminanticum, Prevotella ruminicola, Eubacterium uniforme, and Eubacterium xylanophilum, produce both de-polymerase and glycosidase to digest hemicellulose directly (Dehority, 1993). In addition to the cellulolytic species, Lahnospora multiparus readily digests pectic substances in the rumen (Dehority, 1993).

Bacteria, fungi, and protozoa colonize plant particles near epidermal lesions or stomata and expose their microbial extra-cellular enzymes near the substrate. Intact plant cells do not allow microbial adhesion (Chesson and Forsberg, 1988; Pell and Schofield, 1993). Adherence to plant material also helps to prevent attached microbes from being swept out of rumen with fluids (Tamminga, 1993). Synergistic action among several extracellular enzymes contributes to digestion of cellulose. Indeed, cellulase is not a single enzyme as the name might suggest; rather, cellulase is a complex of at least four enzymes: endo-β-1,4-glucanase, exo-β-1,4-glucanase, cellodextrinase and β-1,4-glucosidase (White et al., 1993). In addition, amorphous cellulose and crystalline cellulose differ in resistance to digestion but can be degraded simultaneously. Endo-β-1,4-glucanase first cleaves the amorphous ends of cellulose exposing oligosaccharides with free ends for exo-β-1,4-glucanase attack. The end product of exo-β-1,4-glucanase

cleavage is cellobiose. Cellobiose is hydrolyzed to glucose by β-1,4-glucosidase, an enzyme found in many bacterial strains that may not have the other components of the cellulase complex (Moore and Hatfield, 1994). Hemicellulose degradation by enzymes parallels digestion of cellulose except that a larger number of enzymes are involved (White et al., 1993). Pectin lyases and pectin esterases are main enzymes responsible for rapid degradation of pectins in rumen (Moore and Hatfield, 1994).

End products of fiber digestion and pathways

Monosaccharides, produced during microbial digestion of cell wall polysaccharides and enzymatic hydrolysis of non-structural polysaccharides, are fermented by ruminal microbes to VFA, CO_2, and CH_4. Pyruvate is an intermediate in the degradation process for all carbohydrates (Fahey and Berger, 1988). The molar proportions of the various fermentation products that are formed with vary with diet type, type of carbohydrate being fermented, type of bacteria involved in fementation, and the ruminal environment during fermentation (Fahey and Berger, 1988). For example, more mature forage usually result in high proportions of acetic acid; in contrast, starch fermentation results in higher proportions of propionate (McDonald et al., 1995). Bacteria that rapidly ferment pectin often produce very high ratios of acetate to propionate.

Conversion of pyruvate to acetate in rumen bacteria can follow one of two pathways. The most common pathway uses pyruvate-formate lyase to convert pyruvate to formate and acetyl-Coenzme A. Formate then is converted to CO_2 and H_2 which, in turn, can produce methane. By a second pathway, pyruvate-ferrodoxin oxidoreductase

produces ferrodoxin plus acetyl-CoA (Russell and Wallace, 1988). Acetyl CoA produced by either pathway is converted to acetate by a reversible phosphotransacetylase reaction (Russell and Wallace, 1988). Butyrate producing bacteria, such as Butyrivibrio fibrisolvens, utilize acetyl CoA in a series of enzymatic steps to produce acetoacetyl-CoA, β-hydroxybutyryl CoA, crotonyl CoA, butyryl CoA and butyrate consecutively (McDonald et al., 1995). Though it can be produced by two separate mechanisms, propionate is produced primarily through enzymes of the Krebs cycle. In this dicarboxylic acid pathway, three enzymes are used to convert pyruvate to propionate. Because propionate a major energy source for tissues in ruminants and the only major VFA used for gluconeogenesis, and because diet plays a major role on the proportion of propionate produced, propionate production has been of major interest for many years (Fahey and Berger, 1988). The three enzymes in the conversion of pyruvate to propionate in the TCA cycle are: 1) phosphoenolpyruvate carboxykinase, that produces oxaloacetate plus ATP from phosphoenolpyruvate, 2) pyruvate carboxylase, that catalyzes the conversion of pyruvate to oxaloacetate, and 3) methylmalonyl-CoA carboxyltransferase, that converts succinate to propionate (Fahey and Berger, 1988). The second major pathway for conversion of pyruvate to propionate, the acrylate pathway, involves stepwise conversion of propionate to lactate, then acrylyl-CoA, then propionyl-CoA, and finally, propionate (Russell and Wallace, 1988).

Effect of pH (Acidity) on Digestibility in Rumen

The rumen is the aqueous environment ideal for fermentation, with a temperature between 36°C and 40°C, water to dilute and flush materials along the tract, and salivary

buffers to maintain the pH of the rumen near neutrality despite continual production of fermentation acids that depress pH. Ruminal pH can range from 5.5 to 7.2 in healthy animals (Owens and Goetsch, 1988). Ruminal pH varies with diet type, typically remaining between 5.5 and 6.5 for concentrate-fed ruminants and between 6.2 and 7.0 for in roughage-fed ruminants. Ruminal pH can fluctuate between meals depending on concentrations of fermentation acids and input of buffers from saliva and of buffers or bases released from feed (Owens and Goetsch, 1988). Increases in rumino-reticular pH are prevented by by salivary buffers ($NaHCO_3$ and Na_2HPO_4) and diffusion of $NaHCO_3$ through the ruminal epithelium in exchange for fermentation acids being absorbed. Because VFA are weak acids with pKa values of 4.7, their absorption from the lumen increases as pH decreases (Breazile and Houghton, 2000). At a neutral ruminal pH, over 90% of VFAs are absorbed as salts. Fermentation products like ammonia produced from feed, and basic feed additives (sodium hydroxide, ammonia) can act as buffers or bases to prevent decreases in ruminal pH.

Breazile and Houghton (2000) outlined that absorption of VFA from the ruminoreticulum involves removal of organic acids from the rumen which helps to elevate pH. In addition, methanogic bacteria use hydrogen ions, a mechanism that also aids in acid removal. Hydrogen ions also are removed from the rumen when used to hydrogenate unsaturated dietary fatty acids. Through converting lactic acid to propionate, Propionobacteria also help to avoid high concentrations of lactic acid, an acid that has a lower pKa than VFA (Breazile and Houghton, 2000).

For many years, in vitro studies have been used to examine the impact of pH on nutrient digestibility in the rumen. Grant and Mertens (1992a) developed in vitro buffer

systems to manipulate ruminal fluid pH over a wide range (5.8 to 6.8) so they could evaluate effects of pH on NDF disappearance rate. Citric and phosphoric acids were used to depress pH whereas a phosphate-bicarbonate buffer was added to elevate pH. Fluid pH was controlled tightly throughout fermentation. A McIlvane solution was substituted for bicarbonate, but pH declined rapidly during fermentation. Alfalfa hay, bromegrass hay, and corn silage were incubated in rumen fluid for 0, 12, 24, 48 and 72 hours. Residual NDF from alfalfa hay was lower at pH 5.8 than at pH 6.8 only at 12 h of fermentation; they interpreted this to suggest that alfalfa was the most rapidly digested forage. NDF disappearance from both bromegrass and corn silage were lower at 5.8 pH than at 6.8 pH at all times sampled. They also examined digestion kinetics including discrete lag time, fractional rate of digestion, indigestible residue, and 96-hour endpoint digestion. Rate of digestion of corn silage NDF was significantly greater at pH 6.8 than at pH 5.8 while rate of digestion of the other two feedstuffs (alfalfa hay and bromegrass) was not different at these two pH levels. However, digestion lag was significantly longer at pH 5.8 than at pH 6.8 for all three feeds. The authors suggested that the difference in lag time among forages may be related to alterations in specific physical and chemical properties of plant tissues that in turn alter the rate or extent of bacterial adhesion that must precede fermentation of fiber. They concluded that more research is needed to explain whether a diet-induced low pH would have an effect of fiber digestion similar to those observed from direct alteration of pH that was used in their in vitro research. One additional concern with in vivo experiments is the wide intra-day fluctuation in ruminal pH that is quite evident in dairy cattle (Grant and Mertens, 1992a).

Grant and Mertens (1992b) also investigated whether addition of raw corn starch would alter in vitro fiber digestion kinetics of alfalfa and bromegrass hays. Medium quality alfalfa and grass hay were incubated with rumen fluid with a pH of 6.31 (basal), with pH reduced to 6.2 and 5.8 by addition of 1 M citric acid, or increased to 6.8 by addition of Goering and VanSoest (1963) buffer. Corn starch addition to alfalfa hay lowered the rate of NDF digestion significantly. They suggested that starch addition increased the amount of indigestible NDF in alfalfa hay. The decreases in NDF digestion and the increase in lag time with lowered pH were curvilinear; decreases in NDF digestion were sharper from added starch when pH was below 6.2. This was interpreted as selective inhibition of cellulolytic microorganisms at a low pH. The authors indicated that fiber depression with addition of starch at 6.8 pH was less drastic than when pH was lower. They speculated that cellulose digesting microbes may preferentially utilize starch; thereby, they digest cellulose less readily at a lower pH. However, simply lowering pH had little effect on alfalfa NDF digestion in their study. In contrast, NDF disappearance from bromegrass hay and corn silage were greatly reduced by lowering pH. Addition of starch did not depress the fiber digestion as much for bromegrass as for alfalfa. Grant and Mertens (1992b) did not speculate on whether the depression in fiber digestion was caused by a decreased population or activity of cellulolytic bacteria or by a direct effect of pH on cellulase activity.

In an earlier study, Mertens and Loften (1980) added purified corn and wheat starch to alfalfa, bermudagrass, orchardgrass and fescue hay at four starch percentage levels: 0, 40, 60 and 80%. Source of isolated starch did not affect fiber digestion even though corn starch differs markedly from wheat starch in solubility and fermentation

characteristics. In this study, starch addition depressed extent of fiber digestion primarily by increasing the lag time. Since the pH was tightly controlled throughout this experiment, extent of fiber digestion was not depressed as much as is often observed with in vivo experiments. Subsequently, Grant (1994) examined the effects of starch source and pH on kinetics and extent of ruminal fiber digestion. Raw sorghum, raw corn, or pure corn starch, each with a different degradation rate, were added to alfalfa hay and bromegrass hay and incubated in vitro at pH values of 5.5, 6.2 and 6.8 . In this experiment, as pH was decreased, lag time increased and fractional rate of NDF digestion was depressed. The depression in fiber digestion at 96 h at the lower pH conditions, as compared to a pH of 6.8 with no starch added, ranged from 44 to 100%. He concluded that forage source, starch source and pH influence all can influence apparent extent of fiber digestion at 96 h..

A very wide range of ruminal pH in vitro (6.8, 6.5, 6.2, 6.0, 5.8 or 5.5) was obtained by adding citric acid to the phosphate-bicarbonate buffer system in Grant and Weidner's (1992) study. In addition to alfalfa hay and bromegrass hay, corn silage was included as a forage substrate. They concluded that a moderate decrease in pH from 6.8 to 6.5 increased lag time but did not decrease rate of NDF digestion. However, when pH was decreased below 6.0, rate of NDF digestion decreased significantly. Data also indicated that forage type might alter response to pH because the depression in extent of NDF digestion at 96 h by lowering the pH was less for bromegrass hay than for alfalfa hay and corn silage. Mertens and Ely (1982) stated that lowering pH below 6.0 will change the ratio of microbial species in rumen and thereby reduce cellulase activity. In vivo, microbes may have time to adapt to altered pH, whereas with a sudden drastic drop

in pH, as occurs with abrupt pH adjustment in vitro, time is insufficient to allow for alterations in and adaptation by the existing microbial population. If nutrient supplies were utilized by soluble carbohydrate digesting bacteria, and if ruminal turnover rate of fiber digesting bacteria increases, slower growth in number of cellulolytic bacteria may not be able to maintain their population in the rumen. Therefore they can not produce high concentration fiber digesting enzymes for optimal fiber degradation.

Erdman (1988), reviewing the buffer requirements of dairy cows, indicated that ruminal pH and dietary ADF were linearly related, presumably due to diet dilution and increased flow of salivary buffers when more ADF was present in the diet. He suggested that the optimal pH for maximum fiber digestion in rumen ranges from 6.4 to 6.8; within this range, dietary buffers do not alter rate of ADF digestion. Hoover's review (1986) focused on chemical factors which influence fiber digestion in ruminants. He noted that addition of only 10 to 15 % of DM as readily fermented carbohydrate impaired fiber digestion (Hoover, 1986). He proposed that reduced fiber digestion was the result of carbohydrate preference, decreased pH, and a reduction in the population of celluloytic bacteria. At a higher ruminal pH, researchers generally consider that starch has a direct effect, presumably independent from pH, that is considered to be a "carbohydrate effect" (Mould and Orskov, 1983, Mould et al., 1983, Miron et al., 1990, 1996, and Miron et al., 1997). But when added starch also reduces pH, then pH depression is considered to be the main factor inhibiting fiber digestion (Hoover, 1986). Mould and Orskov (1983) suggested that the break point between moderate depression in fiber digestion and severe fiber digestion occurred at a pH of 6.0. Fibrolytic enzyme activity remained high when

pH remained above 6.0, but the number of cellulolytic microbes was inconsistent even when pH remained above 6.0 (Hoover, 1986).

Attachment of bacteria to plant cell walls may be responsible for reduced fiber digestion when pH is decreased to about 6.0, but below 6.0, growth and survival of several cellulolytic species supposedly is reduced; their decrease in number, due to extensive wash-out, might explain the cessation of fiber digestion (Hoover, 1986). However, the concept of a reduced population of cellulolytic microbes associated with addition of grain or low pH directly conflicts with direct counts of cellulolytic bacteria in the rumen (Van Gylswyk and Schwartz, 1984). These workers reported that cellulolytic bacteria numbers ranged from 0.1 to 9 x 10^8 per ml whether or not readily fermented carbohydrate was fed; numbers of cellulolytics decreased only when pH fell below 5.2. Consequently, some reason for the reduction in fibrolytic activity other than a reduced population of cellulolytic microbes in the rumen must explain the decrease in extent of fiber digestion noted both in vitro and in vivo with starch or acid addition.

Digestibility Responses with Various Fiber Classes

Various cell wall polysaccharides differ in their rate and extent of digestion due to differences in their structural features. Though cellulose generally is considered to be less completely digested than hemicellulose and pectin, Tamminga (1993) indicated that cellulose was more extensively digested than noncellulosic pollysaccharides. In addition, differences in enzyme activity and substrate concentration can influence digestibility of specific cell wall constituents. So discrepensies still remain about the comparative digestibilities of cellulose and hemicellulose (Merchen and Bourquin, 1994). This may

be due to different types of cellulose or analytical problems in estimating hemicellulose. Most cellulolytic microbes have the multiple enzymes needed to form a cellulolytic system (Hatfield, 1993), but cellulose that is more highly crystalline resists attack except by only one species of cellulolytic bacteria. Ben-Ghedalia and Miron (1984) stated that hemicellulose, as calculated as the difference between NDF and ADF, differs from true hemicellulose when estimated from monosaccharide analysis as developed by Merchen and Bourquin (1994). Van Soest (1993) clarified the issue stating that a neutral detergen solution fails to extract all cell wall carbohydrates and that cinnamyl groups that may be degraded in rumen. He stated that the main purpose for ADF analysis was to isolate the cell wall constituents that are more resistant to digestion. However ADF also includes resistant pentosans, lignin, cutin and acid-detergent insoluble nitrogen; thereby, ADF alone is insufficient to precisely predict indigestibility of forages. Pectic substances are almost completely digested in the rumen. Fiber requirements for dairy cattle still appear to be adequately estimated by NDF content because of its close relationship to production of fat-corrected milk (Varga et al., 1998). If ADF is used for predicting the fiber requirements of dairy cattle, most common feeds such as corn silage, grasses and hays, will have higher predicted requirements than when NDF is used to predict fiber requirements. NDF of forage has been used for estimating energy value of forage and voluntary forage intake (Varga et al., 1998 and Van Soest et al., 1991). Ruminal digestibility of NDF ranges from 11 to 73%, averaging 44%. Varga et al. (1998) indicated that about 15% of total protein in feed is associated with cell walls (Varga et al., 1998). Even though it is not digested, the indigestible proportion of NDF will affect ruminal pool size, increase ruminal fill, and may alter dry matter intake. Three

mechanisms exist for increasing intake of fibrous feeds: increasing the rate of ruminal digestion, increasing passage rate, and increasing ruminal volume (Varga et al., 1998). Susmel et al. (1990) suggested that rates of ruminal degradation of various fiber fractions are correlated with each other even though disappearance of each fraction is independent of its concentration in feed.

Differences in Digestibility of Various Forage Classes

Some 65% of the world's land mass is covered with temporary or permanent pasture; standing pasture plants contain 30 to 80 % fiber (Tamminga, 1993). This makes forage an important source of energy and nutrients for ruminant animals. Botanists classify plants of the plants kingdom into two primary types: grasses and legumes. Generally, when compared with grasses, legumes are more rapidly fermented (Merchen and Bourquin, 1994). Legumes often have a lower proportion of total dry weight as cell wall constituents, but a higher fraction of the cell wall consists of lignin. Nevertheless, because of its restricted location and type, lignin in a legume depresses cell wall digestion less than an equal amount of lignin in a grass (Galyean and Goetsch, 1993). Hemicellulose content also is lower in legumes than grasses. When compared with grasses, legumes, with a lower levels of cell wall constituents, leads to a faster digestion rate, a shorter ruminal retention time, and higher dry matter intake (Merchen and Bourquin, 1994). Galyean and Goetsch (1993) proposed that availability of certain readily fermentable substrates from legumes will improve the microbial environment in the rumen; thereby, substrate preference of bacterial species can decrease adverse effects of microbial population on fiber digestion. Conservation of forages, i.e., harvest and

storage as hay or ensiling, generally results in an increase in the fiber content of forages due both to leaf loss during hay harvest, respiration during drying as hay, or fermentation of cell contents during fermentation in a silage mass. Heating following harvest also can increase the concentration of indigestible fiber (Tamminga, 1993). Forage quality also will vary markedly with stage of maturity at harvest, physical form, i.e., chopped, graund or pelleted, the proportion of concentrate in the diet, and feeding method. However, forage type (legume vs grass) appears to be the single most important variable influencing nutritional value of forage..

Cool season grasses are more rapidly and extensively degraded in rumen than warm season grasses (Galyean and Goetsch, 1993). These grass types differ not only in photosynthetic pathways, with cool season grasses using the C4 pathway and warm season grasses using the C3 pathway, but also in leaf to stem ratio, and tissue morphology. These differences make cool season grasses more readily fermented by microorganisms in the rumen than warm season grasses. With low quality forages, intake often limits intake of feed and energy as well as essential fatty acids;supply of amino acids also may be very low with low quality forage diets depending on the type of forage and its stage of maturity (Galyean and Goetsch, 1993).

Canadian researchers (Beauchemin et al., 1994) conducted an experiment to determine the optimum NDF source and level for lactating dairy cows fed a barley-based diet. Various levels of NDF in the form of alfalfa hay, orchardgrass hay, and corn silage were fed. NDF from orchardgrass hay was less extensively degraded than NDF from either alfalfa hay or corn silage. Shaver et al. (1988) compared alfalfa hay harvested at three different stages of bloom with bromegrass hay and corn silage. Again, digestibility

was lower for NDF from bromegrass hay and corn silage than for NDF from alfalfa hay. The lower extent of NDF digestion for corn silage was attributed to a reduced ruminal pH. Although the potentially digestible NDF was higher for bromegrass than for other roughages tested, the slower fractional digestion rate for NDF for bromegrass suggested that cellulolytic activity was decreased or soluble protein and starch supply might be limiting ruminal digestibility.

Moore et al. (1987) measured in situ disappearance of NDF from wheat straw in the rumen at 24 and 36 h of incubation. Ruminal NDF disappearance from wheat straw was surprisingly high when compared to cottonseed hulls when steers were being fed 65% concnetrate diets. They concluded that low ruminal pH did not limit NDF disappearance from either of these roughages (Moore et al., 1987). With a 90% concentrate diet, adding wheat straw to the diet increased ruminal buffering by enhancing saliva flow; thereby, disappearance of NDF was significantly higher from wheat straw than from cottonseed hulls. Later, Moore and coworkers (1990) noted that addition of wheat straw to the diet increased apparent extent of total tract NDF digestion of milo and alfalfa hay by 46 and 35%, respectively.

In addition to microbial digestion in the rumen, microorganisms in the cecum and lower gut can ferment dietary fiber that escapes ruminal digestion and produce VFA to be absorbed and used as energy sources. However, microbial protein and organic matter formed beyond the small intestine are not digested and absorbed but instead are lost in feces. Thus, it is more efficient energetically for ruminants to digest fiber in the rumen than in the cecum and large intestine (Moore et al., 1990).

Miron and coworkers (1997) treated ground sorghum with 4 % sodium hydroxide; NDF digestion in ruminoreticulum and total tract digestion from this product was 45% greater than for untreated ground sorghum. Mader et al. (1991) included corn silage, alfalfa hay and alfalfa silage as roughage sources in high concentrate diets based on corn grain that was whole (unprocessed), dry-rolled, or as high moisture grain, being either ground or left whole into storage. Ruminal digestion of NDF was significantly lower with the ground high moisture corn diet when the roughage source was corn silage than when the roughage source was alfalfa hay, but no other difference between these three roughage sources was detected. Poore et al. (1991) noted that increasing the wheat straw portion of a wheat straw:alfalfa hay mixture of roughage in a high concentrate diet linearly increased ruminal NDF digestibility.

Rate and extent of fiber digestion of a wide range of forage, grain and by-product feeds from the Pacific Coast of US were investigated by Xu and Harrison (1997). Early and late cut grass silages and alfalfa hay samples had different extents of NDF loss from nylon bags indicating that dry matter degradation and NDF content of the feed will change- withharvest date. Even though it had slightly less NDF and ADF than corn silage, sunflower plus corn silage had a slower NDF degradation rate than corn silage alone; this implies that chemical or physical factors of the fiber source may affect NDF degradation in rumen. Feeds rich in protein or fat had longer lag times than other feeds in their study. They found a close relationship ($r = .66$) between NDF content and ADF content of the feeds tested. NDF degradation of forages in rumen was closely correlated with NDF content and DM degradation. Beside those correlations, NDF degradation of grain and byproduct feeds was inversely related to forage nitrogen content. They

calculated half life times for NDF degradation from various forages in rumen as well as the amount of time needed to clear 50% of the NDF from the rumen.

Comparison of Ankom Technique with Tube Technique

In vitro rumen procedures are designed to obtain apparent digestibility of feeds based on the weight of undigested residues caught on filter paper. The original in vitro estimation technique outlined by Tilley and Terry (1963) utilizes fermentation flasks incubated in water baths with samples swirled by hand. Difficulties in maintaining active ruminal fluid and variability in manual procedures limit the repeatability of the in vitro incubation technique with individual test tubes..

Ankom (Ankom Tech., Turk Hill Rd, Fairport, NY) Daisy^{II} incubator contains four rotating (agitating) 2-L jars (vessels) kept under constant and uniform 39^{0}C temperature. Since it utilizes a large volume of ruminal fluid in tightly controlled environment, it allows researchers to incubate multiple feed samples in nylon bags in a single batch of ruminal fluid. The system is easily accessible for measurement and manipulation of pH and ammonia. One concern noted in our early experiments was that the bags tended to float on the surface of the fluids, a condition that reduces the potential for fluid and microorganisms to pass through the bag and digest the bags' contents. To alleviate this problem, a marble (mean weight 3 to 4 g) was included in each bag that caused them to sink and to increase exposure of the feed to the ruminal fluid in the rotating vessels.

CHAPTER III

Influence of NDF source and ruminal pH on in situ fiber disappearance.

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ABSTRACT

To measure the impact of ruminal pH on in situ disappearance of hemicellulose (NDF minus ADF) and of ADF, three ruminally cannulated heifers (387.5 kg) in a 3 by 3 Latin square experiment were fed a single diet at three different levels of intake in an attempt to obtain three different ruminal pH values. The diet, consisting of 90% concentrate based on ground corn grain, was fed at three levels of feed intake (1% and 1.5% of body weight daily or free choice) with fresh feed provided three times each day. NDF, the residue from extraction with neutral detergent solution devoid of EDTA, from three different fiber sources (alfalfa hay, prairie hay and wheat straw) were placed in dacron bags and incubated in situ for 0, 6, 12, 24, or 96 h. Once bags were recovered, they again were extracted with neutral detergent solution and subsequently with acid detergent solution. NDF disappearance at 96 h ranged from 14 to 33%, being greater ($P < .05$) from wheat straw than from alfalfa hay. Rate of disappearance of hemicellulose and ADF, expressed as a fraction of that disappearing at 96 h were quadratically related to pH with disappearance being minimum at pH values of 5.9 and 5.4, respectively. Stepwise regression revealed that when ruminal pH was below pH of 5.2 to 5.6,depending on the source of fiber, pH had no impact on disappearance of either hemicellulose or ADF.

Above this pH, disappearance of hemicellulose and ADF increased quadratically with alfalfa hay but not with prairie hay or wheat straw. Results indicate that rate and extent of fermentation differ with fiber source, that fiber sources differ in their response to low ruminal pH, and that disappearance of NDF from alfalfa hay decreased in a curvilinear, not an abrupt, fashion as pH dropped below 6.0.
(Key words: NDF, hemicellulose, fermentation.)

INTRODUCTION

To obtain maximum rates and efficiencies of gain and economic efficiency with feedlot cattle, grains comprise the majority of DM in their diets. However, the amount and quality of forage included in such diets can impact productivity. A fiber deficiency can result in various metabolic disorders, including rumenitis, acidosis, and liver abscesses (Marshall et al., 1992). The NRC for Dairy Cattle (1989) suggested that diets should contain a minimum of 25 to 28% NDF of which 75% should come from forage sources based on research by Clark et al. (1997) and Beauchemin (1991). Mertens (1987) also recommended that optimal dietary NDF intake is about 1.1% of body weight daily for dairy cows; with feed intake at 4% of body weight daily, is equal to 27.5% of diet dry matter. Cereal grains, being rich in non-structural carbohydrates, particularly starch, are rapidly fermented in rumen. Starch from barley and wheat is degraded more rapidly than that from corn and milo (Reynolds et al., 1993). When fed as the majority of the diet, starch can drive ruminal pH below 5.5, a pH level where rate of fiber digestion is depressed. Low ruminal pH has been proposed to explain why adding grain to forage diet will decrease feed intake and why forage digestibility is low in concentrate-rich diets

(Caton and Dhuyvetter, 1997). Mertens (1977) reported that forage fiber digestion starts to decline whenever ruminal pH falls below 6.7. Compared to bacterial species that digest starch, ruminal bacteria that digest cellulose are quite sensitive to and inhibited by low rumen pH (Hoover, 1986). Russell (1979) indicated that the population of cellulolytic bacteria in the rumen will decrease whenever pH falls below 5.7 while amylolytic bacteria survive even when pH falls below 5.0 (Russell, 1979). Decreased cellulase activity and microbial attachment, as well as production of inhibitors by starch digesting bacteria, may contribute to diminished cellulolysis at a low pH (Poore et al., 1987). Bourquin et al. (1994) suggested further that cellulolytic microbes may switch from digesting cell walls to digesting readily fermented carbohydrates when pH is low. Grant and Mertens (1992) suggested that the optimal pH for rumen microbes (cellulolytic) is between 6.5 and 6.8. Based on in vitro studies in which pH was adjusted by adding base or acid, they postulated that decreasing pH from 6.8 to 5.8 increases lag time, the period of time from the start of incubation until digestion begins, and decreases rate of NDF digestion. In contrast to in vitro condition, in vivo the ruminal pH will fluctuate between meals and microbes can adapt over time to their environment. Consequently, in vivo digestion cannot be directly predicted from in vitro estimates. Response to low pH also may differ with feedstuff type, as fiber sources differ markedly in both nutrient content and fiber composition (ADF, hemicellulose, lignin).

The objective of this study was to determine the degree to which low ruminal pH will depress rate and extent of in situ disappearance of NDF, ADF, and hemicellulose from several widely fed roughage sources.

MATERIALS AND METHODS

Animals: Three continental crossbreed heifers (mean weight 388 kg) approximately 3 years of age were assigned randomly to a 3X3 Latin square. Heifers were ruminally fistulated and individually penned at the OSU Nutrition and Physiology Center.

Diet: Prepared by OSU Feedmill, the diet consisted of 90% concentrate and 10% roughage (Table 1) based largely on ground corn grain. Concentrate content of the diet was high so that ruminal pH would be depressed. Heifers were assigned randomly to be fed different amounts of feed in each period of the Latin square. Rather than altering the diet to influence ruminal pH, we fed a single diet at three different levels of intake; this was an attempt to remove the impact of diet composition on the microbial population in the rumen that may occur when different ruminal pH conditions are obtained by feeding different diets. The three intake levels were ad libitum (free choice) or restriction of daily DM intake to 1.5% or 1% of body weight. Intake of animals given ad libitum access to feed was slightly restricted (fed at the peak level consumed during the 14 day adaptation period) in order to avoid metabolic disorders and refusal of feed during the in situ measurement period. Heifers were fed 3 times each day (0800, 1600 and 2400) to reduce fluctuations in ruminal pH. Heifers were adapted to their intake level for the first 14 days of each period. Animals had free access to the water throughout the experiment.

Procedure and Analysis: Two forage samples (alfalfa hay and prairie hay) were obtained from 3 feedlots located in the Oklahoma Panhandle on September 1st, 1997. Wheat straw was obtained from OSU Equine Center. These feeds were chopped manually to a mean particle length of 2.5 cm. Approximately 2.0 g of each sample were

placed in separate 6.35 X 13.70 cm dacron bags and bags were heat-sealed. Bags containing samples were dried at 55° for 24 hours. NDF content of each feed was analyzed before the bags were placed in the rumen. Neutral detergent solubles were removed from each feedstuff before the bags were incubated in the rumen. Amount of each forage placed in bags was calculated based on NDF content as reported in the published literature (Carro et al., 1995) as well as the required surface area needed for adequate in situ digestion based on values of 10-20 mg and 15 mg per square cm surface area of the bag proposed by Vanzant (1997) and Carro et al., (1995) respectively. The maximum sample DM:surface area ratio was 10 mg DM:cm^2. Fiber analyses (NDF, ADF) were conducted using an Ankom^{200} (Ankom, Turk Hill St. NY). Duplicate dacron bags containing each of the three feedstuffs were placed in the rumen and allowed to ferment for 96, 24, 12, 6 and 0 hours. To eliminate variability in dacron bag rinsing procedures over time, bags were inserted at 4 different times and retrieved at a single time (0900) that corresponded to 1 h after the previous meal. The 0 h bags were rinsed en mass with bags retrieved from the rumen. Dacron bags inserted at each time interval were combined into a single large net laundry bag to ensure that ruminal location would be similar for all bags within a time period and to simplify recovery of the bags. All bags were rinsed at 39^0 C under running tap water and placed in buckets. Water in each bucket was agitated by hand and changed at 5 minute intervals over a 60 min period. Then, each individual bag was rinsed under a stream of running tap water for another minute. After drying at 55^{0}C for 48 h, bags were weighed and bags containing all samples were again extracted with neutral detergent solution that should remove all attached microbes. After drying and re-weighing to determine residual NDF, bags containing samples were

extracted with acid detergent solution to determine ADF content, again using the Ankom^{200}. Ruminal pH was recorded every 4th h and rumen fluid was obtained, filtered through four layer of cheesecloth, and frozen for subsequent analysis for ammonia N content.

Data were analyzed using the SAS GLM procedure by regressing disappearance of NDF, ADF, and hemicellulose (NDF minus ADF) for individual times against mean ruminal pH for the animal during the 96-h incubation period. Disappearance of NDF for individual feeds was regressed against incubation time to examine differences among feeds. Disappearance rate for NDF expressed as a percentage of fermentable NDF (disappearance at 96 h minus disappearance at 0 h) was calculated by regression of the natural log of residual fermentable NDF against time either including or excluding zero values for disappearance at 0 h. Including the 0 h values gives an estimate of NDF disappearance assuming that time lag prior to the onset of NDF disappearance was nil whereas regressions based on only values from 6h, 12 h, and 24 h incubations permit lag to occur prior to the onset of NDF digestion and lag times were calculated as the difference between the intercept and the 0 h value divided by NDF disappearance rate. NDF disappearance rates were regressed against ruminal pH and ruminal pH squared in an attempt to determine the slope of the relationship between NDF disappearance and pH.

RESULTS AND DISCUSSIONS

Components of fiber in the three feedstuffs incubated in situ are presented in Table 2. Alfalfa hay contained less NDF and ADF than prairie hay or wheat straw. Ruminal pH values as well as extent of NDF, ADF, and hemicellulose disappearance

after various incubation times averaged across feed intake levels and across feedstuffs are presented in Table 3 and Figures 1, 2 and 3. Surprisingly, pH was not significantly altered by level of feed intake as expected. Consequently, effects of intake level on disappearance in this study should be independent of ruminal pH.

Extent of disappearance was greater with the highest than the lowest intake level for NDF at 6, 12, and 96 h of incubation. ADF and hemicellulose disappearance paralleled NDF disappearance. When level of grain in the diet is increased, rate of fiber digestion usually declines (Mould and Orskov, 1983, and Grant, 1994)), however this response may be due to a decrease in ruminal pH. In vitro, addition of starch also decrease rate of fiber digestion (Mould and Orskov, 1983, and Miron et al., 1990)), but in our trial, diet composition and thereby the substrates available for fermentation and pH were not altered by intake level. Through increasing the rate that microbes are forced to multiply in the rumen, the faster ruminal turnover with higher feed intakes may have increased activity of resident microbes though no published literature to support this concept is available.

With the exception of the value for 96 h, disappearance at all hours was greater for alfalfa hay than for prairie hay and wheat straw (Table 3). This was partly due to greater washout at 0 h for alfalfa hay than for other feeds. At 96 h, disappearance of wheat straw NDF was greater than for the other two feeds. If disappearance at 96 h is used as an index of potential extent of fiber digestion, wheat straw had greater potential NDF, ADF, and hemicellulose disappearance than either prairie or alfalfa hay.

Interactions between intake level and disappearance of ADF at 0 h and of NDF and hemicellulose at 12 h were detected as shown in Table 2. Increasing intake level

tended to increase disappearance of alfalfa hay but it had little impact on other feeds. No explanation for this difference is apparent. In contrast, increasing intake level increased rates of NDF and hemicellulose disappearance from wheat straw but not from other feeds at 12 h of incubation. Perhaps the increased intake enhanced liquid turnover and mixing in the rumen; this could increasing exposure of straw-bound fiber-digesting microbes to needed nutrients (ammonia, vitamins) that were lacking in wheat straw but not lacking in prairie hay or alfalfa hay. Schaefer et al. (1988) observed that in situ digestion was increased by higher ruminal ammonia levels with oats grain than other feedstuffs. They suggested that higher ammonia concentrations helped to overcome some spatial limitations in access to ammonia for fiber-digesting microbes.

Rates of NDF and hemicellulose disappearance expressed as a fraction of that potentially digested (96 h minus 0 h) with or without provision for lag time for the different feeds and intake levels are presented in Table 5. Significance of linear effects of intake level on disappearance also is provided for each feed in Table 5. Similarly, disappearance of potentially disappearing fractions is presented graphically in Figures 4, 5 and 6. Disappearance rates with a lag time were generally slightly lower than disappearance rates without provision for a lag time (Table 5) suggesting that some time lag preceded the onset of NDF disappearance, particularly with higher feed intake levels. Chappel and Fontenot (1968) also noted that time lag was lengthened by addition of starch to in vitro cultures. However, no lag time for hemicellulose disappearance was evident in our study at any intake level. This indicates that all components of NDF do not respond similarly to level of feed intake. Disappearance of potentially digested NDF and hemicellulose were consistently higher for alfalfa hay than for other feeds at the

lowest intake level. More rapid disappearance of NDF and hemicellulose will allow more extensive digestion of potentially available fiber when ruminal residence time is limited. Assuming a 2%/h passage rate, less than half of the fermentable fiber from prairie hay would be fermented in the rumen compared to over 75% for alfalfa hay.

Significance of the regressions of rate of disappearance of potentially digestible NDF, ADF, and hemicellulose against ruminal pH are presented in Table 4 together with the feeds for which the regression was highly significant. In all cases, disappearance of fermentable NDF, ADF, and hemicellulose increased as ruminal pH increased though the level of significance was consistently greater for ADF than for either NDF or hemicellulose. Among the feeds tested, only alfalfa hay had a regression against pH that was significant for NDF and for hemicellulose disappearance. These results support the general concept (Grant and Mertens, 1992) that fiber digestion rate is depressed when pH is decreased.

Linear and quadratic effects of pH on NDF disappearance from various feeds are shown in Figure 7. In all cases, disappearance decreased in a curvilinear fashion as pH was decreased though significance of these relationships differed with feed, only being significant for alfalfa hay. At a pH of 5.2, disappearance of NDF was similar for all feeds, but as pH was increased, NDF disappearance increased markedly only for alfalfa hay. This supports the contention that response to ruminal pH differs with the specific feed being measured. In this case, the feed with the highest potential rate of NDF disappearance (alfalfa hay) was altered to the greatest degree by pH depression. Whether this a general rule is not clear.

What are the characteristics of an ideal roughage for supplementing feedlot diets? For dilution of the diet and ruminal contents, high NDF content is preferable. A slow rate of ruminal disappearance has both advantages and disadvantages. Slow ruminal disappearance helps to retain both dilution and "scratch factor" in the rumen for several hours after a meal. Consequently, less NDF needs to be fed. With high roughage diets, a slow rate of ruminal disappearance will increase ruminal bulk and may reduce feed intake, but with the low roughage diets fed to feedlot cattle, excessive bulk should not be a problem. One adverse effect of slow ruminal disappearance is that more undigested fiber must be pushed through the small and large intestines. Erosion of epithelial tissue from the small and large intestine increases as intestinal flow of dry matter and, particularly fiber, increases. Consequently, high intakes of slowly disappearing NDF would be expected to increase both energy and amino acid requirements for replacement of intestinal tissue. Finally, is an increased supply of potentially digestible fiber for large intestinal fermentation desirable or undesirable? Fermentation in the large intestine will retrieve some of the energy from feed nutrients not fermented in the rumen, but nutrients present in the microbial mass generated in the large intestine (protein, vitamins, phosphorus) will be largely lost in feces. Residual starch supply and low pH of the large intestine will limit extent of fermentation in the large intestine of both starch and fiber. This means that recovery of energy from slowly fermented fiber that is more rapidly fermented when pH is high (like alfalfa hay) will be greater when fed with grain sources that supply little starch to the large intestine (wheat, barley, flaked grain).

How might pH dependence of NDF digestion alter ruminal metabolism? Consider the case with alfalfa hay. If ruminal pH is held constant, NDF disappearance

rate should be constant and steady state conditions will exist in the rumen. However, ruminal pH is not constant. If ruminal pH fluctuates during the day, rate of NDF disappearance will vary. As ruminal pH increases with time after a meal or fasting, VFA supply from alfalfa hay will increase and residual NDF will decrease due both to ruminal outflow and an increased rate of fermentation. Hence, although pH dependence of NDF fermentation will enhance steady state conditions for VFA production, it will decrease steady state of ruminal mass and NDF concentration in the ruminal mass. Such fluctuations in ruminal mass and delayed production of VFA might be expected to decrease meal frequency and increase variability in meal size, a condition conducive to both feedlot bloat and acidosis. Again, a roughage ideal for high roughage diets, where ruminal pH is high and relatively constant, may not be ideal for maintaining steady state ruminal conditions and regular meal eating patterns and meal sizes for feedlot cattle.

Results from this research support the idea that fiber fractions as well as different feeds respond differently to a decrease in pH. Xu and Harrison (1996) indicated that disappearance of various fiber fractions was not correlated with NDF degradation. Grant (1994) also noted that the response to lowering pH in vitro was different for alfalfa hay than for bromegrass hay. In agreement with our results, Poore et al. (1991) reported that degradable NDF was digested less from wheat straw than from alfalfa hay (Poore et al., 1991). In comparison to disappearance of NDF in vitro at pH values near neutrality by Grant and Mertens (1992), our disappearance values and rates all are quite low. This presumably is due to the much lower pH of ruminal contents in our study than for ruminal fluid used in their in vitro studies.

IMPLICATIONS

High grain diets in feedlot usually drive the ruminal pH below 6.0. Considering the steady increase in NDF digestion between the pH of 5.0 and 6.0, elevating the pH may be beneficial for feeding strategies. If nutrient supply from other diet ingredients is adequate, low quality forage sources such as wheat straw and prairie hay may be superior to alfalfa hay when added in limited amounts to feedlot diets. This is because these roughages have a slow but steady digestion rate that seems relatively independent of ruminal pHs.

TABLE 1. Composition of diet fed to heifers in experiment I, DM

Ingredient	Percentage
Rolled Corn	70.00
Ground alfalfa hay	17.50
Cottonseed hulls	10.87
Cane molasses	1.50
Trace mineralized salt	0.12

TABLE 2. NDF, ADF and hemicellulose contents of feeds studied in experiment I (% DM).

Feed	Neutral detergent fiber (NDF)	Acid detergent fiber (ADF)	Hemicellulose[1]	Hemicellulose (% of NDF)
Alfalfa Hay	51.8	40.7	11.1	21.4
Prairie Hay	73.3	46.2	27.1	37.0
Wheat Straw	75.9	46.6	29.3	38.6

[1]Hemicellulose = NDF(%) – ADF(%)

Table 3. Impact of intake level and feed source on in situ disappearance of various fiber fractions.

	Intake level, %BW/d			Feed			SEm
	1.0	1.5	2.0	Alfalfa hay	Prairie hay	Wheat straw	
Ruminal pH	5.5	5.8	5.5				
NDF disappearance							
0 h	5.3	5.2	5.2	9.9 [a]	3.5 [b]	2.4 [c]	0.323
6 h	8.5 [b]	9.5 [ab]	10.3 [a]	14.8 [a]	5.8 [b]	7.7 [c]	0.541
12 h	9.9 [b]	10.9 [ab]	12.2 [a]	15.0 [a]	7.0 [c]	11.0 [b]	0.496
24 h	14.5	14.5	14.4	17.2 [a]	10.5 [b]	15.7 [a]	0.791
96 h	26.1 [b]	27.2 [b]	31.0 [a]	27.9 [b]	25.1 [b]	31.3 [a]	1.373
ADF disappearance							
0 h	-1.0	-0.8	1.2	-0.6	0.0	0.0	0.977
6 h	-2.6 [b]	1.4 [ab]	2.7 [a]	-2.7 [a]	2.8 [b]	1.3 [ab]	1.610
12 h	0.7	1.7	2.4	-3.1 [a]	3.0 [b]	5.0 [b]	1.696
24 h	5.7	4.5	5.4	0.3 [b]	6.1 [a]	9.3 [a]	1.598
96 h	15.6 [b]	16.2 [b]	21.7 [a]	11.6 [b]	19.8 [b]	22.1 [a]	1.664
Hemicellulose disappearance							
0 h	-5.7	-5.6	-5.5	-10.9 [a]	-0.6 [b]	-2.6 [b]	0.677
6 h	0.4	2.3	3.2	-0.6 [a]	0.5 [a]	6.0 [b]	1.192
12 h	2.8 [b]	4.0 [ab]	6.4 [b]	-0.3 [a]	2.4 [a]	11.1 [b]	0.955
24 h	10.0	9.7	9.8	4.0 [c]	7.7 [b]	17.8 [a]	1.196
96 h	27.6 [b]	30.0 [b]	33.9 [a]	24.3 [b]	28.2 [b]	38.6 [a]	1.616

[a, b] Means with different superscripts differ ($P < 0.05$).

Table 4. NDF, ADF, and hemicellulose disappearance in response to Interactions of intake with feed source.

Intake, %BW	Feed		
	Alfalfa hay	Prairie hay	Wheat straw
	ADF disappearance at 0 h		
1.0	-4.8	0.1	1.8
1.5	0.3	0.4	-2.9
2.0	2.8	-0.5	1.2
	NDF disappearance at 12 h		
1.0	15.1	6.4	8.2
1.5	15.3	6.5	10.9
2.0	15.6	8.1	13.8
	Hemicellulose disappearance at 12 h		
1.0	0.4	1.3	6.8
1.5	0	1.5	10.6
2.0	-1.1	4.3	15.9

Table 5. Impact of intake level on rate of NDF disappearance in situ.

	Intake level, %BW/d			Linear response
	1.0	1.5	2.0	
	NDF disappearance, %/h			
Alfalfa hay	5.7	1.7	3.5	0.7
Prairie hay	2.2	1.7	1.6	0.15
Wheat straw	2.8	2.8	1.6	0.43
	Hemicellulose disappearance, %/h			
Alfalfa hay	6.8	2	4.1	0.7
Prairie hay	2.3	1.9	1.8	0.33
Wheat straw	3.1	2.9	1.8	0.52
	NDF disappearance, with lag, %/h			
Alfalfa hay	5.5	0	0.9	0.23
Prairie hay	1.9	1.9	0.8	0.07
Wheat straw	2.9	2.6	0.2	0.32
	Hemicellulose disappearance with lag, %/h			
Alfalfa hay	6.8	2	4.1	0.24
Prairie hay	2	2.1	1	0.25
Wheat straw	3.1	2.7	0.3	0.4

Table 6. Impact of pH on NDF, ADF and hemicellulose disappearance in situ

	Linear P<	Feed effect
NDF disappearance	0.04	Alfalfa**
ADF disappearance	0.01	
Hemicellulose dis	0.05	Alfalfa**
NDF dis with lag	0.08	Alfalfa**
ADF dis with lag	0.01	
Hemi dis with lag	0.08	Alfalfa**

**P < 0.05

Table 7. Projected NDF disappearance based on regression

	Alfalfa hay	Prairie hay	Wheat straw
Linear effect	0.64	0.09	0.15
Quadratic eff	-0.05	-0.01	-0.014
PH = 5.0	0.002	0.016	0.022
PH = 5.2	0.019	0.018	0.024
PH = 5.4	0.032	0.018	0.024
PH = 5.6	0.041	0.018	0.024
PH = 5.8	0.046	0.017	0.022
PH = 6.0	0.046	0.016	0.020

CHAPTER IV

Impact of level of feed intake and ruminal pH on in situ disappearance of NDF, ADF, and hemicellulose from several roughage sources

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ABSTRACT

When ruminal pH falls below 6.0, NDF fermentation has been presumed to cease due to reduced attachment and activity of cellulolytic bacteria. This study was designed to examine whether various fiber sources and fiber components responded differently to ruminal pH when ruminal pH remained below 6.0. Three ruminally fistulated crossbred heifers (434 kg) in a 3 by 3 Latin square design were fed a 90% concentrate diet three times daily with DM supply being either 0.9%, 1.4% BW/d, or unlimited (about 2.0% BW/d). Roughages tested included the NDF residues obtained from corn silage (separated into grain and non-grain [stover] portions), cottonseed hulls (CSH), and wheat pasture. These sources contained 13, 69, 87 and 62% NDF and 5, 38, 55, and 24% ADF, respectively. NDF residues from these feeds in dacron bags were suspended within rumen for 0, 6, 12, 24, and 96 h with ruminal pH being monitored every fourth hour. Indigestible residues were analyzed so that rate and extent of disappearance of NDF, ADF, and hemicellulose (NDF minus ADF) could be calculated. Ruminal pH (range = 4.3 to 5.9) was lowest ($P < 0.05$) with the highest feed intake (4.9 vs. 5.3 and 5.5 for unlimited, 0.9, and 1.4% BW/d dry matter intakes). For corn stover and wheat pasture, disappearance of NDF and hemicellulose were unexplainably lowest with the lowest feed

intake. Among these roughage sources, extent of disappearance was highest ($P < .05$) for wheat pasture at all incubation times. NDF disappearance at 24 h was 2.4, 4.6, 12.2, and 29.5% for stover, CSH, grain, and wheat pasture even though ADF disappearance remained extremely low (1, 3, 6, and 10%). Regressed against pH and pH squared, disappearance of NDF, ADF, and hemicellulose at 96 h all increased ($P < .05$) as pH increased. At 24 h, disappearance of NDF and hemicellulose also increased with pH (except for stover). Even though pH remained below 5.9 in this study, extent of in situ disappearance of NDF, due primarily to hemicellulose, at 24 and 96 h generally increased as ruminal pH increased.

Key Words: Fiber Digestion, Rumen, pH,

INTRODUCTION

In a previous trial (Chapter 3), rate and potential extent of NDF disappearance in situ varied with source of roughage, feed intake level, and ruminal pH. Numerous feeds are included in feedlot diets as sources of roughage or fiber. Besides alfalfa hay, prairie hay, and wheat straw that were examined in the previous study, corn silage and cottonseed hulls often are fed to feedlot cattle. Because corn silage includes both grain and stover, these fractions were separated prior to analysis. Wheat forage harvested as hay or silage also is used as a source of roughage or can be grazed as a source of energy and protein for growing ruminants. In contrast to typical roughage sources, wheat forage has a low NDF content and very high digestibility. When fed as the sole source of energy and protein, wheat forage produces very rapid and efficient gains by either grazing or pen-fed cattle (Horn et al., 1983). This study was designed to examine the impact of feed

intake level and low ruminal pH, as found with high concentrate diets, on in situ disappearance of cell wall constituents isolated from three additional sources of roughage and of grain isolated from corn silage.

MATERIALS AND METHODS

Animals: Three continental crossbreed heifers (BW = 434 kg) approximately 4 years of age were assigned randomly to columns of a 3X3 Latin square design. Heifers were ruminally fistulated and individually penned in OSU Nutrition and Physiology Center.

Diet: The diet, prepared by OSU Feedmill, consisted of 90% concentrate and 10% roughage (Table 1) based on ground corn grain. The concentrate content of the diet was high to maintain a low ruminal pH as would be found with cattle fed typical feedlot finishing diets. Heifers received different amounts of this diet in an attempt to achieve three different ruminal pH conditions. These intake levels (DM basis) were 0.9, 1.3% of BW daily, and an unlimited supply of feed. However, feed supply for animals of the latter intake group was equal to the peak daily intake during the 14-d adjustment period; this helped to avoid DM refusal during the experiment and to prevent metabolic disorders. Heifers were fed 3 times each day (800, 1600, and 2400) in an attempt to reduce fluctuations in ruminal pH. Heifers were adapted to their diets for 14-d in each period prior to initiation of in situ measurements. Animals had free access to the water throughout the experiment.

Procedure and Analysis: Two feed samples (corn silage and cotton seed hulls) were obtained from 3 major feedyards located on the panhandle of Oklahoma on September

1st, 1997. Wheat pasture samples were collected from the OSU wheat pasture facility at Stillwater. After drying, the corn silage was separated into grain and forage portions manually. Wheat pasture samples were dried for 72 hours and cut with scissors to an average length of 2.5 cm. Samples were place into 6.35 X 13.70 cm size dacron bags that then were heat-sealed. Samples containing these feeds were dried for 24 h at 55° in a forced-air oven. To examine loss of NDF independent of other feed components, these samples in bags were extracted with neutral detergent solution and the residual NDF was used for in situ measurement. The weight of feed placed in each bag prior to extraction was predicted based on literature values of NDF content of the feed and the amount of NDF desired per square cm of bag surface based on suggestions of Vanzant et al. (1997) and Carro et al. (1995). The approximate sample (NDF) DM:surface area ratio was 10 mg DM:cm^2. Detergent extraction was performed using an Ankom^{200} (Ankom, Turk Hill St. NY) extraction system. Duplicate dacron bags of each of the three feeds were placed into the rumen and retrieved after 96, 24, 12, or 6 h. Bags were inserted at different times and recovered simultaneously to standardize post-incubation washing procedures. In addition to retrieved samples, bags that had not been incubated in the rumen, designated as 0 h bags, were rinsed with the bags removed from the rumen. Mesh laundry bags were used to encase dacron bags to ensuring that ruminal location was similar for all bags and simplify bag recovery. Bags were rinsed en mass in buckets at 39^0 C under running tap water. Water in each bucket was agitated by hand and was changed repeatedly for 60 min. Then each bag was rinsed individually under a stream of tap water for around 1 minute. After drying, the samples were reanalyzed for NDF content and analyzed for ADF using the Ankom^{200}. Ruminal samples were taken every 4th hour, filtered through

four layer of cheesecloth, and analyzed for pH; these samples were frozen for ammonia N analysis at a later time.

The 3 by 3 Latin square, with three heifers, three periods, and three intake levels, served as a main plot with feeds being a sub-plot within the main plot. Weight loss of initial NDF, ADF, as calculated from mean ADF content of feed, and hemicellulose, calculated as the difference between NDF and ADF, at each incubation time was compared among feedstuffs. In addition, the natural log of the indigestible portion of each fiber fraction, calculated as the difference between loss as 96 h and loss at 0 h, was regressed against time to calculate rate of disappearance. Recovery of each fiber fraction at 0 h, 100%, was either excluded or included when calculating rate of disappearance assuming that digestion lag time did or did not precede the onset of fermentation, respectively. Lag time was calculated as the difference between the intercept at 0 h and ln(100) divided by disappearance rate. In addition, regressions of disappearance rates and extents at various times were regressed against ruminal pH and ruminal pH squared in an attempt to determine dependency of fermentation on ruminal pH.

RESULTS AND DISCUSSION

Composition of fiber fractions of feeds tested are presented in Table 2. Note that hemicellulose was calculated as NDF minus ADF with ADF being determined sequentially.

Ruminal pH tended to decrease linearly ($P < .09$) as feed intake was increased (Table 3). This would be expected from higher rates of acid production relative to the

input of buffers and liquid from saliva; the pH at the lowest intake level was much lower than expected although the pH was extremely low with the highest intake level.

Up to 24 h, disappearance of all fiber fractions tended to be lowest with the lowest feed intake level. However, by 96 h, disappearance was lowest with the highest feed intake level. This suggests that the response in ruminal fiber digestion to feed intake level differs with the time period at which disappearance is measured. These results are opposite the classical suggestion that higher intake levels and lower ruminal pH will extend the time lag prior to the onset of fermentation. However, at higher intake levels, one would expect ruminal residence time to be decreased. If so, the increased extent of disappearance of fiber fractions with higher feed intake levels at 12 and 24 h may not translate into any greater extent of ruminal digestion of these fiber components. No explanation for the higher extent of ruminal disappearance at 12 and 24 h with higher feed intakes is apparent. However, with higher intakes, ruminal volume and the strength of ruminal contractions should be greater, and this might increase nutrient flow to microbes attached to fiber in the dacron bags and also may have increased washout of undigested small particles. Further studies to examine effects of intake level on fibrolytic enzyme activity, on the population of species of fiber digesting microbes, and on disappearance of pure cellulose (cotton string) from dacron bags are needed to explain this unexpected observation.

Extent of disappearance of these fiber components after washing of dacron bags (0 h) or ruminal incubation for 6, 12, 24, and 96 h are presented in Table 3. Washout (0 h loss) for NDF and hemicellulose was greater with feeds that contained more hemicellulose. Potential extent of disappearance of all fiber components (24 h loss) was

greatest for wheat pasture, second for corn grain, third for corn stover, and lowest for cottonseed hulls. Results are presented graphically in Figures 9, 10, and 11. As shown in Figure 9, NDF disappearance from dacron bags in rumen was significantly higher for wheat pasture than other feeds after 24 h of incubation. Although wheat pasture NDF disappearance was the highest, NDF from grain portion of the corn silage tended to disappear even faster rate at earlier fermentation times. Disappearance of NDF from corn stover and cottonseed hulls was slow and not as extensive as from the other two feeds.

Hemicellulose disappearance over time (Figure 10) followed a pattern similar to that of NDF disappearance. Disappearance from grain and wheat pasture, both with over 60% of NDF being hemicellulose (Table 2), was greater than from corn stover and cottonseed hulls. Hemicellulose disappearance from cottonseed hulls was less ($P < 0.01$) than from all other feeds at all times measured.

NDF disappearance at 24 hour (Figure 9) was greatest with wheat pasture, with disappearance being considerably greater than for all other feeds. However NDF from grain portion of corn silage was digested more extensively at lower rumen pH (below 6.0), NDF from cottonseed hulls disappeared more than from the grain portion of corn silage. Less than 5% of NDF from the stover portion corn silage disappeared even at the highest ruminal pH. Hemicellulose disappearance paralleled NDF disappearance (Figure 10.). Wheat pasture hemicellulose was digested more extensively than hemicellulose from other feedstuffs. Hemicellulose disappearance from the stover portion of corn silage was the least digestible among these feeds. Since ruminal pH remained below 6.0 throughout the experiment, disappearance of ADF from those roughages was very limited.

NDF disappearance of each individual feed at various incubation times was regressed against ruminal pH (Figures 11, 12, 13, and 14). Extrapolated graphs (Figures 15 and 16) showed that at 24 h and 96 h, disappearance of NDF approached a plateau when pH was above 6.0.

IMPLICATIONS

Variability among forage sources in both content and degradation of NDF, ADF and hemicellulose as well as alteration in degradation rate by low ruminal pH must be considered when devising feeding management strategies. Substitution of common roughages by low quality forages in feedlot will decrease extent of ruminal NDF degradation and decrease the dietary amount needed to maintain normal ruminal health and function.

TABLE 1. Composition of diet fed to heifers in experiment II, DM

Ingredient	**Percentage**
Rolled Corn	70.00
Ground alfalfa hay	17.50
Cottonseed hulls	10.87
Cane molasses	1.50
Trace mineralized salt	0.12

TABLE 2. NDF, ADF and hemicellulose content of feeds studied in experiment II(% DM).

Feed	Neutral detergent fiber (NDF)	Acid detergent fiber (ADF)	Hemicellulose[1]	Hemicellulose (% of NDF)
Grain isolated from corn silage)	12.7	4.3	8.4	66.1
Corn stover (silage minus grain)	69.4	37.7	31.7	45.7
Cottonseed hulls	86.7	54.8	31.9	36.8
Wheat pasture	61.9	24.1	37.8	61.2

[1]Hemicellulose = NDF(%) – ADF(%)

Table 3. Impact of intake level and feed source on NDF, ADF and hemicellulose disappearance.

	Intake level, %BW/d			Linear	Feed				
	0.9	1.3	2.0	Prob P<	Cottonseed Hulls	Corn grain	Corn stover	Wheat pasture	SEm
Ruminal pH	5.34	5.54	4.86	0.09					
NDF disappearance									
0 h	10.1 ^{b}	11.4 ^{ab}	11.8 ^{a}	0.55	4.9 ^{c}	13.4 ^{ab}	14.2 ^{a}	11.7 ^{b}	0.675
6 h	13.4 ^{b}	19.0 ^{ab}	17.3 ^{a}	0.04	5.8 ^{c}	17.9 ^{b}	14.6 ^{b}	28.0 ^{a}	1.342
12 h	16.0 ^{b}	21.3 ^{ab}	19.7 ^{a}	0.07	6.2 ^{d}	21.7 ^{b}	15.5 ^{c}	32.4 ^{a}	1.263
24 h	20.4 ^{b}	27.9 ^{a}	21.4 ^{a}	0.03	9.6 ^{d}	25.5 ^{b}	16.7 ^{c}	41.3 ^{a}	1.230
96 h	44.8 ^{a}	47.8 ^{a}	33.9 ^{b}	0.49	18.5 ^{d}	55.5 ^{b}	25.2 ^{c}	69.4 ^{a}	1.808
ADF disappearance									
0 h	-0.9	0.0	0.9	0.90	0.0	0.0	0.0	0.0	1.874
6 h	0.6	-0.6	1.6	0.73	-1.3	-0.8	2.3	2.1	1.761
12 h	-1.4 ^{b}	3.9 ^{a}	2.1 ^{a}	0.06	0.5	1.2	0.4	4.0	1.351
24 h	2.5 ^{b}	8.0 ^{a}	5.1 ^{a}	0.52	3.0 ^{c}	6.4 ^{ab}	1.0 ^{bc}	10.5 ^{a}	1.896
96 h	31.2 ^{a}	34.8 ^{a}	17.1 ^{b}	0.48	12.3 ^{c}	35.0 ^{b}	12.7 ^{c}	50.8 ^{a}	3.565
Hemicellulose disappearance									
0 h	14.7	15.3	16.1	0.84	8.7 ^{c}	16.1 ^{b}	21.9 ^{a}	14.7 ^{b}	1.155
6 h	17.9 ^{b}	25.4 ^{ab}	22.8 ^{a}	0.06	11.2 ^{c}	21.6 ^{b}	20.8 ^{b}	34.4 ^{a}	1.708
12 h	21.8 ^{b}	26.7 ^{ab}	25.7 ^{a}	0.11	10.7 ^{c}	25.7 ^{b}	23.5 ^{b}	39.1 ^{a}	1.586
24 h	26.9 ^{b}	34.2 ^{a}	26.4 ^{b}	0.01	14.3 ^{c}	29.1 ^{b}	25.0 ^{b}	48.2 ^{a}	1.704
96 h	48.4 ^{a}	51.4 ^{a}	39.1 ^{b}	0.47	23.8 ^{d}	58.2 ^{b}	30.8 ^{c}	72.3 ^{a}	1.685

$^{a, b, c, d}$ Means with different superscripts differ ($P < 0.05$).

Table 4. NDF, ADF, and hemicellulose disappearance in response to interactions of intake with feed source.

Intake, % of BW/d	Feed: Cottonseed hulls	Corn grain	Corn stover	Wheat pasture
	NDF disappearance at 6 h			
0.9	5.4	16.4	13.3	18.3
1.3	5.8	18.3	14.9	36.9
2.0	6.2	18.9	15.5	28.9
	NDF disappearance at 12 h			
0.9	6	17.7	16.4	23.9
1.3	6.6	26.3	15.3	37
2.0	6.2	21.2	14.9	36.4
	ADF disappearance at 12 h			
0.9	0	-10.3	3	1.8
1.3	1.7	8.1	0.4	5.3
2.0	-0.1	5.9	-2.1	4.8
	Hemicellulose disappearance at 6 h			
0.9	10.6	19.3	18.5	23.3
1.3	11.2	23.3	21.9	45.1
2.0	11.7	22.3	22.1	35
	Hemicellulose disappearance at 12 h			
0.9	11	23.7	23	29.5
1.3	10	29.3	23.2	44.3
2.0	11.1	24	24.1	43.6

Table 5. Impact of intake level on rate of NDF disappearance in situ.

	Intake level, %BW/d			Linear effect
	0.9	1.3	2.0	P<
	NDF disappearance, %/h			
Cottonseed hulls	1.8	2.7	0.5	0.23
Corn grain	1.4	1.3	0.9	0.29
Wheat pasture	2.5	2	4.3	0.41
	Hemicellulose disappearance, %/h			
Cottonseed hulls	4.6	2.8	0.2	-
Corn grain	1.2	1.3	0.4	0.06
Wheat pasture	3.0	1.9	6.2	0.32
	NDF disappearance, with lag, %/h			
Corn grain	1.4	1.3	0.9	0.29
Wheat pasture	2.5	3.6	5.2	0.27
	Hemicellulose disappearance with lag, %/h			
Corn grain	1.9	1.5	0.6	0.32
Wheat pasture	3.0	4.3	7.0	0.2

Table 6. Impact of pH on NDF, ADF and hemicellulose disappearance

	Linear P<	Feed effect
NDF disappearance	0.38	Wheat pasture; L .05; Q .06
ADF disappearance	0.66	
Hemicellulose disappearance	0.54	Wheat pasture; L .03; Q .03
NDF disappearance with lag	0.44	
ADF disappearance with lag	0.54	
Hemicellulose disappearance with lag	0.77	

Table 7. Projected NDF disappearance based on regression

PH		Cottonseed hulls	Corn grain	Wheat pasture
	Linear effect	1.785	0.56629	0.48485
	Quadratic effect	-0.1676	-0.05273	-0.0437
5		0.003	0.006	0.018
5.2		0.018	0.012	0.026
5.4		0.020	0.014	0.030
5.6		0.008	0.011	0.030
5.8		-0.017	0.004	0.027
6		-0.056	-0.007	0.021

CHAPTER V

In vitro fiber digestibility responses to ruminal pH and nitrogen adjustments.

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ABSTRACT

Fiber digestion depresses in rumen as ruminal pH decrease. An in vitro experiment was conducted to examine the fiber digestion kinetics with pH and NH_3-N adjustments. Alfalfa hay and corn silage stover samples were incubated either intact or NDF extracted prior to incubation for 12 and 24 hour in a) control, b) nitrogen added, c) pH adjusted and d) nitrogen added and pH adjusted rumen fluids. Increase and decrease in pH influenced 24 h NDF ($P<0.10$) and ADF ($P<0.05$) digestions significantly. NDF extracted forages prior to incubation digested significantly more than intact incubated forages. The decrease in NDF disappearance with decreased pH of forage fed rumen fluid was significantly higher than the increase in NDF disappearance with increased pH of concentrate fed rumen fluid. Rumen ammonia N addition did not improve NDF, ADF or hemicellulose disappearance except with intact incubated forages. Ammonia N effect on individual feeds either intact or NDF extracted was inconsistent. Ruminal pH has a significant role on fiber digestion; however, ammonia effect was not significant.

Key words: NDF, disappearance, in vitro

INTRODUCTION

To achieve maximum daily gain with optimum feed efficiency, finishing cattle in feedlots are fed diets that contain only 5 to 15% roughage. The proper level of forage or roughage with the correct physical characteristics will help to avoid acidosis and to maintain high and steady feed intakes and ruminal function. Yet, roughages contribute little net energy to the diet because fiber digestibility generally is low, particularly with high concentrate diets. Furthermore, rapidly fermented carbohydrates such as starch decrease ruminal pH below 5.8 in the rumen of feedlot cattle; low pH inhibits cellulose digesting microorganisms more than amylolytic bacteria. Although low pH generally has been implicated as the cause of low rates of fiber digestion with concentrate-rich diets, the mechanisms by which rapidly degraded carbohydrates depress fiber fermentation in rumen are not fully understood (Piwonka and Firkins, 1996, Huhtanen and Khalili, 1991).

Hoover (1986) listed a number of factors that might reduce fiber digestion in rumen. He included preference of ruminal microbes for readily rather than slowly fermented (cell wall) carbohydrates as a major contributor for reduced fiber digestion. Secondly, low pH was mentioned. Rapid and extensive fermentation of starch results in high concentrations of VFA in the rumen; pH is depressed by VFA and low pH, in turn, depresses growth and replication and thereby the population of cellulolytic bacteria in the rumen. A third factor relates to bacterial attachment to feed particles. For fiber digestion, bacterial must attach to plant cell walls. Complex chemical and physical structures involved in such attachment may be affected by a decreased ruminal pH (Hoover, 1986). As an example, bicarbonate has been shown to enhance attachment, but bicarbonate concentrations in fluids will drop when pH drops. Low ruminal pH may

have additional adverse effects on animal performance that become evident through reduced appetite, depressed ruminal motility, and reduced microbial efficiencies and yields (Allen, 1997).

The theory that depressed pH alone can explain the reduced rate of fiber digestion in the rumen has been examined primarily with in vitro studies. Addition of organic and inorganic acids to decrease in vitro pH was studied by Grant and Weidner (1992), Grant (1994), and Grant and Mertens (1992). Although decreasing pH alone depressed rate of fiber loss, the reverse response, increasing pH of ruminal fluid from cattle fed concentrate, has not been extensively tested. Further, addition of starch while holding pH constant depressed rate of fiber loss; this observation has resulted in a theory about a direct "carbohydrate effect" that depresses fiber digestion even though pH is not reduced (Mould and Orskov, 1984). Miron et al. (1990 and 1997) replicated this experiment by holding pH at or above 6.5 while adding the starch to the medium. In contrast to expected pH effects, Erdman (1988) suggested that simply buffering the rumen to maintain pH above 6.4 failed to improve extent of fiber digestion by lactating cows.

Colonization, attachment, and activity of bacteria associated with cell wall digestion requires bacterial access to nutrients essential for growth, i.e., NH_3-N and branched-chain fatty acids (McCarthy et al., 1989). Consequently, nutrient supply must be maintained in the ruminal and microscopic (intra-fiber) locations if maximum fiber digestion rate is to be achieved. In the case of "high quality forages," these nutrients, particularly protein, and buffers often are associated with fiber particles whereas with "low quality forages," supply of such nutrients from cell contents usually is low. A shortage of NH_3. -N in the rumen, and particularly in the ruminal raft, will depress

activity of cellulolytic microbes and depress fiber digestion. With low quality forages, any depression in fiber digestion rate usually reduces forage intake.

Alfalfa hay and corn silage are the most common forages fed to feedlot and dairy cattle in the U.S. Since alfalfa hay has higher cation exchange and buffering capacity than corn silage, the depression in ruminal pH typically is less with alfalfa than with corn silage as a roughage (Miron et al., 1996) though several factors may be involved. First, about half the dry matter in corn silage is corn grain. Fermentation acids produced from this starch also adds to the acid load in the rumen, further depressing pH. Most researchers consider that corn silage is only 50% roughage, with the stover fraction being the only portion that contributes "roughage value" to a diet. Secondly, corn silage is much lower in protein than most alfalfa products. Ammonia liberated during fermentation at ruminal pH will act as a base and prevent pH reduction. Finally, organic acids and other organic compounds in alfalfa products give it greater "buffering capacity" that also will help maintain a higher ruminal pH.

The objective of this study was to determine how adjustment in pH and addition of NH_3-N would alter in vitro disappearance of fiber fractions from corn stover and alfalfa hay. In addition, in vitro disappearance of NDF was measured either with or without previous extraction of feeds with neutral detergent solution to test whether presence of cell contents would alter extent of NDF loss.

MATERIALS AND METHODS

Rumen fluid sources: One ruminally cannulated Continental crossbreed heifer (443.5 kg) was used as a source of ruminal fluid for all in vitro runs. For the first two

samplings, the heifer was fed medium quality prairie hay ad libitum with fresh feed added twice daily (0800 and 1600) for 14 d prior to obtaining ruminal fluid with a high pH. The second rumen sampling was two days after the first sampling. Third set of rumen fluids was collected two days after second sampling. Then, the heifer was adapted to a 90% concentrate diet based on rolled corn grain for 14 d to induce a low ruminal pH and again the heifer was ruminally sampled. Fermentation inocula (solid plus liquid) for each in vitro run were collected at 0800 immediately prior to morning feeding through its ruminal cannula. Ruminal fluids were strained through 4 layers of cheesecloth, pH was measured, and the fluid was placed in an insulated container for transport to the laboratory.

Laboratory procedures: The pH of ruminal fluids was measured again, and the fluid was strained through 8 layers of cheesecloth placed in 1 L incubators jars from an Ankom® fermentation system (Ankom, Inc., Turk Hill St., NY). To obtain different initial pH values and different ammonia concentrations, acid or base and water or an ammonia solution was added during an adjustment period of approximately 2 h; jars were held in a water bath at 39 C and purged continuously with CO_2 during this time period. With ruminal fluid with a high initial pH, the pH was either a) unadjusted or b) acidified to reach a pH below 5.7. In addition two levels of NH_3-N were obtained, being either a) unadjusted or b) adjusted by addition of 15-mg ammonia-N/dl to obtain a non-replicated 2 X 2 factorial design. For acidification, a 5% HCl solution was dispensed slowly with mixing into two of the incubation flasks containing 1, 000-ml rumen fluid till pH dropped below 5.7. The pH was measured every 15 min. A similar procedure was followed with addition of 3 ml of 14.3% ammonium chloride solution to add 15 mg NH_3 N/dL. After

the desired pH and ammonia N levels were obtained, the sealed incubation jars were placed in the rotating incubation system at constant temperature (39 ^{0}C).

To measure dry matter and NDF losses during incubation, alfalfa and corn stover separated from corn silage were ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Approximately 0.5 g samples were put in to Ankom in vitro fermentation bags and dried at 55 ^{0}C for 48 hours. Half of the bags were extracted with neutral detergent solution to yield NDF residues within the bags. These bags were either not incubated or incubated for 12 or 24 h within the incubation jars. Non-incubated bags and bags retrieved from the incubation jars at various times were washed under running warm tap water in a bucket until the color of water was clear. Then, the bags were washed individually under running warm water. Bags then were extracted sequentially with neutral detergent and acid detergent solution to estimate residual NDF and ADF of each bag.

Statistical Analysis: The GLM procedure of SAS was used to analyze these data. Main effects [initial pH (high or low), ammonia (added or not), feed source (alfalfa vs. stover), and substrate (intact feed vs. NDF residue of the feed)] and all possible interactions were included in the model with replicate runs at different times providing the error term.

RESULTS AND DISCUSSION

Effects of adjusting the pH and extraction procedures main effects were significant ($P<0.10$). Interactions between initial pH and pH adjustment, extraction method by N addition, and the three-way interaction of feed type by extraction method by adjusted pH also were significant ($P<0.10$). Only these main effects and interactions that

were detected as being significant (P <. 10) will be discussed. This discussion will emphasize disappearance at 24 h.

Compared with disappearance at pH of 6.8, disappearance of all fiber fractions at pH 5.5 tended to be reduced both at 12 and 24 h (Table 2). At 24 h, NDF and ADF disappearance were significantly reduced (P < .05) with less difference in hemicellulose disappearance. Disappearance of ADF at 24 h was over three times as great at 6.8 than at 5.5. However, an interaction between initial pH and pH adjustment (i.e., lowering pH of ruminal fluid from the heifer fed roughage from 6.8 to 5.5 vs increasing pH of ruminal fluid from the heifer fed concentrate from 5.5 to 6.8) was detected (Tables 3, 4, and 5). Increasing pH of ruminal fluid from the heifer fed roughage decreased NDF disappearance at 24 h by about 60% (Table 3), of hemicellulose by 40% (Table 4), and completely inhibited ADF disappearance (Table 5). In contrast, increasing pH of ruminal fluid from the heifer fed concentrate increased ADF disappearance by 40% (Table 4) but tended to decrease (P < .10) hemicellulose disappearance (Table 5) leaving NDF disappearance not significantly altered (Table 3). This would suggest that ruminal microbes fermenting ADF are sensitive to low pH whether from the rumen of cattle fed roughage or concentrate. This would suggest that ruminal microbes fermenting hemicellulose remain active whether the pH is low or high, but any change (increase or decrease) will depress their activity so that hemicellulose disappearance is decreased.

Disappearance of all fiber fractions was greater from the NDF extract of roughage than from the intact roughage (P < .05 for NDF and ADF; P < .10 for hemicellulose). Lower fiber digestion with intact feed than isolated NDF could be a result of preferential utilization of other substrates (cell contents) by fiber digesting microbes or presence of

physical or chemical barriers to digestion that were altered or removed by neutral detergent solution. However, an interaction between substrate and ammonia supplementation also was detected (Tables 6, 7, and 8). Addition of ammonia increased NDF and ADF digestion with intact roughage but not with NDF extract from these roughage sources (Tables 6 and 7). In fact, ammonia addition tended to decrease ADF disappearance of NDF extract of these feeds (Table 7). As ammonia is required for active cellulose digesting microbes, these results might be interpreted to indicate that presence of cell contents may complex with ammonia or fermentation of cell contents may deplete ammonia needed by cellulose digesting microbes.

A three-way interaction between ruminal fluid source, feed source, and feed extraction on 24-h disappearance of NDF and ADF was detected. This was due primarily to low disappearance of NDF and ADF from intact alfalfa hay with rumen fluid from the heifer fed roughage. This might be interpreted to suggest that certain compounds present in cell contents of alfalfa may inhibit ADF digestion.

Finally, an interaction between ammonia supplementation, feed source, and feed extraction on ADF disappearance at 24 h was noted. In this case, supplementing intact alfalfa hay with ammonia markedly depressed ADF disappearance. No explanation for this interaction is apparent.

IMPLICATIONS

Decreasing pH of ruminal fluid from a heifer fed roughage, even in the absence of rapidly fermented carbohydrates, depressed ruminal NDF, ADF and hemicellulose disappearance. However, the converse, increasing pH of ruminal fluid from the heifer

when fed concentrate, did not increase NDF and ADF disappearance. Nitrogen addition may increase depression in NDF digestion of intact feeds, possibly through maintaining a supply of ammonia used by microbes fermenting cell contents of high quality, low protein forages.

Table 1. Percentage of ingredients in dry matter of diets fed to heifer during roughage and concentrate feeding periods.

Ingredient	Roughage diet	Concentrate diet
Prairie hay, chopped	100	10
Corn grain, rolled	0	90

Table 2. Impact of in vitro pH on disappearance of various fiber components from stover and alfalfa hay.

	Initial pH		Substrate		
	5.5	6.8	Intact roughage	NDF extracted	SEm
NDF disappearance, %					
12 h	2.19	2.99	1.99	3.02	1.12
24 h	5.07 [d]	8.13 [c]	4.82 [b]	8.37 [a]	1.40
ADF disappearance, %					
12 h	-0.33	0.77	-0.21	0.66	1.19
24 h	1.69 [b]	5.90 [a]	2.32 [d]	5.27 [c]	1.53
Hemicellulose disappearance, %					
12 h	2.80	4.00	6.05 [d]	9.77 [c]	1.90
24 h	12.88	13.92	10.58 [d]	16.22 [c]	2.38

[a,b] Means in a row within a group with different superscripts differ ($P < .05$).
[c,d] Means in a row within a group with different superscripts differ ($P < .10$).

Table 3. Interaction of ruminal fluid source with pH on NDF disappearance at 24 h.

	Incubation pH		Average
Diet	5.5	6.8	
Roughage	2.54 [b]	9.06 [a]	5.80
Concentrate	7.60 [a]	7.19 [a]	7.40
Average	5.07 [c]	8.13 [d]	

[a,b] Means in a row with different superscripts differ ($P < .05$).
[c,d] Means in a row with different superscripts differ ($P < .10$).

Table 4. Interaction of ruminal fluid source with pH on ADF disappearance at 24 h.

	Incubation pH		Average
Diet	5.5	6.8	
Roughage	-0.49 [b]	5.68 [a]	2.60
Concentrate	3.87 [ab]	6.12 [a]	5.00
Average	1.69 [b]	5.9 [a]	

[a,b] Means in a row with different superscripts differ ($P < .05$).
[c,d] Means in a row with different superscripts differ ($P < .10$).

Table 5. Interaction of ruminal fluid source with pH on hemicellulose disappearance at 24 h.

	Incubation pH		Average
Diet	5.5	6.8	
Roughage	10.05 [b]	17.81 [a]	13.93
Concentrate	15.70 [a]	10.02 [b]	12.86
Average	12.87	13.91	

[a,b] Means with different superscripts differ ($P < .05$).

Table 6. Interaction of feed extraction with ammonia supplementation on NDF disappearance at 24 h.

	Ammonia addition		Average
Substrate	None	+15 mg/dl	
Intact roughage	3.11 [b]	6.54 [a]	4.82 [d]
NDF extract	9.80 [a]	6.95 [a]	8.37 [c]
Average	6.46	6.75	

[a,b] Means with different superscripts differ ($P < .05$).
[c,d] Means in a column with different superscripts differ ($P < .05$).

Table 7. Interaction of feed extraction with ammonia supplementation on ADF disappearance at 24 h.

	Ammonia addition		Average
Substrate	None	+15 mg/dl	
Intact roughage	0.27 [b]	4.38 [a]	2.32
NDF extract	7.20 [a]	3.34 [a]	5.27
Average	3.74	3.86	

[a,b] Means with different superscripts differ ($P < .05$).

Table 8. Interaction of feed extraction with ammonia supplementation on hemicellulose disappearance at 24 h.

	Ammonia addition		Average
Substrate	None	+15 mg/dl	
Intact roughage	9.92	11.23	10.58 [b]
NDF extract	15.53	16.91	16.22 [a]
Average	12.73	14.07	

[a,b] Means in a column with different superscripts differ ($P < .05$).

Table 9. Interaction of ruminal fluid source by feed source by feed extraction on NDF disappearance at 24 h.

Substrate	Roughage diet	Concentrate diet	Average
Intact corn stover	4.34 [ab]	5.92 [ab]	5.13
Extracted corn stover	6.67 [ab]	8.95 [a]	7.81
Intact alfalfa hay	2.76 [b]	6.27 [ab]	4.52
Extracted alfalfa hay	9.43 [a]	8.45 [ab]	8.94
Average	5.80	7.40	

[a,b] Means with different superscripts differ ($P < .05$).

Table 10. Interaction of ruminal fluid source by feed source by feed extraction on ADF disappearance at 24 h.

Substrate	Roughage diet	Concentrate diet	Average
Intact corn stover	3.67 [ab]	2.04 [ab]	2.86
Extracted corn stover	3.19 [ab]	7.46 [a]	5.33
Intact alfalfa hay	-2.10 [b]	5.68 [a]	1.79
Extracted alfalfa hay	5.65 [a]	4.79 [a]	5.22
Average	2.60	4.99	

[a,b] Means with different superscripts differ ($P < .05$).

Table 11. Interaction of ammonia supplementation by feed source by feed extraction on ADF disappearance at 24 h.

	Ammonia addition		Average
Substrate	< 5 mg/dl	> 15 mg/dl	
Intact corn stover	2.79 [ab]	2.92 [ab]	2.86
Extracted corn stover	4.90 [a]	5.75 [a]	5.33
Intact alfalfa hay	5.97 [a]	-2.40 [b]	1.79
Extracted alfalfa hay	1.80 [ab]	8.65 [a]	5.23
Average	3.87	3.73	

[a,b] Means with different superscripts differ ($P < .05$).

CHAPTER VI

CONCLUSIONS AND IMPLICATIONS

Conclusions:

Results presented in this thesis confirm that ruminal NDF and ADF disappearance in situ are depressed when ruminal pH is low. The published literature suggests that supplementation with protein to supply ammonia nitrogen may enhance fiber digestion. Though not detected with NDF residues from alfalfa or intact corn stover, extent of disappearance of NDF, ADF, and hemicellulose from intact alfalfa hay was enhanced by ammonia addition to the incubation medium.

Responses in fiber digestion to a low ruminal pH (below 6.0) differed with forage source and with the fraction of fiber (NDF, ADF, hemicellulose). Depression of disappearance at low pH was greater for ADF and not detectable for hemicellulose, and greater for alfalfa hay than for the other roughage sources tested. Furthermore, response to adjustment in pH differed with source (and initial pH) of ruminal fluid. Decreasing pH of ruminal fluid of roughage-fed animals markedly decreased ADF disappearance, but increasing pH of ruminal fluid of concentrate from concentrate-fed animals failed to increase ADF disappearance. Indeed, increasing pH decreased hemicellulose disappearance. Consequently, factors in addition to ruminal pH must be considered when predicting responses to addition of buffers or bases to a diet.

In the first experiment, rate and extent of in situ disappearance of fiber components of isolated NDF differed among various fiber fractions as well as various roughage sources. Hemicellulose disappearance was closely correlated with ADF disappearance within

specific roughages. When ruminal pH fell below 6.0, in situ fiber disappearance was much slower than when pH was above 6.0. Regression analysis indicated that decreasing pH even when pH was below 6.0 reduced fiber disappearance; this contradicts the concept that fiber digestion is low but constant whenever pH drops below 6.0. In situ disappearance of NDF from wheat straw was greater than from alfalfa hay and prairie hay. Hemicellulose disappearance from wheat straw and alfalfa hay were similar, both being greater than from prairie hay. For ADF disappearance, both prairie hay and wheat straw were superior to alfalfa hay. Consequently, the touted advantage of alfalfa hay over prairie hay and wheat straw as a source of roughage in feedlot diets must be related to the concentration of fiber in the feed, not to the availability of fiber present in the feed.

In the second experiment, in situ disappearance of various fiber fractions and additional feeds were examined across a range in ruminal pH from 5.0 to 6.0. Both NDF and hemicellulose disappearance were much greater for wheat pasture forage and for grain isolated from corn silage than for cottonseed hulls and corn stover. As pH was increased from 5.0 to 6.0, NDF and hemicellulose disappearance were doubled for all feeds except corn stover.

In the third experiment, extent of disappearance of various fiber components after incubation in vitro for 24 h at pH 5.5 and 6.8 was examined. Decreasing pH of ruminal fluid from the heifer fed roughage from 6.8 to 5.5 depressed disappearance of NDF, ADF and hemicellulose. In contrast, elevating of pH of ruminal fluid from the heifer fed concentrate from 5.5 to 6.8 failed to increase disappearance of any of these fiber fractions. Addition of ammonia nitrogen to the in vitro medium failed to increase fiber

disappearance except for intact alfalfa hay. In this study, alfalfa hay and corn stover responded similarly to changes of in vitro pH.

Although rate of ruminal fiber digestion is depressed by low pH with high concentrate diets, rate of passage of particles from the rumen generally is lower with concentrate than with roughage diet. This can compensate slightly for the reduced rate of fiber digestion. Therefore it is improper to predict extent of ruminal fiber digestion from these in situ and in vitro experiments in which only rate of disappearance of fiber fractions has been studied. Though disappearance results underestimate ruminal digestion due to diurnal fluctuations and occasionally higher ruminal pH, experiments measuring ruminal outflow with cattle fed ruminal buffers or having limited feed intake should give more conclusive information about the impact of ruminal pH on extent of ruminal disappearance of specific fiber fractions from various sources of roughages. As both ruminal retention and rate of disappearance of various fiber components may depend on particle size of the fiber source, examination of various fiber sources with different fiber lengths and particle sizes should prove revealing.

Implications:

Concepts developed in this thesis should prove useful for re-evaluating feeding strategies. Knowledge about the interaction between low ruminal pH and kinetics of fiber digestion for specific fiber sources may prove useful for matching forage and starch sources in feedlots and might help to eliminate some of the negative effects of starch on fiber digestion. Perhaps, some index of readily fermented carbohydrate in the diet, both from grain and from cell contents of roughages, can be developed to predict ruminal pH;

this could be combined with passage rate that can be predicted from level of feed intake. From these estimates, the relative extents of ruminal disappearance as well as ruminal presence of ADF and hemicellulose could be predicted. Such an approach should be superior to predicting the nutritive value of a roughage from chemical data, e.g., effective NDF, alone. Nevertheless, rather than grouping all components of fiber as NDF and using that base for comparing various sources of roughage, prediction of roughage value certainly should be improved by 1) considering ADF and hemicellulose contents of fiber separately and 2) incorporating some index of particle size, as used to predict effective NDF. Such information, though less informative than a totally integrated passage rate-digestion rate system for individual forages, should be of practical use in formulating diets for feedlot cattle. Processing methods that retard release of starch from grains in rumen and infrequent meals may increase extent of ruminal digestion of ADF through maintaining a higher pH longer during the day. However, when fiber sources, e.g., alfalfa hay, whose fiber digestion rate is markedly dependent on pH are fed, fluctuating pH may increase gas production and the incidence of bloat. Whether ionophores might reduce the impact of fluctuations in ruminal pH on rate of fiber digestion is not known. Many additional factors related to roughage particle size, bulk density, and chemical composition forages used in feedlot diets deserve attention.

The relative "roughage value" of a forage remains elusive. For inclusion in feedlot diets, roughage sources that have "low quality" due to being slowly fermented and poorly digested, though providing less energy for the ruminant, may be preferable to rapidly and extensively digested forage because they provide a stable and consistent

amount of ruminal bulk and, though stimulating mixing and chewing, will enhance rumination and saliva production.

Today, the feedlot industry utilizes primarily highly digestible and in some cases, high protein forages when formulating diets even though low quality forages may be available. At earlier stages of finishing, high quality long particle forages will supply needed nutrients (protein, energy) and generally will lead to high and consistent feed intakes. However, once steers are adapted to the high concentrate finishing rations, fiber digestion is markedly depressed and the primary function of forage is to dilute the diet to avoid excessive energy intakes and to enhance rumination to help maintain ruminal health and function. For this purpose, low quality forage is equal or superior to high quality forage. Data from this thesis should help to increase interest in substituting alternative, low quality forages or roughages for expensive high quality forages used widely today in commercial feedlot diets.

Future research:

Including buffers, bases, or feeds that yield ammonia (due to its basic properties) to increase pH above 6.0 should enhance activity of the cellulolytic microflora in the rumen when pH is low. This area as well as the impact of diurnal fluctuations in ruminal pH needs further study. Differential responses of various fiber sources to elevated ruminal pH deserves further study. Characteristics of additional fiber components, e.g., organic acids, that may inhibit lactic acid production and acidosis also needs attention as presence in common forage sources may help prevent acidosis. Study of alternative non-

structural polysaccharides such as pectins, galactans and beta-glucans and their alteration through plant selection may help to reduce negative associative effects of low pH on rate and extent of ruminal fiber degradation.

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APPENDIX

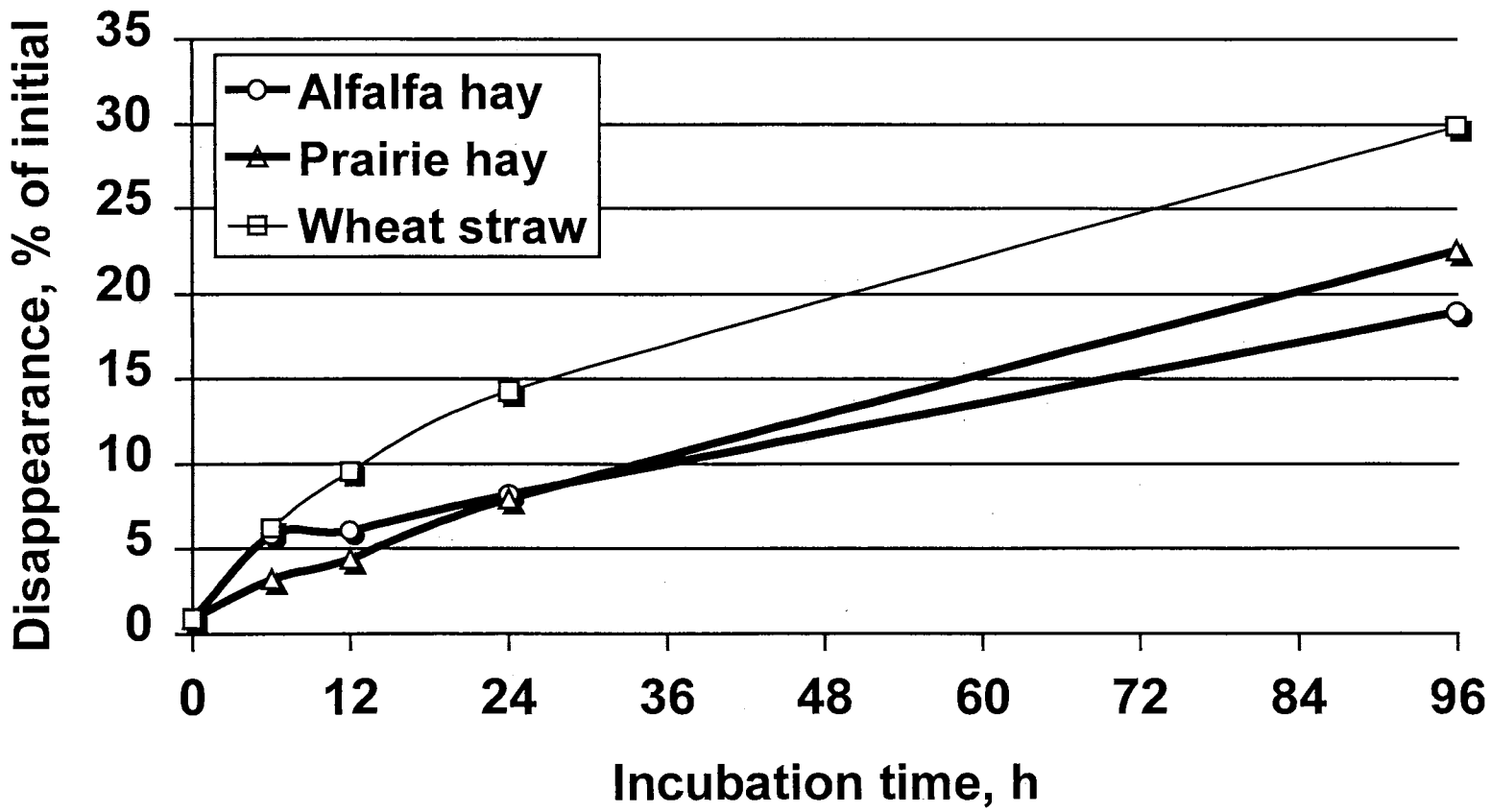

Figure 3. 1. Neutral detergent fiber disappearance from alfalfa hay, prairie hay and wheat straw at various incubation times.

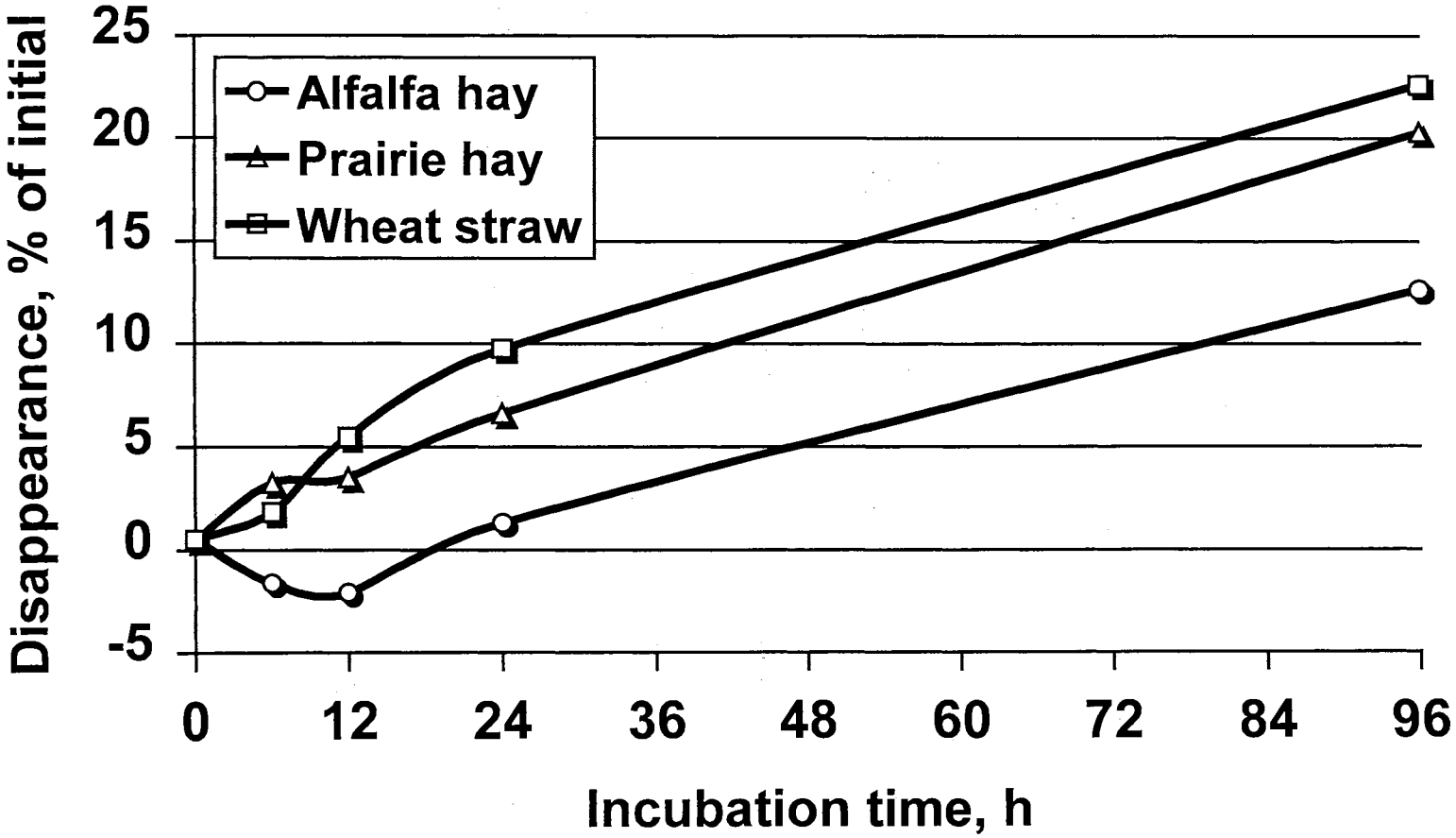

Figure 3. 2. Acid detergent fiber disappearance from alfalfa hay, prairie hay and wheat straw at various incubation times.

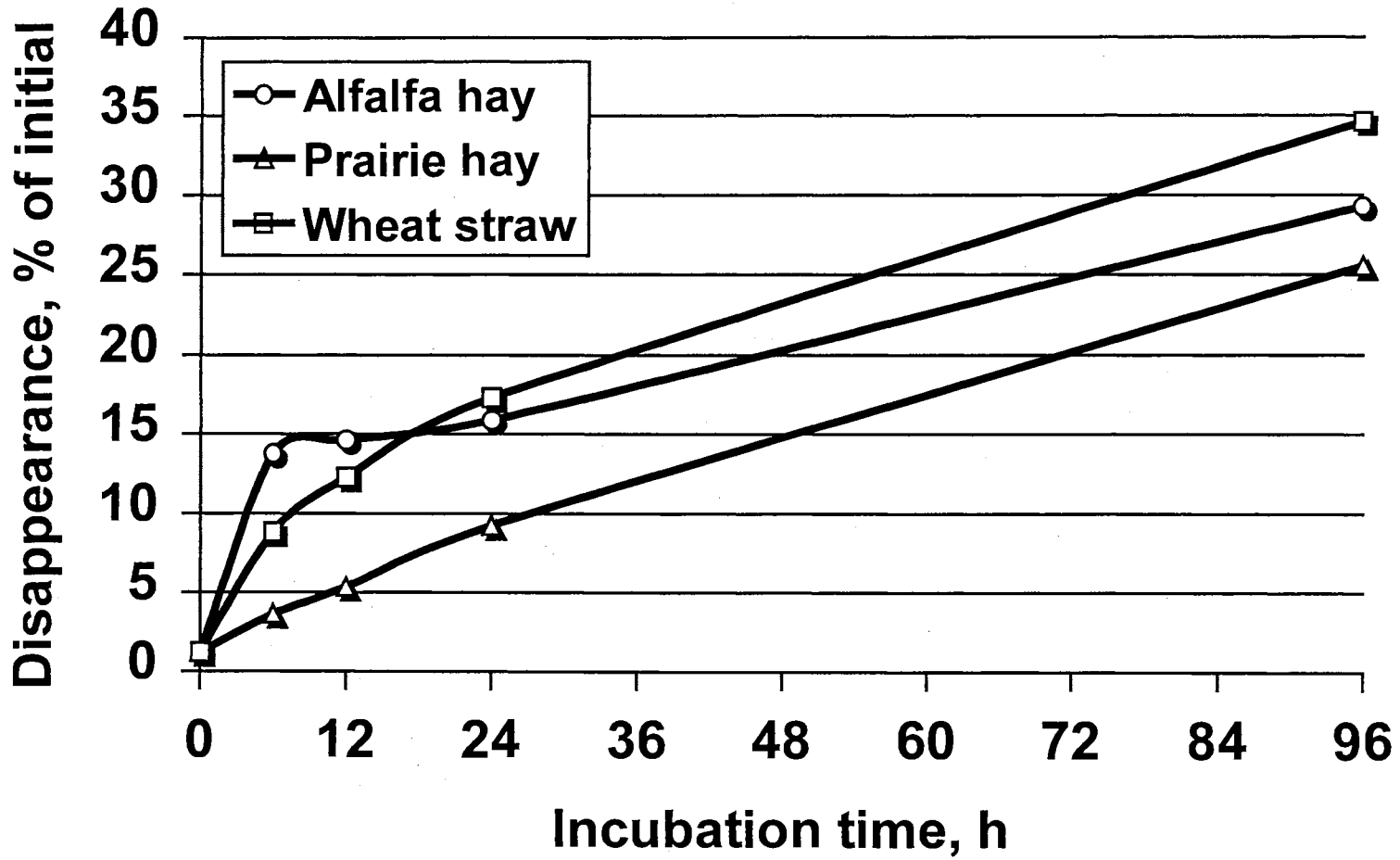

Figure 3. 3. Hemicellulose disappearance from alfalfa hay, prairie hay and wheat straw at various incubation times.

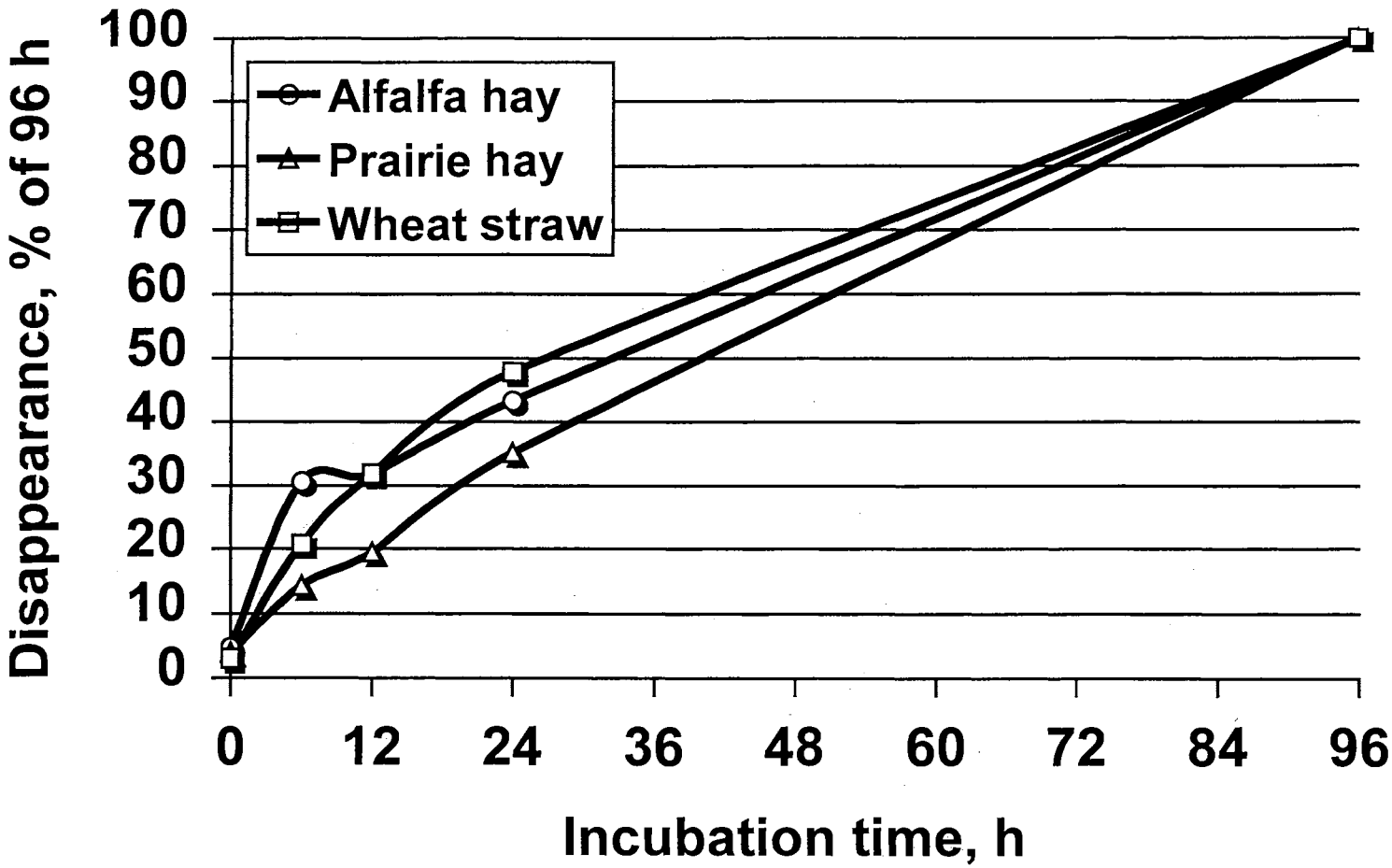

Figure 3. 4. Neutral detergent fiber disappearance from alfalfa hay, prairie hay and wheat straw as a percentage of disappearance after 96 hours.

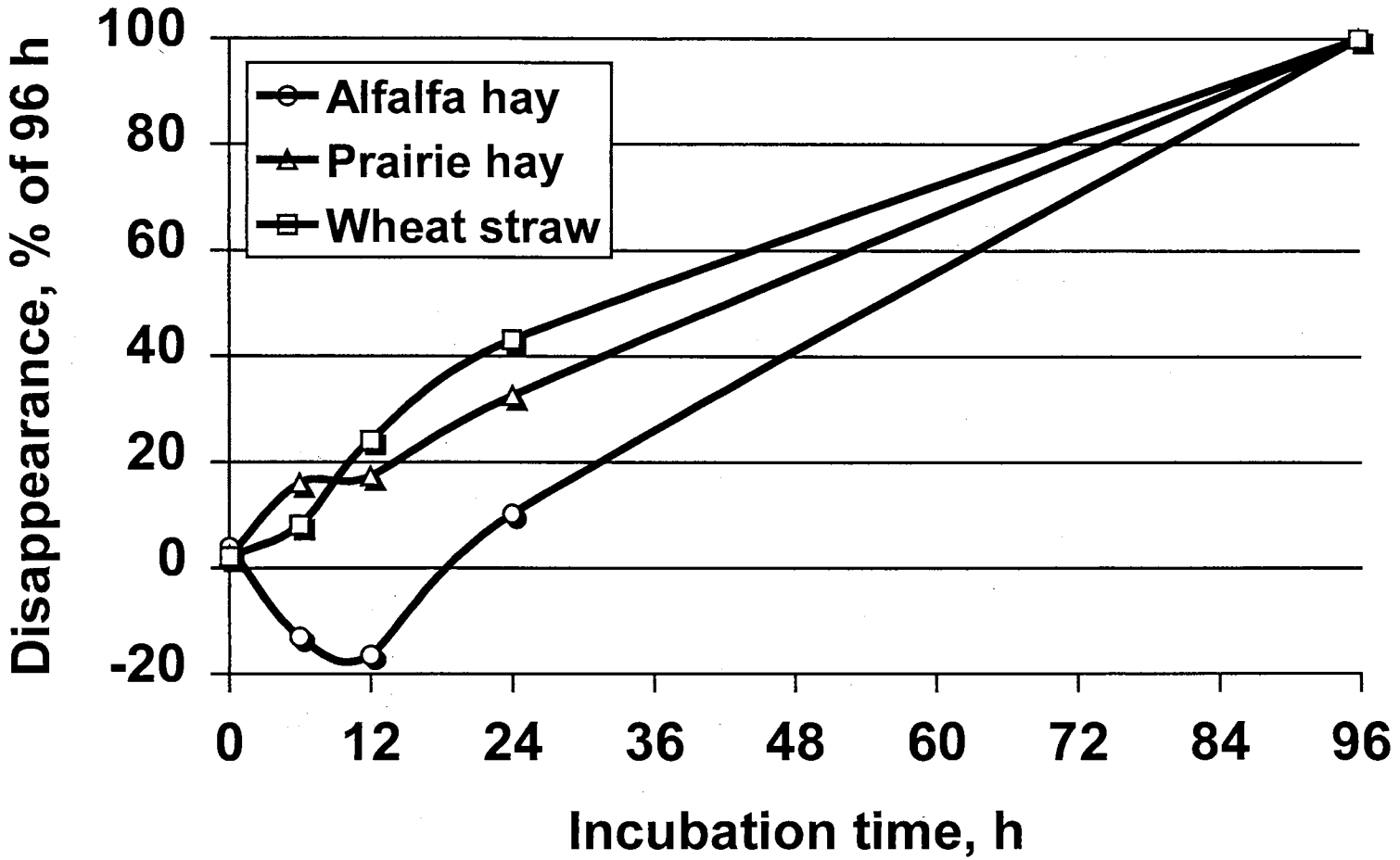

Figure 3. 5. Acid detergent fiber disappearance from alfalfa hay, prairie hay and wheat straw as a percentage of disappearance after 96 hours.

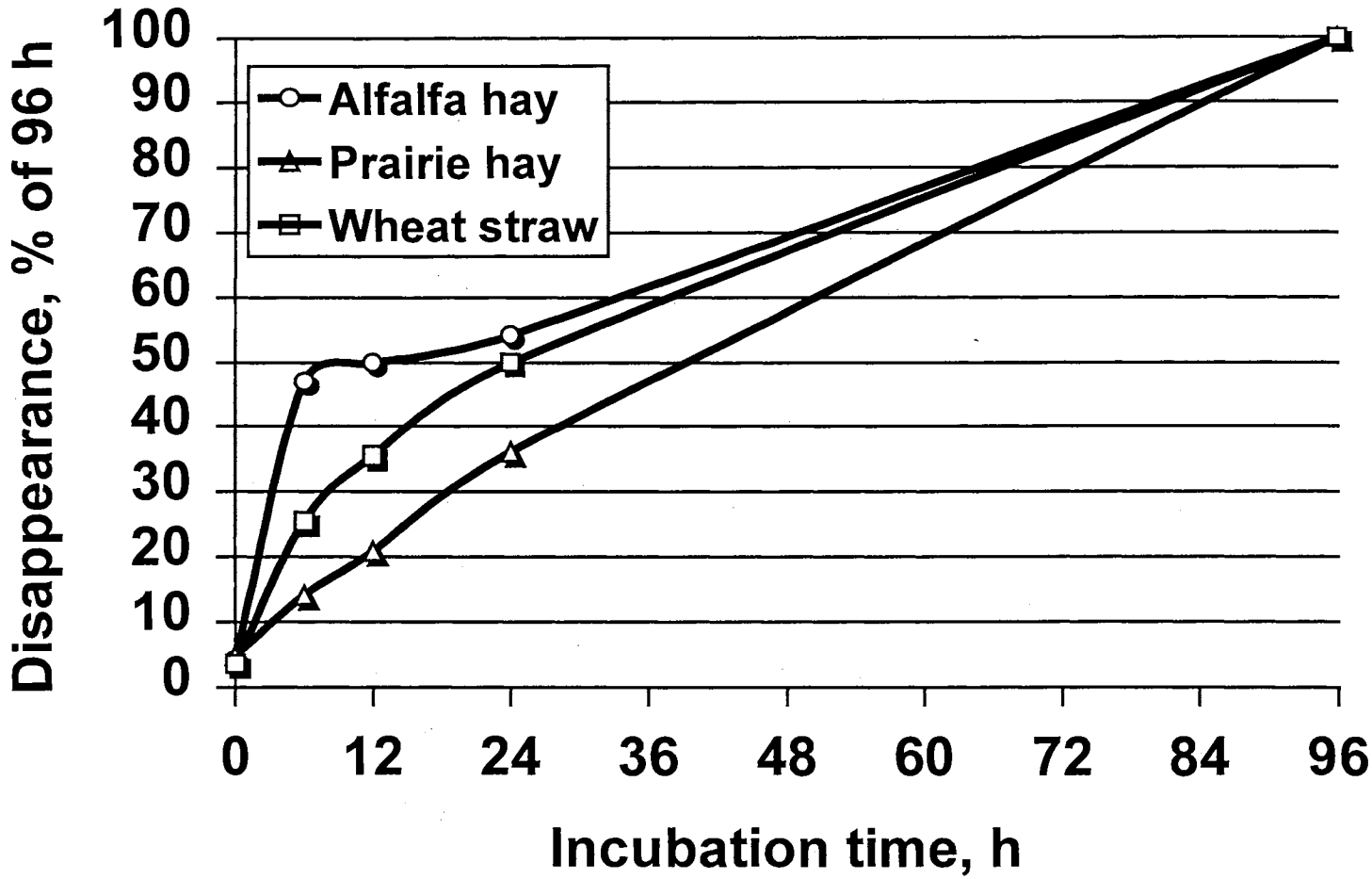

Figure 3. 6. Hemicellulose disappearance from alfalfa hay, prairie hay and wheat straw as a percentage of disappearance after 96 hours

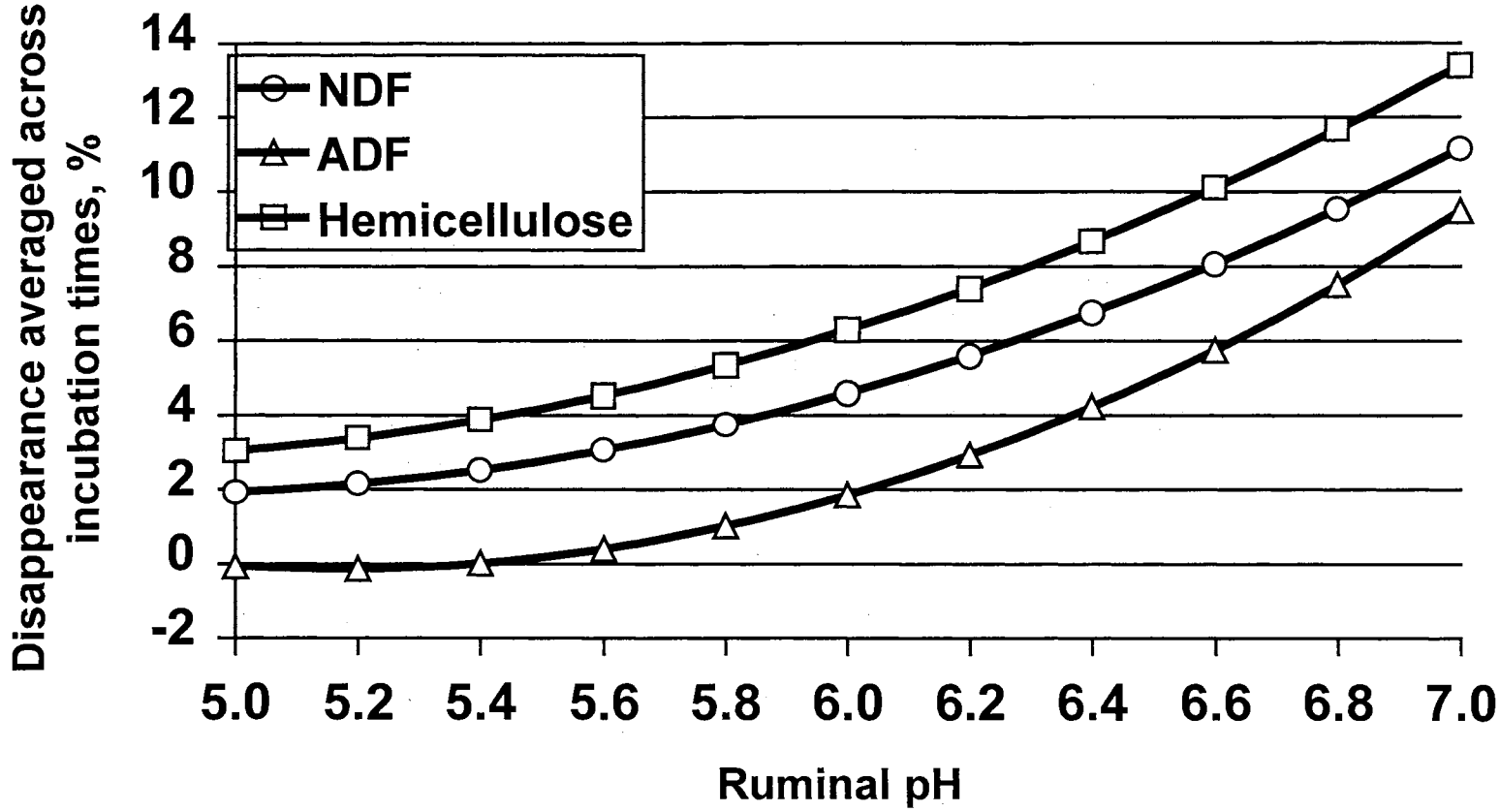

Figure 3. 7. Mean disappearance of neutral detergent fiber, acid detergent fiber and hemicellulose disappearance from alfalfa hay, prairie hay, and wheat straw regressed against mean ruminal pH.

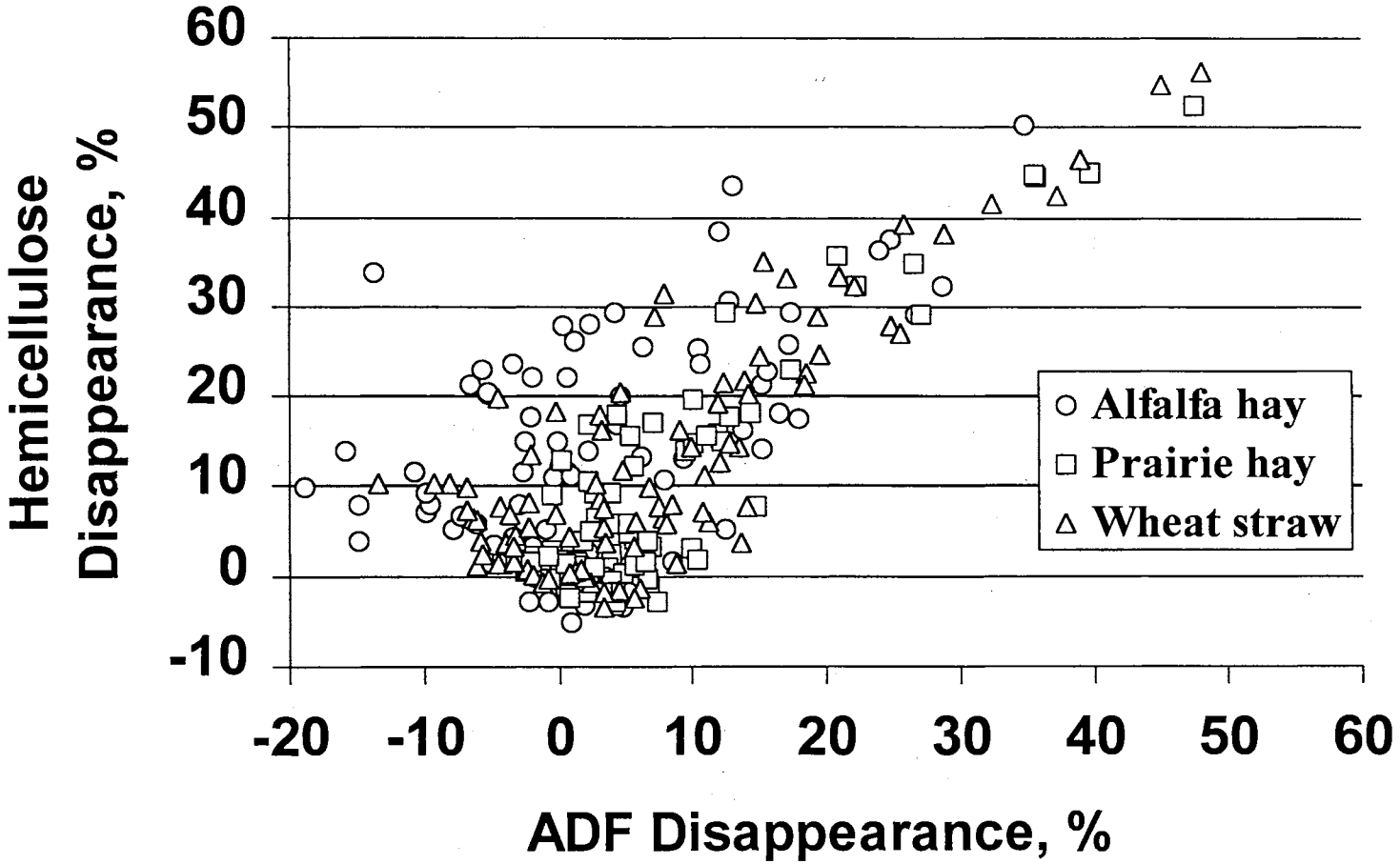

Figure 3. 8. Disappearance of hemicellulose versus disappearance of acid detergent fiber from alfalfa hay, prairie hay and wheat straw.

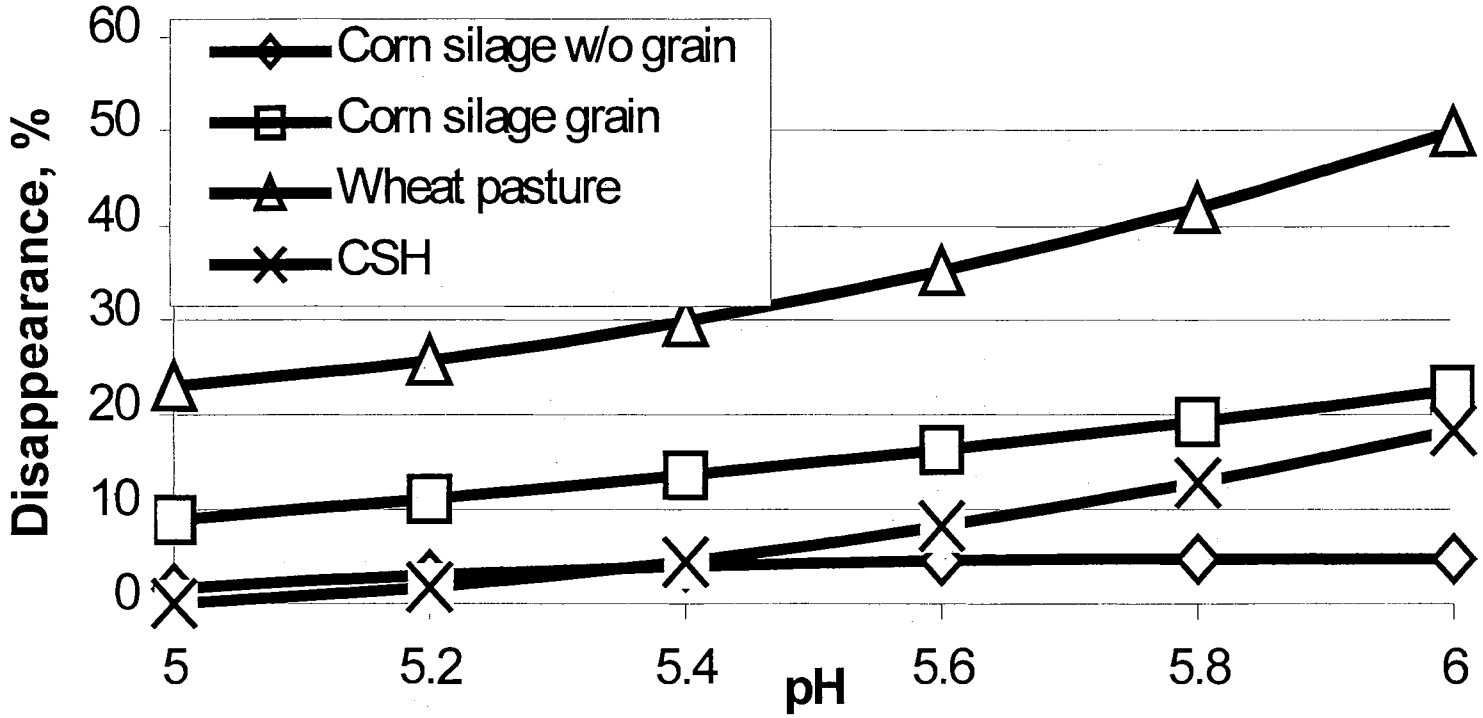

Figure 4. 9. Neutral detergent fiber disappearance at 24 hours from corn stover, grain from corn silage, wheat pasture and cottonseed hulls regressed against mean ruminal pH.

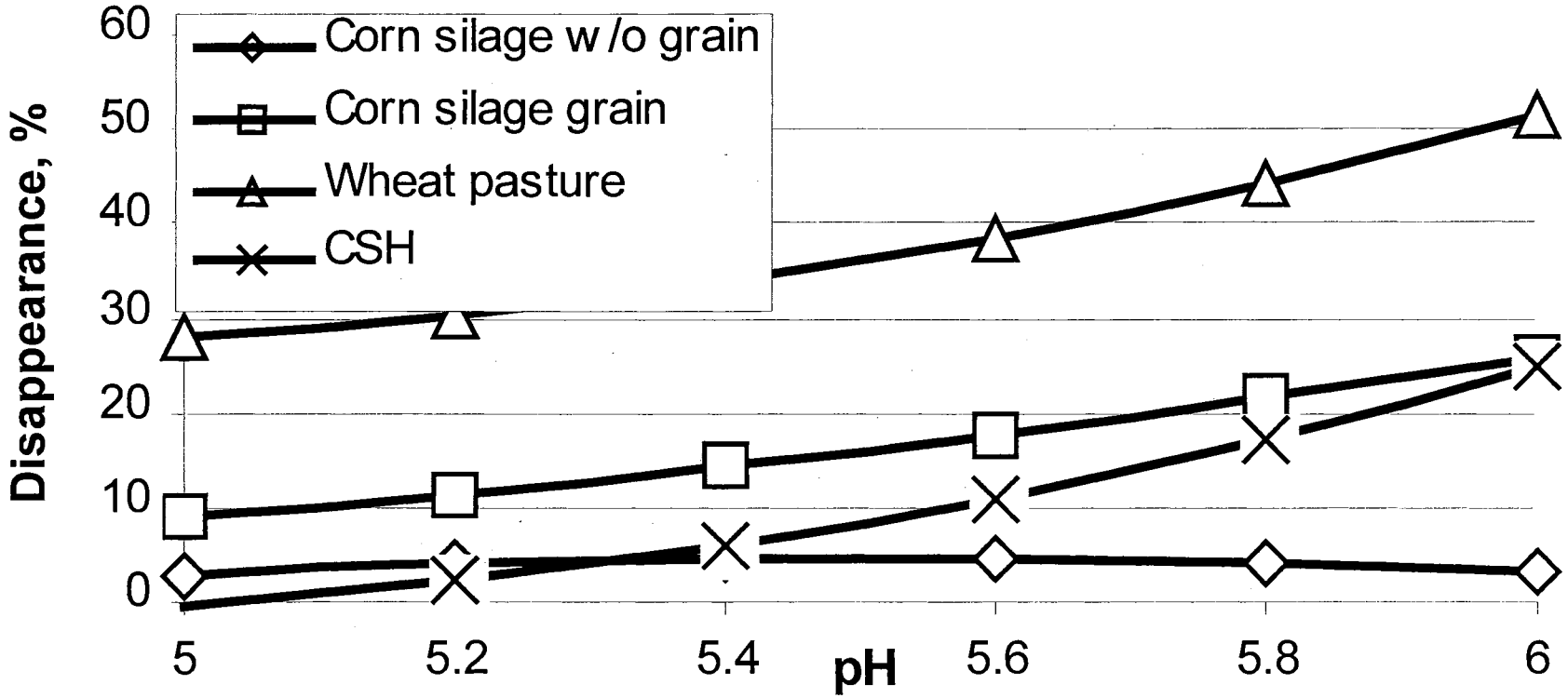

Figure 4. 10. Hemicellulose disappearance at 24 hours from corn stover, grain from corn silage, wheat pasture and cottonseed hulls regressed against mean ruminal pH.

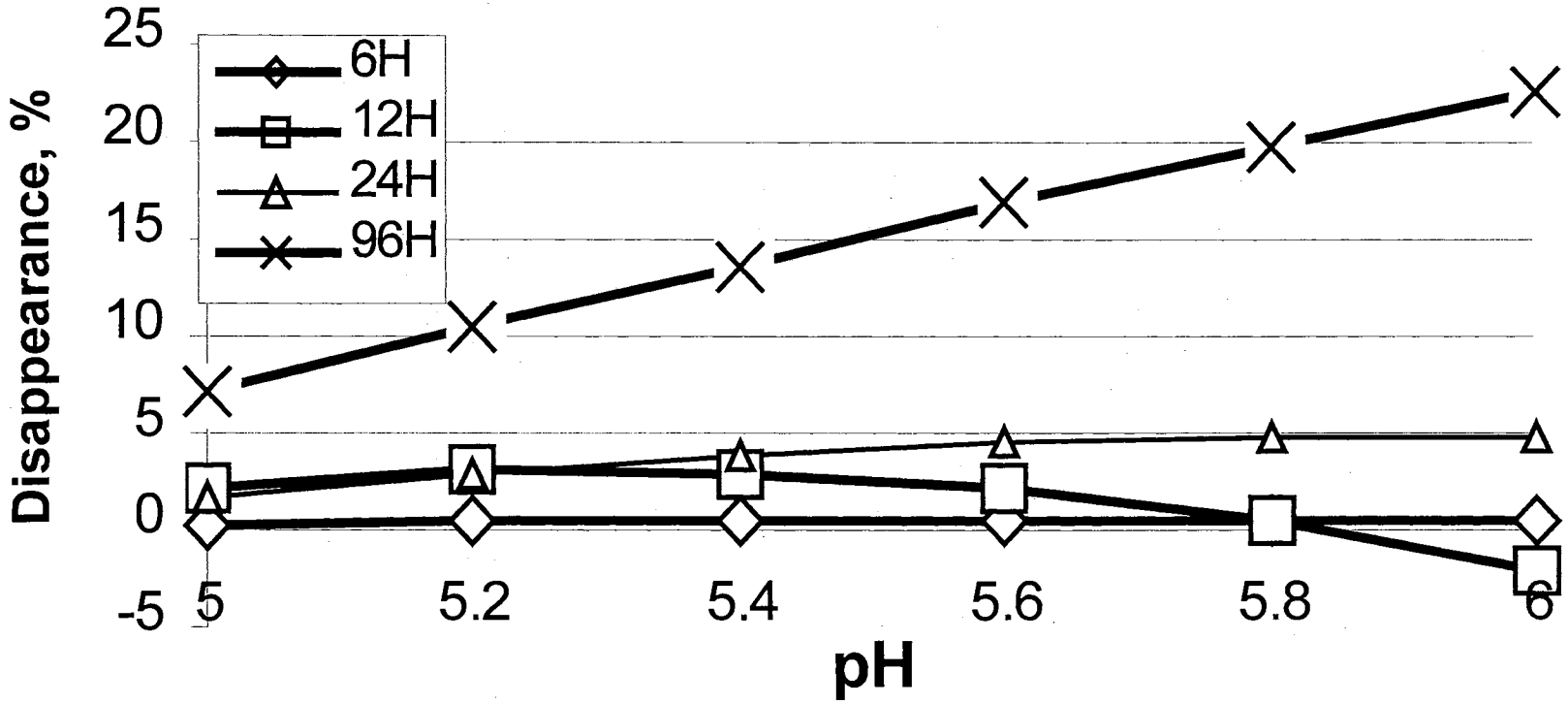

Figure 4. 11. Neutral detergent fiber disappearance from corn stover at various incubation times regressed against mean ruminal pH.

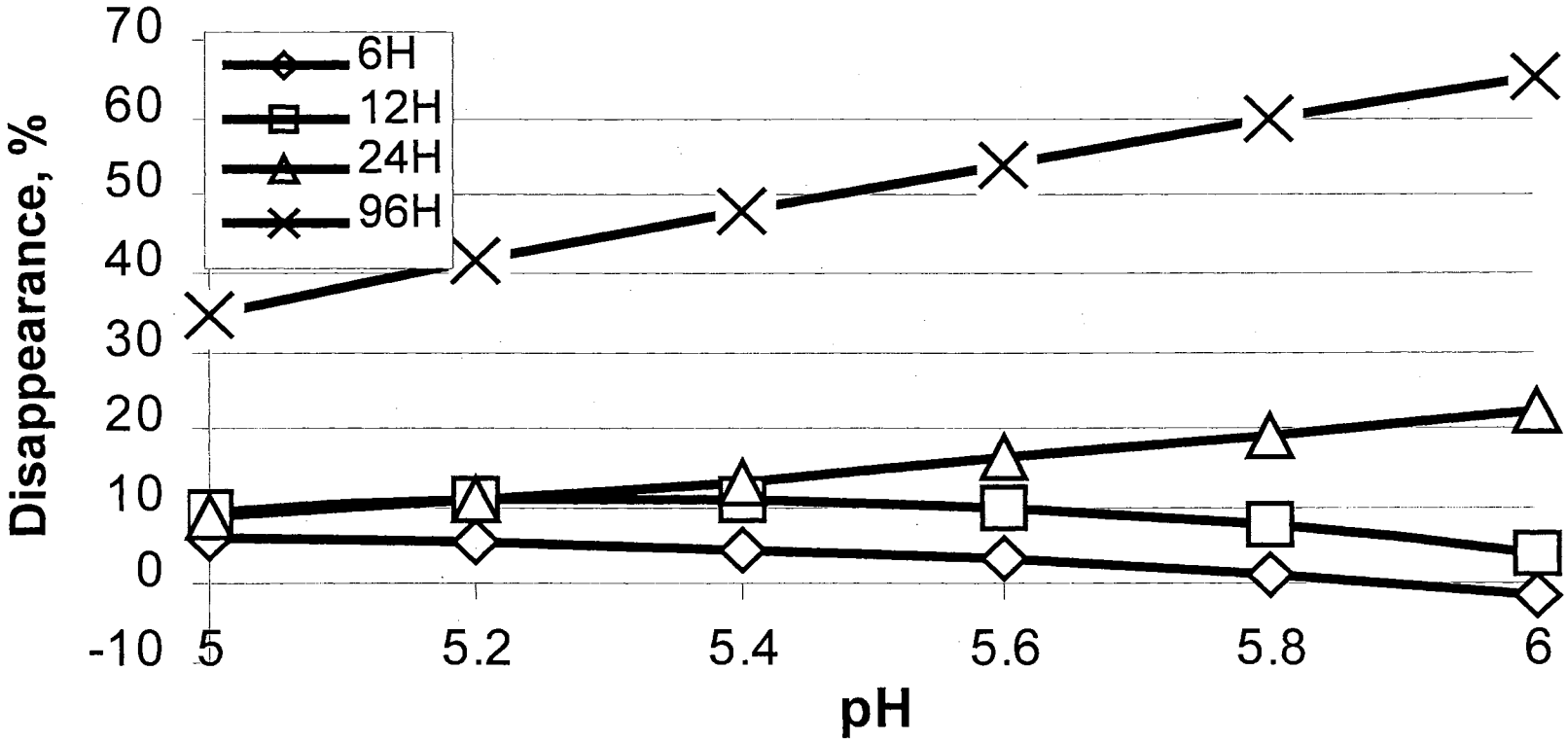

Figure 4. 12. Neutral detergent fiber disappearance from grain from corn silage at various incubation regressed against mean ruminal pH.

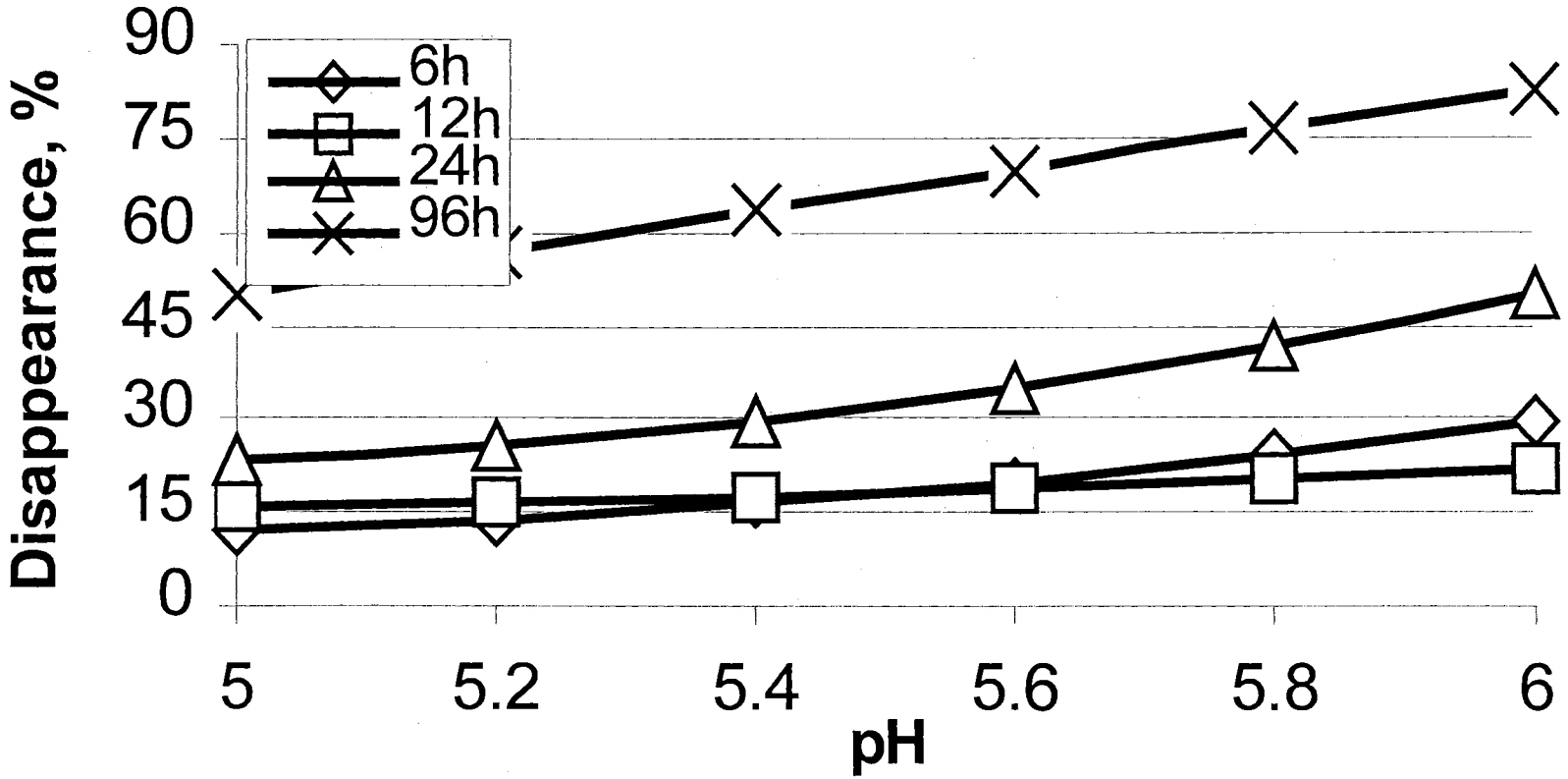

Figure 4. 13. Neutral detergent fiber disappearance from wheat pasture at various incubation times regressed against mean ruminal pH.

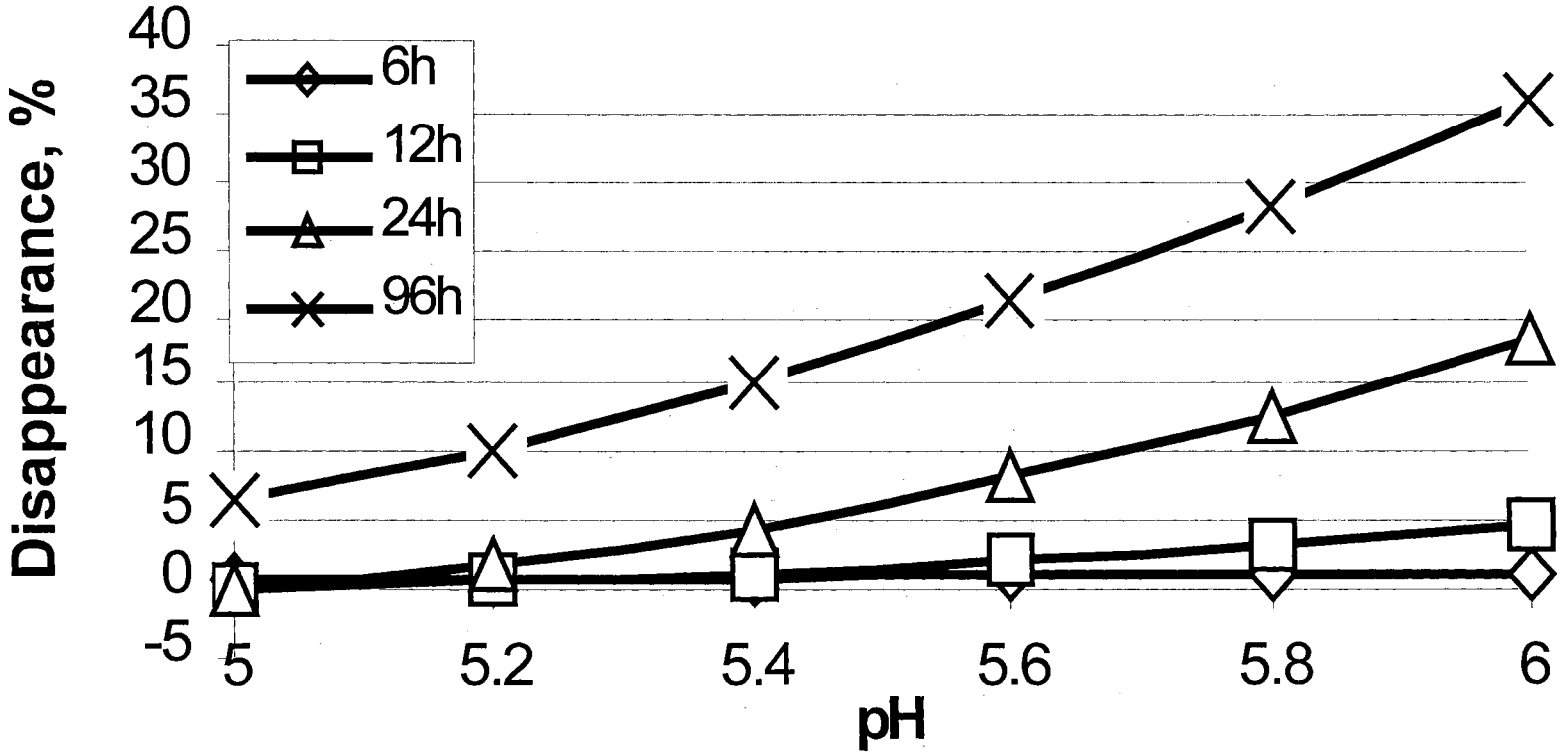

Figure 4. 14. Neutral detergent fiber disappearance from cottonseed hulls at various incubation times regressed against mean ruminal pH.

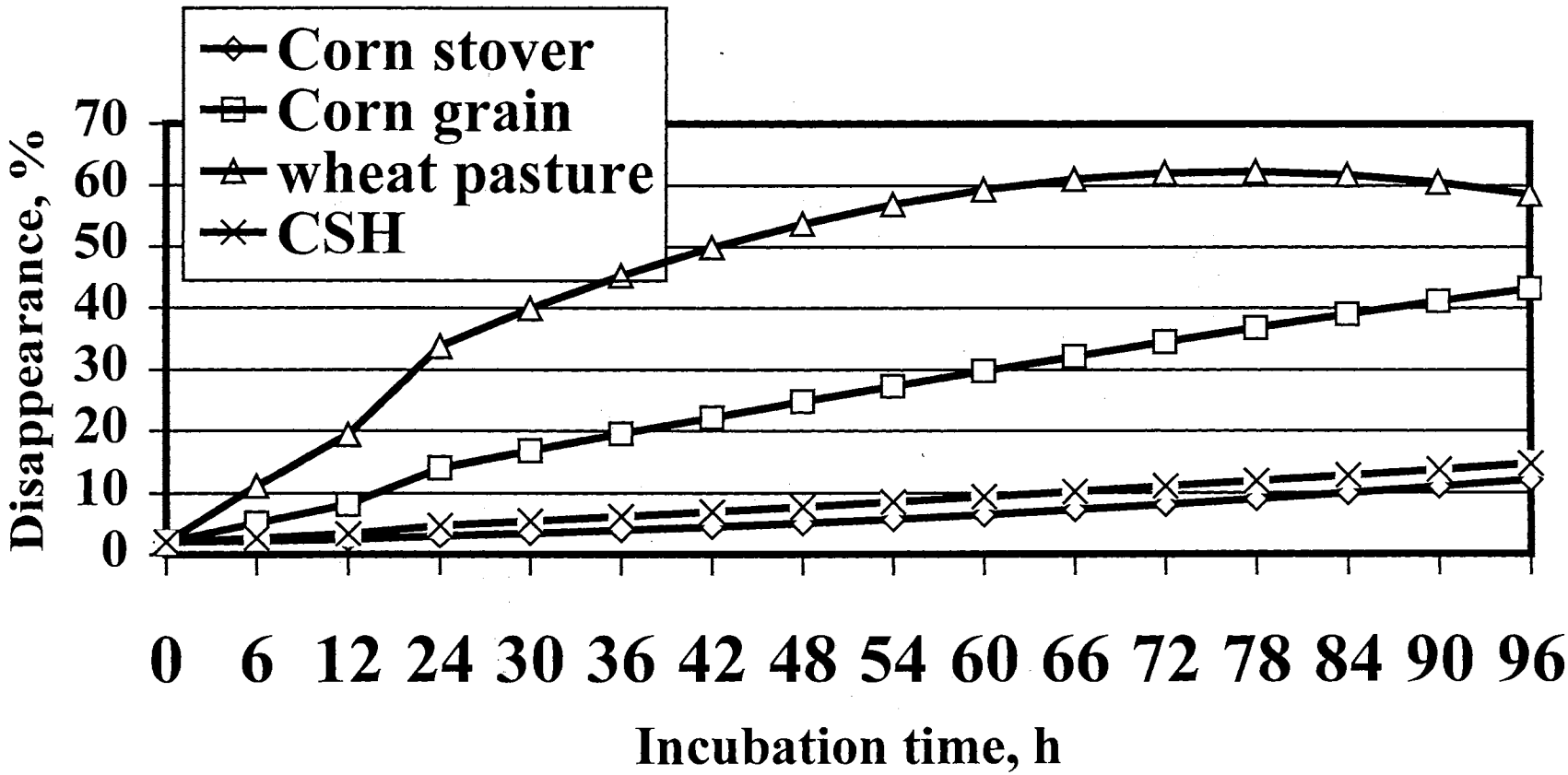

Figure 4. 15. Neutral detergent fiber disappearance from corn stover, grain from corn silage, wheat pasture, and cotton seed hulls after various incubation times.

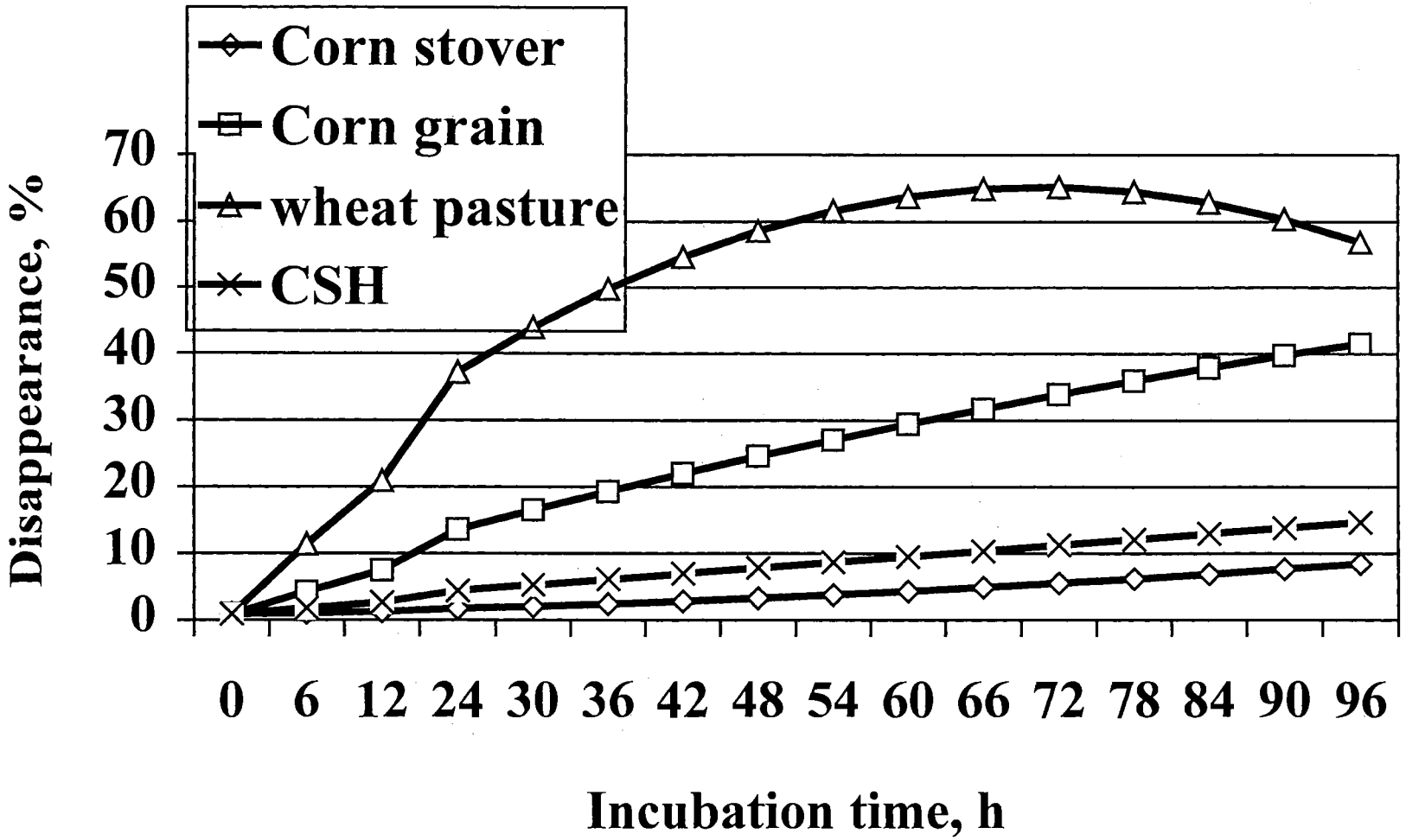

Figure 4. 16. Hemicellulose disappearance from corn stover, grain from corn silage, wheat pasture, and cotton seed hulls after various incubation times.

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