

## MICROBIAL AND NUTRITIVE CHANGES IN FORAGE DURING HARVEST AND STORAGE AS HAY

K.M. Wittenberg

Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada. R3T 2N2

### ABSTRACT

Hay harvest systems have made advancements over the past decades by increasing capacity, reducing labor requirements and improving the quality of the product at feeding. This paper focuses on the biochemical and microbial changes associated with technology advancements most directly aimed at improved quality through understanding quality changes directly associated with moisture removal from the plant and the control of saprophytic fungi. Fungal growth can occur on the standing plant, the wilting crop and during storage; the type of growth being strongly influenced by the micro environment in the field, the swath or the bale. A major problem associated with the control of fungal growth is lack of uniform conditions during harvest and storage of hay. Thus the traditional view has been that forage must be dried to less than 15 - 18% moisture for safe storage, with the assumption that field losses were a lesser risk than storage losses. Physical or chemical conditioning treatments, applied at the time of cutting, have traditionally been viewed as a means to reduce field wilting time. However, these strategies are also a means of creating a more uniform environment within the swath or the bale. Recognition of the micro environment within the bale may be critical to achieve more consistent protection by preservatives applied to minimize adverse fungal growth during storage.

### KEYWORDS

Hay, fungi, forage quality, conditioning, preservative

### ACRONYMS

**ADF** = acid detergent fiber, **ADIN** = acid detergent insoluble nitrogen, **cfu** = colony forming units, **N** = nitrogen, **NDF** = neutral detergent fiber

### INTRODUCTION

Preservation of forage as hay originated as a means to maintain livestock during periods of minimal pasture productivity. The basic harvest processes associated with hay production remained virtually unchanged until the start of the 20th century. At that time, most of the advances in harvest technology were driven by a need to make the harvest, storage and feeding systems associated with hay production more labor efficient. Current trends towards greater livestock numbers on farms, market incentives to maintain high levels of animal productivity on a year round basis and expansion in the world trade of dried forage products have refocused the priorities associated with hay production towards increased capacity and quality.

Hay quality can be described in terms of desirable and undesirable characteristic. Basically, hay having a nutrient profile that closely matches the nutrient requirements for the livestock category to which it is being fed is considered to be of high quality, provided that there are no undesirable characteristics. Undesirable characteristics in hay can include any factors that interfere with animal maintenance and productivity. Factors that reduce hay palatability or intake; reduce nutrient digestibility or availability at the site of absorption; and adversely affect animal health are deemed to be undesirable.

The following discussion will focus on information collected with temperate forage crops. Rotz and Muck (1994) provide a good overview of factors associated with forage DM losses during hay

harvest and storage. The current discussion will focus more on the biochemical and microbial changes associated with hay harvest and storage, and technological advances that may influence these changes.

### CHEMICAL CONSTITUENT CHANGES ASSOCIATED WITH HAY PRODUCTION

Standing plants, when cut, are subjected to enzymatic, microbial and chemical reactions which can change the nutrient composition of the cut plant material during the process of hay harvest and storage. For example, soluble carbohydrates are the principle group of compounds utilized during plant cell and microbial respiration which occurs under aerobic conditions. The process involves a complete oxidation of hexose sugars to yield CO<sub>2</sub>, water and energy (Wilkinson and Hall, 1966):



The energy released from this process may be released as heat or used for synthesis of plant or microbial biomass. The heat generated can help remove forage moisture by evaporation. Undi and Wittenberg (1996b) observed that the bulk of moisture loss from hay stored at 23.7 to 34.7 % moisture occurred between days 4 to 14 of storage, a period when stack temperatures were the highest. In the initial stages of storage, however, the temperature rise associated with aerobic respiration is also important for the initiation of storage microorganism growth (Undi and Wittenberg, in press; Hlödversson and Kaspersson, 1986).

The extent of respiration loss is a function of plant species, rate of drying in the field, wilting and storage temperatures, and moisture content of hay placed into storage. Soluble carbohydrates levels in the hay never approach zero concentrations (Undi and Wittenberg, 1996b; Hlödversson and Kaspersson, 1986) unless the forage has undergone severe heating (Festenstein, 1971). It is likely that new soluble carbohydrates generated from fungal breakdown of structural carbohydrates occurs as the original plant soluble carbohydrates are used up. This would be evidenced by the appearance of glucose and pentose and the loss of fructosan and its breakdown product, fructose.

Forage acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations increase as a result of the enzymatic, microbial and chemical processes occurring during hay harvest and storage. Miller et al. (1967) suggested that the increase in fiber fractions during storage represents a percent replacement, rather than true increase, and is primarily due to loss of non-fiber constituents with little or no loss of fiber. This explanation disregards the presence of fungal biomass, the cell wall of which can contribute to ADF and NDF fractions, as determined using current analysis techniques. Exact determinations are difficult to assess since fungal growth on forage is difficult to replicate on media, however, as much as 10% of molded hay DM may actually be fungal biomass (Bossuyt et al., 1996). This may explain why DM losses measured from carbon dioxide production were higher than losses measured from stored hay balance studies in the work conducted by (Hlödversson and Kaspersson, 1986). A third factor that can contribute to the increased fiber fraction, not directly caused by microorganisms but favoured by high temperatures and high moisture contents, is the nonenzymatic condensation of carbonyl groups to form large indigestible polymers with amine moieties, i.e. the Maillard reaction.

Maillard reaction products have traditionally been estimated by the change in forage acid detergent insoluble nitrogen (ADIN) concentrations. Undi and Wittenberg (1996b) and Colbentz et al. (1996) observed significant increases in stored hay ADIN concentrations even after storage temperatures returned to near ambient levels. It may be that a part of the nitrogen (N) associated with the fungal cell wall may contribute to the changes observed in forage ADIN. The availability of this N is not known.

Preservation of forage as hay is recognized to be superior to preservation as silage with respect to protein quality for the high producing ruminant animal (Petit and Tremblay, 1992). Forage protein value is assessed as the amount of amino acid available for absorption in the animal's small intestine. These amino acids may be derived directly from the dietary protein being offered or may be derived from microbial protein synthesized in the rumen. Forage harvest and storage systems that increase the proportion of forage protein that is undegradable in the rumen without increasing the unavailable protein will result in greater concentrations of dietary protein available for absorption in the small intestine. Michalet-Doreau and Ould-Bah (1992) found that the hay making process, under good harvest conditions, led to a mean increase of 4.0 percentage units of soluble N, total N basis, from the time the plant is cut to the time of baling. Variation in N degradability ranged from 67.8 to 82.9% in the grass and legume hays tested for that study. Michalet-Doreau and Ould-Bah (1992) reported that 91% of the variation in N degradability of hay was determined by the N degradability of the standing crop at the time of harvest, with hay production resulting in a mean decrease of 2.5 percentage units. This decrease in degradability was similar for grasses and alfalfa. The extent of decline in degradability was greater when degradability of the standing crop was high or when harvest conditions were poor. The major reason for decreased degradability was related to a reduction in the rapidly degradable N fraction. A similar study (Broderick et al., 1992), using an *in vitro* technique, confirmed the decrease in protein degradation rates of baled alfalfa hay, harvested under good conditions vs. standing forage when cut at various maturities over three cuttings during each of two years, however, the extent of the decrease was much greater. A recently conducted survey in Western Canada (von Keyserlingk et al., 1996) showed that the soluble N concentrations of alfalfa and grass hays can range from 25 to 63% of total N and that the fractional rate of crude protein degradation for the degradable N in the hays can range from 3.6 to 17.1% h<sup>-1</sup>. These studies indicate a potential use of hay as a source of ruminal escape protein, however, livestock producers and nutritionists require greater ability to control and estimate these properties in order to realize advantages when formulating diets.

Hay harvested and stored at moisture levels that result in heating will affect the protein quality at feeding. Losses associated with Maillard reaction products were previously discussed. Variations in amino acid stability when exposed to heat were observed for orchard grass in some work conducted by Weiss et al. (1986). Of the amino acids deemed essential for ruminants, methionine was relatively stable; isoleucine, tyrosine, histidine, valine, and threonine showed minimal declines (.07 mg g<sup>-1</sup> DM d<sup>-1</sup>); arginine, phenylalanine and leucine showed moderate rates of decline (.13 mg g<sup>-1</sup> DM d<sup>-1</sup>); and lysine showed severe decline (.25 mg g<sup>-1</sup> DM d<sup>-1</sup>) for wet forage during periods of high heat during storage. Along with a decrease in total amino acids, the percentage of remaining amino acids associated with the fiber fractions increased, accounting for the increase in slowly degraded protein previously discussed. The rate of this increase was relatively similar across amino acids with the exception of methionine, for which rate of accumulation was higher and lysine

which had a lower rate of accumulation. Methionine and lysine are the two amino acids most commonly found to be limiting in ruminant diets. These data suggest that hay drying processes and moderate heating may dramatically improve methionine availability to the animal, however, the net benefit for lysine should be evaluated.

Forage analysis for glucosamine, a constituent of chitin found in cell walls of spores and mycelium of fungi, has been used as a means to estimate the total fungal biomass in forage (Wittenberg et al., 1989; Roberts et al., 1987). Species of fungi indigenous to Western Canada, when grown on liquid media have been observed to range from 65 to 100 g glucosamine per kg mycelial DM (Rotter et al., 1989). Therefore, 1 g kg<sup>-1</sup> DM of glucosamine can be equated to 10 to 15 g fungal biomass kg<sup>-1</sup> forage DM. Fungal biomass concentrations measured in stored forage represents the total fungal biomass accumulated from the time the plant is growing, the time spent wilting and time spent in storage. Studies at the University of Manitoba show that glucosamine values can be as low as 0.9 g kg<sup>-1</sup> DM under ideal growing, harvest and storage conditions, with levels as high as 10 g kg<sup>-1</sup> DM for severely molded hay (Wittenberg, 1994). Generally, the highest accumulation of fungal biomass is associated with fungal growth on cut forage lying in the field. Further characterization of this biomass has not been conducted, however, it should be assumed that the biomass contributes to the N and carbohydrate fractions of hays. Availability of these fungal fractions to as protein and energy sources for ruminants consuming them are not known.

#### FACTORS INFLUENCING FUNGAL GROWTH DURING HAY HARVEST AND STORAGE

Many environmental factors are involved in the extent of fungal growth and species succession in hay following cutting, including: water availability, nutrient availability, temperature, pH, gaseous composition and interactions with other micro-organisms.

Water activity ( $a_w$ ) is the ratio of the vapor pressure over the forage to that over pure water at the same temperature and pressure, and is a measure of the water that the microorganism can use for growth or water availability. The most commonly used estimate of water availability used in forage trials is the measurement of moisture content, however, this is not a true estimate of water availability (Albert et al., 1989). Fungal growth can occur at  $a_w$  ranging from .755 to 1.0, however, the nature of growth will vary (Albert et al., 1989). At a  $a_w$  of 1.0, corresponding to a moisture range of 51.7 to 63.3% in alfalfa, fungal growth is characterized by extensive mycelial development, whereas at a  $a_w$  of .88, which corresponds to 23.9 to 31.6% moisture, fungal mycelia growth is not apparent to the unaided eye but fruiting body production occurs. Variation in  $a_w$  and its effect on fungal growth become critical when one considers the high degree of variability in moisture content of alfalfa in windrows and in storage.

The availability of substrate and its effects on fungal growth are not as clearly defined. Although leaves have a higher nutrient density than stems, alfalfa and grass stems had a greater spoilage potential than leaves (Albert et al., 1989; Waite, 1949), with growth initiating at breaks or internodes along the stem. Initial soluble carbohydrate, fiber, or protein concentrations do not have a major impact on population or succession of species in the work completed by Undi and Wittenberg (in press).

In general, fungal growth can occur at a wide range of temperatures, however, most fungi found in stored plant products thrive at a range of 10-40 °C and have optimal conditions at a range of 25-35 °C. Hay produced under conditions where temperatures do not exceed this

optimal range generally is observed to have a diverse range of fungal species, however, conditions resulting in temperatures in excess of 40 °C will cause the species that are not thermotolerant to disappear. Stack temperatures can readily reach 40 °C when forage moisture content at stacking is in the range of 18 to 25%. High ambient temperatures and humidity, and restricted air movement due to bale density or stacking design, will contribute to an increase in peak temperature.

Hydrogen ion concentration (pH) appears to remain near neutral in hay that has undergone molding (Undi and Wittenberg, in press; Duchaine et al., 1995), unless storage moisture content exceeds 40 % when the pH value may rise to 7.0 or more (Gregory et al., 1963). Most fungi grow well over a wide pH range, but will compete poorly with bacteria at pH 7.0 or above when the  $a_w$  is near 1.0.

Little work has been conducted to determine the partial pressure of O<sub>2</sub> in stored hay, however, it is likely that poor air exchange could result in lowered O<sub>2</sub> and elevated CO<sub>2</sub> levels when plant or microbial respiration occurs. Although the fungi causing spoilage in hay are considered obligate aerobes, many are capable of growth at low concentrations of O<sub>2</sub>.

Environmental conditions can influence each of the main phases of the growth cycle. It can affect spore germination (or duration of the lag phase), linear growth of the hyphae and sporulation. The criteria most important to the subsequent development of a micro-organism in a stored product is the minimum condition permitting germination and growth. Any decrease in the rate of growth can influence the ability of one organism to compete with another.

#### FUNGAL SUCCESSION DURING HAY HARVEST AND STORAGE

Epiphytic organisms are generally considered to cohabit with the living plant. The surface of living plants is colonized first by bacteria, but they are soon followed by yeasts and then pathogenic and saprophytic filamentous fungi (Lacey, 1989). The bacteria that dominate may help protect the plant from fungal invasion, and yeasts are thought to provide protection from the effects of visible light.

Field data collected in Manitoba and elsewhere (Lin et al., 1992) indicate high populations, 10<sup>6</sup> to 10<sup>7</sup> colony forming units (cfu)/g<sup>-1</sup> DM, of yeast and mold on the growing plant at the time of cutting. Little species identification work has been conducted to date, however, glucosamine measurements indicate wide ranges in fungal biomass on standing plants and that these ranges appear to be influenced by field growing conditions. Recently conducted screenings of alfalfa at the University of Manitoba indicate that the epiphytic populations of bacteria and yeasts on standing plants, but not molds, also may be influenced by plant genotype.

The process of cutting and conditioning of the cut plant results in the introduction of soil and air borne microbes to the plant material. Microbial populations present at time of cutting, plant physiological stage, temperature and moisture are the main factors determining the predominant fungal species and their relative level of activity during field wilting. These organisms are frequently not identified in molded hay sampled after being placed into storage, however, the mycelia, spores and other end products generated during this period of active growth may still be present. Breton and Zwaenepoel (1991) and other European studies with grasses have identified the following soil borne species to be present on forage during wilting and at baling: *Alternaria*, *Ascochyta*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Fusarium*, *Phaeoseptoria*, and *Phoma*. Local studies with alfalfa

forage have identified the same genera as well as *Aspergillus glaucus*, which is more commonly associated with stored products. Wilting trials have indicated that precipitation events can result in 10 to 100 fold increases in the forage bacterial populations during forage wilting, with little change in the fungal counts (Wittenberg, 1995), although fungal biomass yield has increased.

Visual assessment of forage that has undergone molding during the wilting phase is usually characterized by localized discoloration on plant stems and leaves due to the presence of spores, with increased friability and dustiness evident once the forage is dry. Extent of fungal growth is often difficult to assess visually, however, it should be noted that a single event such as heavy dew or precipitation can double the fungal biomass accumulation on forage during field wilting.

Hay that is stable during storage will have similar fungal plate counts and species diversity as would be observed for the standing crop or wilting forage. The "field" fungi will rapidly disappear upon exposure to high temperatures. A simultaneous rise in "storage" fungal populations will occur. Individuals attempting to determine hay moldiness on the basis of fungal count measurements may, therefore, see little change in cfu g<sup>-1</sup>, even though forage quality decline is evident.

Fungal species that become dominant during storage of hay that has under gone heating thrive at lower  $a_w$  and higher temperatures. European studies with grass hays have identified these fungi to belong to the genera *Aspergillus*, *Emericella*, *Eurotium*, *Humicola*, *Paecilomyces*, *Penicillium*, and *Rhizopus* (Breton and Zwaenepoel, 1991; Kaspersson et al., 1984). University of Manitoba trials (Undi and Wittenberg, in press) have identified many of the same organisms to be present in alfalfa hay baled at moisture levels of 20 to 30%. Typically, the storage fungal population will not have the diversity of species, with one or more species becoming dominant. It is this evolving dominance of specific species that may prove to be one of the most useful tools for describing the degree of microbial activity in stored forage. For example, Lacey (1989) identified presence of such species as *Aspergillus fumigatus* and *A. versicolor* to be an indicator of extensive damage during storage. These studies also suggest that factors influencing the second temperature peak during storage will be a determining factor in the proliferation of a particular dominant species.

Visual assessment of molding during storage is generally related to the presence of intact mycelial mats upon gross examination. However, since storage conditions tend to become xerophilic with time, fungal growth becomes characterized with reduced mycelial development and increased spore formation. Therefore, alternative methods for estimation of fungal activity in the study of hay storage should be encouraged.

Efforts have been made to quantify the adverse effects of fungal biomass in hay by studying its effects on hay palatability, intake and digestion. Presence of fungal biomass in hays having similar nutrient profiles did reduce palatability of the hay (Undi and Wittenberg, 1996a) in young heifers, but did not adversely affect intake in growing steers (Bossuyt et al., 1996) when fed as the sole dietary energy source. The fiber fraction of hay with elevated levels of fungal biomass also appeared to be more digestible compared to less moldy hay of a similar nutrient profile, possibly due to the action of mycelia penetrating the plant cuticle and causing splits or fractures in the xylem and lignified vascular bundles.

Many of the field and storage fungi identified are potential mycotoxin producers. Few field trials in the literature have included assessment for mycotoxins in forage dried and stored as hay. Mycotoxin screening of alfalfa and grass forages baled and stored at moisture ranging from 15 to 30% in various University of Manitoba have been negative. Clear definitions of the storage conditions associated with many of the incidences in which mycotoxin production have been evidenced are lacking, however, most observations have been associated with material exposed to repeated wetting or material stored at moisture levels well in excess of those normally associated with hay production. Some fungi demonstrate very low or no toxin production at a low  $a_w$  even though active growth is still occurring (Corry, 1977). Competition from mixed flora in non sterile products also can retard mycotoxin production by fungi (Denizel et al., 1976).

### TECHNOLOGICAL ADVANCES FOR HAY HARVEST AND STORAGE

The remainder of the discussion will focus on two areas which have received a great deal of attention with respect to use of forage additives to maintain forage quality during harvest and storage. Field curing of cut forage remains the most popular means of drying forage for storage as hay due to relatively lower costs. MacDonald and Clark (1987) provide an excellent review of water and dry matter losses during field drying of hay. The main advantage of conditioning of the crop at cutting is not to improve yield, but to reduce risk of quality loss due to weathering and to improve the moisture uniformity of the dried forage at time of baling. Efforts also have been directed towards the development of hay preservatives, designed to inhibit microbial activity that may adversely affect quality of hay during storage.

**Conditioning processes:** The plant cuticle is a major barrier to water loss in forage plants. Weathering can break down the protective cuticle of the plant as it ages, which may explain the faster drying rates observed for more mature plant material. The epidermis of a plant is not totally intact because of stomata or pores that function to regulate water vapor and  $\text{CO}_2$  exchange between the plant and its environment. Once the plant is cut, progressive water loss and shading will induce these pores to close. Normally, a 20 to 30% water loss will occur prior to stomatal closure, after which the rate of water loss declines by a factor of 8 because water must pass through the plant cuticle. Conditioning processes, therefore, have attempted to either inhibit or delay stomatal closure, or to disrupt the plant cuticle.

Chemicals known to inhibit stomatal closure include sodium azide, fusaric acid and kinetin. A limited number of studies conducted under field conditions indicate variable improvements with the use of these compounds.

Mechanical conditioning treatments to disrupt the plant cuticle; which include crushing, breaking or abrasion of the waxy outer layer of the plant allow direct movement of water from the plant stem interior to the air. Miller and Rotz (1995) reviewed the types of mechanical conditioners available and the crops to which they are best suited. Most recently, attention has been directed towards more severe forms of mechanical conditioning, referred to as superconditioning, maceration or mat making (Savoie et al., 1993; Koegel et al., 1992). Under good drying conditions, this process can allow hay to be baled on the day of cutting. Little work has been completed to determine whether storage concerns for this material are similar or different from that of forage harvested under more conventional means of mechanical conditioning. Increased breakage of the stem, increased availability of cell contents and increased bacterial populations pre baling can influence plant and bacterial respiration during the initial

stages of storage, the range of storage  $a_w$ , and ultimately the fungal activity and species succession in stored hay. Fed to animals, maceration did improve ruminal digestibility, but not total tract digestion of timothy hay (Chiquette et al., 1994), however, the authors felt that more conclusive information was required to establish the potential impact on animal performance.

Thermal treatments, which function by disorganizing the wax structure of the plant cuticle, have not gained popularity due to high operating costs.

Alkaline solutions, prepared by adding monovalent alkaline metal ions to a carbonate solution, cause a dissociation of the carboxyl groups in the cutin matrix of the plant cuticle. As a result, the plant is not able to prevent water movement from its interior to exterior. Solutions made with potassium carbonate ( $\text{K}_2\text{CO}_3$ ), available as granules, granular powder, or granular crystals, have received the greatest amount of attention in field trials. Alkaline solutions (0.18 M or higher) are effective for legumes but not for grasses. The ability of  $\text{K}_2\text{CO}_3$  to improve drying rates with grass-legume mixtures is directly proportional to the proportion of each forage type in the stand at the time of cutting. The recommended application rate will vary with field condition and method of application, but most commonly  $\text{K}_2\text{CO}_3$  solution is applied at 2-3% of forage weight with the location of the spray nozzles and push-bars ahead of the cutters are such that the majority of the solution is directed toward plant stems.

Combining mechanical conditioners and  $\text{K}_2\text{CO}_3$  has an additive effect on drying rates. Crump (1985) found that a combination of mechanical and chemical conditioning was more effective than mechanical conditioning alone in four of six trials, and than chemical conditioning alone in three of six trials.

Potassium carbonate has been combined with sodium carbonate to reduce costs without sacrificing effectiveness, however, current recommendations suggest that the ratio should not be less than 1:1 (Rotz and Thomas, 1985). Potassium carbonate also has been combined with methyl esters and emulsifying agents in an effort to further improve forage drying rates, however, response to these additions is varied. Other forms in which the alkaline metals have been tested include potassium hydroxide or sodium silicate. Chemical drying agents or desiccants that cause disruption of the plant cuticle can cause increased plant susceptibility to fungal invasion during storage (Kraynyk and Wittenberg, 1989) and microbial attack in the rumen (Hong et al., 1988).

Organic phosphates have demonstrated an ability to increase forage drying rates and reduce cell respiration rates but have not been tested under field conditions and could present toxicity problems for livestock. Compounds in this category include tri-n-butyl phosphate and a diphosphate ester.

**Hay preservatives:** Preservative agents are defined to be substances added to food or feed to prevent spoilage. Two types of preservatives commonly used in hay production include fungicides, which kill fungi; and fungistats, which inhibit growth of fungi. Categories of hay preservatives, described on the basis of mode of action, include direct acidifiers, specific antimicrobial agents, bacterial inoculants and nutrients. Most preservatives are applied at the time of baling as either a granular or liquid product, however, some products such as anhydrous ammonia and cold-flo ammonia may be applied shortly after stacking. Desirable criteria of hay preservatives include: an ability to prevent fungal invasion in hay baled and stored above

defined safe moisture levels; an ability to inhibit production of fungal end products (spores and mycotoxins) that have direct adverse effects on hay handlers and livestock; ease of product application, safety associated with product handling, lack of adverse response by animals consuming treated hay; no residue in animal products; and cost recovery.

Organic acids are generally proven to be effective in preventing fungal invasion of moist hay during storage when adequate levels are applied. Studies have been conducted with propionic, acetic, isobutyric and formic acids, however, most commercial acid based preservatives contain propionic acid or a propionic-acetic acid mixture. The amount of acid to apply at baling is dependant upon forage moisture content, laboratory results indicating a minimum of 1.25 kg propionic acid for every 100 kg of water. However, under field conditions, high percentages of acid may be lost at the time of addition requiring that field application rates be increased.

The corrosive nature of acids and high vaporization losses encouraged the industry to refine the original product. Two approaches were taken. One was to produce a dilute acid product, which was less corrosive, but required high application rates to be effective. A second approach was to neutralize the acids. Neutralized propionates include ammonium, calcium or sodium salts of propionic acid or use a buffer that will associate with the free ion of the acid, thus making the product less corrosive and less volatile. Laboratory results indicate that neutralized acids are less effective in preventing fungal invasion of moist hay, however, because they do not volatilize during field application, the recommended application rates are similar to that of the acid that is not neutralized.

Ammonia preservatives, including anhydrous ammonia, cold-flo ammonia and urea, are fungistats. Anhydrous ammonia is the most commonly used of these products and requires that hay be covered in plastic at the time of and after application. Application rates of ammonia depend on hay moisture content, and are recommended to be approximately 2% of forage weight for hay that is 25 to 30% moisture (Thorlacius and Robertson, 1984). Cold-flo ammonia is applied by converting the gas to a liquid and vapor mixture that is metered into a plastic covered stack at similar application rates. The volatile and caustic nature of these fungistats requires extreme user caution. Urea can be applied as a granular at the time of baling or at the time of stacking, however, the release of the active agent, ammonia, is dependant upon the presence of bacterial ureases.

Ammonia will inhibit most yeast and mold growth in baled hay, however, the fact that some bacteria and heat resistant fungi will proliferate in this preservation system can present problems. Hay spoilage is frequently observed in the bale or stack when the ammoniated forage is exposed to condensation or moisture infiltration from rain and snow. Also, the chemical reaction associated with ammoniation can generate enough heat to cause an undesirable browning of the forage. Hay ammoniation, on rare occasions, has been associated with the development of a nervous disorder in livestock consuming high quantities of the treated forage. The factors contributing to this condition, commonly referred to as crazy cow syndrome or bovine bonkers are not known (Nielsen et al., 1993).

Bacterial inoculants are a well accepted tool in forage ensiling systems because they meet all the criteria of a desirable preservative agent. The potential use of inoculants in hay systems is a relatively recent advancement in forage preservation. Many of the early studies focused on lactic acid anaerobes commonly used in silage preservation, including the genera *Lactobacilli*, *Pediococci*, and

*Streptococci*. A wider range of organisms, which includes the genera *Bacilli*, are currently being investigated, based on their ability to survive in the early stages of storage within the bale micro environment and on their ability to inhibit or modify fungal activity.

Although bale characteristics are well defined, the micro environments within the bale are not understood. For example, the move to larger more dense bales often referred to as large round bales, large square bales or densified bales can result in much lower oxygen potentials due to respiration, by either the plant or the indigenous bacterial populations. These physical characteristics, as well as bale stacking immediately after baling make moisture escape (a byproduct of heat generation) more difficult. Therefore, the emergence of pockets that support the high  $a_w$  and high temperatures optimal for microbial growth can be expected even though the average moisture content of the bale would not indicate this. Advancement in our ability to use bacterial inoculants as effective hay preservatives depends upon a better understanding of the creation of these micro environments and the microbial activity supported by them.

Other types of hay additives include sugars, protein and other nutrient supplements, and crude enzyme preparations designed to liberate nutrients from forage material for the purpose of enhanced desirable microbial activity. Antioxidants such as butylated hydroxy anisole, ascorbic acid, propyl gallate and butylated hydroxy toluene are sometimes included in hay additives to maintain the green coloration of hay and to enhance the effect of organic acids.

## CONCLUSION

Growing conditions, harvest conditions and storage conditions can influence the chemical constituents and microbial profile of forage conserved as hay. Research efforts to improve our ability for production of a hay product that will optimize the use of this important energy and protein source in livestock feeding is a well defined need by livestock nutritionists. Many of the improvements made in hay harvest have indirectly led to increased uniformity in the wilting and storage conditions associated with hay production. This increased product uniformity is advantageous from the perspective of product marketing and feeding. However, there is still a need to more clearly understand the processes associated with forage quality decline due to microbial actions. Development of forage plants with increased resistance to microbial attack during harvest, production of additives to control microbial activity, and continued efforts to develop equipment and storage facilities that result in minimal adverse microbial action are critical components to further advances in economic production of high quality hay.

## REFERENCES

- Albert, R.A., B. Huebner and L.W. Davis.** 1989. Role of water activity in the spoilage of alfalfa hay. *J. Dairy Sci.* **72**:2573-2581.
- Bossuyt, C.V., K.M. Wittenberg and G.H. Crow.** 1996. Effect of fungal biomass in alfalfa hay on intake and total tract digestion in growing beef calves. *J. Anim. Sci.* **74**:1336-1342.
- Breton, A. and P. Zwaenepoel.** 1991. Succession of moist hay microflora during storage. *Can J. Microbiol.* **37**: 248-251.
- Broderick, G.A., S.M. Abrams and C.A. Rotz.** 1992. Ruminal in vitro degradability of protein in alfalfa harvested as standing or baled hay. *J. Dairy Sci.* **75**:2440-2446.
- Chiquette, J., P. Savoie and A Lirette.** 1994. Effects of maceration at mowing on digestibility and ruminal fermentation of timothy hay in steers. *Can. J. Anim. Sci.* **74**:235-242.
- Colbentz, W.K., J.O. Fritz, K.K. Bolsen and R.C. Cochran.** 1996. Quality changes in alfalfa hay during storage in bales. *J. Dairy Sci.* **79**:873-885.

- Corry, J.E.L.** 1977. Relationships of water activity to fungal growth. p. 45-82 In: Beuchat, L.R. (ed) Food and beverage mycology, 2nd ed. Van Nostrand Reinhold, New York.
- Crump, S.V.** 1985. Potassium carbonate and forage drying. M.Sc. thesis. Univ. of Alberta, Edmonton, AB.
- Denizel, T.E.J. Rolf, B. Jarvis.** 1976. Moisture-equilibrium relative humidity relationship in pistachio nuts with particular reference to the control of aflatoxin production. *J. Sci. Food Agric.* **27**:1027-1034.
- Duchaine, C., M.C. Lavoie and Y. Cormier.** 1995. Effects of a bacterial hay preservative (*Pediococcus pentosaceus*) on hay under experimental storage conditions. *Appl. Environ. Microbiol.* **61**:4240-4243.
- Festenstein, G.N.** 1971. Carbohydrates in hay on self-heating to ignition. *J. Sci. Food. Agric.* **22**:231- 234.
- Hlödversson, R. and A. Kaspersson.** 1986. Nutrient losses during deterioration of hay in relation to changes in biochemical composition and microbial growth. *Anim. Feed. Sci Technol.* **15**:149-165.
- Hong, B.J., G.A. Broderick and R.P. Walgenbach.** 1988. Effect of chemical conditioning of alfalfa on drying rate and nutrient digestion in ruminants. *J. Dairy Sci.* **71**:1851-1859.
- Kaspersson, A., R. Hlödversson, U. Palmgren and S. Lindgren.** 1984. Microbial and biochemical changes occurring during deterioration of hay and preservative effect of urea. *Swed. J. Agric. Res.* **14**:127-133.
- Koegel, R.G., R.J. Straub, K.J. Shinnors, G.A. Broderick and D.R. Mertins.** 1992. An overview of physical treatments of lucerne performed at Madison, Wisconsin, for improving properties. *J. Agric. Eng. Res.* **52**:183-191.
- Kraynyk, P.D. and K.M. Wittenberg.** 1989. The effect of potassium carbonate, a drying agent, on storage of alfalfa and on the performance of growing lambs. *Forage and Grassl. Conf. Guelph, Canada.* p. 272-276.
- Lacey, J.** 1989. Pre- and post harvest ecology of fungi causing spoilage of foods and other stored products. *J. Appl. Bacteriol. Symposium Supp.* 11s-25s.
- Lin, C., K. Bolsen, B.E. Brent, R.A. Hart and J.T. Dickerson.** 1992. Epiphytic microflora on alfalfa and whole-plant corn. *J. Dairy Sci.* **75**:2482-2493.
- MacDonald, A.D. and E.A. Clark.** 1987. Water and quality loss during field drying of hay. p. 407 - 436. In: *Advances in Agronomy*. Vol. 41. Academic Press
- Michalet-Doreau, B. and M.Y. Ould-Bah.** 1992. Influence of hay making on in situ nitrogen degradability of forages in cows. *J. Dairy Sci.* **75**:782-788.
- Miller, L.G., D.C. Clanton, L.F. Nelson and O.E. Hoehne.** 1967. Nutritive value of hay baled at various moisture contents. *J. Anim. Sci.* **26**:1369-1373.
- Miller, D.A. and C.A. Rotz.** 1995. Harvesting and storage. In: *Forages*, 5th ed. Vol. 1: An introduction to grassland agriculture. Barnes, R.F., Miller, D.A. and Nelson, C.J. (eds.). Iowa University Press, Ames, Iowa.
- Nielsen, P., C. Friis, I. Kraul and C.E. Olsen.** 1993. Disposition of 4-methylimidazole in goats and heifers. *Res. Vet. Sci.* **54**:72-74.
- Petit, H.V. and G.F. Tremblay.** 1992. In situ degradability of fresh grass and grass conserved under different harvesting methods. *J. Dairy Sci.* **75**:774-781.
- Roberts, C.A., K.J. Moore, D.W. Graffis, H.W. Kirby and R.P. Walgenbach.** 1987. Chitin as an estimate of mold in hay. *Crop Sci.*, **27**:783-785.
- Rotter, R.G., A.A. Frolich, R.R. Marquardt and P.A. Mills.** 1989. Estimation of fungal contamination of cereal grains as determined by measuring glucosamine concentration. *Can. J. Anim. Sci.* **69**:235-246.
- Rotz, C.A. and R.E. Muck.** 1994. Changes in forage quality during harvest and storage. p. 828-868. In: G.C. Fahey (ed.). *Forage quality, evaluation, and utilization*. Am. Soc. Agron., Inc. Madison, WI.
- Savoie, P., M. Binet, G. Choinière, D. Tremblay, A. Amyot and R. Theriault.** 1993. Development and evaluation of a large-scale forage mat maker. *Trans. ASAE* **36**:285-291.
- Thorlacius, S.O. and J.A. Robertson.** 1984. Effectiveness of anhydrous ammonia as a preservative for high moisture-hay. *Can. J. Anim. Sci.* **64**:867-880.
- Undi, M. and K.M. Wittenberg.** 1996a. Effect of fungal biomass in moldy alfalfa hay on preference by dairy calves with no previous exposure to moldy feeds. *J. Dairy Sci.* **79**:1250-1254.
- Undi, M. and K.M. Wittenberg.** 1996b. Effect of dry matter content at baling on change in forage constituents during the storage of alfalfa hay. *Can. J. Anim. Sci.* **76**:599-605.
- Undi, M. and K.M. Wittenberg.** Occurrence of fungal species in stored alfalfa forage as influenced by moisture content at baling and temperature during storage. *Can. J. Anim. Sci.* (in press).
- von Keyserlingk, M.A.G., M.L. Swift, R. Puchala and J.A. Shelford.** 1996. Degradability characteristics of dry matter and crude protein of forages in ruminants. *Anim. Feed Sci. Technol.* **57**:291-311.
- Waite, R.** 1949. The relationship between moisture content and molding in cured hay. *Ann. Appl. Biol.* **36**:496-503.
- Weiss, W.P., H.R. Conrad and W.L. Shockey.** 1986. Amino acid profiles of heat-damaged grasses. *J. Dairy Sci.* **69**:1824-1836.
- Wilkinson, R.H. and C.W. Hall.** 1966. Respiration heat of harvested forage. *Trans. Am. Soc. Agric. Eng.*, **47**:424-427.
- Wittenberg, K.M.** 1995. Efficacy of *Pediococcus pentosaceus* for alfalfa forage exposed to precipitation during field wilting. *Can. J. Anim. Sci.* **75**:303-308.
- Wittenberg, K.M.** 1994. Nutritive value of high moisture alfalfa hay preserved with *Pediococcus pentosaceus*. *Can. J. Anim. Sci.* **74**: 229-234.
- Wittenberg, K.M., S.A. Moshtaghi-Nia, P.A. Mills and R.G. Platford.** 1989. Chitin analysis of hay as a means of determining fungal invasion during storage. *Anim.. Feed Sci. Technol.* **27**:101-110.