EFFICACY OF A *PEDIOCOCCUS SP.* BACTERIAL PREPARATION FOR TIMOTHY HAY PRESERVATION

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Suwarno

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EFFICACY OF A PEDIOCOCCUS SP. BACTERIAL PREPARATION FOR TIMOTHY HAY PRESERVATION

BY

SUWARNO

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

ABSTRACT

An experiment was conducted to assess the efficacy of a *Pediococcus sp.* bacterial preparation for preservation of timothy hay baled and stored at high moisture levels. Timothygrass was cut at heading and dried in windrows to achieve a moisture content of approximately 20% (L) or 30% (H). Hay baled at the 30% moisture level was subjected to one of two treatments at the time of baling; H-S was inoculated and H-Con was not inoculated. Hay baled at the lower moisture level was not inoculated (L-Con). The inoculant was a preparation of viable *Pediococcus sp.* bacteria, commercially available as Super-Hay (Biotal, Didsburry, Alberta) and was applied at the rate of 5 X 10^5 CFU/g hay DM. Test bales were stacked outside and protected from precipitation with a tarpaulin. Effect of treatment was determined by measuring bale temperature, change in forage chemical composition during storage, storage dry matter and nutrient retention and fungal biomass assessments. A lamb trial was conducted to compare dry matter intake(DMI) and digestibility of the hays after a 60 d storage period. Temperature measurements during the initial 33 days of the storage period showed higher ($P<0.05$) temperatures for hay in H-Con and H-S relative to L-Con.

Increased field drying time associated with L-Con relative to hays baled at a higher moisture level resulted in higher ($P<0.05$) neutral detergent fiber(NDF), acid detergent fiber (ADF), acid insoluble nitrogent (ADIN) and glucosamine levels but did not influence crude protein (CP) levels. Only NDF and ADF levels were greater ($P<0.05$) in L-Con relative to H hays after a 60 d storage period. Inoculation of H hay did not influence ($P>0.05$) the chemical composition at the end of the storage period. Total mold counts in hay after storage were similar among treatments. No differences were detected in terms of dry matter intake and feed nutrient digestibilities among the three hay treatments.

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ABBREVIATIONS

ADF	acid detergent fiber
ADIN	acid detergent insoluble nitrogen
BW	body weight
°C	degree Celcius
CFU	colony forming units
CP	crude protein
Con	control
DDM	digestible dry matter
DM	dry matter
DMI	dry matter intake
g	gram
gluco	glucosamine
h	hour
H-Con	high-moisture hay, control
H-S	high-moisture hay, inoculated
kg	kilograms
l	liters
L-Con	low-moisture hay, control
ml	milliliters
mg	milligrams
N	nitrogen
NDF	neutral detergent fiber
NH_3	ammonia

VDMI	voluntary dry matter intake
SE	standard error

INTRODUCTION

Timothygrass, a perennial forage plant, is grown throughout the U.S. and Canada. The grass ranks high in productivity, comparable to that of bromegrass and orchardgrass. In Manitoba, the capacity of production ranges from 4150 to 6125 kg DM/ha (Anonymous, 1987).

Besides being used as a pasture plant, timothygrass is also harvested as hay which is forage conserved at relatively low moisture content. In practice, forage is generally baled at 15-20% moisture. It has been reported that baling hay at more than 20% moisture can enhance microflora activity which is responsible for deterioration of the forage during storage, although this approach reduces field losses.

To reduce microflora activity during hay storage several types of additives have been evaluated in earlier studies, including the use of acids, organic salts, and ammonia. More recent studies have attempted to inoculate moist forage with bacterial preparations at the time of baling, however, the majority of the work is conducted with alfalfa as the experimental forage. The results, although variable, demonstrated some potential for bacterial preparations as moist hay inoculants.

This study was undertaken to establish efficacy for a bacterial preparation used to inoculate moist timothygrass forage at the time of baling in terms of storage change and animal intake and digestion responses.

LITERATURE REVIEW

Description and Origin of Timothygrass

Timothygrass (*Phleum pratense L.*) is a perennial, bunch grass, with its origins from Central Europe, northern Africa, western Asia, and Siberia. It was introduced into North America about the year of 1720 by Timothy Hanson, after whom it was named (Clark and Malte 1913). In North America timothygrass is widespread throughout coastal and central Alaska, the southern portion of the provinces of Canada, and the northern portion of the contiguous U.S.(Heath et al. 1985).

Timothygrass stems, which can reach a height of 80 to 110 cm (in Manitoba: 50-100 cm high), are smooth and erect. The leaves are flat-elongated, but short compared with the height of the plant. The spikelets are arranged in a dense, cylindrical, spikelike-inflorescence. Although it can reproduce through seed, timothygrass also reproduces vegetatively by means of tillers, arising from buds at the lower nodes of the stems. Its shallow, fibrous root system makes timothygrass a forage which requires moderately good rainfall conditions (over 400 mm/y) for survival (Walton 1983, Heath et al. 1985, Anonymous 1987).

Timothygrass does not perform well under heat and low

moisture conditions, heavy grazing conditions, salinity and droughts, however, like reed canarygrass, and meadow fescue, timothygrass tolerates flooding (Walton 1983, Anonymous 1987). As a temperate, cool-season perennial forage, timothygrass is adapted to cool, humid climates, and has good winter hardiness (Jerrel and Hanson 1973).

The time required for heading, anthesis, and maturity is affected by daylength. The onset of flowering was substantially reduced when the plant was exposed to short daily illumination (10 h), compared to a long daily period of illumination (14 h-day period). In addition, plants with short-day exposure showed reduced growth compared to those with long-day exposure (Allard and Evans 1941). The optimum temperature for growing timothy under controlled environment ranged from 18.3 to 21.6 C in one trial. Another trial showed day/night temperatures of 15/10 and 21/15 C were optimum (Heath et al. 1985)

Agronomic Appearance of Timothygrass

In the earlier decades,the use of timothy as hay was the primary method for harvest. Newer cultivars resulting from the breeding programs are greatly improved for pasture use. In 1919 timothy and clover made up 45.6% of all hays

harvested in the U. S., but only 21.5% in 1969 (Heath et al 1985).

It generally is grown in a mixture with clover, alfalfa, or birdsfoot trefoil. The first growth is frequently harvested for hay or silage, and the aftermath pastured (Heath et al. 1985). Timothygrass ranks high in productivity among the grasses, comparable to that of bromegrass and orchardgrass. The average yield of a mixture of timothygrass-alfalfa versus bromegrass-alfalfa were 1049.1 and 1365.1 kg DM/h respectively (Dale 1962). However, when timothygrass was grown in pure stands or in mixture with birdsfoot trefoil and red clover, it produced more DM than those of smooth bromegrass and orchardgrass. It was reported that when timothygrass was grown in Wisconsin as pure grass without N fertilization, the three-year average yield of 2 cuttings was 4,560 kg DM/ha. Dry matter yields of smooth bromegrass and orchardgrass handled in a similar manner were only 3,794 and 3,795 kg/ha, respectively. When timothygrass was cultivated in pure stands and fertilized with 90.8 kg N/ha the yield was 10,821 kg hay DM/ha, a little lower than those of orchardgrass (10,982 kg DM/ha), or smooth bromegrass (11,053 kg DM/ha). When timothygrass was grown in mixture with alfalfa-ladino, and with trefoil-red clover, the 3-year average yield of 2 cuttings were 8,466 and 7,182 kg DM/ha, respectively, comparable to that of smooth bromegrass-alfalfa-ladino and

orchardgrass-alfalfa-ladino of 8,485 and 8,050 kg dry matter/ha (Schmidt and Tenpas 1960). In Manitoba, timothygrass growth occurs mostly in the spring through summer periods (June-August), with the capacity of production ranging from 4500 to 6125 kg DM/ha. Dry matter yields of smooth bromegrass and orchardgrass in the same area were reported to range from 5600 to 8800 kg/ha and 3600 to 5000 kg/ha, respectively (Anonymous 1987).

Jung et al (1974) compared the DM yield of six perennial grasses. At high rates of N, timothy and tall fescue showed the best yields with five clippings, kentucky bluegrass with eight clippings, and reed canarygrass, orchardgrass and smooth broomgrass with three clippings. At low rates of N, timothygrass showed the best yields with five clippings, the other four grasses with three clippings, while clipping frequency had little effect on the yield of kentucky bluegrass.

A study conducted in British Columbia (Rode and Pringle, 1986), observed DM yields of timothygrass as a pasture plant under different precipitation levels. Moist conditions in May and dry, hot conditions in June, followed with damp conditions in July 1982 (precipitation was 63.3, 15.0, and 131.2 mm/mo. and average temperature was 8.8, 16.2, and 16.3° C/mo. respectively), resulted in DM yields ranging from 450 kg/ha in June to 860 kg/ha in July. In late June and July, DM yield of

timothygrass averaged 250 kg/ha more than that of meadow foxtail, while they were similar during the other months. The even precipitation distribution during the growing season resulted in uniform DM production of timothygrass during that time period.

Stand persistence and aftermath yields are important in managing timothy for profitable production. Food reserves required to overwinter plants in good condition are influenced by time of harvest and soil fertility. After the first cut the primary haplocorm gives rise to new tillers and secondary haplocorms. The haplocorm is a bulb-like structure at the base of the stem, used by the plant to deposit food reserves. After defoliation new sets of buds form, and the timothygrass overwinters as tertiary shoots (Sheard 1968).

Application of N has been shown to decrease dry weight of primary haplocorms, increase dry weight of secondary haplocorms, and decrease dry weight of tertiary shoots. Poor regrowth of tertiary shoots can be expected if they are low in N. A management system that includes harvesting at the early head stage in combination with high N applications results in a consistent reduction in timothy stands (Heath et al. 1985).

Nutritive Value of Timothygrass

Timothygrass cut for hay will produce maximum DM yields when cut at the postheading stage. Only when some other variable such as lodging is taken into account does timothygrass sometimes produce less at this stage (Heath et al. 1985). Brown et al. (1968), compiled by Lechtenberg and Hemken (1985), compared DM yield (t/ha), digestible DM (%) and digestible CP (%) for different stages of harvest, from prejoint to postbloom, of timothygrass var. Climax stands at three locations. They found that the highest DM yield generally was at the postbloom stage (7.5-10.4 t/ha), and the lowest was at the prejoint stage (2.5-7.4 t/ha). The reverse was true for digestible DM (55.3-56.3 vs 76.4-84.9%) and digestible CP (4.5-4.7 vs 22.7-26.6%) for postbloom relative to prejoint stage, respectively.

Even though the quality of timothygrass can be influenced by location, fertility and cultivar, the stage of harvest is the most important management variable. Colovos et al.(1949) found that early cut hay was able to furnish 3.2 times as much digestible CP and 1.25 times as much metabolizable energy as late cut timothy hay. Crude protein content of timothy can reach as high as 20% DM at the juvenile stage of growth, and decreases down to 7% at the post heading stage (Walton 1983).

Abdalla et al. (1988) reported that the nutrient content

of timothygrass var. Climax (% DM basis) was 13.6, 63.6, 34.6, and 4.96 for CP, NDF, ADF, and acid detergent lignin (ADL), respectively, when grown in monoculture. He also noted that the increase in precipitation just before harvest might be responsible for an increase in the soluble fraction of CP. Application of 144 kg actual N fertilizer/ha after the first harvest was assumed responsible for the increased CP content of the second harvest.

The Purpose of Making Hay

The origin of hay making is not well understood, but the belief is that it has developed very early in history. For instance, the Roman writer Columella described the process about 2,500 years ago. Until the end of the 19th century, hay making practices remained unchanged (Walton, 1983)

Hay making today is still demanding in terms of energy, time and human effort. Only quite recently have a great many new hay-making systems been evolved. This still remains an important area of challenge, since the improvement of hay-making systems could decrease the production cost, while on the other hand, improve the quantity and quality of the product.

The purpose of hay production is to conserve and store

forages at a relatively low moisture content, with minimum dry matter loss, minimum quality loss, minimum expenditure of money and time, and maximum stability of the quality during storage (Walton 1983). MacDonald and Clark (1987) mentioned that the objective of hay making is to conserve the yield and nutritional value of fresh-cut forage by drying it as quickly as possible to a moisture level at which the activity of microbial decomposers is halted.

The moisture content theoretically required to prevent microbial activity is 10-12% (Nash 1978, in MacDonald and Clark 1987), but in practice, dry hay is baled and stored at from 15 to 20% moisture (Jones and Harris 1980, compiled by MacDonald and Clark 1987). Another report by Lechtenberg and Hemken (1985) states that for satisfactory storage, it was recommended that forage moisture content was less than 15%.

Historically, the benefit of forage conservation as hay is that it can be fed to the animal at any time of the year, held as a reserve in case of dry years, for the purpose of animal transports, or can be sold as a commercial feed. More recently, hay has been used throughout the year to feed livestock where zero grazing is practised (Heath et al. 1985). Michalet-Doreau and Ould-Bah (1992) found in their trial that forage conserved as hay was able to increase the percentage of soluble N slightly: 33.0% for hay relative to 29.9% for standing forages, respectively.

Hay is produced extensively throughout the U.S. and Canada, its production being greatest in those areas where ruminant livestock density is greatest. The major reason for feeding hay to animals is to provide energy for maintenance, meat and milk production, work, and other functions (Lechtenberg and Hemken 1985).

Forages Used for Hay

In general, almost all kinds of forages (Gramineae and Leguminoceae families) can be conserved as hay. To reduce the feed-related problems, however, it should be taken into account that some criteria must be met. The first and most important is that the forages are not toxic to the animal. The second and the third are that the forages should be palatable and nutritious. The fourth, but not least important, is that the forages are reasonably manageable; for instance, they can be dried quickly and baled easily.

The most commonly used forage in the legume family is alfalfa (*Medicago sativa L.*), whether used in mixture or pure hays. A great deal of research which uses alfalfa as its material has been conducted. Miller et al (1967) used alfalfa at different moisture levels for hay in their study. Ingalls et al (1977) treated alfalfa hay before storage using

propionic acid. More recently, Wittenberg and Nia (1990, 1991) and Wittenberg (1991) studied alfalfa hay of high moisture content by treating the hay with ammonia or bacterial preparations. Sweet clover (*Trifolium repens L.*), white clover (*Trifolium pratense L.*), alsike clover (*Trifolium hybridum L.*) and other legumes also have been conserved as hay.

A number of grasses have been dried and stored as hay. Smooth broomgrass (*Bromus inermis*), quackgrass (*Agropyron repens*), bermuda grass (*Cynodon dactylon L. Pers*) and timothygrass are some examples. Some research has been conducted using timothygrass as a forage in hay making. For instance Christen et al.(1990), Seoane et al.(1981), Hayhoe and Jackson (1974), studied hays by using timothygrass as one of the forages in their studies.

Procedure of Making Hay

Harvesting the Forage

In much of North America, hay making is the most risky operation undertaken by farmers. A farmer must be aware of the extent of quality loss in forage owing to weather damage or delayed harvesting, because during the harvest process forage is quite perishable. Timeliness of harvest is essential to obtain high-quality hay (Nicholson 1983).

Maturity stage at harvest is the most important single factor determining the digestible dry matter (DDM) percentage of hay, whatever species the forage is (Lechtenberg and Hemken, in Heath et al. 1985). Cool season grasses and legumes often contain 80-85% DDM during the first two to three weeks of spring growth. The DDM declines by 0.3-0.5% daily thereafter until the DDM is less than 50% (Moore and Mott, 1973 in Heath et al. 1985). Maturity at harvest not only affects the DDM, but also influences hay consumption by animals. Using timothygrass and quackgrass hays, Christen et al.(1990) found a significant decrease in voluntary dry matter intake (VDMI) as the maturity of the forages advanced. The VDMI of timothygrass was 2.82% BW/d at the joint stage of growth and down to 2.62% BW/d at the early heading stage. For quackgrass, the figures were 2.74% and 2.53% BW/d, respectively.

Forages are mechanically cut with a mower, and remain on the field as swaths or windrows. A swath is the cut forage lying in the field, while a windrow is a row of forage formed by raking a swath together or formed directly by a mower-conditioner or windrower. A high cut ensures that adequate number of buds are present for rapid regrowth after defoliation, thereby keeping the pasture productive. Also, the longer stubble (4 to 6 inches) holds the swath off the ground, thereby allowing air circulation under the swath or windrow as well as providing a barrier to uptake of soil moisture by the cut forage (Dale et al. 1986).If the plant is cut near the ground level, leaving only a few stem buds, crown buds will develop and grow more slowly than stem buds, resulting in slow regrowth rates (Walton 1983).

Mowers for cutting the standing crop are common to all methods of harvesting. The cutter bar mower is the most common and is used alone or incorporated into assemblies involving windrowers, conditioners, combinations of these two, or forage choppers. Conditioners pass freshly cut hay between smooth or corrugated rollers under pressure. This process crushes and opens the stems so that they dry at a rate approaching that of the leaves. Thus leaves are less likely to become overdry and lost in subsequent handling (Heath at al. 1985). Horizontal rotary mowers of multiple disc type, flail type mowers, and single-knife reciprocating mowers have recently been

introduced (Dale et al. 1986).

Drying

In practice, the crop in the field contains from 90% moisture content by weight for young, immature crops, down to 75% for mature, more fibrous forage (Walton, 1983). Robertson (1983), states that successful storage of hay requires drying to a moisture content of approximately 20%. In order to reach a 20% moisture content, the freshly cut forage is allowed to wilt which may take from a few hours up to several days depending primarily on forage maturity and weather conditions.

Direct windrowing of hay increases field curing time compared to allowing partial curing in a swath before raking into a windrow. However, combination of a conditioner with a windrower reduces windrow drying time to a level which could have been achieved with unconditioned hay in a swath. Windrowing also protects leaves from the rapid overdrying to which they are exposed in a swath (Heath et al. 1985).

Walton (1983), states that the size and compaction of a windrow is important in relation to climatic conditions which exist when the hay is being dried. In addition to the moisture content in the herbage when it is cut, water will also be formed by the oxidation of the plant sugars. A wetter plant could be expected following cutting if a tightly packed

windrow is made. Also, surface drying of a tightly packed windrow results in a large difference in water content between the top and the center of the windrow. This may cause the shedding and shattering of leaves on the surface when the windrow is being handled. Prolonged respiration due to wetter conditions in the center of tightly packed windrow may also result in up to 10% loss of DM.

Drying can be done on the field if the weather permits, and in the stack or barn by artificial drying with heated air and blower (Walton 1983). Alli et al.(1985), compared the changes associated with the field wilting of lucerne and timothygrass in swaths at drying times ranging from 0 to 52.5 hours at St Anne de Bellevue, Quebec. The loss of moisture from lucerne was relatively rapid, dropping from 73 to 33% during the first 10 h following the harvest of the crop, when the weather conditions were excellent. The rain which fell during the following period of drying resulted in a slower rate of drying; during the remaining 42.5 h the moisture content only dropped from 33% to 25%. The loss of moisture from timothygrass 10 h after harvesting dropped from 69% to 38% with identical weather conditions, and dropped from 38% to 22% during the remaining 42.5 h drying.

Water loss from cut forage has been increased by the treatments that disrupt the cuticle and cell membranes. Leshem et al. (1972) showed that chemically treated forage in the

laboratory dried to 50% moisture in approximately one hour and to 40% moisture in approximately two hours. Under the same drying conditions untreated forage dried only to 60% moisture in four hours. Hong et al. (1987) dried alfalfa using potassium carbonate (K_2CO_3) and potassium hydroxide (KOH) as the desiccant. Tested in the laboratory, time required to reach 60% DM content were 14.8, 8.1, and 8.0 h for control, K_2CO_3-treated, and KOH-treated forage, respectively. While time required to reach 80% DM content were 109.4, 46.8 and 45.5 h for control, K_2CO_3-treated and KOH-treated forage. Chemical composition of laboratory dried hay was not affected by drying agent treatment. When dried in the field, the drying times also were significantly enhanced by the drying agents. Average field drying time to reach 80% DM was shortened by 12 h with KOH (from 55.5 , control, to 43.5 h) and 14 h with K_2CO_3 treatment.(55.5 vs 41.3 H, respectively).

The effect of drying method on hay quality depends largely on the length of time required to reach a desired moisture content. Hay crops contain maximum nutrient content at the time of cutting. During curing (drying) metabolic activity, primarily respiration, decreases non-structural carbohydrate concentration and DDM percentage. Drying methods that minimize time needed to dry the forage also will minimize the decrease in DDM. The decrease in DDM under prolonged drying conditions can be attributed to leaching of soluble

nutrients due to rainfall, extensive respiration loss of non-structural carbohydrates, and physical loss of leaves (Klopfenstein et al. 1978, in Heath et al. 1985). Only 57% DDM was left from hay which needed 8-day drying in the field, compared to that of 67% DDM from hay dried with heated air (Reid et al. 1959). Michalet-Doreau and Ould-Bah (1992) compared in-situ degradation of perennial ryegrass. One group was cut and dried for 3 days without rain, another group remained on the ground for 6 days and was harvested after 3 day of rain. They found that rapidly degraded fraction was greater (56.2%) for hay with no rain than for hay subjected to rain (37.5%), as was true for N degradability (82.9 relative to 80.3%, respectively). The reverse were true for slowly degraded fraction (42.8 versus 60.2%, respectively), and undegraded fraction(0.9 versus 2.3%, respectively).

The length of time required to reach the desired moisture content in field drying depends mostly on weather conditions (rainfall,temperature, humidity, soil moisture, solar radiation and wind speed). It is generally conceded that a minimum of 3 good drying days are required to dry hay to the stage where it can be baled (Nicholson, 1981). A day is a good drying day if 2 conditions are met: less than 12.7 mm. of rainfall on the previous day; and the value of the drying index based on potential evaporation is greater than or equal to 4.2 mm (Hayhoe and Jackson, 1974). Tables prepared for

Environment Canada, defined a dry day as a day with less than 1.00 mm of rainfall. The probability of 3 consecutive dry days at Frederickton, New Brunswick, in June and July was only 0.15 (Treidl, 1981, in Nicholson, 1981).

The drying behaviour of plant material is influenced by plant species, stage of growth, leaf-to-stem ratio and the structure and volume of the swath or windrow which act as a barrier to removal of water from plant tissues. The energy for the drying process is derived from the self-heating of the crop through continued respiration. Respiration is assumed to end when the life-supporting plant constituents become exhausted after prolonged exposure at slow drying rate. Solar radiation and wind can elevate the moisture loss from the crop (Klinner and Shepperson 1975). Consequently, temperature, number of sunshine hours, and the prospect of rain at hay-making time are all important in determining harvesting methods and equipment. The rate and the total amount of drying which might be expected in a swath or windrow in a particular environment should match the harvesting method used (Walton, 1983)

Baling and storage

Compressing hay into high-density packages such as bales greatly facilitates its transportation, handling and storage.

Such high-density hay packages are usually formed with relatively dry hay. However, except for storage problems, forming high-density packages of high moisture content hay could be more desirable. It would take advantage of lower field losses associated with high-moisture hay production (Nelson 1972).

It was said by Walton (1983), that the bale is the most widely used method for making hay. The automatic baler machine, which came up into use in about 1940, picks up the swath or windrow and compresses it into a package that is easier to handle than loose hay. In general, two types of balers have been recognized; rectangular balers and round balers (Walton 1983, Dale et al. 1986).

The small rectangular baler that produces bales weighing in the range of 18 to 30 kg. has been the most popular type of machine. Walton (1983), wrote that the traditional bale weighs about 24 kg and has a density about 160 kg/m^3. Balers are available, however, that produce a wide range of rectangular bale sizes and densities, including units that produce a very large rectangular bale weighing 681.8 to 909.1 kg (Dale et al. 1986). The baler takes up hay from the swath or windrow, compresses it into rectangular packages, and ties the packages with either two or three bands of wire, hemp, sisal, or plastic (Heath et al. 1985). Dale et al.(1986) reported further that the large round balers have received relatively

wide acceptance primarily because baling hay with a round baler requires one-half the labor compared to using a small rectangular baler. The large round balers roll the windrow into a large round cylinder and consequently can bale much faster than the small rectangular bales. The large round bales are somewhat impervious to surface water and, therefore, they can be left in the field for a few days following baling (Kjelgaard et al. 1981, in Dale et al. 1986).

In spite of these distinct advantages, large round bales have one serious disadvantage in that such bales frequently result in poor quality hay. Large round bales require lower moisture levels to avoid microbial activity and degradation during storage than is the case with small rectangular bales. Hay baled with a large round baler must be less than 17% moisture to avoid microbial activity and degradation during storage as compared to 20% for the small rectangular bale. Baling hay at a lower moisture level than 20% may result in DM losses as high as 25% during the baling operation (Dale et al. 1986).

Another problem arising from the large round bales is susceptibility to DM loss due to the manner in which they are stored. Experience showed that if the bales are stored outdoors in a climate where freezing and thawing are frequent during winter, losses can be high (Dale et al. 1986). Belyea et al. (1985) reported DM loss of 2% for large round bales

stored indoors, 6% for large round bales stored outside under cover, and 15% for large round bales stored uncovered outside. The large DM losses for bales stored outside uncovered, are due to in part, to penetration of precipitation to the depth of 10 to 25 cm. This loss was accompanied by a feeding loss as high as 13 to 20% (Belyea et al. 1985). Lechtenberg (1978), in Dale et al. (1986), observed DM losses approximating 23% of the initial bale weight after some 9 months of storage on the ground. Elevating the bales onto a crushed rock surface reduced the loss to 14% of their initial weight. Bales stored under cover, off the ground lost 8% of their initial weight. Dale et al. 1986, stated that the high DM loss of large round bale stored without cover, are further amplified by chemical changes that result in serious losses in the nutritive value of the stored hay.

Losses are higher in low-density bales than they are in high-density bales. Kemp (in Nicholson 1981) compared hay harvested in three forms; 450-kg round bales, 340-kg round bales, and 680-kg stacks. Storage DM losses and feed refusals were 13% for the large round bales, 28% for the small round bales and 18% for the stacks. On the basis of a 3-year study, he concluded that large round bales at baling should have a moisture content of 20% or less and should be made as dense as possible for outside storage. Loose bales suffer excessive weather damage especially during winter. Storage of bales of

more than 20% moisture content may result heating in dense bales (Nicholson, 1981).

It is likely that high quality hay is difficult to make with large round baler. A decision to use such a unit is apparently a compromise between the lower manual labor involved and the speed of the baling operation when using a large round baler relative to the quality of hay required by the consuming animals (Dale et al. 1986).

Nutritive value of hay and factors affecting it

Walton (1983), stated that the nutritive value (quality) of hay as forage is the amount of nutrient materials that an animal can obtain from the hay in the shortest possible time. According to Lechtenberg and Hemken (1985), the quality of hay must be determined by those characteristics of the hay that affect consumption and utilization by animals. Therefore, animal production can be viewed as the critical measurement of hay quality.

There are several ways in which hay quality may be measured. First is by chemical analysis, which aims to study nutrient composition of the hay (carbohydrates, proteins, minerals, and vitamins). The second is by measuring the fiber

content of the hay in relation to DDM. A third method is determination of the extent and rate of digestion of hay materials. Fourth, by measuring DMI of the hay for a given animal, which is closely related to liveweight gain (Walton 1983). Raymond (1969), recognized three general categories of feed evaluation: digestibility, consumption and the efficiency with which consumed and digested feed is used for productive purposes.

Many factors affect hay quality and no one factor or characteristic can satisfactorily predict animal production. Important factors that determine hay quality include (1) maturity stage, (2) forage species, (3) chemical composition, (4) leaf:stem ratio, (5) physical form, (6) foreign material, (7) damage or deterioration during harvest and storage, and (8) presence of antiquality constituents such as alkaloids or toxic entities (Lechtenberg and Hemken 1985).

The time of harvesting in relation to the stage of physiological development of the plant has a very pronounced effect on hay quality (Walton, 1983, Seoane et al. 1991). As the plant matures, CP values fall while crude fiber rises. Data from the University of Alberta showed a decrease in CP of smooth bromegrass from 19.2 to 6.7% DM basis, and an increase in crude fiber from 19.8 to 27.2% DM basis, when age increased from 6 to 10 weeks (Walton 1983). Stone et al. (1960) found that first growth, cool-season grasses and legumes often

contained 80-85% DDM during the first two to three weeks of spring growth. The DDM declined by 0.3 to 0.5% unit daily thereafter until DDM was less than 50%. Using timothygrass and quackgrass hays at joint and early heading stages of growth fed to sheep, Christen et al. (1990) found a decrease in CP content from 12.7 to 10.8% for timothygrass and from 19.2 to 13.9% for quackgrass. This was accompanied by an increase in NDF, ADF and ADL in both grasses respectively, followed by a decrease in dry matter intake (DMI) from 2.82 to 2.62% body weight (%BW) for timothygrass and from 2.74 to 2.53 ($P<0.01$) for quackgrass, as the ages advanced. Significant decreases in gross energy intake, crude protein intake and NDF intake due to age of the grasses were also noted. Seoane et al. (1991) showed a constant decrease in nutritive value of timothygrass and bromegrass hays harvested at 4 different stages of growth from vegetative to seed, expressed in terms of digestible DM intake, digestible protein intake, digestible energy intake or TDN intake. Marked differences were observed between forages harvested at the vegetative stage and those harvested at post heading stage. They also found the nutritive values were highly correlated with day of harvest ($r=0.90$ to 0.98 for %CP, $r= -0.74$ to -0.85 for %ADF, and $r=-0.88$ to -0.94 for %ADL).

Differences in quality among species and cultivars at the same maturity and when harvested under similar harvesting and handling schemes have been documented (Minson et al. 1961, in

Maurice et al. 1985). Marten et al. (1976), mentioned that these differences are generally related to differences in structural carbohydrate constituents, leaf percentage, or presence of secondary metabolites that affect digestibility and acceptance of hay by animals. They identified that none of the steers grazed on low-alkaloid reed canarygrass (*Phalaris arundinacea L.*) pastures suffered from diarrhea, while 50% of the population grazed on high-alkaloid reed canarygrass pastures did. Porter et al (1978) compared fiber composition of mutant and normal sorghum. Of 13 mutants evaluated, 10 had a significantly lower lignin content compared to normal sorghum, 6 of these had a lignin content that ranged from 18 to 51% of normal sorghum. Similar results were found by Fritz et al (1981), in sudangrass and grain sorghum mutants. Christen et al. (1990), showed significant differences in CP content between timothygrass and quackgrass hays harvested at 2 stages of growth (12.7 vs. 19.2% at joint stage and 10.8 vs. 13.9% at early heading stage). However, apparent DDM of timothygrass was significantly higher at both stages of growth (70.2 vs. 69.5% and 66.0 vs. 64.8%, respectively). This was probably due, in part, to the lower NDF content of timothygrass relative to quackgrass at both stage of growth (63.2 vs 65.8% and 67.5 vs. 68.3%, respectively).

Major chemical constituents of hay include nonstructural carbohydrates (sugars and starch), CP, minerals, and

structural carbohydrates or fiber (primarily cellulose and hemicellulose). Concentration of non structural carbohydrates in hay may range from less than 5% to greater than 30% of the dry weight. These constituents are highly digestible when fed to ruminants and can also be digested by monogastric animals. For this reason, hays containing relatively large concentrations of nonstructural carbohydrates are generally high-quality hays. The high fiber content of hays restricts their utilization by animals. Rumen bacteria are able to digest cellulose and hemicellulose and convert these constituents into metabolic products useful to the animal. But these bacteria are unable to significantly degrade lignin. In addition, lignin interferes with or limits the capacity of bacteria to digest cellulose and hemicellulose. Thus, the proportion of hay cellulose and hemicellulose to lignin may affect the variability of DDM which may range from 20% to 80% (Heath et al. 1985). Porter et al. (1978), showed that a unit increase in lignin often will result in a three to four-unit decrease in DDM percentage.

Plant leaves, as primary sites of photosynthesis, possess tremendous enzymatic activity. Thus, nonstructural carbohydrate and CP concentrations are generally much higher in leaves than in stems (Walton 1983, Heath et al. 1985). Stems are more fibrous than leaves, have large amounts of vascular tissue, are generally lower in CP and nonstructural

carbohydrates, and are lower in DE than leaves (Heath et al. 1985). The differences between CP percentage of leaf to stem in alfalfa have been documented by Nelson et al. (1989), who identified that at 10% bloom the leaf CP ranged from 27.7 to 29.6% DM basis, while stem CP content ranged from 10.6 to 12.5% DM basis. Youngberg et al. (1972) found similar results by showing that leaf CP concentration in alfalfa often is as high as 27% of DM. Thus, leaf:stem ratio can be used as a measure of hay quality. The change of leaf to stem ratio may, at least partly, be explained by studying grass development (Walton 1983). The major change which takes place in a grass as it ages is the elongation of the stem. The juvenile grass is composed almost entirely of leaves with very short internodes. Waite and Sastry, (1949) measured the leaf:stem ratio, CP, and crude fiber percentages of leaf and stem of timothy harvested at different stages of growth, from May 20 to July 14, 5 harvests with 2-week intervals. They found a decrease in leaf:stem ratios from 2.57 (May harvest) to 0.20 (July harvest), and a decrease of leaf CP from 21.7 to 11.1% DM, a decrease in stem CP from 14.1 to 3.4% DM, and an increase of crude fiber from 19.1 to 30.6% DM for leaf and from 23.5 to 32.4% DM for stem, respectively. These changes arise primarily from the development of the structural carbohydrate materials, consisting mainly of cellulose, hemicellulose and lignin (Walton, 1983). Alfalfa leaves

harvested at the maturity stage may contain 85% DDM, while stems at the same stage of maturity may contain only 45-47% DDM (Sotola 1933). Leaf percentage also is a good predictor of intake by animals (Heath et al. 1985). Leaves, especially legume leaves, are more fragile and are more easily lost in mechanical handling than stems, during hay making. Leaves also dry more rapidly than stems and since they contain more nutrients, are subject to greater nutrient losses during curing than are stems (Heath et al. 1985).

The physical form of hay fed to animals affects two important determinants of animal production: amount of energy animals obtain from a unit of hay and amount of hay consumed. The chemical constituents of hay, that is, the relative proportions of cellulose, hemicellulose, lignin, and nonstructural carbohydrate affects the DDM percentage of hay. Because hay is incompletely and slowly digested and because it is digested in a dynamic system that also involves passage through the digestive tract, particle size and density of the consumed product affect both digestibility and passage (Heath et al. 1985). Mertens and Ely (1979) found that fiber digestibility, which occurs mostly in the rumen, is normally greatest with long, unchopped hay. Grinding to reduce particle size prior to consumption by animals generally hasten passage through the digestive tract (Meyer et al. 1959). Meyer et al. (1959) also noted that voluntary DMI was consistently greater

for ground-pelleted hay than for chopped hay, when fed to fattening lambs, steers, and lactating cows (2.48 vs 3.25 kg/d, 14.0 vs 17.1 kg/d, and 26.4 vs 33.3 kg/d, respectively), however, grinding depressed milk fat content. Voluntary hay consumption by animals often is 10-30% greater for ground hay than for long or coarsely chopped hay (Ronning et al. 1959), and these differences are generally greater for low-quality than for high-quality hays (Heaney et al. 1963).

Aids for Hay Preservation

Microbial growth in moist hay can be prevented by treatment with preservatives (Walton 1983, Heath et al. 1985), which can minimize respiration losses as well (Walton 1983). Ideally, a preservative or fungicide must meet the following criteria: inhibit mold growth in the forage at moisture contents higher than 20%, cheap enough to compete with other methods of preservation, not harmful to the farmer or animals, no decrease in DMI and DMD, and no deleterious side effects, such as residues in milk or meat (Kennedy and Schenk 1954). Examples of the kinds of preservatives used for hay production include acids, ammonia and bacteria preparations.

Acids

Several kinds of acids were used to preserve moist hays. Knapp et al. (1976) reported that propionic acid reduces heating and storage losses and preserves the quality of high-moisture (32.4% H_2O) alfalfa hay when applied at a rate of 10 kg/t DM (1% of forage DM), but lower application rates were not effective. Jafri et al. (1979) identified that a mixture of 70% propionic acid and 30% formalin was effective on alfalfa hay (28% H_2O) at an application rate of 1% of the hay as baled. Davies and Warboys (1982) treated forage of 56.6%DM with 47 g propionic acid/kg forage DM (4.7% of forage DM) and forage of 65.1%DM with 43 g propionic acid/kg DM (4.3% of forage DM). The untreated forage of 65.1%DM was as control. Compared to control, the treated forages had significantly higher water soluble carbohydrate (WSC) concentration (60, 55, and 22 g/kg DM respectively for treated forages of 56.6%DM, 65.1%DM and control), but there were no significant differences in DMI and liveweight gain when fed to sheep. In another experiment, however, they found hay (64.1%DM at baling) treated with 35 g propionic acid/kg DM (3.5% of forage DM), which was higher in WSC concentration compared to field cured hay (93 relative to 57 g/kg DM), and gave a significantly higher value for DMI and liveweight gain (Davies and Warboys 1982). Rotz et al. (1990) observed that propionic acid treated alfalfa hays (22.8 to 29.7% DM at baling) with an

moisture content at baling) and dry hays (14-20% moisture content) with either propionic acid, formic acid, acetic acid, or a mixture of propionic and acetic acid marketed under the trade name Chemstor. The levels of the acids used were between 0.05 to 0.11% of forage, on a fresh weight basis. In general all the acids inhibited mold growth, with the exception of acetic acid applied to clover baled at more than 30% moisture. The acid mixture performed better than pure acid treatments. Nehrir et al. (1978) reported that ewes fed alfalfa hay treated with 1.0% Chemstor gained more weight than those fed heat-dried hay (2.3 relative to 1.3 kg in 10 days). Woolford (1984) reported that formic acid is slightly more effective against actinomycetes than sodium diacetate. Russell and Buxton (1985) treated alfalfa-grass of 19.0 and 15.6% moisture with 1.25 g sodium diacetate/kg wet weight of the forage (0.15% of forage DM). The forages were baled in large round bales and stored for 24 weeks. Although sodium diacetate reduced the concentrations of ADL and ADIN after storage, the additive did not significantly influence the recovery of total DM and DDM.

The use of acids as preservative for moist hay can improve hay quality, however, several precautions should be taken into account. The corrosiveness of the acid, the proper concentration applied, the possibility of residual effect on animal products and the cost of handling are potential

limiting factors that should be considered in the use of acid as hay preservative.

Ammonia

Anhydrous ammonia has been shown to kill mold spores (Bothast et al.1973), and if applied appropriately to high moisture hay, it prevents mold growth during storage (Knapp et al. 1975). High-moisture alfalfa hay treated with ammonia (Weiss et al. 1982) showed a higher CP content than untreated hay, and the ammonia-treated hay was readily consumed by the animal without any serious negative effects.

Koegel et al (1985) treated alfalfa bales of 18 to 50% moisture content with anhydrous ammonia (NH_3). The ammonia was injected to the bales on the basis of percentages of wet weight of forage. The bales of 25.5 to 27.5% moisture contents were injected with 1.1 to 2.0% NH_3. The peak temperature recorded from these bales was 35^{0}C and mold appearance was very rare. Other bales of alfalfa with moisture ranging from 18.6 to 25.1% were treated with NH_3 at rates ranging from 1.3 to 1.6%. The maximum temperature recorded was 32^{0}C which occurred in the first day of storage, and no mold was found. The 27.5% moisture bales were treated with 1.5% NH_3. The maximum temperature observed on the second day was 34°C. Significant mold was found only at isolated locations, such as where bales had touched the concrete floor or at the surface

of the stack, apparently due to condensation at a surface cooler than the interior of the stack. Several bales of the moisture content ranging from 26.0% and up, treated with 1.4 to 2.6% NH_3 approached the temperature of 60°C, observed almost immediately after ammonia injection. These bales showed insignificant molding, but were dark brown in color. Koegel concluded that in general, the ammonia acted as a good mold inhibitor.

Jones et al. (1985) compared anhydrous ammonia (NH_3) and sodium diacetate treatments of mature fescue (Festuca sp.) at 18 to 20% moisture, baled in large, round bales. All treatment groups suffered significant losses in DM during storage. Hay treated with NH_3 showed the lowest DM loss compared to control or sodium diacetate treatment. After 120-d storage,in vitro DMD (IVDMD) and chitin were observed. Chitin is a major component of mold's cell wall, therefore, hay with high chitin concentration is not desirable. In vitro DMD did not change significantly over the storage period in the control group. The largest change in IVDMD was found in the NH_3 group which was 42% before storage and 46% after storage. All hay groups showed a significant increase in chitin during storage,the CC group being the highest of the three.

Similar results for IVDMD were observed by Moore et al. (1985), who found an increase in IVDMD for NDF, ADF, cellulose and hemicellulose in orchardgrass hay baled at 10, 30, or 50%

moisture, treated with ammonia with application rates of 1.2, 2.4 and 3.6% of forage DM, respectively. Wittenberg and Nia (1990) reported that application of NH_3 at the rates of 3.0 and 3.2% forage DM to moist alfalfa hays (23.9% and 29.9% moisture contents at baling), resulted in the highest DM, CP, and NDF retention values during storage relative to untreated forage or forage treated with bacterial preparations, but the application of NH_3 did not improve DMI, DDM, digestible CP, and digestible ADF of the forage. Wittenberg (1991) observed in further trials that NH_3 applied to moist hays, at the rates of 2.8 and 2.5% forage DM, improved the visual assessment of the moist hay for mold and dust relative to dry (15-20% moisture) hay, but chitin concentration as a measure of the extent of fungal invasion was not reduced with the use of the additive.

The trials suggested that in general ammonia is a good hay preservative. One limiting factor of ammonia as a hay preserver is its volatile, undesirable odour which may cause rejection of the hay by animals, or the odour can contaminate the milk of the dairy cows being fed the hay. It is prudent to uncover ammoniated hay for several days prior to feeding. Other limiting factors are the cost of ammonia and its handling.

Inoculants

Trials using bacterial inoculants have been successful to preserve forages as silage (Kennelly and Baars 1990). The one advantage of this kind of preservative is that it is relatively noncorrosive to the implements (Deetz et al. 1989). Some experiments have been conducted using bacterial inoculants as a hay preservative.

Nelson (1989a) treated large round bale alfalfa hay having 64.3, 73.4 or 84.7% DM content with 0.1% wet weight of inoculant, equal to 2 X 10^5 colony-forming units (CFU) of lactic acid-producing bacteria/g DM, *Lactobacillus plantarum* and *Streptococcus faecium*. Inoculant applications increased recovery of NDF at all moisture levels. Inoculant did not affect recovery of CP and IVDMD in 64.3% DM hay, but increased recovery for hay baled at 73.4% DM and decreased recovery of 84.7% DM alfalfa hay. In another trial Nelson et al. (1989b), showed that baling hay in small rectangular bales treated with the same level and the same kind of inoculant, resulted in no significant difference in DMI by wethers. Inoculation of 56.6% DM alfalfa tended to reduce loss of CP during storage of 42 days. Nitrogen digestibility was increased 4% units by inoculation of 56.6% DM hay but was decreased 4% units by inoculation of 73.5% DM hay.

Inoculants may contain either pure culture or a mixed culture of bacteria. Most of the information that has been

obtained is for mixed cultures. Nelson et al. (1989a,b.) used an inoculant containing *Lactobacillus plantarum* and *Streptococcus faecium,* Wittenberg and Nia (1990) used mixed inoculants similar to that used by Nelson et al. (1989a,b). Wittenberg (1991) also used several strains of *Pediococcus pentosaceus* to inoculate alfalfa hay. Limited work has been done with a nonviable *Pediococcus sp.* and *Lactobacillus* fermentation product, which was used as a nutrient source, facilitating desirable microbial activity in hay (Deetz et al. 1989, Wittenberg and Nia 1991). Wittenberg and Nia (1990) treated alfalfa hay baled at 25-30% moisture (H) and 20-25% moisture (M) with three kinds of inoculants. The first was a mixture of *Pediococcus acidilactici* and *Lactobacillus plantarum* inoculant, inoculated to H and M hays at the rate of 0.22 g/kg forage, approximately 0.02% of forage DM (1.8×10^5 CFU/g DM) for the H hay (H-SS) and 0.20 g/kg forage, approximately 0.02% of forage DM (1.6×10^5 CFU/g DM) for the M hay (M-SS). The second inoculant was a mixture of *L. plantarum* and *Streptococcus faecium* with the rate of 9.8 mg/kg forage DM (2.0×10^5 CFU/g DM) for H hay (H-SB) and 8.2 mg/kg DM forage (1.6×10^5 CFU/g DM) for M hay (M-SB), which was approximately 0.001% of forage DM. The third preparation was a product of a lactobacillus fermentation, which was non-viable, applied at a rate of 0.11 ml/kg forage DM for H hay (H-Cul) and 0.09 ml/kg forage DM for M hay (M-Cul). Bales were

stored outside in the stack for 60 days. The use of inoculants gave variable results. Applied to hay baled at 76.1% DM (M-SB), inoculant (SB) resulted in a decrease in acid detergent insoluble N (ADIN) levels post-storage, but there was no effect of this inoculant (SB) on ADIN content in high moisture hay. Inoculant SS applied to M hay (M-SS) did not improve DMI or DMD, however, inoculant SB gave some improvement in these parameters. Application of inoculant Cul to M hay (M-Cul) gave no significant improvement in post storage nutrient values, except for ADIN. However, DDM was improved by this inoculant. Calculation based on field and storage losses and digestibility trial showed that DDM yields (%) were 54.1, 48.5, 49.5, 52.4 and 53.1 for L-Con, M-Con, M-SS, M-SB and M-Cul, respectively. Although DM retention generally was not affected by inoculation in M hay compared to M-Con, soluble N and NDF retentions were increased by inoculations and acid-insoluble N retention was decreased by inoculation, except for M-SS.. Using similar moisture levels of forage, Wittenberg (1991) found that the application of *Pediococcus pentosaceus* preparation at the rate of 5.7 mg/kg DM forage (5.7×10^5 CFU/g DM) to M (20-25% moisture) hay resulted in lower post-storage CP level, but better visual assessment for mold invasion than that of untreated M hay. Inoculation of H (25-30% moisture) hay with a mixture of *Pediococcus acidilactici* and *Lactobacillus plantarum* at the rate of 6.6 mg/kg forage DM

(6.2 X 10^5 CFU/g DM) resulted in a consistent improvement in terms of DM, CP and ADF retention and visual assessment of the bales compared to those of untreated H hay. Post-storage chitin concentration was found to be higher in M-Con than L-Con. Medium-moisture hay inoculated with bacterial preparations contained lower chitin levels than ammoniated hay after 60-day of storage, but was higher than L-Con.

Some species of bacteria for example *Pediococcus sp,* have been produced commercially for forage preservation. Buchanan et al (1975) reported that bacteria of the genus of *Pediococcus* is cocci occurring in pairs or in tetrads as the result of alternate division along the two perpendicular planes. They are non motile, do not form endospores and gram positive microorganisms. The bacteria ferment simple sugars (galactose, maltose, glucose, etc) into lactic acid (Buchanan et al, 1975).

Pediococcus is facultative anaerobic. Some species, for example *Pediococcus cerevisiae* prefers anaerobic conditions while the other species for example *Pediococcus acidilactici* and *Pediococcus pentosaceus,* prefers the appearance of trace amount (<.20 atm) of oxygen (Buchanan et al, 1975).

The temperatures required for optimum growth of this bacteria ranges from 26 to 30°C, while pH requirement for the optimum growth ranges from 6.2 to 6.5 (Anonymous, 1989). It was stated by Brock (1979) that the minimum water activity for

the growth of most cocci is 0.90 (according to Albert et al, 1988, this is similar to about 26.4-36.0% moisture content in alfalfa). Water activity (a_w) is a measure of the water that a microorganism can use for growth and is defined as the ratio of the vapor pressure of water over a substrate to that of pure water at the same temperature and pressure (Albert et al, 1988). The highest value for a_w is 1.0 for pure water and are lower for solutions. Brock (1979) stated that water adsorbed to surfaces may or may not be available to microorganisms, depending on how tightly it is adsorbed and how effective the organism is in removing it.

It is still not well-understood whether the action of bacterial preparations against mold growth is through parasitism or competition. One speculation is that the bacteria compete with the mold for nutrients and oxygen. At the first moments in the stack, oxygen in the stack is used by mold and the living plant cells. At this moment, bacteria preparation is still relatively dormant. As the respiration continues, the O_2 in the stack decreases to a lower level, at which the facultative anaerobic bacteria preparation can tolerate better than mold. At this stage, bacteria begin to accelerate its activity, while the referse is true for mold. At higher moisture content (high water activity), the rate of growth of bacteria is greater than that of mold (Banwart, 1989). Hence at high moisture content bacteria will outgrow

the fungi. During this fastest growth stage, the bacteria might have generated heat that is able to decrease moisture content to a level that can prevent mold activity, or the bacteria might have produced a mycostatic compound that can inhibit mold growth.

The advantages of bacterial inoculant are that it is relatively inexpensive, easy to apply, not hazardious to man, animals and not corrosive for equipment. On the contrary, some disadvantages of inoculant are that it is less reliable and not enough information is available concerning mode of action.

Molds in Hay

Description.

The term mold is a common one applied to certain multicellular, filamentous fungi whose growth on food or organic matter is readily recognized by its fuzzy or cottony appearance. Molds belongs to the division of Eumycetes or true fungi, which has five classes: oomycetes, zygomycetes, basidiomycetes, deuteromycetes and ascomycetes (Alcamo 1991). They lack chlorophyll and are saprophytic which means that they feed on dead organic matter. The main part of the growth commonly appears white but may be colored, dark or smokey. Colored spores are typical of some kinds of mature mold and

these spores may give color to part or all of the growth.

The thallus, or vegetative body, is characteristic of thallophytes, which lacks true roots, stems, and leaves (Frazier and Westhoff 1988). The thallus consists of a mass of branching, interwined filaments, called hyphae. The whole mass of hyphae is called mycelium (Frazier and Westhoff 1988). The hyphae of some molds are full and smooth, but the hyphae of the others are thin and ragged. Microscopic examination of hyphae may be used to identify the genera of the mold. Molds are divided into two groups: septate organisms, for example *Aspergillus sp.*and *Penicillium sp.*, which have cross walls dividing the hyphae into cells; and nonseptate organisms, for example *Rhizopus sp.* and *Mucor,* which have hyphae that appear to consist of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout the length and are considered multicellular. The hyphae of most molds are clear, but some are dark and smokey. Hyphae may appear uncolored and transparent upon microscopic examination, but colored when large masses of hyphae are viewed macroscopically (Frazier and Westhoff 1988).

The cell wall is composed of cellulose and chitin. Chitin is a polymer of acetylglucosamine units, that is, glucose molecules containing amino and acetyl groups. Chitin gives the cell wall rigidity and strength (Alcamo 1991).

Mold can grow from a transplanted pie of mycelium.

Although reproduction of mold is chiefly by means of asexual spores, some molds also form sexual spores. The molds which have both types of reproduction systems are called perfect molds and are classified as either Oomycetes or Zygomycetes if nonseptate, or Ascomycetes or Basidiomycetes if septate. *Fungi Imperfecti* (typically septate) molds which have only asexual spores, are termed imperfect molds (Frazier and Westhoff 1988).

Requirements for growth

In general most molds require less available moisture than do most yeasts and bacteria. It has been claimed that below 14 to 15% moisture levels in flour or some dried fruits will prevent or greatly delay mold growth (Frazier and Westhoff 1988). Cherney et al. (1987) found that at the start of storage, most of the fungi of the *Aspergillus* species were isolated from high-moisture hay (26.4% moisture) rather than from dry hay (13.1% moisture): 159.1 $X10^5$ relative to 68.5 X 10^5 CFU/g DM forage. After 7-day storage, *Aspergillus glaucus*, a xerophytic fungus, grew and sporulated only in the wet hay, without appreciable sporulation of any fungi in the dry hay. Wittenberg and Nia (1991) found similar results to those of Cherney et al (1987), by observing a significantly greater number of total molds comprised primarily of *Aspergillus glaucus*, in moist (20-25% moisture) hay relative to dry (15-

20% moisture) hay after 60-day of storage (68.7 X 10^5 vs. 0.5 X 10^5 organisms/g DM., respectively).

Alcamo (1991) states that most fungi grow best at approximately 25° C. Some fungi, especially the pathogenic ones, thrive at 37° C, however, they also grow on nutrient medium at 25° C. These fungi are termed biphasic (2 phases) or dimorphic (2 forms). Some have a yeast-like phase at 37° C and a mold-like phase at 25° C. Certain fungi grow at still lower temperatures, such as 5° C. Breton and Zwaenepoel (1991) using moist hay, found that *Aspergillus fumigatus* was able to grow at temperatures ranging from 20 to 37° C and from 45 to 65° C with the greatest number found in the range of 20 to 33° C and 55 to 65° C. *Penicillium sp.* grew in the temperature range of 20-45° C, while some *Alternaria sp.* and some other members of *Deuteromycetes* flourished well at 25° C. Some species of fungi, such as *Alternaria alternata* and *Cladosporium cladosporiodes* were found at temperatures up to 61° C. *Aspergillus fumigatus* and some members of *Mucorales* even thrived at temperatures up to 65° C (Breton and Zwaenepol 1991). However, most fungi are mesophilic, growing at temperatures within the range 10-40° C. Others are able to grow at higher temperatures although they are still capable of growing at temperatures within the mesophilic range are generally called thermotolerant. Those that thrive at 45° C but fail to grow below 20° C are termed thermophilic. Fungi

that grow at low temperature may be either called psychrophilic if they are unable to grow at 20° C, or psychotolerant if they are able to grow at low temperature and in the mesophilic range (Smith and Onions 1983).

Fungi have variable pH requirements, pH 2 to 8.5 (Frazier and Westhoff 1988), but almost always grow best in acid conditions, normally at pH 5-6 (Smith and Onions 1983). Some such as *Aspergillus niger* will grow in a very acid environment, pH 2 and below (Smith and Onions 1983, Alcamo 1991). Burnett (1976) reported that environmental pH of the microflora has a correlation with the utilization of the particular carbon compounds by the organisms. For example *Zygorhynchus moelleri*'s cell walls were permeable to glucose and acetate only at pH 6.8, whereas at pH 3.4 the cells were permeable to all the other TCA cycle intermediates, but not to acetate.

Fungi are aerobic, and when grown in tubes or bottles normally obtain sufficient oxygen through the cotton wool plug. Although some cultures grow quite well in the normal incubator which is usually dark, many still grow better in the daylight and some spore better under black light (Smith and Onions 1983).

All microorganisms require water for growth, but the available water needed varies according to species and isolate. In their study Gregory et al. (1963) recognized that

hays baled at about 16% moisture heated little (22-26° C maximum temp. reached) during storage, and contained a small (0.1-0.7 million spore count of fungi/g and 0.4-2 million spore count of Actinomycetes & bacteria/g) but diverse microflora. Hays baled at about 25% moisture heated to approxymately 45°C and molded (12-60 million fungus spores/g and 3-10 million Actinomycetes & bacterium spores/g), mainly with *Aspergillus glaucus*. Hay baled at about 40% moisture became very hot (60-65°C) and contained a large number (9-94 million fungus spores/g and 370-680 million Actinomycetes & bacterium spores/g) of thermophilic fungi, particularly *Aspergillus fumigatus, Absidia* spp., *Mucor pusillus, Humicola lanuginosa* and *Actinomycetes*.

The negative effects of mold

The bacteria and fungal microflora of hays, have been investigated for their toxicological or pathological risks both to animals or man, in relation to pulmonary allergies (Breton and Zwaenepoel 1991). *Aspergillus flavus*, which is able to produce aflatoxin that can cause potential threat to the animals, was found in timothy, meadow fescue and clover samples of hays (Clevstrom and Ljungren 1984), lucerne hay (Magan and Lacey 1986) and alfalfa hay (Wittenberg 1991). Some other members of *Aspergillus* found in hay were *A. fumigatus*, which causes respiratory disease in man and animals, *A.*

nidulans and *A. terreus* (Lacey 1975). Other pathogenic fungi found in fodder were *Aspergillus niger, Scopulariopsis brevicaulis, Absidia ramosa, Absidia corymbifera, Mucor pusillus, Candida krusei, Candida guilliermondii* and *Candida tropicalis* (Lacey 1975). Molds found in moist hay in appreciable number were *Aspergillus glaucus* (Cherney et al 1987, Wittenberg and Nia 1991, Wittenberg 1991), *Aspergillus flavus, Fusarium sp., Penicillium sp., Mucor sp.* and *Rhizopus sp.* (Clevstrom and Ljunggren 1984). To date, no reports of mycotoxins in hay have been documented.

Quantification

According to Breton and Zwaenepoel (1991), microflora in hay can be catagorized into field and storage microflora. The microflora, present in the field when baling, is composed mainly of soil species belonging primarily to the genera *Alternaria, Cladosporium, Colletorichum, Fusarium, Phaesoptoria, Phoma and Ascochyta.* These species are mesophylic, and can be isolated on media at 25° C, and never at temperatures such as 37 or 50° C. When the bale temperature at storage approaches 50° C (after 2nd or 3rd day of storage), the organisms disappear rapidly. Storage microflora, having less diversified thermophilic or thermotolerant characteristics, appear as early as the 36th hour after baling, at a temperature range 37-45° C. They belong to the

genera *Absidia, Rhizopus, Aspergillus,* and *Humicola*. Breton and Zwaenepoel (1991) observed that the total number of storage microflora isolated from each bale could exceed 10^7/g DM forage, a number much higher than that found for field species.

Cultures of living fungi are being used increasingly in industry, research, and for teaching at a wide range of levels (Smith and Onions 1983). These cultures are also useful to identify and quantify fungi that invade hays both before and after storage. There have been many studies in the area of techniques and methods of examining microflora that attack hay. Lacey and Dutkiewicz (1976) used the wind tunnel examination, dilution plate technique and haemocytometer slide count to examine microflora in moldy hay. Dilution plate methods (Flannigan 1973), latex agglutination assay (Kamphuis et al 1989), dichloramphenicol peptone agar (Hocking 1987) and chitin analysis (Roberts et al. 1987, Wittenberg et al. 1989) are some examples.

EFFICACY OF A *PEDIOCOCCUS SP.* BACTERIA PREPARATION FOR TIMOTHY HAY PRESERVATION

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Short title: inoculation of high-moisture grass hay.

Key words : inoculation, hay, storage, mold, DM intake, digestibility.

ABSTRACT

A study was conducted to evaluate the effect of baling timothygrass hay at a high moisture level with or without bacterial inoculation on hay quality. Timothygrass forage was baled at two moisture levels: 15-20% moisture (L-Con), and 20-30% moisture (H). Hay baled at the higher moisture level was subjected to two treatments: H-S, for which *Pediococcus sp.* was applied at the rate of 5 X 10^5 CFU/g hay DM and H-Con which did not receive inoculant. Hays were stored outside, in a tarpaulin covered stack, for 60 and 66 days for L and H hays, respectively. Moisture content at baling did not affect ($P>0.05$) prestorage CP percentage. Prestorage NDF, ADF, ADIN and glucosamine levels were higher in L-Con relative to H hays. Moisture content at baling did not affect ($P>0.05$) poststorage CP levels. Poststorage NDF and ADF levels were greater ($P<0.05$) in L-Con relative to H hays. Inoculation of high-moisture hay did not influence ($P>0.05$) poststorage chemical composition of the H hay. Poststorage CP retention tended to be greater ($P<0.07$) in L-Con relative to H hays. Although the total plate count of microflora was not different among treatments, H hays tended ($P<0.10$) to show a higher amount of *Aspergillus glaucus*, a predominant microflora found in the post storage hays. Moisture content at time of baling and bacterial inoculation of high-moisture timothygrass hay

did not influence (P>0.05) dry matter intake and apparent nutrient digestibilities of hay fed to growing lambs.

INTRODUCTION

Hay is a forage preserved at relatively low moisture levels for the purpose of feeding to animals when fresh forages are not available, or are not desired in a management system. The ideal moisture content required in order to obtain a good quality hay post storage is 20% or less (Robertson 1983). However, hay baled at low levels of moisture can increase harvest DM loss during wilting, baling and stacking. Hay baled at more than 20% moisture content can reduce field DM losses associated with precipitation or leaf shatter, however, storage losses may be enhanced by intense mold growth. Suppressing the mold growth by manipulating the stored hay microenvironment has the potential of decreasing both field and storage losses.

Additives have been applied to moist hays at the time of baling in an attempt to decrease storage losses. Acids and salts(Lacey et al. 1981), urea (Rotz et al. 1990), ammonia (Moore et al. 1985) and fermentation products of bacteria (Deetz et al. 1989, Wittenberg and Nia 1990) have been used experimentally to preserve moist hays. Recent trials (Nelson et al. 1989a,b, Wittenberg and Nia 1990, Wittenberg 1991) have attempted to document the effect of moist alfalfa hay inoculation with bacterial preparations on post storage hay nutritive value as well as DM retention during storage. A very

limited amount of information regarding application of bacterial inoculants to grass harvested for hay production is available. Whether inoculants can be effective for preservation of moist grass hay is, therefore, not yet well understood.

Wittenberg (1991) used 2 strains of viable *Pediococcus pentosaceus* preparation, a lactic acid-forming bacteria, to inoculate moist (21.3 and 28.7% H_2O) alfalfa hay. The levels of the inoculant applied were 5.7 X 10^5 colony forming units(CFU)/g forage DM for 21.3% moisture hay, and 6.2 X 10^5 CFU/g forage DM for 28.7% moisture hay. Although inoculation did not improve post storage crude protein levels and nutrient retentions, it showed improvements in terms of visual assessments for moldiness, colour and dust. No chitin reduction was detected with the inoculation.

The objective of this study is to establish the efficacy of a *Pediococcus sp.* bacterial preparation for preservation of timothygrass hay. A field trial was conducted to evaluate the effects of the inoculant on post-storage nutrient composition and on DM and nutrient retention during storage. A lamb feeding trial was conducted to determine the impact of the inoculant on dry matter intake (DMI) and apparent digestibility of hay constituents when fed to ram lambs.

MATERIALS AND METHODS

Field trial

Two one-hectare paddocks of timothygrass (*Phleum pratense L.*) var. Champ, at 50% heading, were used in this trial. A John Deere 1209 mower conditioner (2.74-m swath width) was used to cut the grass stands into 20 swaths; 10 swaths (4.8 km long) per paddock. Six swaths from each paddock were randomly assigned to 1 of 3 treatments, so that there were 2 swaths per treatment in each paddock. The remaining swaths were used to determine optimum inoculant application rate and tractor speed for baling. The field yield was estimated by cutting 5-m^2 from each paddock prior to cutting with John Deere mower. Forage samples were immediately weighed after the cutting and taken to the laboratory for DM determinations.

Hay treatments included hay baled at 20-30% moisture and inoculated with Super Hay (Biotal Canada Ltd, Didsbury, Alta) (H-S), hay baled at 20-30% moisture without Super Hay (H-Con), and hay baled at 18-20% moisture without Super Hay (L-Con). The Super Hay inoculant contained viable lactic acid-forming bacteria of *Pediococcus sp.* which was applied by soluting 50 g into 10 liters distilled water. This solution contained 2.5 X 10^{11} CFU (colony forming unit) of the bacteria/l. The solution was applied to the forage at the time of baling using a custom-designed applicator capable of delivering 1 of 4

different solutions and equipped with two 0.5-flood jet nozzles positioned to administer the solution onto the feed intake of a John Deere 336 baler. Actual application rates were determined for each swath by measuring the volume of solution placed into and removed from the applicator. The volume of solution used was divided by total forage DM baled for the swath to calculate the application rate. The application rate resulted in 5 X 10^5 CFU/g hay DM.

Four bales were randomly selected from each swath for data collection. The small rectangular bales were stacked within 24 h of baling. Selected bales were tagged, and subjected to core sampling (6 cores per bale, Penn State Core Sampler) and weighed. Core samples from each bale were divided into 2 portions, of which one was frozen immediately (-20 C) for glucosamine analysis while the other was weighed immediately and oven dried (60°C, 48 h). The dried sample was then ground (1 mm) for CP, NDF, ADF, and ADIN determinations. Groups of 4 bales were randomly assigned to the second, third, fourth, or fifth layer in the stack and surrounded by bales of similar treatment. The top and sides of the stack were protected from precipitation by a tarpaulin. Bales remained in the stack for a minimum of 60 days, after which they were weighed and core sampled as before. Core samples were reserved for the analyses previously described as well as for plate counts. The temperature for each test bale was measured daily

for the first 33 days of storage, at 11:30 AM, using a trendicator connected to the bale by a thermocouple.

Bales were opened and scored for dust, mold, and colour by a thorough visual examination of the entire bale content using 3 appraisers. The score range was 0 to 5 for each parameter. For moldiness ranking, 0 was equivalent to no detectable mold and 5 was equivalent to the appearance of a mycelial mat thorough out the bale. For color ranking, 0 represented green appearance resembling standing crop and 5 represented brown, black discoloration of forage. For dust scoring, 0 represented no detectable dust and 5 reflected dust release from all parts of the bale. Following the visual appraisal, hay was chopped and bagged for use in the intake and digestibility trials.

Intake and digestion trials

Thirty crossbred ram lambs, 29.4 ± 2.4 kg live weight and 112.8 ± 1.6 days old were randomly assigned to one of three hay treatments in the intake trial. Lambs were weighed on two consecutive days, at the start and end of the trial. Eighteen lambs (6 per treatment) were randomly accommodated in individual floor crates and the other 12 were placed into individual raised floor crates. Prior to the trial, lambs were given a 7-day period to adjust to untreated timothy hay which was relatively free from mold.

The intake trial was started with a 10-day adaptation period in the individual crates during which time animals were fed their respective hay diets. Feed, a mixture of concentrate and the treatment hays, was offered twice a day, at approximately 9:30 AM and at 3:45 PM. Concentrate was offered at the morning feeding only. The ratio of hay to concentrate was 85:15 on an as fed basis. Animals had access to fresh water and cobalt iodized salt throughout the study. Following the adaptation period, intake was measured for 7 days by weighing the feed offered and refused. Grab samples of the hays and concentrate mixture were also taken daily during the study and composited for analysis.

Following the intake trial, the 12 lambs in raised-floor crates, which represented 4 lambs for each hay treatment, were retained for a digestibility trial which consisted of a 2-day adjustment to 90% of ad libitum intake and a 7-day collection phase. Hay offered, concentrate offered, feed refused and feces were weighed and sampled daily. Daily samples of hay were composited on the basis of treatment and samples of feed refused and feces were collected and composited for individual sheep. Fecal samples were taken twice daily, in the morning before feeding and in the afternoon at the time of feeding. All samples was placed in plastic bags and frozen (-20 C) until ready for DM, CP, ADF and NDF analyses.

Chemical analysis

Dry matter determinations were conducted by drying the samples in a forced air oven (60 C, 48 h). Crude protein determination was done as N X 6.25 with Kjeltec System 1030 Distilling Unit (Tecator AB, Hoganas, Sweden) according to Association of Official Analytical Chemists (AOAC 38.012, 1975). Acid detergent fibre and acid detergent insoluble nitrogen (ADIN) were determined (AOAC 7.074, 1984) using refluxing apparatus (Laboratory Construction Co., Kansas City, MO). Neutral detergent fibre was also determined using the refluxing apparatus according to procedures of Goering and Van Soest (1970) modified to exclude use of decalin and sodium sulfite. Calcium determination was conducted with dry ashing methods (AOAC, 7.103, 1975), solubilizing the ash and measuring the Ca concentration by flame atomic absorption spectrophotometry (Instrumentation Laboratory AA/AE spectrophotometer model 1L-551). Phosphorus determination was conducted colorimetrically with the spectronic 20 (AOAC 12th ed 7.103-105).

Samples for glucosamine were freeze-dried and ground (0.5 mm screen). Glucosamine analysis was done by placing 150 mg ground forage into 19 X 100 mm Kontes vacuum hydrolysis tube (Mandel Scientific, Nisku, Alberta) and adding 4 ml 6 N HCl. The tube was closed with a stopper, air was removed from the tube by evacuation to 750 mm Hg, and the stopcock closed. The

tube was placed in a pre-heated block heater (7 h, 121 C) for hydrolysis. Following hydrolysis, the tube was cooled to room temperature on ice and neutralized with 4.1 ml 25% (w/v) NaOH. Tube contents were diluted in a volumetric flask with 0.2 N sodium citrate buffer(pH 2.2), mixed, and filtered through Whatman No.40 filter paper. The filtered solution was stored (-20 C) until time of analysis.

Plate counts were conducted using frozen, ground forage samples according to the procedure described by Tuite (1969). Potato dextrose agar (PDA) and malt salt agar (MSA) sterilized medium and a 1% peptone sterilized dilution buffer (121°C, 15 min.) were used. All implements used and operation of the plate count were made as sterile as possible. To make a 1 in 10 (10^{-1}) dilution, 90 mL of 1% peptone and 10 g of sample were mixed and homogenized by shaking in a blender for approximately 30 seconds. A 10^{-2} suspension was prepared by adding 1 mL of 10^{-1} suspension to 9 mL of 1% peptone and shaking the mixture 25 times. A 10^{-3} suspension was prepared by taking 1 mL of 10^{-2} suspension and repeating the above procedures. Aliquots of 0.1 ml of the resulting suspensions were spread, using a bent glass rod, over the surface of sterile plates of PDA and MSA (Difco Laboratories, Detroit, MI). Plates were incubated for 5 days at 25°C. Plate colonies were observed and counted with the aid of a colony counter (New Brunswick Scientific, New Brunswick, NJ). Dilutions of 1

X 10^{-2}, 1 X 10^{-3}, 1 X 10^{-4} and 1 X 10^{-5} were conducted, but the results reported were based on the 10^{-2} dilution.

Statistical analysis

Complete Randomized Design (CRD) was used in the field trial for the pre-stacked hay data. Swaths in the fields were used as replicates, each of which had 4 bales as the experimental units. The analysis was done as a one-way analysis of variance using general linear model (GLM) in the Statistical Analysis Systems (SAS Institute, Inc. 1986). Differences between means were determined using Duncan's Means Comparisons (Snedecor and Cochran, 1980) when treatment means were different ($P<0.05$). Least square means were used where missing data occured. The same procedure was applied in the intake and digestibility trials, where individual animals were used as experimental units.

For post-storage hay data, a factorial design was used. The first factor was the treatments and the second factor was the layers in the stack.

RESULTS AND DISCUSSIONS

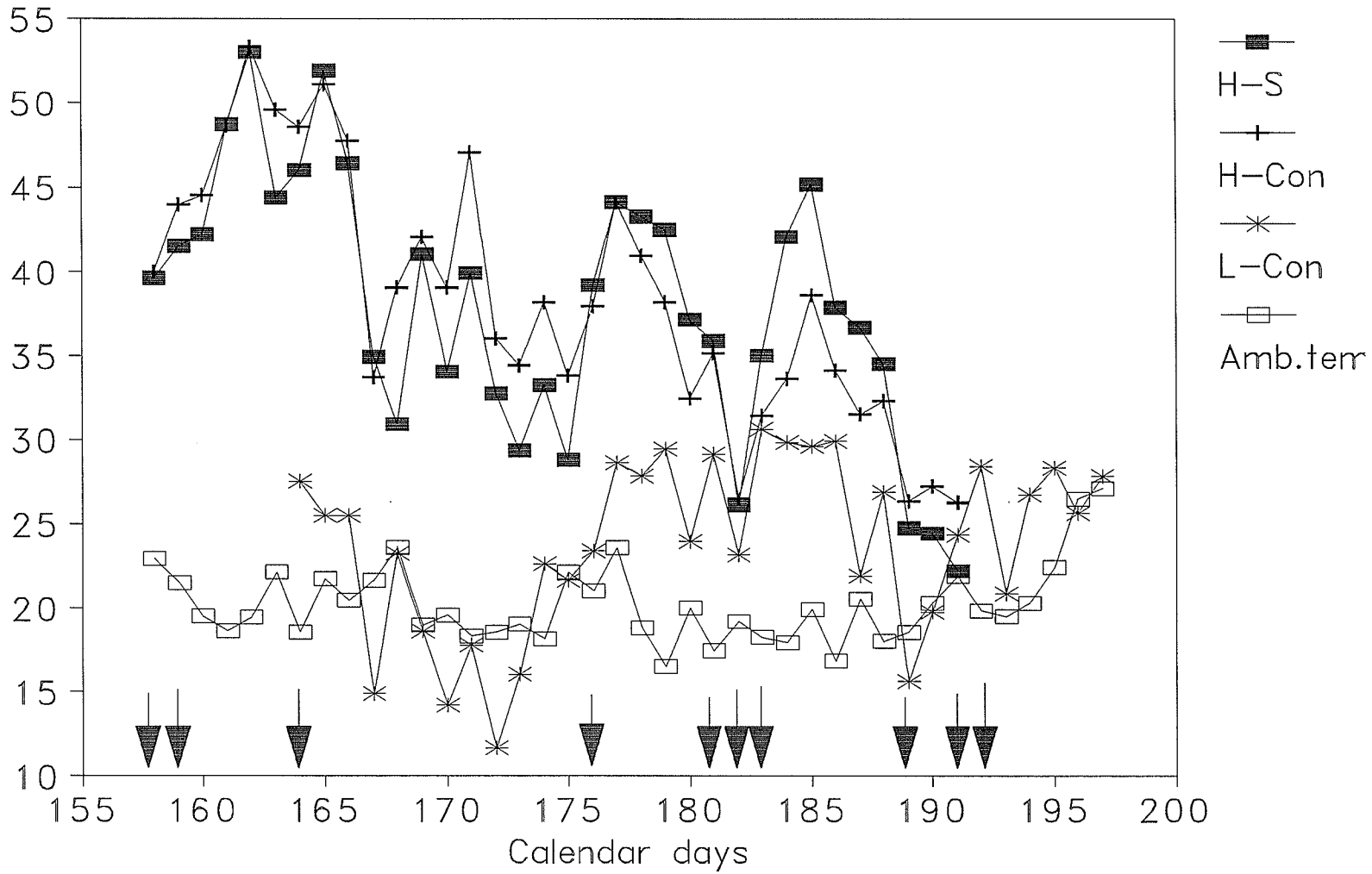
Harvesting conditions

Timothygrass was cut on June 4th, 1991, when it was about midheading. To achieve 30% (H) and 20% (L) moisture contents, the forage was baled approximately 48 h and 192 h after cutting (June 6th and 12th, 1991) respectively. There was no precipitation on H hay while it was being dried. A total precipitation of 22.1 mm occurred during drying of L hay. Average maximum, minimum and mean air temperatures of 24.3, 13.5 and 20.0°C respectively were recorded during the H hay drying period, while the air temperatures in the same order during the drying of L hay were 26.9, 14.7 and 20.5°C, respectively. Precipitation during the drying of L hay was responsible for the long drying time to decrease moisture content from approximately 30% to 20%.

Field Trial

There was a similar pattern of temperature rise and decline during the first 33 d in the stack for H-Con and H-S hays (fig. 1). The first 2 d in the stack resulted in a temperature rise from 40°C to 42 and 45°C for H-S and H-Con hays, respectively. The temperatures peaked at about 54°C on the 4th day of storage (171 calendar day), and began to decrease at day 7 in the stack (calendar day 165).

Figure 1: Storage temperatures for hay baled an stacked at a low moisture level (L-Con, —X—), high moisture level without inoculant (H-Con, —┼—) or with inoculant (H-S, —■—). Ambient temperatures (Amb.temp, —□—) and days with greater than 2mm precipitation (↓) are also recorded.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Calendar days
H-S
H-Con
L-Con
Amb.tem

This result was similar to that of Nelson et al. (1989b), using alfalfa hay baled at approximately 43% moisture with the same bale size, or to that of Russell and Buxton (1985) using hay baled in large, round bales at 19% moisture. Peak temperatures greater than 60°C were detected by Miller et al (1967) and Nelson et al. (1989a), using hays baled at about 26 to 35% moisture in large, high density bales. Currie and Festenstein (1971) stated that heating to 70°C is caused by microorganisms, and is a process that will readily occur in the field or laboratory. Moisture content and density of hay both can affect heat development during storage. Moisture can enhance respiration and microbial activity, while high density can act as a good insulator for heat loss.

Environmental conditions such as aeration (wind), ambient temperature, and relative humidity during storage also have an effect on the relative pattern of self heating of the hay (Currie and Festenstein 1971). A total 250.7 mm precipitation was recorded during 66 day hay storage in this study. Although the tarpaulin used in this study can protect hay from direct precipitation, it is less appropriate for preventing stack penetration of moisture due to high relative humidity. This environmental humidity can cause suppression of moisture loss from the hay, which in turn may have caused increased mold activity.

The temperatures of H hays stabilized at approximately

32-38°C from day 11 to day 28 (calendar day 169 to 186), during which little precipitation was recorded (fig. 1). In general, hay temperatures in this study increased one or two days after the days in which precipitations greater than 2mm were detected. Peak temperatures of about 44°C on calendar day 178 and 45°C on calendar day 186 may have been the result of two consecutive rainy days on calendar d 176 and 177 and four consecutive rainy days, calendar d 181 to d 184. Hay temperatures of 28-29°C, which were similar to ambient temperatures, were identified during the last 3 days of the first 33 days in storage.

This pattern of temperature fluctuation was also identified by Miller et al. (1967) and Russell and Buxton (1985). Whether this phenomenon has a relationship with the stationary growth phase of microorganisms in the hay is not exactly understood.

Inoculation did not lower the temperature of the H hays. The results of previous studies on the effect of bacterial inoculation of moist hays on heat generation were not conclusive. Heat development was decreased by inoculation of 56.6% DM alfalfa hay, but was increased by inoculation of 73.5% DM alfalfa baled as small rectangular bales (Nelson et al. 1989b). When the hay was baled as large round bales, inoculation of 73.4% DM forage resulted in a decrease in hay temperature (Nelson et al. 1989a). It was assumed that

environmental conditions during storage and bale type may have altered rate of moisture loss from the bales, which could have affected growth of spore-forming and clostridia organisms, and, therefore, hay temperature.

Contrary to the H hays, the L hay experienced a decrease in temperature from approximately 28°C to about 12°C during the first 8 days in the stack (calendar day 171). This was followed by an increase to a stable temperature of 29-30°C from calendar day 179 to day 186, after which time a fluctuation from 17 to 28°C followed. A relatively stable temperature of about 27-28°C was recorded during the last 3 days of the first 33 days in storage. Hays baled at the lower moisture level in this study experienced only a little heating, due to lower respiration rate and/or microbial activity. It seemed that precipitation and relative humidity during storage of hay in a tarpaulin covered stack rather than ambient temperature, might be responsible for the temperature changes observed during storage of the hays.

Greater level of NDF and ADF ($P<0.05$) were identified in low moisture hay relative to high moisture hay (table 1). Similarly, Nelson et al. (1989) found an increase in NDF and ADF levels when alfalfa hay was baled at 56.6% relative to 73.5% DM content. Wittenberg and Nia (1990) found no change in forage NDF and ADF contents by increasing DM content at baling in alfalfa hay when weather conditions for drying were

Table 1. Effect of moisture content at baling on chemical composition of timothy forage prior to storage[z]

Item	Treatment		
	L-Con	H-Con	H-S
No. of observation	16	14	16
DM(%)	79.2(0.7)a	69.3(0.7)b	67.2(0.7)b
CP(%DM)	14.7(0.3)	14.6(0.3)	15.0(0.3)
NDF(%DM)	70.0(0.4)a	62.5(0.4)b	62.4(0.4)b
ADF(%DM)	37.5(0.4)a	34.1(0.4)b	34.6(0.4)b
ADIN(%total N)	2.7(0.2)a	1.9(0.2)b	2.0(0.2)b
Glucosamine (mg/gDM)	1.8(0.1)a	1.1(0.1)b	1.1(0.1)b

[z] numbers in parantheses are standard errors of least square means.

a-b, least square means in the same row with different letters are different, $P<0.05$.

good. Rain during the drying of low moisture hay in this trial may have caused leaching of some water soluble constituents of the hay, causing an apparent increase in the percentages of water insoluble fractions of DM. The leaching may be due to loss of permeability of cell wall of the dying and dead plant cells. Similar results were observed by Wittenberg (1991), for low moisture hay which was exposed to rain during the drying, compared to that of medium or high moisture hays without any precipitation during drying. Also, higher ($P<0.05$) glucosamine levels (table 1) for hay baled at the lower moisture level suggests that field drying conditions caused mold invasion in the forage which could result in a loss of the nonstructural carbohydrate components.

Although CP content was not different for hay baled at different moisture levels, the form of nitrogen was affected. The concentration of bound nitrogen, identified as ADIN, was greater ($P<0.05$) for hay baled at the L moisture level than hay baled at H moisture level. Exposure to precipitation during drying of L-Con may be responsible for these differences.

The L hay was baled 6 days after the H hays were baled. Since all experimental bales were removed from the stack at the time of its opening, storage time for the H hays was 6 days longer than that of L hay (table 2).

Mean hay DM after 60 and 66 days of storage were 88.5,

85.9 and 86.9% for L-Con, H-Con and H-S, respectively, indicating that moisture was released from H hays during storage. The intense heating of H hay might have caused migration of physically tied water in H hays.

No difference (P>0.05) was identified in CP levels among the hays post storage. No significant differences (P>0.05) for post storage ADIN levels were identified among treatments. Goering and Van Soest (1972) stated that heat can cause condensation of carbonyl groups of carbohydrates with amino groups of protein to form a dark-colored nitrogenous polymer which accumulates in the lignin fraction of ADF. According to this theory, it was expected that L hay should have had lower post storage ADIN levels than the H hays, because storage temperatures for L hay were low. However, L hay had a greater ADIN level before storage relative to H hays.

Structural carbohydrates, for example hemicellulose and fructosan can be degraded into simple sugars such as glucose and fructose after self heating at 70°C (Festenstein 1971). This was expected to occur in H hays, because several bales showed temperatures more than 65°C. The released simple carbohydrates might have been used by microorganisms, which in turn, decrease the DM content of the hays. It was expected, therefore, that less DM would be recovered after storage from the H hays relative to that of L hay, with the assumption that stacking loss was similar.

Table 2. Effect of moisture content and inoculation at baling on chemical composition of stored timothy hay[z].

Item	Treatment		
	L-Con	H-Con	H-S
No. of observ.	16	15	16
Days in stack	60	66	66
DM(%)	88.5(0.6)a	85.9(0.7)b	86.9(0.6)ab
CP(%DM)	15.3(0.3)	15.0(0.3)	15.8(0.3)
NDF(%DM)	73.0(0.5)a	70.5(0.5)b	70.3(0.5)b
ADF(%DM)	42.0(0.8)a	38.4(0.9)b	40.6(0.8)ab
ADIN(%total N)	5.0(0.4)	3.8(0.5)	4.6(0.4)

[z] figures in the parantheses are standard errors of least square means.
a-b, least square means in the same row with different letters are different, $P<0.05$.

Percentages of NDF and ADF in post storage L-Con hay were greater ($P<0.01$ and $P<0.05$, respectively) than in the comparable H hays. Russell and Buxton (1985) showed a lower ($P<0.05$) NDF and ADF levels in low compared to high-moisture hays after 17 and 39 weeks post-storage. Similar results to that of Russell and Buxton (1985) were observed by Wittenberg and Nia (1990) and Rotz et al. (1990), when weather conditions were good during the drying of all hays used in their trial.

No effect of inoculation on post storage forage chemical composition was observed when the bacterial preparation was applied to moist (H) timothygrass hay in the current study. Previous studies have indicated variability in the improvements of nutrient value for moist alfalfa hays inoculated with bacterial preparations (Nelson et al. 1989a,b; Wittenberg and Nia 1990, 1991, Wittenberg 1991).

A difference ($P<0.05$) was observed for hay DM, CP, ADF and ADIN relative to position in the stack. Stack position, identified as layer, can influence forage storage conditions due to differences in heat dissipation and warming effects relative to the sun's rays. In this study all significant effects could be related back to significant differences observed in bale nutrient contents for the various layers at the time of stacking. This can be expected since bales from a particular swath in the field would be stacked in one layer.

No difference ($P>0.05$) was observed for DM retention for

different moisture contents or for H-Con relative to H-S (table 3). This was in accordance with the findings of Wittenberg and Nia (1990), who observed no difference ($P>0.05$) in DM retention of post-storage alfalfa hay baled at 17.2 (L) and 23.9% (M) moisture.

Low moisture hay tended ($P<0.07$) to retain more CP relative to H hays. On the contrary, the inoculated H hay tended ($P<0.09$) to retain more NDF relative to L-Con.

Inoculation tended ($P<0.08$) to increase ADF retention in H hay. One possible reason was that a higher percentage of simple sugars were used by the bacteria as energy sources in the inoculated H hay relative to H-Con.

Layer in the stack influenced ($P<0.01$) ADF retention. Greater percentages of ADF were retained in the 2 lower layers (106.4% in average) relative to that at upper layers (99.8% in average) in the stack. Pre storage higher moisture levels of forage in the lower layers (69.8% DM in average relative to 74.0% DM) might have been responsible for this phenomenon. Dry matter, CP, NDF and ADIN retentions were not affected by layer ($P>0.05$).

Layer by treatment interaction affected ($P<0.05$) CP, NDF and ADF retentions. This interaction effect suggests that at least two treatments behaved differently in each position in the stack. Crude protein, NDF and ADF retentions were greatest in the upper most layer, layer 5 (103.4, 113.2 and 113.0%

Table 3. Effect of moisture content and inoculation at baling on dry matter and nutrient retention of stored timothy hay[z,y]

Item	Treatment			Level of significance	
	L-Con	H-Con	H-S	layer	layer X trt
No. of oberv.	16	14	16		
Days in stack	60	66	66		
DM(%)	92.3(1.5)	89.0(1.5)	90.7(1.5)	NS	NS
CP(%)	95.9(1.8)	92.0(1.9)	95.5(1.8)	NS	.05
NDF(%)	96.4(1.9)b	100.2(2.0)a	102.2(1.9)a	NS	.05
ADF(%)	103.4(1.9)	99.7(2.0)	105.7(1.9)	.01	.05

[z] Retention =(poststorage weight/prestorage weight) X 100
[y] figures in the parantheses are standard errors of least square means.
a-b, least square means in the same row with different letters are different, P<0.05.

respectively) for H-S hay, and were lowest in the lower most layer (layer 2) for CP and NDF retentions and in layer 4 for ADF retention of the hay. The difference between the highest and the lowest retention values were 9, 7, and 6 percentage points respectively for CP, NDF and ADF. Pre storage moisture contents of H-S hay in layers 5, 2 and 4 were similar (67.2, 67.0 and 69.4% DM, respectively). On the contrary, L-Con hay showed the lowest CP, NDF and ADF retention values (91.4, 91.2 and 99.3% respectively) in layer 5 and greatest in layer 3 for CP and NDF retentions and layer 2 for ADF retention. Actual differences in retention between the highest and lowest were 15, 10, and 9 percentage points for CP, NDF and ADF, respectively. Crude protein, NDF and ADF retentions for H-Con in the layers behaved similarly to those for L-Con. Upper layers, layer 4 and 5, showed the lowest retention of CP, NDF and ADF retentions (90.8, 99.0 and 92.3% respectively) while lower layers, layer 2 and 3, showed the greatest levels. The differences between the greatest and the lowest retention values for H-Con hay were 4, 2 and 7 percentage points respectively, which were similar to the range found in H-S hay. Pre storage DM levels for H-Con hay were 73.6, 71.1, 64.3 and 67.9 in layers 4, 5, 2 and 3, respectively.

The phenomena of the differences in nutrient retentions of the preserved forages can be approached emphirically through DM retention (DM loss) and/or chemical conversions

which occur during hay storage. For L-Con and H-Con hays, lower moisture content in the upper most layer bales before and after storage might have resulted in higher storage DM loss during post storage weighing and core sampling, relative to lower layer bales. This was reflected in the lowest retention rates in the upper most layer in L-Con and H-Con hays, respectively. The differences between the greatest retention values with the lowest were greater for low moisture hay (L-Con) than those for high moisture hay (H-Con). This is because low moisture hay is more susceptible to leaf loss (brittler) than high moisture hay. Retention value of a given nutrient constituent in a forage also is determined by percentage level of the constituent before and after storage, which in turn, influenced by chemical conversions as a result of plant respiration and microbial activity during storage. From this point of view, it is expected that more ADF will be retained in high moisture than low moisture hay, with the assumption that greater amount of DM and percentage of ADF will be generated from high moisture hay. This was reflected, also, in the lower layers of H-Con and L-Con hays.

In general, nutrient retention values in this study were in a similar range with that reported by Nelson et al. (1989a, b) and Wittenberg and Nia (1991).

Post storage forage glucosamine levels were similar ($P>0.05$) among hay treatments. Some visible molding was

evident for all hay treatments (table 4). No significant differences ($P>0.05$) were identified among treatments regarding dust or mold appearances, although H-S hay tended ($P<0.09$) to show more browning discoloration relative to the other hays. This might be related to the ADIN levels observed in the respective hays.

No interaction ($P>0.05$) between layer with treatment was identified for color, dust and mold visual assessments, however, layer showed an effect ($P<0.01$) on color and mold assessments. Lower layers, layer 2 and 3, showed darker (3.28 and 3.07) discoloration relative to upper layers, layer 4 and 5 (2.25 and 2.74, respectively). In accordance with the color, lower layers showed greater mold appearance (3.21 and 2.78) relative to upper layers (1.76 and 2.07, respectively). Higher microenvironment humidity surrounding the lower layers, caused by wet soil surface due to precipitation during storage might have caused greater mold activity in the bales placed in those positions.

Plate scores indicated that the predominant fungi present in all treatments was *Aspergillus glaucus*, a mesophilic microflora. No effect ($P>0.05$) of moisture level or inoculation on the population of this microorganism was identified, although H hays tended ($P<0.10$) to have greater numbers of the fungus. This was in accordance with the finding of Wittenberg (1991) who found no differences in the

Table 4. Effect of moisture content and inoculation at baling on glucosamine levels, visual and plate count assessments of molds in stored timothy hay[z]

Item	Treatment			Level of significance	
	L-Con	H-Con	H-S	lyr	lyrXtrt
Sample number	16	15	16		
Days in stack	60	66	66		
Gluco.(mg/gDM)	3.48(.36)	2.80(.38)	2.74(.36)	NS	NS
Visual assessment					
Color	2.7(0.2)	2.8(0.2)	3.2(0.2)	.01	.05
Dust	2.4(0.2)	2.0(0.2)	2.3(0.2)	NS	NS
Mold	2.6(0.3)	2.3(0.3)	2.6(0.3)	.01	NS
Plate count assessment.[y]					
Aspergillus glaucus	46.6(12.2)	65.8(12.7)	76.3(12.2)	NS	NS
Penicillium sp.	0.0(4.9)	0.3(5.1)	8.3(4.9)	NS	NS
Absidia sp.	4.4(1.8)	2.8(1.9)	2.8(1.8)	NS	NS
Scopulari-opsis	14.6(3.7)a	0.2(3.9)b	1.5(3.7)b	NS	NS
Actinomy-cetes	15.1(4.1)a	0.4(4.3)b	0.0(4.1)b	NS	NS
Yeast and *Bacteria.*	7.3(5.4)	9.0(5.6)	0.0(5.4)	NS	NS
Total	88.0(14.0)	78.6(14.5)	88.9(14.0)	NS	NS

[y] 10^2 CFU/g hay DM.

[z] numbers in parantheses are standard errors of least square means.

a-b, least square means in the same row with different letters are different, $P<0.05$.

number of viable *Aspergillus glaucus* in low compared to high moisture alfalfa hays, but the low moisture hay tended to have a lower number of the species. Gregory et al. (1963) also identified that hay at 16% moisture heated little and contained a small but diverse microflora, and hay at 25% moisture heated to about 45°C and moulded, mainly with *Aspergillus glaucus*.

Penicillium sp. was also found in similar ($P>0.05$) number among treatments. No *Penicillium sp.* were found in L hay and low concentrations were observed for the H hays. Similarly, no significant difference ($P>0.05$) was detected in *Absidia sp.* Breton and Zwaenepoel (1991) found a substantial number (1×10^4 to 6×10^5 CFU/g forage) of *Absidia corymbifera* in forage at the temperature ranging from 20 to 65°C, with the greatest number at 65°C. Gregory et al (1963) also identified that *Absidia sp.* flourished well at 60-65°C. Viewed from the temperature development during storage of the hays, L-con should have had the lowest count of this microflora. Factors other than temperature, for example rewetting of L-Con hay during drying due to rain may have acted as growth catalist of the microflora before storage.

Scopulariopsis sp. and *Actinomycetes* were found in greater number ($P<0.05$) in low-moisture hay relative to high-moisture hay. Gregory et al. (1963), found that hay at about 40% moisture contained large amount of *actinomycetes*. It was

likely that precipitation during the drying of low moisture hay in this study might have enhanced the growth of this fungi before storage. Yeast and bacteria also was found to be distributed with no significant differences (P>0.05) among hay treatments. This was due to all bales were stacked on the pallets without soil contamination, where the organisms live. No actinomycetes, yeast or bacteria were identified in the inoculated high-moisture hay. This was as expected, and might be due to competition toward similarity in the requirement of their growth.

The total number of microorganisms was not different (P>0.05) among hay treatments. These results indicate that, in general, differences in moisture content or inoculation of high moisture timothy hay with the *Pediococcus sp.* used in this study failed to suppress mold growth. No significant effects (P>0.05) of both layer and interaction between treatment with layer were identified on microbial populations in post storage timothy hays. Environmental conditions, and to some extent position in the layers, might have had a greater influence on the microenvironment humidity of the bales, which in turn, could affect microbial growth.

Intake and Digestibility trial

The CP, Ca and P of diet (table 5) met the requirements for growing lamb (National Research Council, 1985).

Table 5. Composition of diet offered to lambs.

Item	% in diet (DM basis)
Diet Ingredients	
Timothy hay, chopped	85.5
Concentrate[z]	14.5
Chemical compositions of diet	
CP	18.1
NDF	63.3
ADF	35.9
Ca	0.44
P	0.37

[z] Concentrate consisted of the following ingredients, as fed basis: ground barley, 21.0%; 44.0% soybean meal, 76.7%; calcium carbonate, 1.3%; cobalt iodized salt, 1.0%.

Table 6.Effect of moisture content and inoculation at baling on DMI and apparent digestibilities of hay by lambs[z]

Item	Treatment		
	L-Con	H-Con	H-S
Dry matter intake:			
No. of observ.	9	10	10
g/d	919 (73.7)	984 (69.9)	912 (69.9)
%BW	3.1(0.3)	3.3(0.2)	3.2(0.2)
Digestibility:			
No. of observ.	4	4	4
DM (%)	63.6(1.2)	67.3(1.2)	65.3(1.2)
CP (%)	66.8(1.5)	70.0(1.5)	66.3(1.5)
ADF(%)	64.8(1.4)	66.1(1.4)	64.6(1.4)
NDF(%)	70.7(1.1)	72.0(1.1)	71.6(1.1)

[z]Figures in the parantheses are standard errors of means.

One lamb in the L-Con group of the intake trial suffered serious diarrhea two days after the collection period began, and was culled from the intake trial.

Therefore only 9 lambs were included in the analysis for L-Con treatment. Dry matter intake was not different ($P>0.05$) among hay treatments (table 6), and in general, the intakes were similar with the recommended level of DMI for growing lambs. Similar results were observed by Miller et al. (1967), Mathison et al. (1975) and Wittenberg and Nia (1990) using alfalfa hay baled at different moisture levels.

No differences ($P>0.05$) were observed in terms of CP, ADF and NDF digestibilities among treatments (table 6). However uninoculated high-moisture hay tended to show the highest DM digestibility ($P<0.10$). Miller et al. (1967) and Wittenberg and Nia (1990) found a decrease ($P<0.05$) in DM digestibility when alfalfa forage moisture was increased at baling. However Miller et al. (1967) stored the bales in the insulated boxes (controlled environment), and Wittenberg and Nia (1990) dried the forage at excellent weather conditions.

Mathison et al. (1975) stated that increased levels of ADIN in hay generally associated with reduced CP digestibility. It has been shown previously that although post-storage CP levels were similar among treatments, ADIN retained tended to be greater in the L-Con and H-S relative to H-Con. This in part, might have caused a tendency to the lower

values of CP digestibilities in L-Con and H-S, relative to H-Con.

In general, inoculation of high-moisture grass hay with a bacterial preparation failed to improve hay quality. Poor weather conditions during the drying of L-Con in this study caused this hay to mold in the field causing quality to be similar to high moisture hays which molded during storage.

CONCLUSIONS

Heat development during storage for high moisture timothygrass hay was higher than for low moisture hay. Inoculation of high-moisture hay failed to depress storage temperature of the hay during storage. Heat development during storage in this trial did not reflect the increase in ADIN levels of the hays.

Low-moisture hay at baling contained higher pre storage and post storage NDF and ADF levels. Inoculation of high moisture timothygrass hay failed to improve post storage nutrient composition of the hay.

Moisture content and inoculation did not influence total post storage microflora count. However, field drying conditions can result in fungal invasion of low moisture hay prior to baling. In addition, position in the layer during storage in high precipitation conditions can affect mold activity.

No differences were identified among hay treatments in terms of voluntary dry matter intake and apparent DM, CP, NDF and ADF digestibilities, when the hays was fed to growing lambs.

LITERATURE CITED

Abdalla, H. O., D.G. Fox and R.R. Seaney. 1988. Protein distribution in four cool season grass varieties alone or in combination with trefoil. J. Anim. Sci. 66:2325-2329.

Albert, R.A., B. Huebner and L.W. Davis. 1988. Role of water activity in the spoilage of alfalfa hay. J. Dairy Sci. 72:2573-2581.

Alcamo, I.E. 1991. Fundamentals of microbiology. 3rd ed. The Benjamin and Cummings Publ. Co., Inc. Redwood City.

Allard, H.A. and M.W. Evans. 1941. Growth and flowering of some tame and wild grasses in response to different photo periods. J. Agric. Res. 62:193-228.

Alli, I., E. Robidas, E. Norozi and B.E. Baker. 1985. Some changes associated with the field drying of lucerne and timothy. Grass forage Sci. 40:221-226.

Anonymous 1987. Forages '87. A Manitoba Agriculture home study course. Lesson2: Selecting forages for your farm.

Anonymous 1989. Catalogue of strains 1989. German collections of microorganisms and cell cultures (Deutsche Sumlung von Mikroorganismen und Zellkulturen. GmbH. Berlin, Germany.

Association of Analytical Chemist. 1975. Official method of Analysis. 12th ed. AOAC, Washington, DC.

Banwart, G. J. 1989. Basic food microbiology. Van Nostrand Reinhold. New York.

Belyea, R.L., F.A. Martz and S. Bell. 1985. Storage and feeding losses of large round bales. J. Dairy Sci. 68:3371-3375.

Breton, A. and P. Zwaenepoel. 1991. Succession of moist hay mycroflora during storage. Can. J. Microbiol. 37:248-251.

Brock, T. D. 1979. Biology of microorganisms. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Bothast, R.J., E.B. Lancaster and C.W. Hesseltine. 1973. Ammonia kills spoilage molds in corn. J. Dairy Sci. 56:241-245.

Buchanan, R.E. and N.E. Gibbons, co-eds. 1975. Bergey's manual of determinative

bacteriology. The William and Wilkins. Baltimore.

Burnett, J.H. 1976. Fundamentals of mycology. Edward Arnold Publ. Ltd. London, Great Britain.

Candlish, E., K.W. Clark and J.R. Ingalls. 1973. Organic acid treatment of hay. Can. J. Anim. Sci. 53:513-518.

Cherney, J.H., K.D. Johnson, J. Tuite and J.J. Volenec. 1987. Microfloral and compositional changes in alfalfa hay treated with sodium diacetate and stored at different moisture contents. Anim. Feed Sci. Technol. 17:45-56.

Christen, A.M., J.R. Seoane and G.D. Leroux. 1990. The nutritive value for sheep of quackgrass and timothy hays harvested at two stages of growth. J. Anim. Sci. 68:3350-3359.

Clark, G.H. and M.O. Malte. 1913. Fodder and pasture plants. Department of Agriculture, Ottawa.

Clevstrom, G. and H. Ljunggren. 1984. Occurence of storage fungi, especially aflatoxin-forming *Aspergillus flavus* in soil, greenstuff and prepared hay. J. Stored Prod. Res. 20:71-82.

Colovos, N.F., H.A. Keener, J.R.Prescott and A.E. Terri. 1949. The nutritive value of timothy hay at different stages of maturity as compared with second cutting clover hay. J. Dairy Sci. 32:659-664.

Currie, J.A. and G.N. Festenstein. 1971. Factors defining spontaneous heating and ignition of hay. J. Sci. Fd. Agric. 22:223-230.

Dale S. 1962. Forage management in the north. W.M.C. Brown Book Co. Iowa, U.S.

Dale S., R.J. Bula and R.P. Walgenbach. 1986. Forage management. Kendall/Hunt Publ. Co., Iowa.

Davies, M.H. and I.B. Warboys. 1982. Evaluation of propionic acid-treated hay as a feed for growing sheep. Grass Forage Sci. 37:165-167.

Deetz, D.A., J.H. Harrison, F.R. Valdez and D.W. Evans. 1989. Impact of noncorrosive forage stabilizers on digestibility of alfalfa hay and lactation performance of dairy cows. J. Dairy Sci. 72:2062-2073.

Festenstein, G.N. 1971. Carbohydrates in hay on self-heating to ignition . J. Sci. Fd. Agric. 22:231-234.

Flannigan, B. 1973. An evaluation of dilution plate methods for enumerating fungi. Lab. Practice 22:530-531.

Frazier, W.C. and D.C. Westhoff. 1988. Food microbiology. 4th. ed. Mcgraw-Hill Book Co, New York.

Fritz, J.O., R.P. Cantrell, V.L. Lechtenberg, J.D. Axtell and J.M. Hertel. 1981. Brown midrib mutants in sudangrass and grain sorghum. Crop Sci. 21:706-709.

Goering, H.K. and P.J. Van Soest. 1970. Forage fibre analysis; Apparatus, reagents, procedures and some applications. USDA Agricultural Handbook No. 379, U.S. Government Printing Office, Washington, DC.

Goering, H.K. and P.J. Van Soest. 1972. Relative susceptibility of forages to heat damage as affected by moisture, temperature and pH. J. Dairy Sci. 56:137-143.

Gregory, P.H., M.E. Lacey, G.N. Festenstein and F.A. Skinner. 1963. Microbial and biochemical changes during the moulding of hay. J. Gen. Microbiol. 33:147-174.

Hayhoe, H.N. and L.P. Jackson. 1974. Weather effects on hay drying rates. Can. J. Plant Sci. 54:479-484.

Heaney, D.P., W.J. Pigden, D.J. Minson and G.I. Pritchard. 1963. Effect of pelleting on energy intake of sheep from forages cut at three stages of maturity. J. Anim. Sci. 22:752-757.

Heath, M.E., R.F. Barnes and D.S. Metcalfe. 1985. Forages: The science of grassland agriculture. 4th ed. Iowa State Univ. Press. Ames, Iowa.

Hocking, A.D. and S. Andrews. 1987. Dichloran chloramphenicol peptone agar as an identification medium for fusarium species and some dematiaceous hyphomycetes. Trans. Br. Mycol. Soc. 89:239-244.

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.

Jafri, S.A., L.J. Bush and G.D. Adams 1979. Chemical preservation of alfalfa hay for dairy cows. J. Dairy Sci. 62:455-458.

Jerrel, B.P. and A.A. Hanson. 1975. Timothy. in Forages: The science of grassland

agriculture. Heath, M.E., D.S. Metcalfe and R.F. Barnes (editors). The Iowa State Univ. Press. Ames, Iowa.

Jones, A.L., R.E. Morrow, W.G. Hires, G.B. Garner and J.E. Williams. 1985. Quality evaluation on large round bales treated with sodium diacetate or anhydrous ammonia. Trans. ASAE 28:1043-1046.

Jung, G.A., J.A. Balasko, F.L. Alt and L.P. Stevens. 1974. Persistence and yield of 10 grasses in response to clipping frequency and applied nitrogen in the Allegheni Highlands. Agron. J. 66:517-521.

Kamphuis, H.J., S. Notermans, G.H. Veeneman, J.H. Van Boom and F.M. Rombouts. 1989. A rapid and reliable method for the detection of molds in food: Using the latex aglutination assay. J. Food Protect. 52:244-247.

Kennedy, W.K. and R.U. Schenk. 1954. The use of fungisides in the preservation of moist hay. Agron. J. 46:252-257.

Kennelly, W.K. and L.A. Baars. 1990. Influence of method of conservation on the nutritive value of forages. Proceedings of the 11th Western Nutrition Conference. Winnipeg. The University of Manitoba, Winnipeg, MB.

Kent, B.A., M.J. Arambel, M.D. Winsryg and J.L. Walters. 1989. Microbial inoculation of alfalfa haylage: Ensiling characteristics and milk production response when fed to early lactation dairy cows. J. Dairy Sci. 72:2325-2330.

Klinner, W.E. and G. Shepperson. 1975. The state of hay making technology; a review. J. Br. Grassl. Soc. 30:259-266.

Klopfenstein, J., C. Dorn, R.L. Ogden, W.R. Kehr and T.L. Hanson. 1978. Field wilted and direct cut dehydrated alfalfa as protein sources for growing beef cattle . J Anim. Sci. 46:1780-1788

Knapp, W.R., D.A. Holt and V.L. Lechtenberg. 1976. Propionic acid as a hay preservatives. Agron. J. 68:120-123.

Knapp, W.R., D.A. Holt and V.L. Lechtenberg. 1975. Hay preservation and quality improvement by anhydrous ammonia treatment. Agron. J. 67:766-769.

Koegel, R.G., R.J. Straub and K.J. Shinners. 1985. Ammonia treatment of large and small hay packages. Trans. ASAE 28:726-730.

Lacey,J. 1975. Potential hazards to animals and man from microorganisms in fodders

and grain. Trans. Br. Mycol. Soc. 65:171-184.

Lacey, J. and J. Dutkiewicz. 1976. Methods for examining the microflora of mouldy hay. J. Appl. Bacteriol. 41:13-27.

Lacey, J., K.A. Lord and G.R. Cayley. 1981. Chemicals for preventing moulding in damp hay. Anim. Feed Sci. Technol. 6:323-336.

Lechtenberg, V.L. and R.W. Hemken. 1985. Hay Quality. in Forages: The science of grassland agriculture. Heath, M.E., R.F. barnes and D.S. Metcalfe (eds). Iowa State Univ. Press. Ames, Iowa.

Leshem, J., R. Thaine, C.E. Harris and R.J. Canaway. 1972. Water loss from cut grass with special reference to hay-making. Ann. Appl. Biol. 72:89-104.

Macdonald, A.D. and E.A. Clark. 1987. Water and quality loss during field drying of hay. Adv. in Agron. 41:407-437.

Magan, N. and J. Lacey. 1986. The effects of two ammonium propionate formulations on growth in vitro of *Aspergillus* species isolated from hay. J. Appl. Bacteriol. 60:221-225.

Marten, G.C., R.M. Jordan and A.W. Hovin. 1976. Biological significance of reed canarygrass alkaloids and associated palatability variation to grazing sheep and cattle. Agron. J. 68:909-914.

Mathison, G.W., L.P. Milligan and R.D. Weisenburger. 1975. Ruminant feed evaluation unit: Effect of baling damp hay on nutritive value. Agron. J. 67:51-53.

Merten, D.R. and L.O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. J. Anim. Sci. 49:1085-1095.

Meyer, J.H., R.L. Gaskill, G.S. Stoewsand and W.C. Weir 1959. Influence of pelleting on the utilization of alfalfa. J. Anim. Sci. 18:336-346.

Meyer, J.H., W.C. Weir, J.B. Dobie and J.L. Hull. 1959. Influence of the methods of preparation on the feeding falue of alfalfa hay. J. Anim. Sci. 18:976-982.

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.

Moore, K.J., V.L. Lechtenberg and K.S. Hendrix. 1985. Quality of orchardgrass hay ammoniated at different rates, moisture concentrations and treatment durations. Agron. J. 77:67-71.

Nash, M.J. and D.L. Easson. 1977. Preservation of moist hay with propionic acid. J. Stored Prod. Res. 13:65-75.

National Research Council. 1985. Nutrient requirements of sheep. Sixth revised edition. National Academy Press. Washington, DC.

Nehrir, H., W.L. Kjelgard, P.M. Anderson, T.A. Long, L.D. Hoffman, J.B. Washko, L.L. Wilson and J.P. Mueller. 1978. Chemical additives and hay attributes. Trans. ASAE 201-205.

Nelson, F.L. 1972. Storage characteristics and nutritive value of high-density native hay bales. Trans. ASAE 201-205.

Nelson, M.L., T.J. Klopfenstein and R.A. Britton. 1989a. Control of fermentation in high-moisture baled alfalfa by inoculation with lactic acid-producing bacteria: I. Large round bales. J. Anim. Sci. 67:1577-1585.

Nelson, M.L., D.M. Headley and J.A. Loesche. 1989b. Control of fermentation in high-moisture baled alfalfa by inoculation with lactic acid-producing bacteria: II. Small rectangular bales. J. Anim. Sci. 67:1586-1592.

Nicholson, J.W.G. 1983. Forage utilization in Eastern Canada. In Proceedings of the National Forage Symposium 1981. Agriculture Canada, Ottawa.

Porter, K.S., J.D. Axtell, V.L. Lechtenberg and V.F. Colenbrander. 1978. Phenotype, fiber composition and in vitro dry matter disappearance of chemically induced brown midrib (bmr) mutant sorghum. Crop Sci. 18:205-208.

Raymond, W.F. 1969. The nutritive value of forage crops. Adv. Agron. 21:1-108.

Reid, J.T., W.K. Kennelly, K.L. Turk, S.T. Slack, G.W. Trimberger and R.P. Murphy. 1959. Effect of growth stage, chemical composition and physical properties upon nutritive value of forages. J. Dairy Sci. 42:567-571.

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.

Robertson, J.A. 1983. Influence of harvesting and conservation practices on forage quality. Can. J. Plant Sci. 63:913-925.

Rode, L.M. and W.L. Pringle. 1986. Growth, digestibility and voluntary intake by yearling steers grazing timothy *(Phleum pratense)* or meadow foxtail (*Alopecurus pratensis*) pastures. Can. J. Anim. Sci. 66:463-472.

Ronning, M., J.H. Meyer and G.T. Clark. 1959. Pelleted alfalfa hay for milk production. J. Dairy Sci. 42:1373-1376.

Rotz, C.A., R.J. Davis, J.W. Thomas and M.S. Allen. 1990. Propionic acid and urea as prservatives for alfalfa hay. Proceedings of Forage and Grassland Conference . Donaldson Brown Center for Continuing Education, Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Russell, J.R. and D.R. Buxton. 1985. Storage of large round bales of hay harvested at different moisture concentrations and treated with sodium diacetate and/or covered with plastic. Anim. Feed Sci. Technol. 13:69-80.

Schmidt, D.R. and G.H. Tenpas. 1960. Comparison of the performance of bromegrass, orchardgrass and timothy in northern Wisconsin. Agron. J. 52:689-691.

Seoane, J.R., M. Cote, P. Gervais and J.P. Laforest. 1981. Prediction of the nutritive value of alfalfa (Saranac), bromegrass (Saratoga) and timothy (Champ, Climax, Bounty) fed as hay to growing sheep. Can. J. Anim. Sci. 61:403-413.

Seoane, J.R., C. Beaulieu, J. Florez and G. Dupuis. 1991. Evaluation of the nutritive value of grass hays for growing sheep. Can J. Anim. Sci. 71:1135-1147.

Sheard, R.W. 1968. Relationship of carbohydrate and nitrogen compounds in the haplocorm to the growth of timothy. Crop Sci. 8:658-663.

Smith, D. and A.H.S. Onions. 1983. The preservation and maintenance of living fungi. Commonwealth Mycological Institute. Surrey, England.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical methods. 7th ed. Iowa State Univ. Press. Ames, Iowa.

Sotola. 1933. The nutritive value of alfalfa leaves and stems. J. Agric. Res. 47:919-945.

Statistical Analysis System Institute, Inc. 1986. SAS user guide: Statistics, version 6 edition. SAS Institute Inc. Cary, NC.

Stone, J.B., G.W. Trimberger, C.R. Henderson, J.T. Reid, K.L. Turk and J.K. Loosli. 1959. Forage intake and efficiency of feed utilization in dairy cattle. J. Dairy Sci. 43:1275-1281.

Tuite, J. (editor). 1969. Plant pathological methods, fungi and bacteria. Burgess, Minneapolis, MN.

Walton, P.D. 1983. Production and management of cultivated forages. Reston Publ. Co. Reston, Virginia.

Weiss, W.P., V.F. Colenbrander and V.L. Lechtenberg. 1982. Feeding daity cows high moisture alfalfa hay preserved with anhydrous ammonia. J. Dairy Sci. 65:1212-1218.

Wilkins, R.J. 1988. The preservation of forages. In World Animal Science: Feed Science. Orskov, E.R. (editor). Elsevier Sci. Publ. B.V. Amsterdam.

Wittenberg, K.M. and S.A. Moshtaghi-Nia. 1990. Influence of anhydrous ammonia and bacterial preparations on alfalfa forage baled at various moisture levels: I. Nutrient composition and utilization. Anim. Feed Sci. Technol. 28:333-344.

Wittenberg, K.M. and S.A. Moshtaghi-Nia. 1991. Influence of anhydrous ammonia and bacterial preparations on alfalfa forage baled at various moisture levels: II. Fungal invasion during storage. Anim. Feed Sci. Technol. 34:67-74.

Wittenberg, K.M. 1991. Preservation of high-moisture hay in storage through the use of forage additives. Can. J. Anim. Sci. 71:429-437.

Woolford, M.K. 1984. The antimicrobial spectra of organic compounds with respect to their potential as hay preservatives. Grass Forage Sci. 39:75-79.

Youngberg, H.W., D.A. Halt and V.L. Lechtenberg. 1972. Diurnal variation in nitrogenous constituents of alfalfa (*Medicago sativa L.*). Agron. J. 64:288-291.

APPENDICES

Appendix 1. Storage temperatures (°C) for hay baled and stacked at low moisture level (L-Con), high moisture level without inoculant (H-Con) or with inoculant (H-S). Ambient temperatures and precipitation (mm) were also included.

Calendar Day	Treatment			Ambient temperature	Precipitation
	H-S	H-Con	L-Con		
158	39.6	40.0		22.9	8.13
159	41.5	43.9		21.5	10.16
160	42.2	44.5		19.5	.51
161	48.7	48.6		18.6	.25
162	53.0	53.3		19.4	0
163	44.3	49.6		22.1	0
164	46.0	48.5	27.5	18.5	45.47
165	51.9	51.1	25.4	21.7	0
166	46.4	47.7	25.4	20.4	.51
167	34.9	33.7	14.9	21.6	0
168	30.9	39.0	23.2	23.5	0
169	41.0	42.0	18.6	18.9	0
170	34.0	39.0	14.2	19.6	0
171	39.9	40.7	17.7	18.3	0
172	32.7	36.0	11.6	18.5	0
173	29.3	34.4	16.0	19.0	0
174	33.2	38.1	22.0	18.1	0
175	28.8	33.8	21.6	22.1	0
176	39.2	37.9	23.4	21.0	45.97
177	44.1	44.1	28.6	23.5	.76
178	43.2	40.9	27.8	18.8	0
179	42.4	38.1	29.4	16.5	0
180	37.1	32.4	23.9	20.0	0
181	35.8	35.1	29.1	17.4	10.92
182	26.1	26.5	23.1	19.2	9.65
183	35.0	31.4	30.6	18.2	10.41
184	42.0	33.6	29.8	17.9	1.78
185	45.1	38.5	29.6	19.9	0
186	37.8	34.1	29.9	19.8	.76
187	36.6	31.5	21.9	20.5	0
188	34.5	32.3	26.9	18.0	0
189	24.7	26.3	15.6	18.5	0
190	24.4	27.2	19.7	20.3	7.11
191	22.2	26.2	24.3	21.9	0
192			28.4	19.8	40.39
193			20.8	19.5	13.21
194			26.7	20.3	0
195			28.3	22.4	0
196			25.6	26.5	0
197			27.8	27.1	0

Appendix 2. Analysis of variance for chemical composition of timothy forage prior to storage.

Parameter	Source	DF	Type III SS	F value	PR>F
CP	TRT	2	0.75927403	0.17	.8474
	ERROR	42	96.69859111		
NDF	TRT	2	1101.17579336	6.62	0.0032
	ERROR	42	3492.32246442		
ADF	TRT	2	103.03339400	18.43	0.0001
	ERROR	42	120.56927656		
ADIN	TRT	2	5.78031184	3.59	0.0362
	ERROR	42	34.62522946		
Glucosamine					
	TRT	2	5.32616358	54.92	0.0001
	ERROR	44	2.13356833		

TRT=treatment

Appendix 3. Analysis of variance for chemical composition of stored timothy hay.

Parameter	Source	DF	Type III SS	F value	PR>F
DM	TRT	2	2.43560316	0.19	0.8280
	LYR	1	128.54414526	20.01	0.0001
	TRT*LYR	2	11.98509091	0.93	0.4017
CP	TRT	2	10.19183997	3.05	0.0585
	LYR	1	16.97408357	10.16	0.0028
	TRT*LYR	2	13.53777615	4.05	0.0250
	ERROR	40	66.83907320		
NDF	TRT	2	50.13631800	6.86	0.0027
	LYR	1	5.71834000	1.57	0.2181
	TRT*LYR	2	24.51485670	3.36	0.0449
	ERROR	40	146.09370374		
ADF	TRT	2	2.53601913	0.12	0.8876
	LYR	1	245.55992572	23.15	0.0001
	TRT*LYR	2	19.47090523	0.92	0.4076
	ERROR	40	424.21646546		
ADIN	TRT	2	13.21138941	2.31	0.1127
	LYR	1	75.58084737	26.39	0.0001
	TRT*LYR	2	14.13939091	2.47	0.0975
	ERROR	40	114.56579942		
Glucosamine					
	TRT	2	2.08123103	0.49	0.6143
	LYR	1	9.02192653	4.28	0.0450
	TRT*LYR	2	4.99647279	1.18	0.3163
	ERROR	41	86.51017470		

TRT=treatment.
LYR=layer.

Appendix 4. Analysis of variance for nutrient retention of stored timothy hay

Parameter	Source	DF	Type III SS	F value	PR>F
DM	TRT	2	119.15621428	1.67	0.2001
	LYR	1	70.92481446	1.99	0.1656
	TRT*LYR	2	126.67526530	1.78	0.1815
	ERROR	41	1459.42928017		
CP	TRT	2	297.04335229	2.81	0.0723
	LYR	1	0.05700286	0.00	0.9740
	TRT*LYR	2	350.95175128	3.32	0.0465
	ERROR	40	1903.25966525		
NDF	TRT	2	277.85328658	2.52	0.0934
	LYR	1	13.25175422	0.24	0.6268
	TRT*LYR	2	482.85410433	4.37	0.0191
	ERROR	40	2207.61274057		
ADF	TRT	2	309.13672032	2.64	0.0838
	LYR	1	297.08658863	5.07	0.0299
	TRT*LYR	2	534.08124479	4.56	0.0164
	ERROR	40	2342.47419904		
ADIN	TRT	2	42260.15728391	1.44	0.2489
	LYR	1	21404.34905689	1.46	0.2342
	TRT*LYR	2	55511.77592299	1.89	0.1640

TRT=treatment.
LYR=layer.

Appendix 5. Analysis of variance for visual and plate count assessments of molds in stored timothy hay

Parameter	Source	DF	Type III SS	F value	PR>F
Visual assessment					
Color	TRT	2	3.17414964	4.26	.0209
	LYR	1	3.89344922	10.45	.0024
	TRT*LYR	2	2.62078468	3.52	.0390
	ERROR	41	15.28272944		
Dust	TRT	2	1.05310014	0.85	.4350
	LYR	1	1.04912691	1.69	.2005
	TRT*LYR	2	2.09162687	1.69	.1976
	ERROR	41	25.41056494		
Mold	TRT	2	3.42417868	1.58	.2176
	LYR	1	12.94243390	11.97	.0013
	TRT*LYR	2	5.29385665	2.45	.0990
	ERROR	41	44.33884307		
Plate count assessment					
Aspergillus glaucus	TRT	2	11692.31953489	2.39	.1047
	LYR	1	2904.24444444	1.19	.2825
	TRT*LYR	2	11063.64295125	2.26	.1175
	ERROR	40	97884.07878788		
Penicillium spp.	TRT	2	328.04611521	0.42	.6599
	LYR	1	47.36821338	0.12	.7295
	TRT*LYR	2	106.94225955	0.14	.8724
	ERROR	40	15621.42803030		
Absidia sp.	TRT	2	73.73308585	0.69	.5063
	LYR	1	237.15533460	4.45	.0411
	TRT*LYR	2	65.07279315	0.61	.5478
	ERROR	40	2130.00075758		
Scopulariopsis spp.	TRT	2	107.45761406	0.24	.7908
	LYR	1	52.69141414	0.23	.6330
	TRT*LYR	2	34.01024374	0.07	.9281
	ERROR	40	9101.22272727		
Actinomycetes	TRT	2	43.81698103	0.08	.9254
	LYR	1	25.28488005	0.09	.7662
	TRT*LYR	2	71.61972991	0.13	.8812
	ERROR	40	11287.91287879		

Appendix 5. Analysis of variance for visual and plate count assessments of molds in stored timothy hay (Continued).

Total	TRT	2	16188.97276102	2.60	.0868
	LYR	1	4081.42806187	1.31	.2591
	TRT*LYR	2	12853.72131094	2.06	.1402
	ERROR	40	124546.90984848		

TRT=treatment
LYR=layer

Appendix 6. Analysis of variance for dry matter intake and apparent digestibilities of timothy hay by lambs

Parameter	Source	DF	Type III SS	F value	PR>F
DMI:					
g/an./d	TRT	2	31076.63873600	0.32	0.7302
	ERROR	26	1269444.93552150		
(%BW)	TRT	2	0.30097800	0.27	0.7691
	ERROR	26	14.75383560		
Digestibility:					
DMD	TRT	2	28.14781667	2.43	0.1432
	ERROR	9	52.11927500		
CP	TRT	2	33.42971667	1.79	0.2213
	ERROR	9	83.96545000		
NDF	TRT	2	3.690050000	0.38	0.6972
	ERROR	9	44.214550000		
ADF	TRT	2	5.846316670	0.37	0.7022

TRT=treatment

Appendix 7. Individual data for nutrient digestibility of feed by lambs

Trt	Sheep #	DM	CP	NDF	ADF
H-S	190	66.31	67.96	69.84	65.37
H-S	270	68.44	69.87	74.55	67.32
H-S	197	61.61	61.06	70.11	60.50
H-S	208	65.01	66.13	72.07	65.03
H-Con	254	65.82	67.65	71.59	64.96
H-Con	201	68.50	72.68	72.22	66.85
H-Con	297	66.10	68.98	71.06	63.85
H-Con	279	68.82	70.84	73.04	68.83
L-Con	214	62.06	65.78	67.65	63.46
L-Con	287	63.01	68.82	69.24	63.45
L-Con	268	61.80	63.07	71.06	62.47
L-Con	204	67.37	69.54	74.73	69.61

Trt = treatment.
H-S = high-moisture hay, inoculated.
H-Con = high-moisture hay, control.
L-Con = low-moisture hay, control.