Laboratory exercise to evaluate hay preservatives


ABSTRACT

Preserving hay is an important component of many forage production systems. Most beginning forage crop courses teach the basic principles of hay preservation. This laboratory exercise is designed to demonstrate the effects of moisture on hay preservation and the use of hay preservation products in a relatively simple laboratory experiment that does not require large amounts of equipment or instructor time. The students pack hay into 0.47-L (1 pt) canning jars. Water is added to hydrate the hay to moisture levels that cause spoilage. Three types of hay preservation products are used to preserve the hay: organic acid, bacterial inoculant, and ammonia. Hay quality is evaluated after 2 wk. This laboratory exercise demonstrates the importance of moisture content of hay at baling, the characteristics of the different types of hay preservatives available, their advantages and disadvantages, and the importance of uniform application of preservation materials. Student reaction to this laboratory was positive.

Utilizing hay preservatives is a management option in many humid areas that allows forage to be baled at elevated moisture levels without excess heating and molding. To package good-quality hay, the forage must be at the proper moisture content at baling. Fresh cut forage may have a moisture content of 800 g kg\(^{-1}\) or greater (Larsen and Rider, 1985). However, hay must be stored at <150 g kg\(^{-1}\) moisture to prevent microbial degradation and heating (Lechtenberg and Hemken, 1985). Cut forage is usually dried in the field to an acceptable moisture content before baling, but dry matter losses and quality deterioration may occur due to rain or inclement weather. Secondly, leaf loss in legume hay can be excessive when hay is handled at moisture contents low enough to prevent spoilage. Leaves contain a major portion of the protein and readily digestible carbohydrates in hay. Baling hay at a higher moisture content reduces drying time in the field, thus decreases the probability of encountering inclement weather. It also improves forage quality in legume hay by reducing leaf loss.

Several preservation agents are available that allow growers to bale hay at a higher moisture content. These include organic acid products, ammonia, and bacterial inoculants. These preservatives must be used properly to make quality hay. They differ in their mode of action for microbial suppression, come in various formulations and concentrations, and are not all equally effective. Field demonstrations of hay preservative use are seldom practical due to lack of haying activity at the time of year when most forage classes are taught, a large time commitment, and the large amount of equipment and materials required. Our objective was to develop a laboratory exercise to teach students about the general types of hay preservatives, the forage moisture contents at which they

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are effective, and to evaluate the organoleptic quality differences between high-moisture content hay that is well preserved and unpreserved hay. This laboratory exercise is designed to help students learn the important concepts of forage preservation by a hands-on experiment.

MATERIALS

For this experiment, we use three different types of hay preservatives: an organic acid, a bacterial inoculant, and ammonia. Several manufacturers market organic acid products of various formulations and concentrations, all of which have a similar mode of action. There are also several brands of bacterial inoculants available. It is not the purpose of the laboratory exercise to evaluate the various brands, although any could be used.

The treatments can be applied to different types of hay (e.g., legume, grass, or legume/grass mixtures). Results of the treatments are essentially the same for the different types of hay, however, good-quality legume hay with plenty of leaves shows the molding and discoloration better. The completion of several sets of the same treatments is recommended to provide replication. We recommend organizing the class into four or five teams, with each team doing one complete set of treatments. The materials listed below are sufficient for one complete set of treatments for one forage using one organic acid (propionic), one bacterial inoculant (Anchor, St. Joseph, MO), and urea. Each complete set consists of nine treatments: air-dried control, 240 g kg⁻¹ moisture content control, 350 g kg⁻¹ moisture content control, propionic acid at 240 g kg⁻¹ moisture content, propionic acid at 350 g kg⁻¹ moisture content, bacterial inoculant at 240 g kg⁻¹ moisture content, bacterial inoculant at 350 g kg⁻¹ moisture content, urea at 240 g kg⁻¹ moisture content, and urea at 350 g kg⁻¹ moisture content.

Materials for one set of treatments:
- 540 g of air-dry forage,
- nine canning jars (0.47 L) with lids that have holes punched in them,
- 0.9 mg of bacterial hay inoculant,
- 1.61 mL of propionic acid,
- 6.8 g of urea, and
- one 30-mL syringe with 10-cm needle.

PRELABORATORY PREPARATION

Calculating the amount of water and preservative needed for each treatment is an excellent learning experience for the student. If class time is not limited, instructors may wish to have their students do their own calculations. These should be checked by the instructor before making the solutions and applying the treatments to the hay. To save time, however, instructors may prepare treatment solutions and calculate the amount of water and treatment solution needed for each treatment prior to the laboratory. Calculations are given below as a guide for the instructors.

If solutions are prepared in advance and only one type of hay is used, the laboratory can be completed in 1 h. Evaluation of the hay and discussion of the efficacy of the preservation products 2 wk after treatment requires about 1 h, depending on the depth of the discussion.

Sixty grams of air-dried forage is used for each of the nine treatments. Air-dried forage is usually assumed to have 100 g kg⁻¹ of moisture. This can be verified by determining the fresh weight of the forage, then heating it to remove the moisture and determining its dry weight, and calculating the moisture content using the following equation:

\[ MC = \frac{FW - DW}{FW} \times 1000 \]

where,
- \( MC \) = moisture content as a percentage of the fresh wt. (g kg⁻¹)
- \( FW \) = fresh wt. of the sample, and
- \( DW \) = dry wt. of the sample.

The calculations described below are based on the assumption that the air-dried forage has a moisture content of 100 g kg⁻¹.

Three of the nine treatments are controls: air-dry control (100 g kg⁻¹ moisture), 240 g kg⁻¹ moisture content control, and 350 g kg⁻¹ moisture content control. The amount of water needed to hydrate the 60-g air-dried forage to 240 or 350 g kg⁻¹ moisture content control is calculated from the dry weight of the sample. The equation for calculating dry weight is:

\[ DW = FW - (FW \times MCI) \]

where,
- \( DW \) = grams dry wt. of sample,
- \( FW \) = grams fresh wt. of sample, and
- \( MCI \) = initial moisture content (decimal form) of the sample.

Thus the dry weight of a 60-g sample of air-dry forage (100 g kg⁻¹ moisture content) or 0.10 moisture content) would be 54 g. Final weight of the forage sample after it is hydrated to 240 or 350 g kg⁻¹ moisture content is calculated as:

\[ FWH = DW/(1 - MCD) \]

where,
- \( FWH \) = grams final fresh wt. of the hydrated sample,
- \( DW \) = grams dry wt. of sample, and
- \( MCD \) = desired moisture content (decimal form) of the sample after hydration.

Thus, the final fresh weight of a 60-g air-dried forage sample hydrated to 240 and 350 g kg⁻¹ is 71.1 and 83.1 g, respectively. Finally, the amount of water needed to hydrate the sample to the desired moisture content can be calculated using the equation:

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1 Mention of trademark does not constitute a guarantee or warranty of the product by the Univ. of Missouri, and does not imply its approval to the exclusion of other products that may also be suitable.
Water needed = FWH - FW

where,
FWH = final grams fresh wt. of the hydrated sample, and
FW = grams fresh wt. of sample.

Because 1.0 g of water is approximately 1.0 mL, the water needed to hydrate a 60-g air-dry sample (100 g kg⁻¹ moisture content) to 240 and 350 g kg⁻¹ moisture content is 11.1 and 23.1 mL, respectively.

Commercial application rates of propionic acid for 240 and 350 g kg⁻¹ moisture content hay are 5 and 15 kg of acid per 1000 kg of fresh forage, respectively. A 60-g air-dry sample requires 0.36 and 1.25 mL of propionic acid when hydrated to 240 and 350 g kg⁻¹ moisture content, respectively. Thus, 0.36 mL of propionic acid mixed with 11.1 mL of water are needed to hydrate a 60-g sample of air-dried forage to 240 g kg⁻¹ moisture content and apply the required amount of preservative. A convenient way to do this is to make a stock solution of 3.2 mL propionic acid to 100 mL of water and apply 11.5 mL of this solution to the sample. The stock solution for the 350 g kg⁻¹ moisture content organic acid treatment consists of 5.4 mL of propionic acid per 100 mL of water, and 24.4 mL of this solution must be applied per treatment.

Anchor hay inoculant is commercially recommended at 6.2 mg kg⁻¹ of fresh forage. Less than 1.0 mg is required per treatment and only 4.0 and 2.2 mg per 100 mL are needed for stock solutions for the 240 and 350 g kg⁻¹ moisture content treatments, respectively.

Urea rates are calculated to deliver 20 g N kg⁻¹ of forage on a fresh weight basis (Henning, 1986). Urea breaks down when applied to moist hay to release two molecules of ammonia. The amount of urea needed is 3.1 and 3.6 g per sample for the 240 and 350 g kg⁻¹ moisture content treatments, respectively. Stock solutions would require 27.9 and 15.6 g of urea per 100 mL of water for the 240 and 350 g kg⁻¹ moisture content treatments, respectively. Urea will increase the volume of the stock solutions, so the amounts needed to hydrate a 60-g air-dried sample to 240 and 350 g kg⁻¹ moisture content and supply the necessary urea are 13.3 and 25.0 mL, respectively.

LABORATORY PROCEDURE

1. Chop the air-dried forage into 3- to 5-cm lengths using scissors or a paper cutter. This procedure can be done by the instructor prior to the laboratory to save time.
2. Pack 60 g of hay into each of nine jars and put on lids that have holes punched in them. About 8 to 10 holes (3-mm) are sufficient. Holes are needed because hay is an aerobic system. The hay needs to be packed tightly as about 60 g of hay in each jar simulates the density of a conventional small rectangular bale of hay weighing 18.2 kg. The amount of forage to fill the jar may need to be increased for very leafy legume hays. Be sure to adjust calculated additions of water and preservative if >60-g of air-dry forage is used.
3. Using the syringe, inject the appropriate amount of stock solution through holes in the lid of the jar in each of the eight jars requiring treatment. Immediately rotate the jars so that the solution is equally distributed over all of the hay. Air-dry hay is slow to absorb moisture at least for a few minutes after rewetting. Complete coverage of the hay with the preservative is as important in this laboratory as it is in the field. Uneven distribution of the preservative solution results in overly wet layers of hay that have higher than desired rates of preservative and dryer layers that contain little moisture or preservative.
4. Store jars at room temperature for about 2 wk. During this time the high moisture hay that is not properly treated will spoil.
5. After 2 wk, open each jar and evaluate the hay sample for presence of mold and/or fungal growth, color changes, and odor. Evaluate the presence of mold and/or fungus by ranking the treatments from 1 to 5, where 1 is the least moldy as represented by the air-dry control and 5 is the most moldy as represented by the 350 g kg⁻¹ moisture content control. Evaluate color by ranking the treatments from 1 to 5, where 1 is the most desirable color as represented by the air-dry control and 5 is the least desirable as represented by the 350 g kg⁻¹ moisture content control. Rate each treatment as having either a fresh hay, a moldy, or an ammonia odor.

DISCUSSION

Microbial degradation of hay requires (i) a substrate for microbial growth (i.e., structural and nonstructural carbohydrates in hay), (ii) viable hay degrading microbes, and (iii) sufficient moisture for microbial growth. As hay degrading microbes are ubiquitous, the moisture content of untreated hay is the determining factor in hay spoilage. The safe moisture content for baling small rectangular bales is between 180 and 220 g kg⁻¹, depending on environmental conditions. Bales continue to lose moisture after baling and reach a safe moisture content of about 100 to 120 g kg⁻¹ during storage. Hay with a high moisture content that undergoes microbial degradation will lose dry matter, have reduced feeding value (because much of the dry matter loss comes from high-feed-quality nonstructural carbohydrates), and be less palatable. Hay preservatives are used to protect hay baled at moisture contents that are too high to prevent microbial degradation.

Visual comparisons of the control treatments demonstrate the effect that moisture content has on the microbial degradation of hay. The 100 g kg⁻¹ moisture content control hay is used as a reference for unspoiled, untreated hay. The 240 g kg⁻¹ moisture content control hay is above the moisture content for safe storage and will show evidence of some microbial growth. Most hay degradation is caused by fungal growth (Festenstein et al., 1965). Students look for filamentous hyphae growing on the sur-
Table 1. Characteristics of hay preservatives.

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Composition</th>
<th>Mode of action</th>
<th>Effective moisture range</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial inoculant</td>
<td>Viable bacteria in a dry, organic (peat) matrix (e.g., Lactobacillus plantarum, L. acidophilus)</td>
<td>Bacteria multiply and produce lactic acid that inhibits mold growth</td>
<td>Up to 250 g kg(^{-1}) moisture content</td>
<td>Low cost per ton of treated hay (&lt; $5L)</td>
<td>Limited moisture range. Chlorinated (i.e., tap) water often cannot be used as a carrier for the inoculant.</td>
</tr>
<tr>
<td>Organic acid</td>
<td>Propionic acid</td>
<td>Propionic acid inhibits growth of molds.</td>
<td>Up to 350 g kg(^{-1}) moisture content</td>
<td>Large effective moisture range.</td>
<td>High cost. Irritating to nasal passages and skin. Will remove paint from bale surfaces contacted, resulting in a greater potential for rusting. Can bleach green color from hay.</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Urea</td>
<td>In the presence of water and urease, urea is degraded to NH(_3) and CO(_2). The moisture in hay serves to retain NH(_3). Ammonia is toxic to molds. Ammonia reacts with water in hay to form NH(_4)OH.</td>
<td>Up to 400 g kg(^{-1}) moisture content</td>
<td>Large effective moisture range. Urea can be applied in dry or liquid form at the time of baling. Urea is relatively nontoxic to equipment operators or hay handlers. Urea is easier to handle and store than anhydrous ammonia. Treated hay has increased N content and greater digestibility than untreated hay.</td>
<td>Treatment requires at least 30 g of urea per kg of hay.</td>
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</tbody>
</table>

face of the hay and small clumps of spore-bearing reproductive stalks (similar to those produced by common bread mold). The hay should have a musty odor. The 350 g kg\(^{-1}\) moisture content control hay is well above the moisture content for proper storage and will be completely covered with microbial growth.

Bacterial inoculants contain live bacteria that multiply in moist hay and produce lactic acid (Table 1). If enough lactic acid is produced, the pH will be reduced and lactic acid concentration will retard further microbial growth. This process is similar to the one that preserves silage. Silage, however, is usually stored at a moisture content of about 650 g kg\(^{-1}\) and the lactic acid production is much greater than that obtained in a hay at 250 g kg\(^{-1}\) moisture content.

Research data supporting the efficacy of bacterial inoculants to preserve hay is not available. Despite this, bacterial inoculants are widely used because of their advantages over other hay preservatives (Table 1). Our experience with using bacterial inoculants in this laboratory is that the 240 and 350 g kg\(^{-1}\) moisture content bacterial treatments look very similar to the 240 and 350 g kg\(^{-1}\) moisture content controls. Some decrease in microbial growth may be observed at 240 g kg\(^{-1}\) moisture content compared to the 240 g kg\(^{-1}\) moisture content control, but the 350 g kg\(^{-1}\) moisture content treatment is usually covered with fungal growth.

Ammonia, when applied to hay, reduces microbial populations that cause degradation (Grotheer et al., 1985). Anhydrous ammonia is usually used in the field. Hay bales are stacked and covered with plastic and anhydrous ammonia is released under the plastic from a tank. The ammonia permeates the hay, killing the microbial population, thus preserving the hay from degradation. Anhydrous ammonia can be toxic and care must be taken while applying it to hay.

Ammonia is also the only preservative that can improve the quality of hay (Table 1). Ammonia reacts with water in the hay to form ammonium hydroxide. Hydroxide ions can increase the digestibility of hay by disrupting covalent bonds between lignin and cellulose and/or hemicellulose (Buettner et al., 1982). This reaction exposes more surface area of cell wall materials to attack by rumen microorganisms, resulting in increased digestibility of the fiber fraction (Faulkner et al., 1985).

As ammonia is effective up to 400 g kg\(^{-1}\) moisture content, the 240 and 350 g kg\(^{-1}\) moisture content urea treatments should be free of fungal growth. The hay samples should smell of ammonia.

Organic acid products are very effective mold inhibitors, are used widely, and can preserve hay up to 350 g kg\(^{-1}\) moisture content (Knapp et al., 1976). However, they have several disadvantages (Table 1). Hay samples treated with organic acids are largely free of fungal growth. Propionic acid may bleach some of the green color from the hay, causing the samples to appear more brown compared to the controls.

This laboratory exercise exposes students to several important principles in the use and application of hay preservatives. These include the importance of moisture content of hay at baling, what happens when hay is baled at too high a moisture content, the characteristics of the differ-
Table 2. Responses of 35 students to an evaluation of the hay preservation laboratory.

<table>
<thead>
<tr>
<th>Question</th>
<th>Mean response†</th>
<th>SE</th>
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</thead>
<tbody>
<tr>
<td>1. The laboratory improved my understanding of the importance of hay moisture content during baling and preservation.</td>
<td>4.6</td>
<td>0.11</td>
</tr>
<tr>
<td>2. The laboratory improved my understanding of the types of hay preservation products available.</td>
<td>4.9</td>
<td>0.06</td>
</tr>
<tr>
<td>3. The laboratory improved my understanding of the difference between well preserved hay and poorly preserved hay.</td>
<td>4.4</td>
<td>0.12</td>
</tr>
<tr>
<td>4. Even though this laboratory did not occur in the field, the jar technique was adequate to help me visualize the effectiveness of hay preservation products.</td>
<td>4.3</td>
<td>0.11</td>
</tr>
<tr>
<td>5. The laboratory helped to clarify the principles of hay preservation that were discussed in class.</td>
<td>4.5</td>
<td>0.09</td>
</tr>
<tr>
<td>6. I would recommend that this laboratory be used again.</td>
<td>4.8</td>
<td>0.06</td>
</tr>
</tbody>
</table>

† 5 = strongly agree, 4 = agree, 3 = neither agree nor disagree, 2 = disagree, 1 = strongly disagree.

ent types of preservatives available, their advantages and disadvantages, and the importance of uniform application of preservation materials. As a result of this exercise, students have an increased understanding of the effectiveness of the three major types of hay preservatives and a better ability to evaluate both the need for the proper application of hay preservatives.

Student response to this laboratory was positive. An evaluation of the laboratory exercise was conducted and 35 students gave their responses (Table 2). Thirty-two of the students strongly agreed or agreed that the laboratory improved their understanding of the importance of hay moisture content during baling and preservation. All of the students strongly agreed or agreed that the laboratory improved their understanding of the types of hay preservation products available. Thirty-one of the students strongly agreed or agreed that the laboratory improved their understanding of the difference between well preserved hay and poorly preserved hay. Thirty-one of the students strongly agreed or agreed that the jar technique was adequate to help visualize the effectiveness of hay preservation products. Thirty-four of the students strongly agreed or agreed that the laboratory helped improve their understanding of the principles discussed in lecture. All of the students strongly agreed or agreed that the laboratory should be used again.

Students were given an opportunity on the evaluation to express concerns or make general comments regarding the laboratory exercise. Two comments for improving the laboratory were to “bring in some fresher hay” and “reduce overcrowding” in the laboratory. The positive comments included, “It was a very accurate experiment because of the serveral groups doing it’’ and “This lab supported the concepts I had learned in class.” One student wrote that “Of all the things I’ve learned in college, I found this evaluation study to be one of the most interesting and practical I’ve been a part of.”

REFERENCES


