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Original Research

Microbial Counts in Forages for Horses—Effect of Storage Time and of Water Soaking Before Feeding

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ABSTRACT

Soaking of hay in water before feeding has become common practice in equine feeding to reduce number of respirable particles or content of nonstructural carbohydrates. It is, however, not known if soaking may increase general microbial load in different forages. A study was therefore performed where microbial composition of silage, haylage, and hay was analyzed before and after soaking in water for 24 hours. As storage time may also influence microbial composition of forages, the soaking procedure was evaluated after two different storage periods. Results showed that soaking increased counts of yeasts, enterobacteria, and lactic acid bacteria (LAB) and decreased mold counts. Although mold counts in hay decreased with soaking (from log 4.7 to log 3.6 colony-forming units [CFUs]/g), soaked hay still contained greater ($P < .001$) numbers of molds compared with silage and haylage presoaking. Counts of enterobacteria increased ($P < .001$) with soaking in silage (from log 1.1 to 2.7 CFU/g) and haylage (from 1.7 to 4.8 CFU/g) but not in hay (log 4.9 and 5.1 CFU/g before and after soaking). Count of yeast and LAB generally increased by soaking ($P \leq .004$). Soaking forage for 24 hours may therefore reduce the hygienic quality of forage. Increased storage time (from 3 to 12 months) resulted in decreased counts of molds in hay (from log 4.8 to 3.5 CFU/g; $P < .001$) and decreased counts of LAB in silage (from log 8.1 to 6.6 CFU/g; $P < .001$) as well as in haylage (from log 6.9 to log 4.8 CFU/g; $P < .001$).

1. Introduction

Soaking hay in water before feeding has become common practice in many equine management systems throughout Western Europe and the United States, in an attempt to either reduce concentration of respirable particles such as mold spores [1–5] or content of water soluble carbohydrates (WSC) [6–9]. The former is practiced for horses suffering from recurrent airway obstruction and similar conditions [1,4], whereas the latter is especially practiced for insulin resistant horses [10], laminitic horses or horses diagnosed with equine metabolic syndrome [7], horses diagnosed with pituitary pars intermedia dysfunction [11], and horses with polysaccharide storage myopathy [12], as they have all been reported to benefit from a diet low in nonstructural carbohydrates such as sugars and starch. However, soaking forages before feeding is laborious and results in wastewater, which may have a high biological oxygen demand [4,5]. It may also be difficult to soak forage in geographic areas with cold climate due to freezing conditions. Also, no information has been found on how soaking influences general microbial load in forage. Addition of water to dry hay may provide dormant microbial spores with renewed possibilities to proliferate [13]. Other forage types are also used for horses, and although silage and haylage are generally regarded as containing lower counts of respirable particles than hay [4,14–16], a comparison of soaked hay and unsoaked haylage reported lower numbers of respirable particles in the soaked hay [17]. Different forage conservation methods may result in different microbial

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challenges, and silage and haylage may contain microbes such as molds with potentially hazardous effects [18,19] and bacteria belonging to *Clostridia* spp. or enterobacteria which may cause toxicosis [20] or gastrointestinal disorders [21,22]. These microbes could proliferate when, for example, water and oxygen availability in silage and/or haylage changes. The effect of soaking on general microbial load in forages conserved with different methods has, to the authors' knowledge, not been reported previously. With this background, a study was designed with the aim of investigating if soaking silage, haylage, and hay in water resulted in increased microbial loads in the forages and if the microbial load before and after soaking was different among the three forage types. As storage itself may bring different microbial challenges for wrapped forages [23] and hay [24], the study included two different periods of storage.

2. Material and Methods

2.1. General

The experiment was performed using laboratory silos for silage and haylage conservation and a conventional high-density hay baler for hay production. A grass-dominated sward in its fourth year, consisting of approximately 0.5 timothy (*Phleum pratense*), 0.4 meadow fescue (*Festuca pratensis*), and 0.1 red clover (*Trifolium pratense*), was harvested on June 15, 2010, in Uppsala, Sweden (59°86′N, 17°64′E, elevation 20 m above sea level, clay-dominated soil type, humid continental climate with average year precipitation of 576 mm/m^2, and average year temperature 6.5°C). The grass was cut using a mower conditioner with flails (Kverneland Taarup 4028; Kverneland, Nyköping, Sweden) and left in 2-m wide rows in the field during wilting. Dry matter (DM) content of the grass during wilting was monitored by taking samples every third hour during daytime (sunrise to sundown) and drying them in a microwave oven for 8–10 minutes (750 W) until no further weight loss. At an approximate DM content of 400 and of 600 g/kg, two thirds of the crop was collected and transported in open 150-L plastic bags from the field to the experimental station (about 3 minutes transport time) for conservation as silage and haylage, respectively. When the remaining crop in the field had reached an approximate DM content of 800 g/kg, hay was baled.

The wilted grass used for silage and haylage conservation was filled in stainless steel silos (25 L volume) and compacted using a stationary hydraulic press. The same filling–compacting–weighing procedure was followed for each silo. For silage, 8 kg of wilted grass was placed into the silos and for haylage 6 kg (which both corresponded to 3.2–3.6 kg DM of the wilted grass and 128–144 kg DM/m^3). The silos were sealed with stainless steel lids, using silicone paste and plastic stretch film to secure tightness between lid and silo. Each lid was equipped with a water-filled fermentation lock to allow gas out of the silo but prevent oxygen inlet and to allow for control of silo tightness (uneven water levels in fermentation lock) during storage. After completing the sealing procedure, silos were kept in room temperature (16°C–18°C) for a total period of 12 months.

Hay was baled using a high-density hay baler (Welger AP 730; Lely Maschinenfabrik GmbH, Wolfenbüttel, Germany) producing rectangular bales sized approximately 70 × 38 × 46 cm. Hay bales were put on a barn-drier and dried with cold air until DM content was 850 g/kg and water activity (a_w) <0.70 (measured at constant room temperature 21°C using a $BaCl_2 \cdot 2H_2O$-calibrated Lufft Duratherm Kontroll hygrometer 5804, Germany). Thereafter, the hay bales were moved from the barn-drier to a farmhouse, where they were placed on wooden pallets and covered with bales of straw. This is common practice in South Sweden and was performed to protect the hay and avoid moisture uptake from the surrounding air during storage. The total storage period was 12 months.

2.2. Sampling and Soaking

Silos and hay bales were opened and sampled after 3 and 12 months of storage. At every sampling occasion, three hay bales and three silos of silage and haylage, respectively, were randomly selected, opened, and sampled (destructive sampling). Samples were used for chemical (reported elsewhere) and microbial analysis, and after sampling, the remaining silage and haylage of the opened silos and the corresponding amount of hay were soaked in 17 -L water/kg DM. Soaking of forage was performed within the 25-L silos mentioned previously (also for hay). The forages were completely submerged in water, and water temperature was 5°C to 7°C (cold tap water) at the start of soaking. The forages were soaked for a total of 24 hours, and water temperature at the end of the soaking period was 14°C to 16°C (approximately 2° below room temperature). After 24 hours of soaking, samples were taken from the forage for microbial analysis.

All sampling was performed using a stainless steel core sampler (1 m × 40 mm in diameter, Almunge smide, Almunge, Sweden) connected to an electrical drilling machine (DeWalt DW006, Tampa, FL). The core sampler was dried and sterilized between each silo, using clean paper towels, ethanol (0.96 w/w), and an open flame.

2.3. Analyses

Forage samples for microbial analysis were prepared by adding 200 mL of sterile (autoclaved for 15 minutes at 121°C) peptone water (Merck KGaA, Darmstadt, Germany) to 20 g of sample. The sample was run twice for 60 seconds at the setting "normal speed" in a laboratory blender (Stomacher Seward 3500; Worthing, West Sussex, UK). Tenfold dilution series were then made using sterile peptone water. Enumeration of lactic acid bacteria (LAB), enterobacteria, clostridial spores, and fungi (yeast and mold) were made from three dilutions. Lactic acid bacteria were cultivated under anaerobic conditions on duplicate Rogosa agar plates (Merck, KgaA, Darmstadt, Germany) for 72 hours at 30°C [25]. Enterobacteria were cultured aerobically on duplicate violet red bile glucose (VRBG) agar plates (Oxoid CM0485; Oxoid Ltd, Cambridge, UK) in sealed plastic bags at 37°C for 48 hours. Cultures were confirmed biochemically with oxidase test by culturing on bromocresol purple lactose agar. Colonies from VRBG agar plates that tested negative on oxidase test were considered to be