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Original Research

Microbial Composition before and after Conservation of Grass-Dominated Haylage Harvested Early, Middle, and Late in the Season

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ABSTRACT

Haylage for horses is often harvested in late plant maturity, which could be associated with an increased risk of impaired hygienic quality in the forage and short aerobic storage stability after bale opening, but knowledge in this area is scant. An experiment was conducted in which the microbial composition was analyzed before and after conservation of primary growth haylage harvested early (June), middle (July), and late (August) in the season during 1 year. The counts of yeast, enterobacteria, and lactic acid bacteria (LAB) in preconserved herbage increased with the advancing harvest time ($P \leq .02$). After conservation, the August haylage had increased counts of enterobacteria (log 4.3 colony-forming unit [CFU]/g) and LAB (log 6.5 CFU/g), compared with the June and July haylage (log $\leq$1.7 CFU/g for enterobacteria and $\leq$5.7 CFU/g for LAB, $P < .001$). The yeast counts were the lowest in the June haylage (log 5.0 CFU/g) compared with the July and August haylage (log $\geq$6.3 CFU/g, $P < .001$). After conservation, the mold counts were lower in the June haylage and greater in the August haylage ($P = .01$). In the preconserved herbage, *Cladosporium cladosporioides* was the most common mold species in June but *Fusarium poae* was in July, and *Mucor fragilis* in August. After conservation, *Penicillium carneum* was the only species found in the June haylage, with *M. circinelloides* most frequently found in the July haylage and *M. hiemalis* and *M. circinelloides* found at similar frequencies in the August haylage. An advanced harvest time resulted in greater counts of enterobacteria, yeast, and LAB and an increased number of mold species in the conserved haylage. The aerobic storage stability of the opened haylage bales measured by temperature was similar among the harvests.

1. Introduction

The use of baled and wrapped forage with dry matter (DM) contents >500 g/kg, also known as haylage [1,2], has increased in recent years, especially for feeding horses in Scandinavian countries. The haylage used for horses in Sweden has often been characterized by late plant maturity at harvest, resulting in long-stemmed and rough forage material [3]. Although this type of haylage might be suitable nutritionally for a large proportion of adult horses [4,5], it could be a challenge to achieve and maintain a high hygienic quality in such forage, because late harvest has been reported to result in an increased microbial load [6,7]. The hygienic quality of forage could be compromised by the presence of undesirable bacteria and fungi [6,8-10], causing various health disturbances in horses and other animals. Molds can cause respiratory health problems in horses owing to spore formation, and mycotoxins can cause mycotoxicosis; thus, the presence of mold should be avoided in feedstuffs [8,10,11].

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Advanced plant maturity at harvest has previously been reported to negatively influence the hygienic quality of haylage [6,7,12]. However, scant information is available on microbial growth in haylage in general. The microbial composition of crops before conservation could influence the microbial composition after conservation, because haylage does not ferment to the same extent as silage [1,2], in which the initial microbial composition of the plants is replaced by lactic acid bacteria (LAB) when ensiling has been successful [13]. Rough plant stems from crops harvested in late plant maturity can also penetrate the stretch film layers surrounding the bales, allowing oxygen inlet, which in turn results in conditions for mold growth [6]. In a previous experiment with laboratory silos [7], delayed harvest resulted in increased yeast and LAB counts in haylage. Increased yeast counts can compromise aerobic storage stability, because yeasts have been found to initiate aerobic deterioration of silage [14]. This is important in equine operations in Scandinavia, because the number of horses fed from the same bale will often be less than five [15-17]. The resulting slow feed out rate of an opened silage or haylage bale can lead to aerobic deterioration of the forage before the entire bale has been eaten.

The aim of the present study was to evaluate the influence of harvest time of the primary growth on microbial composition before and after conservation of the baled haylage, including the effect of harvest time on the aerobic storage stability after bale opening. The hypothesis was that both preconserved herbage and postconserved haylage from harvest in late plant maturity would contain greater microbial counts and a larger number of mold species. It was also hypothesized that the late harvest of haylage would shorten the aerobic storage stability of the opened bales compared with earlier harvests.

2. Materials and Methods

2.1. Experimental Forages

The experiment was performed in Uppsala, Sweden (59°86′N, 17°64′E, elevation 20 m above sea level, clay-dominated soil type, humid continental climate, with an average annual precipitation of 576 mm/m^2 and average year temperature of 6.5°C). The ley used in the experiment was grass-dominated, consisting of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) in equal proportions and red clover (*Trifolium pratense)*. The proportion of red clover increased with increasing plant maturity as the season progressed. At the first harvest (June 8, 2009), the proportion of red clover was 0.003. At the second harvest (July 2, 2009), the proportion of red clover was 0.04, and at the third harvest (August 5, 2009), the proportion of red clover was 0.26. The remaining grass consisted of timothy and meadow fescue in equal proportions at all harvest times. The species proportion measurements used the DM basis of samples taken in the field the same day as mowing.

The field was divided into nine plots equal in size (0.3 hectares each) and shape (rectangular). At each harvest date, three plots were used. From each plot, triplicate bales were produced, resulting in nine bales from each harvest time and three observations for each harvest (the plot was the observational unit). A total of 27 bales were produced. The plots were randomly allotted among the harvest times in June, July, and August, before the first harvest in June. The herbage was cut with a mower conditioner with flails (Kverneland Taarup 4028, Kverneland, Nyköping, Sweden) and left in rows 2 m in width in the field during wilting. The weather at mowing and wilting was sunny and windy at all harvest dates, except for a small rain shower (1-2 mm/m^2) during the night of wilting for the June harvest crop. In July and August, no rainfall occurred during wilting. The average temperature during the June, July, and August harvest was 15°C, 22°C, and 20°C, respectively (Swedish Meteorological and Hydrological Institute, Agricultural weather forecast and weather reports telephone service, personal communication, June 6, 2009, July 2, 2009, and August 5, 2009). Approximate determinations of the DM content were made during wilting by drying samples for 8-10 minutes in a microwave oven (750 W) until no additional weight loss occurred. The herbage was baled at an approximate DM content of 500 g/kg. In June, the crop was tedded 7 and 26 hours after mowing using a conventional hay tedder (Claas WaS 730, CLAAS, KgaAmbH, Harsewinkel, Germany) and then windrowed (Krone KS 3.80-4.20 Vario, Bernard Krone Holding GmbH, Spelle, Germany) and baled (Taarup Bale-in-One, Kverneland Taarup, Nyköping, Sweden) 28 hours after mowing. In July, the crop was wilted for 19 hours and in August for 24 hours before windrowing and baling 2 hours later, using the same equipment used for the June harvest. The baler produced round bales 1.22 m in width and 1.25 m in diameter. The bales were wrapped with 10 layers of white stretch film (Silotite, London, UK) using 0.5 overlap and prestretching the film 1.7 times.

The bales were transported from the field to a storage area at Kungsängen experimental farm (Uppsala, Sweden) within 12 hours after wrapping, where the bales were weighed and measured (circumference and height) to calculate bale density. The bales were stored in two tiers. In early October, the bales were moved to another farm approximately 20 km away for use in a horse feeding trial previously reported [18,19]. In conjunction with this feeding trial, the bales were opened and sampled for the present experiment. Before transport, the bales were weighed for calculation of the weight loss during storage.

2.2. Sampling of Forages and Bale Tightness Measurement

Samples for microbial analysis preconservation were taken aseptically from the swaths in the field. At each harvest time, triplicate samples were taken from the wilted herbage within each of the three plots just before baling, resulting in nine samples from each harvest time (and three observations per harvest, because the plot was the observational unit). All samples were taken aseptically from randomly selected places in the plots, using a pair of scissors (stainless steel) that were sterilized using 0.995 ethanol (wt/wt) and an open flame between each sample. The samples were collected by cutting pieces of the mowed crop, which were then stored in double sterile plastic bags that were sealed and kept in a cooling bag at 6°C-8°C for a maximum of 4 hours before inoculation.