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ORIGINAL ARTICLE

Rebaling of silage and haylage and its effects on forage microbial and chemical composition—A pilot study

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Abstract

Use of big bale silage and haylage can be difficult on farms where daily forage consumption is comparatively low as speed of deterioration of forage after bale opening may be faster than feed-out rate. Production of smaller bales at harvest is possible, but expensive and work-intensive. Therefore, a pilot study of rebaling forage stored in big bales to smaller bales was conducted. Three separate experiments were included, where microbial and chemical composition of silage and haylage was studied before and after rebaling. In Experiment III, residual big bale forage stored and opened together with rebaled forage was included. Results showed that rebaled haylage and silage had higher yeast counts compared to initial forage; however, residual bales in Experiment III had yeast counts similar to rebaled forage, indicating an effect of storage time rather than of rebaling. In Experiment II, mould counts were higher in rebaled compared to initial silage, but not in haylage. Chemical composition was similar in initial and rebaled forage except for ammonia-N. In Experiment III, ammonia-N was higher in rebaled compared to initial and residual forage and was the only chemical variable affected by rebaling. Bale temperature during aerobic storage followed ambient temperature until day 6–8 in Experiment I and until day 14 in Experiment III where ambient temperature was lower. In conclusion, rebaling can be done without large changes in chemical composition of the forage, but yeast and mould counts may be higher in rebaled forage, and this risk should be considered when using this procedure.

KEYWORDS
additive, aerobic stability, bale, forage, horse, yeast

1 | INTRODUCTION

Use of big bale silage and haylage[1] can be difficult on farms where daily forage consumption rate is slower than speed of deterioration of the forage after opening of bales. This is a common problem on Swedish and Norwegian horse farms: over 0.75 of the Swedish horses are kept in stable housing only one to four horses (Persson, 2005), and the average number of horses at a Swedish horse farm is 4.9 (Enhäll, Nordgren, & Kättström, 2012). In Norway, 0.67 of the horse-owners own one to three horses and 0.78 keep

[1]Haylage has been defined as forage containing >500 g DM/kg and stored anaerobically (Finner, 1966; Gordon et al., 1960; Harris et al., 2017; Müller, 2005; Müller, 2018).

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only their own horses in their stable (Vik & Farstad, 2012). The structure of the horse populations in Sweden and Norway can therefore be described as consisting mainly of small stables, with comparably low daily forage consumption. A little less than half of the Swedish and a little more than half of the Norwegian horse-keepers have been estimated to use wrapped forage (haylage and to some extent silage) as the main forage source for their horses during winter feeding (Enhäll et al., 2012; Vik & Farstad, 2012).

In Scandinavia, haylage is commonly produced in wrapped round or square bales containing approximately 300–700 kg forage ("big bales"). Smaller bales (approximately 30–60 kg forage per bale) of haylage and silage are also available on the market, but are expensive as the price per kg DM is up to five times the cost for big bales. Production of big bales is cost- and time effective at harvest, and the entire production chain can be operated by one person, while production of small bale silage and haylage (or hay) usually requires more than one person/operator and considerable time in the manual handling of bales. Also, machinery equipment adapted for field production of small bale silage and haylage at harvest is not readily commercially available.

Opened haylage bales should ideally be consumed within five to six days after opening to avoid deterioration of the forage (Müller, 2005, 2009a, 2009b). Thus, using conventional big bales on horse farms may result in large amounts of forage being discarded which in an inefficient use of resources, or that deteriorated forage is fed which may result in health problems in horses and increased veterinary costs. As a result of this, a concept of rebaling has developed among horse feed producers in the Scandinavian countries, where big bales are produced at harvest, stored for some months and then opened and split (square bales) or rebaled (round bales) into smaller, individually wrapped bales. However, there is a lack of information on how the rebaling procedure affects the microbial and chemical composition of silage and haylage.

A rebaling procedure means that anaerobically conserved forage is exposed to oxygen-rich air, and there is a possible loss of finer forage particles (which may result in lower nutritive value) due to the mechanical handling during rebaling (Honig, 1980). The oxygen exposure may result in increased yeast populations in the forage, similar to effects of air penetration into silage during storage (Jonsson & Pahlow, 1984). Increased yeast numbers may increase the risk of rapid yeast growth and heat production after opening of rebaled haylage bales. Haylage may heat very rapidly after bale opening as water content of haylage is lower (<200–500 g/kg) compared to silage, and therefore, the capacity of haylage to buffer heat is comparatively low (Muck & Pitt, 1993; Savoie & Jofriet, 2003; Wilson, 1999). It may therefore be of interest to consider using additives to counteract microbial changes during a rebaling process.

It is not well-established whether use of additives can delay onset and/or speed of deterioration in haylage bales after opening, but as propionic acid has been successfully used for preservation of moist hay (Buckmaster & Heinrichs, 1993; Coblentz, Coffey, Young, & Bertram, 2013; Easson & Nash, 1978; Nash & Easson, 1977) and moist grain (Jones, 1970; Saastamoinen, 1994), it is of interest to investigate also for use in haylage. Use of propionic acid has also been reported to increase aerobic storage stability in silage (Crawshaw, Thorne, & Llewelyn, 1980). In combination with benzoic acid, it has been shown to prolong aerobic storage stability in opened haylage bales (Müller, 2005), and it may inhibit fungal growth (Thomas, Yu, McGuffey, Tinnimit, & Ferris, 1973; Woolford, 1975a, 1975b). The objective of these experiments was therefore to study effects of a rebaling procedure (with and without use of an additive containing propionic acid and benzoate), on microbial and chemical composition of silage and haylage, as well as on aerobic storage stability after opening of rebaled bales.

2 | MATERIALS AND METHODS

2.1 | Experimental location

The experiments were carried out in Uppsala, Sweden, at 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 per square metre, and average year temperature 6.5°C.

2.1.1 | Experiment I

Grass silage (approximately 470 g DM/kg) harvested from a regrowth crop consisting of predominantly timothy (*Phleum pratense*), with presence of perennial ryegrass (*Lolium perenne*) and meadow fescue (*Festuca pratensis*), and conserved in big round bales (ca 400 kg bale weight) in July 2000 were used for the experiment. The big bales were stored outdoors in a single layer on concrete ground within a fenced bale yard without roof or cover. Four big bales were opened in March (year 2001) and sampled for analysis of chemical (three grab samples per bale pooled to one sample per bale) and microbial (aseptic grab samples, three per bale, pooled to one sample per bale) composition. One round bale at a time was opened, and the haylage was rebaled using a stationary set high-density baler (New Holland 575, Söderberg & Haak maskin AB) which produced small square bales (approximately 70 × 46 × 36 cm). The square bales were individually wrapped with eight layers of stretch film (Triowrap 500 mm; Trioplast AB) using a mini-wrapper (Tellefsdal 404 MiniWrap, Tellefsdal). Stretch film layers were applied with an overlap of 0.50 and pre-stretching the film by 1.70. The rebaled square bales were stored outdoors in a single layer on concrete ground within a fenced bale yard without roof or cover.

The bales were stored for 30 days before the first bales were opened and sampled. Samples for chemical analysis were kept frozen at −18°C until analysis, and samples for microbial analyses were stored at 6–8°C for a maximum of 14 hr before analysis. Six randomly chosen bales were opened once weekly for 6 weeks (26 April to 6 June 2001). Prior to opening of bales, gas entry rate was measured by creating an under pressure inside the bales as described by Müller (2005). After opening rebaled bales, samples were taken and a thermocouple was inserted into the centre of each bale for

measurements of bale temperature once daily for 14 days. The temperature was read using a digital thermometer (type "T" ST-861-AT07 Pentronic). Ambient temperature was measured at the same time of day as bale temperature, using the same device.

2.1.2 | Experiment II

Grass silage (ca 350 g DM/kg) and haylage (ca 550 g DM/kg) harvested in June 2005 from the same primary growth crop consisting of predominantly timothy (*Phleum pratense*) with presence of meadow fescue (*Festuca pratensis*), common couch grass (*Elytrigia repens*) and dandelions (*Taraxacum vulgare*), and conserved in big round bales (about 700 kg bale weight for silage and 460 kg for haylage) were used for the experiment. Silage and haylage was inoculated with a biological silage additive at baling (Lactisil Horse Plus, Medipharm, Kågeröd, Sweden). The additive consisted of lactic acid bacteria (LAB) (*Lactobacillus plantarum* NCIMB 30083, *Enterococcus faecium* NCIMB 11181, *Pediococcus acidilacti* NCIMB 30086 and *Lactococcus lactis* NCIMB 30117) as well as cellulolytic enzymes (Genecor Multifect CEG IUB 3.2.2124, 54,000 HEC/g concentrate), potassium sorbate and sodium benzoate. The dosage recommended by the manufacturer was used, resulting in application of approximately 200,000 CFU LAB, 1.8×10^{-4} g potassium sorbate and 4.2×10^{-4} g sodium benzoate applied per gram silage, and 200,000 CFU LAB/g, 1.6×10^{-4} g potassium sorbate and 3.8×10^{-4} g sodium benzoate per gram haylage. The big bales were stored outdoors in a single layer on concrete ground within a fenced bale yard without roof or cover.

Six silage and four haylage bales were opened for rebaling in December (year 2005) and sampled for analysis of chemical and microbial composition. Samples were taken using a cylindrical (1 m × 40 mm ø) stainless steel core sampler connected to an electrical drill (DeWalt DW006). Six core samples were taken from each bale and pooled to produce one sample per bale. Depth at drilling was approximately 60 cm. The core sampler was sterilized using 0.995 ethanol (w/w) and an open flame between sampling individual bales. One round bale at a time was opened, and the forage was rebaled using a stationary set high-density baler (Lely Welger AP730; Lely Maschinenfabrik GmbH) with original knotters replaced by knotters intended for a large square baler (Lely Welger D 4000; Lely Maschinenfabrik GmbH), which produced small square bales of haylage (approximately 80 × 48 × 36 cm). The square bales were individually wrapped with ten layers of stretch film (Horsewrap 360 mm, Trioplast AB) using a mini-wrapper (Tellefsdal 404 MiniWrap, Tellefsdal). Stretch film layers were applied with an overlap of 0.50 and pre-stretching the film by 1.70.

The bales were stacked on pallets and transported to an experimental farm for feed-out during February to April (year 2006). During the feed-out period, pallets with bales were stored outdoors in an empty bunker silo with concrete ground and roof. Forages were sampled during four days (1st and 17th of March, and 7th and 27th of April) during the feed-out period. On these days, forages were sampled by taking three grab samples per bale (aseptically) at opening and pooling these to produce one sample per bale. In total, four silage and four haylage samples were collected. The samples were divided into two halves for chemical and microbial analysis. Samples for chemical analysis were kept frozen at −18°C until analysis. Samples for microbial analysis were kept at 6°C for a maximum of 24 hr (due to transport time to the laboratory) before inoculation.

2.1.3 | Experiment III

Grass haylage (approximately 560 g DM/kg) harvested from a primary growth crop consisting predominantly of timothy (*Phleum pratense*) with presence of meadow fescue (*Festuca pratensis*), dandelion (*Taraxacum vulgare*) and common couch grass (*Elytrigia repens*), and conserved in big round bales in June 2011 (average bale weight 476 kg, *SD* 4.4 kg) were used in Experiment III. Twelve bales were included, of which six were treated with a chemical additive at baling, and the other six were untreated controls. The additive (Kofa Grain pH 5, Addcon Nordic AS) supplied 6.0×10^{-4} g sodium benzoate, 1.6×10^{-3} g propionic acid and 5.0×10^{-4} g sodium propionate per gram haylage (salts dissolved in water). To avoid the risk of contamination of control bales with additive residues from the inside of the bale chamber, all control bales were baled first and, thereafter, all additive-treated bales. The big bales were stored outdoors in a single layer on concrete ground within a fenced bale yard without roof or cover.

The bales were stored for 6 months, after which rebaling was performed on two subsequent days (in January 2012). Three bales with additive and three control bales were randomly selected from the batch for weighing, sampling, opening and rebaling. Before opening, gas entry rate was measured as described by Müller (2005). Samples were then taken using a cylindrical (1 m × 40 mm ø) stainless steel core sampler connected to an electrical drill (DeWalt DW006). Eight core samples were taken from each bale and pooled to produce one sample per bale. Depth at drilling was approximately 60 cm. The core sampler was sterilized using 0.995 ethanol (w/w) and an open flame between sampling of individual bales. Samples for microbial analysis were stored in sealed, double plastic bags at 6°C for a maximum of 4 hr before analysis, while samples for chemical analysis were frozen at −18°C within 10 min after sampling.

After sampling, the stretch film surrounding the round bales was removed completely and weighed, and the haylage was fed manually into a stationary high-density baler (Welger AP 730; Lely Maschinenfabrik GmbH), producing rectangular bales of size approximately 80 × 48 × 36 cm. The baler was equipped with knotters originally intended for a large square baler (Lely Welger D 4000; Lely Maschinenfabrik GmbH). Each small square bale was then weighed and individually wrapped with ten layers of 360 mm wide stretch film (overlap of 0.50 and pre-stretching the film by 1.70) by using a mini-wrapper (Tellefsdal Miniwrap 404; Tellefsdal). Each bale was marked to be able to track its original round bale, and bales were stored outdoors in a single layer on concrete ground within a fenced bale yard without roof or cover.

Residual round bales (three control and three additive-treated) were left intact for another ten weeks of storage in the same bale yard as

rebaled bales. After 10 weeks (March, 2012), three rebaled bales originating from the same original big bale were randomly selected (within control/additive treatment) for further study, resulting in 18 rebaled bales in total continuing in the experiment (Figure 1). Before opening of rebaled and residual bales, gas entry rate was measured as described by Müller (2005). Bales were then weighed and measured, the wrapping was removed completely and weighed, and the bale surfaces were checked for visible fungal growth. Sampling for analysis of microbial and chemical composition was thereafter performed as described previously (core sampling). All bales were then equipped with a thermocouple inserted into the centre of each bale, and the temperature was read once daily between 11:00 and 13:00 hr, for 14 days. Bale temperature was read using a digital thermometer (type "T" ST-861-AT07 Pentronic; Gunnebo). The ambient temperature was read using the same device and at the same time of day as bale temperature was measured.

2.2 | Chemical analyses

Dry-matter concentration was determined by drying the samples in two steps; first, the samples were dried for 18 hr at 55°C, and then weighed and ground in a hammer mill to pass a 1 mm screen. Afterwards, samples were dried again for 20 hr at 103°C. Crude protein (CP) concentration was measured using the Kjeldahl method (Bremner & Breitenbeck, 1983). Neutral detergent fibre (NDF) content was analysed according to Van Soest, Robertson, and Lewis (1991) as modified by Chai and Udén (1998) using both sodium sulphite and amylase and reported as NDF exclusive of residual ash (aNDFom). Concentration of water soluble carbohydrates (WSC) was analysed using an enzymatic–spectrophotometric method (Larsson & Bengtsson, 1983). Digestibility of organic matter (OM) was estimated by a 96 hr in vitro method (IVDOM), using fresh rumen inocula from two dairy cows (Lindgren, 1979 as corrected in 1983). Content of metabolizable energy (ME) for ruminants (ME_r, MJ kg/DM) was calculated according to Lindgren (1979 as corrected in 1983). The content of ME for horses (ME_h) was estimated from ME_r) using the equation $ME_h = (1.12\ ME_r) - 1.1$ (Lindberg & Ragnarsson, 2010).

Liquid was extracted from the haylage samples by mixing the samples 1:1 with distilled water. The samples were then frozen for 24 hr and thawed before extraction of liquid using a hydraulic press. The liquid was used for measurement of pH (using a glass electrode), concentration of volatile fatty acids (VFA), ethanol and lactic acid (Andersson & Hedlund, 1983). Ammonia-N concentration was determined in the diluted solution by direct distillation using Kjeltec Auto System 1020 (FOSS, Höganäs), and a correction was made for the dilution, using the following formula: g/kg N in forage sample = 1.84 × g/kg N in 1:1 diluted samples) − 0.002 ($r^2 = .95$, $n = 85$) (Ericsson, B. Swedish University of Agricultural Sciences, Uppsala, Sweden; personal communication, 2011). Ammonia-N was expressed as g ammonia-N per g total N.

2.3 | Microbial analyses

Forage samples for microbial analysis were prepared by adding 450 ml of autoclaved (15 min at 121°C) ¼ strength Ringers solution (Merck KGaA) to 50 g sample and running the sample twice for 60 s at the setting "normal speed" in a laboratory blender (Stomacher Seward 3500). Tenfold dilution series were then made using ¼ strength Ringers solution. Enumeration of lactic acid bacteria (LAB), enterobacteria, clostridial spores and fungi (yeast and mould) was made using three dilutions for all forage samples except for big bale samples from Experiment II, where LAB counts were not determined. Lactic acid bacteria were cultivated under anaerobic conditions on duplicate Rogosa agar plates (Merck, KgaA) for 72 hr at 30°C (Carlile, 1984). Enterobacteria were cultivated on duplicate violet red bile dextrose agar plates (Merck, KgaA) in sealed sterile plastic bags at 37°C for 48 hr (Seale et al., 1986). Clostridial spores were enumerated after samples had been placed in 82°C water bath for 13 min, cooled for 15 min in room temperature (19°C) and thereafter the dilutions were spread on reinforced clostridial medium (with the addition of neutral red and cycloserine) agar plates in triplicate (Merck, KgaA), which were incubated anaerobically for seven days at 37°C (Carlile, 1984;Jonsson, 1991;Seale et al., 1986). Fungi were inoculated in triplicate using malt extract agar plates (Merck, KgaA)

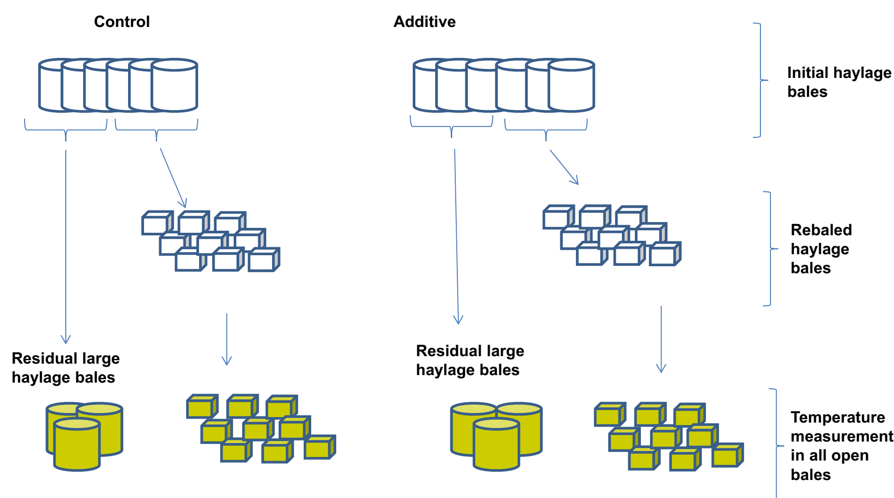

FIGURE 1 Design of Experiment III. Large round bales were produced, six with additive and six controls, and stored for 6 months (initial haylage bales). Three round bales of each treatment were then opened and rebaled into small square bales which were individually wrapped (rebaled haylage bales). After 10 weeks, remaining large round (residual haylage bales) and rebaled small square bales were opened, sampled and left open for 14 days, under which bale temperature was measured [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Experiment I: Average chemical (g/kg dry matter if not otherwise mentioned) and microbial composition ($_{10}$log CFU/g) in initial silage in big round bales, and after being rebaled in small square bales and stored for 30 days

	Initial big round bales (*n* = 4)		Rebaled small square bales (*n* = 36)	
Variable	Average	*SD*	Average	*SD*
Dry matter (g/kg)	469	59.5	482	35.9
Ash	96	29.6	98	14.8
Crude protein	126	8.5	126	8.0
Water soluble carbohydrates	107	29.3	103	21.1
Estimated metabolizable energy for horses, ME_h, MJ/kg DM	9.9	0.27	10.0	0.20
Microbial composition				
Yeast	1.2[a]	1.47	3.7[b]	3.74
Mould	<1.20	—	<1.20	—
Clostridial spores	<1.20	—	1.9	2.12

Abbreviations: *SD*, standard deviation.
[a,b]Yeast counts differed at $p = .007$.

which were incubated at 25°C. Yeasts were counted after 48 hr and moulds after five days (Seale et al., 1986).

2.4 | Statistical evaluation

Variance analysis was performed using SAS 9.3 for Windows (SAS Institute). Microbial values were handled in their logarithmic form. Values below detection levels were transformed to a value corresponding to half the lower detection limit for that particular analysis, before statistical evaluation. Treatment means where $p < .05$ were regarded as statistically different.

Experiment I and II: the general linear procedure was used to evaluate effects of rebaling using the following model:

$$Y_{ij} = \mu + (\text{rebaling})_i + (\text{error})_{ij}.$$

where the error term is the random residual with mean = 0 and variance σ^2.

Experiment I: mixed model procedure was used to evaluate effects of opening week on bale temperature during aerobic storage using the following model:

$$Y_{ij} = \mu + (\text{opening week})_i + (\text{error})_{ij}$$

where the error term is the random residual with mean = 0 and variance σ^2.

Experiment III: the mixed model procedure was used to evaluate the effects of additive treatment, rebaling and interactions between additive treatment and rebaling using the following model:

$$Y_{ijk} = \mu + (\text{additive})_i + (\text{rebaling})_j + (\text{additive} \times \text{rebaling})_{ij} + (\text{error})_{ijk}$$

where the error term is the random residual with mean = 0 and variance σ^2.

3 | RESULTS

3.1 | Experiment I

On average, nine small square bales were produced from each round bale at rebaling. For small square bales, average bale weight was 47 kg (*SD* 6.7), average bale density was 198 kg/m^3 (*SD* 27.9) and average gas entry rate was 111 s (*SD* 100.7). The chemical composition of silage before and after rebaling was similar (Table 1). Microbial composition differed in yeast counts, which were higher in rebaled haylage (Table 1). Average bale temperature during aerobic storage differed ($p > .05$) between opening weeks during Day 0 to 3 and Day 5 to 13 (Figure 2a–f). Average bale temperature roughly followed ambient temperature until day 6–8 during most weeks, where after it deviated (Figure 2a–f).

3.2 | Experiment II

Average bale weight for silage was 66 kg (*SD* 8.5 kg) and for haylage bales 60 kg (*SD* 1.0 kg). Analysed variables were similar in initial and rebaled haylage in Experiment II (Table 2). In silage, counts of yeast and mould were higher in rebaled compared to in initial forage, but all other analysed variables were similar (Table 2).

3.3 | Experiment III

3.3.1 | Small bale weight and measurements

On average, 11–12 small square bales were produced from each round bale at rebaling (Table 3). Average bale weight, volume, density, gas entry rate and stretch film weight and their variation for initial, rebaled and residual bales are given in Table 3. Visible fungal growth was detected at opening of rebaled bales on one bale with additive treatment (covering 0.05 of the bale surface) and on one control bale (covering 0.15 of the bale surface). Sampling of these bales was performed excluding the visibly mouldy surfaces.

3.3.2 | Chemical and microbial composition

Rebaled haylage differed from initial and residual haylage only in concentration of ammonia-N, which was highest in rebaled haylage (Table 4). Rebaled haylage was also higher in yeast counts and lower

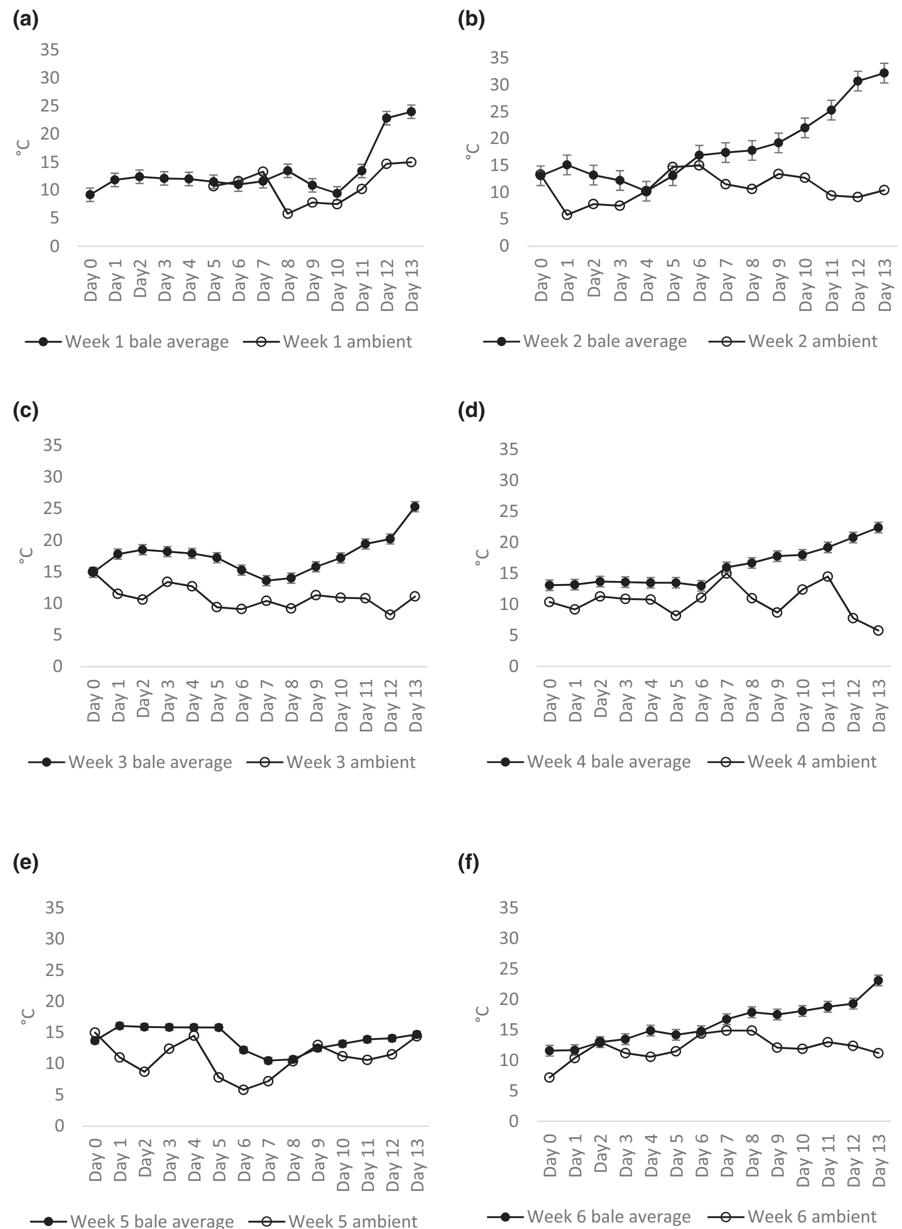

FIGURE 2 (a–f) Experiment I: Average bale and ambient temperature during aerobic storage of opened rebaled haylage bales during 6 weeks (a–f), and each week six bales were opened. Average bale temperature differed between weeks for Days 0–3 and 5–13 ($p < .05$). Error bars show standard error

in concentration of ME_h, IVDOM and propionic acid compared to initial haylage, but did not differ from residual haylage in the same variables (Table 4). Concentration of aNDFom was higher in rebaled compared to residual haylage, but did not differ from the concentration in initial haylage (Table 4).

Rebaled haylage did not differ from initial and residual haylage in mould or enterobacteria counts, although initial haylage had higher mould counts than residual haylage, and residual haylage was higher in enterobacteria counts than initial haylage (Table 4). Interactions among rebaling and use of additive were present only for concentration of propionic acid, where concentration was lowest in rebaled, highest in residual and in between in initial additive-treated forage and the same in all control bales, but differences were very small (Table 4).

Dry-matter content was, in general, higher in additive-treated compared to control haylage (Table 4). Additive-treated haylage had generally higher contents of WSC and propionic acid compared to control haylage (Table 4). Additive-treated haylage also had lower concentration of aNDFom, ammonia-N, lactic and acetic acids, ethanol and lower pH-value (Table 4). Counts of yeast and LAB were, in general, lower in additive-treated compared to control haylages (Table 4).

3.3.3 | Aerobic stability in opened rebaled and residual haylage bales

Average bale temperature in rebaled and residual bales during aerobic storage was higher than ambient temperature but followed its fluctuations (Figure 3). Bale temperature never exceeded the bale temperature at opening over the 14 days of aerobic storage (Figure 3). There was no difference in bale temperature between

TABLE 2 Experiment II: Average chemical (g/kg dry matter if not otherwise mentioned) and microbial composition ($_{10}$log CFU/g) in silage and haylage in initial big round bales at opening in December, and after being rebaled in small square bales (sampled from February to April during feed-out)

	Silage				Haylage			
	Initial big round bales		Rebaled small square bales		Initial big round bales		Rebaled small square bales	
Variable	Average	*SD*	Average	*SD*	Average	*SD*	Average	*SD*
Dry matter (g/kg)	379	47.1	360	35.6	552	6.1	549	13.3
Ash	92	4.1	92	3.1	84	3.1	87	5.4
Crude protein	173	20.9	171	9.1	155	6.9	152	3.7
Estimated metabolizable energy for horses, ME_h, MJ/kg DM	11.5	0.20	11.5	0.14	11.5	0.11	11.5	0.16
Microbial composition								
Yeast	1.4[a]	2.22E-16	3.0[b]	1.05	1.6	0.26	3.0	1.24
Mould	1.4[a]	0.03	2.0[b]	0.52	<1.4	0.00	1.5	0.06
Enterobacteria	1.4	0.03	1.4	0.05	1.5	0.16	<1.4	0.00
Clostridial spores	1.6	0.30	<1.4	0.00	<1.4	0.00	1.4	0.05
Lactic acid bacteria	ND	–	6.6	0.75	ND	–	4.3	0.88

Abbreviations: ND, not determined; *SD*, standard deviation.

[a,b]Yeast counts differed between initial and rebaled haylage at $p = .01$, and mould counts at $p = .03$ in silage, but not in haylage.

TABLE 3 Experiment III: Average bale measurements and tightness of bale wrappings (gas entry rate in seconds) for initial big round bales before rebaling, and for rebaled square bales

Variable	Round bales, before rebaling (*n* = 12)	Rebaled square bales (*n* = 70)
Bale weight, kg (without stretch film)	476 (4.4)	40 (0.7)
Stretch film weight (kg)	2.2 (0.08)	0.3[a] (0.01)
Bale volume (m^3)	1.74 (0.015)	0.13 (0.002)
Bale density (kg dry matter/m^3)	159 (4.0)	179 (5.9)
Gas entry rate (s)	728 (441.4)	51[a] (15.0)
Number of square bales/round bale	–	11.6 (0.52)

Note: Standard error within brackets.

[a]n = 18.

additive-treated and control bales, and no interactions among rebaling and additive treatment were present (data not shown).

4 | DISCUSSION

4.1 | Changes in chemical and microbial composition following rebaling

The most evident change in forage composition attributed to rebaling was increased yeast counts in all three experiments. However, residual and rebaled haylage both had higher yeast counts than the initial haylage. The higher yeast counts may therefore rather be an effect of a longer storage period than of rebaling. Long-term storage (14 months) of wrapped bales has previously been reported to result in higher yeast counts compared to short-term (2 months) storage (Müller, Pauly, & Udén, 2007). On the other hand, yeast counts were similar in haylage samples taken in the autumn and the following spring (from the same forage batch) on commercial horse farms (Müller, Hultén, & Gröndahl, 2011), indicating no effect of storage time on yeast counts. The highest yeast count in the current study was log 5.31 CFU/g, which was higher than average yeast counts (log 4.57 in autumn and log 4.06 CFU/g the following spring) in the study on commercial horse farms (Müller et al., 2011). There are no generally accepted limits for yeast counts in forages, but Kamphues (2005) regarded values higher than log 5–6 CFU yeast/g as indicative of advanced deterioration. On the other hand, 13 of 28 forage samples from horse stables in Bavaria contained >log 6 CFU yeast/g (Sliwinsky, Krabisch, Rosenberger, & Scwarz, 2005), and 0.75 of 28 forage samples from commercial horse farms in Sweden contained at least log 5.7 CFU yeast/g (Müller et al., 2011). In both of these studies, the horses being fed the forages were reported to be healthy. Nevertheless, yeasts are considered as the culprits in starting aerobic deterioration in silage (Jonsson, 1989) and may increase the risk of growth of other microbes which may have higher pathogenicity.

Rebaled silage had higher mould counts compared to initial silage in Experiment II, but the same pattern was not present in haylage. It could have been due to the oxygen exposure of the forage during the rebaling procedure, but it does not explain why mould counts were higher in rebaled silage but not in haylage. In Experiment III, mould counts in rebaled haylage did not differ from initial or residual haylage. Therefore, rebaling could not be concluded to result in increased mould counts in haylage. In previous studies of effects of

TABLE 4 Experiment III: Average composition of haylage in initial round bales before rebaling, in small square bales after rebaling, and in residual intact round bales stored for an equally long time period and at the same place as rebaled haylage

Variable	Initial big round bale haylage	Rebaled small square bale haylage	Residual round bale haylage	*SE*	*p* haylage bale type	Control	Additive	*SE*	*p* additive
Dry matter (g/kg)	556	570	566	6.4	.18	549	579	4.6	<.001
Ash	63	61	63	1.2	.65	63	61	0.8	.07
Crude protein	89	87	88	1.9	.62	88	88	1.3	.88
Water soluble carbohydrates	120	119	120	3.9	.99	110	130	2.8	<.001
Estimated metabolizable energy for horses (ME_h, MJ/kg DM)	9.2[a]	9.0[b]	8.9[b]	0.07	.006	9.0	9.1	0.05	.16
Neutral detergent fibre (aNDFom)	600[a,b]	606[b]	595[a]	4.0	.04	607	593	2.9	.003
In vitro digestible organic matter	735[a]	720[b]	715[b]	4.1	.004	721	726	2.9	.27
Ammonia-N, g/kg total *N*	2.2[a]	3.0[b]	2.6[a]	0.013	<.001	2.8	2.4	0.11	.04
pH	5.61	5.60	5.59	0.15	.49	5.71	5.49	0.009	<.001
Lactic acid	2.4	2.7	2.7	0.20	.44	3.1	2.1	0.15	<.001
Acetic acid	1.1	1.2	1.4	0.11	.15	1.4	1.1	0.08	.05
Propionic acid[1]	1.2[a]	0.9[b]	1.0[b]	0.06	<.001[1]	0.5	1.5	0.05	<.001[1]
Ethanol	11.9	11.1	13.2	1.27	.38	16.5	7.7	0.91	<.001
Microbial composition									
Yeasts	3.21[a]	5.31[b]	5.26[b]	0.267	<.001	5.03	4.15	0.191	.004
Moulds	2.14[a]	1.58[a,b]	1.20[b]	0.253	.05	1.73	1.54	0.182	.47
Enterobacteria	1.52[a]	2.74[a,b]	3.60[b]	0.535	.04	2.64	2.60	0.385	.94
Clostridial spores	1.65	1.52	1.31	0.269	.66	1.73	1.25	0.194	.09
Lactic acid bacteria	5.54	5.33	5.23	0.211	.57	5.65	5.08	0.152	.02

Note: Average composition of haylage with and without additive, means and standard error (*SE*). Chemical composition in g/kg dry matter if not otherwise mentioned. Microbial composition in $_{10}$log colony forming units (CFU)/g forage.

Different superscript letters indicate difference within row (between bale types) at the presented *p*-value.

[1]Interactions among bale type and additive treatment was present at $p = .003$ for concentration of propionic acid: initial haylage control 0.5[a] and additive-treated 1.5[b], rebaled haylage control 0.5[a] and additive-treated 1.3[c], residual haylage control 0.5[a] and additive-treated 1.9[d] g propionic acid per kg dry matter (*SE* 0.009).

storage time on microbial composition of baled and wrapped forage, mould counts have been reported to be similar at 2 and 14 months of storage (Müller et al., 2007), but also tended to ($p = .06$) be higher in autumn samples compared to samples taken in the following spring (Müller et al., 2011). The result in Experiment III, where mould counts were highest in initial (winter) and lowest in residual haylage bales (following spring), seems to be in alignment with the results reported by Müller et al. (2011).

Rebaling could not be concluded to cause increased counts of enterobacteria in haylage, but this could probably be influenced by cleanliness of the place used for rebaling. In a previous study, counts of enterobacteria did not differ in haylage sampled in the autumn and the following spring, and was maximum log 2.4 CFU/g for 0.75 and log 4.6 CFU/g for all of 28 forage samples collected from commercial horse farms in Sweden (Müller et al., 2011).

In Experiment III, rebaled haylage had higher concentration of ammonia-N compared to initial and residual haylage. An increase in ammonia-N could be a result of plant proteolytic enzymes and/or microbial breakdown of amino acids (McDonald, Henderson, & Heron, 1991; Papadopoulos & McKersie, 1983), but no corresponding increase in microbial counts was detected. Both concentration of ammonia-N and the difference between treatments were however low (from 2.2 to 3.0), and may not be biologically important, at least not for the storage time of rebaled forages in this experiment.

In Experiment III, rebaled and residual haylage had lower concentrations of ME_h, IVDOM and propionic acid compared to initial haylage indicating that the differences were not due to the rebaling process. Propionic acid concentration was affected by interactions between rebaling and additive, but these differences were very small.

Rebaled haylage in Experiment III had higher concentration of aNDFom compared to residual but not initial haylage, also indicating it was not the rebaling process (with possible loss of finer forage particles) that caused these differences. Although a rebaling procedure

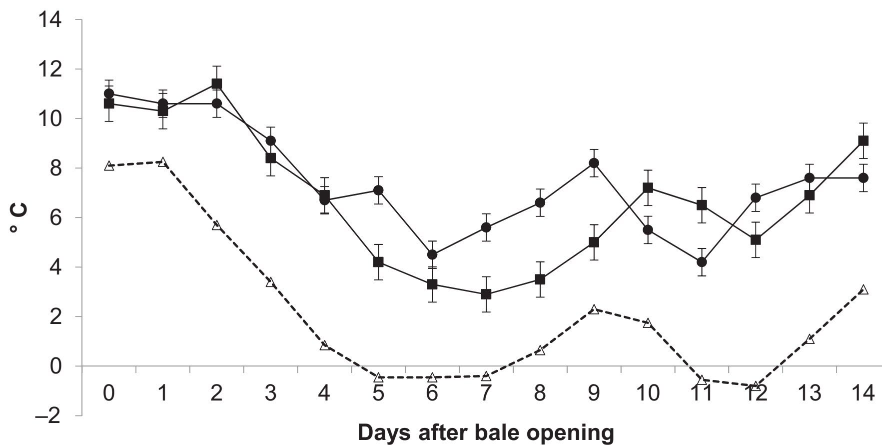

FIGURE 3 Experiment III: Ambient temperature (triangles) and bale temperature in rebaled (squares) and residual (circles) haylage bales during an aerobic storage period of 14 days after opening of bales. Error bars show standard error

inevitably will result in some mechanical loss of forage particles, the results of this study showed that it does not necessarily affect concentration of ME_h, IVDOM or aNDFom.

4.2 | Effect of additive treatment on chemical and microbial composition—Experiment III

As DM content was higher in additive-treated compared to control haylage, the effects that seemed to be due to the additive treatment (such as higher WSC content and lower content of ammonia-N and fermentation products as well as lower counts of yeast and LAB) could also have been due to the higher DM content which have a strong influence on these variables (Müller, 2005; Pahlow & Weissbach, 1996). As all control haylage were baled before all additive-treated haylage (to avoid contamination of control bales with the additive), the difference in DM concentration between treatments could be due to a somewhat longer wilting time for the additive-treated haylage. However, Jaakkola, Saarisalo, and Heikkilä (2010) reported that DM content became higher in haylage (440–560 g DM/kg) treated with propionate-containing additives compared to control haylage or haylage treated with inoculants. This has also been reported in studies where acid additives have been used for silage conservation (McDonald et al., 1991). It may be a result of the inhibitory effect of acids on microbes, minimizing, e.g. heterolactic fermentation and loss of CO_2 during fermentation (Gibbs, Dunrose, Bennett, & Bubeck, 1950). However, additive-treated haylage also had lower pH compared to controls, indicating that the higher DM content in additive-treated haylage was probably not fully explaining the differences. Silage and haylage pH are related to DM content and increases with increasing DM content in silage (Pahlow & Weissbach, 1996) and haylage (Finner, 1966; Gordon, Derbyshire, Wiseman, Kane, & Melin, 1960; Müller, 2005). Rather, a combination of higher DM concentration and additive treatment could explain the more restricted fermentation in additive-treated haylage, compared to the control.

In contrast, addition of propionic acid to hay has been reported to increase moisture content in the hay during storage, probably due to the hygroscopic properties of propionic acid (Coblentz & Bertram, 2012; Coblentz et al., 2013; Rotz, Davis, Buckmaster, & Allen, 1991). As haylage is wrapped in stretch film during storage, the hygroscopic properties of propionic acid may be of interest only after opening of the bales. It is however not yet known whether application of propionic acid is an advantage or disadvantage for the aerobic storage stability of haylage.

4.3 | Bale measurements—Experiment III

Approximately 0.97 of the forage matter in original round bales was recovered in the small bales in Experiment III. A loss of 3% is very low in any forage conservation system (e.g. Buckmaster & Heinrichs, 1993). This loss is probably unavoidable as the extended mechanical handling of the forage is necessary during the rebaling procedure. Lower mechanical losses could probably be expected if large square bales were simply cut into smaller bales and then wrapped, instead of opening and unwinding round bale haylage for rebaling purposes.

Bale weight, bale volume and bale density of small square bales all had low standard variations, implying that it was possible to obtain homogenous bale sizes with the rebaling procedure. This may be important for both producer and purchaser of small bale forage, as uniformity of shape and weight of, e.g. pallets with small square bales stacked on it may affect transportation costs.

4.4 | Aerobic stability

In Experiment I, bale temperature roughly followed ambient temperature until day 6–8, and thereafter bale temperature started to deviate while ambient temperature was fairly stable. This may indicate that 6–8 days after opening rebaled bales the forage may be deteriorated. In Experiment III, temperature in rebaled and residual bales followed ambient temperature during the entire aerobic storage period, the haylage did not heat as the initial bale temperature at opening was not exceeded at any time during the 14 days of aerobic storage. The fast heating after opening of haylage bales described in the literature (Savoie & Jofriet, 2003) did not take place in this experiment. As ambient temperature was

comparably low during the aerobic stability test in Experiment III, this was not surprising.

Due to the different size of small square and big round bales in Experiment III, the distance from the bale surface to the inserted thermocouple in the bale centre differed. However, this did not seem to affect the measured bale temperature as it followed ambient temperature in a similar pattern in both bale types. Additive treatment did not influence bale temperature differently than control treatment, indicating that the additive was not necessary for keeping a sufficiently long aerobic stability in rebaled haylage bales after opening.

Rebaling under the described conditions was not considered to cause short aerobic storage stability of rebaled haylage bales after opening. However, higher ambient temperatures may have another influence on aerobic stability than reported from these experiments.

5 | CONCLUSIONS

Rebaling could be a method for producing small bale silage and haylage from conserved big bales. However, yeast counts may increase in rebaled haylage, and both yeast and mould counts may increase in silage. The same increase may however take place in original bales stored for an equal time period as rebaled bales, during the same storage conditions. Aerobic storage stability after opening of rebaled haylage bales was not impaired by rebaling under these experimental conditions; however, higher ambient temperatures may have a different impact. Use of an acid additive did not improve aerobic storage stability compared to control in this study.

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CONFLICT OF INTEREST

None of the authors have any conflicts of statement to declare. Addcon Nordic AS, Porsgrunn, Norway, provided the additive used in Experiment III in the study, but had no involvement in any part of the study.

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