AGRICULTURAL AND FOOD SCIENCE

Agricultural and Food Science (2025) 34: 113–124

https://doi.org/10.23986/afsci.156983

Management factors affecting preservation quality of grass silage: laboratory evaluation and on-farm case study

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Silage quality significantly influences livestock production costs and environmental impacts. This study assessed the fermentation quality, preservation losses, aerobic stability and microbial quality of grass silages under varying management practices, including compaction methods and additive treatments. In laboratory-scale, timothy and meadow fescue mixture was ensiled in 12-litre silos using three compaction methods (normal, loose, and normal followed by additional delayed compaction) and five additive treatments (control, homofermentative inoculant, heterofermentative inoculant, salt-based additive, and formic and propionic acid-based additive). Silage samples from three farms with aerobic stability issues were also analysed. In laboratory scale, chemical preservatives enhanced aerobic stability and minimized fermentation losses more effectively than biological inoculants. Heterofermentative inoculants increased acetic acid production, enhancing stability, while homofermentative inoculants had minimal impact on most parameters. Farm silages exhibited varied quality, with poor microbial quality linked with fast aerobic deterioration. Effective silage management practices, including oxygen limitation, proper compaction, and appropriate preservative application, are crucial to preventing spoilage and ensuring silage quality.

Key words: silage additive, compaction, aerobic stability, formic acid, lactic acid bacteria

Introduction

Silage is a critical feed resource for ruminant livestock, providing a stable and high-quality source of nutrients throughout the year, and has become the dominant way of forage preservation both in Finland and globally (Wilkinson and Rinne 2018). Effective silage fermentation is characterized by rapid pH decline, inhibition of undesirable microorganisms, and the production of desirable fermentation end-products, such as lactic acid (McDonald et al. 1991).

Microbial spoilage during storage and upon exposure to air can significantly reduce the nutritional value of silage. The use of chemical additives and lactic acid bacteria (LAB) inoculants has been widely studied to improve silage fermentation quality and preservation (Wilkinson and Rinne 2018). Chemical additives, such as short-chain organic acids, can directly reduce the pH of the material and inhibit the growth of spoilage organisms (McDonald et al. 1991), while LAB inoculants can, under optimal conditions, intensify and direct the fermentation (Kleinschmit and Kung Jr 2006, Auerbach et al. 2020). Additionally, salt-based additives, such as sodium benzoate and potassium sorbate, can enhance silage preservation by inhibiting yeast and mould growth, thereby improving aerobic stability (Muck et al. 2018).

In addition to the use of additives, physical factors such as compaction play a crucial role in silage preservation (Rinne and Seppälä 2011). Proper compaction reduces the porosity of the silage mass, thereby limiting the infiltration of oxygen and creating an anaerobic environment conducive to desirable fermentation processes. Studies have shown that improved compaction techniques can significantly enhance silage quality by reducing the risk of aerobic spoilage and nutrient losses during storage (Toruk et al. 2010, Brüning et al. 2017, Tan et al. 2017). In contrast, some studies have reported minimal to negligible impacts associated with varying levels of compaction (Franco et al. 2022a, 2022b). The limited responses to compaction in these studies may have been related to the use of airtight laboratory silos, which emphasizes the need of verifying and validating the results from laboratory-scale experiments with data from larger-scale experimentation.

In agricultural settings, the compaction process of forage at the farm level may encounter various challenges, resulting in divergent compaction levels, including insufficient compaction or delayed compaction that extends to the day after silo filling, followed by the subsequent covering of the silage. In instances where silage production extends late into the night on farms, the final compaction of the silo and the covering of the silage may be postponed until the following day.

Received 13 February 2025 / Accepted 23 April 2025
The Scientific Agricultural Society of Finland

To complement the controlled laboratory experiment and better understand the dynamics of silage deterioration under commercial farming conditions, it is also important to monitor the aerobic stability of silages collected from practical farms. This approach provides real insight into how silage quality and deterioration evolve outside the laboratory, under variable and often suboptimal management conditions.

To elucidate the effects of practically important silage management factors, i.e., level and timing of silage compaction as well as the use of commercially available silage additives relying on different modes of action, we conducted a controlled laboratory-scale experiment where silage preservation parameters were evaluated. We hypothesized that higher compaction improves silage fermentation and reduces spoilage by limiting oxygen infiltration, while additive effectiveness varies by mode of action and compaction strategy. In addition, silages were collected from practical farms, and the evolution of aerobic deterioration of these silages was monitored (case study).

Materials and methods

Laboratory-scale ensiling experiment

Raw material for silage making

Mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grass was harvested from the first cut on 16 June 2020 at the Natural Resources Institute Finland (Luke), Jokioinen, Finland (60°48'N, 23°29'E). A composite sample was obtained from the standing crop before cutting by taking four representative 0.25 m^2 samples, which were manually divided into timothy and meadow fescue to evaluate the botanical composition. The grass was cut using a mower conditioner (JF GMS 3200 Topflex, JF-Fabriken-J Freudendahl A/S, Sonderborg, Denmark and Krone EC 32 CV in front hitch, Maschinefabrik Bernard Krone GmbH, Spelle, Germany), and precision chopped using farm-scale machinery (JF FCT 1350, JF-Fabriken-J Freudendahl A/S, Sonderborg, Denmark). The grass was transported to the laboratory without any additive and pre-dried in a flat dryer with intermittent air blowing without heating until the next day, when the actual ensiling trial was carried out. Raw material samples were taken before treatment application to evaluate chemical composition and microbial quality of the grass before ensiling.

Experimental treatments and procedures

Grass was packed into cylindrical laboratory-scale silos according to a factorial 3 × 5 design comprising three compaction managements and five additive treatments. The three compaction methods used were:

- Normal compaction: manual compaction by dropping an 8-kg lead plummet for ten times after adding a handful of grass into the cylindrical silo.
- Loose compaction: similar to normal compaction, but the lead plummet was dropped only two times after adding a handful of grass into the cylindrical silo.
- Delayed compaction: silos were compacted normally but left open until the next day when they were compacted further by dropping the lead plummet for 20 times.

To evaluate the efficacy of different commercially available silage additives under different compaction methods, five additive treatments were tested as follows:

- Control: negative treatment where tap water was applied to ensure similar physical treatments as for the additives.
- Homofermentative (HO) inoculant: strains of *Lactobacillus plantarum* (DSM 12836; 1k2078; min. 1×10^{11} cfu g^{-1}) and *Pediococcus pentosaceus* (DSM 12834; 1k2103; min. 1×10^{11} cfu g^{-1}) (Bonsilage, Schaumann Agri International GmbH, Pinneberg, Germany) at 1 g ton^{-1} so the dose of each strain to the grass was 1×10^5 cfu g^{-1} fresh grass.
- Heterofermentative (HE) inoculant: strains of *Lactobacillus buchneri* (DSM 13573, 1k20733; min. 1×10^{11} cfu g^{-1}), *Lactobacillus plantarum* (DSM 3676, 1k20731; min. 0.5×10^{11} cfu g^{-1}) and *Lactobacillus plantarum* (DSM 3677, 1k20732; min. 0.5×10^{11} cfu g^{-1}) (Feedtech Silage F600, DeLaval, Tumba, Sweden) at 1 g ton^{-1} so the dose of each strain to the grass was 1, 0.5 and 0.5×10^5 cfu g^{-1} fresh grass, respectively.
- Salt based additive (SA): sodium nitrite, sodium benzoate and potassium sorbate (Safesil Pro, Salinity AB, Göteborg, Sweden) at 5 l ton^{-1} fresh grass.
- Formic and propionic acid-based additive (FPA): formic acid, propionic acid, sodium formate and potassi um sorbate (AIV Ässä Na, Eastman Chemical Company, Oulu, Finland) at 5 l ton^{-1} fresh grass.

After thoroughly mixing and manually homogenizing the raw material to ensure uniform distribution of moisture and nutrients, it was divided into 8 kg batches. Additives were diluted in tap water to a final volume of 10 l ton^{-1} of fresh matter (FM) for all treatments, including the control, in order to increase the total volume of the solution and ensure an even distribution of the additive throughout the raw material. The additives were applied separately for three replicates per treatment by mixing the liquids thoroughly with the grass by hand.

The grass was ensiled into cylindrical laboratory-scale silos with 12 dm^3 capacity. After filling and compacting according to the experimental plan, the silos were covered with a plastic cover, a plastic lid, an 8-kg lead plummet, and a water bag.

The silos were stored at room temperature with protection from light and opened after an ensiling period of 93 days. Prior to emptying, the silos were weighed, and the height of the silage column was measured to determine the density and storage losses. Fermentation loss was calculated from the weight loss during storage multiplying it with a factor of 1.44, accounting for water formation during carbon dioxide production (Knický and Spörndly 2015). Visually deteriorated parts on the surface of the silage were discarded, and the silage was mixed, and samples were taken and analysed for chemical composition, buffering capacity (BC), fermentation quality, microbial counts and aerobic stability using standard methods of Luke laboratory (see Franco et al. 2022b). The propionic acid content of the FPA silages was corrected by subtracting 80% of the amount applied with the additive from the analysed value, reflecting the proportion typically recovered during analysis. The fermentation coefficient (FC) of the raw material was calculated considering the contents of dry matter (DM), water-soluble carbohydrates (WSC) and BC according to the DLG (2020) as follows:

$$FC = \frac{DM\ (g\ kg^{-1}) + [8 \times (WSC\ (g\ kg^{-1}\ DM)\ /\ BC\ (g\ lactic\ acid\ 100\ g^{-1}\ DM)]}{10}$$

The proportion of undissociated acetic acid was calculated using the Henderson–Hasselbalch equation, considering the total acetic acid concentration, pH, and the pK_a value of 4.76.

Aerobic stability measurement

Approximately 500 g of silage was placed into a polystyrene box with internal dimensions of 13.3 cm × 13.3 cm × 10.3 cm, resulting in volume of 1.8 dm^3. Air ingress was allowed into the box. A thermocouple wire was inserted into the middle of the sample and connected to a MicroLite USB data logger. Temperature was recorded at 10-minute intervals for a 360-h follow-up period. Aerobic stability was defined as the time taken for the sample temperature to increase by 2 °C above ambient temperature. The ambient temperature was 19.4±0.43 °C (range: 18.6−20.8 °C), measured using data loggers similar to that employed for the samples.

Statistical analysis

Results were analysed according to a 3 × 5 factorial design (three compaction methods and five additive treatments) using the MIXED procedure of SAS software (SAS Inc. 2002–2012, Release 9.4; SAS Institute Inc., Cary, NC, USA). Compaction method, additive treatment and their interaction were fixed factors in the model, with replicate as a random factor. The normal distribution of the data was tested via the Shapiro–Wilk test using the Univariate procedure. Least squares means and the standard errors of the means were reported per treatment, with significant differences among the treatment means declared at 5% of probability. Pairwise comparisons of the treatments were made with Tukey's test.

Case study: Evaluation of farm silages

Silage collection from farms

Silage samples were collected from three practical dairy farms on 5 November, 2020, in south-western Finland. Farms were selected based on stability issues, as subjectively assessed by the farmers, and all utilized bunker silos. Background information was collected during the silage sampling, including silage raw material, ensiling date, additive use, storage duration, silo opening time, silage feeding rate, frequency of silage removal, silo dimensions, and any specific considerations regarding silage making, storage, and feeding. The density of the silage was subjectively evaluated. The background information is summarized in Table 1.

Table 1. Background information of the farm silages (case study)

	Farm 1	Farm 2	Farm 3
Harvest date in 2020	13–16 June	6 June	8–13 June
Silo opening time	Mid-August	Mid-October	30 July
Plant species	Timothy, tall fescue, red clover, ryegrass, white clover, cocksfoot	Timothy, meadow fescue, red clover, ryegrass, alsike clover, cocksfoot, smooth brome	Timothy, meadow fescue, ryegrass, tall fescue
Silage additive	AIV Ässä Na (formic and propionic acid based)	Josilac Combi (homofermentative + heterofermentative inoculant)	Bonsilage (homofermentative + heterofermentative inoculant)
Harvesting method	Feed wagon	Self-propelled chopper	Precision chopper
Silo dimensions, m			
Width	10	11	10
Depth	50	30	36
Height	2.5	3	3
Subjective compaction	Fairly good	Extremely good	Poor
Rate of silage removal	Daily	Daily	Daily
Silo covering	Silage cover sheet and wood chips	Vacuum sheet, silage cover sheet, green net and car tyres 2 m apart	Silage cover sheet, car tyres evenly distributed
Other remarks	Variable chop length, holes in plastic due to bird activity, some warm spots and dark foul-smelling spots in the silo. During harvesting some loads had probably by accident be collected without additive.	White mould appeared in spots particularly on the surface and edges, but also deeper in the silo	White mould appeared in spots particularly on the surface and edges, but also deeper in the silo, poor palatability

Prior to sampling, the silage temperature in the bunker silo was measured at multiple points (20–30 cm depth from the cutting layer), including the top, middle, and bottom (approximately 50 cm above the silo floor) along the silo face. Additionally, temperature measurements were recorded at the silo edges, approximately 50 cm from the sidewalls. The ambient outdoor temperature was also measured at each farm. Approximately 60 kg of silage per farm was collected from the central section of the silo face to assess its aerobic stability. To obtain silage that was not exposed to air, about 30 cm of silage was first removed and after that, the necessary amount of silage was collected for sampling. In addition, the surface and middle point of the feed in the bunker silo were sampled. The surface sample was taken at a depth of about 30 cm and the DM content of the sample was determined. The samples taken from the middle point were analysed for chemical composition, fermentation quality, aerobic stability, and microbial quality as described for the laboratory-scale ensiling experiment. The silage from Farm 1, preserved with a formic acid-based additive, was also analysed for formic acid content.

Determination of aerobic stability and storage loss

Upon arrival at the Luke laboratory in Jokioinen, Finland, the silage samples were mixed to expose them to air. The aerobic stability analysis was performed similarly as for the laboratory-scale ensiling experiment by packing approximately 600 g of each of the silages in 1.8 dm^3 polystyrene boxes and monitoring them at an ambient temperature of 19.0±0.14 °C (range: 18.2−19.9 °C) over a 15-day period using six replicate samples per farm.

In addition, the aerobic stability of the silages was evaluated using three replicates of 5-kg batches for each farm. The silages were placed in plastic boxes and covered lightly with plastic sheets to prevent drying. The boxes were kept in a hall maintained at 16.1±0.14 °C (range: 15.9−17.0 °C). Samples were collected at 5, 10 and 15 days and analysed for chemical composition, fermentation quality, and microbial quality as described for the laboratory-scale ensiling experiment.

Statistical analysis

The results from the aerobic stability of 5-kg batches over time were statistically analysed using the MIXED procedure of SAS software (SAS Inc. 2002–2012, Release 9.4; SAS Institute Inc., Cary, NC, USA). Fixed factors in the model included the farms, the duration of silage exposure to air, as well as their interaction. The normal distribution of the data was tested via the Shapiro–Wilk test using the Univariate procedure. Given the repeated temperature measurements over time within each silage batch, a repeated measures structure was applied, with time specified as the repeated factor and replicate nested within farm treated as the subject. A compound symmetry (CS) covariance structure was used to model the within-subject correlation. Least squares means and the standard errors of the means were reported for each farm × time point combination, with significant differences among the treatment means declared at 5% of probability. Linear correlations between farm silage temperature and quality parameters during the aerobic exposure were determined using the CORR procedure of SAS software.

Results

Laboratory-scale ensiling experiment

Properties of the ensiled grass

The sward was dominated by timothy, with proportions of timothy and meadow fescue being 0.76 and 0.24, respectively, on a FM basis. The DM content of the grass ensiled was 358 g kg^{-1}, while ash, crude protein (CP) and neutral detergent fibre (NDF) concentrations were 82, 147 and 588 g kg^{-1} DM, respectively. The WSC concentration was 89 g kg^{-1} of DM, and the BC 5.0 g of lactic acid 100 g^{-1}, which combined with the DM content resulted in a fermentation coefficient of 50. The *in vitro* organic matter digestibility (OMD) of the grass was 767 g kg^{-1} OM and subsequently D-value was 704 g kg^{-1} of DM. The counts of yeasts, moulds and aerobic bacteria were 4.4×10^5, 7.3×10^4 and 3.8×10^8 cfu, respectively.

Effects of compaction methods

The effects of additive treatments and compaction methods on the quality of ensiled grass are summarized in Table 2. Expected differences in packing density ($p < 0.001$) were achieved with normal, loose, and delayed compaction. However, the compaction methods had minimal effects on feed quality. The only statistically significant effect of the compaction methods was the slightly higher ($p < 0.001$) fermentation losses in silages with delayed compaction. Further, no interactions were found between the compaction methods and additive treatments.

Effects of additive treatments

The silage additive treatments significantly affected most of the evaluated silage preservation parameters (Table 2). Application of FPA restricted silage fermentation, resulting in higher WSC and lower lactic acid concentration than in the other treatments ($p < 0.001$). Other additives did not differ significantly from each other in these parameters. Inoculation with HO effectively lowered ($p < 0.001$) silage pH due to abundant lactic acid fermentation. Compared to control and HO, FPA produced less while HE and SA more acetic acid ($p < 0.001$). None of the silages contained excessive ethanol concentration, but it was highest in control and HE and lowest in FPA ($p < 0.001$). The OMD was higher ($p < 0.001$) in the FPA treated silage than in the control, HO and SA silages, but the difference to the HE silage was not statistically significant ($p > 0.05$).

No significant differences were found among additive treatments in microbial quality parameters ($p > 0.05$). Aerobic stability was the longest ($p < 0.001$) with FPA and SA treatments, followed by HE, while control and HO treatments had the lowest stability. The SA was highly effective ($p < 0.001$) in inhibiting aerobic spoilage, as SA silages did not heat up during the 360-hour monitoring period.

Table 2. Chemical composition, fermentation quality, aerobic stability, ensiling losses and microbial quality of first cut mixed timothy and meadow fescue silage treated with different silage additives and under different compactions (laboratory-scale ensiling experiment)

	Additive (A)[1]					SEM	Compaction (C)[2]			SEM[3]	*p*-value		
	Control	HO	HE	SA	FPA		N	L	D		A	C	A×C
Dry matter (DM), g kg^{-1}	380	398	404	371	377	10.5	383	379	396	8.1	0.151	0.306	0.921
pH	4.28^{ab}	4.21^{c}	4.30^{ab}	4.25^{bc}	4.34^{a}	0.014	4.27	4.27	4.28	0.011	<0.001	0.762	0.643
Ammonia-N, g kg^{-1} N	56^{ab}	50^{bc}	54^{ab}	61^{a}	42^{c}	2.2	53	53	52	1.7	<0.001	0.781	0.896
Organic matter (OM) digestibility, g g^{-1} OM	0.749^{c}	0.750^{c}	0.759^{ab}	0.753^{bc}	0.761^{a}	0.0017	0.756	0.754	0.753	0.0013	<0.001	0.303	0.106
D-value, g kg^{-1} DM	686^{c}	689^{c}	700^{a}	691^{bc}	698^{ab}	1.9	694	693	692	1.4	<0.001	0.556	0.205
Cellulase solubility, g kg^{-1} OM	0.781^{c}	0.782^{c}	0.793^{ab}	0.786^{bc}	0.796^{a}	0.0020	0.789	0.788	0.786	0.0015	<0.001	0.303	0.106
Chemical composition, g kg^{-1} DM													
Ash	83^{a}	81^{ab}	78^{b}	83^{a}	83^{a}	0.9	82	82	81	0.7	<0.001	0.961	0.913
Water soluble carbohydrates	13^{b}	15^{b}	9^{b}	13^{b}	54^{a}	3.4	22	20	20	2.6	<0.001	0.718	0.878
Ethanol	8.9^{a}	7.1^{b}	7.9^{ab}	3.1^{c}	1.2^{d}	0.34	5.8	5.6	5.6	0.30	<0.001	0.842	0.786
Fermentation quality, g kg^{-1} DM													
Lactic acid (LA)	78.3^{a}	78.4^{a}	75.1^{a}	74.5^{a}	45.9^{b}	1.98	71.2	69.6	70.4	1.53	<0.001	0.773	0.689
Acetic acid (AA)	12.1^{b}	11.8^{b}	16.2^{a}	16.1^{a}	6.3^{c}	0.31	12.5	12.7	12.4	0.24	<0.001	0.657	0.468
Propionic acid	0.09^{b}	0.08^{b}	0.09^{b}	0.09^{b}	2.72^{a}	0.030	0.61	0.65	0.59	0.024	<0.001	0.191	0.237
Corrected propionic acid[4]	0.09^{b}	0.08^{b}	0.09^{b}	0.09^{b}	0.23^{a}	0.026	0.12	0.13	0.11	0.020	0.001	0.661	0.971
Butyric acid	0.05	0.01	0.01	0.01	0	0.014	0.01	0.02	0.02	0.010	0.090	0.436	0.725
Total volatile fatty acids	12.2^{b}	11.9^{b}	16.3^{a}	16.3^{a}	6.6^{c}	0.32	12.6	12.8	12.5	0.25	<0.001	0.624	0.493
Total fermentation acids	90.5^{a}	90.3^{a}	91.4^{a}	90.7^{a}	52.5^{b}	1.94	83.8	82.5	83.0	1.50	<0.001	0.826	0.693
Total fermentation products	99.4^{a}	97.4^{a}	99.3^{a}	93.8^{a}	53.7^{b}	2.17	89.6	88.1	88.5	1.68	<0.001	0.818	0.790
LA/AA ratio	6.50^{a}	6.72^{a}	4.66^{b}	4.64^{b}	7.27^{a}	0.231	5.98	5.78	6.10	0.180	<0.001	0.465	0.604
Aerobic stability (2 °C), hours	117^{c}	135^{c}	272^{b}	360^{a}	312^{ab}	19.8	237	221	259	15.4	<0.001	0.230	0.969
Losses, g kg^{-1} initial DM	19.5^{a}	19.2^{a}	21.4^{a}	12.1^{b}	9.1^{b}	0.76	14.6^{b}	15.6^{b}	18.7^{a}	0.59	<0.001	<0.001	0.599
Density, kg m^{-3}	453	432	447	454	454	9.8	485^{a}	382^{b}	477^{a}	7.6	0.492	<0.001	0.297
Mould score, top surface, from 0–3	2.1^{a}	2.2^{a}	1.9^{a}	0^{b}	2.5^{a}	0.20	1.7	1.9	1.7	0.15	<0.001	0.676	0.692
Yeasts, cfu g^{-1}	1.1×10^{5}	5.2×10^{5}	1.1×10^{3}	8.2×10^{2}	2.4×10^{3}	2.4×10^{5}	3.2×10^{5}	5.9×10^{3}	5.2×10^{4}	1.8×10^{5}	0.470	0.422	0.464
Moulds, cfu g^{-1}	1.4×10^{3}	1.1×10^{3}	8.2×10^{2}	9.9×10^{2}	2.1×10^{4}	9.1×10^{3}	3.7×10^{2}	1.4×10^{4}	5.1×10^{2}	7.0×10^{3}	0.449	0.296	0.499

[1]Control: without additive; HO: Homofermentative inoculant; HE: Heterofermentative inoculant; SA: Salt-based additive; FPA: Formic and propionic acid-based additive. [2]N: Normal compaction; L: Loose compaction; D: Delayed compaction. [3]SEM: standard error of the mean. [4]Corrected by reducing 80% of the amount added in FPA. Values with the same letter in a row within the same group of means are not significantly different (p> 0.05) based on Tukey's test. Each treatment was conducted with three replicates.

Case study: Evaluation of farm silages

Temperature, composition, and preservation quality

During the sampling period, the outdoor temperature was approximately 7 °C. In all three farms, except the top surface silage in Farm 2, the middle point of the silo face was warmer than the bottom, edges, and top surface (Fig. 1).

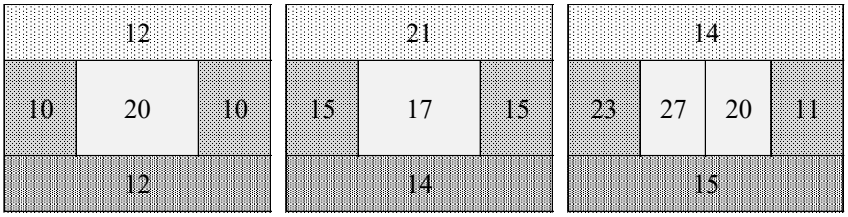

Fig. 1. Temperature (°C) of the silo faces at Farms 1, 2 and 3, respectively, measured on 5 November, 2020. The outdoor temperature at sampling was approximately 7 °C.

The silages varied significantly in DM content (Table 3), with Farms 2 and 3 having higher DM than Farm 1. Consequently, pH, ammonia-N, and soluble nitrogen levels were higher in Farms 2 and 3.

Table 3. Chemical composition, fermentation quality and microbiological counts and aerobic stability of farm silages (case study)

	Farm 1	Farm 2	Farm 3
Dry matter (DM) on the surface of the silo, g kg^{-1}	249	340	303
A sample taken from the middle of the silo face:			
DM, g kg^{-1}	306	503	404
pH	3.90	4.36	4.24
Ammonia-N, g kg^{-1} N	35	48	45
Chemical composition, g kg^{-1} DM			
D-value	723	691	729
Crude protein	101	134	152
Ammonia-N	0.56	1.03	1.09
Soluble nitrogen	7.2	14.5	14.5
Ash	66	83	84
Water soluble carbohydrates	34	72	42
Ethanol	8	3	10
Lactic acid (LA)	57	59	61
Acetic acid (AA)	27	9	23
Undissociated AA	24	6	18
Undissociated AA, g kg^{-1} fresh matter	7.2	3.2	7.1
Propionic acid	1.90[1)]	0.08	0.05
Butyric acid	1.21	0.26	0.02
Total volatile fatty acids	31	10	23
Total fermentation acids	87	69	84
Total fermentation products	95	72	94
LA/AA ratio	2.09	6.53	2.71
Aerobic stability (2 °C), hours	63	242	360[2)]

[1)]The additive used contained propionic acid. When the added propionic acid was subtracted from the analysed, the corrected concentration was 0.10 g kg^{-1} dry matter. [2)]The sample did not heat up during the entire 15-day monitoring period.

Farm 1 exhibited poorer microbiological quality, with higher yeast and mould counts compared to other silages (Fig. 2). Conversely, Farm 3 had a notably lower bacterial count.

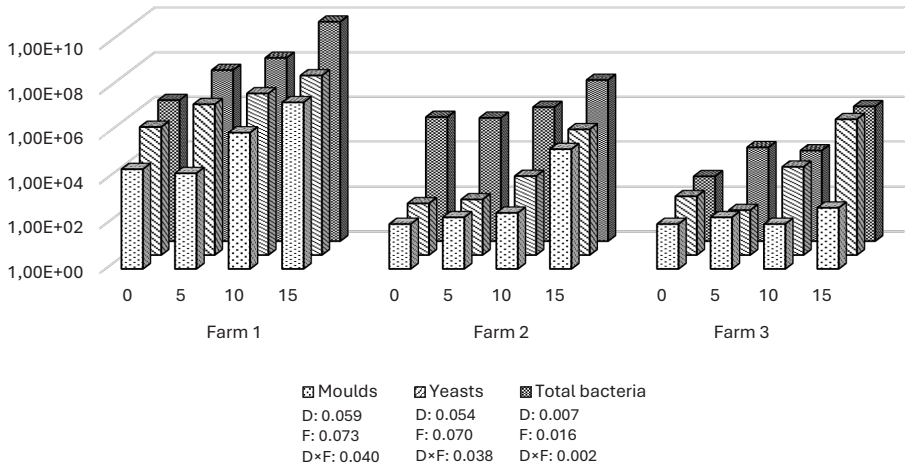

Fig. 2. Microbial quality of silages collected from three different farms (F) and exposed to air during different periods of time (D). Values below moulds, yeasts, and total bacteria represent *p*-values for the effects of D, F, and their interaction D×F.

Aerobic stability

Aerobic stability, measured in small 1.8 dm^3 polystyrene boxes indicated that Farm 3 silage was the most stable, not heating up at all during the 360-hour monitoring period (Table 3). Farm 1 silage warmed up the fastest, reflecting its susceptibility to spoilage, while Farm 2 silage was moderately stable.

Table 4 presents the temperatures and fermentation qualities of silages after 15 days of air exposure of the larger 5 kg batches of the farm silages. Farm 1's silage showed the clearest signs of spoilage, with higher initial counts of mould, yeasts and total bacteria at day 0, followed by a more pronounced increase throughout the days (Fig. 2), accompanied by a decrease in fermentation acids, an increase in pH, and reduced digestibility (Table 4). In contrast, silages from Farms 2 and 3 exhibited minor changes. The analysis revealed a notable increase ($p< 0.001$) in ash content, indicating the depletion of organic matter within the silage samples. This phenomenon was particularly pronounced in the silage from Farm 1, where a concurrent and significant reduction ($p< 0.05$) in organic matter digestibility was observed.

The correlations between the temperature of the silages during aerobic exposure and changes in the chemical composition revealed that with increased temperature, silage DM, ammonia and lactic acid concentrations and digestibility decreased, while pH, propionic and butyric acids as well as microbial counts increased.

Table 4. Temperature, fermentation quality and microbial quality of silages collected from different farms and exposed to air during different periods of time (case study)

Farms (F)	1			2			3			SEM	*p*-value		
Days (D)	5	10	15	5	10	15	5	10	15		D	F	D×F
Temperature	24.4	24.6	24.4	19.1	20.9	24.6	19.2	19.0	19.0	0.28	<0.001	<0.001	<0.001
Difference to ambient temperature	5.21	5.64	5.34	-0.13	1.95	5.54	-0.04	0.01	-0.02	0.285	<0.001	<0.001	<0.001
Dry matter (DM), g kg^{-1}	312	304	286	478	489	502	425	369	366	11.5	0.086	<0.001	0.020
pH	3.93	4.23	5.70	4.38	4.44	4.41	4.27	4.27	4.23	0.293	0.060	0.416	0.033
Chemical composition, g kg^{-1} DM													
Ammonia-N	0.55	0.76	0.64	1.11	1.22	1.23	1.06	1.28	1.21	0.035	<0.001	<0.001	0.309
Ash	65.3	64.8	74.7	83.8	82.9	84.3	80.7	83.3	85.4	0.80	<0.001	<0.001	<0.001
Water soluble carbohydrates	39.7	62.1	35.3	79.5	78.3[a]	84.4	54.4	54.4	55.0	5.95	0.269	0.001	0.089
Acids, g kg^{-1} DM													
Lactic (LA)	60.0	45.1	16.4	61.3	56.5	59.2	61.4	59.6	73.7	4.40	0.023	0.002	<0.001
Acetic (AA)	30.2	22.0	7.9	10.5	8.6	7.7	22.8	24.7	24.7	1.33	<0.001	<0.001	<0.001
Propionic	2.01	1.99	0.66	0.07	0.09	0.10	0.09	0.15	0.11	0.100	<0.001	<0.001	<0.001
Butyric	0.94	1.11	0.23	0.13	0.09	0.07	0.02	0.03	0.03	0.077	<0.001	<0.001	<0.001
Total volatile fatty acids	33.2	25.3	9.1	10.8	8.8	7.9	23.0	24.8	24.9	1.47	<0.001	<0.001	<0.001
Total fermentation acids	93.2	70.4	25.5	72.1	65.3	67.1	84.4	84.4	98.6	5.56	0.002	0.006	<0.001
LA/AA ratio	1.99	2.06	1.81	5.82	6.53	7.70	2.70	2.40	2.98	0.235	0.022	<0.001	0.015
Organic matter digestibility, g g^{-1}	0.773	0.774	0.733	0.747	0.757	0.756	0.797	0.797	0.794	0.0064	0.017	0.001	0.005
D-Value	722	724	679	685	694	693	733	731	726	6.3	0.005	0.003	0.003

SEM: standard error of the mean.

Discussion

Laboratory-scale ensiling experiment

The silage raw material had a typical composition for Finnish grasses, when compared to dairy farm silages from primary growth in 2020, which had on average a DM concentration of 385 g kg^{-1}, and CP and NDF concentrations of 144 and 556 g kg^{-1} DM, respectively, and D-value of 672 g kg^{-1} DM (n = 11 725; Laura Vaarnas, Valio Ltd., personal communication). The fermentation coefficient of 50 indicates that the grass material was easy to ensile (Weissbach et al. 1974, Weissbach 1996).

Reduced porosity of the silage due to proper compaction is recognised as a major factor contributing to proper fermentation and aerobic stability (see e.g. Rinne and Seppälä 2011). Several experiments have also confirmed reduced risk of aerobic spoilage and nutrient losses during storage in response to proper compaction (Toruk et al. 2010, Brüning et al. 2017, Tan et al. 2017). In contrast, previous research from our laboratory failed to demonstrate the effect of compaction on silage quality (Franco et al. 2022a, 2022b) in line with the current experiment. This is most likely an artefact resulting from the experimental techniques aimed at rapidly establishing anaerobic conditions at the beginning of the ensiling, relatively good tightness of the silos without oxygen challenge during the fermentation period, and conduction of the aerobic stability test immediately after silo opening, i.e., without the aerobic exposure of the silo face during the feed out period. Thus, advisory emphasis on proper compaction of the silos should be continued in spite of the current result. This finding also emphasizes the need to conduct experiments in larger scale and utilizing data from real farm conditions, although possibilities to control external factors and include proper replication are hindered.

The effects of chemical and biological additives used influenced silage fermentation in a typical way (McDonald et al. 1991, Muck et al. 2018), although even the control silage without any additive addition could be regarded as well fermented with a pH of 4.28 and ammonia-N of 56 g kg^{-1} total N, and without excessive volatile fatty acid production. The inoculants affected silage fermentation in an expected way with slight boosting of fermentation in the case of HE as evidenced by lower pH, and increased acetic acid production in response to HE. Salt-based additives have typically had minimal effects of silage fermentation quality (Franco et al. 2022a, 2022b, Franco and Rinne 2023), but in the current experiment, SA increased acetic acid concentration compared to control. The application level of SA in the current experiments was higher (5 l ton^{-1}) than recommended by the manufacturer (3 l ton^{-1}), which must be kept in mind when interpreting the results. The restriction of silage fermentation was clear when FPA was applied as evidenced by reduced protein degradation and production of fermentation end products with subsequent higher concentration of residual WSC. Although all silages exhibited low pH, the FPA treated silage had the highest pH, which can be considered typical as it restricts lactic acid formation and has direct antimicrobial effects. Similar trend has been noted previously (Kaewpila et al. 2020, Franco et al. 2022b, Franco and Rinne 2023). However, in cases of poor preservation quality in non-formic acid-treated silages, a lower pH in response to formic acid treatment has been observed (Rinne et al. 2023).

During silage fermentation, the microbes convert nutrients to fermentation end products that preserve the silage, but simultaneously nutrients are lost in the form of carbon dioxide with consequent formation of water (Knický and Spörndly 2015). The microbes in the silage use the highly digestible nutrients of the silage, which the cattle and their ruminal microbes are also able to use to their advantage. The average decrease of *in vitro* cellulase solubility was 23 g kg^{-1} DM in a data set including 52 comparisons of the grass and subsequent silage ensiled using formic acid as additive (Huhtanen et al. 2005). If the microbial activity in the silage is very strong, the amount of losses can increase and this phenomenon could be observed in the current experiment as well with lower weight losses during storage period of the silages treated with chemical additives, and highest *in vitro* OMD of the FPA treated silage.

The differences in the aerobic stability of the experimental silages followed expected patterns. The aerobic stability was the shortest for control and HE, that did not have any protective chemicals against aerobic spoilage microbes. In some cases, untreated silages can be aerobically very stable, if they have been poorly fermented and contain high concentrations of acetic and/or butyric acids (Franco et al. 2022b), but that was currently not the case. The HE inoculant was successful in increasing the concentration of acetic acid and the improved aerobic stability can be linked to it (Driehuis et al. 2001, Wilkinson and Davies 2013). Untreated and inoculated silages are dependent on microbial activity relying on the interaction of the viable microbiome and the substrates available in the grass materials and additionally affected by external factors, such as water activity and temperature (McDonald et al. 1991). For chemical additives, the protective chemicals are provided directly so that the effects could be expected to be more reliable as long as proper amount of chemicals is delivered to the feed, but the effects can be modulated by the characteristics of the biomass preserved. The use of SA has typically shown good ability to improve aerobic stability (Auerbach et al. 2012, Muck et al. 2018, Franco et al. 2022a, Franco and Rinne 2023), and in the current experiment, the SA treated silages did not heat at all during the 360-h follow-up period. The very good performance of SA must be interpreted keeping in mind that the dosage used was 1.7 times higher than commercially recommended. The good aerobic stability of FPA treated silage can be attributed to the propionic acid and sorbate included in it, and has been demonstrated in previous experiments as well (Harrison et al. 1997, Rinne et al. 2023).

The heating of silage is caused by introduction of oxygen that activates aerobic microbes, and their metabolism is observed as silage warming (McDonald et al. 1991). This process begins with yeasts consuming lactic acid and sugars, raising the pH and enabling mould growth, thus accelerating spoilage. Sometimes the concentrations of aerobic microbes in the silages can be directly related to the aerobic stability, such as in the case study reported here, as well as in Kung Jr et al. (1998, 2018), Wilkinson and Davies (2013), and Franco and Rinne (2023). However, in the current case, no statistically significant differences were observed in the yeast or mould counts of the silages, although numerically the yeast count was the lowest in SA in line with good aerobic stability.

Case study: Evaluation of farm silages

It is naturally expected that silages still in the bunkers would have temperatures higher than ambient temperatures, because almost all biological activities are exothermic, that is, they produce heat. In our experiment, all farm silages in the bunkers had temperatures below 30 °C, indicating no significant heating issues, as serious heating problems occur at temperatures above 40 °C (Kung Jr et al. 2018). The temperature measurements showed

that the central parts of the silos were warmer than the peripheral parts, which can be explained by storing heat from summer season and that generated by fermentation, as the silage itself forms effective insulation. Thus, relatively warm silage in the middle of the silo may not always indicate current microbial activity (Kung Jr 2010). The surface of Farm 2 silo showed elevated temperature, which may be linked to the high DM concentration of the silage. High DM grass material is more difficult to compact, and low water activity restricts fermentation so that less fermentation acids are produced resulting in higher pH, which all provide better opportunities for aerobic spoilage microbes to thrive (Wilkinson and Davies 2013). In Farm 3 silo, the left side was clearly warmer than the other parts of the silo demonstrating that the spoilage problems can be sporadic. All the findings indicate that monitoring silo temperatures needs to be done systematically to spot potential problems. Low-cost temperature meters are currently available that can be used for this purpose.

The three silages included in this study showed very different characteristics emphasizing the multifaceted nature of silage quality. Farm 1 silage deteriorated quickly during the aerobic follow-up period, while silages from Farms 2 and 3 remained rather stable. The most obvious reason for the difference between the silages was in the microbial quality as the counts of total bacteria, yeasts and moulds were the highest in silage from Farm 1. The follow-up period of the farm silages under aerobic conditions revealed clear deterioration of all farm silage as evidenced by increased populations of bacteria, yeasts, and moulds, but the chemical composition and OMD remained rather stable in silages from Farms 2 and 3 unlike for Farm 1, which exhibited substantial reductions in lactic and acetic concentrations resulting in increased pH. Also, the digestibility of the feed decreased due to loss of digestible organic matter during the deterioration period.

It was interesting that the relatively high undissociated acetic acid concentration of Farm 1 silage (24 g kg^{-1} DM) could not prevent fast aerobic deterioration of it. According to Wilkinson and Davies (2013), 8 g undissociated acetic per kg FM should protect silage from heating, and that value was not quite achieved for Farm 1 silage, as it remained at 7.2 g kg^{-1} FM. It seems that high acetic acid concentration is not always able to protect silage from heating, as Rinne et al. (2023) reported fast heating of red clover silage with as high acetic acid concentration as around 80 g kg^{-1} DM. However, that conclusion was reversed, when acetic acid was presented as g per undissociated acetic acid (Rinne et al. 2023), which seems to be a more accurate way to evaluate acetic acid in preventing aerobic spoilage.

Conclusions

In silage production, management factors, such as proper compaction combined with the use of silage additives, helps improving the silage fermentation quality of timothy grass. In our experiment, we used additives with different modes of action, including chemical additives and inoculants, as well as different ensiling compaction methods. We found that chemical additives unambiguously outperformed biological inoculants regarding enhancing aerobic stability and reducing fermentation losses. Heterofermentative inoculants also contributed to aerobic stability by increasing acetic acid concentration.

In practical guidelines for silage production on farms, compaction is a particularly important aspect to produce high-quality silage, but in the laboratory scale experiment, the responses were limited regarding compaction. This may be related to the use of airtight laboratory silos. However, at the farm scale, we emphasize that proper compaction remains essential.

The farm silage case study demonstrated great variability in silage stability, which, in some cases, may lead to significant losses in nutritional quality. These losses were linked to insufficient silage packing, underdosing of additive and subsequent poor microbial quality.

Acknowledgement

We are grateful to the farmers for providing access to their silages for this study. This work was supported by ReKiNa project funded by the Centre for Economic Development, Transport and the Environment for Northern Ostrobothnia, Oulu, Finland (grant number 92645/2019), A-Tuottajat Ltd., Eastman Chemical Company, HK Foods Ltd and Snellmanin Lihanjalostus Ltd.

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