Animal Feed Science and Technology 275 (2021) 114869

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effect of acid based additive treatment of low dry matter grass crops on losses and silage quality in bunker silos

Å.T. Randby [a,*], A.K. Bakken [b]

[a] Norwegian University of Life Sciences, Dept. of Animal and Aquacultural Sciences, P.O.Box 5003, 1432, Ås, Norway
[b] Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway

ARTICLE INFO

Keywords:
Bunker silage
Additive treatment
Density
Invisible losses
Fermentation
Clostridium tyrobutyricum

ABSTRACT

The large surface area of bunker silos imposes challenges with heating caused by plant respiration during initial ensiling. This study aimed to explore if application of a formic- and propionic acid-based additive would improve grass silage quality, reduce losses, and increase aerobic stability in bunker silos. At each of three harvests, every second tractor load was filled with either untreated or acid treated precision chopped crop, and ensiled in each of two identical bunker silos, 6 m × 27 m with three 3.5 m high walls, without roof. Each load in both bunker silos was compacted by two packing machines. Initially, an 8.3 t farm tractor worked for 10 min. followed by a 14.5 t wheel loader for 10 min. Silos were filled to approximately half of their capacity.

Due to showers during all three harvests, crop dry matter (DM) concentrations were only 195, 186 and 213 g/kg, respectively. During unloading for feeding, silage DM density and DM concentrations were respectively 7% and 5% higher ($P < 0.01$) in acid treated (A) than in control (C) silage. This was presumably due to early cell rupture caused by the applied acid, and thereby higher effluent release from A than C silage. Additive treatment did not influence the amount of wasted silage. Invisible losses, that included crop respiration, effluent runoff, anaerobic fermentation, aerobic deterioration from the silo face, and gaseous losses were numerically higher in A than C silos on fresh weight basis, but slightly lower on DM basis. The proportion of harvested crop DM that was offered to animals was 837 and 829 g/kg for A and C silage, respectively (NS).

Additive treatment reduced the proportion of non-protein N in total N, restricted silage fermentation to lactic and acetic acid, reduced NH_3-N-values, and increased ethanol fermentation ($P < 0.01$). Silage DM intake index was higher for A than C silage ($P < 0.001$). Aerobic stability was not significantly influenced by additive treatment. The concentration of spores of *Clostridium*

Abbreviations: A, acid treated; AA, acetic acid; AAT, amino acids absorbed in the intestine; ADF, acid detergent fiber; ADIP, acid detergent insoluble protein; ADL, acid detergent lignin; BSP, True buffer soluble protein; C, control; CP, crude protein; DM, dry matter; DOMD, digestible organic matter in dry matter; FW, fresh weight; FA, formic acid; H, harvest number; IP, buffer-insoluble protein; LA, lactic acid; ME, metabolizable energy; NDF, neutral detergent fiber; NDIP, neutral detergent insoluble protein; NE, net energy; NE_L, net energy lactation; NH_3-N, ammonia-nitrogen; NPN, non protein nitrogen; OM, organic matter; OMD, organic matter digestibility; PA, propionic acid; PBV, protein balance in the rumen; SDMI, silage dry matter intake; SEM, standard error of the mean; TA, total acids; TP, true protein; VOS, digestibility of organic matter in rumen fluid *in vitro*; WSC, water soluble carbohydrates.

* Corresponding author.
E-mail addresses: ashild.randby@nmbu.no (Å.T. Randby), Anne.Kjersti.Bakken@nibio.no (A.K. Bakken).

https://doi.org/10.1016/j.anifeedsci.2021.114869
Received 19 October 2020; Received in revised form 30 December 2020; Accepted 3 February 2021
Available online 6 February 2021

tyrobutyricum in spot silage samples from bunker silo faces was low or moderate, and did not differ according to additive treatment. Silo shoulder and side samples contained, however, significantly higher spore concentrations than mid and top samples.

1. Introduction

Along with increases in dairy herd size, the use of bunker silos increases in many countries. Due to the larger surface area, the time from crops are picked up in the field and until anaerobiosis is obtained, is longer in bunker silos than in smaller silos. The time period when plants respire and thereby consume water soluble carbohydrates (WSC), and temperature increases, should be as short as possible. Application of acid in harvested crop retards initial plant respiration, and therefore prevents temperature increases. Prolonged time with plant respiration may also favour yeast multiplication in the crop (Ruxton and McDonald, 1974). When anaerobic conditions are achieved, the frequency of yeast cells decline and *Saccharomyces* spp., that are fermentative but not able to utilize lactate, dominate with a slow anaerobic metabolism dependent on WSC as substrate for fermentation (McDonald et al., 1991). When silage is opened for feeding, and oxygen again is available, yeasts turn to a rapid aerobic metabolism where a wider range of energy sources is used. The products of silage fermentation, such as lactic acid, are themselves substrates for microbial growth (Pahlow et al., 2003).

Low initial crop temperature and high concentrations of WSC favour fermentation by lactic acid bacteria, that is the preferred fermentation pattern (McDonald et al., 1991). When crops are difficult to ensile due to low dry matter (DM) or low WSC concentration, or if ensiling conditions are poor e.g. if sealing is delayed, formic acid-based silage additives may stimulate lactic acid fermentation and restrict acetic, propionic and butyric acid fermentation (McDonald et al., 1991, Randby, 2000). Under more optimal conditions, however, where lactic acid fermentation would dominate even without additive application, formic acidic-based additives restrict fermentation caused by numerous bacteria, which results in reduced concentrations of all fermentation acids including lactic acid. With increasing application rate, this effect increases, and is found to be beneficial in milk production (Jaakkola et al., 2006b).

Formic acid has, however, no antifungal effect like propionic acid (Woolford, 1975). Propionic acid is therefore recommended with increasing wilting rates, where higher pore volumes in silages increase the vulnerability for air ingress. Per kg, formic acid has higher molar concentration of acid, and is cheaper than propionic acid. The antifungal effect of propionic acid increases with decreasing pH (Woolford, 1975). Therefore, silage additives based on a mixture of the two acids are commonly used.

Silage additives based on formic and propionic acids are shown to improve fermentation quality in grass silage, silage intake, milk yield, milk composition, and growth in ruminants (Randby and Selmer-Olsen, 1999; Jaakkola et al., 2006b; Huhtanen et al., 2003, 2007, Krizsan and Randby, 2007).

This study aimed to explore the effects of applying a formic and propionic acid-based silage additive to wet grass crops ensiled in bunker silos. The hypothesis was: Application of a formic- and propionic acid-based additive to grass crops during ensiling in bunkers will improve fermentation quality, reduce losses and increase aerobic stability of the silage.

2. Material and methods

2.1. Experimental design

The study was conducted at the Animal Production Experimental Centre at the University of Life Sciences, Ås, Norway (59°40′N, 10°47′E; elevation 93 m.a.s.l.). In each of three grass crop harvests, of them two primary growths (H1; June 1–2, H2; June 6), and one second regrowth (H3; September 21–22) in 2017, two identical bunker silos, 6 m × 27 m with three 3.5 m high walls, without roof, and with maximum capacity of 300 tonnes fresh crop weight, were filled simultaneously. One silo was filled with untreated crop, and one with acid treated crop.

2.2. Harvested crops

During each harvest, grass crops from two to four fields were harvested. Botanical composition of each field was roughly estimated by visual inspection prior to mowing. Based on weighted averages of harvested crop DM from each field, botanical composition at H1, H2 and H3, respectively, was 71 %, 38 %, and 53 % timothy (*Phleum pratense*), 14 %, 32 %, and 22 % meadow fescue (*Festuca pratensis*), 9 %, 19 %, and 22 % red plus white clover (*Trifolium pratense* and *Trifolium repens*), and 5 %, 11 %, and 3 % of other grass species, forbs and weeds. Harvest 1 was taken at late stem elongation of timothy, i.e. when most shoots had 3–4 palpable nodes and the inflorescence was visible on less than 10 percent of the shoots. Harvest 2 was taken four days later, when timothy had reached the boot stage or early heading, i.e. when about 10 percent of the shoots had visible inflorescences. At the second regrowth (H3) almost none of the grass species was generative, but some red clover shoots flowered.

2.3. Mowing, wilting, additive treatment, and crop transportation

Crops were mown with three mower aggregates placed in butterfly position: A Kverneland Taarup 5087 M (Kverneland Group, Klepp, Norway) without conditioner on each side of the tractor, and a Kverneland Taarup 3632 FT with conditioner in front. The three

windrows were joined to one using Kverneland Taarup 9590 C Hydro rake with TerraLink Quattro Ground Contour System. Due to showers before mowing (H1), shortly after mowing (H2 and H3) or during harvesting (H2), DM concentrations in harvested crops were lower than target concentration 250−300 g/kg. Mean 24 h air temperatures were 11, 15 and 10 °C, for H1, H2 and H3, respectively, and respective maximum day temperatures were 14, 20 and 13 °C. Measured average wilting time in H1, H2 and H3, respectively, were 4, 10 and 27 h before raking and 2, 2 and 2 h following raking. The composite windrows were collected with a 1.8 m wide pick-up on Lely Storm 130 P precision chopper (Lely Industries, Maassluis, The Netherlands), pulled behind a JD 6175 R tractor (Deere & Company, Moline, IL, USA). The flywheel chopping system with 10 blades and 5 blowing paddles gave an expected chop length of 12−44 mm. Every second load was applied GrasAAT Plus silage additive (per kg: 440 g formic acid (FA), 204 g sodium formate, 120 g propionic acid (PA), 15 g benzoic acid; Addcon Nordic, Porsgrunn, Norway), at the harvester at target dosage 4 L/tonne using Cliptonpumpen (Clipton, Vejbystrand, Sweden). Two Metsjø trailers (25 m^3) transported the wilted crop to the silos. All transport tractors with trailers were weighed empty (tare) trice daily, and gross weight was recorded immediately before trailers reversed towards the bunker and emptied the grass load. Every second load, acid-treated or untreated, respectively, were filled into each of two identical bunker silos, 6 m × 27 m with three 3.5 m high walls, without roof. Bunker silos were filled to approximately half of their capacity. Measured additive application rates for the treated bunker silo in H1, H2, and H3, respectively, were 3.9, 4.0 and 4.0 L/tonne.

2.4. Compaction and covering of bunkers

Both bunker silos at each harvest were compacted by two packing machines. Initially, a farm tractor, John Deere 6530 Premium (Deere & Company, Moline, IL, USA) with a 1.90 m wide Norje N106 stone fork in front (Norjes Smidesfabrik, Sölvesborg, Sweden) and weight behind, with a total weight of 8.3 t, worked in each silo. The tractor distributed each trailer load to a thin layer covering the entire crop surface, and initiated compaction. All four tractor wheels were equipped with Michelin Multibib radial tyres (Michelin Multibib, Clermont-Ferrand, France), in front 48 cm wide (480/65 R28) and in rear 60 cm wide (600/65 R38), with 150 kPa air pressure. After approximately10 min., a trailer load was emptied in the other bunker silo, and the tractor moved to initiate compaction in that silo. The other packing machine, a Volvo L90H wheel loader (Volvo Construction Equipment, Gothenburg, Sweden) with a 2.60 m wide Norje N985 silogrip in front, total weight 14.5 t, continued compaction of each load in each silo for further 10 min. All four wheels of the wheel loader were identical, with 52 cm wide Trelleborg C-800 L2 tyres, 20.5 R25 (Trelleborg AB, Trelleborg, Sweden), with 350 kPa air pressure.

In H1 and H3, harvesting for the pair of bunkers lasted one and a half day, and a thin plastic layer (0.04 mm) was placed over the crop surface overnight. In H2 only one day was spent for the same operations. During silo filling, a thick, black plastic with oxygen permeability 180 cm^3/m^2 in 24 h (0.150 mm, Polydress Texaleen Alpha Plus; RKW Agri GmbH & Co. KG, Michelstadt, Germany) was hung over side- and end walls and fastened into the crop. When the last trailer load was filled in each silo, the tractor and the wheel loader both packed an extra of 15 min in each silo. Thereafter a thin 0.04 mm plastic layer with oxygen permeability 940 cm^3/m^2 in 24 h (Polydress transparent PE Vacuum film; RKW Agri GmbH & Co. KG, Michelstadt, Germany) was placed over the entire crop surface and manually fastened in the slot between the crop and the thick plastic hanging on the walls. The plastic from the wall was then turned down to cover the major part of the surface, before another thick 0.150 mm plastic was placed over the entire surface. Old, empty tyres were manually carried into the silos, and placed side by side on the entire top surface to keep plastic down.

Immediately following covering, the height from top of crop surface to top of silo wall was measured with one meter intervals along the two 27 m side walls. This formed the basis for calculation of the volume of ensiled grass crop. The same measurements were done immediately before bunker silos were opened for feeding.

2.5. Opening, unloading and sampling of fresh crop and silage

Six to ten fresh crop portions were grabbed by hand from every trailer load entering the bunker silos. Within trailer load, these were mixed and used for two samples of 500 and 200 g, respectively. The 500 g portions were stored at 4 °C for 0–2 days, when they were dried at 100 °C to constant weight for DM determination. The 200 g portions were stored at -20 °C, and later pooled to 6 or 7 samples per harvest, in total 19 samples, that were freeze dried for chemical analyses.

Following 84 days of storage at average temperature 15.2 °C, the two H1 bunkers were opened on August 24, 2017, and grass silage offered to the university herd until December 12, 2017, in total during 109 days at average temperature 6.4 °C. Following 176 days of storage at average temperature 10.9 °C, the H2 silages were fed from November 29 to May 11, 2018, during 163 days at average temperature -0.5 °C. Following 141 days of storage at average temperature 1.3 °C, the H3 silages were fed from February 9 to April 18, 2018, during 68 days at average temperature -1.8 °C.

Silage was unloaded 3–4 days a week using Triolet TU 180 XL silo block cutter (Triolet Mullos BV, Oldenzaal, The Netherlands) mounted in front of a wheel loader. In advance, tyres on the top surface were manually removed, and the block cutter shore off plastic top film in one meter depth where a silage block was removed. Silage blocks from each silo were weighed, and samples from the removed blocks, in total 1.5−2 kg were stored at -20 °C. Moulded or deteriorated silage that could not be fed to cattle was weighed separately, or the proportion of a weighed block that had to be wasted was visually estimated. The term “offered silage” is used for silage given to livestock as feed. Average daily removal of silage from the face was 14, 10 and 24 cm, in H1, H2 and H3, respectively.

Total precipitation from silo filling and until completed unloading was 650, 919 and 504 mm, giving a maximum of 58, 86 and 48 tonnes of water into the 15−16 m of silo length used in each bunker in H1, H2 and H3, respectively. Because effluent runoff was not measured, effluent was categorized as being part of invisible losses, together with crop respiration, anaerobic fermentation, aerobic deterioration from the silo face, and gaseous losses during formation of surface waste (McDonald et al., 1991).

The frozen silage samples were partly thawed, chopped, and pooled for various purposes. For DM determination, 23–27 pooled samples per silo (250 g-samples in duplicate), each representing 4–5 tonne fresh silage, were dried at 103 °C, weighed warm, and corrected for volatiles (see below). For other analyses, silage samples were pooled to 6 samples per bunker. Each of these 6 composite samples were shared into 3 portions that were later used for (1) analysis of fermentation quality, (2) aerobic stability test, (3) freeze dried for chemical analyses.

2.6. Spot silage samples from bunker silo faces for pH and Clostridium tyrobutyricum analyses

At three time points during unloading of a pair of bunkers, on average 29, 53, and 85 days after silo opening, core samples were taken from four spots of the silo face: (1) mid: at least 0.5 m from top surface and at least 1 m from side walls, (2) side: 0.1−0.5 m from the silo wall and at least 0.5 m from top surface; (3) top: 0.1−0.5 m from top surface and at least 1 m from silo wall; (4) shoulder: 0.1−0.5 m from the silo wall and 0.1−0.5 m from top surface. Cores from the right and left side of the silo face were pooled for side and shoulder samples. The corer was washed in Antibac (ethanol, isopropanol, n-propanol, water) between each sample. Samples were stored at -20 °C until analysis.

2.7. Analytical procedures

2.7.1. Chemical analyses

Silage samples oven dried at 103 °C to constant weight were corrected for volatiles according to NorFor (Åkerlind et al., 2011) and used to calculate the portion of crop DM that was recovered as silage or lost.

Silage samples kept undried were analysed for pH, NH_3-N, organic acids and ethanol by Eurofins Agro Testing Wageningen, The Netherlands. Samples were diluted with distilled water and stored at 4 °C for 24 h before pH was measured with a Termo Orion 420A + pH-meter with Orion 9107BN electrode (Thermo Scientific, Beverly, MA, USA). Ammonia nitrogen was analysed with MAN-TECH PC-titrate (Guelph, ON, Canada) using an Orion ion analyzer 901. For organic acids and ethanol, samples were diluted with demineralized water and stored frozen, then thawed, filtered, diluted and subjected to a clean-up procedure with activated carbon. For lactic acid (LA) and FA the extract was analysed by high pressure liquid chromatography (HPLC) using a separation power column based on polarity, charge and particle size, at 45 °C (mobile phase, 0.0012 M H_2SO_4 at 0.6 mL/min) with a UV spectrophotometric detector. For acetic acid (AA), PA, butyric acid (BA), and ethanol, the extract was acidified and analysed by gas chromatography (GC) after adding internal standards. Carrier gas was helium with constant flow 1.5 mL/min, and temperature gradient 50−250 °C. A flame ionization (FI) detector was used, and quantification was done by comparison with internal standards.

Freeze dried fresh crop and silage samples were equilibrated to room humidity, and milled to pass a 1.0-mm screen (Retsch SM200 cutting mill (Retsch GmbH, Haan, Germany)) prior to analyses of DM (103 °C for 4 h), ash (550 °C for 4 h), water soluble carbohydrates (WSC), crude protein (CP), true protein (TP), buffer-insoluble protein (IP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP) and digestibility of organic matter in rumen fluid *in vitro* (VOS). Analytical methods for these analyses were the same as described by Randby et al. (2020).

2.7.2. Analysis of spores of Clostridium tyrobutyricum in spot silage samples

For analysis of spores of *C. tyrobutyricum* by Eurofins Food and Feed Testing (Jönköping, Sweden), 20 g sample and 180 mL of peptone water (0.1 % peptone and 0.85 % NaCl) were homogenized in a stomacher for 30 s. Approximately 10 mL of the homogenized initial sample dilution was heated in water bath for 12 min at 80 °C, and five following dilutions were prepared from that dilution. From each dilution, 0.1 mL was surface spread on plates with Reinforced Clostridium Agar (RCA; Merck) with addition of neutral red (Sigma) and cycloserine (Sigma). Plates were enumerated after 48 h of anaerobic cultivation at 37 °C. Yellow colonies were counted from dilutions primarily containing 10–100 colonies. When needed, lactate dehydrogenase (LDH) activity was tested on 3 visually similar colonies per sample as described by Jonsson (1990). Detection level was 10 colony forming units (cfu)/g, i.e. 1.00 log cfu/g.

2.7.3. Aerobic stability test

Silage samples of 700 g were placed in perforated plastic bags in perforated polystyrene boxes at 20 °C. The temperature of the silages and the ambient temperature were logged every 2 h for 30 days. Silages were regarded stable until silage temperature reached 2 °C above ambient temperature.

2.8. Calculations

Silage *in vivo* organic matter digestibility (OMD), digestible OM in DM (DOMD), metabolizable energy (ME), net energy lactation (NE_L), metabolizable protein expressed as amino acids absorbed in the intestine (AAT), and protein balance in the rumen (PBV), were calculated as described by Randby et al. (2020). Based on silage concentrations of DM, total fermentation acids, NDF, DOMD, and harvest (primary versus regrowth), silage DM intake (SDMI) index was calculated according to Huhtanen et al. (2007). Analytical results of the feed protein fractions TP, IP, NDIP and ADIP are presented as A, B1, B2, B3 and C, with decreasing solubility and rate of enzyme degradability according to Licitra et al. (1996): Protein fraction A = Non protein nitrogen (NPN, calculated as CP-TP), B1 = True buffer soluble protein (BSP, calculated as TP-IP), B2 = Neutral detergent soluble protein, calculated as IP-NDIP, B3 = Neutral detergent insoluble protein (calculated as NDIP-ADIP (insoluble in neutral detergent but soluble in acid detergent)), C = Acid

Table 1
Chemical composition of fresh grass crops ensiled in bunker silos during three harvests, as weighted averages of dry matter yields from all fields within harvest.

Harvest		DM,	g/kg DM		Protein fractions[1], g N/kg total N					g/kg DM					g/kg	Per kg DM			
	N	g/kg	OM	CP	A	B1	B2	B3	C	WSC	NDF	ADF	ADL	OMD	DOMD	MJ ME	MJ NE_L	g AAT	g PBV
1	5	194	921	177	261	55.5	490	171	22.8	103	526	298	44.2	0.751	691	10.9	6.40	75.3	40.1
2	6	186	930	162	295	30.4	541	111	22.9	118	520	299	9.8	0.756	703	11.1	6.55	76.1	33.3
3	6	213	926	166	323	52.2	444	141	40.2	121	503	291	52.0	0.734	680	10.6	6.25	73.8	39.1

[1] Protein fractions: A = Non protein nitrogen (NPN, calculated as CP-TP), B1 = True buffer soluble protein (BSP, calculated as TP-IP), B2 = Neutral detergent soluble protein, calculated as IP-NDIP, B3 = Neutral detergent insoluble protein (calculated as NDIP-ADIP (insoluble in neutral detergent but soluble in acid detergent)), C = Acid detergent insoluble protein (ADIP).

Table 2
Effect of acidic additive treatment (A) versus untreated control (C) of grass crops for bunker silos during three harvests on fresh grass crop and silage weights, dry matter (DM) concentrations and wet and DM densities.

Harvest	Additive	N	Fresh crop						Silage					
			Volume m^3	Kg	DM g/kg	Kg DM	Kg per m^3	Kg DM per m^3	Volume m^3	Kg	DM g/kg	Kg DM	Kg per m^3	Kg DM per m^3
1	C	1	151	128,100	196	25,103	850	167	140	112,380	186	20,859	802	149
1	A	1	137	134,100	192	25,761	982	189	132	108,520	201	21,833	824	166
2	C	1	180	169,701	185	31,410	944	175	153	138,000	187	25,745	900	168
2	A	1	177	178,260	186	33,216	1010	188	156	140,120	192	26,961	901	173
3	C	1	165	122,400	213	26,083	741	158	149	115,520	206	23,766	774	159
3	A	1	150	124,330	214	26,554	829	177	145	114,820	216	24,778	794	171
1		2	144 [a]	131,100 [a]	194 [a]	25,432 [a]	916 [a]	178	136	110,450 [a]	194 [a]	21,346 [a]	813 [a]	158
2		2	179 [b]	173,981 [b]	186 [a]	32,313 [b]	977 [a]	182	155	139,060 [b]	190 [a]	26,353 [c]	901 [b]	171
3		2	158 [a]	123,365 [a]	214 [b]	26,319 [a]	785 [b]	168	147	115,170 [a]	211 [b]	24,272 [b]	784 [a]	165
SEM			3.33	1672	1.44	361	16.8	2.29	2.78	1496	2.50	65.1	5.80	3.01
P			0.04	0.004	0.01	0.009	0.03	0.09	0.08	0.009	0.046	<0.001	0.009	0.18
	C	3	165	140,067	198	27,532	845	167	147	121,967	193	23,457	825	159
	A	3	155	145,563	197	28,510	940	185	144	121,153	203	24,524	840	170
	SEM		2.72	1365	1.18	295	13.7	1.87	2.27	1221	2.04	53.1	4.73	2.46
	P		0.11	0.10	0.73	0.14	0.04	0.02	0.45	0.68	0.07	0.005	0.17	0.08

Means with different letters differ at $P < 0.05$.

detergent insoluble protein (ADIP).

2.9. Statistical analyses

All data was analysed using SAS (release 9.4, 2002–2012; SAS Institute inc., Cary, NC, USA). Fresh crop and silage weights, densities, losses, and weighted averages of bunker silage chemical composition were analysed using the PROC MIXED procedure by the model: $Y_{ik} = \mu + S_i + H_k + e_{ik}$, where μ = general mean, S_i = effect of additive i, H_k = effect of harvest k, and e_{ik} is the random residual error. All results were presented as least square (LS) means. Due to only 6 observations, the additive × harvest interaction could not be estimated (Tables 2,3).

Silage chemical composition and aerobic stability were analysed using the PROC MIXED procedure by the model: $Y_{ik} = \mu + S_i + H_k + S_i \times H_k + e_{ik}$, where μ = general mean, S_i = effect of additive i, H_k = effect of harvest k, $S_i \times H_k$ = the effect of interaction, and e_{ik} is the random residual error. Means between harvests were separated using the PDIFF statement (Table 4,5).

The frequency of spot samples with detected growth of *C. tyrobutyricum* from the four sampling points in bunker silo faces and from untreated or acid treated silage was analysed with Chi-square test using the PROC FREQ procedure. Additionally, pH, and the number of detected colonies per g sample in bunker silo faces, were analysed using the PROC MIXED procedure by the model $Y_{ikl} = \mu + S_i + H_k + P_l + S_i \times H_k + P_l \times H_k + e_{ikl}$, where μ = general mean, S_i = the effect of additive i, H_k = effect of harvest k, P_l = effect of point in bunker silo face l, $S_i \times H_k$ and $P_l \times H_k$ are effects of interactions, and e_{ikl} is the random residual error. The additive × point in bunker silo face interaction was insignificant for both variables and therefore excluded from the model. Points in bunker silo face: 1. Mid, 2. Side, 3. Top, 4. Shoulder (Table 6).

Counts of *C. tyrobutyricum* were $\log_{10}$ transformed. Negative results (below detection limit) were defined as half of the detection limit, i.e. 5 cfu/g = 0.70 log cfu/g (Vissers et al., 2007). Results were considered statistically significant at $P < 0.05$, and *P*-values between 0.05 and 0.1 were considered to indicate trends.

3. Results

3.1. Bunker silo fresh crop composition, crop and silage weights, densities and losses

Fresh crop DM concentrations were in the range 180–220 g DM/kg (Table 1), and far below the target at 250–300 g DM/kg. Crop concentrations of CP, WSC, NDF, and measures of digestibility and energy, were within normal ranges in forage for dairy cows, and with small differences between harvests. In H1 and H3, 19 loads of crop were filled into each of the two silos, and in H2, 24 loads in each silo. This resulted in a significantly larger silo volume filled up, and higher weight in kg and kg DM per silo, and higher crop density in H2 than in H1 and H3 (Table 2).

Over the three harvests, a 6 % higher silo volume, 165 vs. 155 m^3, was filled up with C crop than with A crop, although 4 % more weight in kg and kg DM was filled into A than C silos (28.5 vs. 27.5 t DM). This resulted in 11 % higher density in kg ($P = 0.04$) and kg DM ($P = 0.02$) in A than in C silos immediately after finished silo filling.

When silos were opened for feeding, silage volumes in A and C silos had shrunk with 7 % and 11 %, respectively since filling. A similar amount of fresh silage weight was emptied from A and C silos, however, due to higher DM concentration in A than C silage, on average 203 vs. 193 g DM/kg ($P = 0.07$), A silos contained 4.5 % more silage DM than C silos ($P = 0.005$). Silage fresh weight (FW) density was numerically 2 % higher, and DM density 7 % higher, in A than C silage ($P = 0.08$).

A higher proportion of harvested crop was offered to animals from H3 compared with H1 and H2, on both FW ($P = 0.04$) and DM

Table 3
Effect of acidic additive treatment (A) versus untreated control (C) of grass crops for bunker silos during three harvests on crop recovered as offered silage and wasted silage, and sum of invisible losses through respiration, effluent, fermentation and aerobic deterioration.

			g/kg crop			g/kg crop DM		
Harvest	Additive	N	Offered silage	Wasted silage	Invisible losses	Offered silage	Wasted silage	Invisible losses
1	C	1	844	33.4	123	799	31.6	169
1	A	1	772	37.4	191	808	39.2	153
2	C	1	789	24.5	187	795	24.7	180
2	A	1	759	26.8	214	784	27.7	188
3	C	1	924	19.5	56.2	892	18.8	88.8
3	A	1	910	13.7	76.5	919	13.8	66.9
1		2	808 [a]	35.4	157 [a]	804 [a]	35.4	161 [a]
2		2	774 [a]	25.7	200 [a]	790 [a]	26.2	184 [a]
3		2	917 [b]	16.6	66.4 [b]	906 [b]	16.3	77.9 [b]
SEM			14.9	2.62	12.9	9.48	3.19	7.98
P			0.04	0.07	0.03	0.02	0.10	0.02
	C	3	852	25.8	122	829	25.0	146
	A	3	814	26.0	160	837	26.9	136
	SEM		12.2	2.14	10.5	7.74	2.60	6.51
	P		0.15	0.96	0.12	0.53	0.66	0.38

Means with different letters differ at $P < 0.05$.

Table 4
Effect of acidic additive treatment (A) versus untreated control (C) of grass crops for bunker silos during three harvests on chemical composition, protein fractions, digestibility, and calculated energy and protein values of silages.

Harvest	Additive		DM	g/kg DM		Protein fractions[2], g N/kg total N					g/kg DM				g/kg	Per kg DM			
		N[1]	g/kg	OM	CP	A	B1	B2	B3	C	NDF	ADF	ADL	OMD	DOMD	MJ ME	MJ NE_L	g AAT	g PBV
1	C	6	186	922	161	576	34.2	291	62.4	36.4	528	336	33.4	0.734	674	10.6	6.19	69.9	39.4
1	A	6	201	927	157	528	27.8	306	96.8	41.7	537	342	30.8	0.734	679	10.6	6.24	70.1	35.7
2	C	6	187	937	148	571	41.9	289	56.8	41.3	548	355	26.4	0.715	668	10.4	6.09	69.6	27.4
2	A	6	192	938	147	552	36.1	299	71.7	41.6	565	373	31.5	0.710	663	10.3	6.03	69.4	26.7
3	C	6	206	927	171	536	34.2	311	74.9	43.3	497	325	38.9	0.716	662	10.3	6.02	69.2	50.4
3	A	6	215	929	168	492	40.5	340	82.4	44.8	494	317	34.9	0.720	667	10.4	6.09	69.5	47.3
SEM			2.78	2.55	3.22	15.1	4.35	11.7	5.70	1.69	9.0	6.3	1.67	0.006	4.17	0.08	0.055	0.21	3.04
P	Harv. × Additive		0.22	0.66	0.93	0.58	0.27	0.69	0.07	0.31	0.57	0.15	0.02	0.73	0.40	0.45	0.44	0.44	0.87
1		12	194 ^{a}	924 ^{a}	159 ^{b}	552 ^{a}	31.0	298 ^{a}	79.6 ^{a}	39.0 ^{a}	533 ^{a}	339 ^{b}	32.1 ^{a}	0.734 ^{a}	676 ^{a}	10.6 ^{a}	6.21 ^{a}	70.0 ^{a}	37.6 ^{b}
2		12	189 ^{a}	937 ^{b}	147 ^{a}	562 ^{a}	39.0	294 ^{a}	64.2 ^{b}	41.4 ^{ab}	557 ^{a}	364 ^{a}	28.9 ^{a}	0.713 ^{b}	666 ^{b}	10.4 ^{b}	6.06 ^{b}	69.5 ^{b}	27.1 ^{a}
3		12	211 ^{b}	928 ^{a}	169 ^{c}	514 ^{b}	37.4	326 ^{b}	78.7 ^{a}	44.1 ^{b}	495 ^{b}	321 ^{c}	36.9 ^{b}	0.718 ^{b}	664 ^{b}	10.4 ^{b}	6.05 ^{b}	69.4 ^{b}	48.9 ^{c}
SEM			1.96	1.80	2.28	10.7	3.07	8.2	4.03	1.20	6.4	4.5	1.18	0.004	2.95	0.05	0.039	0.15	2.15
P			<0.001	<0.001	<0.001	0.009	0.17	0.02	0.02	0.02	<0.001	<0.001	<0.001	0.002	0.01	0.01	0.01	0.009	<0.001
	C	18	193	928	160	561	36.8	297	64.7	40.3	524	339	32.9	0.722	668	10.4	6.10	69.6	39.1
	A	18	203	931	157	524	34.8	315	83.6	42.7	532	344	32.4	0.722	670	10.5	6.12	69.7	36.6
	SEM		1.60	1.47	1.86	8.7	2.51	6.7	3.29	0.98	5.2	3.7	0.96	0.003	2.41	0.04	0.032	0.12	1.76
	P		<0.001	0.21	0.38	0.005	0.58	0.07	<0.001	0.10	0.29	0.31	0.72	0.96	0.63	0.68	0.68	0.62	0.33

Means with different letters differ at $P < 0.05$.

[1] Number of samples. Six samples were analysed per bunker silo, where each of them was a composite sample of subsamples taken 3–4 days per week during feed out.

[2] Protein fractions: A = Non protein nitrogen (NPN, calculated as CP-TP), B1 = True buffer soluble protein (BSP, calculated as TP-IP), B2 = Neutral detergent soluble protein, calculated as IP-NDIP, B3 = Neutral detergent insoluble protein (calculated as NDIP-ADIP (insoluble in neutral detergent but soluble in acid detergent)), C = Acid detergent insoluble protein (ADIP).

Table 5
Effect of acidic additive treatment (A) versus untreated control (C) of grass crops for bunker silos during three harvests on fermentation quality, intake potential and aerobic stability of silages.

Harvest	Additive		g/kg		g/kg N	g/kg DM								Aerobic
		N[1]	DM	pH	NH_3-N	WSC	LA	FA	AA	PA	BA	TA	Ethanol	SDMI	stab.,h[2]
1	C	6	186	4.34	60.9	0.30	81.7	0.77	41.3	2.91	0.59	127	6.9	91.0	173
1	A	6	201	4.21	50.3	0.38	76.1	6.91	28.9	2.45	0.18	115	11.7	95.6	232
2	C	6	187	4.18	60.9	2.62	80.4	0.47	37.3	2.08	0.20	120	6.2	93.8	151
2	A	6	192	4.44	61.4	0.98	39.5	8.96	32.9	3.37	0.17	85	30.7	98.9	181
3	C	6	206	4.03	53.8	10.3	108.5	0.65	27.8	1.14	0.00	138	5.9	90.2	263
3	A	6	215	3.97	41.3	30.5	81.4	10.2	18.7	3.09	0.00	113	6.7	96.5	258
SEM			2.99	0.040	3.20	2.02	5.63	0.535	2.52	0.242	0.169	5.94	1.32	1.12	31.5
P	Harv. × Additive		0.19	<0.001	0.11	<0.001	0.01	0.01	0.30	<0.001	0.43	0.17	<0.001	0.74	0.60
1	12		194 ^{a}	4.28 ^{a}	55.6 ^{a}	0.34 ^{a}	78.9 ^{b}	3.84 ^{a}	35.1 ^{a}	2.68 ^{a}	0.39	121 ^{a}	9.3 ^{b}	93.3 ^{a}	203 ^{ab}
2	12		189 ^{a}	4.31 ^{a}	61.1 ^{a}	1.80 ^{a}	59.9 ^{a}	4.72 ^{ab}	35.1 ^{a}	2.73 ^{a}	0.18	103 ^{b}	18.5 ^{c}	96.3 ^{b}	166 ^{a}
3	12		211 ^{b}	4.00 ^{b}	47.6 ^{b}	20.4 ^{b}	94.9 ^{c}	5.41 ^{b}	23.2 ^{b}	2.12 ^{b}	0.00	126 ^{a}	6.3 ^{a}	93.3 ^{a}	261 ^{b}
SEM			2.34	0.028	2.26	1.43	3.98	0.378	1.78	0.171	0.120	4.20	0.93	0.79	22.3
P			<0.001	<0.001	<0.001	<0.001	<0.001	0.02	<0.001	0.03	0.09	0.001	<0.001	0.01	0.02
	C	18	193	4.18	58.5	4.41	90.2	0.63	35.4	2.04	0.26	129	6.3	91.7	196
	A	18	203	4.21	51.0	10.6	65.6	8.68	26.8	2.97	0.12	104	16.4	97.0	224
	SEM		1.73	0.023	1.85	1.17	3.25	0.309	1.46	0.140	0.098	3.43	0.76	0.64	18.1
	P		<0.001	0.43	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	0.30	<0.001	<0.001	<0.001	0.29

Means with different letters differ at $P < 0.05$.
[1] Composition based on 6 analysed silage samples from each bunker.
[2] Aerobic stability, hours.

($P = 0.02$) basis (Table 3). Invisible losses were significantly lower in H3 than in H1 and H2 both on FW ($P = 0.03$) and DM basis ($P = 0.02$), and the amount of wasted silage tended to be lowest in H3 and highest in H1.

Additive treatment did not influence the amount of wasted silage. On FW basis, the proportion of offered silage was numerically higher in C than A silos, whereas on DM basis this had turned to a small numerical difference in the opposite direction. Invisible losses were numerically higher in A than C silos on FW basis, whereas on DM basis this had turned to a small numerical difference in the opposite direction.

3.2. Silage chemical composition, digestibility, calculated energy and protein values, fermentation quality, and aerobic stability

Significant differences were found among harvests for all components given in Table 4 apart from the proportion of protein fraction B1. Harvest 3 had highest DM, CP, ADL and PBV concentrations, lowest protein fraction A and highest protein fractions B2 and C, and lowest concentrations of NDF and ADF. Harvest 1 had lower protein fraction C, and ADL concentration, than H3, and slightly higher values for all measures of digestibility, energy and AAT than H2 and H3. Additive treatment increased silage DM concentration, and gave lower proportion of protein fraction A and higher proportions of protein fractions B2 and B3 than untreated silage. Application of acidic additive restricted silage fermentation and gave lower NH_3-N-values, lower concentrations of LA and AA, and higher WSC concentration (Table 5). However, extensive ethanol fermentation occurred in A silage, particularly in H2, where concentration of WSC was low, and LA only half of that in C silage, and with very high ethanol concentration, 30.7 g/kg DM. Ethanol concentration was clearly higher in A than C silage also in H1, although LA concentrations were similar and WSC extremely low in both silages. This extensive ethanol fermentation in silage A in H2 was responsible for the significant harvest × additive interactions in pH and concentrations of WSC, LA and ethanol. Concentrations of FA, and partly PA, reflected the applications of the silage additive. Silage intake potential was higher in H2 than in H1 and H3, and higher in A than C silage. Aerobic stability was higher in H3 than in H2, but not influenced by additive treatment.

3.3. Clostridium tyrobutyricum spores and pH in spot silage samples from bunker silo faces

Spores of *C. tyrobutyricum* were detected in 9 of 24 spot silage samples from H3, which was a lower frequency than from H2, where *C. tyrobutyricum* was detected in 18 of 24 samples ($P = 0.03$; Table 6). The number of *C. tyrobutyricum* colonies found per g sample from H1 and H3 was lower than from H2 ($P = 0.002$).

Spores of *C. tyrobutyricum* were most frequently detected in spot silage samples from sides in bunkers, where spores were found in 16 of 18 samples. About a half of samples (9 and 10 of 18) from top and shoulder, respectively, contained *C. tyrobutyricum* spores, whereas mid samples had lowest frequencies of detected spores ($P = 0.008$). The number of *C. tyrobutyricum* colonies detected per g sample was highest in side and shoulder samples, that differed significantly from the number of colonies in top and mid samples ($P < 0.001$). Average pH was lowest mid in bunkers, with only small numerical increases in side and top samples, whereas shoulder samples had significantly higher pH than samples from all other spots ($P < 0.001$). In H1, pH in these shoulder samples was only moderately elevated, and similar to pH in side samples, but in H2 and H3, pH in shoulder samples was clearly higher than from all other spots, which gave a significant sample point × harvest interaction for pH ($P = 0.01$). In H2, pH in spot samples was higher in A than C silage,

Table 6
Effect of sampling point: mid, side, top, shoulder, and acidic additive treatment (A) versus untreated control (C) of grass crops for bunker silos during three harvests, on the frequencies and concentrations of *C. tyrobutyricum* spores, and pH, in spot silage samples from the faces of bunker silos.

Harvest	Sampling point	Additive	Tot.	*C. tyrobutyricum*		
			N	N[1]	Log cfu/g [2]	pH
1			24	14	1.33 [a]	4.42 [a]
2			24	18	2.00 [b]	4.87 [b]
3			24	9	1.09 [a]	4.44 [a]
X[2] or SEM				6.9	0.18	0.13
P				0.03	0.002	0.02
	Mid		18	6	0.83 [a]	4.12 [a]
	Side		18	16	1.98 [b]	4.32 [a]
	Top		18	9	1.17 [a]	4.37 [a]
	Shoulder		18	10	1.92 [b]	5.48 [b]
X[2] or SEM				12.0	0.20	0.14
P	Sampling point			0.008	<0.001	<0.001
P	Sampling point × harvest				0.15	0.01
		C	36	22	1.45	4.55
		A	36	19	1.50	4.60
X[2] or SEM				0.51	0.14	0.10
P	Additive			0.48	0.80	0.73
P	Additive × harvest				0.73	0.03

Means with different letters differ at $P < 0.05$.
[1] Number of samples above detection limit.
[2] Detection limit = 1.00 log cfu/g (10 cfu/g) for *C. tyrobutyricum*. Concentrations in samples below detection limit are set to half of detection limit i.e. 0.70 log cfu/g (5 cfu/g).

whereas the opposite was true in H1 and H3, which was the basis for the additive × harvest interaction for pH ($P = 0.03$).

4. Discussion

4.1. Bunker silo crop and silage weights and densities

During harvesting, the driver of the tractor with the precision chopper judged by eye when each load was filled up. Despite the same standard for judgement was applied for all loads, a slightly higher weight of trailer loads of A than C crop was obtained, and a numerically higher total weight was therefore filled into silo A than silo C in each harvest (Table 2). This suggests that acid-induced crop cell rupture and mass compaction might have occurred already in the trailers. Jaakkola et al. (2006b) found that FA reduced both respiration and proteolysis immediately after application, since WSC concentration was higher and soluble N concentration lower in FA treated than in untreated crop already at the time of silo filling. Significantly higher crop FW and DM densities obtained in A than in C silos immediately after silo filling were probably an effect of the applied acid on plant cells, that have caused an early increase in effluent runoff, as also observed by Bastiman (1976) and Haigh (1999).

A higher total amount of effluent lost from A than from C silos was probably the main reason why total silage weight and volume was equalized between A and C silos when opened, on average 134 days following ensiling. The tendency of higher DM concentration in A than C silages (203 vs. 193 g/kg DM; $P = 0.07$) was also likely caused by a higher release of effluent from A than C silos. The significantly higher amount of silage DM in A than C silos ($P = 0.005$) was partly due to the higher amount of crop DM filled in the silo, and partly due to the higher silage DM concentration. According to Holmes and Bolsen (2009), FW density increases with increasing packing vehicle weight, packing time, depth of silage and with reducing spreading layer thickness. All these factors were identical for A and C silage in the present study, in line with only 2 % numerically higher FW density in A than C silage. However, because silage DM concentration was higher in A than C silage, DM density was 7 % higher with acid treatment ($P = 0.08$). Cell wall degradation caused by application of acid leads to efficient silage consolidation and may increase density (Huhtanen et al., 2013). In order to reduce porosity and air ingress, Holmes and Muck (2007) recommended a FW density $\geq$ 705 kg/m^3 and a DM density $\geq$ 210 kg/m^3. All six bunker silos were within the recommended range for FW density, but below recommended DM density.

4.2. Silage losses

According to Savoie and Jofriet (2003), losses in bunker silos are mainly caused by aerobic conditions during filling, storage and feed out, and to a lesser extent caused by anaerobic fermentation and release of effluent. The very low crop DM concentrations in the present study, caused by showers during all harvests, suggest that losses due to release of effluent and anaerobic fermentation constituted a larger proportion than in most other studies. On both FW and DM basis, the proportion of ensiled crop that could be offered to animals from the three harvests followed crop DM concentrations, with a significantly higher proportion from H3 that had the highest crop DM, than from H1 and H2 (Tables 1,3). The invisible DM loss in H3 was less than half of that in H1 and H2, and wasted silage DM in H3 was about half of that in H1 and H2.

Losses caused by effluent runoff depend on several factors such as crop species, maturity (McAllan et al., 1991), chop length (Messer and Hawkins, 1977, cited by McDonald et al., 1991) and the type of additive (Bastiman, 1976; Mo and Fyrileiv, 1979), and increase strongly with moisture content (Bastiman, 1976) and silo compaction (Reynolds and Williams, 1995). The higher crop DM in H3 has surely diminished DM loss through effluent compared with H1 and H2, and the higher crop weight filled into silos in H2 than in H1 and H3 may have increased pressure and thereby DM lost in effluent. The use of wheel loader for consolidation in all bunkers may have contributed to higher density and DM losses than otherwise expected. Muck et al. (2003) stated that excessive densities in direct cut silages will increase effluent losses.

Due to considerable release of effluent, it was expected that silage was drier than the parent crop, as shown by Randby (1997) with crops of similar DM concentrations ensiled in roofed tower silos. In unroofed silages, however, rainwater enters silos where it partly accumulates and decreases silage DM concentrations (Randby et al., 2020). Across harvests and additive treatment, crop and silage DM concentrations were equal, which suggests that, to some extent, rainwater had accumulated in the present bunker silos.

The numerically higher invisible FW losses from A than C silos during all harvests were in line with the expected higher release of effluent from A silos. Using 60-tonne capacity silos that were rolled, plastic covered and weighted with tyres after silo filling, Kennedy (1990) found 47 % higher FW flow of effluent from grass crops applied FA compared with untreated crops over 5 harvests with 186 g/kg average crop DM. With an even wetter crop, 159 g/kg DM, Jaakkola et al. (2006a) experienced 77 % increased FW loss and 71 % increased DM loss in effluent due to FA treatment, using pilot scale silos. In the present study, an expected increase in invisible DM loss in A compared with C silos due to increased effluent release could not be confirmed in the observed invisible DM losses, that in average were higher in C than A silos, 146 vs 136 g/kg DM. The higher DM concentration in A than C silage, that was apparent in all three harvests, and also found by Kennedy (1990), 200 vs 192 g DM/kg, and Jaakkola et al. (2006a), 178 vs 160 g DM/kg, in FA treated vs untreated silage, respectively, suggest that more effluent was released from A than from C silages, and that invisible losses caused by other factors such as aerobic respiration and anaerobic fermentation in C silos have more than outweighed the increased losses due to effluent release in A silos. An increase in losses in C silos due to aerobic respiration during filling, storage and feeding may be expected, because acidic additives restrict initial plant respiration and thereby keep temperatures down (McDonald et al., 1991). Kennedy (1990) found slightly higher total DM losses in untreated than in FA treated silage, 238 vs 227 g/kg, although DM loss arising from effluent was 50 % higher in FA treated silage, 35 vs 23 g/kg. Also, Jaakkola et al. (2006a) found higher total DM losses in untreated than in FA treated silage, 117 vs 99 g/kg, although DM loss arising from effluent was 71 % higher in FA treated silage, 68 vs 40 g/kg.

Of all factors affecting quality of conserved forage, postharvest respiration is the greatest (Van Soest, 1994). In poorly sealed silos, respiration loss of hexoses and other substrates may be so extensive as to leave insufficient substrate for fermentation (Woolford, 1984). The slightly higher invisible DM loss in A than C silo in H2 could probably be related to extensive ethanol fermentation in A silage. Fermentation of glucose to ethanol by yeasts produce CO_2 with 48.9 % DM loss with virtually no loss of energy (McDonald et al., 1991). In a laboratory scale study, Driehuis and Van Wikselaar (2000) found 62.8 g/kg DM loss in silages where ethanol was the dominating fermentation product, and 24.4 g/kg DM loss in silages where lactic acid dominated.

4.3. Silage chemical composition, digestibility, energy and protein values, fermentation quality, and aerobic stability

The contents of silage chemical components presented in Table 4 differed significantly among harvests as should be expected when crops from different fields, with slightly different botanical composition, maturity stages, and DM concentrations were harvested. In H1 and H2, but not in H3, CP was considerably lower in silage than in crop. This might be related to larger loss of effluent in H1 and H2, because effluent DM contains a higher CP concentration than DM in the parent crop and the resulting silage (Randby, 1997). The lower proportions of protein fractions A, and higher proportions of protein fractions B2 and B3 in A than in C silage, was in line with consistently lower NPN proportions in CP found in silages applied FA than in those left untreated, as found by Randby and Selmer-Olsen (1997) and Jaakkola et al. (2006a, b). Proteolysis is predominantly the result of plant enzyme activity, that may be restricted by applied acid, whereas further degradation of amino acids is brought about by microbial activity (McDonald et al., 1991).

Application of FA-based additives to direct cut or wilted grass silages causes a drop in the pH by direct acidification, restricts fermentation of WSC, and reduces acetic acid concentration and proteolysis (Muck et al., 2018). These typical effects of FA based additives were found in the present study, however, the effects on pH, WSC, and proteolysis were not apparent in H2, where A silage was extensively ethanol fermented, with 30.7 g ethanol/kg DM (Table 5). Yeasts are tolerant of formic acid, and high yeast counts leading to high ethanol contents have previously been noted in FA treated silages (Henderson et al., 1972). Ethanol fermentation in silage is undesirable because it consumes sugar without contributing to reduction of pH or to preservation of nutrients (McDonald et al., 1991), and because it may impart feed flavor to raw milk (Randby et al., 1999). Ethanol concentrations in the range 10−25 g/kg DM have frequently been found in untreated silage and in silage treated with a low or moderate dose of a FA based additive (Randby, 2000, 2002, Jaakkola et al., 2006b; Randby, 2010, Seppalä et al., 2016), but ethanol concentrations may be reduced when higher doses of the same additives are applied (Jaakkola et al., 2006b; Randby, 2010). Driehuis and Van Wikselaar (2000) documented up to 63 g ethanol/kg DM in high DM untreated grass silage in laboratory silos, and Randby (1997) found 42 g ethanol/kg DM in wet FA treated silage in tower silo.

The fermentation quality was in general high in the present study, with very low BA concentrations and low NH_3-N values. The more extensive fermentation in C than in A silage, shown by higher concentrations of LA plus AA, was the reason why silage intake potential, SDMI index, was lower (Table 5). Restricted silage fermentation, as obtained in A silage despite the low DM concentration, increases silage intake that can be realized as increased milk yield or a "concentrate sparing effect" (Huhtanen et al., 2013).

Wilkinson and Davis (2012) recommend 7 d (168 h) as a target for aerobic stability of silage. This was obtained for 5 of 6 silages in the present study. Mann and McDonald (1976) found that silages applied PA, or mixtures of FA and AA, or FA, AA and PA, were more stable than untreated silage, in line with Randby (2002, 2010), who also found that increasing application rates of acids increased aerobic stability. In the present study, however, the aerobic stability of A silage was not significantly higher than in C silage. This was in line with Seppälä et al. (2016), who experienced similar aerobic stability in untreated and acid treated silages that had fermentation characteristics similar to the present silages. According to Wilkinson and Davis (2012), a high DM content and high yeast counts are risk factors for poor aerobic stability, whereas high concentrations of AA and BA minimize risks. Contrary to this, the three least stable silages in the present study were those with highest AA + BA concentrations and lowest DM concentrations (Table 5). The H2 silages, that were significantly less stable than H1 and H3 silages, had lower daily feed out rate, only 10 cm, versus 14 and 24 cm in H1 and H3, whereas Savoie and Jofriet (2003) recommend $\geq$ 20 cm. Feed out rate might have influenced stability, although average air temperature during the 163 days of feed out in H2 was only −0.5 °C.

4.4. Clostridium tyrobutyricum spores and pH in spot silage samples from bunker silo faces

If a stable, low pH is not rapidly achieved in silage, clostridial activity may give a secondary BA fermentation that is stimulated by low crop DM and WSC contents, and high buffering capacity (McDonald et al., 1991). With direct cut grasses, this problem has long been avoided by application of a strong acid. In recent times, higher DM and WSC contents have often been attained by rapid wilting, and growth of *C. tyrobutyricum* is now more often a result of poor compaction that allows oxygen penetration and growth of yeasts that increases pH and temperature, and reduces aerobic stability (Vissers et al., 2007). Vissers et al. (2007) suggested that farmers should aim for < 3 log spores/g silage, and never exceed 5 log spores/g, in order to obtain < 3 log spores/L in farm tank milk. Although the crops in the present study were wet, sufficiently low pH was obtained by a predominating lactic acid fermentation in C silage, and a more restricted fermentation in A silage. The low concentrations of butyric acid and the moderate levels of *C. tyrobutyricum* spores in silage (Table 6) document that spore forming *Clostridia* constituted a minor problem in the present study. However, poor compaction of vulnerable spots in the silo was a challenge, where samples from sides and shoulders had clearly elevated *C. tyrobutyricum* concentrations compared with samples from the mid. Although wet crops are easily consolidated, plastic coverage of silages is seldom perfect and may ease growth of undesirable microorganisms such as yeasts and *C. tyrobutyricum* in vulnerable spots.

5. Conclusions

Postharvest respiration was expected to be severe in bunker silos due to their large surface area. This challenge was, however, smaller than anticipated in untreated silage, possibly due to the small and wet masses filled into only half-full silos during a relatively short time period, with lower air temperatures than is usual in many other countries. In acid treated silage, WSC, initially preserved by the respiration inhibiting acid, was probably utilized by yeasts that produced higher silage ethanol concentrations than wanted. The hypothesis that application of a formic- and propionic acid-based additive to grass crops during ensiling in bunkers would reduce losses and increase aerobic stability of the silage could not be confirmed with significant results. Numerically, however, total DM losses were slightly lower, and aerobic stability slightly higher, in treated silage, so these hypotheses could not either be rejected. The hypothesis regarding improved fermentation quality after acid application was to some extent confirmed by the fact that application of the additive gave silage with increased intake potential that might increase animal production in systems with limited concentrate allowance. However, the increased ethanol concentration obtained in treated silage was not wanted, and suggested that acidic silage additives should contain a higher proportion of propionic acid or other acids that restrict yeast growth and ethanol fermentation. The observed differences in silage chemical composition and fermentation quality might primarily result from reduced crop temperature during initial ensiling caused by restricted plant respiration, and further to reduced activity of plant enzymes and microbes, due to the applied acid. The obtained results are, however, only applicable to the crops studied.

Funding

The work has been financed by the Norwegian Research Funding for Agriculture and the Food Industry, The County Governor of Rogaland, and the industry partners Addcon Nordic AS, Agromiljø AS, Animalia, Fiskå Mølle, FK Agri, FK Rogaland Agder, Felleskjøpet fôrutvikling, Norske FK, Nortura, Strand Unikorn, TINE Rådgiving og medlem and Yara Norge.

CRediT authorship contribution statement

Å.T. Randby: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing. **A.K. Bakken:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Åkerlind, M., Weisbjerg, M.R., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M., Volden, H., 2011. Feed analyses and digestion methods. In: Volden, H. (Ed.), Norfor - The Nordic Feed Evaluation System. EAAP publication No. 130, Wageningen Academic Publishers, The Netherlands, pp. 41–54 chapter 5.

Bastiman, B., 1976. Factors affecting silage effluent production. Expl. Husb. 31, 40–46.

Driehuis, F., Van Wikselaar, P.G., 2000. The occurrence and prevention of ethanol fermentation in high-dry-matter grass silage. J. Sci. Food Agric. 80, 711–718.

Haigh, P.M., 1999. Effluent production from grass silages treated with additives and made in large-scale bunker silos. Grass Forage Sci. 54, 208–218.

Henderson, A.R., McDonald, P., Woolford, M.K., 1972. Chemical changes and losses during the ensilage of wilted grass treated with formic acid. J. Sci. Food Agric. 23, 1079–1087.

Holmes, B.J., Bolsen, K.K., 2009. What's new in silage management?. In: Proc. XVth International Silage Conf. Madison, Wisconsin, USA, pp. 61–76.

Holmes, B.J., Muck, R.E., 2007. Packing bunkers and piles to maximise forage preservation. Proceedings of the 6th International Dairy Housing Conference. American Society of Agricultural and Biological Engineers, Publication, St Joseph, MI, USA, 701P0507e. Minneapolis, 16–18 June 2007.

Huhtanen, P., Nousiainen, J.I., Khalili, H., Jaakkola, S., Heikkila, T., 2003. Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci. 81, 57–73.

Huhtanen, P., Rinne, M., Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal 1, 758–770.

Huhtanen, P., Jaakkola, S., Nousiainen, J., 2013. An overview of silage research in Finland: from ensiling innovation to advances in dairy cow feeding. Agric. and Food Sci. 22, 35–56.

Jaakkola, S., Kaunisto, V., Huhtanen, P., 2006a. Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass Forage Sci. 61, 282–292.

Jaakkola, S., Rinne, M., Heikkilä, T., Toivonen, V., Huhtanen, P., 2006b. Effects of restriction of silage fermentation with formic acid om milk production. Agric. Food Sci. 15, 200–218.

Jonsson, A., 1990. Enumeration and confirmation of *Clostridium tyrobutyricum* in silages using neutral red, D-cycloserine, and lactate dehydrogenase activity. J. Dairy Sci. 73, 719–725.

Kennedy, S.J., 1990. Comparison of the fermentation quality and nutritive value of sulphuric and formic acid-treated silages fed to beef cattle. Grass Forage Sci. 45, 17–28.

Krizsan, S.J., Randby, Å.T., 2007. The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as sole feed. J. Animal Sci. 85, 984–996.

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358.

Mann, E.M., McDonald, P., 1976. Effect of formalin and lower volatile fatty-acids on silage fermentation. J. Sci. Food Agric. 27, 612–616.

McAllan, A.B., Jacobs, J.L., Merry, R.J., 1991. Factors influencing the amount and pattern of silage effluent production. Europ. Grassl. Federation. Braunschweig Landbauforschung Völkenrode, Sonderheft 123, 368–370.

McDonald, P., Henderson, N., Heron, S., 1991. The Biochemistry of Silage, 2nd ed. Chalcombe Publications, Marlow, Bucks, UK, p. 340.

Mo, M., Fyrileiv, E., 1979. Methods of estimating ensiling losses. Acta Agric. Scand. 29, 49–64.
Muck, R.E., Moser, L.E., Pitt, R.E., 2003. Postharvest factors affecting ensiling. In: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Agronomy Monograph, 42. Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, pp. 251–304 chapter 6.
Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung Jr., L., 2018. Silage review: recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980–4000.
Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F., 2003. Microbiology of ensiling. In: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Agronomy Monograph, 42. Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, pp. 31–93.
Randby, Å.T., 1997. Quality of silage and silage effluent as influenced by storage of effluent in tower silos. Acta Agr. Scand. Sect. A, Animal Sci. 47, 9–19.
Randby, Å.T., 2000. The effect of some acid-based additives applied under various ensiling conditions. Grass Forage Sci. 55, 289–299.
Randby, Å.T., 2002. Effect of acid-based additives on fermentation quality, aerobic stability, and fungal growth in round bale silage, stored with a tight or perforated plastic cover. Grassland Sci. Europe 7, 226–227.
Randby, Å.T., 2010. The effect of Na-buffered acid-based additives on wilted roundbale grass silage. Grassland Sci. Europe 15, 545–547.
Randby, Å.T., Selmer-Olsen, I., 1997. Formic acid treated or untreated round bale grass silage for dairy cows. In: Jambor, V. (Ed.), 8Th Int. Symp. Forage Conservation, Brno Research Institute of Animal Nutrition, Pohorelice. Czech Republic, pp. 160–161, 29. Sept-1. Oct. 1997.
Randby, Å.T., Selmer-Olsen, I., 1999. Formic acid treated or untreated round bale grass silage for steers and dairy cows. Nordisk Jordbruksforskning 81, 276–277.
Randby, Å.T., Selmer-Olsen, I., Baevre, L., 1999. Effect of ethanol in feed on milk flavour and chemical composition. J. Dairy Sci. 82, 420–428.
Randby, Å.T., Halvorsen, H.N., Bakken, A.K., 2020. Losses and grass silage quality in bunker silos compacted by tractor versus wheel loader. Anim. Feed Sci. Technol. 266 art.no. 114523.
Reynolds, A.M., Williams, A.G., 1995. A model of silage consolidation and effluent flow. J. Agric. Eng. Res. 61, 173–182.
Ruxton, I.B., McDonald, P., 1974. Influence of oxygen on ensilage 1. Laboratory studies. J. Sci. Food Agric. 25, 107–115.
Savoie, P., Jofriet, J.C., 2003. Silage storage. In: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Agronomy Monograph, 42. Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, pp. 405–467 chapter 9.
Seppälä, A., Heikkilä, T., Mäki, M., Rinne, M., 2016. Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass Forage Sci. 71, 458–471.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, second edition. Cornell University Press, p. 463.
Vissers, M.M.M., Driehuis, F., Te Giffel, M.C., De Jong, P., Lankveld, J.M.G., 2007. Concentrations of butyric acid bacteria spores in silage and relationships with aerobic deterioration. J. Dairy Sci. 90, 928–936.
Wilkinson, J.M., Davis, D.R., 2012. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1–19.
Woolford, M.K., 1975. Microbiological screening of the straight chain fatty acids (C1-C12) as potential silage additives. J. Sci. Food Agric. 1975 (26), 219–228.
Woolford, M.K., 1984. The silage fermentation. In: Microbiology Series, 14. Marcel Dekker, inc., NY, p. 350.