AGRICULTURAL AND FOOD SCIENCE

Agricultural and Food Science (2022) 31: 187–197

https://doi.org/10.23986/afsci.115381

Effect of inoculants of different composition on the quality of rye silages harvested at different stages of maturity

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Winter rye (*Secale cereale* L.), one of the small-grain winter annuals, can be used as a cover crop for protection against soil erosion for absorption of unused soil nitrogen, and for cattle feed by preserving as silage. The experiment was conducted with the objective to evaluate the potential of the blend of homofermentative and hetero- and homofermentative lactic acid bacteria (LAB) as a rye silage additive. Early-cut rye (at boot stage, wilted) and whole-crop rye (at milk and soft dough stages of grain) were ensiled in laboratory mini-silos with (1) a blend of homofermentative LAB strains containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502), and *Lactococcus lactis* (*NCIMB30117*), (2) a blend of hetero- and homofermentative LAB strains containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502), and *Lactobacillus buchneri* (DSM22501), or (3) a blend of hetero- and homofermentative LAB strains containing *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). They were compared to ensiling without additive. After 60 days of fermentation at room temperature, mini-silos were opened, sampled for proximate analysis, forage hygiene, fermentation profile, and subjected to an aerobic stability (AS) test. Although the addition of homofermentative LAB strains was effective in reducing fermentation losses, it impaired the aerobic stability of rye silages. The combination of hetero- and homofermentative LAB strains was effective in reducing the aerobic deterioration of the rye silages by supporting a low pH value and inhibiting the proliferation of yeast and moulds.

Key words: aerobic spoilage, additives, bacteria strains, *Secale cereale*, dry matter loss

Introduction

Cover crops or double-cropping, used to protect against soil erosion, is becoming increasingly important. Implementation of double-cropping is generally recognised as an accessible in-field method for reducing N leaching from fields by 40–70% compared to winter-bare fallow (Tonitto et al. 2006). Cover crops add organic matter to the soil, reduce the nitrate loss to groundwater and surface water, reduce soil erosion, suppress weeds, and keep valuable nutrients in the soil, hereby increasing the sustainability and reducing the environmental impact of the production system (Everett et al. 2019). Harvested at the boot (early-cut) or at the soft dough (whole-crop) stages of grain, double-cropped winter annuals can increase total dry matter (DM) yield per hectare up to 33% compared with a single crop of corn (Jemison et al. 2012). Moreover, this technique can decrease N leaching, reduce the phosphorus loss, and increase income over feed cost (Ranck et al. 2020). Like other small grain winter annuals (wheat, triticale, and barley), whole-crop rye can be a valuable forage for silage production; it was found to be a good forage source for many ruminants (Kennelly and Weinberg 2003) and could be fed to lactating dairy cows (Harper et al. 2017). Auerbach and Theobald (2020) showed poor fermentation quality in early-cut whole plant rye reflected by a high content of acetic acid, butyric acid, and ammonia-N despite the high content of water-soluble carbohydrates at harvest. According to Weissbach and Honig (1996), a lack of nitrate in whole-crop cereals (milk stage or later) may explain the fermentation of butyric acid despite high fermentability coefficients (FC).

Because of the hollow nature of the stem and high porosity, whole-crop rye silages are prone to aerobic deterioration after silo opening. Application of the right silage inoculant can facilitate a successful fermentation process and inhibit aerobic deterioration during feed out (Wilkinson and Muck 2019). Generally, LAB in inoculants can be either homofermentative such as *Lactobacillus plantarum*, *Enterococcus faecium*, and various *Pediococcus* species that mainly convert crop sugars into lactic acid leading to a rapid decrease in silage pH, or heterofermentative such as *Lactobacillus buchneri* and other species capable of converting crop sugars and lactic acid into acetic acid and 1,2-propanediol during silo storage and increase the aerobic stability of the silages. *Lactobacillus brevis* is an obligate heterofermentative LAB species that produces acetate from sugar as well as supports aerobic stability. Using inoculants that combine a mixture of homo- and hetero LAB should contribute to fast reduction of pH, control of the early active fermentation period by suppressing enterobacteria, clostridia, and other

Received 14 March 2022 / Accepted 8 August 2022
The Scientific Agricultural Society of Finland

microorganisms thus reducing proteolysis, the DM losses caused by fermentation, and improvement of the aerobic stability after the active silage fermentation period (Oliveira et al. 2017, Muck et al. 2018). Moreover, inhibiting the growth of undesirable microorganisms in silage (fungi, most of the yeasts, and some groups of bacteria) is an important aspect of using combined hetero- and homofermentative LAB (Auerbach and Theobald 2020). There are only few published studies (Lee et al. 2004, Auerbach et al. 2020, Auerbach and Theobald 2020, Paradhipta et al. 2020) on the fermentation pattern, DM losses during fermentation, and the aerobic stability of early-cut and whole-crop rye harvested at different stages of maturity. The experiment was motivated by the lack of information and evidence on the preservation of early-cut and whole-crop rye by fermentation as silage and the use of an additive containing a combination of LAB strains in the literature.

Our hypothesis was that due to different behaviour of the strains used in combined LAB inoculants, treatment with an inoculant could differently affect the fermentation characteristics, dry matter loss, the microbial population, and the aerobic stability of early-cut or whole-crop rye silage ensiled in a mini-silo.

Materials and methods

The ensiling procedure

Rye (*Secale cereale* L.) was harvested at three stages of maturity from the same field and location: (1) the early-cut harvest (slightly wilted, 24 h before ensiling) was reaped on 10 May 2019, when forage contained 273.6 g kg^{-1} DM, (2) the whole-crop harvest (milk stage of grain) was brought in on 19 June 2019, when forage contained 390 g kg^{-1} DM, and (3) the whole-crop harvest (soft dough stage of grain) was gathered on 26 June 2019, when forage contained 457 g kg^{-1} DM. To avoid contamination with soil, forages were harvested by hand at the height of 10 cm and chopped to about 2 cm using a laboratory-type chopper. Prior to ensiling, rye forage was separated into four piles, and four additive treatments for each maturity stage were applied: control (T0) with no additive (only tap water added) and three commercial (Chr. Hansen A/S, Denmark) LAB inoculants: (1) a homofermentative SiloSolve® MC (T1) containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502), and *Lactococcus lactis* (NCIMB30117), at the proportion 40:30:30, (2) a hetero- and homofermentative SiloSolve® AS200 (T2) containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502), and *Lactobacillus buchneri* (DSM22501), at the proportion 20:30:50, and (3) a hetero- and homofermentative SiloSolve® FC (T3) containing *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037), at the proportion 50:50. The products were applied at the dose of 150 000 CFU g^{-1} forage. The application rate of the inoculant was calculated according to the target dose and the actual bacterial concentration in the products. Additive-treated carefully mixed forage was weighed for each mini-silo and packed tightly into 3-l glass jars by hand with periodic tamping. Each jar was filled with 2000 ± 52 g, 1600 ± 56 g, and 1500 ± 54 g, or at density 184 ± 4 kg/m^3, 213 ± 7 kg/m^3, and 239213 ± 9 kg/m^3 of rye forage at early-cut (wilted) and whole-crop (milk and soft dough stages of grain), respectively. Ten experimental silages were prepared for each vegetation stage and for each treatment: five for the chemical and microbiological analyses and five for the aerobic stability test after the targeted fermentation period. Silages were stored for 60 days in a dark room at an ambient temperature (20–22 °C). The fermentation period of 60 days was chosen based on the study of Kleinschmit and Kung (2006). In this study, the positive effect of *L. buchneri* on the aerobic stability of silages was reported after 56 days of storage. Samples of the water and suspension used for inoculation were collected, and the total number of LAB was counted immediately (within an hour) using the ISO method 15214. The analysis was consistent with the expected number of LAB in the suspension (Table 1).

Table 1. The number of lactic acid bacteria in the inoculum suspension

Rye	Date	T0	T1	T2	T3
Early-cut (wilted)	10 May 2019	<1.0 × 10	1.5×10^8	1.5×10^8	1.5×10^8
Whole-crop (milk stage)	19 June 2019	<1.0 × 10	1.5×10^8	1.5×10^8	1.5×10^8
Whole-crop (soft dough stage)	26 June 2019	<1.0 × 10	1.5×10^8	1.5×10^8	1.5×10^8

Sampling and measurements

For each vegetation stage, five samples representing the fresh material prior to ensiling were randomly obtained from the chopped rye herbage. Forage samples were subjected to chemical and microbiological analyses. After 60 days of ensiling, five mini-silos per treatment were opened for the analyses of the nutrient composition and

fermentation quality. Five mini-silos represented five repetitions. For the aerobic stability test, the other five mini-silos per vegetation stage and per treatment were used, where five mini-silos represented five repetitions. The temperature was measured by using data loggers that recorded temperature readings every six hours from the thermocouple wires placed in five replicates, 1200 g silage representative samples aerated in open polystyrene boxes. A thermocouple wire was inserted into the geometric centre of the silo. The boxes were kept in a constant room temperature (20 ± 0.5 °C). Aerobic stability was considered lost when the temperature of the ensiled material exceeded the temperature of the room by 3 °C. Therefore, the aerobic stability test lasted 30, 14, and 15 days for early-cut (boot stage) and whole-crop rye (milk and soft dough stages), respectively. At the end of the aerobic stability test, losses during exposure to air were calculated based on the silage weight before and after the aerobic stability test. The increase in temperature, the change in the pH value, the DM content, the fresh weight loss, and the numbers of yeasts and moulds during aerobic exposure were used as indicators of aerobic spoilage.

Chemical and microbiological analyses

Samples of fresh rye and rye silage were processed, and DM and the nutrient content (crude protein, crude fibre, water-soluble carbohydrates (WSC), crude ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), and fermentation products (pH, lactic acid, acetic acid, propionic acid, butyric acid, ammonia nitrogen, and alcohols) were determined as described previously by Jatkauskas et al. (2013). The dry matter (DM) content measured by oven drying was consequently corrected for volatile compounds (DM_c) and was calculated according to the method described by Weissbach (2009): $DM_c = DM_n + (1.05 - 0.059\ pH)\ FA + 0.08\ LA + 0.77\ PD + 0.87\ BD + 1.00\ OA$ (g kg^{-1} FM) for the early-cut of rye and $DM_c = DM_n + 0.95\ FA + 0.08\ LA + 0.77\ PD + 1.00\ OA$ (g kg^{-1} FM) for the whole-crop rye (milk and soft dough stages) grain, where: FA – fatty acids (C_2…C_6), PD – 1,2-propanediol, BD – 2,3-butanediol, LA – lactic acid, and OA – other alcohols (C_2…C_4). Different equations for the DM content correction were used due to the differences between early-cut and whole-crop rye (either without or with grain, respectively). Fresh weight losses during the ensiling period were determined by weighing the silos after filling and after 60 days of storage. Given the DM content of the forage and silage, the DM loss was calculated using the following equation: Dry matter loss = (DM at ensiling – DM_c silage) / DM at ensiling. The buffer capacity of rye was determined according to Playne and McDonald (1966). A chemical analysis of fresh forages and silages was performed in two repetitions for each five replications (mini-silages) and presented on a DM basis. Samples of rye and silage were analysed for LAB (ISO 15214:2009), yeasts, and moulds (ISO 21527-1:2008).

Statistical analysis

Data from the microbial counts were transformed to $\log_{10}$. The statistical analysis of the results included a completely randomised design using the general linear model (GLM) procedure (SAS, 9.4) with treatment as a fixed effect for each maturity stage separately. A pair-wise comparison between LSMEANS was performed by the Tukey's test, when a significant F-test ($p < 0.05$) was detected.

Results

Characteristics of rye forage

The basic nutrient content and the microbiological characteristics of rye forage prior to ensiling and before additive application are presented in Table 2. Wilted early-cut (boot stage) and whole-crop (milk and dough stages) rye had a DM content of 273.6, 390.2, and 457.1 g kg^{-1}, respectively, and it contained 238.2 g, 132.4 g, and 117.6 g WSC kg^{-1} DM and 99.0 g, 85.3 g, and 78.9 g crude protein kg^{-1} DM, respectively. The buffering capacity of rye forage at all three vegetation stages was low (25.2–21.2). The numbers of the initial yeasts and moulds in fresh early-cut (boot stage) and whole-crop (milk and soft dough stages) forages were high, 5.26 and 4.25; 6.20 and 5.84; and 6.36 and 6.03 log $CFUg^{-1}$, respectively. The number of epiphytic LAB in rye forage was low at <4 log CFU g^{-1}.

Table 2. Characteristics of rye forage at different stages of vegetation before ensiling

Parameters	Vegetation stage					
	Early-cut (boot)		Whole-crop (milk)		Whole-crop (soft dough)	
	Mean	SD	Mean	SD	Mean	SD
Ensiled forage density, kg/m^3	184.7	4.24	213.1	7.52	238.8	8.85
DM, g kg^{-1}	273.6	2.73	390.2	1.73	457.1	2.82
In DM, g kg^{-1}						
Crude protein	99.0	3.04	85.3	2.63	78.9	2.10
Crude fat	23.7	1.18	19.9	2.35	20.4	1.36
WSC	238.2	13.48	132.4	11.99	117.6	6.56
ADF	233.8	11.85	379.1	19.79	335.4	11.82
NDF	431.7	9.76	612.6	16.86	554.1	16.84
Starch	–	–	37.2	6.22	101.6	6.73
pH	5.73	0.06	5.47	0.07	5.44	0.10
BC, meq 100 g^{-1} DM	25.2	1.15	22.8	0.97	21.2	1.40
Yeast, $\log_{10}$ CFU g^{-1}	5.26	0.27	6.20	0.40	6.36	0.33
Mould, $\log_{10}$ CFU g^{-1}	4.25	0.34	5.84	0.27	6.03	0.14
LAB, $\log_{10}$ CFU g^{-1}	1.79	0.31	3.25	0.21	3.27	0.20

DM = dry matter; WSC = water soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre; BC = buffer capacity; FC = fermentation coefficient; CFU = colony-forming units; LAB = lactic acid bacteria; SD = standard deviation

Characteristics of ensiled early-cut rye (boot stage)

The changes in the nutrient content and in the yeast and mould counts of silage on day 60 of storage are shown in Table 3. Corrected for volatiles, all three inoculant-treated silages (T1, T2, and T3) contained a significantly higher DM and crude protein content compared with control (T0) silage. Inoculants T1 and T2 preserved a larger amount of WSC compared with control (T0) or T3 treatment ($p < 0.05$). The number of LAB was significantly increased and the numbers of yeasts and moulds were significantly decreased by all three inoculant treatments. The strongest effect was observed in silages T2 and T3.

Table 3. Nutrient composition and microbial characteristics of early-cut (boot stage) rye silage

TR	DM_c[1], g kg^{-1}	g kg^{-1} DM_c					$\log_{10}$ CFU g^{-1} of FF		
		CP	CF	WSC	ADF	NDF	LAB	Yeast	Mould
T0	252.8^c	96.3^a	201.7	42.3^c	260.5^a	455.1^a	5.38^b	1.88^a	2.19^a
T1	261.6^b	99.9^b	195.4	100.0^b	255.5^a	448.0^a	7.13^a	1.37^b	1.78^b
T2	259.1^b	99.9^b	198.1	89.4^b	257.5^a	451.9^a	7.31^a	1.12^c	1.55^c
T3	261.4^b	98.8^b	199.3	50.1^c	254.4^a	452.6^a	7.46^a	1.06^c	1.48^c
SEM	1.320	0.973	3.882	4.536	2.072	3.251	0.22	0.06	0.06

TR = treatment; T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM_c = dry matter corrected for volatiles; CP = crude protein; CF = crude fibre; WSC –=water-soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre, CFU = colony-forming units; FF = fresh forage; LAB = lactic acid bacteria. Means with different superscripts within columns differed significantly at $p < 0.05$.

Inoculant application had an effect on the silage fermentation profile (Table 4). During fermentation, the use of homofermentative LAB (T1) resulted in the highest concentration of lactic acid ($p < 0.05$) and the lowest concentration of acetic acid ($p < 0.05$), the lowest pH value ($p < 0.05$), and the lowest DM losses ($p < 0.05$). Application of hetero- and homofermentative LAB (T3) caused the highest concentration of acetic acid ($p < 0.05$) between the treatments. All three inoculant treatments suppressed the formation of ammonia-N, alcohols, and butyric acid.

Table 4. Fermentation characteristics and dry matter losses of early-cut (boot stage) rye silage

TR	pH	Ammonia-N, g kg^{-1} total N	g kg^{-1} DM$_c$				
			LA	AA	Alcohols	BA	DM loss
T0	3.91^{a}	50.27^{a}	52.37^{a}	22.28^{a}	25.94^{a}	7.01^{a}	93.7^{a}
T1	3.62^{b}	32.63^{b}	91.50^{b}	8.23^{b}	11.37^{b}	0.70^{b}	53.2^{b}
T2	3.69^{c}	36.45^{c}	79.91^{c}	25.49^{a}	12.66^{bc}	0.74^{b}	63.6^{c}
T3	3.74^{d}	39.03^{c}	73.97^{c}	45.87^{c}	13.74^{c}	1.42^{b}	57.8^{bc}
SEM	0.016	1.130	2.298	1.330	0.499	0.324	2.078

TR = treatment; T0 = control, T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM$_c$ = dry matter corrected for volatiles; LA = lactic acid; AA = acetic acid; BA = butyric acid. Means with different superscripts within columns differed significantly at $p < 0.05$.

The aerobic deterioration of silages was evaluated by observing temperature dynamics inside the silages, the pH value, and the number of yeasts and moulds at the end of the aerobic stability test (Table 5, Fig. 1). Untreated (T0) and homofermentative LAB-treated (T1) silages started deteriorating after 120 and 98 hours, respectively, of aerobic exposure and peaked on the pH value, fresh weight loss, and the number of yeasts and moulds at the end of the aerobic stability test when compared with T2 and T3. As expected, the application of hetero- and homofermentative LAB (T2) extended aerobic stability beyond T0 and T1 (approximately three times, 369 h) with a significantly reduced number of yeasts and moulds as well as the DM loss. The application of hetero- and homofermentative LAB (T3), however, extended aerobic stability approximately six times (688 h) and showed the lowest pH value, fresh weight loss, and the number of yeasts and moulds between all inoculant treatments.

Table 5. Characteristics of the aerobic stability of early-cut (boot stage) rye silage

TR	pH at the end of test	DM, g kg^{-1}	Weight loss, %	AS^1, h	Highest temp., °C	Log$_{10}$ CFU g^{-1} of FF	
						yeast	mould
T0	8.09^{a}	211.4^{b}	10.10^{a}	120.0^{c}	31.5	6.86^{a}	8.36^{a}
T1	8.48^{a}	225.0^{a}	9.88^{a}	98.4^{c}	29.7	6.75^{a}	7.26^{b}
T2	8.05^{a}	221.3^{a}	8.83^{b}	369.6^{b}	25.6	5.73^{b}	5.04^{c}
T3	5.56^{b}	224.0^{a}	3.51^{c}	687.6^{a}	23.0	3.95^{c}	3.22^{d}
SEM	0.159	3.373	0.362	18.969	-	0.224	0.247

TR = treatment; T0 = control, T1 = 1.5 × 105 CFU g^{-1}. *L*. plantarum, *E*. *faecium*, *L*. *lactis*; T2 = 1.5 × 105 CFU g^{-1}. *L. buchneri*, *L*. *plantarum*, *E*. *faecium*; T3 = 1.5 × 105 CFU g^{-1} *L*. *buchneri*, *L*. *lactis*; SE = standard error; DM = dry matter; AS = aerobic stability; CFU = colony-forming units; FF = fresh forage; ^{1}number of hours needed for the silage to reach a sustained temperature higher than 3 °C above ambient. Means with different superscripts within columns differed significantly at $p < 0.05$.

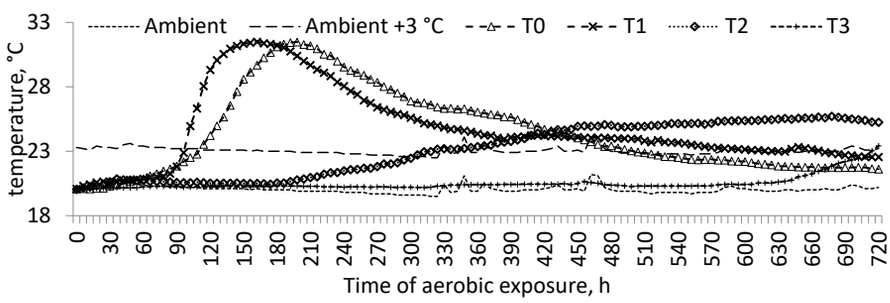

Fig. 1. Temperature changes in early-cut (boot stage, slightly wilted) rye silage during the aerobic exposure period (T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*)

Characteristics of ensiled whole-crop rye (milk stage of grain)

The nutrient content and the microbial characteristics of silage at day 60 of storage are presented in Table 6. When compared with control (T0), all three inoculant-treated silages (T1, T2, and T3) contain a significantly higher DM corrected for volatiles. Inoculant T3 preserved a lower amount of WSC ($p < 0.05$) compared with control (T0) or T1 and T2 treatments. All three inoculant treatments significantly increased the number of LAB , and the number of yeasts was significantly decreased ($p < 0.05$) in T2 and T3 inoculant-treated silages; the number of moulds decreased ($p < 0.05$) in all three inoculant treatments compared to control (T0). The strongest effect was observed in T3 silages.

Table 6. Nutrient composition and microbial characteristics of whole-crop (milk stage of grain) rye silage

TR	DM_c[1], g kg^{-1}	g kg^{-1} DM_c					Log_{10} CFU g^{-1} of FF		
		CP	CF	WSC	ADF	NDF	LAB	yeast	mould
T0	368.0^{c}	75.6^{b}	328.4^{b}	39.3^{b}	418.4^{b}	576.3^{b}	5.54^{a}	3.07^{a}	2.74^{a}
T1	378.5^{b}	77.6^{b}	317.3^{c}	45.2^{b}	410.2^{b}	560.4^{c}	6.71^{b}	3.00^{a}	2.03^{b}
T2	376.7^{b}	77.2^{b}	322.2^{bc}	39.8^{b}	407.2^{b}	564.1^{c}	7.78^{c}	2.18^{b}	1.70^{c}
T3	375.5^{b}	77.8^{b}	319.4^{c}	21.5^{c}	404.1^{b}	560.1^{c}	7.96^{c}	1.16^{c}	1.12^{d}
SEM	1.399	0.641	2.231	2.448	5.177	4.393	0.14	0.07	0.05

TR = treatment; T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM_c = dry matter corrected for volatiles; CP = crude protein; CF = crude fibre; WSC = water-soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre; CFU = colony-forming units; FF = fresh forage; LAB = lactic acid bacteria. Means with different superscripts within columns differed significantly at $p < 0.05$.

Inoculant application had an effect on the silage fermentation profile (Table 7). During fermentation, the use of homofermentative LAB (T1) resulted in the highest concentration of lactic acid ($p < 0.05$) and the lowest concentration of acetic acid ($p < 0.05$), the lowest pH value ($p < 0.05$), and the lowest DM losses ($p < 0.05$). Application of hetero- and homofermentative LAB (T3) caused the highest concentration of acetic acid ($p < 0.05$) between all treatments. All three inoculant treatments suppressed the formation of ammonia-N, alcohols, and butyric acid.

Table 7. Fermentation characteristics and dry matter losses of whole-crop (milk stage of grain) rye silage

TR	pH	Ammonia-N, g kg^{-1} total N	g kg^{-1} DM_c				
			LA	AA	Alcohols	BA	DM loss
T0	4.36^{a}	68.68^{a}	10.59^{a}	9.16^{a}	16.50^{a}	10.58^{a}	77.8^{a}
T1	3.66^{b}	36.95^{b}	54.66^{b}	9.58^{a}	10.27^{b}	0.32^{b}	39.8^{b}
T2	3.72^{b}	35.60^{b}	40.30^{c}	15.32^{b}	9.25^{bc}	0.38^{b}	44.9^{bc}
T3	3.86^{c}	35.41^{b}	33.09^{d}	35.81^{c}	6.99^{c}	1.58^{b}	49.8^{c}
SEM	4.36^{a}	1.104	1.583	1.223	1.038	0.508	3.299

TR = treatment; T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM_c = dry matter corrected for volatiles; LA = lactic acid; AA = acetic acid; BA = butyric acid. Means with different superscripts within columns differed significantly at $p< 0.05$.

The aerobic deterioration of silages was evaluated by observing temperature dynamics inside silage, the pH value, and the number of yeasts and moulds at the end of the aerobic stability test (Table 8, Fig. 2). Homofermentative LAB-treated silage (T1) began to deteriorate only 52 h upon aerobic exposure, while it took control (T0) and hetero- and homofermentative LAB (T2) silages 168 h and 132 h, respectively, to reach a temperature of more than 3 °C above ambient. At the end of the aerobic stability test, T0 and T1 silages reached a high pH value, fresh weight loss, and the number of yeasts and moulds. Application of hetero- and homofermentative LAB (T3) maintained aerobic stability for 310 h and showed the lowest pH value, fresh weight loss, and the lowest number of yeasts and moulds between all inoculant treatments.

Table 8. Characteristics of the aerobic stability of whole-crop (milk stage of grain) rye silage

TR	pH	DM, g kg^{-1}	Weight loss, %	AS[1] h	Highest temp., °C	Log$_{10}$ CFU g^{-1} of FF	
						yeast	mould
T0	6.10^{a}	328.2^{a}	4.77^{a}	168.0^{a}	29.7	6.24^{a}	7.68^{a}
T1	7.76^{b}	338.5^{b}	5.35^{a}	51.60^{b}	32.2	6.82^{b}	7.00^{b}
T2	7.97^{b}	340.5^{b}	5.18^{a}	132.0^{c}	30.0	5.46^{c}	5.92^{c}
T3	5.09^{c}	343.0^{b}	1.71^{b}	310.0^{d}	23.8	3.51^{d}	2.97^{d}
SE	0.135	2.733	0.372	8.325	-	0.165	0.080

TR = treatment; T0 = control; T1 = 1.5 × 10^{5} CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM = dry matter; AS = aerobic stability; CFU = colony-forming units; FF = fresh forage; [1]number of hours needed for the silage to reach a sustained temperature higher than 3 °C above ambient. Means with different superscripts within columns differed significantly at $p < 0.05$.

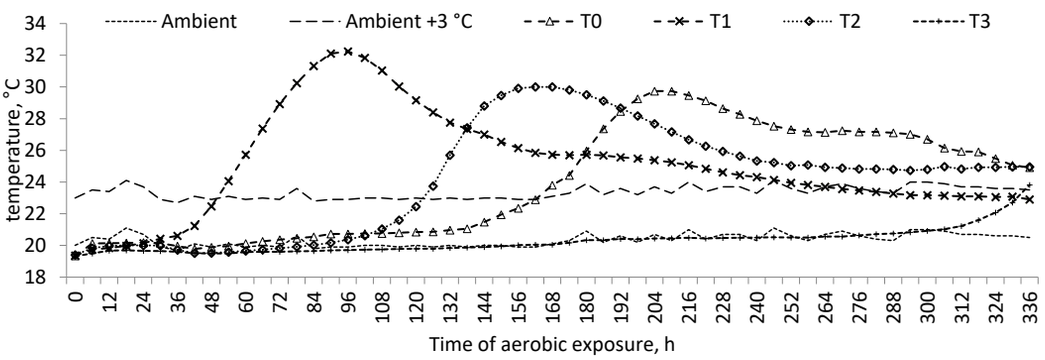

Fig. 2. Temperature changes in whole-crop (milk stage of grain) rye silage during aerobic exposure period (T0 = control; T1 = 1.5 × 10^{5} CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. lactis*)

Characteristics of ensiled whole-crop rye (soft dough stage of grain)

The nutrient content and the microbial characteristics of silage at day 60 of storage are presented in Table 9. When compared with control silage (T0), all three inoculant-treated silages (T1, T2, and T3) contain significantly higher DM content corrected for volatiles. Inoculant T3 preserved a lower amount of WSC ($p < 0.05$) compared with control (T0) or T1 and T2 treatments. The number of LAB was significantly increased, and the numbers of yeasts and moulds were significantly decreased by all three inoculant treatments. The strongest effect was observed in silages T2 and T3.

Table 9. Nutrient composition and microbial characteristics of whole-crop (soft dough stage of grain) rye silage

TR	DM$_c$[1], g kg^{-1}	g kg^{-1} DM$_c$					Log$_{10}$ CFU g^{-1} of FF		
		CP	CF	WSC	ADF	NDF	LAB	yeast	mould
T0	432.9^{b}	70.3^{b}	291.3^{b}	23.2^{b}	352.3^{b}	504.7^{b}	5.67^{a}	3.45^{a}	2.90^{a}
T1	446.7^{c}	73.5^{c}	285.9^{b}	42.5^{c}	346.4^{a}	493.3^{b}	7.16^{b}	3.02^{b}	2.07^{b}
T2	444.1^{d}	71.8^{d}	287.7^{b}	24.9^{b}	346.9^{a}	502.3^{b}	8.21^{c}	2.89^{b}	1.70^{c}
T3	445.9^{cd}	70.0^{bd}	286.3^{b}	17.6^{d}	344.4^{a}	501.4^{b}	8.58^{c}	1.18^{c}	1.12^{d}
SEM	0.806	1.011	3.096	1.587	5.173	8.333	0.18	0.06	0.04

TR = treatment; T0 = control; T1 = 1.5 × 10^{5} CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^{5} CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM$_c$ = dry matter corrected for volatiles; CFU = colony-forming units; FF = fresh forage; CP = crude protein; CF = crude fibre; WSC = water-soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre; LAB = lactic acid bacteria. Means with different superscripts within columns differed significantly at $p < 0.05$.

Inoculant application had an effect on the silage fermentation profile (Table 10). The use of homofermentative LAB (T1) and hetero- and homofermentative LAB (T2) resulted in the highest concentration of lactic acid ($p < 0.05$), and T1 silage showed the lowest DM loss ($p < 0.05$). The lowest concentration of acetic acid ($p < 0.05$) was detected in control (T0) and homofermentative LAB (T1) silages. Hetero- and homofermentative LAB silage (T3) caused the highest concentration of acetic acid ($p < 0.05$) between all treatments. All three inoculant treatments suppressed the formation of ammonia-N, alcohols, and butyric acid.

Table 10. Fermentation characteristics and dry matter losses of whole-crop (soft dough stage of grain) rye silage

TR	pH	Ammonia-N, g kg^{-1} total N	g kg^{-1} DM$_c$				
			LA	AA	Alcohols	BA	DM loss
T0	4.41^a	51.32^a	12.58^a	5.89^a	12.52^a	6.93^a	75.2^a
T1	3.70^b	32.73^b	34.57^b	5.65^a	8.32^b	0.65^b	29.7^b
T2	3.77^c	34.97^b	32.74^b	10.69^b	5.25^c	0.71^b	36.1^c
T3	3.96^d	39.06^c	7.78^c	29.26^c	6.78^d	0.73^b	36.8^c
SEM	0.014	1.106	1.030	0.997	0.482	0.265	1.555

TR = treatment; T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM$_c$ = dry matter corrected for volatiles; LA = lactic acid; AA = acetic acid; BA = butyric acid. Means with different superscripts within columns differed significantly at $p < 0.05$.

The aerobic deterioration of silages was evaluated by observing temperature dynamics inside the silages, the pH value, and the number of yeasts and moulds at the end of the aerobic stability test (Table 11, Fig. 3). Homofermentative LAB-treated silage (T1) began to deteriorate 65 h after aerobic exposure, and it took control (T0) and hetero- and homofermentative LAB (T2) silages 176 h and 116 h, respectively, to reach a temperature of more than 3 °C above ambient. At the end of the aerobic stability test, T1 and T2 silages reached the highest pH value, fresh weight loss, and the largest number of yeasts and moulds. Application of hetero- and homofermentative LAB (T3) supported aerobic stability for almost 340 h and showed the lowest pH value, fresh weight loss, and the lowest number of yeasts and moulds between all inoculant treatments.

Table 11. Characteristics of the aerobic stability of whole-crop (soft dough stage of grain) rye silage

TR	pH	DM, g kg^{-1}	Weight loss, %	AS^1, h	Highest temp., °C	Log$_{10}$ CFU g^{-1} of FF	
						yeast	mould
T0	6.23^a	396.5^a	5.29^a	176.4^a	29.8	7.60^a	8.06^a
T1	7.17^b	407.4^b	6.44 ^{b}	64.8^b	29.9	8.34^a	7.26^b
T2	7.63^c	409.7^b	5.96^{ab}	116.4^c	28.2	7.71^a	7.05^b
T3	4.32^d	411.5^b	2.51^c	339.6^d	23.3	4.78^b	2.24^c
SEM	0.149	3.303	0.289	11.392	-	0.255	0.214

TR = treatment; T0 = control; T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*; SEM = standard error of means; DM = dry matter; AS = aerobic stability; CFU = colony-forming units; FF = fresh forage; ^{1}number of hours needed for the silage to reach a sustained temperature higher than 3 °C above ambient. Means with different superscripts within columns differed significantly at $p < 0.05$.

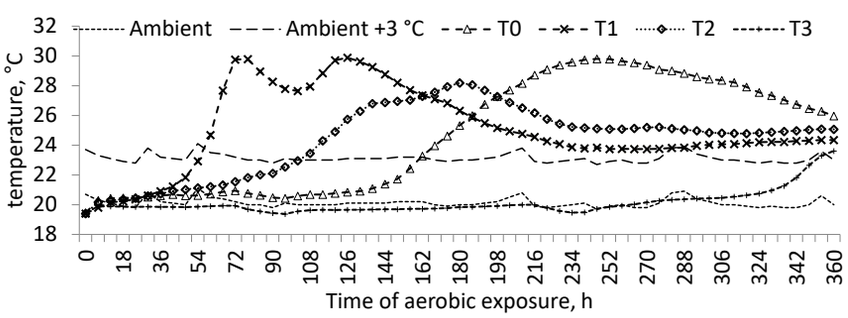

Fig. 3. Temperature changes in whole-crop (soft dough stage of grain) rye silage during aerobic exposure period (T0 – control, T1 = 1.5 × 10^5 CFU g^{-1} *L. plantarum*, *E. faecium*, *L. lactis*; T2 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. plantarum*, *E. faecium*; T3 = 1.5 × 10^5 CFU g^{-1} *L. buchneri*, *L. lactis*)

Discussion

At the time of ensiling, early-cut (boot stage, slightly wilted) rye forage contained 274 g kg^{-1} DM and 99 g kg^{-1} DM crude protein and was similar to that studied by Kim et al. (2001) and Auerbach and Theobald (2020). The DM content increased with increasing maturation of rye up to 390 and 457 g kg^{-1} for whole-crop rye at milk and soft dough stages, respectively. The increase in the DM content during maturation was accompanied by a decrease in crude protein and WSC content. Micek et al. (2001) reported the same tendencies in relation to the stage of maturity of rye forage. However, as the DM content increased with maturation of whole-crop rye, the buffering capacity slightly decreased. On this account and regarding Auerbach and Theobald (2020), early-cut and whole-crop rye forage was considered to be a moderately easy to easy to ensile crop. The number of epiphytic LAB was low and reached only 1.79–3.27 log10 CFU g^{-1}. The number of yeasts and moulds above 5 log10 CFU g^{-1} and above 4.5 log10 CFU g^{-1}, respectively, represent typical values for the Lithuanian climatic conditions.

Untreated (T0) silage underwent butyric fermentation or contained a considerable amount of butyric acid, had a high pH value, a high ammonia-N concentration (high proteolysis), and a high DM loss. This can be attributed to a slow acidification rate caused by a low number of epiphytic LAB (1.79–3.27 log10 CFU g^{-1}) and a low nitrate level of the crop, although this parameter was not measured. These findings agree with the observations by Auerbach et al. (2013), who indicated that under these ensiling conditions, clostridia could thrive during the initial stage of fermentation.

Compared to control (T0), homofermentative (T1), and hetero- and homofermentative LAB (T2) treatments resulted in a significantly higher DM content (corrected for volatiles) for early-cut and whole-crop rye silages. As expected, the WSC content in all silages was reduced during fermentation. The residual WSC content was the highest for the T1 treatment among all vegetation stages of rye indicating more effective WSC utilisation by homofermentative LAB. Inoculation with *L. buchneri* in combination with homofermentative LAB (T3) resulted in a lower content of residual WSC in silages. Similar results were reported by Kleinschmit and Kung (2006). The homofermentative LAB treatment (T1) produced a typical effect on silage quality at all three vegetation stages of rye: more lactic acid, less acetic acid (for early-cut rye), less ethanol, and lower proteolysis (lower ammonium-N content) compared to control (T0). A positive effect of T1 treatment was also manifested by a lower pH value of this silage and a lower content of butyric acid in it. The homofermentative LAB treatment (T1) resulted in the lowest DM losses among all inoculant treatments during the storage period. Our previous observations (Jatkauskas et al. 2018) and results reported by other authors (Basso et al. 2014, Borreani et al. 2018, Muck et al. 2018) confirm the findings of this study. According to Schmidt and Kung (2010) and Muck et al. (2018), homofermentative LAB usually promote lactic acid fermentation by shifting fermentation products towards lactic acid and away from ethanol and acetic acid fermentation products with a decreased DM loss as the main benefit.

Homofermentative LAB-based silage additives sometimes render silages less stable when they are exposed to air, because lactic acid is not a strong antifungal agent and the production of antifungal compounds (acetic acid) in the silages is limited (Danner et al. 2003, Kung 2009). This effect was also observed in the current experiment: the aerobic stability of T1 silages treated with homofermentative LAB were clearly reduced compared to the control (T0) and the other (T2 and T3) treatments. Moreover, at the end of the aerobic stability test, T1 silages manifested the fresh weight loss and the number of yeasts and moulds close to the control (T0) silage at all three maturity stages of rye. Auerbach et al. (2013) indicated that the high residual sugar content may stimulate the extent and the rate of yeast survival and growth in the silages exposed to air and reduce aerobic stability of these silages.

Irrespective of the rye crop maturity stage, hetero- and homofermentative LAB-treated silages (T2 and T3) had a higher content of lactic acid than non-inoculated (T0) silages, but lower than T1-treated silage. Muck et al. (2018) and da Silva et al. (2019) observed that heterofermentative LAB produce more acetic acid and increase the DM loss when compared with homofermentative LAB. The results of the present study are in line with these observations, where T2 and T3 silages showed higher DM losses, when compared with T1 silages. The heterofermentative pathway, however, is usually connected with increased fermentation losses compared to homofermentative pathway (McDonald et al. 1991). The highest content of acetic acid was detected in T3 silages. A capability of a LAB strain to increase the aerobic stability of silage is directly related to its ability to inhibit the growth of microorganisms that deteriorate the silage and causes silage heating when it is exposed to air. Research by Driehuis et al. (2001) showed that addition of *L. buchneri* reduced the survival of yeasts during the anaerobic phase of silage fermentation and inhibited the growth of yeasts during exposure of silage to air. Tabacco et al. (2011) indicated the power of *L. buchneri* to inhibit the aerobic deterioration of silages through the fermentation of lactic acid to acetic acid and the inhibition of yeast and clostridial growth.

The results of our experiment show that rye silages inoculated with a blend of hetero- and homofermentative LAB (*L*. *buchneri* and *L*. *lactis*, T3) had the highest values of aerobic stability and LAB counts, the lowest fresh weight loss, and the lowest number of yeasts and moulds at mini-silo opening and at the end of aerobic exposure at all three vegetations stages. The significant decrease in the number of yeasts and moulds in T3 silage can be ascribed to the significantly highest acetic acid content in T3 silage among all inoculant treatments. Auerbach et al. (2013) and Kleinschmit and Kung (2006) reported that acetic acid can reduce the number of yeasts in silages, and the extent of this effect depends on the content of acetic acid. The growth-inhibiting effect of yeasts and moulds remained during the aerobic exposure phase and consequently resulted in a significantly decreased aerobic deterioration. Under aerobic conditions, T3-inoculated rye silages had a markedly lower pH value, fresh weight loss, and heat production than control and other inoculant treatments used at all three stages of maturity of rye.

Conclusions

Inoculation of early-cut and whole-crop rye harvested at milk or soft dough stages of grain with LAB blends affected silage fermentation properties and aerobic stability traits depending on their intended behaviour. The homofermentative inoculant treatment improved the fermentation profile and reduced DM losses but impaired aerobic stability and increased fresh weight loss during the period of exposure to air. The product containing homoferementative *Enterococcus faecium*, *L*. *plantarum*, and heterofermentative *L*. *buchneri* improved the fermentative profile and reduced DM losses at all stages of maturity, but aerobic stability was improved only in early-cut rye silage. This strain combination can be recommended if rye is harvested at the early growth stage in double-cropping systems. The dual-purpose inoculant containing *L*. *buchneri* and *L*. *lactis* supported good fermentation with reduced DM losses and ensured long-lasting protection against aerobic deterioration caused by yeasts and moulds of rye silages at early-cut and whole-crop maturity.

Results of this experiment should support and promote the use of the combination of these LAB strains in the ensiling whole plant of rye as forage for ruminants independently from the actual maturity stage of the crop.

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