Original Paper

The effect of biological additive on the fermentation quality of whole-crop rye silage

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The aim of this research was to find out the changes in fermentation parameters of whole-crop rye silage after adding the biological additive. Two variants of rye silage were used in the experiment: variant C (control without additive) and variant A (silage with the addition of additive). The wilted whole-crop rye was treated with strains of lactic acid bacteria (*Pediococcus acidilactici*, *Lactobacillus paracasei* and *Lactococcus lactis* $1.25 \cdot 10^{11}$ CFU g^{-1}) in a dose of 2 g of additive + 25 ml of water per 1 ton of matter. Both silage variants were ensiled with a vacuum pack device and after 2 months of storage, average samples were taken to determine the dry matter content, fermentation products, acidity of water extract, pH and the degree of proteolysis by appropriate methods. Treatment of rye silage with a microbial silage additive affected the quality of rye silage by a statistically significant (P <0.05) higher content of lactic acid and acidity of water extract. The lower content of acetic acid, alcohols, pH valueand the degree of proteolysis were also statistically significant (P <0.05). The results confirmed the positive effect of the addition of *Pediococcus acidilactici*, *Lactobacillus paracasei* and *Lactococcus lactis* on the quality of the fermentation process of rye silage.

Keywords: rye, silage, biological silage additives, fermentation quality

1 Introduction

The main priority for ruminants and farmers is continuous access to a quality forage base (Arasu et al., 2014). Silage is an important way of preserving fresh feed and its conditions significantly affect the fermentation properties and aerobic stability of silage (Bíro et al., 2020; Huyen et al., 2020). Rye (*Secale cereale*, L.) is one of the winter cereals that meets the fiber needs of ruminants, and its cultivation improves soil properties and water quality (Paradhipta et al., 2020; Moore et al., 2014). Unlike other winter cereals, it is characterized by resistance to low temperatures, adaptation in infertile soil, and higher growth rates (Kim et al., 2017; Paradhipta et al., 2020). Rye silage contains an average of 64 g kg^{-1} DM crude protein, 478 g kg^{-1} DM acid-detergent fiber and 763 g kg^{-1} DM neutral-detergent fiber. In general, silages are characterized by relatively high stability over a longer period of time. Long-term stability and fermentation quality can be achieved mainly by adding biological silage additives during silage preparation (Arasu et al., 2014). These silage additives are usually divided into two groups as homofermentative (^{ho}LAB) and heterofermentative (^{he}LAB) strains of lactic acid bacteria (Juráček et al., 2018). Therefore, the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and *Weissella* are most commonly associated with silage (Arasu et al., 2014). According to Oliveira et al. (2017), bacterial inoculants are used to prevent the growth or

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survival of pathogens, to maintain the nutritional quality of feed and to improve the fermentation of silage. The use of inoculants also improves nutrient digestibility (Lee et al., 2018). Several studies have shown that the application of homofermentative LAB strains can positively affect the content of crude protein, neutral-detergent fiber and acid-detergent fiber depending on the amount of inoculant (Choi et al., 2015, 2016; Kim et al., 2017; Lee et al., 2018). The positive effect of ^{ho}LAB on fermentation parameters of rye silages was also confirmed (Kim et al., 2017; Choi et al., 2015, 2016; Auerbach et al., 2020; Auerbach and Theobald, 2020; Haag et al., 2016. According to the results of several studies (Haag et al., 2016; Kim et al., 2017; Morais et al., 2017; Lee et al., 2018; Paradhipta et al., 2020; Auerbach et al., 2020), the use of heterofermentative LAB (^{he}LAB) strains increased the concentration of acetic acid and decreased the content of lactic acid, while the pH value decreased compared to the control silage. While Paradhipta et al. (2020) and Joo et al. (2017) achieved positive results in the chemical composition of silage using ^{he}LAB, Kim et al. (2017) and Lee et al. (2018) found an adverse effect. However, in all of these studies, *in vitro* digestibility increased.

The aim of this work was to find out the effect of biological silage additive in the composition of *Lactobacillus lactis*, *Pediococcus acidilactici* and *Lactobacillus paracasei*, on the fermentation parameters of whole-crop rye silage.

2 Material and methods

In cooperation with farm Bzince pod Javorinou, a whole-crop rye (*Secale cereale*, L.) of the Borfuro variety was ensiled. Rye was harvested using a field chopper Class Jaguar 850 in the flowering stage and after 48 hours of wilting, cut to a theoretical cut length of 20 mm. The wilted matter was ensiled in two variants: C (control) and A (with the addition of an additive). In variant A, a biological additive (*Pediococcus acidilactici*, *Lactobacillus paracasei*, *Lactococcus lactis*, containing active bacteria $1.25 \cdot 10^{11}$ CFU g^{-1}) was applied on the matter in a dose of 2 g of additive + 25 ml of water per 1 ton of matter. The wilted matter of each variant ($n = 3$) was ensiled using a vacuum pack device MSW Motor Technics type MSW-VPM-900K into silage units (bags). Silage units (bags) were stored in the Laboratory of Feed Preservation at the Department of Animal Nutrition, FAFR SUA in Nitra, under the conditions with a constant temperature 22 ±2 °C. After the 2 months of storage, silage units (bags) of both variants were opened and average samples for chemical analyzes were taken. In the rye silage samples, the dry matter content was determined by drying (at 103 ±2 °C) and the content of acetic acid, butyric acid, lactic acid and formic acid was determined by ion electrophoresis in the water extract of silage (EA 100 analyzer). The portion of lactic acid from the total acid content (%) was determined by calculating: (lactic acid/total acid content) × 100. The total N content was determined by the Kjeldahl method (Kjeltec, TECATOR) and the NH_3 content by titration acidimetrically by the Conway microdiffusion method. According to the formula, the degree of proteolysis (DP) = (NH_3-N/total N) × 100 (in %) was calculated. The pH value was determined electrometrically, the alcohol content by microdiffusion method – iodometric titration and acidity of water extract by alkalimetric titration of water extract to pH 8.5: in mg KOH 100 g^{-1} feed. The content of fermentation products (FP) was calculated according to the formula (FP) = volatile fatty acids + lactic acid + alcohols. The results were statistically evaluated a processed by IBM SPSS 26.0. The descriptive statistics by Oneway Anova and differences between the control and experimental variant by Independent Samples *T*-Test were expressed.

3 Results and discussion

In the rye silage with the addition of a biological additive, a statistically significant (P <0.05) higher dry matter content was found (Table 1). However, Kim et al. (2017), Lee et al. (2018) and Auerbach et al. (2020) found a lower dry matter content compared to control silage after the ^{ho}LAB application. Consistent with Haag et al. (2016), Choi et al. (2015, 2016, 2017) and Auerbach and Theobald (2020) a statistically significant (P <0.05) higher concentration of lactic acid was recorded in silages with additive compared to untreated silage. There were not statistically significant (P >0.05) differences in the formic acid content. Significant (P <0.05) lower acetic acid content was registered in the silage with the microbial inoculant. Also, Auerbach and Theobald (2020) confirmed a lower acetic acid content (P <0.05) in an experimental variant of the rye silage. In contrary, the results from Lee et al. (2018) showed that the acetic acid content of the treated silage increased, although not significantly (P >0.05). According to the results by Paradhipta et al. (2020), the content of lactic and acetic acid increased (P <0.05) after the treating rye silage with ^{he}LAB. Arasu et al. (2014) stated, that the addition of ^{ho}LAB, and also the simultaneous production of lactic and acetic acid, increased the aerobic stability and inhibited the growth of microscopic fungi. Similarly, Lee et al. (2018) did not find the presence of butyric acid in both rye silage variants. In contrast, Choi et al. (2016) showed a decrease (P <0.05) in this acid in treated silage (DM 591 g kg^{-1}) compared to the control rye silage (DM 575 g kg^{-1}). The ratio of lactic acid to acetic acid was higher (P <0.05) in our silage treated with biological additive, but it tend to be lower (P >0.05) using ^{ho}LAB in the experiment by Lee et al. (2018). The portion of lactic acid in the total acid content was higher than

Table 1 The fermentation quality of whole-crop rye silage

Parameter	Unit	C		A	
		mean	S.D.	mean	S.D.
Dry matter	g kg^{-1}	332.27*	1.20	375.63*	1.59
Lactic acid	g kg^{-1}of DM	151.30*	1.18	172.54*	1.72
Formic acid	g kg^{-1}of DM	1.78	0.11	1.81	0.04
Acetic acid	g kg^{-1}of DM	17.58*	1.29	5.11*	0.17
Butyric acid	g kg^{-1}of DM	nd	–	nd	–
Ratio of lactic to acetic acid	ratio	8.65*	0.62	33.80*	0,81
Portion of lactic acid**	%	88.66	–	96.15	–
Acidity of water extract	mg KOH^{y}	2351.00*	85.60	3160.83*	57.00
pH	–	4.13*	0.02	3.78*	0.01
Alcohols	g kg^{-1}of DM	31.64*	2.54	28.37*	3.34
Fermentation products	g kg^{-1}of DM	202.30*	0.80	207.83*	1.85
Degree of proteolysis	%	8.76*	0.97	4.91*	0.76

C – control; A – with biological inoculant; S.D. – standard deviation; DM – dry matter, nd – not detected; ** from total acid content, ^{y}per 100 g of silage; * values with the identical index in row are significant at *P* <0.05

88% in variant C and A, with a more favorable portion in silages treated with a biological additive. Compared to Auerbach and Theobald (2020), homofermentative inoculant had impact on the portion of lactic acid as well (from 79.84% to 85.17%), and differences were statistically significant. Silages treated with the biological additive (A) with a statistically significant (*P* <0.05) lower pH value were characterized by a statistically significant (*P* <0.05) higher acidity of water extract. These results were also achieved by Alba-Mejía et al. (2016), where biological additive addition (^{ho}LAB + ^{he}LAB) caused a statistically significant (*P* <0.05) higher acidity of water extract from 1,366.00 mg KOH to 1,636.7 mg KOH in grass silage. On the other hand, Juráček et al. (2018) showed a statistically significant (*P* <0.05) lower acidity of water extract using homofermentative and heterofermentative strains of LAB in grass silages. The decrease in pH in current experiment was also statistically significant, and these results were also confirmed by Choi et al. (2015, 2016, 2017). Decrease tendency in pH (4.97 vs. 4.90) was also reported by Kim et al. (2017). According to Adesogan (2014), sufficient content of organic acids and lower pH can prevent the development of undesirable microorganisms. The alcohol concentration decreased by 3.27 g kg^{-1} compared to the control variant. The application of ^{ho}LAB also reduced the alcohol content in other studies, in Auerbach and Theobald (2020) at the level *P* <0.05 and in Haag et al. (2016) inconclusive. In contrast, the results from Auerbach et al. (2020) are in opposite, where alcohol content increased (*P* >0.05), while the dry matter content of rye silage in control variant was 419 g kg^{-1} and in treated variant 413 g kg^{-1}. Factors that affect the formation of fermentation products include the addition of silage additives in addition to the storage time and the process of ensiling (Herrmann et al., 2011). Statistically significant (*P* <0.05) higher value of fermentation products was recorded in silages of variant A, which was mainly due to the higher content of preserving lactic acid. Auerbach et al. (2020) detected fermentation products other than n-propanol and propionic acid. The addition of a biological additive significantly (*P* <0.05) inhibited the degree of nitrogenous decomposition (degree of proteolysis) in the whole-crop rye silage. Presented result is consistent with the statement by Huyen et al. (2020), that lowering the pH (≤4.0) during ensiling inhibits proteolysis.

4 Conclusions

The results of this experiment confirmed the beneficial effect of biological additive (*Lactobacillus lactis*, *Pediococcus acidilactici*, *Lactobacillus paracasei*) on fermentation parameters of whole-crop rye silage. The inoculation with homofermentative lactic acid bacteria contributed to a statistically significant (*P* <0.05) increase of lactic acid and decrease of acetic acid content. The positive effect on portion of lactic acid from total acid content and better ratio of lactic to acetic content was found. After that, the addition of biological additive significantly (*P* <0.05) decreased pH value, which is determining factor of conservation. The content of alcohols and the degree of proteolysis was also significantly (*P* <0.05) decreased in a positive way. Then, in the silage with biological additive significantly (*P* <0.05) higher acidity of water extract and content of fermentation products was found. Treatment of ensiled whole-crop rye with a biological additive on the base of homofermentative lactic acid bacteria improved the quality of the fermentation process of silage.

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