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Original research paper

THE INFLUENCE OF BONSILAGE FORTE ON FERMENTATION AND AEROBIC STABILITY DURING ALFALFA ENSILING

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ABSTRACT: The effects of adding new commercial silage inoculant on fermentation dynamics and aerobic stability of alfalfa silage was examined under laboratory conditions. Poly-propylene containers of 1.8 dm^3 were used for ensiling. Containers were divided in two groups that were opened on days 14 and 49 for sampling. Value of pH of alfalfa decreased faster and to a greater extent in inoculated samples compared to control samples, which is important for conserving of nutrients of the silage. Concentration of lactic acid was significantly ($p<0.05$) higher in inoculated samples and this parameter indicates efficient fermentation. Absence of butyric acid in inoculated samples confirms that Clostridial secondary fermentation did not take place and that used inoculant acts efficiently against Clostridia. Aerobic stability of inoculated samples, as measured by the release of CO_2 after exposure to air, was three times higher compared to control samples.

Key words: *silage, alfalfa, Bonsilage Forte, fermentation, aerobic stability*

INTRODUCTION

Ensiling is a preservation method for moist forage crops. It is based on solid-state lactic acid fermentation under anaerobic conditions whereby lactic acid bacteria (LAB) convert water–soluble carbohydrates (WSC) into organic acids, mainly to lactic acid. As a result the pH decreases, and the moist crop is preserved (Weinberg and Ashbell, 2003). A moist crop can support the growth of a wide range of microorganisms, most of which will degrade the crop's nutrient value to livestock. However, ensiling generally controls microbial activity by a combination of an anaerobic environment and a natural fermentation of sugars by lactic acid bacteria (LAB) on the crop (Muck, 2010).

Legumes are difficult to ensile successfully without an additive (Carpintero et al., 1969; Ohshima et al., 1979). This is especially true for alfalfa (*Medicago sativa L.*). McDonald et al. (1991) considered that the difficulties in ensiling legumes were attributable to three factors. Firstly, they are highly buffered; secondly, they tend to have low water–soluble carbohydrate (WSC) contents; and thirdly, they often have low dry-matter (DM) content.

The number of LAB present on alfalfa plants at harvest may be too low to ensure rapid and efficient preservation, and therefore silage inoculants have been developed to improve the nutritive value of silages and to reduce risks during ensiling (Čabarkapa, 2010a).

The possible beneficial effect of LAB inoculants depends on their composition, concentration and the properties of the crop being ensiled (Tengerdy et al., 1991).

Regarding the effect of bacterial inoculant, some authors (Weinberg et al., 1988; Tengerdy et al., 1991; Masuko et al., 2002) indicated that inoculation of the alfalfa silages with LAB enhanced the silage quality, while others reported no effect (Lindgren et al., 1983; Ohyama et al., 1973). The aim of this study was to determine effects of adding a silage inoculant with a new combination of LAB strains to alfalfa, grown under south–eastern European conditions, on fermentation dynamics and aerobic stability of silage.

MATERIAL AND METHODS

Fifth–cut alfalfa (cultivar Banat) was harvest in 2010 in province of Vojvodina, Serbia, using a precision silage chopper to obtain a theoretical 20 mm chop length. Investigated inoculant *Bonsilage Forte* (Schaumann, Agri Austria GmbH) contains combination of homo–fermentative strains of *Lactobacillus paracasei* (DSM 16245), *Lactobacilus lactis* (NCIMB 30160) Pediococcus acidilactici (DSM 16243). It was used as an inoculant by mixing 0.30 g with 0.1 dm^3 of water (to provide 2.5×10^5 colony forming units of LAB per gram of fresh maize), and spraying over 30 kg of alfalfa. The application rate was in accordance with the level of LAB in the inoculant as determined by the manufacturer. In order to add the same amount of moisture as in the treated alfalfa, the control was treated by spraying 0.1 dm^3 of water on 30 kg alfalfa.

The alfalfa (280.6 g/kg DM) was compactted into 1.8 dm^3 transparent containers made of polypropylene co–polymer and equipped with a special water valve to enable gas release, as proposed by Palić et al. (2011). Each container was filled with approximately 0.65 kg (wet mass) alfalfa without a headspace, and a packing density of approximately 360 kg/m^3 was obtained.

A total of 12 containers were divided into two groups. Each group consisted of three control containers, without inoculant, and three inoculated samples, with *Bonsilage Forte* added. Containers were stored in dark room at a temperature of 21–25 ^0C.

On day 0, fresh alfalfa was collected for subsequent chemical analysis. First group was opened on day 14 and second on day 49 day for determination of pH, dry matter (DM), crude protein (CP), ammonia nitrogen (NH_3–N), lactic acid (LA) and volatile fatty acids (VFA). The pH, DM and CP were determined following the procedures of AOAC (2005). The NH_3–N, LA acid and VFA were determined according to standard methods (Die Chemische Untersuchung von Futtermitteln, 1997). On day 49 silages were also subjected to an aerobic stability test conducted according to procedure of Ashbell et al. (1991).

Statistical Analysis System STATISTICA (2011) was used for analyzing variations (ANOVA) and least significant differences. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Dry matter and crude protein content of the fresh alfalfa and silage samples is presented in the table 1. It can be seen that there was no significant difference between control (C) and inoculated samples (BF).

Initial pH value of alfalfa (6.43) decreased to a greater extent in inoculated samples and was about 5.0 on day 49, compared to control samples where it was slightly below 6.0 (Figure 1). Also, pH drop of inoculated silage in the first days of ensiling was faster, which is important for conserving of nutrients of the silage by inhibiting spoilage microorganisms (present in the natural plant microflora) and hetrofermentative bacteria (enterobacteria) and promoting homofermentative lactic–cid bacteria. Value of pH is a key criterion to evaluate silage fermentation. Generally, the lower the pH, the better preserved and more stabile is the silage (Seglar, 2003).

Fermentation parameters of experimental silages (lactic acid, acetic acid, butyric acid and ammonia nitrogen concentration) are shown in the table 2. Concentration of lactic acid was significantly higher in inoculated samples and its concentration was doubled on day 49 compared to day 14. Generally, the presence of high lactic acid levels indicates efficient fermentation and minimal dry matter loss (Seglar, 2003).

Table 1.
DM and CP content in fresh alfalfa and experimental silages

Analysis/Sample			Sampling day		
			0	**14**	**49**
***DM** (g/kg DM)*					
Fresh alfalfa			280.6		
Silage	C		-	237.5 ± 8.2 [a]	254.9 ± 10.6 [a]
		BF	-	237.5 ± 5.5 [a]	246.9 ± 5.6 [a]
***CP** (g/kg DM)*					
Fresh alfalfa			63.3		
Silage	C		-	50.9 ± 2.7 [a]	61.8 ± 3.8 [a]
	BF		-	53.2 ± 1.7 [a]	61.0 ± 3.2 [a]

C - without inoculant
BF - with inoculant
Results are given as mean ± standard deviation
[ab]Means with different superscripts in the same column are significantly different (P<0.05)

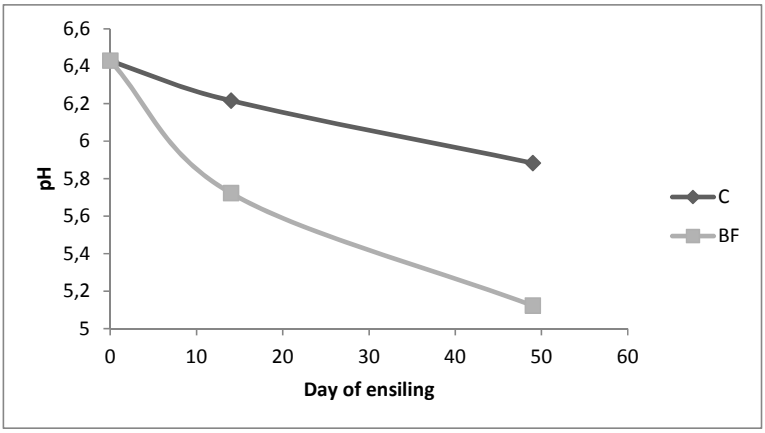

Figure 1. pH of fresh alfalfa (day 0) and experimental silages
(C - without inoculant; BF - with inoculant)

Table 2.
Fermentation parameters of experimental silages

Analysis/Sample			Sampling day		
			0	**14**	**49**
***Lactic acid** (g/kg DM)*					
Fresh alfalfa			2.2		
Silage	C		-	3.1 ± 0.1 [a]	6.6 ± 0.5 [a]
		BF	-	5.1 ± 0.3 [b]	10.6 ± 1.6 [b]
***Acetic acid** (g/kg DM)*					
Fresh alfalfa			3.1		
Silage	C		-	8.7 ± 0.4 [a]	11.8 ± 1.6 [a]
		BF	-	11.7 ± 0.8 [b]	17.6 ± 0.9 [b]
***Butyric acid** (g/kg DM)*					
Fresh alfalfa			0.0		
Silage	C		-	0.04 ± 0.05 [a]	0.08 ± 0.9 [a]
	BF		-	0.00 [a]	0.00 [a]
***NH_3-N** (g/kg DM)*					
Fresh alfalfa			0.7		
Silage	C		-	1.7 ± 0.0 [a]	2.1 ± 0.1 [a]
	BF		-	1.6 ± 0.1 [b]	1.8 ± 0.0 [b]

C - without inoculant
BF - with inoculants
Results are given as mean ± standard deviation
[ab]Means with different superscripts in the same column are significantly different (P<0.05)

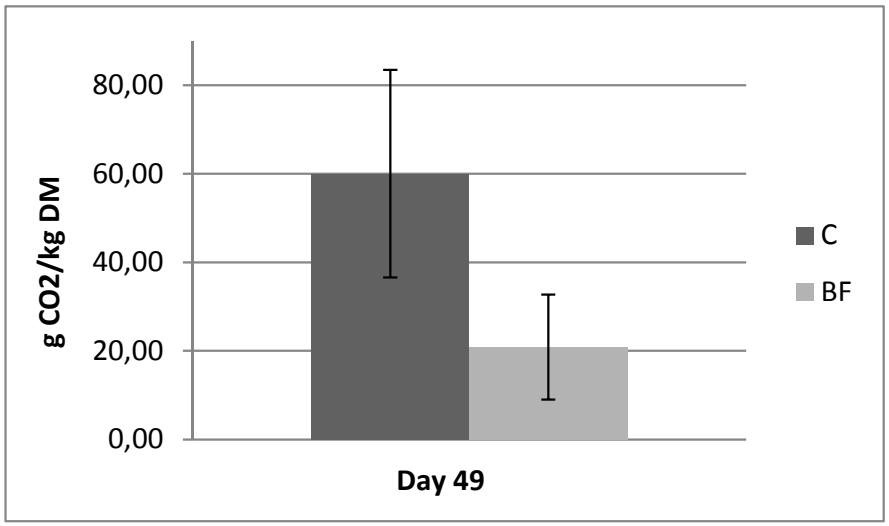

Figure 2. Aerobic stability of experimental silages on day 87 (C – without inoculant; BF–with inoculant)
Results are given as mean ± standard deviation

Acetic acid concentration was significantly higher in inoculated samples which have positive effect on silage quality since this acid is important for inhibition of growth of moulds and yeasts (McDonald et al., 1991). The effect of acetic acid on fungal growth is related to the undissociated concentration in solution. Thus a given concentration of acetic acid becomes more inhibitory to yeasts and molds as silage pH decreases (Čabarkapa et al. 2010b).

Presence of butyric acid is the result of Clostridial activity (Seglar, 2003). Butyric acid was detected only in control samples. Clostridia can grow at lower pH values than enterobacteria making them more difficult to control (Čabarkapa et al. 2010b). If pH value cannot be decreased deep enough the Clostridia spores germinate and degrade lactic acid to butyric acid.

Concentration of ammonia nitrogen (NH_3–N) increased through the experiment, but it was significantly lower in inoculated samples. Higher ammonia indicates protein brake down from proteolytic enzymatic activity (Seglar, 2003).

Results of aerobic stability test on day 49 (Figure 2), expressed by released CO_2 upon exposure to air, showed positive effect of inoculants on aerobic stability of silage. Amount of released CO_2 was three times lower in inoculated samples compared to control.

CONCLUSIONS

In this investigation, rapid drop of pH occurred in the first days of ensiling and it was much stronger in inoculated samples. Value of pH continued to decrease through whole period of ensiling for both control and inoculated samples, and reached much lower value in inoculated samples.

Lower pH in inoculated samples probably inhibited protein degradation and therefore concentrations of ammonia–nitrogen were lower in those samples, which demonstrates positive effect of Bonsilage Forte on nutritive value of silage.

Addition of inoculants resulted in a signifycantly higher lactic concentration than in the control samples. Lactic acid is the strongest of all silage acids and its presence drops pH more effectively than other volatile fatty acids.

Absence of butyric acid in inoculated samples confirms that Clostridial seconddary fermentation did not take place and that Bonsilage Forte acted efficiently against Clostridia.

The aerobic stability of silage, as measured by the release of CO_2 after exposure to air, was significantly improved by addition of BF.

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УТИЦАЈ БАКТЕРИЈСКОГ ИНОКУЛАНТА (*BONSILAGE FORTE*) НА ФЕРМЕНТАЦИОНЕ КАРАКТЕРИСТИКЕ И АЕРОСТАБИЛНОСТ СИЛАЖЕ ЛУЦЕРКЕ

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Сажетак: У овом истраживању испитиван је утицај додатка комерцијалног силажног инокуланта (*Bonsilage Forte*) на ферментационе карактеристике и аеро-стабилност силаже луцерке. За силирање су коришћене полипропиленске посуде запремине 1,8 dm^3. Посуде су подељене у две групе. Прва група је отворена ради узорковања 14-ог а друга 49-ог дана експеримента. Вредност pH је у инокулисаном узорку опала брже и у већој мери у односу на контролне узорке што је важно за очување хранљивих материја силаже. Концентрација млечне киселине била је значајно већа у инокулисаним узорцима, што указује на ефикасну ферментацију у тим узорцима. Одсуство бутерне киселине у инокулисаним узорцима потврђује да није дошло до секундарне ферментације Клостридија и да Bonsilage Forte ефикасно делује против Клостридија. Количина ослобођеног CO_2 након изагања силаже ваздуху, која је мерило аеробне стабилности силаже, била је три пута мања у инокулисаним узорцима и указује на њихову већу аеростабилност у поређењу са контролом.

Кључне речи: *силажа, луцерка, Bonsilage Forte, ферментација, аеростабилност*

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