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EFFECT OF BACTERIAL INOCULANTS APPLICATION AND SEEDING RATE ON COMMON VETCH-OAT SILAGE QUALITY

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Abstract: The experiment was carried out to evaluate the possibility of ensiling common vetch – oat mixtures sown at five different seeding rates. Two effects were studied: seeding rate of common vetch and oat in the mixtures and application of bacterial inoculant. The pH, DM (Dry Matter) content, ammonia nitrogen, soluble nitrogen, lactic, acetic and butyric acids were determined on silages. DLG method was utilized for classification the silage quality. Common vetch and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia, and were tested at five different mixture rates: pure vetch, 25% vetch + 75% oat, 50% vetch + 50% oat, 75% vetch + 25% oat and pure oat. Application of bacterial inoculant affected higher content of ammonia nitrogen and acetic acid ($P< 0.05$), but lower content of soluble nitrogen ($P< 0.05$). Depending on the seeding rates of common vetch and oat, 75 : 25 common vetch – oat silage had the highest content of lactic acid and the lowest content of butyric acid. Contents of DM, pH and ammonia nitrogen were similar in all silages ranged from 307.2 to 318.5 g kg^{-1}, from 4.27 to 4.54 and from 16.1 to 19.1% ΣN, respectively. According to the DLG method for silage quality evaluation, similar quality grades were founded.

Key words: common vetch – oat silages, quality of fermentation

Introduction

Good quality silage and hay are important for the nutrition of ruminants, as well as for the quality and safety of dairy products. The conservation process involves many steps that should be managed carefully to ensure good quality. This starts in the crop composition, continues with harvest, ensiling, and feed out

management and is influenced by additives. Silage quality depends on many factors. In terms of the nutritive value of the forage, the crop composition at harvest has a major impact on the ensiling process and quality of silage (*Buxton and O´Kiely, 2003*). *Đorđević et al. (2011)* reported that bacterial-enzyme additives reduce fiber and increase the concentration of sugar and lactic acid and digestibility of silage. *Bijelić et al. (2015)* concluded that bacterial inoculants reduced crude protein content, ammonia nitrogen, acetic acid and pH value and increased the proportion of lactic acid relative to the acetic acid.
Experience with cultivation of legume –cereal mixtures for silage is limited, and there have been no enough studies on different mixtures for this purpose. The aim of the present study was to evaluate the fermentative characteristics of common vetch-oat silages depending on different seeding rate in the mixtures and application of inoculant.

Material and methods

Common vetch and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac-Serbia (21º 19′ 35″ E, 43º 34′ 58″ N). The experiment was designed with three replication according to a randomized complete block. The common vetch:oat mixtures were ensiled in the experimental containers holding 130 dm^3, with three replications. After compaction, silomass was covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load. Bacterial inoculant *BioStabil Plus* which contained homo-fermentative lactic acid bacteria (*Enterococus faecium* and *Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram was added, and ensiled in containers for 45 days (a_1 – treatment with bacterial inoculant; a_2 – treatment without bacterial inoculant). The common vetch and oat were tested at five different mixture rates: b_1) 100% common vetch + 0% oat; b_2) 25% common vetch + 75% oat; b_3) 50% common vetch + 50% oat; b_4) 75% common vetch + 25% oat and b_5) 0% common vetch + 100% oat. Plant samples were taken at forming the first pods on 2/3 plants of common vetch.

The DM content was determined in the silage, the degree of acidity (pH), ammonia and soluble nitrogen, content of acetic, butyric and lactic acids. In order to provide more realistic estimates, DLG method for evaluating the quality of silage was used (*Đorđević et al., 2003*).

The experimental data were analyzed by a two-way analysis of variance for silage samples using a model that accounted for the main effects of addition of inoculant and common vetch : oat mixtures. Effects were considered significant at $P<0.05$ level. The significance of differences between arithmetic means was tested by LSD test.

Results and discussion

The results of fermentation characteristics in common vetch-oat silages are presented in Table 1. The higher numerical content of DM was found for the inoculant treatment (313.3 g kg^{-1}) than in treatment without bacterial inoculant (311.9 g kg^{-1}). However, differences were not significant. Depending on the seeding rate of common vetch and oat in the mixtures, DM content increased from 307.2 g kg^{-1} in pure common vetch silage to 318.5 g kg^{-1} in pure oat silage.

Table 1. Conservation characteristics of common vetch – oat silages

Factors		DM	pH	NH_3-N	H_2O-N	AA	BA	LA
A	B	g kg^{-1}		%ΣN	%ΣN	g kg^{1}DM	g kg^{1}DM	g kg^{1}DM
a_1	b_1	301.3^{d}	4.48^{c}	18.1^{b}	58.5^{d}	53.3^{b}	5.3^{b}	139.2^{b}
	b_2	315.7^{b}	4.61^{b}	19.0^{b}	58.3^{d}	36.9^{f}	5.4^{b}	126.5^{c}
	b_3	312.6^{b}	4.25^{e}	19.8^{a}	48.0^{f}	60.9^{a}	5.4^{b}	118.9^{c}
	b_4	317.0^{b}	4.32^{d}	19.7^{a}	61.6^{c}	55.3^{b}	1.4^{d}	97.2^{d}
	b_5	319.7^{a}	4.17^{f}	17.1^{c}	65.7^{a}	47.7^{c}	3.9^{b}	72.5^{e}
Average A_1		**313.3^{NS}**	**4.36^{B}**	**18.7^{A}**	**58.4^{B}**	**50.8^{A}**	**4.3^{NS}**	**110.9^{NS}**
a_2	b_1	313.0^{b}	4.60^{b}	16.0^{d}	59.7^{d}	51.0^{c}	5.1^{b}	133.0^{b}
	b_2	302.7^{c}	4.13^{f}	19.3^{a}	63.7^{b}	49.7^{c}	3.1^{c}	89.2^{d}
	b_3	312.0^{b}	4.85^{a}	17.6^{c}	59.6^{d}	43.1^{e}	2.2^{c}	79.6^{e}
	b_4	314.7^{b}	4.22^{e}	17.4^{c}	55.2^{e}	45.2^{d}	6.2^{a}	148.3^{a}
	b_5	317.3^{b}	4.89^{a}	15.0^{d}	64.2^{b}	47.6^{c}	4.1^{b}	95.4^{d}
Average A_2		**311.9^{NS}**	**4.54^{A}**	**17.1^{B}**	**60.5^{A}**	**47.3^{B}**	**4.2^{NS}**	**109.1^{NS}**
Average B_1		**307.2^{C}**	**4.54^{A}**	**17.0^{B}**	**59.1^{B}**	**52.1^{A}**	**5.2^{A}**	**136.1^{A}**
Average B_2		**309.2^{C}**	**4.36^{B}**	**19.1^{A}**	**61.0^{B}**	**43.3^{D}**	**4.3^{B}**	**107.9^{C}**
Average B_3		**312.3^{B}**	**4.55^{A}**	**18.7^{A}**	**53.8^{D}**	**52.0^{A}**	**3.8^{C}**	**99.3^{D}**
Average B_4		**315.8^{A}**	**4.27^{B}**	**18.5^{A}**	**58.4^{C}**	**50.2^{B}**	**3.8^{C}**	**122.7^{B}**
Average B_5		**318.5^{A}**	**4.53^{A}**	**16.1^{C}**	**64.9^{A}**	**47.6^{C}**	**4.0^{B}**	**83.9^{E}**

a_1- treatment with bacterial inoculant; a_2- treatment without bacterial inoculant; b_1- silage made from pure common vetch; b_2- silage made from 25 : 75 common vetch – oat mixture; b_3- silage made from 50 : 50 common vetch – oat mixture; b_4 - silage made from 75 : 25 common vetch – oat mixture; b_5 - silage made from pure oat; AA – acetic acid; BA – butyric acid; LA – lactic acid; Different letters denote significantly different means ($P< 0.05$)

The pH of silage with the inoculant was lower (4.36) than the pH of silage without inoculant (4.54). The highest pH values were determined in the silages of

pure common vetch, pure oat and 50:50 common vetch oat mixture. According to *Weissbach (1996)* pH values below 4.2 with 200 g DM kg^{-1} and below 4.45 with 300 g DM kg^{-1} are needed to obtain well-fermented and stable silage. Results of the present study comply with these requirements.

The ammonia nitrogen ratios in common vetch-oat silages were very high (18.7% ΣN in silage with bacterial inoculant and 17.1% ΣN in silage without inoculant). Depending on the seeding rate in the common vetch-oat mixture, the ammonia nitrogen ranged from 16.1% ΣN in pure oat silage to 19.1% ΣN in the 25:75 common vetch-oat silage. It implies on significant activity of proteolytic bacteria. Legume crops, such as vetch species have high protein and low carbohydrate content effect difficulties for fermentation of silage and these protein is rapidly degraded resulted in high ammonia nitrogen *(Balabanli et al., 2010).* So, protein can be inhibit acid to neutralize and prevent pH fallings. The presence of ammonia nitrogen in silages without or with very low level of butyric acid can be explained with the activity of plant enzymes *(McDonald, 1981).* Higher content of the soluble notrogen was recorded in silage without inoculant (60.5% ΣN) and differed significantly from the silage with inoculant (58.4% ΣN). In pure oat silage the ratio of soluble nitrogen of 64.9% ΣN is above the permitted value wich is 60% ΣN *(Ensilage, 1978),* whereas in all other silages ratio of soluble nitrogen was below or equal to the permitted value.

Table 2. Evaluation of silages quality

Factors		DLG method of evaluation		
A	**B**	**Score**	**Class**	**Quality**
a_1	**b_1**	46	I	Very good
	b_2	45	I	Very good
	b_3	46	I	Very good
	b_4	46	I	Very good
	b_5	43	II	Good
a_2	**b_1**	46	I	Very good
	b_2	47	I	Very good
	b_3	43	II	Good
	b_4	47	I	Very good
	b_5	43	II	Good

a_1- treatment with bacterial inoculant; a_2- treatment without bacterial inoculant; b_1- silage made from pure common vetch; b_2- silage made from 25 : 75 common vetch – oat mixture; b_3- silage made from 50 : 50 common vetch – oat mixture; b_4 - silage made from 75 : 25 common vetch – oat mixture; b_5 - silage made from pure oat

Bacterial inoculation caused high content of the lactic acid and the acetic acid, and low content of butyric acid content (Table 1). The lactic acid bacteria

may be classified as homofermentative or heterofermentative based on their by-products of sugar fermentation. Homofermentation gives only lactic acid as the end product of glucose metabolism. In heterofermentation equimolar amounts of lactic acid, carbon dioxide and ethanol or acetic acid are formed from glucose via the phosphoketolase pathway *(Tyrolová and Vyborná, 2011)*. In the present study, the added heterofermentative bacteria might have utilised water soluble carbohydrates more effectively than the homofermentative bacteria. The highest lactic acid conten was determined in pure common vetch silage (136.1 g kg^{-1} DM), followed by silage from 75:25 common vetch-oat mixture (122.7 g kg^{-1} DM). The lactic acid contents decreased as the DM content of the silage increased, with the exception of 75:25 common vetch-oat silage (Table 1). This is consistent with observations reported by *Muck et al. (2003)*. These authors suggest that a high DM content depresses the total amount of fermentation in silages, resulting in a higher final pH and lower concetration of fermentation acids, particularly lactic acid. In this study, quality classification of the silages by DLG scores resulted that silages prepared from common vetch-oat mixtures have very good or good quality (Table 2).

Conclusion

According to the results in performed investigations, it could be concluded that bacterial inoculant application did not significantly have an effect on fermentative process during ensilaging of common vetch-oat mixtures. Only the amount of soluble nitrogen in the treatment with inoculant was below permitted value, while the amounts of ammonia nitrogen were much higher than permitted value, and it implies on significant activity of proteolitic bacteria. However, obtained results show that silage from 75:25 common vetch-oat mixture had the lowest pH, ammonia nitrogen and buturyc acid content and the highest content of lactic acid. And according to the DLG method of evaluation almost all silages achieved very good and good quality. Common vetch-oat mixtures could be successfully ensiled with and without bacterial inoculant.

Uticaj primene bakterijskih inokulanata i strukture smeše na kvalitet silaže grahorice i ovsa

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Rezime

Istraživanje je sprovedeno da bi se procenila mogućnost siliranja smeša grahorice i ovsa posejanih u pet različitih odnosa. Ispitivana su dva faktora: udeo semena grahorice i ovsa u smeši i primena inokulanta pri siliranju. Sadržaj suve materiije, pH, sadržaj amonijačnog i rastvorljivog azota, kao i sadržaj sirćetne, buterne i mlečne kiseline je utvrđen u silaži. Za ocenu kvaliteta silaže je korišćena DLG i metoda po Weissbach-u. Ogled je postavljen na eksperimentalnom polju Instituta za krmno bilje u Kruševcu, Srbija, i ispitavanja su obuhvatila pet različitih smeša: čist usev grahorice, 25% grahorice + 75% ovsa, 50% grahorice + 50% ovsa, 75% grahorice + 25% ovsa i čist usev ovsa. Rezultati suobrađeni kao dvofaktorijalni ogled, analizom varijanse korišćenjem modela koji objašnjava uticaj structure smeše i primene inokulanta na kvalitet silaže. Primena bakterijskog inokulanta je uzrokovala veći sadržaj amonijačnog azota i sirćetne kiseline ($P< 0.05$), ali niži sadržaj rastvorljivog azota. Smeša u kojoj je odnos grahorice i ovsa bio 75:25 sadržala je najveći udeo mlečne kiseline i najmanji udeo buterne kiseline. Sadržaj suve materije, pH i amonijačnog azota je bio sličan u svim silažama i kretao se od 307,2 do 318,5 g kg^{-1}, od 4,27 do 4,54 i od 16,1 do 19,1% ΣN, respektivno. Na osnovu ocene kvaliteta silaže prema DLG i Weissbach metodi utvrđen je približan kvalitet ispitivanih silaža.

Ključne reči: silaža grahorice i ovsa, kvalitet fermentacije

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