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EFFECT OF SUPPORTING CULTURE AND INOCULANT ON QUALITY OF COMBINED VETCH SILAGES WITH OATS AND ITALIAN RYEGRASS

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Abstract

The biomass of mixture of winter vetch K-10 and supporting crops (oats and Italian ryegrass) were ensiled in Institute for forage crops in 2012. Ensilaging was conducted in the form of two factorial experiment 2 x 2 in three replications: the first factor A – type of supporting culture (a_1 - oats, a_2 - Italian ryegrass) and the second factor B - application of bacterial inoculants (b_1 - no inoculant, b_2 - with the addition of inoculant). The suitability for ensiling and the chemical composition of the initial material and silages were established by laboratory methods, and the energy value of the silages (NE_L and NE_M) was calculated by computation. The lactic acid fermentation process was monitored via the level of dry matter content, pH, ammonia and soluble nitrogen and lower fatty acids. Silages were evaluated/scored according to the methods of Deutsche Landwirtschafts-Gesellschaft (DLG) and Flieg. The biomass of Italian ryegrass is more suitable for silage because it has a better WSC/BC ratio. It was found that the type of supporting culture significantly influenced the level of CP in silages, with a mixture of vetches and Italian ryegrass, compared to the mixture with oats had by 41.5 gkg^{-1}DM more CP (171.9:130.4), but at the same time had significantly higher crude fat content, Ca and P. The inoculants had no significant impact on changes in the chemical composition of silage, except for the reduction of the crude fat content and increase of BEM. Italian ryegrass contributed to greater production of lactic and acetic acids compared to the oats. The inoculant had a significant effect on the decreased production of butyric acid in average by about 6.0 gkg^{-1} (7.85:1.86). All silage treatments with inoculant, according to the methods of DLG and Flieg, were given/scored more points in relation to the treatment without inoculant.

Key words: *silage, vetches, inoculant, fermentation, proteins*

Introduction

One of the most common problems of modern animal husbandry is the continuous provision of sufficient quantities of high-quality plant proteins in animal nutrition. The fastest and easiest way to meet the protein needs of animals in the diet is the use of large amounts of concentrated feed, but this way is also the most expensive, which greatly affects the profitability of livestock production. These expensive proteins in concentrated feed can be very effectively replaced through production of quality fodder legume crops in the form of hay, haylage or silage. One of the potential solutions to partially overcome this problem and compensate a substantial portion of protein in the diet of animals is cultivation of annual forage legumes as a source of high quality plant protein. Among the most important crops in the group of annual forage legumes are winter and spring varieties of vetches, whose cultivation is of great importance generally in agriculture in and livestock sector from the point of feeding of farm animals. There are numerous ways of utilization of vetch biomass from the use as green forage to the form of hay, haylage, silage and straw. However, unlike some other annual legumes, vetch can be used for grazing (Mikić et al., 2006). In addition,

economic and ecological aspects of growing vetch are very important. The vetch legume species supplying soil with a certain amount of nitrogen through nitrogen fixation, which provides part of the need for this mineral nutrient for cultivation of the subsequent crops, which reduces the use of expensive nitrogen fertilizers.

In animal nutrition, vetch as roughage can be used in several ways, but the best way recommended is ensiling of vetch in mixtures with small grains. The justification for this attitude is reflected in the fact that during the ensiling of vetch the losses of the highest quality plant parts (leaf, husk) are the lowest, and therefore the loss of nutrients is lower than in preserving by drying in the form of hay. Well prepared silage can be used during the entire period of the year, thus avoiding frequent change of diet composition which is a very important fact in ruminant nutrition. Earlier mowing of green biomass in the flowering stage or early forming of the first pod, the sowing of subsequent crops (e.g. corn of short growing season) is enabled so that the same plot can be used two times in one productive year for the production of animal feed.

Results of some studies suggest that it may still get relatively good quality silage from pure vetch crops with the prior wilting (Muck and Gostiša, 1963-b), with the use of inoculants based on lactic acid bacteria, or chemical preservatives (Đorđević and Dinić, 2007). In order to obtain a good quality silage, the last few years has been working on the application of bacterial inoculants containing selected homo and heterofermentative strains of lactic acid bacterial cultures, aimed at better utilization of sugars from biomass ensile and directing the flow of fermentation in the right direction, towards reducing the content of harmful, such as butyric acid to increase the content of lactic or acetic acid.

Vetch can be grown as a pure culture, but because of the tendency towards flattening and deterioration due to rotting of the highest quality plant parts, it is commonly grown in mixtures with some supporting crop such as small grains (Karagić et al., 2011). In this way the flattening and decay are reduced, but a biomass is obtained more suitable for conservation/preservation by method of ensiling due to higher content of easily fermentable sugars and lower buffer capacity in relation to the pure vetch crop.

The aim of this study was to investigate the effect of type of supporting culture and inoculant on the quality and process of lactic acid fermentation of vetch silage combined with oats and Italian ryegrass.

Materials and Methods

The experiment was conducted in 2012 at the Institute of forage crops, Kruševac (Serbia). Mixtures of winter vetch K-10 with oats, and Italian ryegrass were sown on a plot of 4 m^2 in three replications. The biomass was harvested when the vetch was in the phase of forming the first pod. The yield of biomass, suitability for ensilaging, botanical composition of mixture and quality of starting material was determined. The biomass is ensiled in plastic containers volume 65dm^3. Experiment was conducted as two-factorial 2 x 2 in three replications. The first factor A – type of supporting culture (a_1 - oats, a_2 - Italian ryegrass) and the second factor B - application of bacterial inoculants (b_1 - no inoculant, b_2 - with the addition of inoculant).

As inoculant, BioStabil Plus of Austrian company Biomin was used. The inoculant contains homo fermentative lactic acid bacteria (*Lactobacillus plantarum* i *Enterococcus faecium*) and hetero fermentative lactic acid bacteria (*Lactobacillus brevis*). These bacteria provide better use of water-soluble carbohydrates (WSC) in the biomass, optimal pH and aerobic stability of silage after opening the silo. Opening of experimental containers and sampling of silage was done 90 days after ensiling. Chemical analyses of the starting material and silage were performed in the laboratory of the Institute for Forage Crops, Kruševac, according to standard methods (AOAC, 2002).

In starting material, parameters of biomass suitability for ensilage- buffer capacity (BC), monosaccharides (MS) and water-soluble carbohydrates (WSC) were determined using laboratory methods. Also, in starting material and silage, the chemical composition was determined using analytical methods. Chemical composition analyses included dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), NDF, ADF, Ash, Ca, P, and NFE values were calculated. The chemical analyses in silage of the process of lactic acid fermentation were performed, where pH, ammonium (NH_3-N) and soluble nitrogen (H_2O-N), content of organic acids, acetic, butyric and lactic acids were determined. The quality of silage was evaluated by the methods DLG and Flieg (Đorđević and Dinić, 2003). Energy values (NE_L i NE_M) were calculated using methods by Obračević (1990).
The analysis of variance was conducted using the results of chemical analysis of silage quality. The statistically significant differences for all tested parameters between treatments were tested by LSD test (Statsoft, 2006).

Results and discussion

By cutting green forage mixture in the stage of forming of first vetch pods, cultivar K-10 the average yield of mixtures, the share of the components in biomass and their chemical composition were determined. It was found that the average realized yield of green mass of the mixture of vetch with oats was 50.2 t tha^{-1} (13.90 tha^{-1} DM), with the share of vetch, in percentages, in relation to the oats observed in the green mass was 59:41, i.e. observed in DM 63:37. Mixtures with Italian ryegrass had an average yield of 42.5 tha^{-1} green mass (11.09 tha^{-1} DM) with an average ratio of vetch to Italian ryegrass 68:32 in green biomass, i.e. 71:29 observed in DM.
The chemical analysis of the starting material of cut biomass has shown that the level of crude protein (CP) in a mixture with the Italian ryegrass was relatively higher by 39.7 gkg^{-1}DM (170.9:131.2) compared to a mixture with oats, whereas in absolute terms, the achieved level of protein per hectare was higher by as much as 72 kg (1823:1895 $kgha^{-1}$). Chemical analyses of biomass have shown that the level of CP in mixtures with Italian ryegrass was, relatively, higher by 39.7 gkg^{-1}DM (170.9:131.2) compared to the mixture with oat. Slightly lower values of CP 119.1 gkg^{-1}DM in the mixture of vetch and oats in a similar biomass ratio of 58:42 has been obtained by Lithourgidis et al. (2006). In both mixtures somewhat higher level of CF was observed from 380.4 to 387.1 gkg^{-1}DM which can be interpreted as the response of crops to prolonged drought and extreme weather conditions in 2012. Other chemical parameters of both mixtures were quite uniform, and one could see that the mixture of Italian ryegrass had more EE 43.7:30.8 gkg^{-1}DM, while the mixture with oats was richer in calcium by about 2.3 gkg^{-1}DM (Table 1).

Table 1. Suitability for ensilage and chemical composition of starting material gkg^{-1}DM

The starting material	**Buffer capacity meq Lactic acid (LA) 100g^{-1} DM**			**Monosaccharides gkg^{-1}DM**		**WSC gkg^{-1}DM**		**Ratio WSC / BC**	
Vetch K-10	82.29			46.60		59.40		0.72	
Oat	63.08			66.70		75.60		1.19	
Lolium	31.61			64.00		85.50		2.71	
The chemical composition of starting material gkg^{-1}DM									
Mixture	DM	CP	CF	EE	Ash	NFE	ADF	NDF	Ca	P
Vetch / lolium	261	170.9	387.1	30.8	102.4	308.8	505.5	630.3	9.5	3.9
Vetch / oat	277	131.2	380.4	43.7	105.9	338.8	481.5	699.7	7.2	3.2

The analysis of the individual components of the mixture showed that vetch as a main crop in the mixture had a high BC 82.29 meq LA100g^{-1} DM and low level of WSC 59.40 gkg^{-1}DM. This ratio WSC/BC of only 0.72 is unfavourable for silage, so it is not possible to obtain high-quality silage from pure vetch crop, which is consistent with the statements of Dinić et

al., (2008). Supporting cultures (oats and Italian ryegrass) have significantly lower BC and higher level of WSC resulting in more favourable WSC/BC ratio (1.19 and 2.71 respectively). The results of WSC/BC ratio in Italian ryegrass, although favourable, are significantly lower than the results that are obtained by Dinić et al. (2002), which may be due to slightly later harvesting or influence of weather conditions on the chemical composition and content of certain substances in the biomass of Italian ryegrass. Consequently, the resulting mixtures of vetch with oats, especially with Italian ryegrass had significantly better WSC/BC ratio compared to a pure vetch crop.

The results of the chemical composition of silages are shown in Table 2. It was established that the type of supporting culture significantly influenced the level of CP in the silage, where the mixture of vetch and Italian ryegrass had more CP by 41.5 gkg^{-1}DM in relation to the mixture with oats (171.9:130.4) as a result of a greater share of oats, and a higher CP content in the biomass of Italian ryegrass. The achieved level of CP in the mixture with oats of 131.4 gkg^{-1}DM was significantly lower than the values stated by Mikić et al., (2006).

Table 2. The chemical composition and energy values (NE_L i NE_M) of silage gkg^{-1}DM

Mixture	Inoculant	CP	EE	CF	NFE	NDF	ADF	Ash	Ca	P	NE_L	NE_M
(a_1) Vetch/ Oat	(b_1)Without	128.7	41.0	387.0	336.9	676.6	493.7	106.4	7.27	3.10	4.65	4,40
	(b_2)With	132.1	33.4	384.2	348.1	669.7	503.0	102.2	6.60	2.80	4.66	4.40
(a_2) Vetch/ Lolium	(b_1)Without	172.7	56.1	378.4	277.4	603.8	490.9	115.3	8.60	4.03	4.37	3.99
	(b_2)With	171.1	37.0	376.6	308.5	601.2	497.5	106.8	9.77	4.07	4.33	3.95
Average for A_1		**130.4^b**	**37.2^b**	**385.6^a**	**342.5^a**	**673.2^a**	**498.4^a**	**104.3^a**	**6.94^b**	**2.95^b**	**4.65^a**	**4.40^a**
Average for A_2		**171.9^a**	**46.6^a**	**377.5^a**	**292.9^a**	**602.5^b**	**494.2^a**	**111.1^a**	**9.18^a**	**4.05^a**	**4.35^b**	**3.97^b**
Average for B_1		**150.7^a**	**48.6^a**	**382.7^a**	**307.1^b**	**640.2^a**	**492.3^a**	**110.8^a**	**7.94^a**	**3.57^a**	**4.51^a**	**4.20^a**
Average for B_2		**151.6^a**	**35.2^b**	**380.4^a**	**328.3^a**	**635.5^a**	**500.3^a**	**104.5^a**	**8.18^a**	**3.44^a**	**4.49^a**	**4.18^a**

Significance LSD 0,05

Italian ryegrass, as a supporting crop was directly influenced by the chemical composition of silage, so in this mixture significantly higher EE, Ca and P were observed, i.e. decreased values of NFE and NDF compared to the mixture with oats. The inoculant had no significant impact on change in the chemical composition of silage, except on the decrease of EE and increase of NFE. Type of supporting culture and inoculant did not have a significant impact on changes in the content of CF, ADF and Ash. The energy value of the silage was significantly different depending on the type of supporting culture and its share in the mixture, so the NE_L and NE_M values of the silage made from mixture with oats were significantly higher than in the silage obtained from mixtures with Italian ryegrass. This may be explained by partial participation of milky-waxy oats grain in the biomass, which increased the energy value of oats as a supporting culture.

Table 3. The parameters of the fermentation process in silages

Mixture	Inoculant	DM gkg^{-1}	pH	% NH_3-N/pN,	% H_2O-N/pN,	Acetic acid gkg^{-1}DM	Acetic acid %TA	Butyric acid gkg^{-1}DM	Butyric acid %TA	Lactic acid gkg^{-1}DM	Lactic acid %TA
(a_1) Vetch/ Oat	(b_1)Without	286.7	5.07	18.51	63.71	55.73	40.1	6.83	4.9	76.40	55.0
	(b_2)With	280.0	4.77	12.94	51.14	57.73	45.0	0.03	0.1	70.50	54.9
(a_2) Vetch/ Lolium	(b_1)Without	266.7	4.87	17.66	61.72	72.23	40.7	8.87	5.0	96.37	54.3
	(b_2)With	270.0	4.56	14.73	50.63	92.93	45.4	3.70	1.8	107.97	52.8
Average for A_1		**283.4^a**	**4.92^a**	**15.73^a**	**57.42^a**	**56.73^b**	**-**	**3.43^a**	**-**	**73.45^b**	**-**
Average for A_2		**268.4^b**	**4.71^a**	**16.19^a**	**56.18^a**	**82.58^a**	**-**	**6.28^a**	**-**	**102.17^a**	**-**
Average for B_1		**276.7^a**	**4.97^a**	**18.09^a**	**62.72^a**	**63.98^a**	**-**	**7.85^a**	**-**	**86.38^a**	**-**
Average for B_2		**275.0^a**	**4.66^a**	**13.84^b**	**50.88^b**	**75.33^a**	**-**	**1.85^b**	**-**	**89.23^a**	**-**

Significance LSD 0,05., %TA- share of total acids

Based on the analysis of the parameters of the fermentation process (Table 3), we can conclude that both these mixtures contained relatively satisfactory average level of dry matter (268.4 to 286.7 g/kg), with a significantly higher dry matter content in the mixture with oats

which can be interpreted as the result of different rates of maturation of supporting crops (oats and Italian ryegrass) in mixtures with vetch. The addition of inoculant did not significantly affect the changes of dry matter in silage. The degree of acidity in all tested treatments varied in the range typical for this type of silage from 4.56 to 5.07, which is similar to the results for pH that were obtained by Kasapović et al. (1994) for silage mixture of vetch and oats. Type of supporting culture and adding the inoculant had no significant influence on the change in pH in any of the treatments. Noticeable are the relatively lower pH values of the treatments with the addition of the inoculant compared to the treatments without it (4.66:4.97), indicating the necessary application of the inoculant in the process of ensiling of biomass. The mixture with Italian ryegrass had slightly lower pH, i.e. more acidic silage compared to the mixture with oats (4.71:4.92), indicating that the biomass of Italian ryegrass had a better WSC/BC ratio. The inoculant has contributed to the reduction of protein degradation in the process of ensiling and obtained significantly lower values of ammonia nitrogen and water soluble nitrogen in the silage with the addition of the inoculant (Table 3). The biomass of supporting crops had no significant effect on the concentration of ammonia- and soluble nitrogen expressed as total nitrogen. The type of supporting culture had a significant impact on the production of lactic and acetic acids in the silages, hence, in the silage mixture with Italian ryegrass, in average, more acetic acid by 25.85 gkg^{-1}DM was produced (82.58:56.73), or more lactic acid by 28.72 gkg^{-1}DM (102.17:73.45) in relation to the silage obtained from the mixture of oats. Larger production of acids in a mixture with the Italian ryegrass may be due to somewhat lower levels of dry matter in the starting material, but also a more favourable ratio of the total sugars, and the buffer capacity of the supporting crop relative to the oats. Significantly lower production of butyric acid was observed in silage with the addition of inoculant in relation to silage without inoculant (1.85:7.85) gkg^{-1}DM, which had positive effects on the quality of silage. This trend of decreasing production of butyric acid is a consequence of increase of the number and of the competitiveness of homofermentative and heterofermentative lactic acid bacteria compared to butyric bacteria, and of better utilization of easy fermentable sugars by lactic acid bacteria.

The quality of silages was assessed by methods according to Flieg and DLG (Table 4), which are based on an assessment of the relative shares of lactic, acetic and butyric acids in the total acid content, whereas DLG method, as a relevant parameter for the assessment, takes into account the pH value of silage (Đorđević and Dinić, 2003).

Table 4. The silage quality assessed

Mixture	**Inoculant**	**FLIEG**		**DLG**	
		Poeni	**Klasa**	**Poeni**	**Klasa**
(a_1) Vetch / Oat	(b_1) Without	65	II	36	III
	(b2) With	80	I-II	39	II
(a_2) Vetch / Lolium	(b_1) Without	65	II	37	II
	(b_2) With	70	II	39	II

According to the Flieg method, all treatments were evaluated and categorized as II class - good quality, with silage of vetch with oats with the addition of inoculant, according to the number of points, is on the border between very good and good quality - I and II classes. Both treatments with the inoculant had more points compared to the treatments without inoculant (80:65 mixtures with oats, 70:65 mixtures with Italian ryegrass). Rating method according to DLG, placed treatments without the inoculant on the verge between class III (36 points) and class II (37 points) - satisfactory and good quality. Treatments with inoculants were rated class II, with both treatments with inoculants receiving 39 points.

Conclusion

Vetch as an annual forage legume can be successfully grown and ensiled in combination with oats and Italian ryegrass as a supporting culture, to provide a relatively good quality silage. Ensiling of such mixtures reduces the nutrient losses in relation to the method of preserving by drying, and the quality obtained is similar to the quality of the starting material. In this way, a significant portion of plant proteins needed to meet the needs of specific types and categories of animals can be provided. Chemical composition and content of certain nutrients, especially protein, depends largely on the type of supporting culture. Adding bacterial inoculants to the biomass significantly affects the increase of competitiveness of lactic acid bacteria in relation to butyric bacteria, and thus to the greater production of lactic and acetic acids, and reduced production of butyric acid.

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