Original paper

THE EFFECT OF ADDITION OF LUCERNE BIOMASS AND NPN SUBSTANCES ON QUALITY OF GRAPE POMACE SILAGE

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Abstract

Grape pomace (GP) of white varieties without stalks was ensiled by the method of single factor trial in the two treatments: in the first treatment, with the addition of lucerne biomass (L) of the last cut, which was harvested at the stage of forming pods with equal shares in the weight ratio and in the second with the addition of Benural S (B) in the amount of 1%. Chemical analyses were conducted on GP and L to determine the suitability of biomass for silage and to determine the chemical composition and nutritional value and the process of lactic acid fermentation. It has been found that the biomass of GP had 2 times higher concentration of water-soluble carbohydrates (WSC 140.5:69.4 $gkg^{-1}DM$) relative to L, and a lower buffer capacity (BC) what makes it 10 times more favourable for ensiling (ratio WSC/BC 13.1 : 1.3). GP, as compared to L, had lower contents of CP, CF, and a lower nutritional value expressed in NE_L and NE_M units and a higher content of crude fat. Silage with the equal share of GP + L compared to silage with GP + B had a slightly lower CF and significantly less crude fat and ash, especially Ca. More favourable ratio Ca : P (2.93 : 1) was established in silage GP + L compared to 10.1:1 silage GP + B. Silages GP + L in the fermentation process were scored/rated one class higher according to the DLG and Zelter assessment methods, compared to the silages GP + B.

The aim of the study was to investigate the possibility of ensiling grape pomace with equal proportion of biomass of lucerne and added NPN substances and to determine the detailed chemical composition, nutritive value and silage quality on the basis of the process of lactic acid fermentation.

Key words: *Benural S, ensilability, grape pomace, lucerne, NE_L and NE_M, silage quality*

Introduction

Providing feed for ruminants in Serbia has been a growing problem in recent years, both because of rising prices of production inputs (oil, fertilizers, pesticides) and climate changes and droughts in the years 2012 and 2013. Decreased production of animal feed can cause a reduction in the number of ruminant animals (cattle and sheep) and therefore reduction in the production of milk and meat.

This problem of lack of fodder can be partly alleviated by using additional products of food industry (sugar, beer, alcohol, juice, etc.). By-products are usually characterized by a high water content, rapid fermentation and perishability and present real environmental problems because they pollute water and soil (Nikolić and Jovanović, 1986). Dehydration of these products requires a large amount of energy and it is economically unprofitable. Some by-products can be used as raw material for making the compost, but the most cost effective and sustainable solution is processing and preservation/conservation so that they can be used as part of the animal diet.

GP, as a by-product of wine and fruit juice industry, is a major problem for storage and for the environment. Large quantities of obtained pomace, hundreds of tons annually, must be stored in a safe, environmentally friendly, sustainable and cost-effective manner. One way that meets the aforementioned criteria is its preservation and the use as silage for animal nutrition.

GP is the residue produced by pressing the grapes and juice extraction and consists mainly of fruit skin, seeds, insoluble fruit parts and of the juice remains. Approximately 22-25 % of fresh pomace is obtained from the total amount of grapes, and it contains 40-50 % dry matter (Pavličević et al., 1988). The content of nutrients in the GP is variable and depends on the grape variety, processing technology, climate and soil conditions of production. In nutrition of ruminants, fresh, dried and ensiled grape pomace can be used. It is not possible to use fresh pomace as animal feed in the long run due to rapid fermentation and spoilage. In previous years, dried GP was used in the nutrition of ruminants, primarily for cattle fattening (Nikolić et al., 1980; Zeremski, 1982, Stojanović et al., 1989), but today this kind of feed is not cost effective because of the high cost of energy required for dehydration. For this reason ensiling GP becomes the most economical solution for its long-term storage and use.

Considering that the GP is a typical carbohydrate feed, the goal of this study was to determine the possibility of increasing the CP content in order to optimize the nutrition of ruminants. The best solution is to add biomass of perennial legumes, but the problem occurs at the time when grape is processed (September and October) when there is very little lucerne. Another possibility, extremely simple and cheap, is to add a non-protein source of nitrogen to the silage or diet. Symbiotic bacteria in rumen use this nitrogen for the synthesis of their own proteins of high biological value, which will be digested and utilized in the small intestine of ruminants together with protein in the diet (Đorđević and Dinić, 2011). Potential problem for the using value of GP silage could be insufficient aerobic stability due to the very broad WSC and BC ratio and improper unload and low daily consumption.

Material and methods

GP of white varieties (Rkatsiteli and Chardonnay) without stalks was taken from the production facility "Rubin" Kruševac immediately after pressing and was ensiled according to the method of single factor trial in the two treatments a) with the addition of lucerne biomass (L) of the last cut, which was harvested at the stage of forming pods with the weight ratio of 1:1, and b) with the addition of Benural S (B) in the amount of 1%. Ensiling was carried out on September 15th 2012, on a private farm in the village of Pepeljevac in experimental containers holding 130 litres.

Benural S containing 42 % of urea, 56 % of bentonite and 2 % of sulphur was used as the NPN substance. Bentonite allows slower release of ammonia in the rumen, and thereby a more efficient utilization by rumen micro-organisms. It binds gases and toxic substance and contains some important elements of the alkali (K, Na, Mg etc.). Sulphur from Benural S enables more efficient synthesis of amino acids methionine and cystine.

The parameters relevant to the suitability of biomass for silage were determined in the initial material. WSC were determined colorimetrically by the method of DuBois et al. (1956), while BC was determined according to the method of Weissbach (1967). The following parameters were determined in GP and silage: content of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), NDF, ADF, ash and content of macro elements (Ca and P).

The samples of silage were taken 90 days after ensiling, and at the same time the chemical analysis of the process of lactic acid fermentation was performed. The degree of acidity (pH) and the content of soluble nitrogen and ammonia, acetic, butyric and lactic acids were determined in the silage. Chemical analyses were conducted in the laboratory of the Institute of Forage Crops, Kruševac, according to the standard methods (AOAC, 2002). In the assessment of the quality of silage, three methods were applied: DLG, Zelter and Weissbach (Đorđević and Dinić, 2003). The results of chemical analysis were processed by the analysis of variance while the statistical significance of differences was tested using the LSD test.

Results and discussion

The suitability of the initial material for ensiling

In order to ensure successful lactic acid fermentation and stable silage without the presence of butyric acid, the WSC/BC ratio should be equal to or greater than 3.0 (Weissbach, 1967). The tested silages showed favourable share of DM (380 gkg^{-1}), low BC (0.7 meq LA - lactic acid100g^{-1}DM), high concentration of monosaccharides (117 gkg^{-1}DM) and WSC (140.5 gkg^{-1}DM) and a very favourable WSC/BC ratio (13.12) (Table 1). Such favourable WSC/BC ratio in GP suggests that it may be successfully ensiled and can serve as a supplement to biomasses that are difficult for ensiling, such as perennial legumes. However, silages made from easy ensiled biomasses can often subsequently ferment due to residual WSC in the silage and insufficient generated fungicidal acids (acetic, propionic, etc.). In contrast to GP, withered lucerne biomass is extremely poor in monosaccharides and WSC, and has an unfavourable SC/BC ratio (1.32), indicating that it cannot be successfully ensiled on its own. Wilted lucerne biomass has a high content of DM as a result of wilting and mowing at the stage of forming the pods. The suitability of the lucerne biomass for ensiling in this study is slightly greater than in the research by Dinić et al. (2012). The results of these studies show WSC content in the GP decreased by 28.5 gkg^{-1}DM (169 gkg^{-1}DM) compared to the results of Alipour and Rouzbehan (2007) and more than 2 times lower than in the studies of Zheng et al. (2012), which could be interpreted as a result of favourable climatic conditions and varietal characteristics.

Table 1. *Parameters of suitability of grape pomace and lucerne biomass for ensiling*

Biomass	DM, gkg^{-1}	BC, meq LA100g^{-1} DM	Monosah. gkg^{-1}DM	WSC gkg^{-1}DM	Ratio WSC/BC
Alfalfa wilted	410	52.4	42	69.4	1.32
Grape pomace, fresh	380	10.7	117	140.5	13.1

Legend: DM – dry matter; BC – buffer capacity; LA – lactic acid., WSC – water-soluble carbohydrates

Chemical composition and nutritive value of the initial material and silage

Chemical composition and nutritional value of organic matter of the initial material of GP and lucerne biomass harvested in the last stage of pod formation were different in all tested parameters particularly in the content of ether extract (92.1 : 18.2 gkg^{-1}DM) what can be interpreted by the high concentration of oil in the grape seeds released after milling with a sieve (Table 2).

It is considered that fat present in ruminant diet containing GP or GP silage is not available to animals because it is imprisoned in seeds. A significant difference in the content of CP in favour of lucerne (29 gkg^{-1}DM) was determined, as well as nutritional value expressed in NE_L and NE_M units, also in favour of lucerne. Statistically significant differences were determined between silages for all tested parameters, except for the content of CP and CF. Similar values for the concentration of CP and CF were the result of late cutting of lucerne. Had the cutting of lucerne been done in a timely manner (at budding stage) the concentration of CP, minerals and nutritional value would have been higher and the content of structural carbohydrates significantly lower (Dinić et al., 1997).

Higher NFE, NDF, ADF values and nutritional value expressed by NE_L and NE_M were determined in silage GP + L (Table 2). Lower nutritive value of silages GP + B was the result of lower digestibility coefficients, primarily high share of lignin in biomass and silage from white grape pomace (Zalikarenab et al., 2007).

Table 2. *Chemical composition and nutritive value of the initial material and silages, gkg^{-1} DM*

Treatments	CP	CF	EE	NFE	NDF	ADF	NE_L, MJ kg^{-1}SM	NE_M, MJ kg^{-1}SM
	The starting material							
GP	128.4	330.6	92.1	395.9	625.6	590.4	2.87	2.17
L	157.4	360.1	18.2	371.2	560.4	470.7	4.57	4.29
	Silage							
GP+L	141.9a	339.2a	60.76b	403.2a	621.7a	586.0a	3.69a	3.13a
GP+B	145.a	331.4a	94.9a	332.2b	573.3b	503.6b	2.74b	2.06b
LSD 0.05	3.74	8.97	6.72	13.09	23.59	9.04	0.09	0.08

GP– grape pomace; L–alfalfa biomass; B–benural S; CP– crude protein; CF–crude fiber; EE – ether extract

The concentration of mineral substances in the silage with the equal shares of GP + L (54.9 gkg^{-1}DM) is almost two times lower than in silage GP + B (96.1 gkg^{-1} DM) and this difference was statistically significant (Table 3). This difference was the result of high concentrations of bentonite, urea and sulphur in Benural S. In addition, a higher concentration of Ca in silage GP + B was determined, as well as less favourable (broader) Ca : P ratio.

Table 3. *Concentration of mineral substances in the initial material and silages, gkg^{-1} DM*

Treatments	Ash	Ca	P	Ratio Ca:P
The initial material				
Grape pomace	53.0	5.0	2.6	1.92
Alfalfa	93.1	14.1	2.5	5.64
Silage				
Grape pomace + Alfalfa	54.9b	7.33b	2.5a	2.93b
Grape pomace + Benural S	96.1	24.5a	2.43a	10.11a
LSD 0.05	10.46	2.33	0.19	1.71

Concentration of calcium of 7.33 gkg^{-1} DM in the silage GP + L can meet cows\` nutritional needs according to NRC recommendations (1989 and 2001) (0.43-0.66% and 0.57-0.67%), and Đorđević et al. (2009), while the concentration of P of 2.5 gkg^{-1}DM did not meet the requirements. The concentration of P in the dry period of cows should be 0.3-0.4% and during lactation from 0.38 to 0.55% in the dry matter of the ration. The concentration of Ca in the silage GP + B was three times higher compared to the cows' requirements.

Biochemical changes occurring during ensiling process

It was established that very good silages were obtained on the basis of sensory perception expected on the basis of the advantages of GP for ensiling (WSC/BC ratio 13.1), which contributed to the successful ensiling of lucerne. High DM content, favourable H values, favourable share of ammonia and soluble nitrogen were established particularly in silage GP + L, the absence or traces of butyric acid and a favourable ratio of lactic and acetic acids (Table 4). It is a well-known fact that DM levels above 300 gkg^{-1} can prevent the activity of butyric acid and other undesirable bacteria that cause decay and spoilage of silage and to reduce the losses in the form of effluent - silage juice (Ensilage, 1978, Đorđević and Dinić, 2003), which was confirmed by the results of the present study.

Table 4. *Parameters of biochemical changes in silages*

Treatments	DM	pH	% NH_4-N /ΣN	% H_2O-N /ΣN	Acids in silage					
					Acetic		Butyric		Lactic	
					gkg^{-1}DM	% u TA	gkg^{-1}DM	% u TA	gkg^{-1}DM	% u TA
GP+L	413.3a	3.80b	2.89b	23.77b	24.6b	37.19	0.0b	0.00	41.3b	62.81
GP+B	353.3b	4.98a	10.01a	60.71a	37.5	42.48	0.77a	0.87	50.2a	56.65
LSD 0,05	52.35	0.255	1.69	9.37	6.84	-	0.092	-	8.55	-

The degree of acidity (pH 3.80) of silage GP + L was at the level of acidity which can be realized in whole maize silage or silage with easy ensiled plant material and was evaluated as the maximum score for this parameter, as well as the share of ammonia nitrogen in relation to total nitrogen (%NH_4-N/NΣ) and water-soluble in relation to total nitrogen (%H_2O-N/ΣN). The silage GP + B was found to have higher values of pH (lower acidity) as well as the share of NH_4-N/ΣN and % H_2O-N/ΣN which had negative impact on its quality. Most of the methods for assessing the quality of silage allocate maximal number of points for the pH in the range of 3.5 to 4.8 (depending on the level of dry matter), for the NH_4-N/NΣ up to 10%, for the H_2O-N/ΣN up to 50%, butyric acid up to 4kg^{-1}DM of silage, and acetic acid up to 40% of relative share in relation to the total acid (Ensilage 1978).

Table 5. *Evaluation of the silage quality by various methods*

Treatments	DLG		ZELTER		WEISSBACH	
	Points	Class	Points	Class	Points	Class
GP + L	47	I	16	II	70	II
GP + B	39	II	14	III	65	II

In order to assess the quality of silage three methods were used: DLG, which is based on the degree of acidity and the relative proportion of lactic, acetic and butyric acids, Zelter's method which assesses the percentage of NH_4-N/NΣ and the content of acetic and butyric acids in fresh silage, and Weissbach method, which scores the content of butyric acid gkg^{-1}DM, % NH_4-N/ΣN, pH in relation to the level of DM, and points are deducted, if the total content of acetic and butyric acids is in the excess of 40 gkg^{-1}DM and based on share of moulds in silage. The results of quality evaluation of silage are shown in Table 5. The silage GP + L in relation to the silage GP + B was scored one class higher based on the DLG and Zelter methods, while according to Weissbach method, it was classified into the same class, but with a greater number of points (70:65).

Conclusion

On the basis of the conducted research on ensiling GP with biomass L from final cut in a weight ratio of 1:1 and ensiling GP with 1% Benural S the following can be concluded:

GP in relation to L has 5 times lower buffer capacity (10.7: 52.4 g LA/100gDM), 2 times higher content of WSC (140.5:69.4gkg^{-1}DM), i.e. 10 times more suitable for ensiling compared to lucerne (WSC/BC ratio = 13.1:1.32).

Biomass L compared to the GP had a higher content of CP, CF, nutritional value expressed in NE_L and NE_M units, higher mineral content and unfavourable (broader) ratio of Ca : P.

Silages GP + L compared to silages GP + B had higher values for NDF, ADF, and higher nutritional value and lower value for the content of EE, ash and Ca.

Silage GP + L compared to silages GP + B had more favourable values for the acidity (pH), for the lower values of ammonia and soluble nitrogen in relation to total nitrogen, and more favourable relative share of acids, i.e. were scored one class higher based on DLG and Zelter's methods.

The general conclusion is that the GP can be successfully ensiled together with lucerne biomass to provide somewhat better silage compared to silage supplemented with NPN substances. The diet in ruminant nutrition will comprise parts of both silages.

Acknowledgments

The present study is part of the project (TP 31057) financed by the Ministry of Education and Science of the Republic of Serbia.

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