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IMPACT OF NON-PROTEIN NITROGEN SUBSTANCES ON GRAPE POMACE SILAGE QUALITY

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Abstract: In this study grape pomace was ensilaged without and with the addition of NPN substances (Benural) at the dose of 0.5, 1.0 and 1.5% of the amount of husk and with the addition of inoculant based on homo and hetero fermentative lactic acid bacteria. The greatest effect on the nutritional value had application of Benural, especially in increasing the CP content from 126.9 to 178.3 g kg^{-1}DM, an increase of over 40%. Application of Benural increased the ammonia and soluble nitrogen several times, but even with the maximum addition of Benural percentage of ammonia nitrogen in the total nitrogen reached only slightly above 5% NH_3-N/Σ N (5.38%), while the percentage of soluble nitrogen in total nitrogen was 28.29%. The application of inoculants generally had no significant impact, both on the chemical composition and the fermentation process.

Key words: grape pomace, NPN substance, protein fractions, silage quality

Introduction

By-products are generally characterized by high water content, rapid fermentation and easy spoilage. Grape pomace is the residue after pressing grapes and juice extraction and consists mainly of epidermis, seeds, fruit and insoluble parts of the rest of the juice. Nutrient content of grape pomace is variable and depends on the grape variety, processing technology, the climate and soil conditions of production. For feeding of ruminants grape pomace can be used fresh, dried and ensiled. The use of fresh pomace for animal feeding in the long run is not possible due to rapid fermentation and spoilage. Dried grape pomace has been used in ruminant nutrition especially in fattening cattle *(Nikolić et al., 1980, Zeremski, 1982, Stojanović et al., 1989)*, but today it is not profitable since energy required for drying is too expensive. Ensilage of grape pomace is the best solution today.

Taking into account that the grape pomace is poor in protein and rich in soluble carbohydrates, it is necessary to apply the procedure to increase the crude protein (CP), in order to optimize the nutrition of ruminants. One extremely simple and inexpensive method is to add a suitable source of non-protein nitrogen in the silage or meal. Symbiotic bacteria in the rumen will use the added nitrogen to synthesize their own proteins of high biological value, which will be digested and used in the small intestine of ruminants together with proteins from the meals *(Đorđević and Dinić, 2011)*. In accordance with this fact, the experiment is planned with an addition of commercially available non-protein nitrogen in the silage to increase the amount of CP.

The aim of this study was to investigate the possibility of ensiling grape pomace with the addition of different doses of NPN substances and inoculants based on lactic acid bacteria, and to determine detailed chemical composition and nutritive value of silage.

Material and methods

Grape pomace without stems from white grape "Rkaciteli" taken immediately after pressing from the "Rubin" Kruševac and ensiled by the method of two-factorial experiment (4 x 2) with three replications. Silage was conducted in the experimental containers holding 130 liters. The containers was loaded with sand layer thickness of 15 cm. The containers was open 65 days after ensilaging, and two examples was taken for analysis. The first research factor (A) is the addition of NPN commercial substance called "Benural" to pomace in four concentrations, and the second factor (B) is the addition of inoculants on two levels: A_1 B_1 – grape pomace without Benural and inoculant; A_1 B_2 – grape pomace with inoculant; A_2 B_1 – grape pomace with 0.5% Benural; A_2 B_2 – grape pomace with 0.5% Benural and inoculant; A_3 B_1 – grape pomace with 1.0% Benural; A_3 B_2 – grape pomace with 1.0% Benural and inoculant; A_4 B_1 – grape pomace with 1.5% Benural; A_4 B_2 – grape pomace with 1.5% Benural and inoculant (homofermentative-*Enterococcus faecium* and *Bacillus plantarum*, and heterofermentative-*Bacillus brevis*; concentration of 5×10^{10} cfu g^{-1})

As NPN substance was used Benural S which contains 42% urea, 56% bentonite and 2% sulfur. In the grape pomace and silage following parameters were determined: dry matter content (DM), crude protein (CP), crude fiber (CF), crude fat (EE), NDF, ADF, lignin. In the silage DM content, the degree of acidity (pH), ammonium and soluble nitrogen, content of acetic, butyric and lactic acids was determined. Chemical analisys were performed in the laboratory of the Institute for Forage Crops, Kruševac according to standard methods (AOAC, 2002). DM digestibility was determined by enzymatic method according *De Boevar et al. (1986)*. Data were processed by the analysis of variance (COSTAT) in a randomized block design. The significance of differences between arithmetic means was tested by LSD test.

Results and discussion

Quality forage is a decisive factor in the production of milk and meat of ruminants. Balance meals with regard to forage to concentrate ratio, then the content of nutrients: protein, minerals and vitamins, especially the relationship between energy and protein, is also a very significant impact on the quality of ruminant nutrition. The content of nutritive substances in the starting material and in the control silage (treatment A_1B_1) is similar and the differences are small (Table 1). CP content increases from the control silage (126.9 g kg^{-1}DM) to the silage with the highest level of Benural (178.3 g kg^{-1}DM). Nutritional value of grape pomace is significantly lower than other forages, resulting from high proportion of structural carbohydrates (CF, NDF and ADF). CF level is above 300 g kg^{-1}DM, and the concentration of NDF and ADF was also high at 650 g kg^{-1} DM and 590 g kg^{-1} DM, respectively (Table 1).

Table 1 Organic matter composition of starting material and silages, g kg^{-1} DM

Starting material		Crude Proteins	Ether extract	Crude Fibre	NFE	NDF	ADF
Grape pomace		118.4	91.3	339.6	417.0	651.4	490.8
Silages							
Level of Benural	Inoculalant	CP	EE	CF	NFE	NDF	ADF
A_1	B_1	125.7	109.1	346.2	365.8	628.6	594.7
	B_2	128.2	102.5	329.5	385.2	676.6	589.2
A_2	B_1	140.8	93.8	319.1	391.5	647.3	586.8
	B_2	139.7	98.5	325.1	401.1	657.5	609.1
A_3	B_1	153.4	94.5	327.4	359.6	673.4	587.2
	B_2	157.1	100.9	310.1	377.0	636.9	546.7
A_4	B_1	177.2	102.3	299.3	373.7	634.4	576.5
	B_2	179.3	94.1	323.3	346.7	640.3	607.5
$\overline{X}$ A_1		126.9^{d}	105.8^{a}	337.9	375.5^{ab}	652.6^{a}	591.9^{a}
$\overline{X}$ A_2		140.3^{c}	96.1^{a}	322.1	396.3^{a}	652.4^{a}	597.9^{a}
$\overline{X}$ A_3		155.3^{b}	97.7^{a}	318.7	368.3^{ab}	655.2^{a}	566.9^{a}
$\overline{X}$ A_4		178.3^{a}	98.2^{a}	311.3	360.2^{b}	637.3^{a}	592.0^{a}
$\overline{X}$ B_1		149.3^{a}	99.9^{a}	323.0	372.6^{a}	645.9^{a}	586.3^{a}
$\overline{X}$ B_2		151.1^{a}	99.0^{a}	322.0	377.5^{a}	652.8^{a}	588.1^{a}
Significance for A		*	ns	ns	*	ns	ns
Significance for B		ns	ns	ns	ns	ns	ns

ns – no significance; * ($p<0,05$); ** ($p<0,01$)

Nutritional value of raw material in this study is considerably lower compared to the research of many researchers, which is defined by much higher lignin content and a high proportion of lignin in the NDF (Table 2). The concentration of CP in this study in the starting material and in the control silage was within results of *Stojanovic et al. (1989)*, *Zalikarenab et al. (2007)*, *Alipour and Rouzbehan (2007)* and *Bahrami et all. (2010)*. The higher values are found by *Zheng et all. (2012)* and *Mirzaei-Aghsaghali et al. (2011)*, who showed the highest value of 172.7 g kg^{-1}DM, which matches the results of these studies with the addition of 1.5% Benural. CF content in the starting material and in the silage in this study is within the above-cited results which values are above 300 g kg^{-1}DM. Significantly lower value (228 g kg^{-1}DM) was found by *Mirzaei-Aghsaghali et al. (2011)* and two times lower values were found by *Zalikarenab et al. (2007)*. The concentration of lignin in this study was almost two times higher than in most other research. These differences in the content of CF and lignin can be interpreted by varietal characteristics of grape, climatic conditions, the quality of the separation stems and quality of juice removal. For example DM content of grape pomace for the study was 400 g kg^{-1}DM, and this value in *Mirzaei-Aghsaghali et al. (2011)* was 225 g kg^{-1}DM. Results for NDF and ADF in this study is within the above-cited results. Significantly lower values for these parameters were determined by *Zalikarenab et al. (2007)*, and in particular *Bahrami et al. (2010)*, who showed 471 g kg^{-1}DM for NDF and 312 g kg^{-1}DM for ADF.

Table 2 Digestibility of silage from grape pomace, lignin content and share of lignin in NDF (%)

Levels of Benural	Inoculalant	Digestibility	Lignin	Lignin % in NDF
A_1	B_1	33.65	39.29	62.09
	B_2	32.75	39.15	58.73
A_2	B_1	33.18	37.98	59.07
	B_2	33.92	38.43	58.75
A_3	B_1	33.43	38.88	58.34
	B_2	34.61	37.10	57.84
A_4	B_1	35.03	37.29	58.80
	B_2	34.03	38.07	58.48
$\overline{X}\ A_1$		33.20	39.22	60.41
$\overline{X}\ A_2$		33.55	38.20	58.91
$\overline{X}\ A_3$		34.02	37.99	5.,09
$\overline{X}\ A_4$		34.53	37.68	58.64
$\overline{X}\ B_1$		33.82	38.36	59.58
$\overline{X}\ B_2$		33.83	38.19	58.45
Significance for A		ns	ns	
Significance for B		ns	ns	

ns – no significance; * (p<0,05); ** (p<0,01)

Sampling of silage was done 90 days after ensiling when chemical analyses of the process of lactic acid fermentation were performed. It was found that very good silage were obtained. The high DM content (400 g kg^{-1}DM) is established. It is known that levels of DM above 300 g kg^{-1} DM prevents the butyric acid and other undesirable bacteria that cause spoilage and decay of silage *(Ensilage, 1978, Djordjevic and Dinić, 2003)*, as shown by the results of this study because in any of the treatment has not been established presence of butyric acid (Table 3). It was found that increasing the dose of Benural caused statistically significant reduction in dry matter content, while inoculant differences were not statistically significant.

Table 3 Parameters of biochemical changes in silages, g kg^{-1} DM

Benural Level	**Inoculalant**	**DM gkg^{-1}**	**pH**	**NH_3-N/ΣN, %**	H_2O-/ΣN, %	**Acetic acid**	**Butyric acid**	**Lactic acid**
A_1	B_1	417	3.78	0.99	4.81	19.8	0.0	26.9
	B_2	417	3.76	0.97	4.87	20.4	0.0	24.3
A_2	B_1	403	3.85	2.82	12.02	20.6	0.0	27.5
	B_2	407	3.89	3.13	13.87	21.8	0.0	23.6
A_3	B_1	400	3.95	4.08	21.19	30.4	0.0	15.0
	B_2	392	4.02	4.26	23.67	22.7	0.0	18.7
A_4	B_1	392	4.08	5.52	27.98	20.2	0.0	25.0
	B_2	396	4.00	5.23	28.59	20.6	0.0	26.8
$\overline{X}\ A_1$		417^a	3.77^d	0.98^d	4.84^d	20.1	0.0^a	25.6^a
$\overline{X}\ A_2$		405^{ab}	3.87^c	2.98^c	12.94^c	21.2	0.0^a	25.5^a
$\overline{X}\ A_3$		396^b	3.98^b	4.17^b	22.43^b	26.5	0.0^a	16.8^b
$\overline{X}\ A_4$		394^b	4.04^a	5.38^a	28.29^a	20.4	0.0^a	25.9^a
$\overline{X}\ B_1$		403^a	3.91^a	3.39^a	16.49^a	22.7	0.0^a	23.6^a
$\overline{X}\ B_2$		403^a	3.92^a	3.35^a	17.75^a	21.4	0.0^a	23.3^a
Significance for A		*	*	*	*		ns	*
Significance for B		ns	ns	ns	ns	ns	ns	ns

ns - no significance, * ($p < 0.05$), ** ($p < 0.01$)

It is noted that the increase in the content of Benural helps to reduce the degree of acidity. Silage with pH values up to 4.20 are scored high marks, even for the lower DM content of silage *(Ensilage 1978)*. Different concentration of Benural caused statistically significant difference in the degree of acidity of the treatments, while the use of inoculants had no effect on the degree of acidity (Table 3). The presence of lower fatty acids in silages (lactic, acetic and butyric) is an indicator of the successful lactic acid fermentation. Earlier it was pointed out the absence of butyric acid and relatively favorable ratio of lactic and acetic acids. In the present study, the application of inoculant did not have statistically significant impact on the content of the lower fatty acids (lactic, acetic and butyric), which can be explained by rapid decrease of pH to around 4.0.Application of Benural at 1% provided the greatest amounts of acetic and the lowest content of lactic acid, which was statistically significant.With increasing addition of NPN substances the proportion of ammonia and soluble nitrogen in the total nitrogen content of silage (% NH3-N / Σ N) increases from the control silage to silage with 1.5% Benural, but the values are small (max 5.38%). It was found that the increase in the content of Benural in the silage induced increase of % NH3-N / Σ N and% H_2O-N / N Σ, and the differences are statistically significant, while the use of inoculants had no effect on these values Table 3).

Conclusion

On the basis of the examination of ensiling grape pomace with addition of Benural and inoculants based homo and hetero fermentative lactic acid bacteria it can be concluded: Grape pomace can be successfully ensiled without additives. Add NPN substances contributed to a significant increase in crude protein, ammonia and soluble nitrogen, whereas there was no significant degradation of the protein in the silage. The application of inoculants generally had a significant impact on both the chemical composition and the fermentation process.

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Uticaj dodavanja neproteinskih azotnih supstanci na kvalitet silaže komine grožđa

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Rezime

U istraživaniima komina grožđa je silirana bez i uz dodatak NPN supstanci (Benural) u količini od 0,5; 1,0 i 1,5% od količine komina i sa dodatkom inokulantana na bazi homo i heterofementativnih mlečno-kiselinskih bakterija.. Najveći efekat na hranljivu vrednost imala je primena NPN, posebno u povećanju

sadržaja SP od 126,9 na 178,3 gkg^{-1}SM. Primena NPN supstanci je uticala na povećanje amonijačnog i rastvorljivog azota nekoliko puta, ali i pri najvećoj količini dodatog NPN supstanci udeo amonijačnog azota u ukupnom azotu je neznatno prešao vrednost od 5% (%NH3-N/ΣN 5,38) dok je udeo rastvorljivog azota u ukupnom azotu iznosio 28,29%. Primena inokulanata uglavnom nije imala značajnog uticaja, kako na hemijski sastav, tako i na proces fermentacije.

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