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Effect of addition of NPN substances and inoculants on fermentation process and nutritive value of corn silage

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Abstract: Biomass of maize (hybrid ZP 735) in the stage of waxy grain/kernel maturity was ensiled without additives, with the addition of inoculants and with the addition of Benural in an amount of 1% of the silo mass. Biomass of the whole maize plant can be successfully ensiled without additives. Adding Benural, in the amount of 1% of the biomass of the whole maize plant, provided increase in crude proteins of nearly 50%. Adding inoculants and Benural provided for increase in the production of lactic and acetic acid by 44-47% and 49-74%, respectively, and thus a higher aerobic stability of silage, with a favourable balance of these acids (2-3:1). Application of inoculants reduced the level of ADF and increased the nutritional value expressed in net energy (NE_L and NE_M units), and provided higher RFV and greater consuming ability. The general conclusion is that non-protein nitrogen substances (NPN) should be added to the maize biomass during the ensiling in order to increase the content of CP, as well as the inoculants based on homo- and

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heterofermentative bacteria in order to increase the production of lactic and acetic acids and ensure the aerobic stability of silage and its higher nutritional value.

Key words: silage, benural, inoculants, proteins, fermentation.

Introduction

Maize is the most common forage crop grown in Serbia with different possibilities of use. In the first place it is grown in order to produce concentrated animal feed, to a lesser extent for silage or feeding as maize forage. Through ensiling and use of silage in ruminant nutrition, a higher nutritional value of 30-60% is provided compared to its use in form of grain/kernel (Dinić and Đorđević, 2005).

Examinations and analyses of fodder/animal food on farms have shown a lack of protein in the diet of ruminants in lowland areas, primarily as a result of poor quality forage and the high proportion of maize in the form of grain/kernel, silage and maize stover (Dinić et al., 2011). One way of solving the protein deficiency in the diet of cattle (ruminants) is adding NPN substances in maize silo mass.

The principle of use of NPN substances is based on the fact that these substances decompose in the rumen of ruminants, influenced by microbial urease, to NH_3 and CO_2 . Ammonia reacts with keto acids and amino acids occur, that are used by microorganisms to build their own proteins. Microbial protein, in the abomasum and small intestine, breaks down to free amino acids, through the activity of enzymes, which are then absorbed in the small intestine and used by the host animal. However, urea hydrolysis is a rapid process and is usually much faster than the utilization of ammonia for microbial protein synthesis. Therefore, the concentration of ammonia after consuming a meal is rapidly growing, with excess ammonia passing through the rumen wall into the blood stream, and from the bloodstream into the liver. In the case of high concentration of ammonia in the rumen, its absorption by the animal leads to the intoxication of the animal (Đorđević and Dinić, 2011). To avoid these problems Benural S was applied, comprising about 2.5 times less urea and large amount of bentonite that slows the release of ammonia. Hoffman (1999) lists several guidelines for the application of urea, and three are emphasized in the present paper: no addition of urea at ensiling of the whole maize plant if the level of DM is low (below 30%); then when the DM level is high (above 40%) and do not feed the breeding heifers of weight below 400 lb (180 kg).

The aim of this paper is to examine the possibility of improvement of whole maize plant biomass with the addition of "Benural S" and inoculants to increase

CP content and nutritive value of silage, and thus provide higher production of milk and meat.

Materials and methods

Biomass of maize (hybrid ZP 735) in the stage of waxy grain/kernel maturity from year 2011, was ensiled according to the single factorial method, in three replicates. The study included three treatments: first: ensiled whole maize plant biomass without additives (ZM-C), the second treatment: ensiled maize biomass with the addition of inoculants (ZM + I) and third treatment: ensiled maize biomass with the addition of 1% Benural (ZM + B). Ensiling experiment was carried out in experimental dishes/containers of 250 dm³ volume.

Shortly before cutting of maize, the number of plants ha⁻¹ was determined, also the average mass of a single plant and the share of the ear, grain/kernel and cob in the mass of whole plant. Based on the number of plants and mass of a single plant, the biomass yield per hectare was established. In order to determine the quality of cutting of maize biomass, a modified separator was used (Kononoff et al., 2003) with three sieves with perforations (19 mm, 8 mm and 2 mm) and 4 fractions - particle size.

BioStabil Mays of the Austrian company Biomin, containing homofermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and heterofermentative lactic acid bacteria (*Bacillus brevis*) at a concentration of 2.5×10^{10} CFU per gram of dry product, was used as an inoculant.

Benural S, containing 42% urea, 56% bentonite and 2% sulphur was used as NPN substance. Unlike pure urea, this product contains bentonite, which allows slower release of ammonia in the rumen and more efficient use of the rumen microorganisms; it binds gases and some toxic substances and contains some important alkaline elements (K, Na, Mg, etc.). Sulphur present in Benural S enables efficient synthesis of some essential amino acids containing this macro element (methionine, cystine).

Chemical analyses of samples of initial material (whole maize plant, grain/kernel and cob) and of maize silage were conducted in the laboratory of the Institute of forage crops in Kruševac, according to standard methods (AOAC, 2002).

The following parameters were determined analytically in the biomass of whole maize plant and silage: dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (lipids), NDF, ADF, ash, Ca and P, and nitrogen free extracts were calculated (NFE). In the silage, the following were determined: DM content, the degree of acidity (pH), ammonia nitrogen (NH₃-N), the content of

acetic, butyric and lactic acid. For the assessment silage quality, three methods were used: DLG, Zelter and Weissbach (Đorđević and Dinić, 2003). Nutritional value expressed in units of NE_L and NE_M was calculated according Obračević (1990) and Schroeder (1994), and digestibility coefficients of nutrients according Glamočić (2002). On the basis of chemical composition (CP, ADF, NDF) and dry matter digestibility (DMD) an assessment was made of the relative feed value (RFV) according to the standards of quality for legume and grass - American Forage and Grassland Council (Schroeder, 1994). The possibility of consumption is calculated using the formula $DMI \% BM = 120 : \%NDF$.

The results of the chemical analysis were processed using the variance analysis, and statistical significance of differences was tested using the LSD test (StatSoft, 2006).

Results and Discussion

Characteristics of the biomass of whole maize plant

In the harvest, average of 49.200 plants ha^{-1} was achieved, with the average mass of single plant of 970g, and the average yield of silo mass of 47,724 $kg ha^{-1}$. The average ear mass was 350 g with a share in the fresh mass of 36.08%, grain/kernel mass 260g with a share of 26.80% and cob mass was 90 g per plant⁻¹ with a share of 9.28% in relation to the whole plant.

Ensiling of maize biomass was carried out using single-row forage harvester with the quality of cut given in Table 1.

Table 1. Cutting length of corn plant and optimal cutting length for silage making

Fractions	Replicates						Average	The optimal length of the cuts
	I		II		III			
	g	%	g	%	g	%		
> 19mm	150	13.04	165	22.30	200	21.74	19.03	3-8
> 8 do 19 mm	540	46.96	300	40.54	370	40.22	42.57	45-65
> 2 do 8 mm	400	34.78	250	33.78	300	32.61	33.72	30-40
< 2mm	60	5.21	25	3.38	50	5.43	4.67	< 5
Σ	1150	99.99	740	100.00	920	100.00		

Quality of maize biomass chopping for silage should be such that each grain/kernel is damaged, the length of slices is 7-10 mm, provided that the forage harvester is not equipped with grain/kernel crusher which allows the length of

the cuts of 20 mm. Such quality work ensures fine chopping, and thus successful compaction, good fermentation and consumption by animals. The quality of forage harvester used in these studies is somewhat poorer and has a fraction above 19 mm, significantly more (19.03%), and it is preferable that this fraction is 3-8% (Table 1).

Chemical composition and nutritive value of silage and biomass

The chemical composition of the starting material and maize silage is shown in Table 2. A low concentration of CP in the biomass is an essential characteristic of the biomass of whole maize plant. In the whole maize plant biomass and silages without benural CP content was similar - slightly higher than 60 g kg⁻¹DM, in silages with benural, it was 90.3 g kg⁻¹DM, which was an increase of almost 50% (Table 2). Statistical analysis revealed a highly significant difference in CP content between the silages with benural and other silages. Nutrient content in the grain/kernel is characteristic of the data presented in absolute dry matter (CP 99.6 gkg⁻¹DM, CF 31.8 gkg⁻¹DM, fat 52.8 gkg⁻¹DM and low concentration of Ca and average concentration of P). Results of the analysis of the chemical composition of the cob indicated the same low nutritional value at the level of wheat straw.

Table 2. Hemical composition of biomass and whole plant corn silage, gkg⁻¹DM

Quality parameter	CP	CF	Lipids	NFE	NDF	ADF	Ash	Ca	P
Starting biomass									
Whole plant	64.9	245.3	28.6	610.4	-	-	50.8	5.4	2.3
Grein	99.6	31.8	52.8	800.4	-	-	15.4	0.6	3.1
Cob	13.2	395.5	19.4	564.6	-	-	7.4	1.8	0.7
Silage									
ZM - C	61.3 ^b	234.6 ^a	24.9 ^b	638.9 ^a	627.8 ^a	385.1 ^a	40.3 ^b	4.87 ^a	2.67 ^a
ZM + I	63.2 ^b	227.1 ^a	26.8 ^{ab}	650.3 ^a	610.3 ^a	310.0 ^c	33.5 ^b	4.17 ^b	2.60 ^a
ZM + B	90.3 ^a	236.6 ^a	29.8 ^a	593.0 ^b	641.4 ^a	351.5 ^b	50.3 ^a	4.87 ^a	2.67 ^a
LSD 0,05	6.79	31.75	3.69	38.17	36.31	19.22	8.09	0.44	0.22
LSD 0,01	10.29	48.11	5.60	57.84	55.01	29.12	12.27	0.68	0.33

Next to the drive letter of the parametar values in the subscript refers to the LSD 0.05.

CF content in the biomass was slightly higher compared to silages and can be interpreted as the influence of microorganisms and their enzymes that occur in the fermentation process. Differences in the CF content between silage treatments were not statistically significant. The lowest values for the NFE were established in silage ZM + B, and occurred as a result of increase of the share of CP with addition of Benural, the differences were statistically significant. It is interesting to observe that the values of the ADF in silage ZM + I were significantly lower than in silage ZM + B, especially compared to the ZM-C and can be interpreted as the activity of lactic acid bacteria and their enzymes. The differences established between treatments were statistically significant. The differences established in ash content between silage ZM + B and the other treatments were statistically significant and occurred as a result of added Benural, which contains bentonite which is a mineral supplement (Table 2).

Table 3 Indicators of nutritional value, relative feed value and the possibility of consuming silage

<i>Treatments</i>	NE_L <i>MJkg⁻¹DM</i>	NE_M <i>MJkg⁻¹DM</i>	<i>RFV</i> <i>%</i>	<i>DMI</i> <i>% BW</i>
ZM-C	6.00 (4.84)	6.12 (5.10)	85.74	1.91
ZM+I	6.08 (5.72)	6.23 (6.14)	100.95	1.97
ZM+B	5.93 (5.23)	6.02 (5.57)	89.22	1.87

Legend: Data in parentheses are expressed in units of the NE_L and NE_M by Schroder (1994) calculated via ADF

The nutritive value of silages is shown in Table 3. Indicators NE_L , NE_M , RFV and CDM indicate that the most favourable values were established in silage ZM + I. It was found that silage ZM + I had a slightly higher energy value than silage ZM + B and ZM+ C, according to Obračević (1990) and significantly higher according to Schoreder for NE_L by 0.49 and 0.88 $MJkg^{-1}DM$ and NE_M 0.57 and 1.04 $MJkg^{-1}DM$. Relative feed value (RFV) was also the highest in silages ZM + I and was higher in relation to silage ZM + B and ZM + C by 11.73 and 15.21%, respectively. The possibility of consuming silage DM in relation to % of body mass was similar, although also for this parameter, the highest value was determined in silage ZM + I (Table 3).

The content of CP, in the present studies, in silages without additives was significantly lower than the results stated by Lauer (2013) of 84-86 $gkg^{-1}DM$, which can be explained by greater application of nitrogen fertilizers in the United States and perhaps greater share of leaf in maize biomass. Also, in the studies by the same author, slightly lower CF values (22-23%) can be observed, significantly lower NDF (46-53%) and ADF (26-30%) values and lower values

for Ca and P, and ash content within the limits. Higher nutritional value of maize biomass (NEL 6.63 to 6.64 MJ) in years 2011 and 2012 are stated in the Annual report of the Centre for Animal Husbandry (Enzinger, 2012). In the same report, higher content of CP (77-75 gkg⁻¹DM), lower crude fibre content (198-203 gkg⁻¹DM) and lower ash content (35-34 gkg⁻¹DM) are stated. Higher nutritional value as expressed in the NEL and NEM, and higher protein content (7.88% of DM) in the biomass of maize in the stage of milky-wax grain maturity, compared to the present results, have been reported by Bokan et al. (2004). Significantly lower results in maize silage compared to the results of these studies in the content of CP and CF (4.22 and 7.98% of DM), and even more in the content of Ca and P (1.20 and 1.80% in the DM) are reported by Stojković et al. (2012).

Biochemical changes in the process of ensiling

The process of lactic acid fermentation of maize silage took place as expected because the maize biomass falls into the category of easy ensiling plant material, suitable for ensiling and often serves as the carbohydrate component in the process of ensiling of heavy ensiling biomasses (Dinić et al., 1998). DM content in silages was over 300gkg⁻¹, which is considered to be extremely favourable, and the differences between silages were negligible and statistically not significant (Table 4). The acidity of silage was very good, the highest acidity (pH 3.74) was detected in the silage prepared using the inoculants, and the lowest acidity (pH 4.20) in the control silage.

Table 4. Biochemical parameters of the silage

Treatmans	DM gkg ⁻¹	pH	NH ₃ -N/ΣN %	Acids in silages, gkg ⁻¹ DM					
				Acetic		Butyric		Lactic	
				gkg ⁻¹ DM	%	gkg ⁻¹ DM	%	gkg ⁻¹ DM	%
ZM-C	316.7 ^a	4.20 ^a	3.10 ^b	26.0 ^b	29.66	0.0 ^a	0.00	61.7 ^b	70.34
ZM+I	306.6 ^a	3.74 ^b	3.19 ^b	38.7 ^a	30.40	0.0 ^a	0.00	88.6 ^a	69.60
ZM+B	310.0 ^a	3.92 ^{ab}	10.41 ^a	45.3 ^a	32.79	0.0 ^a	0.00	90.8 ^a	67.20
LSD 0.05	22.08	0.28	1.28	12.95	-	--	--	30.09	-
LSD 0.01	33.47	0.43	1.94	19.64	-	--	--	45.59	-

Legend: letter next to the parameter value in the subscript refers to the LSD 0.05.

The percentage of ammonia nitrogen in relation to the total nitrogen is an indicator of protein degradation in the process of ensiling. In this study, % NH₃-N/ΣN is extremely low, less than 5% for silages ZM+C and ZM+I. On the basis

of this parameter alone, silages get max score when the share of $\text{NH}_3\text{-N}/\Sigma\text{N}$ is up to 10% (Ensilage, 1978). Higher value of % $\text{NH}_3\text{-N}/\Sigma\text{N}$ in silage ZM + B compared to other silage treatments was expected, and differences between the said silage on one hand and silages ZM + C and ZM + I on the other hand were highly significant.

Based on the results presented in Table 4, it can be concluded that the lactic acid fermentation process took place in the desired direction. The presence of butyric (undesirable) acid was not detected and the ratio of lactic and acetic acids was favourable. Application of inoculants and Benural resulted in increased production of lactic and acetic acids compared to control silage, lactic acid from 61.7 to 88.6 and 90.8 gkg^{-1} DM and acetic acid from 26.0 to 38.7 and 45 gkg^{-1} DM, and highly statistically significant differences between the control silage and other silages were identified, in favourable ratio (2-3:1).

For the assessment silage quality, three methods were applied: DLG, which is based on the pH value and the relative ratio of acetic, butyric and lactic acids, Zelter method based on the % of $\text{NH}_3\text{-N}/\Sigma\text{N}$, the content of acetic and butyric acids in fresh silage and Weissbach method which is based on the content of butyric acid, % of $\text{NH}_3\text{-N}/\Sigma\text{N}$, pH value in relation to the concentration of DM, based on the total content of acetic and butyric acids and possible presence of mould.

Silages were scored as the first class based on DLG and Weissbach methods, with almost max number of points realized, and based on the Zelter method silages were scored as class III and class II quality (Table 5). We believe that the Zelter method is very strict in regard to the parameter of acetic acid, in particular since it is preferable that the content of acetic acid in silage is higher since it leads to a greater aerobic stability of silage. In support of this conclusion is also a new method of Kaiser and Weiss (2005) used for assessing the quality of silage. Based on this method, all silages would be scored as Class II, which is considerably higher score than according to Zelter method. That is why today inoculants based on homo- and heterofermentative lactic acid bacteria are increasingly used.

Table 5 Quality evaluation of whole plant corn silage using different methods

Treatments	DLG		ZELTER		WEISSBACH	
	points	class	points	class	points	class
ZM-C	50	I	17	II	100	I
ZM+I	48	I	14	III	100	I

The results of these studies which suggest the lactic acid fermentation process showed the same tendency as the results by Dinić et al. (2001) in ensiling maize biomass with the addition of 0.0, 0.25 and 0.50% of urea. Starting

from the control silage, pH increased (acidity decreased) from 3.68 to 4.15, the share of ammonia nitrogen, acetic and butyric acid increased, also of lactic acid. A similar tendency of changes in the pH values and other parameters are listed by Weissbach and Laube (1964) (citation by Čobić et al., 1983).

Conclusion

Biomass of whole maize plant can be successfully ensiled without additives. Addition of Benural in the amount of 1% of the maize plant biomass provided increased crude protein content close to 50%. Adding of inoculant and Benural provided for increase of the production of lactic and acetic acids, 44-47% and 49-74%, respectively, and thus a higher aerobic stability of silage. Application of inoculants reduced the level of ADF and increased the nutritional value expressed in net energy (NE_L and NE_M units), and provided higher RFV and greater consumption ability.

The general conclusion is that the NPN substances should be added to the biomass of whole maize plant during ensiling, in order to increase the content of CP, and inoculant to ensure the anaerobic stability of silage and higher nutritional value.

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UTICAJ DODAVANJA NPN SUPSTANCI I INOKULANATA NA PROCES FERMENTACIJE I HRANLJIVU VREDNOST KUKURUNE SILAŽE

- originalni naučni rad -

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Rezime

Biomasa kukuruza (hibrid ZP 735) u fazi voštane zrelosti zrna silirana je bez aditiva, sa dodatkom inokulanta i sa dodatkom Benurala u količini od 1% od silomase. Biomasa kukuruza cele biljke može se uspešno silirati bez aditiva. Dodavanje Benurala, u količini od 1% u biomasi cele biljke kukuruza, obezbeđuje povećanje sirovih proteina blizu 50%. Dodavanje inokulanta i benurala obezbeđuje povećanju produkciju mlečne za 44-47% i sirćetne kiseline za 49-74%, a time i veću aerobnu stabilnost silaže, uz povoljan odnos ovih kiselina (2-3:1). Primena inokulanta smanjuje nivo ADF i povećava hranljivu vrednost izraženu u neto energiji (NE_L i NE_M jedinicama), kao i obezbeđuje veću RHV i veću mogućnost konzumiranja. Opšti je zaključak da biomasi kukuruza u vreme siliranja treba dodavati neproteinske azotne supstance (NPN) u cilju povećanja sadržaja SP i inokulant na bazi homo i heterofermentativnih bakterija u cilju veće produkcije mlečne i sirćetne kiseline i obezbeđivanja aerobne stabilnosti silaže i veće hranljive vrednosti.

Ključne reči: silaža, benural, inokulant, proteini, fermentacija.