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

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Abstract: Biomass of maize (hybrid ZP 735) at the wax-ripe stage was ensiled without additives, with the addition of inoculants and with the addition of Benural at 1% of silage. Biomass of the whole maize plant can be successfully ensiled without additives. Adding Benural at 1% of the biomass of the whole maize plant provided an increase in crude proteins by nearly 50%. The addition of inoculants and Benural led to a 44-47% and 49-74% increase in the production of lactic and acetic acids, respectively and, thus, to a higher aerobic stability of silage, with a favourable balance of these acids (2-3:1). The use 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 maize biomass during ensiling in order to increase CP content, and that inoculants based on homo-and heterofermentative bacteria should be added in order to increase the production of

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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. It has a wide range of uses, and is primarily grown for concentrated animal feed and 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 as grain/kernel (Dinić and Đorđević 2005).

Examinations and analyses of fodder/animal feed on farms have shown a lack of protein in ruminant diets in lowland areas, primarily as a result of poor quality forage and a high proportion of maize as grain/kernel, silage and maize stover (Dinić et al. 2011). One way of solving protein deficiency in cattle (ruminant) diets is by adding NPN substances to maize silage.

The principle underlying the use of NPN substances is based on the fact that these substances decompose in the rumen of ruminants, due to the effect of microbial urease, to NH_3 and CO_2. Ammonia reacts with keto acids to form amino acids 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 meal ingestion rapidly increases, with excess ammonia passing through the rumen wall into the blood stream, and from the bloodstream into the liver. In the case of high concentrations 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 a large amount of bentonite that slows down 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 weighing below 400 lb (180 kg).

The aim of this paper is to examine the possibility of improving whole maize plant biomass by the addition of "Benural S" and inoculants to increase CP content and nutritive value of silage, and thus ensure a high level of milk and meat production.

Materials and methods

Biomass of maize (hybrid ZP 735) at the wax-ripe stage obtained in 2011 was ensiled according to the single factorial method, in three replicates. The study included three treatments: ensiled whole maize plant biomass without additives (ZM-C), ensiled maize biomass with the addition of inoculants (ZM + I) and ensiled maize biomass with the addition of 1% Benural (ZM + B). The ensiling experiment was carried out in 250 dm^3 experimental dishes/containers.

Shortly before cutting, the number of maize plants ha^{-1}, the average mass of a single plant and the ear, grain/kernel and cob percentages of the whole plant were determined. Based on the number of plants and mass of a single plant, biomass yield per hectare was established. In order to determine the cutting quality 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 four 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 a slower release of ammonia in the rumen and a more efficient use of rumen microorganisms; it binds gases and some toxic substances and contains some important alkaline elements (K, Na, Mg etc.). The sulphur present in Benural S enables efficient synthesis of some essential amino acids containing this macro element (methionine, cystine).

Chemical analyses of samples of the initial material (whole maize plant, grain/kernel and cob) and of the 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 the 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 (NH_3-N), the content of acetic, butyric and lactic acids. Silage quality was assessed using three methods: DLG, Zelter and Weissbach (Đorđević and Dinić 2003). The nutritional value expressed in NE_L and NE_M units was calculated according to Obračević (1990) and Schroeder (1994), and digestibility coefficients of nutrients according to Glamočić (2002). On the basis of the chemical composition (CP, ADF, NDF) and dry matter digestibility (DMD) an assessment was made of the relative feed value (RFV) according to quality standards for legumes and grasses - 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 the whole maize plant

At harvest, the average of 49,200 plants ha^{-1} was achieved, with the average mass of a single plant of 970g, and the average silage yield of 47,724 kg ha^{-1}. The average ear, grain/kernel and cob mass were 350 g, 260 g and 90 g per $plant^{-1}$, accounting for 36.08%, 26.80% and 9.28% of the whole plant.

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

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

Fractions	Replicates						Average	Optimal cutting length
	I		II		III			
	g	%	g	%	g	%	%	%
> 19mm	150	13.04	165	22.30	200	21.74	19.03	3-8
> 8 do19 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		

The 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 a grain/kernel crusher which allows a cutting length of 20 mm. This ensures fine chopping and, hence, successful compaction, good fermentation and consumption by animals. The quality of the forage harvester used in this study 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 the whole maize plant. In the whole maize plant biomass and silages without benural, the CP content was similar - slightly higher than 60 g kg^{-1}DM; in silages with benural, it was 90.3 g kg^{-1}DM, which was an almost 50% increase (Table 2). The 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^{-1}DM, CF 31.8 gkg^{-1}DM, fat 52.8 gkg^{-1}DM 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. Chemical composition of biomass and whole plant maize silage, gkg^{-1} DM

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
Grain	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

The letter next to the parameter values in the superscript refers to an LSD at 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 CF content between silage treatments were not statistically significant. The lowest values for NFE were established in ZM + B silage, and occurred as a result of an increase in CP percentage after Benural treatment. The differences were statistically significant. It is interesting to observe that ADF values in ZM + I silage were significantly lower than in ZM + B silage, especially compared to 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 ZM + B silage 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

Treatments	**NE_L** ***$MJkg^{-1}DM$***	**NE_M** ***$MJkg^{-1}DM$***	***RFV*** ***%***	***DMI*** ***% BW***
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 expresed in NE_L and NE_M units 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 ZM + I silage. It was found that ZM + I silage had a slightly higher energy value than 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 for NE_M by 0.57 and 1.04 $MJkg^{-1}DM$. Relative feed value (RFV) was also the highest in ZM + I silage and was higher in relation to 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 the highest value was determined in ZM + I silage (Table 3).

The content of CP, in the present study, in silages without additives was significantly lower than the results stated by Lauer (2013) of 84-86 $gkg^{-1}DM$, which is due to the greater application of nitrogen fertilizers in the United States and perhaps greater leaf percentage 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 was within the limits. Higher nutritional values for maize biomass (NEL 6.63 to 6.64 MJ) in 2011 and 2012 are stated in the Annual Report of the Centre for Animal Husbandry (Enzinger 2012). In the same report, a higher content of CP (77-75 $gkg^{-1}DM$), a lower crude fibre content (198-203 $gkg^{-1}DM$) and a lower ash content (35-34 $gkg^{-1}DM$) were reported. Higher nutritional values as expressed in NEL and NEM, and a higher protein content (7.88% of DM) in the biomass of maize at the stage of milk-wax grain maturity, compared to the present results, were 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) were reported by Stojković et al. (2012).

Biochemical changes during ensiling

Lactic acid fermentation of maize silage took place as expected because maize biomass is easy to ensile, suitable for ensiling and often serves as the carbohydrate component during 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 non-significant (Table 4). Silage acidity 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 maize silage

Treatments	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: the letter next to the parameter value in the superscript refers to an LSD at 0.05.

The percentage of ammonia nitrogen in relation to the total nitrogen is an indicator of protein degradation during ensiling. In this study, % NH_3-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 proportion of NH_3-N/ΣN is up to 10% (Ensilage 1978). The higher value of % NH_3-N/ΣN in ZM + B silage compared to other silage treatments was expected, and differences between this silage and ZM + C and ZM + I were highly significant.

The results presented in Table 4 suggest that lactic acid fermentation took place as desired. The presence of butyric (undesirable) acid was not detected and the ratio of lactic and acetic acids was favourable. The use of inoculants and

Benural resulted in an increased production of lactic and acetic acids compared to control silage, the increase being from 61.7 to 88.6 and 90.8 gkg^{-1} DM for lactic acid and from 26.0 to 38.7 and 45 gkg^{-1}DM for acetic acid, and highly statistically significant differences between the control silage and other silages were identified, at a favourable ratio (2-3:1).

Silage quality was assessed using three methods: DLG, which is based on pH value and the relative ratio of acetic, butyric and lactic acids, Zelter method based on the % of NH_3-N/ΣN, the content of acetic and butyric acids in fresh silage and Weissbach method which is based on the content of butyric acid, % of NH_3-N/ΣN, 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 first class based on DLG and Weissbach methods, with almost max number of points scored, 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 higher aerobic stability of silage. This conclusion is also supported by a new method of Kaiser and Weiss (2005) used for assessing the quality of silage. Based on this method, all silages were scored as Class II, which is a considerably higher score than according to Zelter method. This is why inoculants based on homo-and heterofermentative lactic acid bacteria are increasingly used today.

Table 5 Quality evaluation of whole plant maize 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
ZM+B	49	I	13	III	95	I

The results of this study suggesting the lactic acid fermentation process showed the same tendency as did 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 and the proportion of ammonia nitrogen, acetic, butyric and lactic acids also increased. A similar tendency of changes in 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. The addition of Benural at 1% of maize plant biomass provided an almost 50% increase in crude protein content. The addition of the inoculant and Benural led to an increase in the production of lactic and acetic acids, 44-47% and 49-74%, respectively, and thus to higher aerobic stability of silage. The use 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 NPN substances should be added to whole maize plant biomass during ensiling in order to increase CP content, and an inoculant should also be added to ensure anaerobic stability of silage and a higher nutritional value.

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UTICAJ DODAVANJA NPN SUPSTANCI I INOKULANATA NA PROCES FERMENTACIJE I HRANLJIVU VREDNOST KUKURUZNE 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 povećanu produkciju mlečne kiseline 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) 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.