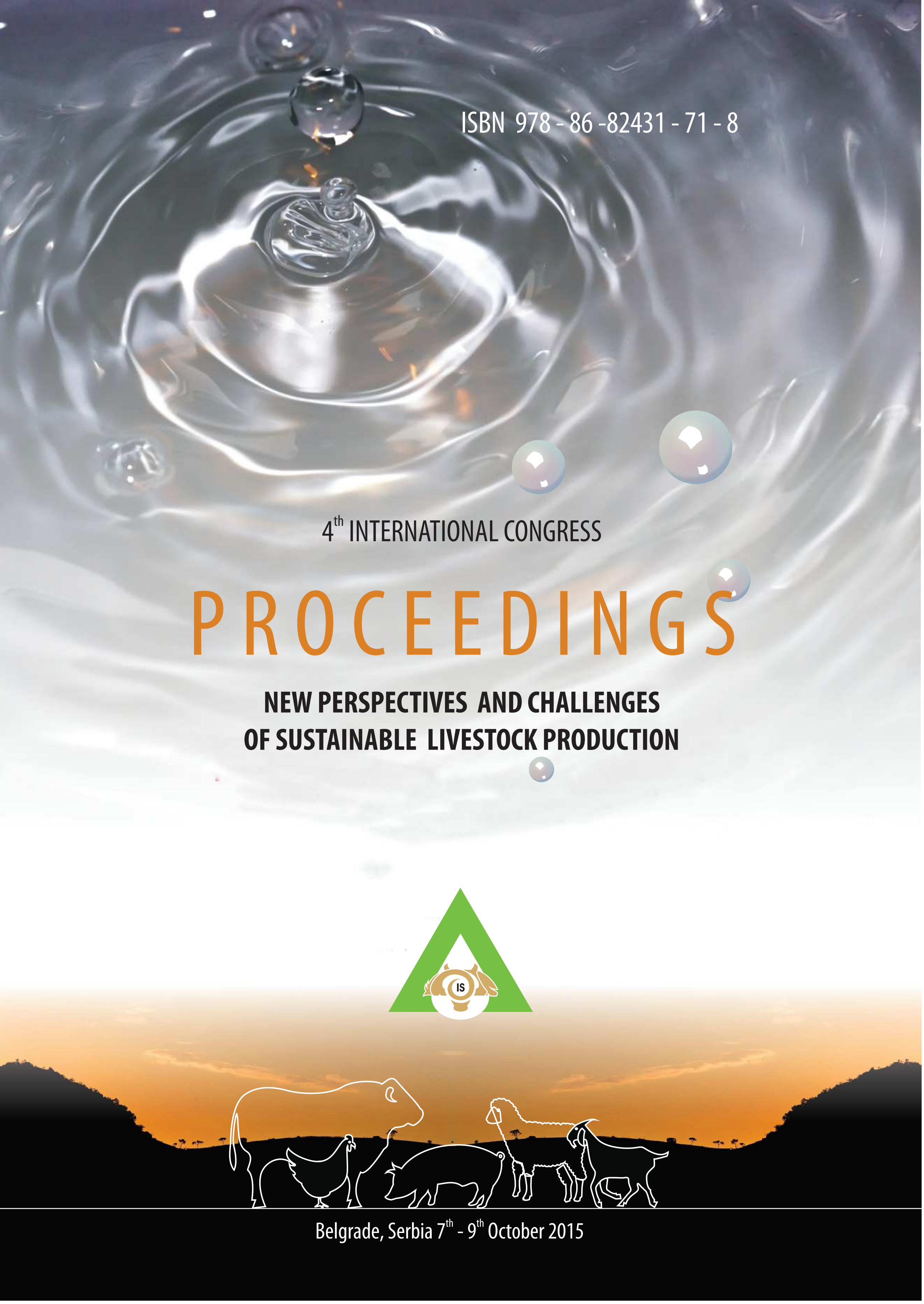

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EFFECTS OF NITROGEN FERTILIZATION AND USING OF INOCULANT ON NUTRITIVE VALUE AND FERMENTATION CHARACTERISTICS OF WHOLE CROP MAIZE SILAGE

Z. Bijelić[1], V. Mandić[1], D. Ružić-Muslić[1], Z. Tomić[1], V. Krnjaja[1], V. Petričević[1], M. Gogić[1], W. de Souza Filho[2]

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Invited paper

Abstract: The aim of our study was to examine the effect of N fertilization and treatment of silo mass with bacterial-enzyme inoculant on chemical, fermentative and nutritional characteristics of whole maize plant silage, and to determine the correlation between certain quality parameters. The experiment was carried out on experimental field and in the experimental laboratory of the Institute for Animal Husbandry. Corn was planted in the experimental field in four repetitions. The effect of four different nitrogen rates of 0, 60, 120, 180 kg N ha^{-1}, add to the corn crops during the growing season was studied. The silage was prepared in the laboratory and stored in vacuum bags. One part of the chopped mass was ensiled using the bacterial-enzyme inoculant, and the other partn without inoculant. Silage was stored at room temperature for 90 days. After 90 days, silage was sampled for chemical analysis. Fertilization with nitrogen in the amount of 120 kg N ha^{-1} resulted in an increased content of CP, CFT. N fertilization had a positive impact on the content of ME and NEL. The highest content of ME and NEL were determined in treatments with 120 and 180 kgN ha^{-1} of 10.3 MJ kg^{-1} ME and 6.0 MJ kg^{-1} NEL. The treatment with bacterial-inoculants considerably reduced the content of CP, NH_3-N, acetic acid, the pH value and increased the proportion of lactic acid relative to the acetic LA/AA.

Key words: nitrogen, bacterial-enzyme inoculant, whole crop maize silage

Introduction

In the intensive agricultural production and ruminant nutrition corn silage is very valuable voluminous energy feed. It is represented in livestock production throughout Europe and America. In Serbia it dominates in ruminant nutrition for many years. Compared to other forage silages, silage from whole maize plant has high energy content in dry matter, high palatability, high sugar content and easier technological procedure of preparation *(Forouzmand et al., 2005).*

For optimum production of maize it is necessary to add large amounts of N in the form of nitrogen fertilizers. Supplemental fertilization with N significantly affects the yield increase. *Amanullah (2010)*, in examination of the effects of different levels of nitrogen fertilization on the biomass yield concluds that the biomass yield significantly increases with the addition of N in an amount of 150-200 kg ha^{-1}. According to research by *Zhang et al. (2015)* fertilization of corn with N in amounts of 0, 79, 147, 215 and 375 kg N ha^{-1}resulted in the increase of average yield of fertilized treatments from 17 to 20%, except in case of > 215 kg N ha^{-1}ofnitrogen which did not lead to increasing yields. In addition to the yield, N fertilization affects the chemical composition of green mass (fresh forage). In studies of *Shaeffer et al. (2006),* nitrogen fertilization shows little effect on forage quality variables except for CP concentration. However, studies of *Islam et al. (2012*) show nitrogen fertilization not only increasing the crude protein content, but also the dry matter content, metabolizable energy and in vitro dry matter digestibility and reducing the content of the ADF. As for the easily soluble carbohydrates (WSC) as the primary substrate for the growth and development of fermentable lactic acid bacteria, some studies suggest that their content in plants is reduced by adding N fertilizer (*Almodares et al., 2009),* while some studies report their increase *(Islam et al., 2012).*

In order to achieve a good quality silage it is necessary in the initial stages of fermentation to achieve a rapid reduction of the pH, to avoid the occurrence and growth of harmful microorganisms, to avoid losses of dry matter and increase aerobic stability of silage. This can be achieved by the addition of bacterial and bacterial-enzyme inoculants *(Jatkauskas et al., 2012).* These supplements will lead to reduced fiber content, increasing the concentration of sugar and lactic acid and increasing the digestibility of silage (*Đorđević et al., 2011*). In addition, microbial additives act on animal performance by increasing the nutritional value of the silage, and some strains of LAB can survive in the gastric juice (rumen fluid) and play the role of buffer thus maintaining the activity of a cellulase enzyme and thereby increasing the digestibility (*Weinberg et al., 2003*), and therefore the consumption and animal performance *(Ando et al., 2006).*

The dry matter content in plants must be adequate, because in the silo mass which has a higher dry matter content, the effect of inoculants is smaller, i.e. it decreases with increasing dry matter content (*Comino et al., 2014*). Optimum value of DM should be around 35% when the best balance is achieved between starch as

a carrier of energy value and soluble sugars needed to produce enough lactic acid and lowering the pH (*Horrocks and Vallentine, 1999*).
The aim of our study was to examine the effect of N fertilization and treatment of silo mass with bacterial-enzyme inoculant on chemical, fermentative and nutritional characteristics of whole maize plant silage, and determine the correlation between certain quality parameters.

Materials and Methods

Maize hybrid (NS 6030 and ZP 666) was planted on 12th April 2013 at a planting rate of 64,900 plants ha^{-1} at the experimental field of the Instutute for Animal Husbandry, Belgrade, Serbia (44° 49′ 10″ N, 20° 18′ 45″ E). The experimental field was divided into four blocks to obtain four replication per treatment and each block was split into four plots for the four treatment of nitrogen fertilization (0, 60, 120, 180 kgN ha^{-1}). Fertilization was done on 15th May 2013. with mineral fertilizer KAN (27%N). Maize was harvested in August, as whole-crop (cutting hight 20 cm) and chopped to a theoretical cut lenght of 10 mm. From each plot chopped mass was divided on two parts. One part was untreated and second treated with bacterial-enzyme inoculant Sill-All 4x4+WS (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Propion bacterium acidi propionici*, α-amilaza, celuloza, beta-glukanaza, ksilanaza). The inoculant was applied at recomended rate of 5g t^{-1} fresh maize material. The chopped forages from each plot was than ensiled in the 200 x 300 x 0.9 cm vacuum plastic bags with package mashine. Before filling of plastic bags, fresh maize forage samples were taken for chemical anallisys (Table 1.) The silages were conserved at room temperature for 90 days. After period od 90 days silages were opened and contents were sampled to determine the DM content, chemical and fermentable composition.

Table 1. The chemical composition of maize forages at ensiling

level of fertilization	0	60	120	180
DM (g kg^{-1})	431.3	472.9	448.4	477.4
CP (g kg^{-1} DM)	71.6	78.6	81.4	104.7
NDF (g kg^{-1} DM)	496.8	495.2	552.7	510.7
ADF (g kg^{-1} DM)	260.4	268.1	283.2	266.5
WSC (g kg^{-1} DM)	40.2	49.5	23.6	19.0
BC (Meq/100 gDM)	42.7	39.3	41.5	40.2

DM-dry matter; CP-crude protein; NDF-neutral detergent fiber; ADF- acid detergent fiber; WSC-water soluble carbohydrates; BC-buffering capacity

Chemical analysis

Dry matter content was determined by drying the samples at 105°C overnight. Crude protein content was determined according to Kjeldahl *(AOAC 1990),* content of crude fat using the Soxhlet method and ash by heating the dry samples in anoven at 550°C for 2h. Neutral detergent fiber (NDF) and acid detergent fibre were analysed according to Van Soest method. Water soluble carbohydrate (WSC) content was determined using the method of Luff-Schoorl. Ammonia nitrogen was determined by the distillation method using a Kjeltec 1026 analyser and the pH value was measured with a Hanna Instruments HI 83141 pH meter. Lactic acid (LA) and volatile fatty acids [acetic (AA) and butyric acid (BA)] were quantified by a gas chromatographic system (GC-2014, Shimadzu, Kyoto, Japan) equipped with flame-ionization detector and auto sampler and injection system, using a NukolTM (30m × 0.53mm × 0.5μm) capillary column (Supelco, Sigma-Aldrich Co.) *(Faithfull 2002).* Total digestible nutrients value (TDN), relative feed value (RFV) and values of metabolic energy (ME) and net energy for lactation (NEL) were calculated using the following steps:

TDN (%)= (-1,291 x ADF) + 101.35
RFV (%) = DDM (%) x DMI (%) x 0.775
DDM (%) = Digestible Dry Matter = 88.9 - (0.779 x % ADF)
DMI (%) = Dry Matter Intake = 120 / (% NDF)
Calculation of TDN and RFV was done according to *Harrocks and Vallentine (1999).*
ME(MJ kg^{-1}) = (0.01715 x dCP) + (0.03766 x dCf) + (0.0138 x dCF) + (0.01464 x dNFE)
NEL(MJ kg^{-1}) = ME x kl
kl = 0.6 x (1+ 0.004 x (q-57)) x 0.9752
q (%) = (ME / GE) x 100
GE(MJ kg^{-1}) = (0.02414 x CP) + (0.03657 x Cf) + (0.02092 x CF) + (0.01699 x NFE)
CP - crude protein (g kg^{-1});
Cf - crude fat (g kg^{-1});
CF – crude fibre (g kg^{-1});
NFE – nitrogen free extracts (g kg^{-1});
d – digestible
q - metabolizability coefficient

The experimental data ME and NEL were calculated by formula according *Obračević (1990).*

Statistical analysis

The experimental data were processed by the method of analysis of variance (two factor experimental design), applying the programme ANOVA and means were compared using t test.

Results and Discussion

The chemical composition and energetic characteristics of treated and untreated whole maize plant silage is shown in Table 2. Fertilization treatments had a significant impact on the content of crude protein, crude fat, metabolic energy and net energy for lactation. Nitrogen fertilization led to an increase in crude protein content. However, this increase was determined only in the treatment with 120 kgN ha^{-1}. Nitrogen fertilization that is > 120 kgN ha^{-1} reduced the crude protein content in the silage. The content of crude fat also increased by the addition of N mineral fertilizer up to 120 kgN ha^{-1}. Further adding of nitrogen led to a reduction of crude fat in the silage. N fertilization had a positive impact on the content of ME and NEL. The highest content of ME and NEL were determined in treatments with 120 and 180 kgN $ha^{-1}$of 10.3 MJ kg^{-1} ME and 6.0 MJ kg^{-1} NEL.

Table 2. Nutritional (g kg^{-1} DM) and energetic characteristics (MJ kg^{-1}) of maize silage after 90 days of conservation

Lev. of fertil.	0		60		120		180		F effect	I effect	FxI effect
Treatm	C	T	C	T	C	T	C	T	P value		
DM	423.7	430.3	463.5	469.4	446.4	440.6	474.3	467.0	0.235	0.906	0.302
Ash	28.5	30.4	27.8	27.3	31.4	29.6	29.3	31.0	0.061	0.705	0.344
CP	55.4	48.1	58.6	53.9	80.1	71.8	79.4	76.2	<0.001	0.036	0.906
CFT	23.8	23.5	25.0	19.3	24.5	25.3	20.8	18.4	0.003	0.059	0.120
NDF	504.6	537.6	503.8	508.6	510.9	491.8	477.1	532.1	0.567	0.089	0.088
ADF	281.8	279.7	279.7	276.0	291.6	281.6	250.3	278.2	0.161	0.671	0.245
TDN%	66.9	66.5	67.2	63.9	66.2	68.2	66.5	65.1	0.524	0.386	0.219
RFV%	132.4	121.9	134.2	115.7	133.9	137.8	124.2	119.0	0.080	0.060	0.242
ME	10.2	10.2	10.0	10.2	10.3	10.2	10.3	10.3	0.007	0.915	0.221
NEL	5.9	5.9	5.8	5.9	6.0	5.9	6.0	5.9	0.033	0.801	0.189

C-untreated; T-treatment with inoculant; F-effect of fertilization; I-effect of inoculation; FxI- effect of interaction; DM-dry matter; CP-crude protein; CFT- crude fat; NDF-neutral detergent fibre; ADF- acid detergent fibre; TDN-total digestible nutrient; RFV-relative feed value; ME-metabolizable energy; NEL-net energy for lactation

In the research by *Islam et al. (2012)* N fertilizer has also led to a significant increase in the content of crude protein and metabolizable energy in the silage. Increasing the content of CP in the treatments with fertilization can be explained primarily by increasing the crude protein in the plant before ensiling, while the increase in ME is explained by the increasing content of CP, and reduction in ADF and NDF *(Asgharzadeh et al. 2013).*

A significant effect of adding the inoculant was reflected only in the content of crude protein. Namely, the addition of lactic acid bacteria and enzymes to the silo mass led to a reduction in the concentration of crude protein in the silage. Also, in the research of *Ruiz-Perez et al. (2012)* and *Dupon et al. (2012),* crude protein content decreased with the addition of the bacterial inoculant, while in the study by *Vakily et al. (2011)* it was uniform.

The other investigated parameters shown in table 2 had not significantly changed in relation to the studied factors. According to *NRC (2001),* corn silage with over 40% dry matter contains 65.4% TDN, 44.5% NDF, 27.5% ADF and 4.0% ash. The values obtained were similar to TDN values of *NRC (2001),* and the values for NDF and ADF slightly higher, indicating that the silage was prepared at a later stage of maturity.

In table 3 are presented fermentation end product and the pH value of maize silage after 90 days of conservation. N fertilization had a significant impact on fermentable features of the silage, however, in certain tested parameters, such as lactic acid, the differences depending on the level of N fertilization can be observed. The treatments with 60 and 120 kgN ha^{-1} had higher lactic acid content compared to the control. This is confirmed by the research of *Namihira et al. (2010)* who has concluded that N fertilization is important silage quality factor and significantly increases the content of lactic acid to the fertilization level of 150 kg N ha^{-1}.

Table 3. Fermentation end product (g kg^{-1} DM) and pH value of maize silage after 90 days of conservation

Level of fertilization	0		60		120		180		F effect	I effect	FxI effect
Treatment	C	T	C	T	C	T	C	T	P value		
NH_3-N†	51.6	47.7	58.9	50.1	68.2	44.2	64.3	50.1	0.155	0.002	0.033
Lactic acid	69.2	67.6	76.6	72.4	72.7	72.2	68.1	67.4	0.181	0.311	0.593
Acetic acid	12.2	7.9	13.9	9.7	13.5	12.3	12.4	8.8	0.210	0.001	0.674
LA/AA	5.9	8.9	6.4	7.9	5.4	6.2	5.6	8.2	0.064	<0.001	0.247
Butyric acid	0.4	0.4	0.6	0.4	0.8	0.6	0.6	0.3	0.315	0.202	0.840
pH	3.9	3.8	3.9	3.8	4.0	3.8	3.9	3.8	0.363	<0.001	0.889

†-(g kg^{-1} TN); C-untreated; T-treatment with inoculant; F-effect of fertilization; I-effect of inoculation; FxI- effect of interaction; LA/AA-lactic acid/acetic acid

Treatment with bacterial-enzyme inoculants had a significant impact on the content of ammonia nitrogen, acetic acid, pH value and LA/AA. Silages treated with inoculants showed a lower content of ammonia nitrogen and according to its content, which is less than the limit 7-10% (*Đorđević and Dinić, 2003*), they are classified as high quality silages. Also, the content of acetic acid and pH were lower in silages with inoculant. *Rota et al. (2012)* in their research also report lower content of acetic acid and pH in silages treated with *Pediococcus pentosaceus* and *Lactobacillus plantarum*. However, in recent years, the opinion prevailed that the acetic acid, particularly acid created during the anaerobic phase of lactic acid, is essential for the preservation of aerobic stability of silage. The hetero-fermentative bacteria that are integral part of some bacterial inoculants sare responsible for this. For this reason, *Comino et al. (2014)* reported a higher content of acetic acid in the silage with inoculant as the inoculant used, in addition to homo- also had hetero-fermentative bacteria such as *Lactobacillus buchner*. Otherwise, the content of acetic acid from a control treatment is similar to the content of the present study. The level of acetic acid in the investigated silages was satisfactory (<3-4%) and the ratio LA/AA (>3:1), indicating a good fermentation in the control and the treatment with inoculant.

According to *Đorđević and Dinić (2003),* pH value of silage should be in the range from 3.8 to 4.5. If the pH is lower than 3.8 it is less consumed. In our research the pH value of silage from the control and treatment was 3.8-4.0.

The results of correlation coefficients between tested chemical and fermentable parameters (Table 4) showed a significant negative correlation between the dry matter content in silage and content of lactic, acetic, butyric acid, TDN and RFV. Also, negative correlation was established between the ash content and the ADF with TDN and RFV.

Table 4. Correlation analysis of chemical and fermentable parameters of the silage

	NH_3-N	pH	LA	AA	BA	TDN	RFV	ME	NEL
DM	-0.08	-0.28	-0.49*	-0.54*	-0.33*	-0.06	-0.36*	0.17	0.21
Ash	-0.13	-0.14	-0.02	0.03	-0.03	-0.44*	-0.32*	0.17	0.10
CP	0.22	0.19	0.21	0.41*	0.31*	0.41*	0.19	0.40*	0.28
CFT	-0.10	0.40*	0.24	0.38	0.31	-0.06	-0.02	-0.14	-0.12
NDF	-0.08	0.05	-0.01	-0.04	0.10	-0.53*	0.31*	-0.12	-0.20
ADF	-0.02	0.07	-0.18	-0.10	-0.00	-0.97*	-0.60*	-0.19	-0.26

*- Marked correlations are significant at $p < 0.05$; DM-dry matter; CP-crude protein; CFT- crude fat; NDF-neutral detergent fibre; ADF- acid detergent fibre; TDN-total digestible nutrient; RFV-relative feed value; ME- metabolizable energy; NEL-net energy for lactation; LA- Lactic acid; AA- acetic acid; BA-butyric acid

Increase of the NDF content decreased TDN and increased RFV. The crude protein content was significantly positively correlated with certain fermentable parameters. Thus, increase in the crude protein content in the silage increased the content of acetic, butyric acid, TDN, RFV and ME: r=0.41, r=0.31 and r=0.41, r=0.40 respectively.

With maturation of plants the dry matter content increased, and therefore the NDF and ADF, causing the decline in crude protein and metabolic energy. Such high fibre and low protein concentrations decrease overall digestibility and RFV (*Asgharzadeh et al., 2014*).

Conclusion

On the basis of the examined factors of the quality and nutritive value of silage from whole maize plant, we concluded that nitrogen fertilizer contributed favourably to the quality and energy value of silage, but only to the level of nitrogen of 120 kgN ha^{-1}. Further adding of nitrogen had a depressive character. N fertilization led to a significant increase in crude protein, metabolizable energy and net energy for lactation. It also led to a minimum increase in the content of lactic acid.

Application of inoculants had a significant impact on the improvement of the quality of the fermentation, particularly in the reduction of the content of ammonium nitrogen, acetic acid and the pH. However, the silages from control treatments also had satisfactory investigated parameters of quality and nutritional value so that both can be classified as good quality silage.

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Efekat đubrenja azotom i inokulanta na hranljivu vrednost i fermentabilne karakteristike silaže od cele biljke kukuruza

Z. Bijelić, V. Mandić, D. Ružić-Muslić, Z. Tomić, V. Krnjaja, V. Petričević, M. Gogić, W. de Souza Filho

Rezime

Cilj naših istraživanja je da ispitamo efekat đubrenja N i tretmana silo mase bakterijsko-enzimskim inokulantom na hemijske, fermentativne i nutritivne karakteristike silaže od cele biljke kukuruza, kao i da utvrdimo korelacionu zavisnost pojedinih parametara kvaliteta. Ogled je izveden na oglednom polju i u eksperimentalnoj laboratoriji Instituta za stočarstvo. Kukuruz je posejan na oglednom polju u četiri ponvljanja. Ispitivan je uticaj četiri različite doze azota od 0, 60, 120, 180 kgN ha^{-1}, dodate u toku vegetacije kukuruza. Silaža je pripremana

u laboratoriji. Silaža je pripremana u vakum kesama. Jedan deo iseckane mase siliran je sa bakterijsko-enzimskim inokulantom, a deo bez dodatog inokulanta. Silaža je čuvana na sobnoj temperaturi 90 dana. Nakon devedeset dana silaža je uzorkovana za hemijske analize. Đubrenje sa količinama azota do 120 kgN ha^{-1} dovelo je do povećanja sadržaja CP, CFT. Đubrenje azotom imalo je pozitivan uticaj na sadržaj ME i NEL. Najvećim sadržajem ME i NEL odlikuju se tretmani sa 120 i 180 kgN ha^{-1} od 10.3 MJ kg^{-1} ME i 6.0 MJ kg^{-1} NEL. Tretman bakterijsko-enzimskim inokulantima je značajno smanjio sadržaj CP, NH_3-N, sićetne kiseline, pH vrednost i povećao udeo mlečne kiseline u odnosu na sirćetnu LA/AA.

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