

EFFECTS OF ADDED GROUND CORN GRAIN ON THE NUTRITIONAL VALUE AND PROTEIN DEGRADABILITY OF AN ENSILED MIXTURE OF COMMON VETCH AND OATS

**Bojan D. Stojanović^{1*}, Dragan Ž. Stanojević¹, Vesna M. Davidović¹,
Aleksandra M. Ivetić², Stamen B. Radulović³,
Blagoje M. Stojković¹ and Ivana M. Grujić¹**

¹University of Belgrade, Faculty of Agriculture,
Nemanjina 6, 11000 Belgrade, Serbia

²The Institute for Science Application in Agriculture,
Bulevar despota Stefana 68b, 11000 Belgrade, Serbia

³University of Belgrade, Faculty of Veterinary Medicine,
Bulevar oslobođenja 18, 11000 Belgrade, Serbia

Abstract: The aim of the study was to determine the effects of the addition of 5% or 10% of ground corn grain (CG) on the ensiling properties, nutritional value, and protein degradability of a forage mixture of common vetch and oats (VO). The addition of CG significantly increased the dry matter content of the silages ($p < 0.01$), while the crude protein (CP) content decreased by 4.7% and 9.3% in the VO+5% CG and VO+10% CG treatments, respectively. The addition of CG to ensiled forage resulted in higher values of lactic acid concentration (4.5% and 6.8%), especially when the proportion of milled grain was higher ($p < 0.05$), and the pH of the silages was significantly lowered ($p < 0.01$). The use of ground CG in ensiled legume-cereal forage provided higher net energy (NE) concentration values, which were significant ($p < 0.05$) for NE for meat production. The ammonia-N concentration (protein fraction A1) was significantly lower ($p < 0.05$) in the VO+5% CG and VO+10% CG treatments (15.8% and 21%, respectively), and also the soluble protein (CP fraction A2) by 8.2% and 12.1% ($p < 0.01$). Protein degradability was affected by the supplementation of ensiled forage with CG ($p < 0.05$ for $K_p = 5\%/h$ and $p < 0.01$ for $K_p = 8\%/h$), with the lowest degradability observed in VO+10% CG silages. It is suggested that the supplementation of vetch-oat forage with ground corn grain, especially at a higher proportion (10% vs. 5% of wet weight), has a favorable effect on fermentation parameters, the proportion of rumen undegradable protein (RUP) in CP, and the nutritional value of the obtained silage.

Key words: corn, legume-cereal silage, protein fractions, protein degradability, fermentation parameters.

*Corresponding author: e-mail: arcturas@agrif.bg.ac.rs

Introduction

Common vetch (*Vicia sativa* L.) is an annual legume commonly used in forage mixtures with cereal small grains, such as oats (*Avena sativa* L.) under rainfed conditions. The vetch-oat mixture is characterized by the highest forage dry matter (DM) yield and a higher CP content compared to the vetch-barley and vetch-triticale mixtures (Nájera et al., 2016). The ensiled mixture of common vetch and oats has the highest CP content, compared to mixtures of common vetch and barley, wheat, triticale or rye, also with the lower values for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Balabanli et al., 2010). This is another reason for the high rate of silage obtained with common vetch and oat intercrop.

Good silage fermentation depends on the rapid proliferation of lactic acid bacteria, which mainly ferment water-soluble carbohydrates. Legumes and legume-cereal forages are difficult to preserve because of their low water-soluble carbohydrate content (Kristensen, 1992). The buffering effect of legumes may be an additional constraint to rapid acidification (Cussen et al., 1995) in legume crops and legume-cereal bi-crops. Obtaining quality silage and avoiding losses during ensiling can be achieved by treating with silage additives such as carbohydrate sources that stimulate fermentation (McDonald et al., 1991). In general, the addition of fermentable carbohydrates results in the accelerated growth of lactic acid bacteria, the more rapid development of acidic conditions and an early elimination of coliform bacteria (Kung et al., 2003). Corn grain contains adequate water-soluble carbohydrates for fermentation to lactic acid, has a low buffering capacity, and a high dry matter and energy content (Topps and Oliver, 1993). The inclusion of legumes in grass forage raises the pH and ammonia nitrogen of silages, while the increase in pH and $\text{NH}_3\text{-N}$ content is lower in the silages treated with the additive – ground corn (Sibanda et al., 1997). According to the report by Weiss and Underwood (2009), the addition of grain to the hay-crop forage increases the energy and dry matter (DM) content of the silage.

Forages, especially legumes, are an important source of protein for ruminants, but their protein is often poorly utilized because it is extensively degraded during ruminal fermentation, and this may be the most limiting factor for high quality forage legumes (Tremblay et al., 2003). The changes in protein quality during ensiling of legumes and grass forages can affect intake, protein utilization and productivity of dairy and beef cattle (Guo et al., 2008). During ensiling, plant endopeptidases catalyze proteolytic reactions converting most of the soluble forage protein to non-protein nitrogenous (NPN) compounds (Nsereko and Rooke, 2000). Supplementing ensiled forage with a source of readily fermentable carbohydrates can result in a greater pH reduction than in untreated silages and also in a lower $\text{NH}_3\text{-N}$ concentration (Jones, 1988; McDonald et al., 1991; Adesogan et al., 2004).

Although there is much information on the effects of numerous additives on mono-crop silages, little is known about the suitability of ground corn grain as an additive for legume-cereal bi-crop silages. The aim of this study was to measure the effects of adding ground corn grain on improving the fermentation process in silages and on the nutritional value and CP degradability of an ensiled forage mixture of common vetch and oats.

Material and Methods

Common vetch (*Vicia sativa* L. cv. Novi Beograd) and oats (*Avena sativa* L. cv. Rajac) were intercropped at respective seed rates of 70 and 30 kg/ha in late March 2022 in a field located in the western region of Serbia, in the vicinity of Loznica (44°53' N, 19°49' E) on loamy soil. The field was fertilized with 30 t/ha of cow manure, which was incorporated before plowing in the fall, and with 100 kg/ha of compound fertilizer (N, 15; P, 15; K, 15) before sowing. The area has a continental, semi-arid to semi-humid climate. The temperature and precipitation for April, May and June 2022 were 11.3, 18.7 and 23.2°C and 57.8, 31.2 and 171.5 mm, respectively.

The forage of vetch and oats was harvested when the vetch formed first pods (end of the third week of June), with a rotary mower about 5 cm above ground level. The cut herbage was left to wilt overnight *in situ*, and after the wilting period the plant material was chopped to about 1.5 cm particle length with a forage chopper. The wilted and chopped forage was ensiled untreated (VO) or after treatment with 50 or 100 g/kg (wet weight) of ground corn grain (VO + 5% CG or VO + 10% CG). The dried corn grains were ground in a hammer mill (2-mm sieve size) before being mixed with the chopped, wilted legume-cereal plant material. The forage was preserved using a small-scale silage fermentation system. All feeds were ensiled without the use of inoculants in anaerobic glass jars with a capacity of 1.5 l, each jar containing 650 to 700 g of forage mass. The silages were stored at room temperature (22–29°C) for 3 months. Five replicates each of untreated and treated silage were prepared to obtain a total of 15 laboratory silos. Representative samples of the wilted vetch-oat herbage were frozen (-20°C) for subsequent laboratory analysis.

The chemical analysis was conducted in the Laboratory of Animal Nutrition at the Faculty of Agriculture of the University of Belgrade. The samples of wilted plant material and silages were dried for 48 h at 50°C in a forced-air oven for chemical composition analysis. All samples were ground through a 1-mm sieve on a small sample cutting mill (Kinematica PX-MFC 90D). The ground samples were analyzed according to the official methods (AOAC, 2002). The analytical DM content of the samples was determined by drying at 105°C for 16 h (method 967.03), the ash content by combustion at 600°C for 2 h (method 942.05), the CP

content by the Kjeldahl method (method 2001.11), the ether extract content by extraction with diethyl ether (method 920.39), the crude fiber content by the Henneberg and Stohmann method (method 978.10), the NDF content was determined using heat-stable α -amylase (method 2002.04) and the ADF content according to the method 973.18. The water extracts of the ensiled forage were analyzed for pH (Owens et al., 1999) (Table 1).

Table 1. The chemical composition (g/kg DM) of the vetch-oat forage and corn grain used for ensiling.

Ingredient	Vetch-oat forage	Ground corn grain
Dry matter	287.6	874.6
Crude protein	134.3	86.9
Ether extract	35.1	35.8
Neutral detergent fiber	617.8	109.6
Acid detergent fiber	465.2	39.1
Crude fiber	345.1	33.2
Nitrogen-free extract	370.8	833.4
Ash	114.7	10.7

The CP was divided into five fractions (A1, A2, B1, B2 and C) based on ruminal degradation rates according to the Cornell Net Carbohydrate and Protein System – CNCPS v6.5 (Higgs et al., 2015). Within the determined fractions, A1 represented ammonia (as CP equivalents), A2 – soluble true protein (soluble protein minus A1), B1 was buffer insoluble protein minus neutral detergent insoluble protein (NDIP), B2 – NDIP minus acid detergent insoluble protein (ADIP) and ADIP – the C fraction. Rumen degradable protein (RDP) was estimated using digestion rate constants according to CNCPS v6.5 (Van Amburgh et al., 2015) with an assumed passage rate (Kp) of 5%/h and 8%/h (Sniffen et al., 1992).

The CVB (2018) model was used to estimate the concentration of net energy for lactation (NE_{lac}) and meat production (NE_{meat}) of silages in ruminant nutrition.

Analysis of variance using JASP v.0.15 (JASP Team, 2021) was performed to evaluate the effects of the level of ground corn grain addition on the chemical composition and nutritional values, as well as on the protein fractions and degradability of the ensiled mixture of common vetch and oats. The LSD test was used to estimate the significance of the differences between the means of the treatments. The effect of treatment was considered significant if $p < 0.05$.

Results and Discussion

To investigate the effect of adding ground corn grain to ensiled vetch-oat forage on the chemical composition, fermentation parameters, protein degradability and nutritional value of the silages, the grain addition rates applied were within the range commonly used in previous studies with other cereals (Jones et al., 1990; Harrison et al., 1994).

The chemical analysis showed the good quality of the experimental silages (Table 2).

The addition of ground corn significantly increased the dry matter content ($p < 0.01$). The obtained values for the higher DM content (9.1% and 21.9%) in ensiled vetch-oat forage treated with ground corn grain were expected due to the high DM content of CG. These results are consistent with earlier studies by Jones et al. (1990) in which ensiled ryegrass forage was supplemented with rolled barley. The increase in DM content may also contribute to less favorable conditions for undesirable fermentation and possibly promote the rapid proliferation of lactic acid bacteria (Jones, 1988). According to Pys et al. (2002), the addition of rolled grain to ensiled forage increases DM content and also acts as an absorbent additive.

The CP content decreased significantly ($p < 0.05$) after the addition of 5% or 10% of ground corn grain (4.7% and 9.3%) to the ensiled vetch-oat forage, which can be attributed to the significantly lower protein content in the DM of the corn grain used compared to the vetch-oat forage.

Table 2. The chemical composition (g/kg DM) and the net energy content of the silages.

Ingredient	Silage						p-values
	VO	SEM	VO+5% CG	SEM	VO+10% CG	SEM	
Dry matter, %	299.4 ^c	1.9	326.5 ^b	0.6	365.1 ^a	0.3	<0.01
Crude protein	137.2 ^a	2.8	130.7 ^b	0.6	124.5 ^c	0.6	0.04
Ether extract	36.0	0.6	35.3	0.6	35.5	0.3	0.60
Neutral detergent fiber	636.0 ^a	3.9	595.6 ^b	2.8	576.7 ^c	2.1	<0.01
Acid detergent fiber	490.4 ^a	4.3	464.0 ^b	3.0	444.2 ^c	3.1	<0.01
Crude fiber	357.2 ^a	6.5	343.4	9.4	322.8 ^b	4.4	0.02
Nitrogen-free extract	354.3 ^c	1.5	371.2 ^b	2.0	395.8 ^a	1.8	0.02
Ash	115.3	0.6	114.4	0.3	114.0	0.3	0.13
Lactic acid	53.2 ^b	0.7	55.6	1.1	56.8 ^a	1.0	0.02
Acetic acid	26.3	1.1	28.2	1.3	27.8	1.0	0.46
Butyric acid	2.7	0.4	2.5	0.4	2.5	0.4	0.95
pH	4.58 ^a	0.02	4.39 ^b	0.02	4.28 ^c	0.03	<0.01
NElac, kJ/kg DM	5028.9	15.8	5043.4	16.2	5075.1	4.8	0.13
NEmeat, kJ/kg DM	4882.7 ^b	22.4	4906.3	23.7	4954.7 ^a	7.0	0.03

VO – common vetch-oat; VO+5% CG – common vetch-oat + 5% ground corn grain; VO+10% CG – common vetch-oat + 10% ground corn grain. ^{a, b, c} Values within the same row with different letter superscripts differ significantly ($p < 0.05$).

The application of CG in vetch-oat forage at a dosage of 5% and 10% decreased ($p<0.01$) the concentration of neutral detergent fiber (NDF, 6.4% and 9.3%) and acid detergent fiber (ADF, 5.4% and 9.4%) in the silage, and in contrast, the content of nitrogen-free extract (NFE) was 4.8% and 11.7% higher ($p<0.05$). The obtained values could be fully explained by the lower fiber content of the added corn grain as a feed with a particularly high starch content. This is consistent with reports by Jones (1988) that the addition of ground oats or barley to ryegrass forage increases the silage DM and reduces the fiber content of the silage. Harrison et al. (1994) also found that the addition of barley increased the DM and starch content of ensiled alfalfa forage and decreased the fiber content (NDF and ADF).

The treatment of the ensiled forage with CG resulted in higher values for lactic acid concentration (4.5% and 6.8%), especially when the proportion of milled grain was higher ($p<0.05$). The concentrations of acetic and butyric acid did not differ significantly between the treatments. The effect of the addition of CG to the vetch-oat plant mass on the improvement of the fermentation quality of the obtained silages was demonstrated by the significantly lower pH values observed ($p<0.01$). The results obtained show that lactic acid and acetic acid were present in high concentrations, while butyric acid was present in very low concentrations. Since lactic acid is the major organic acid responsible for most of the pH reduction in silage, its presence is favored over other fermentation products in silage (Umana et al., 1991). The addition of ground CG at 5% and 10% of fresh weight resulted in a significantly greater pH reduction than with non-supplemented silage. The results of this study are consistent with the reports of Harrison et al. (1994) that the addition of rolled barley improved the fermentation of alfalfa by lowering the pH and reducing the acetate concentration. In general, a lower pH ensures better preservation and higher stability of the silage. This indicates that corn grain-treated vetch and oat forage has the potential to produce silages of acceptable quality. The effect of milled grain addition to ensiled forage probably cannot be attributed to the hydrolysis of the grain starch, and it is more likely that the 3–4% simple sugars present in the grain significantly affected fermentation or that the other carbohydrate fractions such as β -glucan were hydrolyzed to yield sugars for fermentation (Jones, 1988). The total content of readily fermentable simple sugars in dry corn kernels averages 4.15% (3.31% sucrose, 0.28% each of glucose, fructose, and raffinose) (Kereliuk et al., 1995).

The use of ground CG in ensiled legume-cereal forage provided higher values for net energy concentration (net energy for lactation – NE_{lac} and meat production – NE_{meat}), whereas NE_{meat} was significantly higher ($p<0.05$) in VO+10% CG silage. The increase in the net energy concentration of the supplemented silages is consistent with the lower fiber and the higher NFE content of these silages.

The crude protein fractions of the analyzed silages according to the Cornell Net Carbohydrate and Protein System are shown in Table 3.

Table 3. The CNCPS crude protein fractions (% CP) of the silages.

Fractions	Silage						p-values
	VO	SEM	VO + 5% CG	SEM	VO + 10% CG	SEM	
A1	14.53 ^a	0.67	12.24 ^b	0.65	11.48 ^b	0.59	0.01
A2	49.86 ^a	0.78	45.76 ^b	0.62	43.85 ^b	0.66	<0.01
B1	15.16 ^b	1.66	23.15 ^a	1.95	27.16 ^a	1.90	<0.01
B2	12.28	0.12	12.13	0.50	11.03	0.54	0.12
C	8.17 ^a	0.35	6.72 ^b	0.26	6.48 ^b	0.24	<0.01

VO – common vetch-oat; VO+5% CG – common vetch-oat + 5% ground corn grain; VO+10% CG – common vetch-oat + 10% ground corn grain. ^{a, b} Values within the same row with different letter superscripts differ significantly ($p < 0.05$).

The $\text{NH}_3\text{-N}$ concentration (protein fraction A1) was significantly lower ($p < 0.05$, 15.8% and 21%) in silages where the ensiled forage was supplemented with 5% or 10% ground CG. Soluble protein (fraction A2) was also significantly lower ($p < 0.01$) in silages treated with 5% or 10% CG (8.2% and 12.1%). The significantly higher values ($p < 0.01$) for the moderately degradable protein fraction (B1) were found in ensiled vetch-oat forage with 5% or 10% CG added (52.7% and 79.2%). There were no significant differences in the content of the B2 fraction between the treatments. Protein fraction C, which is not available to the animals, was significantly reduced ($p < 0.01$) by the addition of ground corn to the ensiled forage (17.8% and 20.7%).

The research results regarding the significant reduction in protein fractions A1 ($\text{NH}_3\text{-N}$) and A2 (soluble protein) when supplementing vetch-oat forage with CG are consistent with the findings of Harrison et al. (1994), where the addition of ground barley to the alfalfa forage prior to ensiling (in ranges of 50–150 g/kg wet weight) significantly affected fermentation and resulted in lower pH and lower levels of ammonia-nitrogen, acetate and propionate, while the amount of lactate was increased. According to Sibanda et al. (1997), silages treated with ground corn had a lower ammonia-N content than the control grass or grass-legume silages. Jones (1988) also found that the addition of ground oats or barley to ensiled ryegrass forage significantly improved fermentation and led to a significant reduction in the ammonia-N content and pH of the silages. Spörndly (1986) also reported that the addition of 5% oat flakes improved the fermentation quality of the silage, which was confirmed by the reduction in pH and ammonia-N content, this reduction being accompanied by a progressive increase in lactic acid content. The availability of readily fermentable carbohydrates may be a determining factor for the fermentation rate and the reduction of pH and proteolysis in silages (Adesogan et al., 2004). The addition of molasses to ensiled forage resulted in a greater pH reduction and a lower $\text{NH}_3\text{-N}$ concentration than in silages without molasses (McDonald et al., 1991). This was probably due to the fact that a higher sugar concentration led to a rapid drop in pH, which inhibited the deamination and

decarboxylation of amino acids. The increased silage DM content could also reduce the soluble N content and improve the proportion of rumen undegradable protein (RUP) in the CP. Kung et al. (2018) report that ensiled forages with a lower DM content have higher concentrations of soluble N and $\text{NH}_3\text{-N}$ than drier silages, as fermentation is more robust overall in the former.

Due to the reduced contents of fiber fractions (NDF and ADF) in ensiled vetch-oat with milled corn grain, the lower values of CP fractions connected with cell wall components (B2 and C) were observed. Accordingly, the supplemented silages showed increased values for the moderately degradable protein fraction (B1).

Protein degradability was affected by the treatment of the ensiled forage with CG ($p < 0.05$ for $K_p = 5\%/h$ and $p < 0.01$ for $K_p = 8\%/h$), with the lowest degradability observed in ensiled forage with 10% ground CG (Table 4).

Table 4. The crude protein degradability (RDP, % of CP) of silages.

Silage	K_p			
	5%/h		8%/h	
	Mean	SEM	Mean	SEM
VO	76.84 ^a	0.18	71.32 ^a	0.23
VO+5% CG	76.52	0.10	70.38 ^b	0.10
VO+10% CG	76.18 ^b	0.12	69.79 ^c	0.14
p-values	0.02		<0.01	

VO – common vetch-oat; VO+5% CG – common vetch-oat + 5% ground corn grain; VO+10% CG – common vetch-oat + 10% ground corn grain; K_p – ruminal passage rate. ^{a, b, c} Values within the same column with different letter superscripts differ significantly ($p < 0.05$).

The protein degradation during ensiling results from the high activity of the cellular proteolytic enzymes of the plants and the process of bacterial deamination of free amino acids and amides (Vagnoni et al., 1997). The significantly lower CP degradability of silages with added milled corn grain could be due to the more effective restriction of the cellular proteolysis of the protein by a significant improvement in the lactic acid concentration and a stronger pH reduction (Charmley and Veira, 1990). Dried corn grain is characterized by a higher RUP content (45.4% CP) compared to other cereals (barley 36.8% and wheat 32.5% CP) (Chrenkova et al., 2018), which could also contribute to a higher proportion of RUP in supplemented silages. A higher RUP content in silages with corn grain ensures an improved biological value of the silage protein and a more efficient use of the consumed N (Dijkstra et al., 2013). It also increases the amounts of amino acids flowing from the rumen to the duodenum in ruminants.

Conclusion

The results of this study indicate that the addition of ground corn grain to common vetch-oat forage has the potential to provide well-preserved silage. When vetch-oat plant mass was ensiled with added corn grain, especially at higher levels (10% of fresh weight), the production of lactic acid dominated, resulting in a lower pH compared to the non-added forage. The net energy content of the silage was increased by adding milled grain before ensiling vetch-oat forage. The addition of ground corn grain to ensiled legume-cereal forage decreased the proportion of $\text{NH}_3\text{-N}$ and soluble protein and improved the RUP to CP ratio of the silage. Considering the chemical composition and nutritional value, it is suggested that supplementation of vetch-oat forage with ground corn grain, especially at a higher proportion (10% vs. 5% of wet weight), has a favorable effect on fermentation parameters, the ratio of RUP to CP and the nutritional value of the silage obtained.

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EFEKTI DODAVANJA KUKURUZNE PREKRUPE NA HRANLJIVU VREDNOST I RAZGRADIVOST PROTEINA SILIRANE SMEŠE GRAHORICE I OVSA

**Bojan D. Stojanović^{1*}, Dragan Ž. Stanojević¹, Vesna M. Davidović¹,
Aleksandra M. Ivetić², Stamen B. Radulović³,
Blagoje M. Stojković¹ i Ivana M. Grujičić¹**

¹Univerzitet u Beogradu, Poljoprivredni fakultet,
Nemanjina 6, 11000 Beograd, Srbija

²Institut za primenu nauke u poljoprivredi,

Bulevar despota Stefana 68b, 11000 Beograd, Srbija

³Univerzitet u Beogradu, Fakultet veterinarske medicine,
Bulevar oslobođenja 18, 11000 Beograd, Srbija

R e z i m e

Istraživanje je obavljeno u cilju utvrđivanja efekata dodavanja 5% ili 10% prekrupe zrna kukuruza (engl. *corn grain* – CG) na kvalitet, hranljivu vrednost, i razgradivost proteina silaže dobijene siliranjem smeše grahorice i ovsa (engl. *vetch-oat* – VO). Korišćenje prekrupe zrna kukuruza značajno je povećalo sadržaj suve materije u silaži ($p<0,01$), dok je smanjenje sadržaja sirovih proteina iznosilo 4,7% odnosno 9,3%, za tretmane VO+5% CG i VO+10% CG. Uključivanje prekrupe zrna kukuruza u siliranu biljnu masu smeše grahorice i ovsa rezultiralo je povećanjem koncentracije mlečne kiseline (za 4,5% i 6,8%), što je bilo i statistički značajno ($p<0,05$) pri većem učešću samlevenog zrna, kao i značajnim smanjenjem pH vrednosti silaža ($p<0,01$). Dodavanje samlevenog zrna kukuruza pri siliranju smeše leguminoze i žitarice, obezbedilo je veću koncentraciju neto energije u dobijenim silažama, pri čemu je ovo povećanje bilo značajno ($p<0,05$) za sadržaj neto energije za proizvodnju mesa. Koncentracija amonijačnog-N (A1 frakcija sirovih proteina) bila je značajno smanjena ($p<0,05$) kod tretmana VO+5% CG i VO+10% CG (15,8% odnosno 21%), a takođe je značajno smanjen (8,2% i 12,1%, $p<0,01$) i sadržaj rastvorljivog proteina (A2 frakcija sirovih proteina). Dodavanje kukuruzne prekrupe značajno je uticalo na razgradivost sirovih proteina u silažama ($p<0,05$ za $K_p=5\%/h$, i $p<0,01$ za $K_p=8\%/h$), pri čemu je najmanja razgradivost proteina utvrđena kod tretmana VO+10% CG. Može se zaključiti da dodavanje prekrupe zrna kukuruza pri siliranju biljne mase smeše grahorice i ovsa, naročito u većoj količini (10% naspram 5% silirane mase), ima pozitivan efekat na parametre fermentacije, učešće frakcije nerazgradivog proteina (engl. *rumen undegradable protein* – RUP) u sirovom proteinu, kao i na hranljivu vrednost dobijenih silaža.

Ključne reči: kukuruz, silaža smeše leguminoza i žitarica, frakcije proteina, razgradivost proteina, fermentacioni parametri.

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* Autor za kontakt: e-mail: arcturas@agrif.bg.ac.rs