

SILAGE FERMENTATION CHARACTERISTICS OF GRASS-LEGUME MIXTURES HARVESTED AT TWO DIFFERENT MATURITY STAGES

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Abstract: The objective of our study was to investigate the diversity of individual parameters of quality of grass-legume mixture silages harvested in two stages of crop utilization and the impact of the interaction of studied factors on the quality. Legumes as an important source of protein are very difficult to ensilage. However, in mixture with grasses their fermentable characteristics are improved. During the growth and development of plants, their chemical composition changes, hence their ability and suitability for ensiling also changes. In regard to the studied factors, the phase of exploitation had a highly significant impact on almost all quality parameters in both years. By delaying the harvest period, dry matter content in silage has significantly increased and the level of crude protein and $\text{NH}_3\text{-N}$ decreased. In regard to the content of lactic acid, the studied silages fall into category of good quality silages. Its content in the first year ranged from 24.3-31.5 in the early harvest stage and from 27.9-36.2 g kg^{-1} DM at the late harvest stage, and in the second year from 27.4 to 31.4 in the early harvest stage and from 28.2-31.9 g kg^{-1} DM at the stage of late harvest. According to the content of acetic and butyric acids, studied silages can also be considered as silages of good quality.

Key words: silage, grass-legume mixtures, maturity stage, quality

Introduction

Silage quality depends on many factors such as plant species, implemented agro-technical measures and practices, preparation time, applied inoculants, especially stages of plant development in which it is used for the preparation of silage. *McEniry et al. (2013)* state that the two most important pre-ensiling factors responsible for the quality of silage are plant species and stage of plant development at the time of harvest. At certain stages of development plants are

characterized by different chemical composition. Thus, in the younger stages of development plants contain more protein, less dry matter, higher buffer capacity, which results in great losses in the silage dry matter (King *et al.*, 2012) and the incidence of clostridial fermentation. However, plants in later stages of development are also characterized by reduced buffering capacity, reduced concentration of easily soluble sugars, higher content of dry matter and structural carbohydrates, lignin (Keady and O'Kiely, 1998) as well as the incidence of problems in compaction of forage, resulting in silage of poorer quality with a lower concentration of lactic acid, higher pH and butyric acid content, reduced digestibility (Vranić *et al.*, 2008). By delaying the harvest period in the study of Knežević *et al.* (2009) higher content of dry matter, and the ADF and NDF, NH₃-N and acetic acid, as well as pH value of the silage were achieved. Also, Dawson *et al.* (2002), by harvesting the plants in the later stages, obtained silages with substantially higher content of acetic acid, NDF and NH₃-N, but lower pH values and digestibility. In studies of Keady *et al.* (2000), delayed harvest period resulted in silage with high content of dry matter, NDF, ADF, ADL, cellulose and hemicellulose, and reduced content of crude protein and butyric acid.

Legumes as an important source of protein are very difficult to ensilage due to the high buffering capacity and low concentrations of easily soluble sugars. In mixture with grasses their fermentable characteristics are improved, so that silage of adequate quality can be obtained. Also different grass species have different ensiling abilities, depending on the content of WSC and buffering capacity. In the study of the mixture of red clover with perennial and Italian ryegrass and cocksfoot, Wyss (2004) concluded that the ryegrasses had positive, and cocksfoot negative impact on silage quality. According Knotek (1997), crude protein content in cocksfoot ranges from 142.4-130.7, soluble carbohydrates 52.9-52.3 g kg⁻¹, the buffer capacity from 1.34 to 1.59, in tall fescue CP content is 113.7, soluble carbohydrates 89.9 g kg⁻¹, the buffer capacity of 2.19, which means that tall fescue is more suitable for silage.

The aim of our study was to investigate the diversity of individual parameters of quality of grass-legume mixture silages harvested in two stages of crop utilization and the impact of the interaction of studied factors on the quality.

Materials and Methods

The trial was performed at the Institute for Animal Husbandry in Zemun, Belgrade. The experiment was set in a semi random block system in four replications. The main plot size was 10 m². The survey covers lucerne as a pure crop (A) and its mixtures with cocksfoot (50 : 50) - B1, cocksfoot and tall fescue (33.3 : 33.3 : 33.3) - B2 and cocksfoot, tall fescue and sainfoin (25 : 25 : 25 : 25) -

B3. One half of the basic plot was harvested in the bud stage (early harvest), the other half in the stage of 50% flowering of lucerne (late harvest).

The second cut was used for the preparation of silage. After cutting the biomass was weighed, wilted and used for the preparation of silage in experimental silos of 10 dm³ volume. For the purpose of good fermentation before filling the silo, the material was treated with micro-biological preparation - mixture of four homofermentative strains of lactic-acid fermentation (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Streptococcus faecium*, *Lactobacillus salivarius*) and four different enzymes (cellulases, hemicellulase, amylase, xylanase), in the amount of 10g + 2l H₂O t⁻¹ of green mass. Sampling of silage was performed 90 days after the silos were closed.

At the end of the fermentation process the silages were analysed. Dry matter was determined by oven-drying over the night at 105°C. Crude protein was determined according to Kjeldahl. The concentration of ammonia-N was analysed by steam distillation method with a Kjeltex 1026 analyser. Lactic, acetic and butyric acid were determined by Flieg method. pH value was measured with a Hanna Instruments HI 83141 pH meter.

Data were analysed as a completely randomized design using the generalized factorial ANOVA of the software package STATISTICA 8 (StatSoft, Inc. 2007), where mixture type and harvest time were included as fixed factors. Means with a significant F-value were tested with Fisher's LSD test.

Results and Discussion

The dry matter content of silage is one of the important parameters that determines the success of fermentation of the ensiling material. The low level of DM in the silage is a sign of poor fermentation and such silage has a high pH value, less lactic and acetic acids and often high values of butyric acid due to the development of butyric bacteria. Excess DM is also not desirable, because due to more difficult compaction of ensiling material the aerobic processes are extended, resulting in oxidative losses and the occurrence of mould.

In our research, in the first year, the level of DM was quite high. Significant differences were observed only in phases of exploitation. In the early harvest stage, the DM content was lower than in the later stage of harvesting. Also in the second study year, the level of dry matter of silage depended only on the crop utilization phase. DM values were lower than in the first year of the study and ranged from 311.3 to 416.4 g kg⁻¹. Also, in the phase of early harvest lower DM content was obtained compared to the late harvest stage. According *Knotek (1997)*, in order to make good quality silage it is necessary to produce silage from wilted material which contains DM of 320-380 g kg⁻¹, or according to *Đorđević et al. (2001)*, the dry matter content of plant material should be above 35% in order to

ensure successful fermentation. According to these findings, all silages obtained in the second year of research had a satisfactory level of DM, while the silages of the first year had a slightly higher content of DM than expected for successful fermentation.

Table 1. The quality of grass-legume silages, depending on the structure of the mixture and utilization phase in 2008

Quality paramet. (gkg ⁻¹ SM)	Early harvest				Late harvest				level of significance		
	A	B1	B2	B3	A	B1	B2	B3	mix. type	harv time	inter act.
Dry matter	415.4	416.9	429.3	360.8	403.4	459.3	430.6	433.1	ns	*	ns
Crude protein	179.1	170.0	173.0	165.1	146.2	148.9	152.1	149.7	ns	**	ns
NH ₃ -N†	112.8	109.9	120.6	123.5	103.9	105.6	96.1	100.4	ns	**	ns
soluble N	504.1	465.6	463.7	501.0	550.1	515.2	425.8	419.6	**	ns	**
pH	4.8	4.8	4.8	4.8	4.5	4.4	4.6	4.7	ns	**	ns
lactic acid	31.5	26.6	27.3	24.3	35.7	36.2	27.9	29.5	**	**	*
acetic acid	15.0	18.6	14.9	19.7	8.9	9.2	11.2	10.3	**	**	**
butyric acid	0.00	0.04	0.00	0.07	0.00	0.02	0.00	0.15	*	ns	ns

†- g kg UN; A-lucerne in monoculture; B1-mixture of lucerne and cocksfoot; B2- mixture of lucerne, cocksfoot and tall fescue; B3- mixture of lucerne, cocksfoot, tall fescue and sainfoin.

The crude protein content depended primarily on the phase of exploitation, and in the second year of research on the structure of the mixture and the mixture x phase interaction. The early harvest phase is characterized by a significantly higher content of CP compared to the phase of late harvest. This can be explained by the fact that in the early stages of exploitation share of leaves was equal to or greater than the share of stems, while in the later stages of the exploitation the share of stems was relatively higher than the share of leaves, and therefore at this stage the level of crude protein was lower and the crude fiber content higher (*Di Marco et al., 2002*). Similar to our research, *Keady and O'Kiely (1998)*, recorded in their research significantly higher (185 g kg⁻¹ DM) CP content of the grassland silage, prepared from the earlier harvested material, compared to the later harvest silage (143 g kg⁻¹ DM). *Dinić et al. (2008)* also confirmed the presence of significant differences between the stages of heading and flowering in tall oatgrass. Tall oatgrass silage prepared in the heading stage had 156.0 g kg⁻¹ DM of crude protein and prepared in the flowering stage, 138.2 g kg⁻¹ DM.

In the second year of research, the mixtures had a significant impact on the content of CP. Pure lucerne crop silage and mixture with lucerne and sainfoin had

higher CP content than the other two mixtures, which is in line with the findings of *Tekeli and Ates (2005)*, that the content of the CP in the mixture increases with increasing proportion of legumes. The differences are significantly more visible in the silage from the first harvest phase than from the second.

Table 2. The quality of grass-legume silages, depending on the structure of the mixture and utilization phase in 2009

Quality paramet. (gkg ⁻¹ SM)	Early harvest				Late harvest				level of significance		
	A	B1	B2	B3	A	B1	B2	B3	mix. type	harv time	inter act.
Dry matter	311.3	317.6	320.1	340.7	371.3	380.6	416.4	387.2	ns	**	ns
Crude protein	176.6	156.9	154.9	172.5	148.1	146.8	149.1	153.6	**	**	*
NH ₃ -N†	115.4	116.6	104.8	91.3	90.4	95.8	91.5	98.1	ns	**	*
soluble N	598.9	621.0	600.3	542.9	589.6	580.2	558.0	592.8	ns	ns	*
pH	4.6	4.6	4.6	4.6	4.5	4.6	4.5	4.6	ns	ns	ns
lactic acid	28.8	27.6	27.4	31.4	28.6	31.9	29.9	28.2	ns	ns	**
acetic acid	10.7	11.8	11.4	10.3	15.7	13.1	13.7	14.0	ns	**	ns
butyric acid	0.02	0.02	0.02	0.04	0.09	0.11	0.19	0.02	ns	**	ns

†- g kg UN; A-lucerne in monoculture; B1-mixture of lucerne and cocksfoot; B2- mixture of lucerne, cocksfoot and tall fescue; B3- mixture of lucerne, cocksfoot, tall fescue and sainfoin

One of the most important indicators of the quality of fermentation is the amount of NH₃-N, as an indicator of the degradation of proteins. Significant differences in the content of ammonia nitrogen were observed in different phases of exploitation. In the early harvest phase, NH₃-N content of the silage was significantly higher than in the silage of the late harvest phase. *Muck (1988)* found, by ensiling lucerne with different degrees of humidity, that the content of non-protein N and ammonia decreased with increase in dry matter, which explains the phenomenon that the higher content of NH₃-N in the first compared to the second phase of the research. Similar to our research, *Kuoppala et al. (2008)* and *King et al. (2012)* have established that the content of NH₃-N is significantly higher in silages from earlier phases of exploitation. However, *Knežević et al. (2009)*, regardless of the dry matter content of silage from different phases of exploitation, have established that the content of NH₃-N is higher in silages made from plants harvested in the later stages of development (maturity stage, growth stage).

In the course of fermentation, organic acids accumulate in the silage. The most valuable is lactic acid, which lowers the pH, has the bactericidal effect on harmful microorganisms and provides aerobic stability of silage. The content of

lactic acid was highly dependent on all examined parameters only in the first year of research. Its content ranged in the first year from 24.3-31.5 in the early stage of harvest and of 27.9-36.2 g kg⁻¹ DM at the late harvest stage, and in the second year from 27.4-31.4 in the early harvest stage and of 28.2-31.9 g kg⁻¹ DM at the stage of late harvest. According to *Dorđević and Dinić (2003)*, average 3-7% of lactic acid is contained in good quality silage. According to *Dorszewski (1997)*, these values are slightly lower from 2.28-3.90%. The studied silages in regard to the content of lactic acid fall into category of good quality silages.

In addition to lactic acid, during the fermentation process, also the acetic acid is produced. If the content of acetic acid is up to 5.5% of the dry matter, it is considered to be good quality silage (*Dorđević and Dinić, 2003*). The content of acetic acid in the investigated silages ranged from 8.9-18.6 in the first year and from 10.3-15.7 g kg⁻¹ DM in the second year. As in the case of the lactic acid content, in regard to the content of acetic acid, silages can be considered as good quality silages.

Butyric acid is undesirable fermentation product and an indication of the presence of clostridia in silage. It is usually not present or present in small concentrations. A large number of silages of the first year showed no butyric acid, as opposed to the second year silages, where the content ranged from 0.02 to 0.19 g kg⁻¹. According to *Huhtanen et al. (2012)*, wilting to 400 g kg⁻¹ of dry matter acts preventively in the production of butyric acid. Considering that in the first year dry matter content of the silage was higher, then this could explain this fact. Good quality silages should have less than 0.1% butyric acid (*Seglar, 2003*). All studied silages showed a lower content than the mentioned value, which is necessary for good quality silage.

Conclusion

Based on the obtained results of the study of two exploitation phases on the the quality of grass-legume silages, it can be concluded that the preparation of grass-legume mixture silages harvested in the later stages of growth and development are better from the standpoint of quality. Such silages had higher level of dry matter, higher content of lactic acid and less ammonia nitrogen. However, important deficiency in preparation of silages in the later stages (maturity stage) is significantly lower crude protein content. By increasing the degree of wilting good silages from earlier maturity stage could be produced, with high crude protein content, dry matter, lactic acid and a low content of NH₃-N.

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Fermentabilne karakteristike travno-leguminoznih silaža košenih u dve različite starosne faze

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Rezime

Cilj naših istraživanja je bio da ispitamo uticaj faze zrelosti useva u vreme kosidbe različitih travno-leguminoznih smeša, kao i interakciju faktora faza-vrsta smeše na pojedine parametre kvaliteta silaža od tih smeša. Leguminoze kao važni izvori proteina se vrlo teško siliraju. U smeši sa travama njihove fermentabilne karakteristike se popravljaju. U toku rasta i razvika biljaka menja se njihov hemijski sastav, pa i sposobnost za siliranje. Od ispitivanih faktora, faza iskorišćavanja imala je visoko značajnog uticaja na gotovo sve parametre kvaliteta u obe ispitivane godine. Odlaganjem vremena kosidbe značajno je povećan sadržaj suve materije u silaži i smanjen sadržaj sirovih proteina i $\text{NH}_3\text{-N}$. Ispitivane silaže po sadržaju mlečne kiseline spadaju u silaže dobrog kvaliteta. Njen sadržaj u prvoj godini kretao se od 24,3-31,5 u ranoj kosidbi i od 27,9-36,2 g kg^{-1} SM u kasnoj kosidbi i u drugoj godini od 27,4-31,4 u ranoj fazi i od 28,2-31,9 g kg^{-1} SM u kasnoj fazi košenja. Po sadržaju sirćetne i buterne kiseline, ispitivane silaže se takođe mogu okarakterisati kao dobrog kvaliteta.

References

- DAWSON L.E.R., KIRKLAND R.M., FERRIS C.P., STEEN R.W.J., KILPATRICK D.J., GORDON F.J. (2002): The effect of stage of perennial ryegrass maturity at harvesting, fermentation characteristics and concentrate supplementation on the quality and intake of grass silage by beef cattle. *Grass and Forage Science*, 57,255-267.
- DI MARCO O.N., AELLO M.S., NOMDEDEU M., VAN HOUTTE S. (2002): Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ and in vitro). *Animal Feed Science and Technology*, 99, 37-43.

- DINIĆ B., ĐORĐEVIĆ N., SOKOLOVIĆ D., LAZAREVIĆ D., CVETKOVIĆ M. (2008): Tall oat grass (*Arrhenatherum elatius*) silage quality dependences on stage of growth and wilting. Proceeding of 13th International Conference of forage conservation, Nitra, 126-127.
- DORSZEWSKI P. (1997): Quality and stability of silages produced from grass-clover mixtures. Proceedings of the 8th International symposium Forage Conservation, 116-117.
- ĐORĐEVIĆ N., KOLJAJIĆ V., DINIĆ B., GRUBIĆ G. (2001): Postupci konzervisanja i efekti korišćenja lucerke. *Arhiv za poljoprivredne nauke* 62:285-292.
- ĐORĐEVIĆ N., DINIĆ B. (2003): Siliranje leguminoza, Institut za istraživanja u poljoprivredi SRBIJA, Beograd, 226.
- HUHTANEN P., JAAKKOLA S. AND NOUSIAINEN J. (2012): An overview of silage research in Finland: from ensiling innovation to advances in dairy cow feeding. Proceedings of the XVI International Silage Conference Hämeenlinna, Finland, 2-4 July 2012, 16-33.
- KEADY T.W.J., O'KIELY P. (1998): An evaluation of potassium and nitrogen fertilization of grassland and date of harvest on fermentation, effluent production, dry matter recovery and predicted feeding value of silage. *Grass and Forage Science*, 53, 326-337.
- KEADY T.W.J., MAYNE C.S., FITZPATRICK D.A. (2000): Prediction of silage feeding value from the analysis of the herbage at ensiling and effect of nitrogen fertilizer, date of harvest and additive treatment on grass silage composition. *Journal of Agricultural Science, Cambridge*, 134,353-368.
- KING C., MC ENIRY J., RICHARDSON M. AND O'KIELY P. (2012): Ensilage characteristics of perennial ryegrass grown under two nitrogen fertiliser inputs and red clover, each harvested at five dates in the primary growth. Proceedings of the XVI International Silage Conference Hämeenlinna, Finland, 2-4 July 2012, 194-195.
- KNEŽEVIĆ M., VRANIĆ M., PERČULIJA G., KUTNJAK H., MATIĆ I., TESKARA M. (2009): Utjecaj roka košnje travno-djetelinske smjese na kemijski sastav i kvalitetu fermentacije silaže. *Mljekarstvo*, 59 (1), 49-55.
- KNOTEK S. (1997): Qualitative criteria of preserved forage and their effects on animal nutrition. Proceeding of the 8th International scientific symposium „Forage Conservation” 37-49.
- KUOPPALA K., RINNE M., NOUSIANINEN J., HUHTANEN P. (2008): The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. *Livestock Science*, 116 (1-3), 171-182.
- MC ENIRY J., KING C., O'KIELY P. (2013): Silage fermentation characteristics of three common grassland species in response to advancing stage of maturity and additive application. *Grass and Forage Science*, 69, 393-404.

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- MUCK R.E. (1988): Factor influencing silage quality and their implications for management. *Journal Dairy Science*, 71, 2992-3002.
- SEGLAR B. (2003): Mycotoxin effects on dairy cattle. Proceedings of the Minnesota Dairy Health Conference, 119-136.
- TEKELI S.A., ATES, E. (2005): Yield potential and mineral composition of white clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinaceae* Schreb.) mixtures. *Journal of Central European Agriculture*, 6, 1, 27-34.
- VRANIĆ M., KNEŽEVIĆ M., BOŠNJAK K., PERČULIJA G., KUTNJAK H. (2008): Utjecaj roka košnje travno-djetelinske smjese na *ad libitum* konzumaciju, *in vivo* probavljivost i balans dušika silaže. *Mljekarstvo*, 58 (4), 357-369.
- WYSS U. (2004): Ensilability of different grass-clover mixtures. *Grassland Science in Europe* 9: 966-968.

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