Original scientific paper - Izvorni znanstveni rad UDK: 631.115

Effects of additive application upon *ad libitum* intake, *in vivo* digestibility and nitrogen balance of alfalfa haylage

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Received - Prispjelo: 02.04.2009.
Accepted - Prihvaćeno: 09.07.2009.

Summary

The research objective was to determine the effect of the additive Sill-All application on *ad libitum* intake, *in vivo* digestibility and nitrogen balance of alfalfa haylage. About 40 % alfalfa plants was ensiled at the phonological flowering stage into plastic foil-wrapped bales without or with additive (2 L t^{-1} plant mass). No statistically significant differences were found between alfalfa ensiled without additive and alfalfa ensiled with additive for the content of dry matter (DM) (632 g and 631 g DM kg^{-1} fresh sample, respectively). Alfalfa ensiled without additive contained 921 g organic matter (OM) kg^{-1} DM, which was significantly higher ($P<0.001$) compared to alfalfa ensiled with additive (902 g OM kg^{-1} DM). Alfalfa ensiled without additive contained 141 g crude proteins (CP) kg^{-1} DM, which was significantly higher ($P<0.001$) compared to alfalfa ensiled with additive (139 g CP kg^{-1} DM). Alfalfa ensiled with additive contained significantly less acid detergent fibers (ADF) ($P<0.001$) compared to alfalfa ensiled without additive (445 g kg^{-1} DM and 456 g kg^{-1} DM, respectively) and had a lower pH value ($P<0.001$) (5.29 and 5.56, respectively). No statistically significant differences were found between the studied feeding treatments for *ad libitum* intake of fresh ration and DM ration, for the measured parameters of digestibility and N balance. It was concluded that addition of the additive to alfalfa haylage led to significant changes in chemical composition; however, changes in chemical composition had no impact on measured biological parameters (ad libitum intake, in vivo digestibility and nitrogen balance).

Key words: alfalfa haylage, additive, *ad libitum* intake, *in vivo* digestibility, N balance

Introduction

In comparison with grasses, legumes have a higher content of crude proteins (CP), higher concentration of organic acids and more minerals, but a lower content of dry matter (DM), water soluble carbohydrates and high buffering capacity (McDonald, 1991; Castle et al., 1983), so additive application is necessary to achieve the desired course of fermentation. Use of additives is based on the assumption that a sufficient amount of lactic acid will not be produced fast enough on the substrate being ensiled under natural conditions.

Tengerdy et al. (2008) ensiled wilted and unwilted alfalfa with additives containing lactic acid fermentation bacteria and enzymes. Ninety days following the onset of ensiling, an increase was recorded in *in vivo* digestibility of the fibers of alfalfa ensiled without wilting, but not of alfalfa ensiled wilted. Enzymatic treatment including a combination of cellulase, hemicellulase, pectinase and lactic acid fermentation bacteria of the genera *Pediococ-*

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cus, *Lactobacillus* and *Streptococcus* was the most efficient. Mader et al. (1985) ensiled alfalfa at the beginning of flowering (35 % DM) with an additive. The additive enhanced anaerobic fermentation, increased *in vitro* digestibility of DM (from 57 % in untreated to 60.6 % in treated alfalfa), increased silage acidity (from 4.98 to 4.83 in treated silage), but had no effect on daily gain, intake and feed conversion in heifers. Effects of cellulase and hemicellulase addition on the content of neutral detergent fibers (NDF) in silage prepared from orchard grass (*Dactylis glomerata)* were investigated by Nadeau et al. (2000). Addition of 20 ml cellulase kg^{-1} grass mass reduced the NDF content by 30 %. These additives increase the supply of bacteria with water soluble hydrocarbons, whereby more lactic acid is produced and silage is better preserved. Besides, they degrade cell walls of ensiled forage, making forage more digestible. Silage digestibility affects the intake and production characteristics of dairy cows in such a way that highly digestible and well preserved silage has higher intake, supports higher milk production and raises the milk protein levels (Bosh et al., 1992; Cushnahan et al., 1996).

The research hypothesis was that alfalfa ensiled with additives has higher acidity, more CP, less ADF, less ammonium nitrogen (NH_3-N), higher *ad libitum* intake, better digestibility and higher nitrogen balance compared to alfalfa haylage ensiled without additives. The research objective was to determine the *ad libitum* intake, *in vivo* digestibility and nitrogen balance of alfalfa haylage ensiled without or with additives in feeding wether sheep.

Material and methods

Alfalfa haylage

Alfalfa was cut at the flowering stage of about 40 % plants. Cut plant mass was wilted on the ground to a DM content of about 50 % and pressed into cylindrical bales of 125-cm diameter using a John Deere 575 baler. Sill-All additive composed of 4 bacterial species (*Enterococcus faecium, Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus salivarius*) and 4 enzyme species (cellulase, hemicellulase, pectinase and amylase) was diluted in water (10 g of additive diluted in 2 L of water) and evenly sprayed over forage at baling (2 L t^{-1} plant mass).

Bales were wrapped in a 1.2-m wide net, than in 4 layers of plastic foil 50 cm wide and 0.025 mm thick, and were left to ferment in a roofed area of the Grassland Centre on Mt. Sljeme. Four bales of an average mass of 600 kg were separated for research purposes.

Feeding treatments

Two feeding treatments were tested, alfalfa ensiled without additive and alfalfa ensiled with additive. Using a chopper, alfalfa haylage was chopped into 3-5 cm lengths. Chopped material for the following week feed was pressed into plastic bags (about 20 kg of haylage per bag) and stored at a temperature of 4 °C until use.

Experimental animals

The trial included 4 Charolais wether sheep, aged about 18 months, body mass around 43.5 kg (39.5 kg-47.5 kg). Prior to the trial, animals were treated against internal and external parasites. Temperature of the trial area was maintained at 15 °C, light was provided in the period from 8.00-20.00 hours with constant ventilation. Upon 10 days of adaptation to feed, *ad libitum* feed intake was monitored for 4 days, followed by digestibility monitoring for 7 days.

During period of adaptation to feed, animals were kept in individual boxes (1.5 m x 2.2 m), while they were kept in individual cages (136 cm x 53 cm x 148 cm) in the period of measuring *ad libitum* feed intake, digestibility and N balance. They were fed twice a day (8:30 and 16:00 h) equal amounts of feed, designed to ensure a refusal margin of 10-15 % each day. Amounts of feed offered and feed refused, outputs of urine and feces were recorded on a daily basis throughout the measuring period. Samples of feed offered, feed refused, feces and urine were stored at a temperature of 4 °C until the end of each of the 4 trial periods, when they were stored at -20 °C until chemical analyses.

Body weight was determined by weighing animals on days zero, ten, fourteen and twenty-one of each of the four trial periods. Animals were weighed on electronic scales (TRU-TEST Ltd, Model 703B).

Chemical analyses

Content of DM (g kg^{-1} fresh sample) was determined by sample drying in a fan-assisted oven (ELE International) at a temperature of 60 °C to constant mass. Dried samples were then ground into 1-mm particles using a hammer mill (Christy, Model 11) and further used for chemical analyses. Laboratory DM was determined by drying 5 g of sample at a temperature of 105 °C over 4 hours (ISO 6496).

Organic matter (OM) content (g kg^{-1} DM) was determined by combustion of ca 5 g of sample in a muffle furnace (Nabertherm) at 550 °C for 3 hours (ISO 5984).

Nitrogen concentration in sample N was determined by the Kjeldahl method (ISO 5983) using digestion and automatic distillation/titration units (Gerhardt). Nitrogen concentration in sample CP was calculated by multiplying the N concentration by a factor of 6.25. pH value was determined in the extract obtained from ca 10 g of fresh silage and 100 mL distilled water using a WTW pH meter 315i.

Acid detergent fibers (ADF) were determined according to the method of Van Soesta et al. (1991) by boiling samples in neutral and acid detergents. Ammonium nitrogen (NH_3-N g kg^{-1} total nitrogen) was measured by the Bremner and Keeney method (1965). Ration intake (*ad libitum*), *in vivo* digestibility and nitrogen balance were determined as described earlier (Knežević et al., 2007, Vranić et al., 2008).

Statistical analyses

Data were processed by the statistical program SAS (SAS Institute, 1999) using GLM and MIXED procedures.

Results and discussion

Table 1 shows the average chemical composition of alfalfa haylage ensiled without or with additive.

Alfalfa haylage ensiled without additive and alfalfa haylage ensiled with additive were thoroughly wilted on the ground prior to baling and had similar DM contents (Table 1). DM content of 400-500 g kg^{-1} fresh sample has been recommended for alfalfa haylage (Frame et al., 1988). Alfalfa plants lose moisture much more rapidly from leaves than from stalks, so delayed wilting on the ground may cause considerable losses of nutrients due to leaf fall. In dependence on the applied technology and weather conditions, losses in alfalfa DM yield may reach 40 %, which is twice as much as losses of DM yield in the production of meadow hay (Nash, 1985).

CP levels determined in alfalfa haylage ensiled without additive and in alfalfa haylage ensiled with additive (Table 1) are in accordance with the wide range of CP levels in alfalfa of 129-324 g kg^{-1} DM, but are lower than the average CP content of 193 g kg^{-1} DM reported by Frame et al. (1998). One of the reasons is alfalfa cutting at the 40 % of plants

Table 1: The average chemical composition of alfalfa haylage (g kg^{-1}DM, unless otherwise stated)
Tablica 1: Prosječan kemijski sastav sjenaže lucerne (g kg^{-1} ST)

Chemical parameter Kemijski parametar	Alfalfa haylage/Sjenaža lucerne		SEM	Sig.
	Alfalfa without additive Lucerna bez aditiva	Alfalfa with additive Lucerna s aditivom		
Dry matter (g kg^{-1} fresh weight) Suha tvar (g kg^{-1}svježeg uzorka)	632	631	7,23	NS
Organic matter/Organska tvar	921^{a}	902^{b}	3,12	***
Crude proteins/Sirovi proteini	141^{a}	139^{b}	0,39	***
KDV/ADF	456^{a}	445^{b}	2,81	***
pH	5,56^{a}	5,29^{b}	0,02	**
NH_3-N (g N kg^{-1} total N)	91,4	95,5	1,95	NS

ADF, acid detergent fibre. [ab] Values within the same row with different superscripts differ significantly (***, $P<0.001$), NS, $P>0.05$. SEM, standard error of the mean
KDV, kisela detergent vlaknina/[ab] Vrijednosti u redovima označene različitim slovima signifikantno su različite (***, $P<0,001$), NS, $P>0,05$. SEM, standardna greška srednje vrijednosti

flowering, while the time recommended for the production of high quality forage is the flowering stage of about 10 % of plants (Sheaffer et al., 1988). Besides, delayed wilting of alfalfa on the ground prior to baling, led to loss of leaf mass and to lower quality of forage in terms of reduced CP content.

Treated alfalfa haylage had a significantly lower CP content (P<0.001) compared to untreated alfalfa haylage. This is probably a result of more intensified proteolysis of alfalfa haylage ensiled with additive resulting in a relative increase of NH_3-N content, though not statistically significant, compared to alfalfa ensiled without additive. This is corroborated by the research results of Charbel et al. (2005), who report that additives can enhance proteolysis in ensiled plant material, which is reflected in reduced protein levels and increased concentration of non-protein nitrogen in silage.

Alfalfa haylage ensiled with additive contained significantly less ADF (P<0.001) compared to alfalfa haylage ensiled without additive. Additive Sill-All contains the enzymes cellulase and hemicellulase. Influence of the cellulase and hemicellulase application on NDF and ADF in grass silage prepared from orchard grass was studied by Nadeau et al. (2000), who applied 20 mL cellulase kg^{-1} grass mass and recorded a NDF reduction by 30 %. These additives degrade cell walls of ensiled forage and increase the supply of bacteria with water soluble hydrocarbons, whereby more lactic acid is produced and silage is better preserved, with a concurrent reduction of the fiber content in silage; this effect was observed in this research as well. Further, Jones (1992) reports a lower ADF content in silage made of perennial ryegrass treated with Sill-All by comparison with ADF content in untreated silage, which also indicates intensified hydrolysis of plant walls due to enzymatic activity in treated silage.

The positive effect of adding enzymes that degrade cell walls during ensiling of wilted plant mass can be reflected in increased compaction of plant

Table 2: Voluntary intake, *in vivo* digestibility and N balance of alfalfa haylage ensiled without and with an additive

Tablica 2: *Ad libitum* konzumacija, *in vivo* probavljivost i balans N sjenaže lucerne silirane bez dodatka aditiva i s dodatkom aditiva

Parameter Parametar	Alfalfa haylage/Silaža lucerne Alfalfa without additive Lucerna bez aditiva	 Alfalfa with additive Lucerna s aditivom	SEM	Sig.
Voluntary intake/Konzumacija po volji				
Fresh/Svježe (kg d^{-1})	2.89	2.86	0.95	NS
Fresh/Svježe (g kg^{-1} $M^{0.75}$ d^{-1})	105	103	2.86	NS
Dry matter/Suha tvar (kg d^{-1})	1.81	1.82	0.42	NS
Dry matter/Suha tvar (g kg^{-1} $M^{0.75}$ d^{-1})	66	65	1.37	NS
Digestibility/Probavljivost (g kg^{-1} ST/DM)				
Dry matter/Suha tvar	515	499	13.00	NS
Organic matter/Organska tvar	533	518	13.98	NS
D-value/D-vrijednost	489	468	13.59	NS
Crude proteins/Sirovi proteini	662	665	8.75	NS
Balans N (g d^{-1})				
N intake/Konzumirano N	40.9	40.4	1.01	NS
N output in faeces/N izlučen fecesom	14.1	13.7	0.46	NS
N output in urine/N izlučen urinom	22.8	22.7	1.30	NS
N balance/N balans	4.0	4.0	1.57	NS

D-value, digestibility of organic matter in the dry matter; NS, P>0.05. SEM, standard error of the mean

D-vrijednost, probavljiva organska tvar u suhoj tvari; NS, P>0,05. SEM, standardna greška mass in the silo, which in the case of ensiling poorly wilted or unwilted plant mass may increase nutrient losses due to more intensive leaching of silage juice.

The pH value is an important parameter for assessment of fermentation quality (Knežević et al., 2009). Alfalfa haylage ensiled with additive had a significantly lower pH value ($P<0.001$) compared to alfalfa haylage ensiled without additive, which is in agreement with the results obtained by Madera et al. (1985), who determined lower pH values in alfalfa ensiled with additives (4.83) compared to control alfalfa (4.98). Silages produced with the application of biological additives have generally lower pH values, lower NH_3-N concentration and higher levels of total acids generated through fermentation. Besides lowering the level of pH values of plant mass ensiled with additives, additives can also speed up decrease of pH values. Ensiling high DM alfalfa, Charbel et al. (2005) measured a pH value of 5.9, which decreased to pH 4.5 three days after ensiling with additives whereas pH value of control alfalfa did not decrease below pH 5 for 16 days after ensiling.

Jones (1992) investigated the effect of applying Sill-All to grass silage and determined faster acidification; pH 4 was recorded 4 days earlier than in untreated silage. The positive effect of Sill-All on the quality of silage fermentation was also recorded by Jian-Hau et al. (2000), who corroborated it with lower pH value and lower NH_3-N levels in treated silage.

In this study, no differences in NH_3-N contents were found between alfalfa haylages. Treated haylage had a relatively higher NH_3-N content compared to untreated haylage, which might be attributed to intensified proteolysis due to additives (Charbel et al., 2005).

Table 2 shows the *ad libitum* intake, *in vivo* digestibility and N balance of alfalfa haylages studied.

No differences were determined between alfalfa haylage ensiled without additive and alfalfa haylage ensiled with additive regarding *ad libitum* intake, digestibility of measured parameters and N balance.

These findings are in agreement with earlier studies, in which additives composed of the enzymes cellulase, hemicellulase, pectinase and lactic acid bacteria had no effect on alfalfa silage digestibility (Tengerdy et al., 2006) or on DM intake in lamb feeding (Jones, 1992). Application of additives containing lactic acid fermentation bacteria and enzymes did not affect wilted alfalfa digestibility (Tengerdy et al., 2008), daily gain, intake and feed conversion either (Mader et al., 1985).

Effects of the enzymatic additives application on DM and fiber digestibility are more frequently negative than positive, which may be explained by their mechanism of action (Muck and Kung, 1997). Namely, application of enzymes that degrade fibers causes also reduction of their levels in silage, which also applies to the more readily digestible fractions of fibers (cellulose), leaving in silage fiber fractions that decompose more slowly and less intensively or do not decompose at all (hemicellulose, lignin, pectins, gums, ß glucanes, fructanes). For this reason, improvement of fermentation in the silo and reduction of fiber content due to additive activities are more expressed in plant mass ensiled at an earlier maturity stage which contains less fibers than more mature crops where cell wall hydrolysis is slower due to more intensive lignification, and consequently the fiber content reduction is less expressed compared to the untreated silage.

Jian-Hau et al. (2000) determined a positive effect of Sill-All upon milk production of dairy cows, which produced 0.9 kg more milk per day compared to dairy cows fed silage without additives; in standard lactation of 305 days, this means 283 kg more milk produced per dairy cow.

Comparison of silages prepared with addition of microbial inoculants and untreated silages shows that microbial additives were efficacious in 50 % cases regarding production parameters of feed intake, conversion and milk production; it is therefore necessary to know the conditions under which an additive will be most efficient as well as which type of additive should be used with respect to ensiling conditions. Kung and Muck (1997) report that the positive effect of additive application on gain, intake and milk production is less expressed for enzyme-treated silage compared to silage treated with microbial inoculants. Similarly to bacterial inoculants that require certain conditions for their growth, enzymatic inoculants require certain conditions for their maximal activity. Most cellulase enzymes require pH 4.5 and 50 ºC temperature for optimal activity (Kung and Muck, 1997), so this is another reason for non-appearance of positive effects of additives on the monitored biological parameters.

Conclusions

Additive Sil-All reduced the ADF levels and pH value in alfalfa haylage. Additive application had no effect on measured biological parameters of alfalfa haylage (*ad libitum* intake of alfalfa haylage, *in vivo* digestibility of measured parameters and nitrogen balance).

Utjecaj dodatka aditiva na ad libitum konzumaciju, in vivo probavljivost i balans dušika sjenaže lucerne

Sažetak

Cilj istraživanja bio je utvrditi utjecaj dodatka aditiva "Sill All" na *ad libitum* konzumaciju, *in vivo* probavljivost i balans dušika sjenaže od lucerne. Lucerna je silirana u fenološkoj fazi cvatnje oko 40 % biljaka u bale ovijene plastičnom folijom bez dodatka aditiva ili s dodatkom aditiva u količini od 2 L t^{-1} biljne mase. Nisu utvrđene statistički značajne razlike između lucerne silirane bez aditiva i lucerne silirane s dodatkom aditiva u udjelu suhe tvari (ST) (632 g i 631 g ST kg^{-1} svježeg uzorka, respektivno). Lucerna silirana bez aditiva sadržavala je 921 g organske tvari (OT) kg^{-1} ST, što je bilo statistički značajno više (P<0,001) u odnosu na lucernu siliranu s dodatkom aditiva (902 g OT kg^{-1} ST). Lucerna silirana bez aditiva sadržavala je 141 g sirovih proteina (SP) kg^{-1} ST, što je bilo statistički značajno više (P<0,001) u odnosu na lucernu siliranu s aditivom (139 g SP kg^{-1} ST). Lucerna silirana s aditivom sadržavala je statistički značajno manje kiselih detergent vlakana (KDV) (P<0,001) u odnosu na lucernu siliranu bez aditiva (445 g kg^{-1} ST i 456 g kg^{-1} ST, respektivno) i nižu pH-vrijednost (P<0.001) (5,29 i 5,56 respektivno). Nisu utvrđene statistički značajne razlike između ispitivanih hranidbenih tretmana u *ad libitum* konzumaciji svježeg obroka, ST obroka, u mjerenim parametrima probavljivosti, niti u balansu N. Zaključeno je da je u ovom istraživanju dodatak aditiva sjenaži lucerne doveo do značajnih promjena kemijskog sastava, ali promjene kemijskog sastava nisu utjecale na mjerene biološke parametre (*ad libitum* konzumaciju, *in vivo* probavljivost i balans dušika).

Ključne riječi: sjenaža lucerne, aditiv, *ad libitum* konzumacija, *in vivo* probavljivost, balans N

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Acknowledgement

The authors are grateful to Alltech Hrvatska for supporting this research.