

Effect of Dry Matter Content and Inoculation on Ruminal Protein Degradability in Alfalfa Silages

Research Article

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ABSTRACT

The aim of the experiment was to determine the effect of different dry matter content and addition of silage inoculant on the ruminal protein degradability. Alfalfa herbage was collected from two cuts and the fresh matter was sampled two times during each cut (with different dry matter content). During the preparation of experimental micro-silages, the chopped forage was treated with a microbial inoculant or it was left untreated (control). The *in sacco* method was used for the measurement of ruminal protein degradability. The different dry matter content significantly ($P < 0.05$) affected the rate of ruminal protein degradation in alfalfa silages. The average values of ruminal protein degradability of alfalfa silages were at lower dry matter content of $70.72 \pm 1.243\%$, at higher dry matter content $73.69 \pm 0.554\%$ in the first cut. The ruminal protein degradability was significantly ($P < 0.01$) different in the second cut. The alfalfa silages had average value of ruminal protein degradability at lower dry matter content of $69.19 \pm 1.184\%$, at higher dry matter content of $72.74 \pm 0.637\%$. The applied ensiling additive had no statistically significant influence on the ruminal protein degradability in alfalfa silages. In first cut, it was measured in control silages as $72.08 \pm 1.620\%$ and in inoculated silages as $72.33 \pm 1.901\%$. In second cut, the average values of ruminal protein degradability of control silages were $71.56 \pm 1.833\%$ and of inoculated silages as $70.37 \pm 2.014\%$. The using of bacterial inoculant had no significant effect of ruminal protein degradability, but improved the fermentation process quality.

KEY WORDS alfalfa silage, ruminal protein degradability, silage additive.

INTRODUCTION

Alfalfa silages represent one of main components in feeding rations for ruminants. Apart from their chemical composition, the nutritive value of silages prepared from wilted alfalfa depends on the quality of the fermentation process. In terms of chemical composition, the quality of alfalfa silages is determined namely by the content of protein and fiber. The content of crude protein ranges in clover and alfalfa silages around 200 g/kg DM (dry matter) (Wilkinson, 2005). Metabolizable protein requirements are in ruminants covered from two main sources: from the mi-

crobial protein synthesized in rumen and from the fodder protein, which is not digested in rumen (so-called by-pass protein) (Broderick, 1995). Advanced systems of dairy cow nutrition aim at the most precisely as possible estimated flux of individual protein fractions into small intestine (Richter and Trinacty, 2010).

Crude protein of alfalfa silages are degraded in the rumen at various amounts. According to Huhtanen (2010), most fodders are characterized by the high value of the ruminal protein degradability (hereinafter RPD). Regarding the low content of by-pass protein (ca. 10%), alfalfa silages represent the main source of protein degraded in the rumen

(Wilkinson, 2005). Richter and Trinacty (2009) indicate the average RPD value of alfalfa silages to be 82.3%. According to Kowalski *et al.* (2010) the RPD value is affected by the vegetative stage of fodder at harvest and also by the method of conservation. Low quality of crude protein is typical in silages made of low DM forage. Kramer *et al.* (2010) claim that the quality of crude protein in low DM forages can be favorably affected by the application of ensiling additives. According to Luchini *et al.* (1997) the dry matter content of matter at ensile has a great effect on protein and fermentation products profile. Muck *et al.* (1990) claim that the increasing dry matter content affects on the reduction of fermentation. In terms of crude protein, the content of non-protein nitrogen decreases. The optimal dry matter content of alfalfa at ensiling is according to from 35 to 45%. Tyrolova and Vyborna (2008) indicate the optimal dry matter content was as 35-38%.

MATERIALS AND METHODS

Experimental material and preparation of experimental silages

The experiment was conducted on trial plots operated by the Research Institute of Fodder Crops in Troubsko where alfalfa (*Medicago sativa* L., var. Palava) was grown in the first production year.

The first cut was carried out at pre-blooming stage; the second cut was made at the beginning of flowering. After the sward cutting, the matter was left for different lengths of wilting (1st wilting length was ca. 20 hours and 2nd wilting length was ca. 30 hours). Then was the wilted matter cut (theoretical particle length ranging from 20-40 mm). Subsequently, the chopped forage was conserved into experimental micro-silos. We use a commercial bacterial inoculant in our experiment (recommended for alfalfa and other crops). The dose of used inoculant was 5 g/t of matter, CFU (colony forming unit) 30×10^9 (composition: *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus buchneri*, *Enterococcus faecium* and *Pediococcus spp.*). Control silages without any treatment were prepared in parallel with the inoculated variants. Matter packing was made by using the pneumatic. Each silage variant was prepared in three repetitions.

The experimental micro-silos were opened after about 70 days and the silages were sampled for the establishment of their DM contents (at 105 °C). During the sampling, the individual silages were subjected to sensory judgement. Subsequently, the samples were dried at 55 °C, ground and analyzed for the content of basic nutrients and energy according to AOAC (1990) Official methods.

Extracts from the silages contained in all experimental micro-silos served for the assessment of the fermentation process quality. DM content of silages was calculated by

using equations for correction according to Kacerovsky *et al.* (1990).

Analysis of fodders for ruminal protein degradability

RPD was assessed by using the "in sacco" method. The prescribed amount of silage sample (40 g) was sealed in a special bag (50×25 mm) and inserted into the rumen of the cannulated steer (live weight of 750 kg, the diet consisting of (0.5) kg of barley meal, grass hay *ad libitum*, mineral mixtures). The samples were collected after 18 hours of incubation and further analyzed for the content of crude protein. The rate of crude protein degradation was established and calculated as a weight loss of crude protein in the silage sample before and after the incubation.

Statistical analysis

The experimental data were statistically *Evaluated* by using the Statistica CZ program with Tukey test used to establish the significance of differences at two levels of significance ($P < 0.05$) and ($P < 0.01$). Means were reported with their corresponding standard errors.

RESULTS AND DISCUSSION

Effect of different dry matter content

First cut

The average DM (dry matter) content of the initial matter at the time of ensiling was in first taking 401.5 g/kg fresh matter (hereinafter FM) and in second taking 479.2 g/kg FM. The nutritive value of initial matter is shown in Table 1.

Table 1 Nutritive value of fresh (1), wilted (2) and high wilted (3) alfalfa in the 1st cut

Matter	DM	CP	RPD	EE	Ash	ADF	NDF
1	230.2	181.8	72.59	33.9	81.2	299.4	331.2
2	401.5	192.7	71.43	25.5	83.8	355.7	400.8
3	479.2	181	70.24	22.7	83.2	355.8	395.2

DM: dry matter (g/kg fresh matter); CP: crude protein (g/kg DM); RPD: crude protein degradability (% of total CP); EE: ether extract (g/kg DM); Ash: (g/kg DM); ADF: acid detergent fiber (g/kg DM); NDF: neutral detergent fiber (g/kg DM).

Table 2 Chemical composition of silages prepared from forage with different DM content

Silage	DM	DM	CP	EE	Ash	ADF	NDF
Control	Lower	412.7	158.7	55.2	92.3	355.5	429.6
	Higher	471.3	159.0	38.8	92.5	397.0	443.6
Inoculated	Lower	374.9	162.3	62.0	92.5	355.1	434.8
	Higher	462.3	169.7	72.6	91.9	409.0	463.9

DM: dry matter (g/kg fresh matter); CP: crude protein (g/kg DM); EE: ether extract (g/kg DM); Ash: (g/kg DM); ADF: acid detergent fiber (g/kg DM); NDF: neutral detergent fiber (g/kg DM).

The different dry matter content had a significant effect ($P < 0.01$) on RPD of silages. The increased DM content due to longer wilting led to increased RPD values. In case of

shorter wilting time (i.e., lower DM) was the RPD value of $70.72 \pm 1.243\%$; longer wilting time (i.e., higher DM) of $73.69 \pm 0.554\%$. Control silages had the RPD value at the lower dry matter content as $70.63 \pm 0.997\%$, at the higher dry matter content as $73.53 \pm 0.265\%$. In case of inoculated silages, the RPD increased, due to higher dry matter content, significantly ($P < 0.05$). The average RPD value was in the silage at lower dry matter content of $70.63 \pm 0.997\%$, at higher dry matter content of $73.87 \pm 0.698\%$.

Second cut

The average DM content of the initial matter amounted after 20 hrs wilting and 30 hrs wilting as 396.3 g/kg FM and 463.9 g/kg FM, respectively. Nutritive value of the initial matter from the second cut is presented in Table 3, and for silages in Table 4.

Table 3 Nutritive value of fresh (1), wilted (2) and high wilted (3) alfalfa in the 2nd cut

Matter	DM	CP	RPD	EE	Ash	ADF	NDF
1	190.9	194.4	73.02	28.7	82.3	344.3	397.8
2	396.3	183.5	71.01	22.1	86.4	389.9	427.8
3	463.9	215.6	71.91	25.6	83.4	365.9	409.7

DM: dry matter (g/kg fresh matter); CP: crude protein (g/kg DM); RPD: crude protein degradability (% of total CP); EE: ether extract (g/kg DM); Ash: (g/kg DM); ADF: acid detergent fiber (g/kg DM); NDF: neutral detergent fiber (g/kg DM).

Table 4 Chemical composition of silages prepared from forage with different DM content

Silage	DM	DM	CP	EE	Ash	ADF	NDF
Control	Lower	418.4	167.3	51.5	83	382.9	436.5
	Higher	405.5	168	50.2	76.3	384.2	445.8
Inoculated	Lower	402.4	159.7	57.1	81.8	393.3	439.8
	Higher	432.1	166	50	74	401.8	466

DM: dry matter (g/kg fresh matter); CP: crude protein (g/kg DM); EE: ether extract (g/kg DM); Ash: (g/kg DM); ADF: acid detergent fiber (g/kg DM); NDF: neutral detergent fiber (g/kg DM).

The different dry matter content at ensile had a highly significant effect ($P < 0.01$) on RPD. Similarly as in the first cut, the increasing dry matter led to increased RPD (69.19 ± 1.184 ; $72.72 \pm 0.637\%$, respectively). RPD was affected in the control silages significantly ($P < 0.05$) by different dry matter. The average rate of RPD was $69.92 \pm 1.077\%$ (at lower dry matter) and $73.21 \pm 0.379\%$ (at higher dry matter). Statistically significant differences ($P < 0.05$) in the RPD of alfalfa silages due to the different dry matter content were detected in the inoculated silages as well: $68.46 \pm 0.063\%$ (at lower dry matter); $72.28 \pm 0.487\%$ (at higher dry matter).

Mustafa *et al.* (2000) compared RPD values in pea and alfalfa silages. Average RPD values were 87.9% and 88.9% in alfalfa and pea silages, respectively. Verbic *et al.* (1999) compared the rate of RPD in non-wilted, wilted to lower

DM content and wilted to higher DM content grass stands. The authors inform that RPD was 85.5%, 79.6% and 75% in the non-wilted stand, grass stand wilted to lower DM content and grass stand wilted to higher DM content, respectively. Their experiment with the grass stands points to a decreasing RPD trend with the increasing DM content.

Aufrere *et al.* (2000) observed the RPD values in fresh alfalfa, silage and hay.

Those authors reported RPD was the lowest in hay (66%) in comparison with fresh alfalfa (84%). Peltekova and Broderick (1996) indicated non-significant difference in RPD between alfalfa silage and hay. Hawkins *et al.* (1970) confirmed in their trial the tendency of decreased RPD values at increasing dry matter content as well. The wilting of alfalfa led in our experiment to the decrease in ruminal protein degradability in comparison with fresh matter (1st cut: 72.59%; 71.43%; 70.24%; 2nd cut: 73.02%; 71.91%; 71.01%).

Ruggieri *et al.* (2001) observed that wilting resulted in the decreased crude protein fraction soluble in rumen, which leads to decreased RPD. Grabber (2009) compared the ruminal protein degradability of clover and alfalfa silages in dependence on biomass harvest date. Different vegetative stages showed to have a significant influence on RPD in clover silages (term 1: 74.30%, term 2: 2.96%) but also in silages prepared from the wilted alfalfa (term 1: 86.70%, term 2: 84.83%).

According to Kohn and Allen (1995), the alfalfa RPD value decreased due to the ageing of the stand (from 77% to 63%). Based on the results of their experiments, they claimed that alfalfa silages reach higher RPD values as compared with hay, suggesting that the higher dry matter content does not always have to result in the decreased degradability of crude protein.

Hristov and Sandev (1998) informed that the degradation of crude protein in alfalfa silages highly correlated with the amount of non-protein nitrogen and free amino acids in silages, which often connects with a high degree of proteolysis.

Effect of the ensiling additive

First cut

The effect of inoculation on the RPD was not significant. The average RPD value of control silages was as $72.08 \pm 1.620\%$, and of inoculated silages was as $72.33 \pm 1.901\%$. The significant differences were not even detected at the level of particular dry matter contents. The average RPD value of low dry matter control silages was $70.64 \pm 0.997\%$, and in inoculated silages was $70.81 \pm 1.442\%$. In case of higher dry matter silages was found out as $73.51 \pm 0.265\%$ (control) and $73.87 \pm 0.698\%$ (inoculated), respectively.

Second cut

The effect of used bacterial inoculant was not even confirmed in second cut. It was measured the RPD of $71.56 \pm 1.833\%$ in control silages, and in inoculated silages was as $70.37 \pm 2.014\%$. The significant differences were not even evident inside the particular silage group (within various dry matter contents). In the group of low dry matter silages showed control silages $69.92 \pm 1.077\%$, inoculated $68.46 \pm 0.763\%$. The high dry matter silages which were untreated had the RPD value $73.21 \pm 0.379\%$, treated by the bacterial inoculant $72.28 \pm 0.487\%$. The average RPD value of silages from first and second cut are presented in Tables 5.

Table 5 The RPD values of particular variants (%)

Cutting	DM	Control	Inoculated
1. Cut	Low	70.64 ± 0.997	70.81 ± 1.442
	High	73.51 ± 0.265	73.87 ± 0.698
2. Cut	Low	69.92 ± 1.077	68.46 ± 0.063
	High	73.21 ± 0.379	72.28 ± 0.487

DM: dry matter (g/kg fresh matter).

[Aufreder et al. \(2000\)](#) compared RPD values in control silages and in silages chemically treated with formic acid. These authors recorded average RPD values in the control silages and in the formic acid treated silages at 84% and 80%, respectively. [Rizk et al. \(2005\)](#) compared RPD values of untreated and inoculated alfalfa silages.

According to these authors, the matter inoculation had no significant effect on the RPD of alfalfa silages, which amounted on average to 65%. [Petit and Flipot \(1990\)](#) deduced the same conclusion like previous authors and claim that the inoculation had no effect on silage chemical composition, however, it affected significantly the share of nitrogen soluble fraction, which was lower in inoculated silages.

[Hristov and Sandev \(1998\)](#) informed that the crude protein degradation has a high correlation with the amount of non-protein nitrogen and free amino acids in silage. The high content of those substances in silages is connected with the high proteolysis degree. In both cuttings, the degree of proteolysis was significantly ($P < 0.05$, $P < 0.01$) affected by inoculation.

In case of first cut, the average value of proteolysis was in control and inoculated silages as $6.91 \pm 1.050\%$, $5.48 \pm 0.525\%$, respectively. In second cut, it was measured as $8.09 \pm 0.871\%$ in control silages, and in inoculated silages was as $5.68 \pm 0.162\%$. The inoculation led in our trial to decreased ammonia concentration (and thus proteolysis as well) in silages in comparison with control silages in both cuttings, however, the values of RPD were not affected by inoculation. In our experiment, the non-significant effect of inoculant use on RPD values could be caused by the late vegetation stage at matter harvest. Many authors ([Yu et al. 2004](#); [Griffin et al. 1994](#)) claimed that the late vegetation stages are associated with the decrease of crude protein content together with nitrogen soluble fraction. It is likely that the effect of a bacterial inoculation on RPD values will more evident in silages, made from alfalfa plants at early vegetation stages, with a higher content of crude protein including rapidly and potentially degradable fraction. [Tyrolova and Vyborna \(2008\)](#) confirmed the positive effect of silage additive on fermentation quality. [Kramer et al. \(2010\)](#) claim that the quality of crude protein in alfalfa silages can be affected favourably by the application of ensiling additives.

These authors point to a positive influence of the ensiling additive on the fermentation process quality in alfalfa silages that is characterized exactly by the degree of proteolysis. The indicators of silage fermentation quality are mentioned in Tables 6 and 7.

Table 6 The fermentation process quality of alfalfa silages in 1st cut

DM	Variant	PH	NH ₃	LA	AA	PA	BA	TVFA	Eth	AWE	DP
Lower	Control	4.44	0.19	7.85	1.92	0.00	0.00	9.77	0.44	1962.7	6.02
	Inocul.	4.27	0.19	11.02	2.00	0.00	0.00	13.02	0.34	2110.3	5.93
Higher	Control	4.39	0.24	11.39	2.47	0.11	0.26	14.22	0.33	2341.3	7.80
	Inocul.	4.31	0.17	11.32	0.88	0.10	0.27	12.57	0.21	2311.0	5.03

NH₃: ammonia (% of DM); LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid; TVFA: total volatile fatty acid; Eth: ethanol (all in % of DM); AWE: acidity water extract (mg KOH/100 g of silage); DP: degree of proteolysis (% of CP).

Table 7 The fermentation process quality of alfalfa silages 2nd cut

DM	Variant	PH	NH ₃	LA	AA	PA	BA	TVFA	Eth	AWE	DP
Lower	Control	4.73	0.24	10.87	2.89	0.00	0.00	13.76	0.00	1730.3	7.35
	Inocul.	4.41	0.17	9.76	1.50	0.00	0.00	11.26	0.00	2006.3	5.61
Higher	Control	4.78	0.29	8.66	3.34	0.00	0.00	12.00	0.07	1754.7	8.83
	Inocul.	4.37	0.19	9.72	1.46	0.00	0.00	11.18	0.01	2143.7	5.74

NH₃: ammonia (% of DM); LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid; TVFA: total volatile fatty acid; Eth: ethanol (all in % of DM); AWE: acidity water extract (mg KOH/100 g of silage); DP: degree of proteolysis (% of CP).

CONCLUSION

The aim of this work was to judge upon the effect of different dry matter content and inoculation on values of ruminal degradability of crude protein contained in alfalfa silages. The increasing dry matter content caused a higher rate of ruminal protein degradability in both cuts. The using of bacterial inoculant had non-significant effect on RPD in silages. Both groups of silages showed out the similar values of RPD. The inoculation of alfalfa matter led to an improvement in the quality of fermentation process.

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