

The *in vitro* protein degradability of legume and sudan grass forage types and ensiled mixtures

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Abstract: This study examined the *in vitro* crude protein (CP) degradability of wilted, dried for hay and ensiled *Medicago sativa*, *Trifolium pratense* and *Sorghum sudanense*, as well as of ensiled legume-sudan grass mixtures. The rumen degradable protein (RDP) was estimated using the latest Cornell net carbohydrate and protein system (CNCPS v6.5) and with the *Streptomyces griseus* protease assay (48 h of incubation). Sudan grass forages were the lowest in moderately degradable CP and the highest in CP fraction C, while red clover showed the least values for the soluble true protein like alfalfa forages for slowly degradable protein bound in neutral detergent fibre (NDF). Ensiling of legume-sudan grass mixtures decreases N-NH₃ content by 12.2 and 5.1% in comparison with alfalfa and red clover silages, respectively. The RDP values for sudan grass (fresh, wilted, hay) were lower by 7.2 to 7.9% or 10.4 to 15.7% (CNCPS or *S. griseus* procedure) and up to 0.8 or 5.3 to 9.7% in comparison with alfalfa and red clover, respectively. The reduction of protein degradability in ensiled legume-sudan grass mixtures was 5.5 or 6.1% and 1.5 or 3% compared to alfalfa and red clover silages, respectively. Due to higher rumen undegradable protein content, the sudan grass and legume-sudan grass mixtures may be efficiently used to improve the protein utilization in ruminant nutrition.

Key words: Alfalfa, protein fractions, red clover, ruminants, sudan grass.

Introduction

The accuracy of diet formulation for ruminants demands true rumen degradability parameters for crude protein (CP) in the ration. The efficiency of nitrogen conversion in milk production is 18 to 30%, and in meat production 10 to 20%, which is far below the potential of cattle, which exceeds 40% (9). Overfeeding of dietary nitrogen due to the inaccurate prediction of the animal requirements and dietary content of degradable and undegradable protein leads to inefficient ruminal utilization of feed N, which is related directly to NH₃ emission from cattle manure (15). These losses are caused on the one hand by rapid and excessive degradation of plant protein, and improving the efficiency of nitrogen utilization can be achieved by reducing the degree of protein degradation in the rumen (8). For these reasons, many studies have focused on strategies to improve nitrogen balance in milk and meat production based on the legume and grass forages (5, 24, 9). Solubility and degradability of forage protein change during wilting and preservation (6). The changes in protein quality during

hay-making, wilting and ensiling of legume and grass forages can affect intake, protein utilization and productivity of dairy and beef cattle (13).

Legume forages are an important source of protein for ruminants, but its protein is often poorly used because it is extensively degraded during ruminal fermentation, and this may be the most limiting factor of high-quality forage legumes (26). Considering the higher content of NDF and higher values for slowly degradable protein bound in NDF which are usually greater in grasses than in legumes (7), grass and legume-grass forages may have positive effect and reduce the degradability of dietary CP. For that reason, the sudan grass forages could be important for improving the profile of CP in ruminant diets where the ensiling of legume-sudan grass mixtures may be of particular interest. Sudan grass has become an important crop for the production of forage mass for grazing, silage and hay, for dairy and beef producers in areas with lack or not well distributed rainfall throughout a year (23). Sudan grass is currently the only warm-season grass occasionally overseeded into alfalfa in late spring or summer, when

alfalfa has been severely damaged from winter flooding and it is too late to plant other (cool season) species (3).

The objective of this study was to quantify the crude protein fractions and degradability of different types of alfalfa, red clover and sudan grass forages as well as the effects of ensiling legume-sudan grass mixtures on reduction of forage protein degradability. To analyze and compare the composition and degradability of CP, new fractionation scheme CNCPS v6.5 (Cornell Net Carbohydrate and Protein System) as well as the *Streptomyces griseus* protease assay were used.

Material and Methods

Forage material and sample preparation: The trial included wilted, dried for hay and ensiled alfalfa, red clover and sudan grass, as well as ensiled alfalfa-sudan grass and red clover-sudan grass mixtures. Forage material used in this study included: the first cut sudan grass (*Sorghum sudanense* (Piper) Stapf., cultivar Srem, Institute of Field and Vegetable Crops, Novi Sad) harvested at the early-heading stage; the third cut alfalfa (*Medicago sativa* L., cultivar NS Mediana ZMS V, Institute of Field and Vegetable Crops, Novi Sad) at late bud stage; the third cut red clover (*Trifolium pratense* L., cultivar K-17 Institute for Forage Crops Kruševac) at 10% bloom stage. The experiment was carried out during the 2016, plants were harvested from fields with pure stands located in the central region of Serbia, near Velika Plana (44°17'N, 21°05'E). Sudan grass was cut at a height of 100 mm while alfalfa and red clover at 50 mm by a sickle bar mower. For the wilting treatment, the fresh herbage samples were allowed to wilt for 8 h during a sunny day at 24 to 37°C. The herbage material was placed in a thin layer on a clean plastic sheet, and a twice for a wilting time the herbage was manually tedded. Herbage was dried for hay in conditions described for wilting treatment, whereby the plant material stayed outdoor and overnight, with manual turning twice a daily to promote drying, until a dry matter (DM) content of ≥ 850 g/kg was reached. For ensiling treatment, after wilting time of 8 h the plant material was chopped to approximately 1.2 cm particle length with a simple straw chopper. A required part of crop material was used for the preparation of alfalfa-sudan grass and red clover-sudan grass mixture (1:1 ratio, as fed basis). All herbage was ensiled without the use of inoculants in anaerobic glass jars of 1.5 l capacity, each jar contained 600 to 700 g of herbage mass. Silages were stored at room temperature for 4 months. For each forage species and mixture, five laboratory silos with ensiled plant material were prepared.

Analytical procedures: All samples were analyzed in the Laboratory of the Animal Nutrition at the Faculty of Agriculture, University of Belgrade. The samples of the fresh and wilted plant material and silages were dried at

50°C in a forced-air oven for 48 h, for the analysis of chemical composition. All samples were ground through a 1 mm diameter screen on a small-sample cutting mill (Kinematica PX-MFC 90D). Ground samples were analyzed according to the Official Methods (2). DM levels of samples were determined by drying at 105°C for 16 h (method 967.03). Ash was determined by combustion at 600°C for 2 h (method 942.05). The CP content was determined by Kjeldahl method (method 2001.11) using K_2SO_4/Cu catalyst-Kjeltabs S 3.5, on the Kjeltac Auto 1030 Analyzer-Tecator System. Ether extract content was determined by extraction using diethyl-ether in the Soxhlet apparatus (method 920.39). NDF content was determined using heat-stable α -amylase (A3306 Sigma Chemical Co., St Louis, MO, USA) according to the Official Method 2002.04, without use of sodium sulphite, and without correction for ash content. The ADF was determined according to the Official Method 973.18 without correction for ash content. Water extracts of ensiled forage (22) were analyzed for pH using an HI 9017 Microprocessor pH Meter Hanna Instruments.

Separating of CP into five fractions (A1, A2, B1, B2 and C) based on characteristics of degradability was done according to the CNCPS v6.5 (14). Within determined fractions, A1 represents NH_3 (as CP equivalents), A2 soluble true protein (soluble protein minus A1), B1 buffer insoluble protein minus neutral detergent insoluble protein (NDIP), B2 NDIP minus acid detergent insoluble protein (ADIP), and ADIP the C fraction. The NH_3 -N analysis was performed following the procedure of AOAC 941.04 with N determination by Kjeldahl method. The other fractions of CP were determined according to the standardizations of Licitra et al. (19). For soluble true protein determination, the borate-phosphate buffer having pH 6.7 and sodium azide was used. The content of all proteins in the CNCPS are calculated as $N \times 6.25$. Rumen degradable protein was estimated from fractions A1, A2, B1 and B2, using digestion rate constants of 200%/h, 10 to 40%/h, 3 to 20%/h and 1 to 18%/h (27), with assumed passage rate (Kp) of 5%/h (24).

Enzymatic *in vitro* procedure for simulated rumen protein degradation was conducted using *Streptomyces griseus* protease (type XIV, Sigma Chemical Co., Catalog No. P5147) contained 4.0 U/mg, according to the protocol described by Coblenz et al. (4). Triplicate forage samples containing 15 mg of N were incubated in 40 ml of a borate-phosphate buffer solution (pH 7.8 to 8.0) for 1 h at 39°C. Subsequently, 10 ml of separate protease solution with an enzyme activity of 0.33 U/ml was added to the borate-phosphate buffer plus forage (resulted in final enzyme concentration of 0.066 U of activity/ml and a ratio of 0.22 U/mg N) and incubated for 48 h. The fixed ratio of units of enzyme/N was reached by considering the content of CP in analyzed forages. Following incubation, the samples

were vacuum filtered through Whatman #541 filter paper. To calculate enzyme protein degradability (EPD) the following equation was used:

$$\text{EPD (\%)} = (1.0 - (\text{N in residue (mg)} / \text{N in sample (mg)})) \times 100.$$

Statistical analysis: A factorial ANOVA procedure (The General Linear Model Analysis) using the STATISTICA v.6 (25) was conducted to assess the effects of crop (alfalfa, red clover and sudan grass) and type of forage (fresh, wilted or hay), as well as one-way ANOVA for evaluating the effect of ensiled forage crop or binary mixtures, on N fractions contents and *in vitro* crude protein degradability. Differences among treatment means were tested for significance using TUKEY multiple comparison test. Linear regression using the CURVEEXPERT PROFESSIONAL v.2.6.3. (16) was applied to compare RDP estimates for analyzed forages, with the values obtained from the enzymatic procedure as the independent variable and the values based on CP fractionation as the dependent variable, and with relationships described using the coefficient of

determination (R^2). Overall differences between treatment means were considered to be significant when $P < 0.05$. Confidence level for regression models was $P < 0.01$.

Results

Different forage treatments had a negligible effect on the levels of DM components. The CP was substantially lower in sudan grass than in red clover and particular alfalfa forage (Table 1). However, sudan grass forage had the highest fiber levels (NDF and ADF). The DM content of silages was similar to the wilted material. Sudan grass silage was significantly lower ($P < 0.001$) in CP and higher in NDF ($P < 0.05$) and ADF ($P < 0.05$) content in comparison to ensiled legumes, while ensiled legume-sudan grass mixtures were intermediate according to chemical composition.

The pH values of silages were determined as 4.61, 4.87, 4.73, 4.80 and 4.69 for sudan grass, alfalfa, red clover, alfalfa-sudan grass and red clover-sudan grass mixture, respectively.

Table 1. Dry matter (DM) and chemical composition of forages (g/kg DM).

Forage	DM		CP		NDF		ADF		EE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Sudan grass										
Fresh	209.8	5.5	98.4	4.5	599.2	7.1	383.1 ^a	2.2	30.7	1.4
Wilted	301.8	5.1	96.1	3.2	623.7	9.9	397.7	5.2	32.9	1.2
Hay	851.6	0.1	100.4	0.7	625.8	2.8	420.2 ^b	6.1	29.1	1.1
P	-		0.995		0.481		0.003		0.840	
Alfalfa										
Fresh	239.2	6.8	226.6	1.6	443.0	1.8	315.2 ^a	4.7	29.0	1.4
Wilted	389.5	4.4	229.3	4.6	438.7	5.1	335.5	9.7	33.2	1.2
Hay	875.0	2.4	224.3	0.3	458.8	1.4	349.4 ^b	7.7	31.6	0.7
P	-		0.980		0.849		0.008		0.731	
Red clover										
Fresh	248.4	6.1	174.9	2.3	477.1	4.5	357.2	5.4	33.8	3.2
Wilted	369.8	4.9	175.2	3.1	487.3	5.6	359.3	9.4	31.1	1.5
Hay	867.8	1.9	176.5	1.4	488.0	1.5	353.5	3.8	32.1	0.4
P	-		1.00		0.831		0.952		0.986	
Silage										
Sudan grass	313.9 ^a	7.0	108.4 ^a	0.3	616.9 ^a	10.1	412.8 ^a	4.8	33.5	1.1
Alfalfa	396.9 ^b	6.1	234.7 ^b	2.1	451.5 ^b	9.8	349.2 ^b	3.3	36.5	1.4
Red clover	378.6 ^{b,d}	7.1	178.9 ^c	1.5	504.7 ^c	11.7	362.9 ^{b,c}	2.2	36.6	1.4
AF×SG	347.2 ^c	7.1	150.2 ^d	2.3	547.5 ^d	13.0	378.5 ^c	3.9	32.9	1.5
RC×SG	354.0 ^{c,d}	6.9	119.1 ^a	3.0	563.3 ^d	9.8	383.7 ^c	5.5	34.0	1.1
P	0.037		<0.001		0.020		0.046		0.883	

AF×SG: Alfalfa-sudan grass mixture; RC×SG: Red clover-sudan grass mixture; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; EE: Ether extract; SEM: Standard error of the mean; ^{a,b,c,d}: Values within a same column with different numerical superscripts differ significantly ($P < 0.05$).

Table 2. The CNCPS crude protein fractions of fresh and wilted herbage and hay (g/kg CP).

CP fraction/ Forage	Sudan grass		Alfalfa		Red clover		P
	Mean	SEM	Mean	SEM	Mean	SEM	
Fraction A1							
Fresh	2.5	0.4	4.3	0.3	4.1	1.3	1.00
Wilted	3.7	0.5	7.2	0.3	5.1	0.8	0.999
Hay	5.9	0.7	9.2	0.6	5.2	1.0	0.979
P	0.998		0.997		1.00		
Fraction A2							
Fresh	357.1 ^x	7.2	322.0 ^x	1.1	262.5 ^y	4.8	<0.001
Wilted	385.0 ^x	17.9	356.6 ^x	6.2	277.9 ^y	2.8	<0.001
Hay	385.6 ^x	10.7	343.4 ^x	8.6	296.4 ^y	5.2	0.031
P	0.585		0.286		0.313		
Fraction B1							
Fresh	337.4 ^x	8.3	491.4 ^y	2.6	502.7 ^y	4.5	<0.001
Wilted	333.9 ^x	15.5	476.7 ^y	9.9	497.5 ^y	4.3	<0.001
Hay	309.2 ^x	12.2	474.8 ^y	17.2	459.0 ^y	9.1	<0.001
P	0.890		0.999		0.318		
Fraction B2							
Fresh	189.3 ^x	7.4	127.5 ^y	4.5	169.2 ^x	1.3	<0.001
Wilted	168.3 ^x	2.3	117.7 ^y	5.4	162.6 ^x	1.8	<0.001
Hay	175.7 ^x	1.0	122.1 ^y	9.0	170.1 ^x	9.5	<0.001
P	0.235		0.984		0.998		
Fraction C							
Fresh	113.7 ^{a,b,x}	3.0	54.8 ^{a,y}	2.8	61.6 ^{a,b,y}	4.3	<0.001
Wilted	109.1 ^{a,x}	2.3	41.8 ^{b,y}	2.7	56.9 ^{a,z}	3.7	<0.001
Hay	123.6 ^{b,x}	1.8	50.5 ^{a,b,y}	2.1	69.3 ^{b,z}	2.0	<0.001
P	0.002		0.009		0.016		

SEM: Standard error of the mean; ^{a,b}: Values within a same column with different numerical superscripts differ significantly (P<0.05); ^{x,y,z}: Values within a same row with different letter superscripts differ significantly (P<0.05).

Table 3. The CNCPS crude protein fractions of silages (g/kg CP).

Ensiled herbage	CP fractions									
	A1		A2		B1		B2		C	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Sudan grass	67.4 ^a	5.2	504.0	7.0	186.6	12.7	142.3 ^a	4.6	99.7 ^a	2.2
Alfalfa	83.8 ^b	4.3	539.3 ^a	7.5	222.0	10.7	115.0 ^b	6.4	39.9 ^b	2.3
Red clover	72.6	6.4	488.1 ^b	13.6	219.3	12.2	166.8 ^a	1.8	53.2 ^c	1.6
AF×SG	73.6	4.1	509.2	10.3	195.4	11.6	142.1 ^a	3.1	79.7 ^d	2.2
RC×SG	68.9	6.1	489.8 ^b	7.3	197.3	17.4	162.9 ^a	3.8	81.1 ^d	2.1
P	0.046		0.013		0.643		0.035		0.007	

AF×SG: Alfalfa-sudan grass mixture; RC×SG: Red clover-sudan grass mixture; SEM: Standard error of the mean; ^{a,b,c,d}: Values within a same column with different numerical superscripts differ significantly (P<0.05).

Crude protein fractions for the analyzed forages according to the Cornell Net Carbohydrate and Protein System are shown in Table 2 and Table 3.

Concentration of NH₃-N in analyzed forage species was a quite similar, with a pronounced increase for silage, whereas sudan grass silage showed the lowest and alfalfa the highest value (P<0.05). The lowest concentration of

protein fraction A2 was found in fresh and wilted red clover (P<0.001) and in hay (P<0.05) as well as in ensiled red clover and red clover-sudan grass mixture, especially compared to alfalfa silage (P<0.05). Ensiled sudan grass and alfalfa-sudan grass mixture had lesser values for soluble protein in comparison to alfalfa silage by 6.55 and 5.58%, respectively. The crude protein fraction B1 was

higher in legumes ($P < 0.001$) than in sudan grass forage (fresh, wilted and hay). The notably less content of B1 fraction was determined in silages. Sudan grass and red clover showed greater values of fraction B2 than alfalfa forages ($P < 0.001$). The higher content of moderately degradable protein fraction ($P < 0.05$) was also determined in ensiled legume-sudan grass mixtures relative to alfalfa silage. Protein fraction C which is unavailable to the animals was significantly different between crops, with the largest values for sudan grass ($P < 0.001$), and also was higher in red clover than alfalfa forages ($P < 0.001$). Sudan grass showed the greatest content of C fraction which is a result of the highest ADF level.

Table 4 provides mean RDP values for fresh and wilted herbage and for hay. Protein degradability was greatly influenced by forage crops ($P < 0.001$ and $P < 0.05$, according to different procedures) with the highest determined degradability in alfalfa and the lowest in sudan grass. Protein degradability of sudan grass forages was lower by 7.2 to 7.9% or 10.4 to 15.7% (CNCPS or *S. griseus* procedure) and up to 0.8% or 5.3 to 9.7% in comparison with alfalfa and red clover, respectively. Sudan grass showed a lesser protein degradability than red

clover forages. There was a lesser RDP content in red clover relative to alfalfa forages (6.5 to 7.5 or 4.3 to 6.6%). Increased RDP was estimated according to CNCPS procedure in wilted sudan grass and alfalfa ($P < 0.01$ and $P < 0.05$) as in sudan grass wilted herbage and hay ($P < 0.01$) using the *S. griseus* procedure. Ensiled plant material was characterized with the substantially higher RDP values (Table 5).

The lowest RDP values ($P < 0.05$) were in ensiled sudan grass and red clover-sudan grass mixture, while the estimated ruminal protein degradability was the highest in alfalfa silage ($P < 0.05$), and the determined RDP values for red clover and alfalfa-sudan grass mixture were intermediate.

Table 6 shows the relationship between rumen degradable protein obtained using a *Streptomyces griseus* protease incubation and according the Cornell protein fractionation procedure. Estimated RDP values for the protease assay compared to crude protein fractionation were lower by 6.9 to 14.2% for different forage types, and also by 6.0 to 14.0% for different forage species and mixtures.

Table 4. Protein degradability of fresh and wilted herbage and hay (% of CP).

Forage	Sudan grass		Alfalfa		Red clover		P
	Mean	SEM	Mean	SEM	Mean	SEM	
CNCPS procedure							
Fresh	65.11 ^a x	0.27	70.18 ^a y	0.14	65.65 ^x	0.23	<0.001
Wilted	66.62 ^b x	0.37	72.04 ^b y	0.18	66.62 ^x	0.11	<0.001
Hay	65.30 ^a x	0.04	70.90 ^a y	0.28	65.57 ^x	0.25	<0.001
P	0.005		0.024		0.052		
<i>S. griseus</i> procedure							
Fresh	52.37 ^a x	0.45	62.10 ^y	0.42	57.98 ^z	0.55	0.009
Wilted	57.50 ^b x	0.77	64.14 ^y	0.80	61.41 ^y	0.89	0.017
Hay	56.62 ^b x	0.59	63.45 ^y	0.98	59.78 ^x	1.25	0.033
P	0.006		0.716		0.060		

SEM: Standard error of the mean; ^{a,b}: Values within a same column with different numerical superscripts differ significantly ($P < 0.05$); ^{x,y,z}: Values within a same row with different letter superscripts differ significantly ($P < 0.05$).

Table 5. Protein degradability of silages (% of CP).

Ensiled herbage	CNCPS procedure		<i>S. griseus</i> procedure	
	Mean	SEM	Mean	SEM
Sudan grass	71.06 ^a	0.21	64.19 ^a	0.55
Alfalfa	77.50 ^b	0.14	72.12 ^b	0.48
Red clover	72.41 ^c	0.20	69.16 ^{b,c}	0.24
AF×SG	73.23 ^c	0.18	67.75 ^{a,c}	0.54
RC×SG	71.33 ^a	0.10	67.08 ^{a,c}	0.56
P	0.026		0.043	

AF×SG: Alfalfa-sudan grass mixture; RC×SG: Red clover-sudan grass mixture; SEM: Standard error of the mean; ^{a,b,c}: Values within a same column with different numerical superscripts differ significantly ($P < 0.05$).

Table 6. Linear regression ($Y = a + b \times X$) of forage RDP (% of CP) estimated by enzymatic degradation (X) and by CP fractionation (Y).

Forage	<i>a</i>	<i>b</i>	R ²	SE
Fresh	39.161	0.48	0.73	1.31
Wilted	31.094	0.61	0.53	1.95
Hay	30.573	0.61	0.59	1.83
Silage	23.410	0.73	0.72	1.31

a, b: Values in linear regression equation; R²: Coefficient of determination; SE: Standard error.

Discussion and Conclusion

Applied forage treatments did not significantly affect the chemical composition. A slight increase of fiber fractions in wilted herbage and hay was probably due to the result of losses associated with respiration (20) and for ensiled material due to the decrease in water soluble carbohydrate (1).

The CNCPS crude protein fractions were markedly influenced by the crops and forage types. Reduced soluble fraction in red clover may be related to the activity of polyphenol oxidase and subsequent formation of quinones which form complexes with proteins, decreasing their availability for proteases of plants and rumen microorganisms (10). Alfalfa had much lower B2 content due to its low level of NDF as stated by Edmunds et al. (6) for white clover. Values for slowly degradable protein bound in NDF are usually greater in grasses than in forage legumes at comparative growth stages (24). The nonsignificant increase of NH₃-N and fraction A2, and somewhat reduced values for other protein fractions (primarily B1 fraction) in wilted herbage and hay was likely due to the result of proteolysis (11, 13). Soluble true protein was the main fraction in silage, which was combined with a particular decrease in B1 fraction, probably due to the extensive degradation by plant proteases (22). Ensiling had a tendency to reduce content of fraction C.

Protein degradability values significantly differed among forage crops. The largest RDP values for alfalfa can be ascribed to high proportion of soluble true protein (A2) relative to red clover, higher B1 fraction than sudan grass, and its low cell wall associated CP fraction (B2+C). Coblenz et al. (4) also reported the higher RDP estimates for alfalfa hay than that for grass hay. Lower protein degradability of the sudangrass than red clover forage found in the present study was likely due to the significant differences in content of moderately degradable and cell wall associated protein. Increased RDP values in wilted forage were in accordance with findings of Krawutschke et al. (17), which explained the decrease in RUP content in wilted legume herbage by negative correlations with the level of NPN and moderately degradable protein. The substantially higher RDP values in silages are due to the CP fraction distribution, where the soluble true protein is

the main fraction in silage, which is combined with a multiple increase in ammonia N. However, one of the important finding is the significant reduction of CP degradability in ensiled legume-sudan grass mixtures, especially relative to alfalfa silage. The reduction of protein degradability by ensiling alfalfa-sudan grass mixture was 5.5 or 6.1% compared to alfalfa silage as well as 1.5 or 3.0% for ensiled red clover-sudan grass mixture relative to red clover silage. Lower forage and ration RDP content can significantly contribute to reducing ruminal ammonia-N concentration and improve nitrogen use efficiency (5).

The observed estimates for ruminal protein degradation according to the *S. griseus* protease procedure and CNCPS fractionation were highly related with each other. The enzymatic degradation using commercial protease (48 h *S. griseus* assay) yields respective RDP estimates that are close to the known *in vivo* values (4), and explains a large portion of the variation observed from the *in situ* technique (21). Grabber (12) also found lower estimates of RDP by enzyme *in vitro* assay relative to the values from CNCPS fractionation according to Sniffen et al. (24).

The results from this study indicate the potential importance of using the sudan grass forages in order to increase the content of rumen undegradable protein (RUP) in the rations of ruminants. The sudan grass is characterized by a significantly lower crude protein degradability especially relative to the alfalfa forages, and also by a lower RDP content in comparison to red clover. Ensiling of legumes in combination with sudan grass improved CP fractions distribution that significantly reduces the protein degradability in comparison with pure legume silages. Red clover also showed an advantage relative to alfalfa forage according to the content and ratio of individual CP fractions and degradability. Positive effect of ensiling legume-sudan grass mixtures on reducing the crude protein degradability compared to legume silages, may be used for improvement of N utilization in ruminant nutrition. Degradable protein determined by an enzymatic procedure using *S. griseus*, although with slightly lower values, is in good agreement with predictions using the latest CNCPS v6.5 fractionation scheme.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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