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PROTEIN DEGRADABILITY OF GRASSLAND FORAGE UNDER SIMULATED ROTATIONAL SPRING GRAZING

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Abstract: A cutting experiment was conducted to analyze the changes in the crude protein (CP) fraction content and in the estimated ruminal protein degradability of forage, obtained in conditions of simulated rotational spring grazing on permanent grassland. The field trial was conducted on permanent pasture during 2015 and included three cuttings as a simulated rotational spring grazing. For determination of protein degradability of pasture forage, the fractionation of the CP according to Cornell Net Carbohydrate and Protein System (CNCPS v6.5) and the *Streptomyces griseus* protease assay were used. Relative to CP, no significant differences were found among cuts for ammonia N content (A1 fraction) and for protein fraction C which is completely unavailable to the animals. Values for soluble true protein (A2 fraction) and cell wall-associated protein, which is acid detergent soluble (B2), were significantly increased ($p<0.05$) while a significant reduction ($p<0.05$) of the moderately degradable protein (B1) content was determined during the growing season. The lower rumen degradable protein (RDP) content of grassland herbage was obtained in the second cut which was significant ($p<0.05$) according to the CNCPS procedure. Obtained high solubility and degradability of CP in pasture require adequate content of readily available carbohydrates in rations for grazing ruminants to provide efficient utilization of consumed protein.

Key words: ruminants, pasture, protein, fractions, *in vitro* degradability.

Introduction

Currently, models used to balance rations for ruminants emphasize the need to consider ruminal protein degradability. The pasture, which is the main ingredient of ruminant rations, may supply a significant portion of the total crude protein (CP) content of the diet. Hence, there has been an interest in protein degradability of pasture. Excessive protein degradation in the rumen may be the most limiting

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factor of pasture usage (Stojanović et al., 2014). As a consequence of the high rumen degradability of grassland forage protein, a great part of the N may be lost by excretion in the urine (Merchen and Bourquin, 1994). There is interest in identifying factors that influence the rate and extent of ruminal degradation of forage proteins (Broderick, 1995).

The pasture is characterized by a very high content of rumen degradable protein (RDP). Increased rumen undegradable protein (RUP) concentration is highly ranked for improving the nutritive value of forage (Tremblay et al., 2002). An optimal ratio of rumen degradable protein to RUP is ranked as the second criteria to improve the nutritive value of forages for dairy cattle (Smith et al., 1997). Protein degradation of grassland forages is highly variable and depends on botanical composition, plant maturity and growing period (Rayburn, 1991).

The determination of protein degradability of grassland forages and the changes which may occur during the growing season are important for defining the grazing strategies, to increase the protein use efficiency, and to decrease the N losses (Stojanović et al., 2016).

The fractionation of feed protein and estimation of protein degradability according to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992) are well accepted for the characterization of protein quality in ruminant nutrition. One of the generally accepted *in vitro* methods for protein degradability analysis is the *Streptomyces griseus* protease assay (48 h of incubation) (Krishnamoorthy et al., 1983). However, additional work is needed to characterize the grassland herbage crude protein.

The objective of this study was to determine the extent of variation in the CP fraction content and the protein degradability of forage from the simulated rotational spring grazing on permanent grassland.

Material and Methods

The trial was conducted on the natural pasture during the spring of 2015 and included three cuttings as simulated rotational grazing. The study site was located in the western region of Serbia, near Šabac (44°40′ N, 19°39′ E). The experimental design, grassland management and sampling method were described elsewhere (Stojanović et al., 2018). The field trial was established on the pasture which had been exploited permanently for dairy cattle grazing, by the method of an RCB design of plots (5 × 2 m) in 5 replications. There were three cuts (1 May, 24 May and 19 June) in the part of the vegetation season, before the summer drought period. In the 1st, 2nd and 3rd cuts, the botanical composition was as described in Stojanović et al. (2018).

Herbage samples were analyzed in the Laboratory for the Animal Nutrition at the Faculty of Agriculture, University of Belgrade. Chemical analysis was

performed according to the procedure of AOAC (2002). Separating of CP into five fractions (A1, A2, B1, B2 and C) based on characteristics of degradability was conducted according to the Cornell Net Carbohydrate and Protein System – CNCPS v6.5 (Higgs et al., 2015) using standardizations of Licitra et al. (1996). Within 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. Calculated rumen degradable protein was estimated from fractions A1, A2, B1 and B2, using digestion rate constants of 200%/h, 27.3%/h, 15%/h and 5%/h (Van Amburgh et al., 2015), with an assumed passage rate (Kp) of 5%/h (Sniffen et al., 1992).

An *in vitro* enzymatic procedure for simulated rumen protein degradation was conducted using *Streptomyces griseus* protease (type XIV, Sigma Chemical Co., Catalog No. P5147) and contained 4.0 U/mg, according to the protocol described by Coblentz et al. (1999). Triplicate forage samples containing 15 mg of nitrogen were incubated for 48 h in a borate-phosphate buffer solution with added protease (a final enzyme concentration of 0.066 U of activity/ml and a ratio of 0.22 U/mg N). The fixed ratio of units of enzyme/N was reached by considering the content of CP in analyzed forages. To calculate enzyme protein degradability (EPD), the equation EPD (%) = (1.0 - (N in residue (mg)/N in sample (mg))) × 100 was used.

An ANOVA procedure using the STATISTICA v.6 (StatSoft, 2003) was conducted to assess the effects of different cuttings on the CP fraction content and the ruminal protein degradability of herbage from permanent grassland during the spring growth. Differences among treatment means were tested for significance using the LSD test. The statistical significance was determined at $p<0.05$. The linear regression was applied to compare RDP estimates of the protease assay, with the values based on CP fractionation as the dependent variable, and described relationships using the coefficient of determination (R^2).

Results and Discussion

Fresh forages contained a high proportion of soluble CP (A1+A2 fractions, from 40.15 to 50.85%) and a relatively low proportion of moderately degradable protein (B1 fraction) for all harvests across the spring growth. Relative to CP, no significant differences were found between cuts during the analyzed spring grazing period for ammonia N content (A1 fraction) and for protein C fraction which is completely unavailable to the animals, whereas the values of A2, B1 and B2 fractions significantly differed (Table 1).

Considering the previously determined changes in the share of grasses (35, 22 and 15%), legumes (39, 43 and 37%) and forbs (26, 35 and 48%) across the cuttings (the 1st, 2nd and 3rd cuts, respectively) (Stojanović et al., 2018), obtained

results are in accordance with the findings of Elizalde et al. (1999), where the non-protein N content of forages was not affected by forage species (grass and legumes) and sampling dates.

Table 1. The crude protein fractions of forage (%) according to the Cornell Net Carbohydrate and Protein System.

Cuts	CP, % DM	Fractions				
		A1	A2	B1	B2	C
1.	15.30±1.20	3.29±0.47	36.86±1.70^{a}	41.48±2.32^{a}	11.26±0.33^{a}	7.12±1.42
2.	13.72±1.66	3.25±0.73	41.34±1.68^{b}	33.15±0.67^{b}	13.71±0.51^{b}	8.55±0.32
3.	14.55±1.38	3.63±0.55	47.22±0.66^{c}	29.22±0.62^{c}	12.17±0.22^{c}	7.76±0.64

± standard deviation; ^{a,b,c} means in the same column with different superscripts differ ($p<0.05$) significantly; CP – crude protein; A1, A2, B1, B2, C – crude protein fractions according to the Cornell Net Carbohydrate and Protein System.

Values for soluble true protein (A2 fraction) and for cell wall-associated protein (B2) that is soluble in acid detergent were significantly increased ($p<0.05$) while a significant reduction ($p<0.05$) of a moderately degradable protein (B1) content was determined with advancing of the vegetation season. The A2 protein fraction was higher by 12.2 and 28.1% and the B2 fraction by 21.8 and 8.1% in the herbage obtained from the second and third harvests relative to the first one, whereas the B1 fraction content was reduced by 20.1 and 29.6%. A displayed trend may be explained by a noted significant increase of neutral detergent fiber – NDF (39.09, 48.61 and 43.61% DM) and acid detergent fiber – ADF (25.54, 31.22 and 28.61% DM) content in analyzed forage during the spring grazing period (Stojanović et al., 2018). The CP fraction content was also influenced by a marked increase of the percentage of forbs that was noted together with the reduced share of grass species, while the percentages of legume species were similar or slightly increased during the spring grazing period (Stojanović et al., 2018). Rayburn (1991) has reported that protein solubility is greater in mixed mostly legume and legume forage than in grass and mixed mostly grass forage, whereby solubility decreases by 0.25 units/unit NDF. According to Solati et al. (2017), insoluble CP soluble in neutral detergent (fraction B1) was the largest fraction in legume and grass herbage, whereas a significant decline in this fraction was observed in white clover and alfalfa across the spring growth. Obtained values for the B1 fraction are more approximate to those of Sniffen et al. (1992) for grass pasture, and are lower relative to Elizalde et al. (1999) for legume and grass herbage, where this fraction was the largest CP fraction.

The determined B2 fraction (including N in the NDF but soluble in acid detergent) content corresponded with the earlier reported values by Abdalla et al. (1988) for grazed mixed pastures (13.0% CP) and by Sniffen et al. (1992) for grass

pastures during the spring (10.0% CP). Unavailable or bound proteins, which are insoluble in acid detergent (C fraction), are slightly above the upper values for legume and grass (4.6 and 6.6% of CP) forage according to Cherney et al. (1997), probably due to a high share of forbs in the pasture.

Table 2. The herbage crude protein degradability (%).

Method of estimation	Cuts		
	1.	2.	3.
CNCPS procedure	71.10±0.98[a]	69.83±0.45[b]	71.45±0.49[a]
S. griseus procedure	65.78±3.32	62.83±2.14	64.60±2.85

± standard deviation; [a,b,c] means in the same row with different superscripts differ ($p<0.05$) significantly.

Observed results indicate the high ruminal degradability of the CP in fresh forage. Ruminal degradable protein (RDP) values for the examined forages obtained in conditions of simulated rotational spring grazing on permanent grassland are shown in Table 2. According to the CNCPS procedure, the determined protein degradability was significantly influenced ($p<0.05$) by the growing period with the lowest RDP content in the herbage obtained from the second cut. The concentration of rumen degradable protein (% CP) was lower by 1.79 and 2.27% for the herbage from the second harvest relative to the first and the third ones. A lower protein degradability value is likely a result of the increased concentration of B2 and C protein fractions due to higher fiber content (NDF and ADF) in the second cut. Observed results are in accordance with the research of Rayburn (1991), where it was found that the protein degradability decreased by 0.088 units/unit NDF. With the advancing of the spring season, there were no especially large differences in herbage RDP content between cuts, despite the displayed trend of a significant increase of the soluble true protein concentration (% CP) and decreasing the B1 fraction that is more slowly degraded in the rumen. This is likely due to increasing the cell wall-associated protein fractions (B2+C) that are characterized by limited ruminal degradability or are completely undegradable (Higgs et al., 2015). Our findings are in the range reported by Cone et al. (2004) for rumen undegradable protein in different grass samples after 3 weeks of the regrowth period (23.1–37.4 or 34.9% CP) and by Grabber (2009) for RUP in herbage of different legume species (25.6–33.2% CP).

Estimated RDP values of herbage of different cuts using the *S. griseus* procedure did not differ significantly (probably due to a higher variation between individual replications), but a reduced level of ruminally degraded protein (% CP) was found also in forage obtained from the second harvest (4.48 and 2.74%, compared to the first and third harvests, respectively).

Values for RDP from the protease assay compared to CP fractionation were lower by 7.5–9.0% for different harvests. The relationships between the rumen degradable protein obtained with a *Streptomyces griseus* protease incubation and with the Cornell protein fractionation procedure are shown in Table 3.

Table 3. A linear regression of rumen degradable protein (% CP) estimated by enzymatic degradation (x) and by crude protein fractionation (y).

Equation	*a*	*b*	R^2	SE
Parameters	54.07	0.26	0.60	0.64

a, b – linear regression parameters; R^2 – coefficient of determination; SE – standard error.

The determined values for the ruminal protein degradation of grassland forage obtained from different cuttings according to the CNCPS fractionation and *S. griseus* protease procedure were highly related. The lower estimates of RDP by the *in vitro* enzyme assay (*S. griseus*) relative to values from CNCPS fractionation are supported by the results of Grabber (2009a) for red clover forages, where estimates were also highly related. Coblentz et al. (1999) obtained somewhat lower values for RDP based on protease treatment, compared to those determined by the *in situ* procedure, for alfalfa and grass hay.

Conclusion

The estimations of ruminal protein degradability and protein escape are necessary for an adequate diet formulation. The protein degradability of the analyzed forage from the simulated rotational spring grazing on permanent grassland was generally high, with lower values for the herbage obtained from the second cut. A dominant protein fraction in the herbage of the first harvest was the moderately degradable protein (B1), whereas in the second and third harvests that was the soluble true protein (A2). Estimates of RDP by the *in vitro* enzyme assay were somewhat lower relative to the CNCPS protein fractionation procedure. The determined high solubility and degradability of CP in the pasture indicate that rations for grazing ruminants should have an optimal content of readily fermentable carbohydrates to provide efficient utilization of consumed N.

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RAZGRADIVOST PROTEINA ZELENE MASE SA TRAVNJAKA U USLOVIMA PROLEĆNE PREGONSKE ISPAŠE

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R e z i m e

Istraživanje je obavljeno u cilju utvrđivanja promena u sadržaju frakcija sirovog proteina (SP) i ruminalne razgradivosti proteina zelene mase dobijene košenjem prirodnog travnjaka u uslovima koji su odgovarali rotacijskoj pregonskoj ispaši tokom prolećne sezone. Poljski ogled je izveden na permanentnom pašnjaku tokom proleća 2015. godine i uključivao je tri otkosa koji su odgovarali ciklusima ispaše. Za determinisanje razgradivosti proteina zelene mase, frakcionisanje sirovog proteina je obavljeno prema proceduri *Cornell Net Carbohydrate and Protein System* (CNCPS v6.5), kao i primenom *in vitro* metode korišćenjem *Streptomyces griseus* proteaze. U odnosu na SP, nisu utvrđene značajne razlike između otkosa – ciklusa ispaše u pogledu sadržaja amonijačnog N (frakcija A1), kao i u pogledu sadržaja proteinske frakcije C, koja je potpuno nedostupna životinjama. Sadržaj rastvorljivog pravog proteina (frakcija A2) i proteina vezanog za ćelijski zid, koji je rastvorljiv u kiselom deterdžentu (frakcija B2) se značajno povećavao ($p<0,05$), dok se sadržaj umereno razgradive frakcije proteina (B1) značajno smanjivao ($p<0,05$) tokom prolećne sezone vegetacije. Najmanja vrednost za ruminalnu razgradivost i učešće RDP (protein razgradiv u rumenu) u SP zelene mase sa pašnjaka utvrđena je u drugom otkosu, a ova razlika je bila značajna ($p<0,05$) kada je ruminalna razgradivost proteina determinisana korišćenjem procedure CNCPS. Utvrđeno visoko učešće rastvorljive frakcije SP i visoka ruminalna razgradivost SP zelene mase sa pašnjaka ukazuju na potrebu podrobnijeg balansiranja obroka za preživare na paši u pogledu sadržaja lako razgradivih ugljenih hidrata, a u cilju obezbeđenja efikasnog iskorišćavanja konzumiranog proteina.

Ključne reči: paša, protein, frakcije, *in vitro* ruminalna razgradivost.

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