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ESTIMATION OF NUTRITIVE VALUE AND PROTEIN DEGRADABILITY OF *TRIFOLIUM REPENS* AND *POA PRATENSIS* AS THE DOMINANT PASTURE SPECIES, UNDER SIMULATED ROTATIONAL GRAZING

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SUMMARY

A cutting trial was conducted to determine the nutritional value, crude protein (CP) fractions distribution and CP ruminal degradability of white clover (*Trifolium repens* L.) and Kentucky bluegrass (*Poa pratensis* L.) as the dominant species of permanent grassland. The legume and grass herbage was obtained in the field experiment carried out on pasture throughout a spring growth season where rotational grazing was simulated using three consecutive cuts, which were compared. The fractionation of CP, according to Cornell Net Carbohydrate and Protein System (CNCPS v6.5), was used to predict protein degradability of the legume and grass forage. A significant ($p < 0.05$) lowering of the CP content in the legume and grass herbage was found, while NDF (neutral detergent fiber) and ADF (acid detergent fiber) significantly increased ($p < 0.05$) as the spring growth season progressed. A significant decrease ($p < 0.05$) of fractions A1 and A2 was noted in the legume and grass herbage, with a significant ($p < 0.05$) increase of CP fractions B2 and C as the vegetation season progressed. A higher rumen degradable protein (RDP) content ($p < 0.05$) of white clover and Kentucky bluegrass herbage was obtained in the first harvest, compared with the second and third one. Significantly larger values ($p < 0.05$) of net energy were observed in the white clover and Kentucky bluegrass forage obtained from the first cut, compared with later regrowths, and white clover was characterized by a higher energy content of 12.4–33.1% (dry matter basis), relative to Kentucky bluegrass.

Key words: pasture, white clover, Kentucky bluegrass, ruminants, protein quality, energy value

INTRODUCTION

Grassland vegetation covers about 1.5 million ha, accounting for 27% of the total agricultural area of the Republic of Serbia, and it is the most common type of the agroecosystem, whereas permanent grasslands are the main source of forage for grazing animals (Simić et al., 2015). The pasture forage species determine the grazing management needed to optimize animal production (Stojanović et al., 2014). White clover (*Trifolium repens* L.) is one of the main legume components, while Kentucky bluegrass (*Poa pratensis* L.) is one of the significant grass components; both of them create grazing grasslands in Serbia (Aćić, 2019). In grasslands that are not heavily fertilized with nitrogen (N) or manure, legumes are the major source of N for grass growth and a significant determinant of forage quality and animal performance. Legumes, such as clovers, tend to be higher in forage quality than grasses at the same maturity because they are higher in protein, lower in fiber and are digested more rapidly in the rumen, stimulating a higher intake (Rayburn, 2007). White clover is a valuable pasture plant widespread in the native grasslands, and white clover content of about 25–30% of herbage dry matter over the season is optimal for yield and pasture quality

(Adamović et al., 2005). Kentucky bluegrass is one of the most persistent components of grasslands, particularly for soil of medium fertility and moisture, where the access to light is sufficient (Helgadóttir et al., 2002). This grass species is a valuable pasture plant in conditions of frequent and less intense grazing (50% removal) (Bryan et al., 2000). During the season, the species contents vary greatly, with the lowest levels of white clover in the spring and higher levels in the summer, which has a positive effect on the yield distribution during the whole grazing season (Rayburn, 2007). Kentucky bluegrass decreases its contribution to the sward regrowth throughout the summer, as a consequence of precipitation deficiency and high average air temperatures (Warda & Stamirowska-Krzaczek, 2009). Important indicators of the nutritive value for grazing forages are the content of crude protein (CP) and nonprotein N, neutral detergent fiber (NDF) and acid detergent fiber (ADF) as well as the available energy concentration (Stojanović et al., 2014). Forage quality depends on seasonal climate variations, which is evident in forages harvested at similar maturity stages (Buxton & Casler, 1993). The more rapid decline in forage quality with the time typical for spring growth, compared with summer growth, causes the interaction of increasing temperatures with the advancing maturity (Van Soest, 1994).

The pasture, as the main ingredient of ruminant rations, may supply a significant portion of the total crude protein content of the diet. Excessive protein degradation in the rumen may be the most limiting factor of pasture usage (Stojanović et al., 2019). Protein degradation of grassland forages is highly variable and depends on the botanical composition, plant maturity and growing period (Rayburn, 2007). Determination of protein degradability of grassland forages and the changes that occur during the growing season are significant for defining the grazing strategies, increasing the protein use efficiency, and decreasing the N losses (Stojanović et al., 2019).

In order to meet the nutritional requirements of grazing animals, throughout the plant growing season, it is necessary to determine the nutrient content, protein fraction distribution and pasture energy value. For this reason, a cutting experiment was conducted during the spring growing season to determine the changes in the chemical composition and nutritional value of white clover and Kentucky bluegrass as the dominant companion legume and grass species in the analyzed permanent grassland, in conditions of simulated rotational grazing with three consecutive cuts.

MATERIAL AND METHODS

The research was carried out on a permanent pasture in spring 2015 in the western region of Serbia, in the vicinity of Šabac (44°40' N, 19°39' E), and it included three cuts as simulated rotational grazing. The experimental design, grassland management and sampling method were described elsewhere (Stojanović et al., 2018). The field trial was established on the pasture that had been exploited permanently for dairy cattle grazing. Five samples from 5×2 m plots were taken in three cuts, as a simulation of grazing. The three cuts (1 May, 24 May and 19 June) with high biomass production were compared during the part of the vegetation season before the summer drought period. In the first, second and third cuts, the botanical composition was determined by estimation of the floristic share and covering. Considering the botanical composition of pasture by covering, legumes had a higher share (37–43%), while grasses were represented by 15–35%, whereas white clover and Kentucky bluegrass were predominant among the legume (>90%) and grass (>90%) components. Forbs were represented by 28–42%, including *Taraxacum officinale*, *Stellaria media*, *Plantago lanceolata*, *Cichorium intybus*, *Rumex spp.*

The main characteristics of the soil and weather conditions are presented in Table 1. and 2. The experimental field soil had low P content. According to the meteorological data, the season was very humid, with the highest amount of precipitations in May.

Table 1. Chemical properties of soil

Soil properties	pH		OM (%)	AL-P ₂ O ₅ mg/100 g	AL-K ₂ O mg/100 g	Total C (%)	Total N (%)
	CaCl ₂	H ₂ O					
0-20 cm	5.07	5.73	4.31	1.98	11.51	1.37	0.16

Legend: OM – organic matter; AL-P₂O₅ – ammonium lactate available phosphorus; AL-K₂O – ammonium lactate available potassium

Table 2. Meteorological data

Months	Jan	Feb	Mar	Apr	May	Jun	Average/Total
Temperatures, °C	2.5	2.7	7.1	11.9	18.2	20.7	10.5
Precipitation sum, mm	59.8	65.1	37	26	93.1	20.8	301.8

After cutting, white clover and Kentucky bluegrass were hand-picked from fresh forage samples and immediately packed in plastic bags, and placed in a cooled portable refrigerator transported to the laboratory for analysis. The fresh plant material samples were dried at 50 °C for 48 h and ground to pass through a 1 mm screen on a small-sample cutting mill (Kinematica PX-MFC 90D) for analysis of the chemical composition. Chemical analysis was conducted in the Laboratory for Animal Nutrition at the Faculty of Agriculture, according to the AOAC (2002) procedures. The DM content was determined by drying at 105 °C for 16 h (method 967.03), the ash was determined by combustion at 600 °C for 2 h (method 942.05), the CP content was determined by the Kjeldahl method (method 2001.11), the ether extract content was determined by extraction using diethyl-ether (method 920.39), the NDF content was determined using heat-stable α -amylase (method 2002.04) and the ADF according to the method 973.18. Separation of CP into five fractions (A1, A2, B1, B2 and C) based on ruminal degradation rates was performed according to the Cornell Net Carbohydrate and Protein System – CNCPS v6.5 (Higgs et al., 2015), where the ammonia content was measured following the procedure of AOAC 941.04 and the other fractions of CP were determined according to the standardizations of Licitra et al. (1996). Within the 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, 34.2%/h, 15%/h and 7%/h for the legume forage, and 200%/h, 29.8%/h, 15%/h and 6%/h for the grass forage (Van Amburgh et al., 2015), with an assumed passage rate (Kp) of 5%/h and 8%/h (Sniffen et al., 1992).

To estimate the concentration of the net energy for lactation of forage DM in dairy cattle nutrition, the NRC (2001) model was used, as well as INRA (Sauvant et al., 2004) model (by applying equations for dehydrated forages) to predict the values of net energy for ruminants.

The analysis of variance using Statistica v.6 (StatSoft, 2003) was performed to evaluate the effects of consecutive harvests on the chemical composition and nutritional values, as well as on protein fractions and degradability of the main legume and grass components of herbage from the permanent grassland. In order to estimate the significance of differences among the treatment means, the LSD test was used. The effect of the treatment was considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

This research showed a significant effect of the consecutive cuts during the spring growing season on white clover and Kentucky bluegrass herbage quality. The results for the chemical composition of herbage for three harvests from 1 May to 19 June are presented in Table 3.

Table 3. The chemical composition (% DM) of *Trifolium repens* and *Poa pratensis* from consecutive spring harvests of the permanent grassland

Cuts	DM	CP	NDF	ADF	NFC	EE	Ash
<i>Trifolium repens</i>							
I	18.07 ^c	26.25 ^a	22.86 ^c	19.15 ^c	39.20 ^a	4.34 ^a	8.97
SEM	0.14	0.57	0.42	0.13	0.37	0.07	0.24
II	19.33 ^b	21.88 ^b	36.06 ^a	29.03 ^a	32.60 ^b	4.37 ^a	8.73
SEM	0.26	0.30	0.35	0.21	0.59	0.07	0.16
III	20.93 ^a	18.91 ^c	33.49 ^b	26.31 ^b	37.68 ^a	3.55 ^b	8.70
SEM	0.29	0.47	0.23	0.23	0.64	0.05	0.09
<i>Poa pratensis</i>							
I	21.61	13.51 ^a	48.04 ^b	25.70 ^b	28.65 ^a	4.43 ^a	7.12
SEM	0.23	0.08	1.77	0.58	1.43	0.05	0.21
II	22.43	8.51 ^c	60.61 ^a	36.57 ^a	21.89 ^b	3.51 ^b	6.97
SEM	0.62	0.47	1.36	0.84	1.26	0.14	0.11
III	22.42	10.17 ^b	56.35 ^a	34.41 ^a	24.98	3.30 ^b	6.84
SEM	0.28	0.41	1.87	1.57	2.37	0.14	0.05

Legend: DM – dry matter (%); CP – crude protein; NDF – neutral detergent fiber; ADF – acid detergent fiber; NFC – nonfiber carbohydrates; EE – ether extract; SEM – Standard Error of the Mean; ^{a, b, c} - Means in the same column with different superscripts differ ($p < 0.05$)

An increase in the legume and grass herbage DM content was found during the analyzed spring grazing period, which was significant ($p < 0.05$) for white clover. For both clover and grass herbage, the obtained CP values significantly decreased ($p < 0.05$) during the spring growing season. The CP content of the third cut was lower by 28.0 or 13.6% relative to the first or second cut of white clover, whereas CP of the second harvest of Kentucky bluegrass was by 37.0 and 16.3% lower compared with the first and third one. On the other hand, an increase of the fiber content (NDF and ADF) was observed during the spring grazing period, with the highest values for the second cut ($p < 0.05$), where the lowest concentration of nonfiber carbohydrates – NFC ($p < 0.05$) was noted as well. Besides, the NDF of the second cut was higher by 57.7 and 7.7% and by 26.2 and 7.6% for white clover and Kentucky bluegrass, respectively, compared with the first and third cut. A similar trend was determined for ADF, comparing the second with the first or third harvest, where the values increased by 51.6 or 10.3% and by 42.3 or 6.3% for the legume and grass forage, respectively.

The white clover forage is characterized by high CP concentration, relatively low concentration of NFC and high digestibility, especially compared with grasses (Beever, 1993). Steg et al. (1994) reported that white clover had about 200 g/kg DM lower NDF content in comparison with ryegrass, and concluded that hemicellulose content of grasses and clover increased during the season. Higher values, compared with the current results, were reported in the study of Bryan et al. (2000) for the CP content in grazed Kentucky bluegrass herbage, where the CP content ranged between 15.1 and 16.6% DM, while the NDF content was between 47.8 and 49.7% DM, and ADF between 30.9 and 32.9% DM. Findings in the current research are in accordance with the study of Mandaluniz et al. (2015), which reported decreased CP content and increased NDF and ADF content for grazing herbage mass during the spring grazing period. Furthermore, Marshall et al. (2004) found the greatest differences in the CP and nonfiber carbohydrates contents between the early cuts of white clover (1–3), while these differences were less evident in the later cuts. Pelletier et al. (2010) reported that the summer regrowth of grasses showed lower CP content than the spring growth, and the decrease varied from 11% for Kentucky bluegrass to 30% in Timothy (*Phleum pratense* L.). According to these authors, the ADF content of the summer regrowth was higher compared with the spring growth for grass and legume species, and this increase amounted to 14.5% for Kentucky bluegrass and 9.2% for red clover. Tas et al. (2006) also found that the concentration of CP in perennial ryegrass forage was the highest for the first harvest compared with later summer harvests. According to Bakken et al. (2016), the grass–clover herbage from the first cut was higher than the second cut in concentrations of water-soluble carbohydrates. The current research confirmed the negative relationship between the fiber (NDF and ADF) and nonfiber carbohydrate concentrations in the grass and legume forages, earlier determined by Zhao et al. (2008). Similarly, Steg et al. (1994) observed a marked decrease in nonstructural carbohydrates in white clover and ryegrass herbage during the season.

Table 4. The CNCPS crude protein fractions (% CP) of *Trifolium repens* and *Poa pratensis* forage from the permanent grassland, for three harvests during the May– June interval

Cuts	Fractions				
	A1	A2	B1	B2	C
<i>Trifolium repens</i>					
I	2.76 ^a	38.12 ^a	49.57 ^a	6.28 ^c	3.27 ^b
SEM	0.14	1.18	1.02	0.35	0.25
II	2.94 ^a	37.62 ^a	34.66 ^b	16.62 ^a	8.16 ^a
SEM	0.17	1.97	2.17	0.45	0.26
III	2.17 ^b	29.64 ^b	47.98 ^a	12.31 ^b	7.90 ^a
SEM	0.20	2.05	2.26	0.36	0.07
<i>Poa pratensis</i>					
I	2.24 ^a	41.21 ^a	40.69	13.10 ^b	2.76
SEM	0.03	0.56	0.76	1.09	0.13
II	1.23 ^b	28.45 ^b	43.93	17.55 ^a	8.84
SEM	0.15	2.47	2.63	0.67	0.18
III	1.54 ^b	34.73 ^b	40.90	15.19	7.64
SEM	0.13	2.27	3.06	0.41	0.26

Legend: A1, A2, B1, B2, C – crude protein fractions according to the Cornell Net Carbohydrate and Protein System; SEM – Standard Error of the Mean; ^a, ^b, ^c – Means in the same column with different superscripts differ ($p < 0.05$)

The CP fractions distribution is shown in Table 4. The legume and grass forage contained a moderate proportion of soluble CP (A1+A2 fraction), and these values for white clover ranged between 31.8 and 40.9% CP, whereas for Kentucky bluegrass they were between 29.7 and 43.5% CP. A significant decrease ($p < 0.05$) was found in the

legume and grass herbage ammonia nitrogen (fraction A1) and the fraction A2 content with advancing of the vegetation season. In the third cut of white clover, the CP fraction A1 was lower by 21.4 and 26.2%, and the fraction A2 was lower by 22.2 and 21.2%, compared with the first and second cuts. The A1 and A2 fractions of Kentucky bluegrass herbage were lower by 45.1 and 20.1%, and by 31% and 18.1%, respectively, for the second cut compared with the first and third cuts. Similarly, Bakken et al. (2016) reported a higher proportion of the soluble CP fraction of fresh grass-clover forage in the first cut compared with the second cut. The CP soluble fraction for the first harvest in early spring is higher than for the second mid-spring harvest (Keim et al., 2013). In the current research, the crude protein fraction B made up the largest proportion of the CP in white clover and Kentucky bluegrass. The consistent effect of consecutive cuts during the spring growth season on moderately degradable protein (fraction B1) content was not determined. As the spring growth season progressed, there was a significant ($p < 0.05$) increase of CP fractions B2 and C, which coincided with the increased fiber content. In general, Kentucky bluegrass herbage contained a higher proportion of the CP fraction B2 than white clover, and it was likely due to greater values for NDF. The highest contents of B2 and C fractions were found in the second cut, whereas the values for B2 fraction were more than double or by 35.0%, and by 34 or 15.5% higher for clover and grass, respectively, compared with the first and third cut, while C fraction values were more than twofold or by 3.3%, and more than threefold or by 15.7% higher, respectively. These findings are consistent with the study of Keim et al. (2013), where it is reported that the insoluble and potentially degradable fraction of CP (B fraction) of grass-legume herbage (*Lolium perenne* and *Trifolium repens*) was higher for the first cut relative to the second cut. Steg et al. (1994) found that the average rumen undegradable fraction of CP was lower in white clover compared with perennial ryegrass, and that the early cut had a lower undegradable protein fraction than late cuts. According to the same authors, the proportion of ADIN (C fraction) in total N was especially affected by season, whereas the early cut from ryegrass and white clover had lower values than the late cut.

Table 5. The crude protein degradability (RDP, %CP) of *Trifolium repens* and *Poa pratensis* forage from the permanent grassland, for consecutive spring harvests

Cuts	Kp	
	5%/h	8%/h
<i>Trifolium repens</i>		
I	76.79 ^a	68.80 ^a
SEM	0.38	0.41
II	71.38 ^b	63.68 ^b
SEM	0.37	0.43
III	71.15 ^b	63.15 ^b
SEM	0.31	0.39
<i>Poa pratensis</i>		
I	75.63 ^a	67.29 ^a
SEM	0.34	0.36
II	68.75 ^c	60.45 ^c
SEM	0.27	0.36
III	70.78 ^b	62.62 ^b
SEM	0.02	0.11
Legend: Kp – passage rate; SEM – Standard Error of the Mean; ^a , ^b , ^c - Means in the same column with different superscripts differ ($p < 0.05$)		

Lower RDP content ($p < 0.05$) was obtained as the pasture season progressed (Tab. 5). White clover CP degradability was reduced by 7.0 and 7.3% or by 7.4 and 8.2% for the second and third harvest, relative to the first one, when assumed ruminal passage rate (Kp) was 5%/h or 8%/h, respectively. For Kentucky bluegrass herbage, RDP values were reduced by 9.1 and 6.4% or by 10.2 and 6.9%, for the second and third, compared with the first cut, with Kp values of 5%/h or 8%/h, respectively. Lower CP degradability and higher determined RUP in the second and third cut were likely due to a higher relative proportion of CP insoluble fraction (B fraction). Chaves et al. (2006) reported that an increase in RUP content of grasses was probably the result of increased N linked to the cell walls. According to Steg et al. (1994), the proportion of the rumen undegradable fraction in CP is higher for grass than for clover. The crude protein ruminal degradability was lower for the second harvest compared with the first harvest of grass-legume pasture, with higher rumen undegradable protein (RUP) content (27.6 relative to 21.8% CP) (Keim et al., 2013). The lower ruminal protein degradability could be related to climate factors such as temperature, light and rainfall,

whereas the protein degradability is negatively related to higher temperatures (Salaun et al., 1999). The early spring harvested perennial ryegrass had greater proportion of rumen degradable protein and neutral detergent fibre in comparison with cultivars harvested during the summer (Douglas et al., 2020). Tas et al. (2006) found a negative correlation ($R^2 = 0.70$) between the contents of RUP and CP of perennial ryegrass forage, and that higher content of crude protein increased both the proportion of RDP and the Kd-degradation rate of CP.

Table 6. The net energy content (MJ/kg DM) of *Trifolium repens* and *Poa pratensis* herbage from the permanent grassland, for three harvests during the May–June interval

Cuts	NE _L	NEm-rum.	NEg-rum.	NEL-rum.
<i>Trifolium repens</i>				
I	7.61 ^a	9.64 ^a	6.91 ^a	8.06 ^a
SEM	0.09	0.07	0.06	0.06
II	6.31 ^b	7.85 ^c	4.95 ^c	6.56 ^c
SEM	0.01	0.05	0.05	0.04
III	6.41 ^b	8.20 ^b	5.39 ^b	6.85 ^b
SEM	0.09	0.03	0.04	0.02
<i>Poa pratensis</i>				
I	6.53 ^a	8.58 ^a	5.77 ^a	7.17 ^a
SEM	0.05	0.09	0.10	0.07
II	5.21 ^c	6.53 ^b	3.72 ^b	5.46 ^b
SEM	0.08	0.14	0.13	0.11
III	5.68 ^b	6.91 ^b	4.09 ^b	5.77 ^b
SEM	0.07	0.25	0.26	0.21

Legend: NE_L – Net energy lactation for dairy cows (NRC, 2001); NEm-rum., NEg-rum., NEL-rum. – Net energy maintenance, growth and lactation for ruminants, respectively (INRA, Sauvant et al., 2004); SEM – Standard Error of the Mean; ^{a, b, c} - Means in the same column with different superscripts differ ($p < 0.05$)

Significantly higher values ($p < 0.05$) of NE were observed in the white clover and Kentucky bluegrass forage obtained from the first cut, compared with later regrowths (Tab. 6). Higher NE content of the first cut of white clover ranged between 20.6 and 39.6% or 17.6 and 28.2% relative to the second and third cut, respectively, whereas for Kentucky bluegrass, these values ranged between 25.3 and 55.1% or 15 and 41.1%, respectively. The lowest NE contents of the legume and grass were found in the second cut, and in general, white clover was characterized by higher energy content (12.4–33.1%) compared with Kentucky bluegrass. Considering the chemical composition, and determined higher concentration of NDF and ADF in the first and second regrowth, lower energy concentration could be expected, likely due to the reduced DM digestibility. Pelletier et al. (2010) found that DM digestibility was lower in the summer regrowth than the spring growth of grass and legume forage, and this decrease was more prominent in forage grasses than in legumes (up to 19 and 7%, respectively). According to Bakken et al. (2016), the concentration of net energy lactation was higher in the first than in the second cut of grass-clover sward, whereas Steg et al. (1994) reported that clover had a higher content of rumen degradable OM than grass. Dry matter degradability of grass-legume pasture tended to decrease from the first to the second harvest, due to the changes in the cell walls to cell contents ratio (Keim et al., 2013). In addition, Steg et al. (1994) reported increased values of rumen undegradable OM fraction with the season for both grass and clover.

CONCLUSION

The significant effect of consecutive cuts as simulated rotational grazing on white clover and Kentucky bluegrass herbage nutritional value and protein degradability was found during the spring growing season. The CP content was significantly reduced as the season progressed, whereas the increase in the fiber content (NDF and ADF) was observed for different cuts. For both legume and grass forage, a significant decrease in CP fractions A1 and A2 was found with the progress of the vegetation season, while fractions B2 and C were increased. As the growth season progressed, the CP degradability of white clover and Kentucky bluegrass forage significantly declined. The NE content was significantly higher for the first cut, compared with the later regrowths of legume and grass.

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