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YIELD OF CRUDE PROTEIN AND RATE OF ACCUMULATION IN THE DRY MATTER IN A NATURAL GRASS ASSOCIATION USED IN PASTURE AND HAYMAKING REGIME IN THE CONDITIONS OF THE CENTRAL BALKAN MOUNTAIN

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Abstract

The experiment was conducted on a natural meadow type Chrysopogon gryllus - Agrostis capillaris, widespread in the mountainous regions of Bulgaria. Two regimes of use (PR-pasture and HR-haymaking) of grassland in the following variants concerning the harvesting period were studied: PR1 (Control: 31 May - 09 June); PR2 (10 June - 19 June); PR3 (20 June - 29 June) and HR1 (Control: 30 June - 9 July 9); HR2 (10 July - 19 July); HR3 (20 July - 31 July). It was found that the method and period of harvest in the natural mountain association affects the increase in yield and crude protein content. In the pasture and haymaking regime of use, the growth rate of the indicators was the highest, respectively, the highest crude protein production was registered during the harvesting period on 10-19 June (67.42%) and 20-30 July (129.16%), while the highest crude protein content was registered on 20-29 June (55.07%) and 20-30 July (69.95%). The highest increase in dry matter yield was registered in the second decade of June (10-20) for pasture use (14.42%) and at the end of July (20-31) for haymaking (35.77%).

***Key words**: natural grassland, Chrysopogon gryllus - Agrostis capillaris, pasture and haymaking regime of use, crude protein yield.*

INTRODUCTION

Natural plant communities in the mountain regions of Bulgaria are an important feed resource for farm animals. The monitoring of bioproductive potential of natural grasslands related to meadow and pasture ecotypes, which are an essential natural fodder reserve, is directly related to the animal breeding for agriculture. Various measure on the surface are applied such as restorative mowing (Huhta et al., 2001). Mowing at different stages, different ways and systems of fertilizing and regrowth (Cosentino et al., 2002; Kulakov et al., 2015) in these areas stimulate the development of valuable species and increase the beneficial effects on grass vegetation (David et al., 2002). Properly applied management practices, including environmentally friendly ones (Kulakov & Sedova, 2013), as well as combined measures such as lawn cleaning and fertilizing with organic or mineral fertilizers (Kozhouharov & Lingorsky, 2012) are essential and have a direct impact on the chemical composition of grasslands (Popescu and Churkova, 2015; Mrázková et al., 2020).
The careful and purposeful approach among them is a prerequisite for better distribution of components in plant communities and production of feed biomass with high economic value (Vîntu et al., 2011; Fattahi & Ildoromi, 2011; Zziwa et al., 2012). The choice of an appropriate mowing date and the technology for the utilization of the aboveground mass has a beneficial effect on the botanical composition, productivity and longevity of meadow and pasture vegetation (Stevanovıć et al., 2008; Pellegrini et al., 2010).
The sharp reduction in the number and types of perennial meadow grasses requires a purposeful and regulated selection of agrotechnical events for the maintenance and implementation of systems of use. In pasture and haymaking, the time of grazing and mowing affects the yield and variability in the floristic composition of the grassland (Kemp & Michalk, 2005). Knowledge of these patterns aims to create conditions for better development of more

valuable species in different botanical groups (Bovolenta et al., 2008) and increase the quality of fodder biomass. Grass communities respond to changes in the environment both through species turnover and through intraspecific biological and morphological changes (Volf et al., 2016). Species of local origin form highly productive and long-lasting grasslands (Naydenova & Mitev, 2008). The mode of use may be associated with their limited distribution or create conditions for their more stable share in the grassland over the years. Pasture harvesting stimulates lower-growing species (Li et al., 2013), and haymaking of grass cover provides more favorable conditions for the development of high-growing species from the group of grasses and some legumes.

The aim of the experiment was to monitor the rate of accumulation in the crude protein yield, in the natural mountain grass association, in pasture and haymaking regime of use.

MATERIALS AND METHODS

The experiment was conducted in the period 2013-2017, on a meadow of *Chrysopogon gryllus-Agrostis capillaris* type, at the foot of the Central Balkan Mountains (latitude N – 42° 54' 37', longitude E – 24° 41' 31" and 515 m), Bulgaria. The change in crude protein yield was monitored in two main modes of use and three harvest periods (early, medium-early and late with corresponding dates). The design of the selected experimental site covers a total area of 300 m^2 and includes a model by the block method in 4 replications for with a size of the experimental (randomized) plot of 10 m^2.

The experiment includes the following variants:

I. Pasture harvesting (PH):

PH1 - Pasture (Control) from 31st May to 9th June

PH2 - Pasture from 10th June to 19th June

PH3 - Pasture from 20th June to 29th June

II. Hay-making harvesting (HH):

HH1 - Mowing (Control) from 30th June to 9th July

HH2 - Mowing from 10th July to 19th July

HH3 - Mowing from 20th July to 31st July

The periods of pasture and hay-making harvesting were conducted in the phenophase of tasseling/ear formation and in the phenophase of flowering of bunch grasses and common bentgrass until the initial phases of insemination at the edificators of the grass community.

After harvesting in stages at each of the periods/dates of study, the grass samples (each weighing 1 kg) were collected and dried in laboratory conditions at 105°C, and recalculated for an area of 1 ha based on the dry matter content. The crude protein yields were obtained by grass samples, who had been pre-prepared by drying and grounding in an electro-mechanical grinder. The samples were taken from each of the studied variants/harvesting dates.

The crude protein content (CP, %) was determined by the *Kjeldahl* method (according to BDS - ISO-5983).To decompose the organic matter, the sample was boiled with H_2SO_4 in the presence of a catalyst. The acidic solution was alkalified with sodium hydroxide solution (NaOH). The ammonia was distilled and collected in a certain amount of sulfuric acid (H_2SO_4), the excess of which was titrated with a standard solution of sodium hydroxide NaOH. Alternatively, the separated ammonia was distilled off in excess of H_3BO_3 solution and then titrated with HCl or H_2SO_4 solution.

Crude protein yield (kg/ha) was calculated as the product of dry matter yield (kg/ha) and crude protein content in (g/kg).

Botanical changes in grassland (%) were determined by: analysis of fresh grass samples prepared by weight method, taken immediately before mowing the experimental randomized plots, establishing the percentage of the main botanical groups (grasses and legumes) and motley grasses (total).

The experimental area (natural mountain meadow) was used without management, so in the autumn of the third experimental year we applied stockpile combine fertilizing with $P_{120}N_{80}$.

The data processing to track the dynamics of crude protein on two bases (chain and constant) is with the program *StatSoft Inc.* (2010). STATISTICA (*data analysis software system), version 10.* Retrieved from statsoft.com.

Agroclimatic characteristics in the area of the experiment.

The climate in the studied region (The Central Balkan Mountain region) part of the mountain range of the Balkan Mountains is humid continental. During the experimental years, it has an impact on the development of natural grass communities, which manage to form one regrowth of grass mass per year. In 2014, the vegetation and autumn-winter precipitation marked the highest amount (1164.9 mm) compared to the other experimental years, but this did not significantly affect the growth of legumes in the studied grassland (Figure 1). The relative difference in the precipitation amount in 2015 (922.7 mm) and 2017 (983.2 mm), as well as in 2013 (807.3 mm) and 2016 (837.0 mm) compared to the maximum value of the feature varied from 18.5 to 26.2% and from 39.2 up to 44.3%, as the distribution of precipitation was most favorable for sustainable growth of grass species during the last two experimental years (2016 and 2017).

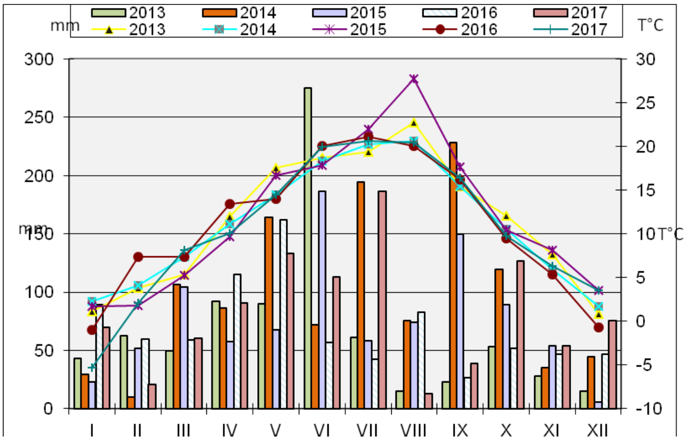

Figure 1. Average monthly temperatures (°C) and monthly precipitation amounts (mm) of 2013-2017

For the experimental period, in 2015 were registered the highest average air temperature (11.9°C) and the highest average temperature for July, August and September (22.4°C), which did not have a significant impact on the productivity of the plant community. The relative difference in average air temperatures for 2013 (11.4°C), 2014 (11.0°C) and 2016 (11.1°C) varied from 0.9 to 3.6%. The temperature regime data show that the lowest average annual temperature (10.5°C) was in 2017, which in turn favorably affects the growth and development of more unstable protein component in grass communities (legumes stimulate their growth more intensively in combination from lower air temperature and higher humidity in late spring and summer months). The study of the data shows that in the fifth experimental year, the active vegetation of plants in the grasslands begins at the highest average temperature in March (8.1°C) compared to other experimental years and this in combination with abundance and distribution of precipitation provoked good quality of grass vegetation.

RESULTS AND DISCUSSIONS

Yield and increase rate in the amount of crude protein.

Crude protein yield is an aggregate indicator important in assessing natural grass associations and systems to improve and maintain them. It combines the two main indicators of productivity and quality of biomass produced, mainly through the amount of crude protein produced, which is very important for animal husbandry and determines the quality of grass biomass.

On average over the five-year experimental period, haymaking yields resulted in a higher yield of crude protein (278.0 kg/ha) compared to pasture (240.7 kg/ha) (Table 1). Among the haymaking variants, the most productive is the one with harvesting/mowing in the period from 10-19 July (323.0 kg/ha). In pasture use, the highest yield of crude protein (287.0 kg/ha) was obtained in the variant with the latest harvesting of grassland in the period of 20th June - 29th June.

Table 1. Yield of crude protein (kg/ha) in pasture and hay-making harvesting, over the years and average for the period

Variants	2013	2014	2015	2016	2017	2013-2017
PH1	192.2	78.3	82.1	144.6	341.3	159.3
PH2	176.0	194.9	218.2	269.8	496.1	275.8
PH3	201.8	170.8	268.5	314.9	442.7	287.0
Average	**190.0**	**148.0**	**189.6**	**243.1**	**426.7**	**240.7**
HH1	172.4	120.4	135.8	524.5	323.0	259.0
HH2	278.4	222.9	203.1	462.6	436.9	323.3
HH3	125.5	305.4	221.8	424.8	198.2	251.6
Average	**192.1**	**216.2**	**186.9**	**470.6**	**319.4**	**278.0**

The maximum amount of crude protein for the first year was found in the second period (HH2) of hay-making harvest with 278.4 kg/ha, while the lowest yield of 125.5 kg/ha was obtained in the third hay-making period (HH3). In the pasture regime, the lowest yields were obtained in the second year of the study (2014) with 78.3 kg/ha in variant PH1, as the average for the year of the three terms of pasture harvesting was 148 kg/ha.

In the third experimental year (2015), the most significant quantitative accumulation of crude protein was in the third period of pasture mowing (PH3) – 268.5 kg/ha. The first period of pasture harvesting (PH1) had the slightest accumulation rate with 82.1 kg/ha. The average annual yield of crude protein in the third experimental year (189.6 kg/ha) obtained during the pasture harvesting periods (PH1, PH2 and PH3) was 1.4% higher than the hay harvesting periods (186.9 kg/ha).

A sharp positive reversal in the dynamics of the amount of crude protein (CP) was obtained in the fourth experimental year (2016). The quantitative accumulation of protein was significant in both pasture and hay-making periods of mowing. The results show that the peak of the quantitative indicator during the first three periods for pasture harvesting reached 314.9 kg/ha, obtained in the time frame of harvesting 20.06-29.06 (PH3).

Table 2. Increase rate (%) on the amount of crude protein (on a constant basis), by years and average for the period

Variants	2014	2015	2016	2017	**Average increase rate**
PH1	-59.23	-57.27	-24.75	77.63	-15.91
PH2	10.69	23.94	53.27	181.78	67.42
PH3	-15.34	33.05	56.06	119.38	48.29
Average	**-22.10**	**-0.21**	**27.95**	**124.58**	**32.56**
HH1	-30.17	-21.20	204.25	87.38	60.07
HH2	-19.94	-27.04	66.15	56.95	19.03
HH3	143.37	76.76	238.55	57.97	129.16
Average	**12.56**	**-2.69**	**145.00**	**66.27**	**55.29**

This is confirmed by the relative values in the increase of crude protein calculated on a constant basis (model tracking the dynamics of change in relative values of crude protein in subsequent experimental years compared to the first year), where according to the values of the attribute the indicated variant (PH3) exceeded the first year by 56.06% and 17.30% in a direct comparison on the model year compared to year (chain basis). On average for the fourth year of the pasture harvesting period, the quantitative indicator (crude protein yield) registered a positive difference on a constant basis compared to the first year by 27.95%, while on a chain basis the increase reached 28.23% (Tables 2 and 3). The upward trend continues with regard to the periods of hay-making (HH1, HH2), where crude protein yields reached maximum values (HH1 on June 30-July 9) with 524.5 kg/ha and the increase on a constant basis was 204.25% compared to the first year, and on a chain basis with 286.09%.

On average for the fourth year, the increase of constant and chain bases was from 145.0% to 151.77%.

In the last (fifth) experimental year (2017) the tendency for progressive increase of crude protein yields compared to the previous one was preserved. The yield of crude protein was 33.6% higher in the variants with pasture harvesting than hay harvesting. The average yield for the year was 426.7 kg/ha, as the largest increase (496.1 kg/ha) of the indicator was measured in the second period of pasture harvesting (PH2 - 10.06. - 19.06.). The increase in crude protein yield on a constant basis was 181.78%, and on a chain basis it was 83.4%. In the haymaking regimes, the most significant increase rate of crude protein was in the first hay-making regime (HH1 - June 30-July 9). On a constant basis, the increase rate was 87.38%. The values are negative (-38.41%) compared to the previous year when calculating the chain basis.

Table 3. Increase rate (%) of the amount of crude protein (on a chain basis), over the years and average for the period

Variants	2014	2015	2016	2017	**Average increase rate**
PH1	-59.23	4.81	76.11	136.07	39.44
PH2	10.69	11.97	23.67	83.84	32.54
PH3	-15.34	57.15	17.30	40.58	24.92
Average	-22.10	28.09	28.23	75.52	27.43
HH1	-30.17	12.86	286.09	-38.41	57.59
HH2	-19.94	-8.87	127.72	-5.54	23.34
HH3	143.37	-27.37	91.53	-53.34	38.55
Average	12.56	-13.55	151.77	-32.13	29.66

During the study period, the increase in crude protein yield, calculated on a constant basis, marked significant differences depending on the harvesting method. The average value of the indicator for the variants of pasture harvesting (32.56%) is a positive value, lower by 69.8% compared to hay-making of grasslands (Table 4). For PH1 (May 31-June 9) the average value is negative (-15.91). The highest increase rate of yield was in PH2 (June 10-June 19) (67.42%).

The results obtained on a chain basis are completely opposite (Table 3), where the average value of the indicator in the variants with pasture harvesting is negative (-22.10), and in hay-making harvesting is positive (12.56). The reason for this is the negative values (-59.23 and (-15.34) of the indicator for the pasture variants at the beginning (PH1 - 31 May - 9 June) and at the end of June (PH3 - 20 June - 29 June).

Regarding the crude protein content, the average value (27.30%) calculated on a constant basis was lower compared to the protein yield (32.56%), where in PH1 increase values are negative (-9.80%).

The percentage increase in protein on a chain basis (23.81%) was significantly higher compared to the negative value of protein yield (-22.10).

In terms of dry matter yield, the increase calculated on a constant basis (0.79%) and on a chain basis (15.65%) is less significant compared to the increase in protein content and protein yield.

In **hay-making harvesting** the average increase in protein yield (55.29%) was higher by 22.73% compared to pasture harvesting. The values for all variants are positive as the highest values were registered in the grasslands of variant HH3 (July 20-July 31). This is significantly influenced by the higher increase in crude protein content in the specific harvesting regime.

The increase in the dry matter yield in hay-making harvesting, calculated on a constant basis with 13.76%, was significantly higher compared to that in pasture harvesting. The results obtained on a chain basis are completely opposite, where the values in the increase of the dry matter yield are about 2 times lower. There was a proven accumulation of crude protein in a direct comparison (each against each other) of the experimental years.

In conclusion, the comparison in the increase of indicators, such as crude protein yield, crude protein content and dry matter yield shows that during hay-making the increase in the values of these quality traits is significantly higher compared to pasture harvesting.

The higher increase in crude protein yield is due to the higher increase in crude protein content in biomass, determined by the higher share of legume grass species in the botanical composition of the grassland.

Table 4. Average increase (%) of crude protein yield, crude protein content and dry matter yield, as a percentage of constant basis (compared to the first year) and chain basis (compared to previous years)

Variants	Crude protein yield		Crude protein content		Dry matter yield	
	Constant basis	Chain basis	Constant basis	Chain basis	Constant basis	Chain basis
Pasture harvesting						
PH1	-15/91	-59.23	-9.80	20.01	-12.15	18.64
PH2	67.42	10.69	50.55	29.30	14.42	18.36
PH3	48.29	-15.34	55.07	26.23	-1.60	14.95
Average	**32.56**	**-22.10**	**27.30**	**23.81**	**0.79**	**15.65**
Hay-making harvesting						
HH1	60.07	-30.17	56.15	65.78	4.62	8.57
HH2	19.03	-19.94	12.94	24.48	6.55	8.20
HH3	129.16	143.37	69.95	20.89	35.77	8.91
Average	**55.29**	**12.56**	**42.63**	**31.03**	**13.76**	**6.63**

Significantly lower increase in dry matter yield shows that the yield of crude protein (as a total indicator combining productivity and quality indicators) is more significantly influenced by quality indicators, such as crude protein content and the share of legume species in the established grasslands.
The data in Figures 2 and 3 follow the increase dynamics of the legume component. Legume species significantly dominate the botanical composition of the final experimental years (2016 and 2017), both in pasture and hay-making harvesting periods. In the first year of pasture harvesting (for PH1, PH2 and PH3) the share of legumes was respectively 2.0%, 0.0%, 0.0%, while in the second 0.0%, 6.7% and 5.4%. In the fourth experimental year (2016), the amount of legumes increased to 22.6%, 44.8% and 45.0%, respectively. These values of legumes, to some extent, explain the sharp rise in the amount of crude protein.

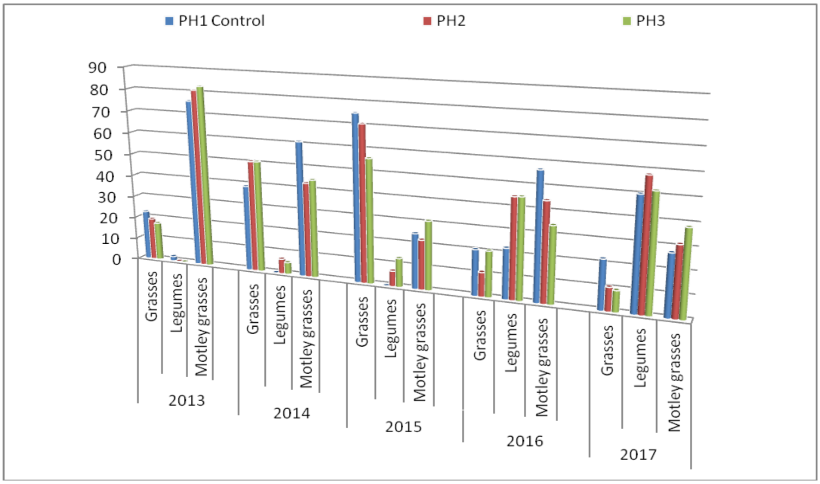

Figure 2. Botanical composition over the years and main plant groups on natural grassland of *Chrysopogon gryllus* L.-*Agrostis capillaris* L. type in pasture harvesting periods

The high content of legumes in the grass mass is directly related to the high content of crude protein (Nilsdotter-Linde et al., 2016; Kovtun et al., 2020) and minerals (P, K, Ca, Mg) in the forage mass.

In the first three experimental years, grasses dominated both in pastures and hayfields. After additional fertilizing (combined mineral fertilizing), the increase, quantity and species diversity of legumes increased and affected the content of crude protein in biomass.

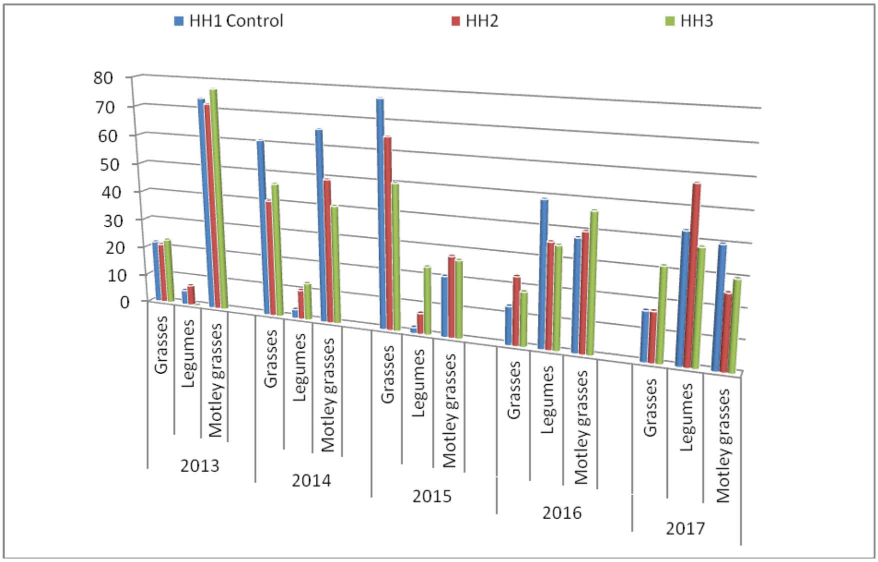

Figure 3. Botanical composition over the years and main plant groups on natural grassland of *Chrysopogon gryllus* L.-*Agrostis capillaris* L. type in pasture harvesting periods

A similar increase rate can be observed in the hay-making regime (Figure 3), where in the periods (HH1, HH2 and HH3) the content of legumes in the first year was 5%, 7.0% and 0.0%, respectively. After the applied agrotechnical events and regimes of use, the increase of the protein component (the group of legumes) in the last year of the experiment (2017) was 43.1%, 57.7% and 38.5%, respectively. The third hay-making variant (HH3 from July 20 to July 31) is particularly impressive, where the most harmonious increase in the relative amount of legumes was found throughout the experimental period. For this variant, the results in the years are: 2013 (0.0%), 2014 (12.9%), 2015 (23.3%), 2016 (34.8%) and 2017 (38.5%). Legumes significantly increase the quality of grass biomass.

CONCLUSIONS

It was found that the method and period of harvesting in the natural mountain association affected the increase in **the crude protein yield**. The value of the indicator in pasture harvesting (32.56%) is a positive value (on a constant basis) and lower by 69.8% in hay-making. On a chain basis, the production of CP, in biomass on 31.May-09.June (-59.23) and 20.June-29. June (-15.34) was negative, which determines the low value of the indicator (-22.10%) in pasture harvesting. Hay-making harvesting of grasslands (in the period July 20-July 31) had a positive effect on the increase in the yield of CP (12.56%).

The increase in **the content of CP** in the regimes of use on a constant basis is respectively 19.3% (27.30-IP) and 29.7% (42.63-CP) lower than that of CP yield. The rate of increase in the content of CP in pasture harvesting, on a chain basis (23.81%) is significantly higher compared to protein yield. The hay-making regime allows a more balanced ratio in the values of the indicators of yield and content of CP (12.56: 31.03).

In pasture harvesting of the grassland, the increase in **dry matter yield** established on a constant basis (0.79%) is significantly lower compared to haymaking (13.76%). The opposite trend is observed when established on a chain basis. The values of the indicator are higher in pasture (15.65%) compared to hay-making harvesting of grassland.

The increase in the studied indicators shows that the increase in crude protein yield during hay-making harvesting is significantly higher compared to pasture harvesting. The increased share of legumes is a prerequisite for higher protein content and higher quality biomass.

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