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Chemical composition, mineral content, "*in vitro*" gas production and relative feed value of *Betonica bulgarica* Degen et Neič.

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Abstract

Gerdzhikova, M., Pavlov, D., Grozeva, N., Mladenova, Ts., Krastanov, J. & Angelova, T. (2020) Chemical composition, mineral content, "*in vitro*" gas production and relative feed value of *Betonica bulgarica* Degen et Neič. *Bulg. J. Agric. Sci., 26 (Suppl. 1), 48-57*

Chemical composition and mineral content of biomass from four populations of *Betonica bulgarica* Degen et Neič. were determined. The average crude protein content in the biomass was 77.41 g.kg^{-1} of dry matter (DM); crude fat – 7.77 g.kg^{-1} DM; crude fibre – 260.16 g.kg^{-1} DM; ash – 50.47 g.kg^{-1} DM and nitrogen free extracts (NFE) – 604.19 g.kg^{-1} DM. The content of nitrogen, calcium, phosphorus and magnesium in the biomass of *B. bulgarica* was close to the meadow grasses. The content of potassium is lower. The content of sodium, iron and manganese was significantly lower. Structural fibre components were an average for neutral detergent fibre (NDF) 44.82 % and acid detergent fibre (ADF) 39.93 % close to that of alfalfa and legume grasses. "*In vitro*" gas production of *B. bulgarica* biomass at 24 hour was average 257.55 dm.ml^{-1} and at the 48 hour – 270.35 dm.ml^{-1} (CO_2 and CH_4), which is close to the group of legume and cereal meadow grasses. The relative feed value (RFV) of *B. bulgarica* biomass is close to perennial legumes and exceeded alfalfa (*Medicago sativa* L.) with 6 to 33 %. Regression equations were developed for advanced determining the quantity of gas production at 24 and 48 hours through the metabolizable energy (ME), MJ.kg^{-1} DM and the Relative feed value through the neutral detergent fibre.

Keywords: *Betonica bulgarica;* chemical composition; mineral content; detergent fibre; "*in vitro*" gas production; relative feed value

Introduction

Betonica bulgarica formed populations of open terrain in oak belt on a grey, brown forest and humic carbonate soils. Up to day, the type was mostly interesting for floristic studies. Data for his propagation in recent decades is published by Koeva (1970), Bondev et al. (1995), Grozeva et al. (2004), Nikolov et al. (2007), Genova (2011). Assessment of the status of its populations in the Eastern Balkan Range and its requirements to the soil conditions are published by Grozeva et al. (2014). Experiments were made for cultivation of the species in the laboratory (Panayotova et al., 2014, 2015). The morphological variability was determined (Grozeva et al., 2016). In the literature there is not enough data on chemical, mineral composition and feeding value in *B. bulgarica* biomass.

The objective of this study was to determine the chemical composition, digestibility, "*in vitro*" gas production and relative feed value of *B. bulgarica* as a component of grassland ecosystems.

Material and Methods

Aerial parts of *B. bulgarica* were collected from four populations on the territory of Sinite kamani Natural park as follows: Ablanovo – N 42° 42.638′, E 26° 17.262′, 540 m a.s.l.; Upper lift station – N 42° 43.100′, E 26° 21.619′, 1015 m a.s.l.; Microyazovir – N 42° 42.852′, E 26° 22.654′, 945 m a.s.l.; Slancheva polyana N 42° 43.252′, E 26° 21.668′, 1001 m a.s.l.

Chemical composition of *B. bulgarica* above ground biomass was determined by Weende method (AOAC, 1984). The nitrogen content was determined by Keldahl; phosphorus – by colorimetry; potassium and sodium – by flame photometry; calcium, magnesium, manganese, iron and copper with Atomic absorption spectrophotometer Perkin Elmer ANALYST-800 AA SPECTROMETER.

The analysis of the NDF and ADF were carried out through Fiber, Analizer 2000 Ankom, USA. Digestibility was determined using the "*in vitro*" analysis under Spanghero et al. (2010). Neutral detergent fibre (NDF), % and Acid detergent fibre (ADF), % were determined by Ankom F57 filter bags (ANKOM Technology Corporation, Fairport, NY, USA). All samples were incubated by Ankom F57 filter bags (ANKOM Technology Corporation, Fairport, NY, USA). Each sample contains the feed and rumen liquid content of fistulated animals (cows) fed with ration consisting of the investigated feeds. The quantity of gas production (CO_2 and CH_4) in different feed sources was defined in the methodology for conducting analyses with Gas Production System/Ankom ® RF ANCOM Tech Co., Fairport, NY, USA at 24 and 48 hours.

Gross energy value (GE), MJ.kg^{-1} DM was determined on the base of the chemical composition and equations for energetic value of feeds by Todorov et al. (2007). Metabolizable energy (ME), MJ.kg^{-1} DM was determined by the methodology of ANKOM through stomach – intestinal gas production system. By the ratio ME/GE, factor (q) was calculated, used additionally for calculation of Feed units for milk (FUM/kg DM) by equation: FUM=GE.(0.075+0.039.q) and Feed units for growth (FUG/kg DM) by equation FUG=GE.(0.0382+0.104.Q). Net energy for lactation, described as NE, MJ.kg^{-1} DM was calculated by equation NE=FUM.6 by Todorov et al. (2007).

The relative feed value (RFV) was defined by Undersander & Moore (2002); Jeranyama & Garcia (2004); Stallings (2006); Boga et al. (2014) by the formula:

- RFV (relative feed value) = (DMI. % DDM) / 1.29.
- DMI (dry mater intake) = 120 / (% NDF).
- DDM (digestible dry mater) = 88.9 - (0.779. % ADF).

Results

Mineral content

The results, reflecting the content of trace elements in the dry matter of *B. bulgarica* above ground biomass indicate that the nitrogen content ranges from 10.79 g.kg^{-1} DM to 13.91 g.kg^{-1} DM, the average 12.39 g.kg^{-1} DM (Table 1). Higher levels were found in plants from the populations in Slancheva polyana and Upper lift station. The content of calcium in *B. bulgarica* was average 7.64 g.kg^{-1} DM. A little higher was in plants from Upper lift station and Microyazovir, but did not find significant differences between populations. Above ground biomass of *B. bulgarica* contains phosphorus in small quantities – from 1.1 to 2.0 g.kg^{-1} DM, an average of 1.85 g.kg^{-1} DM. Potassium values were an average of 12.35 g.kg^{-1} DM. The highest content of potassium was in the plants from the population in Upper lift station – 13.01 g.kg^{-1}, and the lowest in Slancheva polyana – 10.97 g.kg^{-1} DM. The sodium content was on average 0.093 g.kg^{-1} DM. With higher sodium content was the biomass of plants from the population in Slancheva polyana. The content of magnesium was averaging 3.52 g.kg^{-1} DM. The values are similar in the different populations. The variation between different populations was from 3.17 to 3.96 g.kg^{-1} DM.

Table 1. Mineral content of *B. bulgarica*, g.kg^{-1} DM

Populations	N	Ca	P	K	Na	Mg
Ablanovo	11.20	7.46	2.00	12.79	0.093	3.17
Upper lift station	13.65	7.87	1.80	13.01	0.088	3.47
Microyazovir	10.79	7.71	1.90	12.62	0.091	3.96
Slancheva polyana	13.91	7.52	1.70	10.97	0.099	3.46
Average	12.39	7.64	1.85	12.35	0.093	3.52

Analysis on the content of trace elements indicates that the biomass of *B. bulgarica* contains average iron - 58.02 mg.kg^{-1} DM; manganese - 53.09 mg.kg^{-1} DM and copper - 7.73 mg.kg^{-1} (Table 2).

Table 2. Mineral content of *B. bulgarica*, mg.kg^{-1} DM, trace elements

Populations (Location)	Fe	Mn	Cu
Ablanovo	55.15	49.38	7.74
Upper lift station	56.36	51.06	7.86
Microyazovir	58.78	54.41	7.80
Slancheva polyana	61.79	57.52	7.51
Average	58.02	53.09	7.73

Chemical composition

B. bulgarica above ground biomass contains crude protein 67.41 g.kg^{-1} to 86.91 g.kg^{-1}, average 77.41 g.kg^{-1} DM (Table 3). A higher content was found in plants from the population in Slancheva polyana, and the lowest – in those of the population in Microyazovir.

Table 3. Chemical composition of *B. bulgarica*, above ground biomass, g.kg^{-1} DM

Populations (Location)	Crude protein	Crude fat	Crude fibre	Ash	NFE
Ablanovo	70.00	7.75	254.47	46.09	621.69
Upper lift station	85.32	8.41	242.69	55.23	608.35
Microyazovir	67.41	7.20	271.69	45.69	608.00
Slancheva polyana	86.91	7.72	271.78	54.87	578.72
Average	77.41	7.77	260.16	50.47	604.19

The content of crude fat in the dry matter of the biomass was average 7.77 g.kg^{-1} DM, with variation from 7.20 to plants from the population Microyazovir and to 8.41 g.kg^{-1} – for those of the population in Upper lift station.

The contents of crude fibre in *B. bulgarica* ranged from 242.69 to 271.78 g.kg^{-1} DM. The average for the four populations was 260.16 g.kg^{-1} DM. Higher levels were found from plants of the populations in Slancheva polyana and Microyazovir. Mineral substances (ashes) in the biomass of *B. bulgarica* vary from 45.69 to 55.23 g.kg^{-1} DM. On average, for the studied populations, their content was 50.47 g.kg^{-1} DM. Nitrogen free extracts (NFE) occupy the highest amount from all substances in the biomass of *B. bulgarica.* Average content was 604.19 g.kg^{-1} DM. Between populations from different areas small differences were observed – from 578.72 to 621.69 g.kg^{-1} DM.

Fibre composition, digestibility, dry mater intake, energy value, relative feed value and "in vitro" gas production

In dried condition the biomass of *B. bulgarica* has normal color and smell similar to those of all meadow plants. The quantity of neutral detergent fibre (NDF) averaged 44.82% (Table 4). With lower values was the biomass of *B. bulgarica* of the population in Microyazovir – 41.04% and higher is biomass of the population in Slancheva polyana – 49.72%. Acid detergent fibre (ADF) was an average 39.93%, with variation of 38.46 to 41.27 %. In this index the lowest and highest values are established in the same populations as well as the NDF. The ratio of the NDF to ADF averaged 1.12 %. The greatest one was in plants from the population in the Slancheva polyana – 1.20%, and the lowest in plants of the populations in Ablanovo and Microyazovir – 1.07 %.

Table 4. Content of fibre components, digestibility, dry mater intake, energy value, relative feed value and "*in vitro*" gas production of *B. bulgarica*

Parameters	Ablanovo	Upper lift station	Micro yazovir	Slanche va polyana	Average	*Medicago sativa* L. (standard)
NDF, %	43.47	45.04	41.04	49.72	44.82	53.00
ADF, %	40.54	39.45	38.46	41.27	39.93	41.00
NDF, %/ ADF, %	1.07	1.14	1.07	1.20	1.12	1.29
DDM, %	57.32	58.17	58.94	56.75	57.79	56.96
DMI, g.kg^{-1}	2.76	2.66	2.92	2.41	2.68	2.26
GE, MJ.kg^{-1} DM	17.86	17.79	17.91	17.90	17.87	18.36
ME, MJ.kg^{-1} DM	12.32	11.34	9.31	10.76	10.93	9.30
NE, MJ.kg^{-1} DM	7.53	6.79	5.32	6.36	6.50	5.28
FUM	1.26	1.13	0.89	1.06	1.08	0.88
FUG	1.35	1.19	0.86	1.08	1.12	0.86
RFV	122.7	120.2	133.6	106.2	120.0	100.0
GP 24 h, dm.ml^{-1}	283.77	279.63	206.83	259.97	257.55	
GP 48 h, dm.ml^{-1}	313.21	284.52	210.24	273.44	270.35	

Total digestibility of biomass averaged 57.79% with fluctuation of 56.75 to 58.94%. With the highest digestibility was the biomass from the population in Microyazovir, and the lowest was that of the population in Slancheva polyana. Feed consumption (DMI) averaged 2.68 $g.kg^{-1}$ live weight. With a higher DMI value was the biomass from Microyazovir, while in the other three populations the values are much closed. Gross energy (GE), $MJ.kg^{-1}$ DM from biomass averaged 17.87 $MJ.kg^{-1}$ DM. The difference in energy value of biomass in areas are small and non-essential. The metabolizable energy (ME), $MJ.kg^{-1}$ DM, of *B. bulgarica* biomass, measured by Ankom biogas technology average 10.93 $MJ.kg^{-1}$ DM. A bit more is the energy value of the biomass from the population in Ablanovo – 12.32 $MJ.kg^{-1}$, while the smallest was to that of the population in Microyazovir – 9.31 $MJ.kg^{-1}$ DM. Net energy (NE), $MJ.kg^{-1}$ DM averaged 6.50, with variation from 5.32 to 7.53. Feeding value calculated as Feed units for milk (FUM) was on average 1.08 and Feed units for growth (FUG) was 1.12. These values are a bit bigger for plants in Ablanovo and smaller in those from Microyazovir. The relative feed value (RFV) of *B. bulgarica* biomass was average 120, calculated on the basis of the biomass of alfalfa (*Medicago sativa* L.) in flowering phase accepted for standard - 100 with content of ADF – 41 % and NDF 53 % (Jeranyama & Garcia, 2004). Related feed value was higher of the population in Microyazovir, and the lowest from the population in Slancheva polyana. Similar results for RFV-120.1 with ADF 40.5 % and NDF 44.5 % are derived from the *Lotus corniculatus* L. by Boga et al. (2014). This shows that the relative feed value of *B. bulgarica* is similar to other perennial herbaceous legume crops.

Comparison among *B. bulgarica* and *Medicago sativa* in flowering stage adopted for standard shows that the biomass of *B. bulgarica* contains less NDF and ADF. DDM is almost the same, but the consumption DMI is bigger. GE, $MJ.kg^{-1}$ DM is almost the same, but ME, $MJ.kg^{-1}$ DM; NE, $MJ.kg^{-1}$ DM; FUM and FUG of *B. bulgarica* was a little higher, which presumably define a good nutritional value as a geed of good quality. Investigation influence of fibre composition on the rate of degradability established by “*in vitro*” gas production is modern and very important way for estimation feeding value, widely used of many authors for exact evaluation of different forages groups and precise determination of the final effect of feed application (Menke et al., 1979; Menke & Steingass, 1988; Blümmel & Becker, 1997; Getachew et al., 2004).

The gas production (GP) after incubation of biomass at 24 hours reported by the method of “*in vitro*” gas production was an average 257.55 $dm.ml^{-1}$, with fluctuations of 206.83 $dm.ml^{-1}$ up to 283.77 $dm.ml^{-1}$. More gas was produced from the biomass of *B. bulgarica* from the population in Ablanovo and less in Microyazovir. The total quantity of evolved gas at 48 hours is an average 270.55 $dm.ml^{-1}$. Variation here is from 210.24 to 313.21 $dm.ml^{-1}$, with the largest amount obtained from the population in Ablanovo, and lower from the population in Microyazovir.

Table 5. Specific indices related to approximate determination of gas production

Parameters	Ablanovo	Upper lift station	Microyazovir	Slancheva polyana	Average
Specific GP (GP 48 h, $dm.ml^{-1}$/48 h)	6.53	5.93	4.38	5.70	5.63
Speed of degradation and gas formation (GP 48 h, $dm.ml^{-1}$/ GP 24 h, $dm.ml^{-1}$), %	110.37	101.75	101.65	105.18	104.97
GP 48 h, $dm.ml^{-1}$/ ADF %	7.73	7.21	5.47	6.63	6.77
GP 48 h, $dm.ml^{-1}$/ NDF %	7.21	6.32	5.12	5.50	6.03
GP 48 h, $dm.ml^{-1}$/ DDM, %	5.46	4.89	3.57	4.82	4.69
GP 48 h, $dm.ml^{-1}$/ ME, $MJ.kg^{-1}$ DM	25.42	25.09	22.58	25.41	24.73
GP 48 h, $dm.ml^{-1}$/RFV	2.55	2.37	1.57	2.58	2.25

Specific indices in gas production

The specific gas production from *B. bulgarica* determined at 48 hour was average 5.63 $dm.ml^{-1}$ for 1 hour (Table 5). The speed of gas formation (the ratio of the quantity of gas formations in 48 hours to 24 hours) is the average 104.97 %.

Gas production in 48 hours was 6.77 $dm.ml^{-1}$ to 1% ADF and 6.03 $dm.ml^{-1}$ to 1% NDF; 4.69 $dm.ml^{-1}$ to 1% digestible dry matter; 24.73 $dm.ml^{-1}$ to 1 $MJ.kg^{-1}$ DM metabolizable energy and 2.25 $dm.ml^{-1}$ to 1 unit of relative feed value.

Correlation and regression dependences

Comparison among the results obtained for the composition of *B. bulgarica* biomass demonstrates that between chemical composition, mineral content, nutritional value and morphological parameters correlation dependencies exists (Table 6).

Table 6. Correlations among the parameters

Parameters	Protein g. kg^{-1}	Ash, g. kg^{-1}	NDF %	ADF,%	DDM, %	DMI, g. kg^{-1}	Gas 24 h, dm ml^{-1}	Gas 48 h, dm ml^{-1}	ME, MJ. kg^{-1}	RFV	Height, cm	Leaf area, cm^2	N,g. kg^{-1}	K,g ,kg^{-1}	Na, g kg^{-1}	Mg, g. kg^{-1}	Mn mg. kg^{-1}	Cu, mg. kg^{-1}	Fe, mg. kg^{-1}
Protein, g.kg^{-1}	**1.00**																		
Ash, g.kg^{-1}	**0.99**	**1.00**																	
NDF, %	**0.86**	**0.80**	**1.00**																
ADF, %	**0.51**	**0.41**	**0.83**	**1.00**															
DDM, %	**-0.51**	**-0.41**	**-0.83**	**-0.99**	**1.00**														
DMI, g.kg^{-1}	**-0.88**	**-0.82**	**-0.99**	**-0.83**	**0.83**	**1.00**													
GP 24 h, dm.ml^{-1}	**0.48**	**0.43**	**0.44**	**0.64**	**-0.64**	**-0.50**	**1.00**												
GP 48 h, dm.ml^{-1}	**0.32**	**0.26**	**0.39**	**0.70**	**-0.70**	**-0.44**	**0.97**	**1.00**											
ME, MJ.kg^{-1}	**0.20**	**0.14**	**0.25**	**0.60**	**-0.60**	**-0.30**	**0.95**	**0.99**	**1.00**										
RFV	**-0.83**	**-0.76**	**-0.99**	**-0.89**	**0.89**	**0.99**	**-0.54**	**-0.51**	**-0.37**	**1.00**									
Height, cm	**-0.14**	**-0.18**	**-0.11**	**0.33**	**-0.33**	**0.06**	**0.80**	**0.87**	**0.93**	**-0.02**	**1.00**								
Leaf area, cm^2	**-0.38**	**-0.45**	**-0.17**	**0.39**	**-0.39**	**0.14**	**0.60**	**0.75**	**0.81**	**0.04**	**0.92**	**1.00**							
N, g.kg^{-1}	**1.00**	**0.99**	**0.86**	**0.51**	**-0.51**	**-0.88**	**0.47**	**0.32**	**0.20**	**-0.83**	**-0.14**	**-0.38**	**1.00**						
K, g.kg^{-1}	**-0.49**	**-0.42**	**-0.80**	**-0.67**	**0.67**	**0.77**	**0.09**	**0.06**	**0.19**	**0.76**	**0.48**	**0.32**	**-0.49**	**1.00**					
Na, g.kg^{-1}	**0.27**	**0.16**	**0.70**	**0.78**	**-0.78**	**-0.67**	**0.01**	**0.11**	**0.01**	**-0.70**	**-0.22**	**0.02**	**0.27**	**-0.93**	**1.00**				
Mg, g.kg^{-1}	**-0.28**	**-0.21**	**-0.41**	**-0.76**	**0.76**	**0.46**	**-0.94**	**-0.99**	**-0.98**	**0.53**	**-0.85**	**-0.78**	**-0.28**	**0.02**	**-0.22**	**1.00**			
Mn, mg.kg^{-1}	**0.37**	**0.34**	**0.54**	**0.20**	**-0.20**	**-0.49**	**-0.52**	**-0.54**	**-0.65**	**-0.44**	**-0.86**	**-0.71**	**0.37**	**-0.86**	**0.68**	**0.48**	**1.00**		
Cu, mg.kg^{-1}	**-0.39**	**-0.30**	**-0.79**	**-0.80**	**0.80**	**0.76**	**-0.06**	**-0.13**	**-0.02**	**0.78**	**0.25**	**0.05**	**-0.40**	**0.96**	**-0.99**	**0.23**	**-0.71**	**1.00**	
Fe, mg.kg^{-1}	**0.41**	**0.38**	**0.59**	**0.27**	**-0.27**	**-0.55**	**-0.45**	**-0.47**	**-0.59**	**-0.50**	**-0.82**	**-0.68**	**0.41**	**-0.90**	**0.72**	**0.41**	**0.99**	**-0.76**	**1.00**

DDM correlates negatively very well with ADF (r=-0.99). DMI and RFV also correlate negatively with NDF (r= -0.99). Gas production at 24 and 48 h correlates positively with metabolizable energy (r=0.95, r=0.99).

On the basis of correlation regression equations were developed for advance approximate determination of the quantity of gas formations and the relative feed value (Table 7). Gas production at 24 h and 48 h could be estimated by metabolizable energy as independent variable. Relative feed value could be predicted by Neutral detergent fibre with linear equation. Coefficient of determination R = 0.952-0.999 is enough high and equations are statistically significant at P<0.05-0.01.

Table 7. Regression equations

Parameters	R*	SEE	F	P<
Gas production 24 h, $dm.ml^{-1}$				
Y=-35.12+26.77 ME	0.952	6.0	19.6	0.047
Gas production 48 h, $dm.ml^{-1}$				
Y=-102.778+34.130 ME	0.999	3.6	89.8	0.0109
Relative Feed Value				
Y=257.5012-3.0537 NDF	0.988	0.29	111.393	0.00886

*R – coefficient of determination, SEE – standard error, F – ratio, P< – statistical significance. ME – metabolizable energy, $MJ.kg^{-1}$ DM, NDF – neutral detergent fibre, %

Principle component analyses and distribution of populations and observed parameters

The Principal component analysis for the distribution of *B. bulgarica* populations shows that the population in Microyazovir differ the strongest of the three other populations and was negative on both factors (Fig. 1). The population in Slancheva polyana is positive on the first factor, describing 46.45% of variations. The populations in Ablanovo and Upper lift station are positive on the second factor, describing 35.24% of variations, but were negative in the first factor.

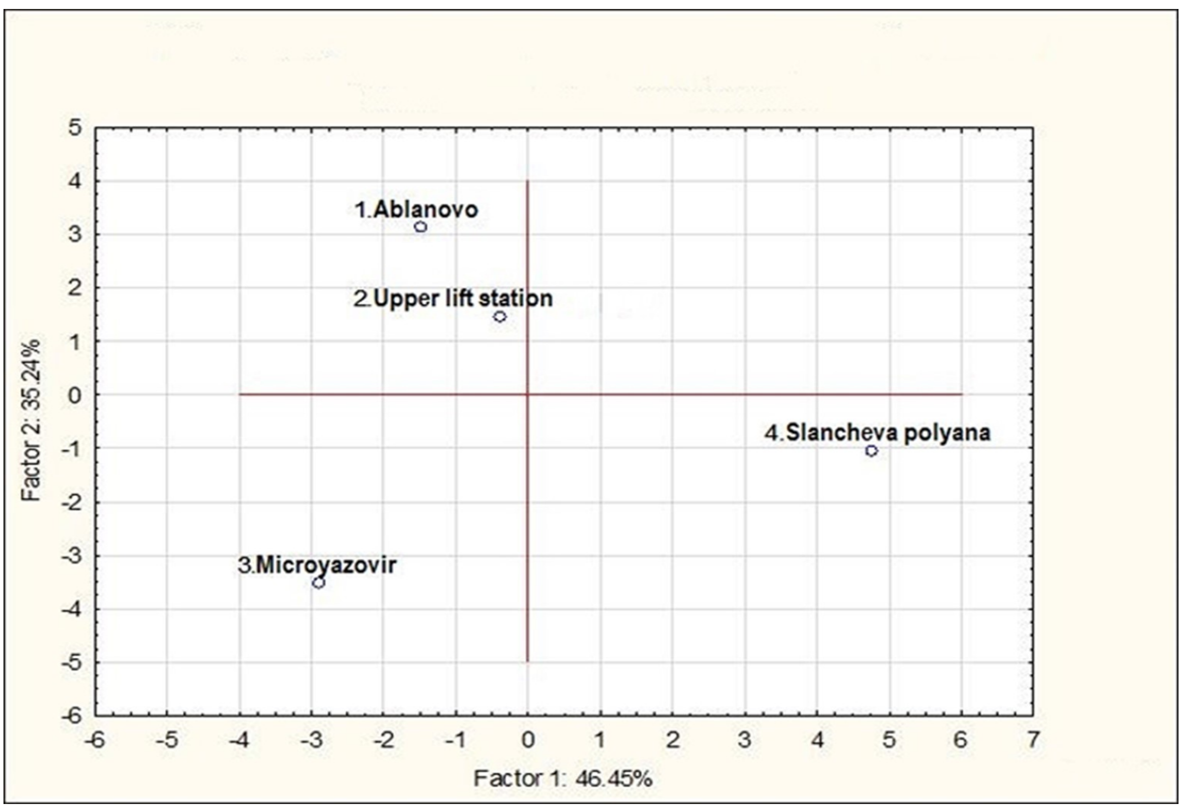

Fig. 1. PCA for the distribution of populations of *B. bulgarica*

The Principal component analysis for the 24 indicators shows that nine of the indicators are positive in the two factors: content of protein, fat, ash, NDF, ADF, gas production at 24 and 48 hours, metabolizable energy, and nitrogen content (Table 8). The content of calcium, magnesium, relative feed value, digestibility and consumption are negative on both factors. Other parameters are positive and negative to first or second factor.

Table 8. PCA distribution of parameters

Parameters	Factor 1	Factor 2
Crud protein, g.kg^{-1}	0.809485	0.15565
Crude fat, g.kg^{-1}	0.239074	0.67753
Crude fiber, g.kg^{-1}	0.262900	-0.7739
Ash, g.kg^{-1}	0.744022	0.11975
NFE, g.kg^{-1}	-0.881503	0.46608
NDF, %	0.987871	0.13481
ADF, %	0.802064	0.46436
DDM, %	-0.802064	-0.4644
DMI, g.kg^{-1}	-0.976728	-0.1892
GP 24 h, dm.ml^{-1}	0.311343	0.94061
Gas 48 h, dm.ml^{-1}	0.273316	0.95812
ME, MJ.kg^{-1}	0.129785	0.98555
RFV	-0.966986	-0.2539
Height, cm	-0.231187	0.95514
Leaf area, cm^2	-0.238586	0.79665
N, g.kg^{-1}	0.810214	0.15414
Ca, g.kg^{-1}	-0.318659	-0.2142
P, g.kg^{-1}	-0.822422	0.33315
K, g.kg^{-1}	-0.886073	0.34654
Na, g.kg^{-1}	0.780851	-0.1596
Mg, g.kg^{-1}	-0.309457	-0.9284
Mn, mg.kg^{-1}	0.652207	-0.7541
Cu, mg.kg^{-1}	-0.858925	0.15172
Fe, mg.kg^{-1}	0.705096	-0.7028
Populations	0.629337	-0.7754

Discussion

The protein content is dependent on the biology features of the species, and also by the factors of the environment. Climatic conditions during the year have a big influence on the chemical composition. The average crude protein content in *B. bulgarica* biomass established in our investigation – average 77.41 g.kg^{-1} DM is very similar to that found by Imbrea et al. (2011) in *Stachys officinalis* (L.) Trevis. (=*Betonica officinalis* L.) – 7% (70 g.kg^{-1} DM). The quantities of raw fat and mineral substances (ashes) were with lower values, and the NFE and the crude fibers with higher values in *B. bulgarica*.

The comparison between the content of mineral elements in biomass of *B. bulgarica* with the data of Todorov et al. (2007) for pasture grass indicates that the content of nitrogen, calcium, phosphorus and magnesium in the biomass of *B. bulgarica* comes close to the pasture grass. The content of potassium in *B. bulgarica* is lower. Significantly lower is the sodium content.

Differences in the amount of trace elements between plants from different populations exists. The highest content of iron and manganese and low copper was found in plants from population Slancheva polyna. The lowest content of iron and manganese is registered for those of the population in Ablanovo. In the population in Upper lift station has the highest content of copper. Content of copper of plants of *B. bulgarica* is the same as the other of the meadow species. There are large differences in the amount of iron and manganese. *B. bulgarica* biomass contains three times less iron and twice manganese compared to the biomass of meadow hay (Todorov et al., 2007).

In assessing the value of the forage plants in addition to the indicators related to the content of protein, fat, fibre, NFE and mineral ingredients, indicators relating to the structure of the fibre components (content of acid and neutral detergent fibre, "*in vitro*" digestibility, energy conversion, the amount of gas formations) have an essential influence for final evaluation of feeding value of the plants. These indicators allow to determine more completely the relative feed value.

The comparison between data obtained on fibre components, digestibility and energy conversion of *B. bulgarica* and other herbaceous forage indicates that the content of the NDF and ADF in *B. bulgarica* biomass is close to that of the perennial herbaceous forage cereal and leguminous plants (Todorov et al. 2007). Naydenova et al. (2005a; 2005b; 2014) obtained similar results for fibre components in new varieties of perennial grasses grown alone and in mixture: NDF – 54.5%; ADF – 29.97% and digestibility – 71.88%. Our results are similar to the results obtained by Yucel & Avci (2009) for the vetch: ADF – 40.11 %, NDF – 46.64 %, digestibility – 57.58%, consumption – 2.58. Established by Ammar et al. (2010) values of NDF, ADF and gas production at 24 hours for oats, vetch, clover and mixtures of oats with vetch and clover are lower than our results. The differences in the content of the structural fibre components between *B. bulgarica* and perennial legumes (*Lotus corniculatus* L.) are very small (Naydenova et al., 2013). Significantly higher digestibility – 80.12 % is established in a new varieties of spring field pea forms (Naydenova & Todorova, 2009). Higher digestibility values were obtained in the initial phases of development of soybean for bulky feed from different maturity groups (Naydenova et al., 2005a, 2005c, 2008). Higher crude protein content 74 $g.kg^{-1}$ DM, lower content of NDF 584 $g.kg^{-1}$ DM, higher digestibility 67 % and metabolizable energy – 7.4 $MJ.kg^{-1}$ DM established López et al. (2005) in the straw of annual legumes compared to that of cereals. The NDF, ADF and digestibility of *B. bulgarica* differ from those established by Kafilzadeh & Maleki (2012) for the straw of four variety chickpeas (*Cicer arietinum* L.). Fibre components are with lower values in the biomass of *B. bulgarica* and digestibility is higher.

The quantity of gas production of *B. bulgarica* biomass comes close to the meadow hays and the perennial legumes grasses. Similar performance has received Yancheva et al., 1997a; 1997b). This is very important and confirms that the biomass of *B. bulgarica* differ compared to coarse fodder plant and ranks it along with the valuable forage plants like alfalfa and perennial cereals. The highest relative feed value of *B. bulgarica* biomass obtained for the population in Microyazovir could be explained with the morphological structure of the plants. According to Grozeva et al. (2016) and Gerdzhikova et al. (2015) the populations of *B. bulgarica* have different height of stems and different leaf area (Table 9). Location and the altitude had a big influence on the growth and morphological parameters of the plants. The population in Microyazovir had the lowest stems with average height 46.39 cm and smaller leaves area - 116.40 cm^2. The lower stem and leaves area is the reason for the lower content of the structural fibre component leading to the higher relative feed value of *B. bulgarica* biomass of this population compared to other populations. Structural fibre components in biomass of *B. bulgarica* determined it high relative feed value. It overtakes the alfalfa in flowering phase with 6.2 % to 20.9 %, an average of 20 %, which ranks her in the group of good forage grasses.

Table 9. Morphological parameters concerning to chemical composition and feeding value

Morphological parameters	Ablanovo	Upper lift station	Micro yazovir	Slancheva polyana	Average
Stem height, cm	73.93	60.84	46.39	50.18	57.80
Specific leaf area of 1 plant, cm^2	251.67	135.68	116.40	128.73	158.10

In terms of environmental pollution and the reduction of gas emissions from ruminants in feeding the bulky and concentrated feeds, established specific indices showing the quantitative characteristics (NDF, ADF, digestibility, energy value and relative feed value), could be successfully used for approximate calculation of the amount of gas formations in optimizing of feeding process.

The relatively small difference in the quantity of gas formations at 48 hours versus 24 hours – 104.97% – confirms that *B. bulgarica* biomass possess good nutritional value and is degraded (normal) as forage with high quality. In rough feeds this parameter is very high – 140 % which shows that degradation is very low at the beginning and very quickly to 48 hour. The difficult initial degradation due to the high NDF and ADF is reason for very slow but more continuing gas formation and emission in the environment.

Established in this investigation correlations among the structure fibre components NDF, ADF with DDM, DMI, RFV and gas production with metabolizable energy, developed linear regression equations is very important way to predict the amount of gas production on the base of metabolizable energy and to optimize diets in animal nutrition with reducing the gas emission process.

Conclusions

In conclusion, as a result of the research can be said that: the biomass of *B. bulgarica* contains average crude protein 77.41 g.kg^{-1} DM; crude fat 7.77 g.kg^{-1} DM; crude fibre 260.16 g.kg^{-1} DM; NFE 604.19 g.kg^{-1} DM and mineral substances 50.47 g.kg^{-1} DM. The content of nitrogen, calcium, phosphorus and magnesium is close to the meadows grass. The content of potassium, sodium, iron and manganese is lower.

In the biomass of *B. bulgarica* contents of structural fibre components is on average NDF 44.82 % and ADF 39.93 % and is close to that of alfalfa and cereal grass. In incubation "*in vitro*" *B. bulgarica* biomass gas formation at 24 hour is average 257.55 dm.ml^{-1}, and at the 48 hour 270.35 dm.ml^{-1} gas (CO_2 and CH_4), which is close to the group of meadows and grasslands feed.

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