

# BASIC CHEMICAL COMPOSITION AND ENERGY NUTRITIONAL VALUE OF FODDER BIOMASS FROM ARTIFICIAL ECOSYSTEMS

**Tatyana Bozhanska, Boryana Churkova, Teodora Mihova**

Research Institute of Mountain Stockbreeding and Agriculture, 5600 Troyan, Bulgaria  
Corresponding author: Tatyana Bozhanska, [tbozhanska@mail.bg](mailto:tbozhanska@mail.bg)  
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**Abstract:** In 2014-2016 at the Research Institute of Mountain Stockbreeding and Agriculture- Troyan, we followed the change in the qualitative composition of dry biomass from two-component grass mixtures. The grasslands with the mixture of *Trifolium repens* L. - *Poa pratensis* L. have the highest crude protein content (151.6 g kg<sup>-1</sup> DM), mineral substances (75.6 g kg<sup>-1</sup> DM) and crude fat (33.9 g kg<sup>-1</sup> DM). The legume grass associated with perennial ryegrass forms forage matter with the highest carbohydrate amount (434.9 g kg<sup>-1</sup> DM) and the lowest amount of crude fiber (269.8 g kg<sup>-1</sup> DM). The biomass of *Lotus corniculatus* L. and *Festuca rubra* L. is the richest in Ca (19.1 g kg<sup>-1</sup> DM), and the mixture of blue hybrid alfalfa - cock's foot has the highest dry matter content (909.7 g kg<sup>-1</sup> DM). The biomass of blue hybrid alfalfa - cock's foot (16.99 MJ/kg DM) and red clover - meadow fescue (16.96 MJ/kg DM) is with the highest caloric values. Both mixtures have almost identical values regardless of the different botanical composition and the predominant component in the grassland. The mixture of *Trifolium pratense* L. with *Phleum pratense* L. has the highest energy nutritional value (FUM - 0.71 kg DM and FUG - 0.66 kg DM), and the biomass of the blue hybrid alfalfa - cock's foot registered the lowest amount of exchange energy (7.30 MJ/kg DM), as well as the smallest number of feed units (FUM – 0.67 kg DM and FUG – 0.61 kg DM) in the dry matter. For the experimental period with the lowest values of gross energy (16.70 MJ/kg DM) are the mixed grasslands of *Trifolium repens* L.

**Key words:** legume and grass mixtures, fodder quality

## Introduction

In mountainous and foothill areas of Bulgaria, artificial hay and pasture ecosystems are an important feed for ruminants. The sowing of two-component mixtures, consisting of medium perennial and grass meadows species (Churkova, 2007; Naydenova, 2008; Mitev and Naydenova, 2008; Mihovski and Sabeva, 2011)

are a factor for obtaining high yield and quality biomass (Stosic *et al.*, 2003; Lazarevic *et al.*, 2004; Vasileva, 2011). An important advantage in mixed cultivation of forage grasses is species diversity and balanced grassland quality. Alfalfa, bird's-foot-trefoil, red and white clover are the legume components used to create temporary meadow and grazing grasslands in Bulgaria (Mihovsky, 1995). Meadow grasses are a major resource for feeding animals in the natural or artificial ecosystem. Therefore, the exact analysis of the main chemical composition (Mihovski and Goranova, 2006) is essential for the evaluation of nutritional value and effective use (Naydenova and Katova, 2013; Bozhanska, 2017a) of the obtained feed. In this connection, Slavkova *et al.* (2017) report a direct relationship between the general body state of ruminants and the protein, calcium and phosphorus content of grazing grassland.

The composition of dry matter in mixed crops is characterized by a lower content of fiber components compared to individual grass and legume monocultures (Naydenova *et al.*, 2015), and the energy value of grass biomass gives a real estimate of the productive potential and a balance between the quantity and the quality of components in artificial grass associations (Bozhanska, 2017b).

The purpose of this study is to assess the quality of feed biomass derived from artificial ecosystems, including basic chemical composition and energy nutritional value of two-component grasslands.

## Material and Methods

The experiment was set in the spring of 2014 at the Research Institute of Mountain Stockbreeding and Agriculture - Troyan, using the block method in four replications with the size of the harvest plot of 5 m<sup>2</sup>. The study period is three years. Experimental variants are:

1. Bird's-foot-trefoil (*Lotus corniculatus* L.) + Red fescue (*Festuca rubra* L.)
  2. White clover (*Trifolium repens* L.) + Perennial ryegrass (*Lolium perenne* L.)
  3. White clover (*Trifolium repens* L.) + Kentucky bluegrass (*Poa pratensis* L.)
  4. Red clover (*Trifolium pratense* L.) + Timothy-grass (*Phleum pratense* L.)
  5. Blue hybrid alfalfa (*Medicago sativa* L.) + Cock's foot (*Dactylis glomerata* L.)
  6. Red clover (*Trifolium pratense* L.) + Meadow fescue (*Festuca pratensis* L.)
- Soil preparation includes autumn plowing of self-sown fallow ground at a depth of 22-25 cm and repeated spring disc, milling and rolling before and after sowing. The sowing of the field experiment was carried out manually, scattered, at the optimum agro-technical period (middle of March) on a pre-created solid bed at a depth of 0.5-1.0 cm. Sowing rates were calculated on the basis of 100% seed germination as for bird's-foot-trefoil and white clover is 1.2 kg/da, for red clover - 1.5 kg/da, and for blue hybrid alfalfa - 2.5 kg/da. Grasses in the grass mixtures are sown with a 2.5 kg/da sowing rate. The ratio of components in mixtures is 50:50. Immediately after sowing, sown areas are rolled for better seed contact between

soil and seeds and their joint germination.

Seeds of the most common cultivars of legumes and meadow grasses were used: Bird's-foot-trefoil – cv. *'Leo'*, White clover – cv. *'Huia'*, Red clover – cv. *'Altaswede'*, Blue hybrid alfalfa, a local population of Troyan origin and Red fescue – cv. *'Ryder'*, Meadow fescue – cv. *'Laura'*, Cock's foot – cv. *'Loke'*, Kentucky bluegrass – cv. *'Sobra'*, Timothy-grass – cv. *'Erecta'* and Perennial ryegrass – cv. *'Belida'*.

Fighting weeds during vegetation is mechanical in order to avoid further chemical interference with plants.

The grasslands were cut in the beginning of the blossoming phase of the legumes and tasseling/ear formation of grass crops.

The chemical composition of the dry feed is analyzed according to *Weende* analysis: Crude protein (CP, g kg<sup>-1</sup>) according to *Kjeldahl* (according to BDS/ISO-5983); Crude fiber (CF, g kg<sup>-1</sup>); Crude fat (Cft, g kg<sup>-1</sup>) (according to BDS/ISO-6492) - by extraction into a *Soxhlet* extractor; Ash (g kg<sup>-1</sup>) - (according to BDS/ISO-5984) degradation of the organic matter by gradual burning of the sample in a muffle furnace at 550°C; Dry matter (DM, g kg<sup>-1</sup>) - empirically calculated from % moisture; Nitrogen-free extractable substances (NFE)=100-(CP, % + CF, % + Cft, % + Ash, % + Moisture, %) converted to g kg<sup>-1</sup>; Calcium (Ca, g kg<sup>-1</sup>) - Stotz (Complexometric) and Phosphorus (P, g kg<sup>-1</sup>) - with vanadate-molybdate reactive according to Gericke and Kurmis - spectrophotometer (agilent 8453 UV - visible Spectroscopy System) measuring in the area of 425 nm.

The nutritional value of the feed was assessed by the Bulgarian system as Feed Unit for Milk (FUM) and Feed Unit for Growth (FUG) and calculated on the basis of equations according to the experimental values of CP, CF, Cft and NFE, recalculated by the coefficients for digestibility by *Todorov (2010)*: Gross energy (GE, MJ/kg DM)=0,0242\*CP+0,0366\*Cft+0,0209\*CF+0,017\*NFE-0,0007\*Zx and Exchangeable energy (EE, MJ/kg DM)=0.0152\*DP (Digestible protein)+0.0342\*Dft (Digestible fat)+0.0128\*DF (Digestible fibers)+0.0159\*DNFE (Digestible Nitrogen-free extractable substances)-0.0007\*Zx.

Data was analyzed with ANOVA (Anova: Two-Factor Without Replication) and means comparison with a Least Significant Difference at 5% level of probability (LSD at P<0.05). The studied sources of variation included: the composition of the components in the mixtures, the conditions of the year and the age of the grass.

## Results and discussion

### Basic chemical composition of perennial grass-legume mixtures.

The chemical composition of the feed gives a real idea of its nutritional value. The most prominent in this regard is the crude protein content in the dry matter composition. The data from the chemical analysis (Table 1) in the year of sowing

indicate that the values of this quality index are highest in the feed of the mixture of bird's-foot-trefoil - red fescue (165.2 g kg<sup>-1</sup> DM) and the lowest in the grassland of red clover with timothy-grass (137.9 g kg<sup>-1</sup> DM).

**Table 1. Basic chemical composition of perennial grass-legume mixtures by years and average for the period 2014-2016 (g kg<sup>-1</sup> DM)**

Variants	DM	CP	Cft	CF	Ash	NFE	Ca	P
<b>2014</b>								
Bird's-foot-trefoil+Red fescue	874.8	165.2	39.3	287.8	64.0	318.5	20.6	2.0
White clover+Perennial ryegrass	871.4	162.5	40.4	279.1	65.5	323.9	16.1	0.9
White clover+Kentucky bluegrass	855.7	139.0	41.8	260.8	71.0	343.1	14.3	2.4
Red fescue+Timothy-grass	863.3	137.9	37.8	255.3	53.0	379.3	15.3	2.2
Blue hybrid alfalfa+Cock's foot	879.2	145.2	49.5	282.4	77.7	324.4	18.2	1.3
Red fescue+Meadow fescue	872.3	141.0	54.0	262.0	90.3	325.0	20.2	1.6
<b>2015</b>								
Bird's-foot-trefoil+Red fescue	929.3	127.8	22.0	310.1	74.5	400.3	9.0	1.9
White clover+Perennial ryegrass	913.8	106.8	25.4	313.6	78.0	396.7	4.8	4.1
White clover+Kentucky bluegrass	905.9	156.5	27.8	227.5	89.0	413.5	11.5	2.7
Red fescue+Timothy-grass	921.9	109.3	20.4	296.2	72.1	429.6	4.8	4.3
Blue hybrid alfalfa+Cock's foot	925.1	99.6	15.2	391.3	64.9	359.0	4.8	3.3
Red fescue+Meadow fescue	918.7	124.2	14.8	331.1	62.6	391.1	9.1	3.5
<b>2016</b>								
Bird's-foot-trefoil+Red fescue	910.9	119.3	22.2	322.0	76.4	371.1	27.7	4.7
White clover+Perennial ryegrass	910.1	163.8	17.4	216.6	77.4	434.9	25.5	5.1
White clover+Kentucky bluegrass	903.4	159.4	32.2	340.9	66.7	304.2	16.8	4.5
Red fescue+Timothy-grass	916.5	121.1	8.8	296.9	65.2	424.5	14.4	5.3
Blue hybrid alfalfa+Cock's foot	924.7	99.8	14.6	365.2	68.7	376.4	9.9	4.5
Red fescue+Meadow fescue	906.2	138.7	32.2	350.6	65.1	319.5	13.5	5.5
<b>2014-2016</b>								
Bird's-foot-trefoil+Red fescue	905.0	137.4	27.8	306.6	71.6	371.1	19.1	2.8
White clover+Perennial ryegrass	898.4	144.4	27.7	269.8	73.6	434.9	15.5	3.4
White clover+Kentucky bluegrass	888.3	151.6	33.9	276.4	75.6	304.2	14.2	3.2
Red fescue+Timothy-grass	900.6	122.8	22.3	282.8	63.4	424.5	11.5	3.9
Blue hybrid alfalfa+Cock's foot	909.7	114.9	26.4	346.3	70.4	376.4	11.0	3.1
Red fescue+Meadow fescue	899.1	134.6	33.7	314.6	72.7	319.5	14.3	3.5
<b>Mean</b>	<b>900.2</b>	<b>134.3</b>	<b>28.6</b>	<b>299.4</b>	<b>71.2</b>	<b>371.8</b>	<b>14.3</b>	<b>3.3</b>
<b>SD</b>	<b>7.2</b>	<b>13.6</b>	<b>4.5</b>	<b>28.9</b>	<b>4.2</b>	<b>53.1</b>	<b>2.9</b>	<b>0.4</b>
<b>LSD<sub>0,05</sub></b>	<b>6.2</b>	<b>23.0</b>	<b>9.1</b>	<b>53.0</b>	<b>13.9</b>	<b>41.0</b>	<b>5.7</b>	<b>0.9</b>

The relatively high protein values in the first vegetation can be explained by the

fact that the yield is mainly formed from high protein legumes. In a second vegetation, the indicator levels range from 99.6 g kg<sup>-1</sup> DM to 156.5 g kg<sup>-1</sup> DM and are significantly lower for all variants (exception makes the mixture of white clover - Kentucky bluegrass) because of the higher participation of the grasses in the grassland and respectively in the cut forage mass. In the third experimental year, the limited participation of alfalfa (4.6%) in the cock's foot variant determined the grassland with the lowest crude protein (99.8 g kg<sup>-1</sup> DM), and the legume crop share (81.5%) in dry white clover and perennial ryegrass biomass increased the protein content of the mixture to 163.8 g kg<sup>-1</sup> DM.

In the year of sowing, the protein content of the mixture of white clover with perennial ryegrass (162.5 g kg<sup>-1</sup> DM) is 23.5 g kg<sup>-1</sup> DM higher than in its mixture with Kentucky bluegrass (139.0 g kg<sup>-1</sup> DM). The values obtained relate to the type of the grass component and its contribution to grassland, as well as to the dry matter content of the reduced biomass from both mixtures.

The perennial ryegrass is a grass forage crop with great competitive ability and fast development. In the first experimental year, the plant biomass prevails over the legume representative. Quite the opposite is the ratio between grasses: legumes in the mixture of white clover - Kentucky bluegrass, where the share of *Trifolium repens* L. is 21.43% above that of the Kentucky bluegrass. The levels for crude protein content in both grasses, which were found in foreign studies (Lemežienė *et al.*, 2007; Bostan *et al.*, 2010), also differ for ryegrass is about 9% and for Kentucky bluegrass is over 14%. Interestingly, the high protein content in the mixture of white clover - perennial ryegrass blend is associated with a higher percentage of crude fiber in the dry matter, and in the mixture of clover with Kentucky bluegrass, the amount of crude protein and crude fiber is lower by 16.9 and 7.0%. This trend is the opposite of the experimental year 2015, when the mixture of white clover - Kentucky bluegrass recorded the highest values of crude protein and lowest crude fiber content (227.5 g kg<sup>-1</sup> DM). The crude protein amount in dry biomass of white clover and perennial ryegrass is lower by 49.7 g kg<sup>-1</sup> DM and the crude fiber and dry matter index values are higher by 86.1 and 7.9 g kg<sup>-1</sup> DM versus its mixture with Kentucky bluegrass. In a third vegetation, the participation of grass crops in white clover grasslands was below 10%, which affected the percentage of protein in the feed mass. Its mixtures with *Lolium perenne* L. has a higher dry matter content, richer in protein and poorer in crude fiber than those of *Poa pratensis* L.

In the year of sowing, the difference in dry matter content and crude protein in the forage matter of red clover is minimal (1-2%). *Trifolium pratense* L. exhibits better adaptability and resistance with meadow fescue compared to timothy-grass. Legume culture dominates in spring and summer regrowth of the crop and ensures higher values of the protein fraction, as well as better take-up and absorption of animal feed. Here too, as in the case of white clover mixtures, the following regularity is observed: the higher protein content (141.0 g kg<sup>-1</sup> DM) is combined

with a higher fiber content of 262.0 g kg<sup>-1</sup> DM (red clover - meadow fescue) and lower protein values (137.9 g kg<sup>-1</sup> DM) are combined with a lower percentage of fibers - 255.3 g kg<sup>-1</sup> DM (red clover – timothy-grass). The differences between the values of the indicators are statistically unproven, which gives little significance to the established ones. The low protein trend - low fiber and vice versa - remains in the second and third years of the plant life cycle, but the percentage between 13.6 and 14.5% (CP) and 11.8 to 18.1% (CF). This atypical manifestation is due to the botanical peculiarities of crops in the grass association, in this case the uniform response of legume crop to wheat and grassland use.

The blue hybrid alfalfa - cock's foot is the only grasshopper whose components in the year of sowing shows almost equal share participation in the grassland, and in the second and third year alfalfa, which is also a major source of protein, records an insignificant presence (2-4%) in plant biomass. The resulting crude protein content in the dry matter is 145.2 g kg<sup>-1</sup> DM (2014), 99.6 g kg<sup>-1</sup> DM (2015) and 99.8 g kg<sup>-1</sup> DM (2016). The comparatively dry climate in 2015 determined a higher content of dry matter and crude fiber (925.1 and 391.3 g kg<sup>-1</sup> DM) in the feed of the mixture versus the first (879.2 and 282.4 g kg<sup>-1</sup> DM) and a third (924.7 and 365.3 g kg<sup>-1</sup> DM) vegetation. Given the data from the analysis, the expected nutritional value of the grass is low.

The dry matter, crude fats and fibers, the mineral and nitrogen-free extractable substances, the macro-elements phosphor and calcium are indicators characterizing the main chemical composition of the herb mixtures included in the study, whose values are regularly increased from first to third vegetation.

Lipids are the most concentrated source of energy for the living organism, improving the flavor of the feed, facilitating the dissolution and absorption of fat-soluble vitamins A, D, E and K. In a first vegetation their dry matter content ranges from 37.8 g kg<sup>-1</sup> DM (red clover - timothy-grass) to 54.0 g kg<sup>-1</sup> DM (red clover - meadow fescue). In the second experimental year the feed from the red clover mixture - meadow fescue leaves for all variants of this indicator (14.8 g kg<sup>-1</sup> DM), and third, together with the white clover grass - Kentucky bluegrass registers the highest values (32.2 g kg<sup>-1</sup> DM).

In the sowing year, the mineral content of the dry matter is 53.0 g kg<sup>-1</sup> DM (red clover - timothy-grass) to 90.3 g kg<sup>-1</sup> DM (red clover - meadow fescue). The amount of ash in the red clover and fescue variant decreases with the age of the grass in the second and third vegetation and the values of the indicator are the lowest (62.6 and 65.1 g kg<sup>-1</sup> DM). For comparison, in the plant biomass of white clover mixtures, we observe a backward dependence associated with increasing the amount of mineral substances.

In the first and second experimental year the mixed grassland of red clover and timothy-grass has the highest content of nitrogen-free extractable substances in the dry matter (379.3 and 429.6 g kg<sup>-1</sup> DM) and in third the mixture of white clover - pasture ryegrass (434.9 g kg<sup>-1</sup> DM) In the second and fifth variants we observe an

increasing regularity of the indicator from first to third vegetation.

The difference between the maximum (20.6 g kg<sup>-1</sup> DM - bird's-foot-trefoil + red fescue) and minimum (14.3 g kg<sup>-1</sup> DM - white clover + Kentucky bluegrass) value of the macro-element Ca in the grasslands of grass and legume mixtures in 2014 is 41.3%. In a second vegetation, only in the plant biomass of the mixture of white clover - Kentucky bluegrass we observe an insignificant increase in the amount of calcium. In the other variants, the trend is decreasing and totally opposite to the third vegetation when the content of this element in the feed of all experimental mixtures is increased, while the grassland of bird's-foot-trefoil - red fescue has a maximum value of 27.7 g kg<sup>-1</sup> DM.

Spectrophotometric measurement showed a significant increase in phosphorus content in the dry matter of two-component grass mixtures from first to third vegetation. The maximum values for the indicator are the variants with white clover with a Kentucky bluegrass (2.4 g kg<sup>-1</sup> DM for 2014) and perennial ryegrass (4.1 g kg<sup>-1</sup> DM for 2015), as well as the grassland with red clover - meadow fescue (5.5 g kg<sup>-1</sup> DM for 2016).

On average, during the study period with the highest protein content (151.6 g kg<sup>-1</sup> DM), the amount of mineral substances (75.6 g kg<sup>-1</sup> DM) and crude fat (33.9 g kg<sup>-1</sup> DM) white clover - Kentucky bluegrass. This is also the grassland with the lowest dry matter content (888.3 g kg<sup>-1</sup> DM) and nitrogen-free extractable substances (304.2 g kg<sup>-1</sup> DM). *Trifolium repens* L. associated with grazing ryegrass forms a feedstock with the highest carbohydrate mass (434.9 g kg<sup>-1</sup> DM) and the lowest amount of raw fiber (269.8 g kg<sup>-1</sup> DM). The plant biomass of bird's-foot-trefoil and red fescue is the richest in the macro-element Ca (19.1 g kg<sup>-1</sup> DM) and the poorest P (2.8 g kg<sup>-1</sup> DM) than the other mixtures included in the experiment.

The plants of *Dactylis glomerata* L. predominate by over 75% of the blue hybrid alfalfa feed, and the herbaceous herb is determined as the highest fibrous (346.3 g kg<sup>-1</sup> DM) respectively corresponding to the lowest amount of crude protein (114.9 g kg<sup>-1</sup> DM) and with the highest dry matter content. On average, for 2014-2016, this blend is also the poorest element of Ca (11.0 g kg<sup>-1</sup> DM).

### **Potential energy nutrition value of mixtures of grasses and legumes.**

The current biomass quality assessment is mainly based on the energy nutrition value and is determined by the feed unit of milk and growth. The feed value is determined based on the chemical composition - crude protein content, fat, fiber, nitrogen-free extractable substances and digestibility ratios, reported by *Todorov (2010)*. The total energy value of mixtures varies from 16.18 MJ/kg DM to 16.87 MJ/kg DM - Table 2.

**Table 2. Energy nutrition value of mixtures of grasses and legumes by year and average for the period 2014-2016**

<b>Variants</b>	<b>GE</b>	<b>EE</b>	<b>FUM</b>	<b>FUG</b>
<b>2014</b>				
Bird's-foot-trefoil+Red fescue	16.87	7.37	0.68	0.62
White clover+Perennial ryegrass	16.75	7.37	0.68	0.62
White clover+Kentucky bluegrass	16.18	7.23	0.67	0.61
Red fescue+Timothy-grass	16.50	7.52	0.70	0.64
Blue hybrid alfalfa+Cock's foot	16.74	7.32	0.67	0.61
Red fescue+Meadow fescue	16.39	7.24	0.67	0.61
<b>2015</b>				
Bird's-foot-trefoil+Red fescue	17.18	7.70	0.71	0.65
White clover+Perennial ryegrass	16.81	7.49	0.69	0.63
White clover+Kentucky bluegrass	16.59	7.80	0.73	0.68
Red fescue+Timothy-grass	16.89	7.70	0.71	0.66
Blue hybrid alfalfa+Cock's foot	17.25	7.28	0.67	0.60
Red fescue+Meadow fescue	17.12	7.57	0.70	0.64
<b>2016</b>				
Bird's-foot-trefoil+Red fescue	16.74	7.36	0.68	0.62
White clover+Perennial ryegrass	16.52	7.91	0.74	0.69
White clover+Kentucky bluegrass	17.33	7.34	0.67	0.60
Red fescue+Timothy-grass	17.10	7.82	0.73	0.67
Blue hybrid alfalfa+Cock's foot	16.98	7.32	0.67	0.61
Red fescue+Meadow fescue	17.38	7.44	0.68	0.62
<b>2014-2016</b>				
Bird's-foot-trefoil+Red fescue	16.93	7.47	0.69	0.63
White clover+Perennial ryegrass	16.70	7.59	0.70	0.65
White clover+Kentucky bluegrass	16.70	7.46	0.69	0.63
Red fescue+Timothy-grass	16.83	7.68	0.71	0.66
Blue hybrid alfalfa+Cock's foot	16.99	7.30	0.67	0.61
Red fescue+Meadow fescue	16.96	7.42	0.68	0.62
<b>Mean</b>	<b>16.9</b>	<b>7.5</b>	<b>0.7</b>	<b>0.6</b>
<b>SD</b>	<b>0.1</b>	<b>0.1</b>	<b>0.0</b>	<b>0.0</b>

GE - gross energy - MJ/kg DM; EE - exchange energy - MJ/kg DM; FUM - feed unit for milk - number in kg of dry matter; FUG - feed units for growth - number in kg of dry matter.

In the year of sowing, the share of cereal crops in the grass: blue hybrid alfalfa - cock's foot, predominates over legumes (34.6%) by about 3%. Data are opposite for the second and third experimental years. Traces of alfalfa are minimal, whereas *Dactylis* L. species occupy 93-95% of the volume of plant biomass in the mixture.



Botanical composition of grassland is a factor that affects the content of fiber fractions in the composition of the forage vegetation, the degradation of which becomes a major source of energy for ruminants. Cock's foot is a high-energy forage grass (Naydenova et al., 2015; Naydenova and Vasileva, 2016). Its mixtures with blue hybrid alfalfa are distinguished with the highest gross energy in the second (17.25 MJ/kg DM) vegetation from the experimental period when the excess over the mean value of the indicator is 0.26 MJ/kg DM. In a third vegetation, gross energy values (16.98 MJ/kg DM) of the variant are identical to the average for the period. On average for the period, the legume and grass grassland of blue hybrid alfalfa - cock's foot has the highest energy.

In the year of experiment establishment, fodder biomass from the mixture of red clover - timothy-grass has the highest energy content that regulates the metabolic processes in the animal organism (7.52 MJ/kg DM) and the highest values of feed units for milk (FUM - 0.70 in kg DM) and growth (FUG - 0.64 in kg DM). In the second and third experimental years, the maximum values of these indices are respectively the mixtures of white clover with Kenthucky bluegrass (EE - 7.80 MJ/kg DM; FUM - 0.73 in kg DM, FUG - 0.68 in kg DM) and perennial ryegrass (EE - 7.91 MJ/kg DM, FUM - 0.74 in kg DM, FUG - 0.69 in kg DM). The excess over the average for the experimental period is minimal.

The nutritional value of feed depends on the predominant botanical composition of the grassland. The good distribution of legume and grass component in the mixture of red clover - meadow fescue - influenced the energy nutrition value of the grassland, by increasing in direct ratio its basic indicators from the first to the third vegetation. Red clover as a legume component dominates in that variant for the entire study period, and the high energy nutritional value of the mixture is influenced by the higher average crude fiber content in the dry matter of the meadow fescue (4.4%) compared to timothy grass (Kostov and Pavlov, 1999). The forage mass in the variants with *Trifolium pratense* L. is characterized by an increasing trend of GE and EE amount from the first to the third vegetation. The values of both indicators in the mixture of red clover - meadow fescue in the second and third experimental years show higher exceed compared to the average for the period: 0.16 MJ/kg DM (2015) and 0.42 MJ/kg DM (2016) - GE and 0.15 MJ/kg DM (2015) and 0.02 MJ/kg DM (2016) - EE. As opposed to gross and exchange energy, the harvested forage mass of the mixture of red clover - meadow timothy has a higher net energy (except for the first vegetation of the plant development), and the values of FUM and FUG in the second and third vegetation are close to the average for the experimental period.

The total energy value of plant matter of bird's-foot-trefoil and red fescue marks a maximum (17.18 MJ/kg DM) in a second vegetation. The excess over the average for 2014-2016 is 0.25 MJ/kg DM. In the third vegetation, the indicators: GE, EE, FUM and FUG of the variant have lower values than the average for the period.

The higher temperatures combined with lower rainfall rates in 2015 and 2016

affected the process of becoming wooden and the fiber content of mixed grasslands. Fiber composition is determinant for the digestibility, quality and nutritional value of the feed (Fahey and Hussein, 1999; Brink et al., 2007) and the main source for the observed increase in the amount of gross and exchange energy in the feed of all grass mixtures in the second and third experimental year.

Against the background of data characterizing energy nutrition of feed in the period 2014-2016 year, the harvested biomass of blue hybrid alfalfa - cock's foot (16.99 MJ/kg DM) and red clover - meadow fescue (16.96 MJ/kg DM) have the highest calorie. Both mixtures have almost identical values regardless of the different botanical composition and the predominant component in the grassland. The energy nutrition value of grass mixtures is a major factor determining the quality of the feed to satisfy the animals' need for food and the production of products of animal origin. The forage from the other observed grasslands is characterized by lower overall energy and high protein content. For the same period, the average value of energy useful for the normal course of physiological needs in the animal organism is highest in the grasslands of red clover - timothy-grass (7.68 MJ/kg DM) and white clover - perennial ryegrass (7.59 MJ/kg DM). This unidirectional data is also maintained in terms of the number of feed unit for milk (0.71 in kg DM for variant 4 and 0.70 in kg DM for variant 2) and growth (0.66 in kg DM for variant 4 and 0.65 in kg DM for variant 2).

For the experimental period, the mixed grassland of *Trifolium repens* L. has the lowest values of gross energy (16.70 MJ/kg DM) energy, and the forage biomass of blue hybrid alfalfa - cock's foot has registered the lowest amount of exchange energy (7.30 MJ/kg DM) as well as the smallest number of feed units (FUM - 0.67 in kg DM and FUG - 0.61 in kg DM) in the dry matter.

## Conclusions

The analyzed dry matter of the mixture of white clover - Kentucky bluegrass has the highest content of crude protein (151.6 g kg<sup>-1</sup> DM), mineral substances (75.6 g kg<sup>-1</sup> DM) and crude fat (33.9 g kg<sup>-1</sup> DM). *Trifolium repens* L. associated with perennial ryegrass forms a forage mass with the highest carbohydrate amount (434.9 g kg<sup>-1</sup> DM) and the lowest amount of crude fiber (269.8 g kg<sup>-1</sup> DM). The plant biomass of bird's-foot-trefoil and red fescue is the richest in the macro-element Ca (19.1 g kg<sup>-1</sup> DM), and the grassland with blue hybrid alfalfa - cock's foot has the highest dry matter content (909.7 g kg<sup>-1</sup> DM).

In the period of study, the harvested biomass of blue hybrid alfalfa - cock's foot (16.99 MJ/kg DM) and red clover - meadow fescue (16.96 MJ/kg DM) has highest caloric values. Both mixtures have almost identical values regardless of the different botanical composition and the predominant component in the grassland. The feed of the mixture of *Trifolium pratense* L. with *Phleum pratense* L. has the

highest energy nutritional value (FUM - 0.71 in kg DM and FUG - 0.66 in kg DM), while the forage biomass in the variant of blue hybrid alfalfa - cock's foot register the lowest amount of exchange energy (7.30 MJ/kg DM), as well as the smallest number of feed units (FUM – 0.67 in kg DM and FUG – 0.61 in kg DM) in dry matter.

The mixed grasslands of *Trifolium repens* L. have the lowest gross energy (16.70 MJ/kg DM).

## Osnovni hemijski sastav i energetska nutritivna vrednost krmne biomase iz veštačkih ekosistema

Tatyana Bozhanska, Boryana Churkova, Teodora Mihova

### Rezime

U periodu od 2014. do 2016. godine, u Istraživačkom institutu za planinsko stočarstvo i poljoprivredu - Trojan, pratili smo promenu kvalitativnog sastava suve biomase iz dvokomponentnih travnatih smeša. Pašnjaci sa mešavinom *Trifolium repens* L. - *Poa pratensis* L. su imali najviši sadržaj sirovog proteina (151,6 g kg<sup>-1</sup> SM), mineralnih supstanci (75,6 g kg<sup>-1</sup> SM) i sirove masti (33,9 g kg<sup>-1</sup> SM). Krmna trava koja se povezuje sa višegodišnjim ljuljom stvara krmu sa najvišom količinom ugljenih hidrata (434,9 g kg<sup>-1</sup> SM) i najmanjom količinom sirovih vlakana (269,8 g kg<sup>-1</sup> SM). Biomasa biljaka *Lotus corniculatus* L. i *Festuca rubra* L. je najbogatija u Ca (19,1 g kg<sup>-1</sup> SM), a smeša plave hibridne lucerke - ježevice ima najveći sadržaj suve materije (909,7 g kg<sup>-1</sup> SM). Biomasa plave hibridne lucerke - ježevice (16,99 MJ / kg SM) i crvena detelina – livadski vijuk (16,96 MJ / kg SM) je imala najviše kalorijske vrednosti. Obe mešavine imaju gotovo identične vrednosti bez obzira na različiti botanički sastav i pretežnu komponentu u travnjacima. Mešavina *Trifolium pratense* L. sa *Phleum pratense* L. ima najvišu energetska hranljivu vrednost (FUM - 0,71 kg SM i FUG - 0,66 kg SM), a biomasa plave hibridne lucerke – ježevice imala je najmanju količinu energije za razmenu (7,30 MJ / kg SM), kao i najmanji broj jedinica za ishranu (FUM - 0,67 kg SM i FUG - 0,61 kg SM) u suvoj materiji. U navedenom eksperimentalnom periodu, sa najnižim vrednostima bruto energije (16,70 MJ / kg SM) bila je smeša travnjaka *Trifolium repens* L.

**Ključne reči:** mešavine krmnih biljaka i trava, kvalitet krme

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