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Effect of organic fertilizing on the composition of fiber components of cell wall and nutritional value of bird's-foot-trefoil (*Lotus corniculatus* L.) forage

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Abstract

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During the period 2021-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture-Troyan, a scientific research experiment was conducted with bird's-foot-trefoil (*Lotus corniculatus* L.), variety Targovishte 1., in the following fertilizing variants: 1. Control – untreated; 2. Blago 5 at dose of 300 ml/da; 3. Blago 5 at dose of 600 ml/da; 4. Fertileader Axis at dose of 500 ml/da; 5. Fertileader Axis at dose of 1000 ml/da. The fiber composition of the cell walls was determined by applying the detergent analysis. The nutritional value of the forage was calculated by gross and exchangeable energy, feed units for growth and milk, and *in vitro* enzymatic digestibility of dry matter by a two-phase pepsin-cellulose method. It was found that fertilization over the years has been shown to affect the values of neutral and acid detergent fibers, cellulose and the degree of lignification. A higher effect on these indicators was recorded by the biofertilizer Fertileader Axis, applied at a dose of 1000 ml/da, and applied at a dose of 500 ml/da, it reduced the levels of acid detergent fibers and cellulose compared to the control variant. The higher nutritional value was reported in fertilizing with Blago 5 at dose of 300 ml/da, whose feed has feed units for milk 0.86% and for growth 0.82%. Fertilization with Fertileader Axis at dose of 500 ml/da registered 68.77% feed digestibility.

Keywords: bird's-foot-trefoil; biofertilization; structural fiber components; nutritional value; digestibility

Introduction

Bird's-foot-trefoil is a perennial grass species, whose forage is economically efficient and of high quality, providing rational and healthy nutrition for ruminants (Chourkova, 2012). Fertilizing with organic fertilizers of meadow legume crops is a major agrotechnical measure for improving the productivity and quality of forage (Churkova and Churkova 2022; Churkova, 2023). The fiber components of the cell walls of forage plants, are a major criterion for determining the energy nutritional value and the forage digestibility (Guertin, 1987). Forage quality is largely influenced by the genetic characteristics of varieties (Churkova et al., 2016), climatic changes and the composition of grass species in the grassland (Karabulut et al., 2006). Crude protein decreases from the beginning of the vegetation to the end, and crude fiber content increases with increasing plant height (Kaplan et al., 2009). The content of fiber components in bird's-foot-trefoil increases until the full flowering phase, after which its intensity decreases (Naydenova et al., 2013). The cellulose in the individual stages of the development of bird's-foot-trefoil has low values and decreases as the vegetation progresses.

The economic efficiency of bird's-foot-trefoil forage largely depends on the nutritional value of the forage, and this is related to the introduction of new environmentally

friendly practices (Larbi et al., 2010a), for sustainable forage grass production (Vasileva and Vasilev, 2012; Vasileva, 2014). Such an improvement measure concerning the forage quality, according to Bozhanska (2020), is the application of organic fertilizers on the grassland of bird's-foot-trefoil.

The basic chemical composition, composition of cell wall components and digestibility, are essential to evaluate their nutritional value and their efficient utilization. Applied Fiber Detergent Analysis (Van Soest et al., 1991) is a basic method for determining nutrient digestibility (Adesogan et al., 2006). It, in turn, affects the productivity of animals (Mertens, 2011). The nutritional value of forage is an indicator of its quality and determines the production cost price. Acid-detergent fibers are a criterion for calculating forage digestibility and neutral-detergent fibers for its assimilation (Ward and Ondarza, 2008; Dewhurst et al., 2009).

Fibers are major cell wall constituents of hemicellulose, cellulose, and lignin. Reduced fiber content in the cell wall improves forage intake (Wróbel and Zielewicz, 2019).

Lignin favours the binding of cellulose fibers, strengthens cell walls, and stimulates plant growth. Lignification is determined by the age of the plants. The higher the lignin content, the worse the forage quality. Low lignin content increases forage digestion and absorption by animals (Srinivasa Reddy et al., 2005). In connection with the ever-increasing organic production, information on the effect of fertilizing with organic fertilizers on the nutritional value of bird's-foot-trefoil forage is insufficiently studied.

The aim of the study is to determine the influence of organic fertilizers Blago 5 and Fertileader Axis, on the composition of the fiber components of the cell walls, the nutritional value and the forage digestibility of bird's-foot-trefoil, and to propose an appropriate type of fertilizer and dose of fertilization.

Material and Methods

The experimental data presented in the scientific study, are for the period 2021-2023. The experiment was conducted in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture-Troyan, with bird's-foot-trefoil, variety Targovishte 1, on light grey pseudopodzolic soil. The humus content of the soil is 1.25% in the 0–20 cm layer and 1.35% in the 0–40 cm layer (Table 1). Regarding the content of absorbable phosphorus and potassium, the values of the indicators are, respectively, 5.29 and 23.06 mg/100 for the layer of 0–20 cm, and in the layer of 0–40 cm they are: 0.73 and 19.0 mg/100. The availability of available nitrogen in the soil is low. In the soil horizon from 0–20 cm, where the root system of plants is mainly located, the amount of the macroelement is 12.67 mg/100, and in the horizon from 0–40 cm – 11.52 mg/100. The soil reaction is acidic at pH_{H2O} = 4.10 and pH_{KCl}= 3.80–3.70.

Table 1. Agrochemical analysis of light grey pseudo–podzolic soils (0–40 cm)

Soil layer, cm	pH		Σ N – NH_4 + NO_3	P_2O_5	K_2O	Humus
	H_2O	KCL	mg/kg	mg/100		%
0–20	4.10	3.80	12.67	5.29	23.06	1.25
0–40	4.10	3.70	11.52	0.73	19.00	1.35

The research experiment was set up according to the block method in four replications with a harvest plot size of 5 m^2. Fertilization is carried out in the following variants: 1. Control – untreated; 2. Blago 5 at dose of 300 ml/da; 3. Blago 5 at dose of 600 ml/da; 4. Fertileader Axis at dose of 500 ml/da; 5. Fertileader Axis at dose of 1000 ml/da.

Bird's-foot-trefoil was sown at a seed rate of 0.12 kg/ha^{-1}. The introduction of the fertilizer was carried out by means of a sprayer with a capacity of 6 ml. Harvesting was carried out in the bud-formation – the beginning of the flowering phase and two regrowths were harvested annually. Blago 5 organic fertilizer contains Nitrogen (N) 2.18%, Phosphorus (P_2O_5) 10.3%, Total potassium (K_2O) 17%, Magnesium (MgO) 0.5%, Manganese (Mn) 0.5%, Zinc (Zn) 0.58%, Copper (Cu) 1.0%, Boron (B) 0.5%, Molybdenum (Mo) 0.08%, Cobalt (Co) 0.13%, Humic and fulvic acids not less than 2%, Organic lake sapropel substance less than or equal to 67%, pH 6.5 – 7.5.

Fertileader Axis is a product with systematic action with the following chemical composition: Nitrogen content – 3%; Phosphorus 18%; Zinc – 5.7%; Manganese – 2.5%.

The samples were taken immediately before harvesting the grasslands in the bud-formation – the beginning of flowering phase. Before analysis, the dried samples were ground with a mill through a sieve with a mesh size of 1 mm. The structural carbohydrates of the cell walls were determined on each sample, and the nutritional value and *in vitro* enzymatic digestibility of the dry matter were calculated.

The fiber composition of the cell walls was determined as a percentage and included: Neutral detergent fibers; Acid detergent fibers and Acid detergent lignin were determined, according to the detergent analysis of Van Soest and Robertson (1976); Degree of lignification (expressed as a percentage ratio of ADL/NDF); Hemicellulose (calculated by NDF – ADF); Cellulose = ADF – ADL.

The nutritional value of the forage was assessed according to the Bulgarian system as Feed units for milk (FUM)

and Feed units for growth (FUG), and calculated based on equations, according to the experimental values of CP, CFr, CF, and NFE, recalculated by the digestibility coefficients proposed by Todorov et al. (2010), as follows: Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*CF + 0.0209*CFr + 0.017*NFE – 0.0007*Zx.; Exchangeable energy (EE, MJ/kg DM) = 0.0152*DP + 0.0342*CF + 0.0128*DF + 0.0159*DNFE – 0.0007*Zx.

Feed unit for milk (FUM) = EE*(0.075 + 0.039*q).

Feed unit for growth (FUG in kg DM) = EE* (0.04 + 0.1*q).

In vitro enzymatic digestibility of dry matter (%) was determined according to the method of Aufrere (1982), by a two-phase pepsin-cellulose method. In the first phase, a pre-treatment with pepsin-hydrochloric acid was conducted to digest the protein, and in the second, the feed residue was treated in an acidic environment with the cellulose enzyme to digest the cellulose.

Statistical treatment of the data is represented by average value (X), standard deviation (SD). Statistical processing of the data was performed to establish the evidence of differences (LSD at 0.05), using data from each year and each regrowth for each indicator. For this purpose, the software products Analysis Toolpak for Microsoft Excel 2010 and Statgraphics Plus v.2.1 were used.

From Figure 1, where the average monthly temperatures are presented, it is clear that the first (2021-11.0°C) and the second (2022-11.6°C) years do not differ significantly in terms of average values for the year. The third (2023) was the warmest compared to the other two years, when the average temperature for the year was 12.6°C, and for the growing season 16.3°C.

The amount of precipitation (Figure 2) is the lowest in 2022 (379.9 mm). The amount of precipitation in the period

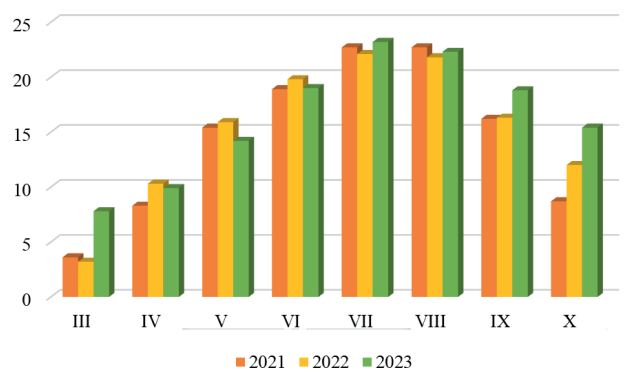

Fig. 1. Average monthly temperatures (°C) for the vegetation period (March-October) 2021–2023

of active plant vegetation (the months of March-October in the years of the experiment), is as follows: 405.5 mm (2021) and 379.9 mm (2022), and 523.3 mm (2023). The data show a significant decrease in the values in the second year, compared to the precipitation amount in the first and third experimental years.

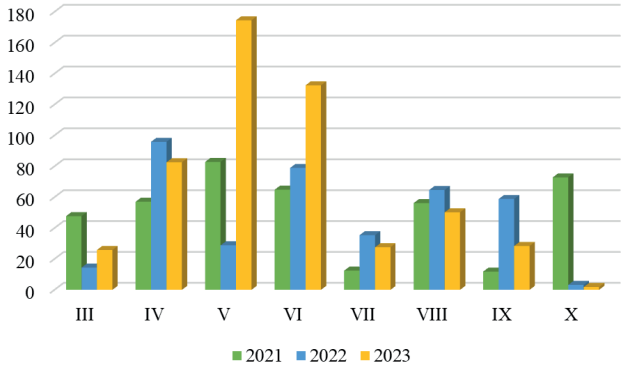

Fig. 2. Average monthly precipitation amounts (mm) for the vegetation period (March-October) 2021–2023

Results and Discussion

Influence of organic fertilizing on the content of the structural fiber components, in the cell walls of the plant biomass of bird's-foot-trefoil over the years and average, for the period 2021 – 2023

The concentration of NDF, ADF, hemicellulose and lignin in the dry matter of the forage mass from bird's-foot-trefoil, depending on the application of organic fertilizers at different fertilizer rates, are decisive for the forage digestibility.

In the first experimental year (Table 2), low values of neutral detergent fibers from the control variant were registered by applying Blago 5, at a dose of 600 ml/da (354.4 $g.kg^{-1}$DM), and Fertileader Axis at a dose of 1000 ml/da (376.0 $g.kg^{-1}$DM). The average value of this indicator is X = 39.43%, the standard deviation SD = 3.40. The effect of applied organic fertilizers and their doses on the content of acid detergent fibers is very well demonstrated. The values for all variants were lower than those of the control and ranged from 218.7 to 313.1 $g.kg^{-1}$DM, with an average degree of variability according to the variation coefficient (VC = 13.15%). Except for fertilizing with Fertileader Axis at a dose of 1000 ml/da (9.45%), the concentration of acid detergent lignin was higher than that of the control. The average acid detergent lignin reported was 11.08% compared to 9.87% for the control. Hemicellulose showed an increase at all doses of fertilizer application compared to the control.

Table 2. Structural fiber components of the cell walls of bird's-foot-trefoil with applying organic fertilizers in 2021 (g.kg^{-1}DM)

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification
Control (C)	390.2	313.1	98.7	77.2	214.4	252.9
Blago 5 300 ml/da	406.2	301.2	123.3	105.0	177.8	303.7
Blago 5 600 ml/da	354.4	273.0	108.9	81.4	164.1	307.4
Fertileader Axis – 500 ml/da	444.8	218.7	128.7	226.1	90.0	289.3
Fertileader Axis – 1000 ml/da	376.0	284.3	94.5	91.7	189.8	251.3
X	394.3	278.1	110.8	116.3	167.2	280.9
SD	34.0	36.6	14.9	62.3	47.0	27.2

*NDF – Neutral–detergent fiber; ADF – Acid–detergent fiber; ADL – Acid–detergent lignin; Hemicellulose; Cellulose; Degree of lignification

Of interest is the increase in hemicellulose in applying Fertileader Axis at a dose of 500 ml/da (226.1 g.kg^{-1}DM), compared to 77.2 g.kg^{-1}DM for the control. Organic fertilizers in the year of sowing had a positive effect on the cellulosic fraction of bird's-foot-trefoil. The organic fertilizers reduced the cellulose content, which in the treated variants was from 90.0 g.kg^{-1}DM to 189.8 g.kg^{-1}DM, and in the control 214.4 g.kg^{-1}DM. The low levels of cellulose in the fertilized variants also determine the low average value X = 167.2 g.kg^{-1}DM. The degree of lignification in applying Fertileader Axis at a dose of 1000 ml/da is close to the control (251.3 g.kg^{-1}DM), and in all other variants, it is higher. This is also reflected in the calculated average value, which is X = 280.9 g.kg^{-1}DM compared to 252.9 g.kg^{-1}DM for the control variant. Higher relative air humidity determines lower ADF and NDF values related to higher *in vitro* digestibility of organic matter (IVOMD). Therefore, the obtained results are based on the peculiarities of the climate during the year, formed under higher humidity.

In the second experimental year (2022), the organic fertilizers registered high levels of detergent fibers according to variants that depend on the high precipitation amounts affecting plant growth, and the biological features of the bird's-foot-trefoil (Table 3). The lowest levels for NDF (442.2 g.kg^{-1}DM), ADF (341.6 g.kg^{-1}DM) and ADL (88.9 g.kg^{-1}DM) were measured in the forage mass of grassland fertilized with Fertileader Axis at a dose of 1000 ml/da at 477.0 g.kg^{-1}DM (NDF), 381.6 g.kg^{-1}DM (ADF), and 116.9 g.kg^{-1}DM (ADL) for the non-fertilized variant. The obtained data prove the effect of fertilizing with bioproducts on the composition of the cell walls. Except for the grassland treated with Blago 5 at a dose of 600 ml/da, all the other variants of the experiment on these indicators had lower values. The average value of neutral detergent fibers was 400.8 g.kg^{-1}DM and of acid detergent was 280.0 g.kg^{-1}DM, of acid detergent lignin was 106.8 g.kg^{-1}DM.

Organic fertilizers lowered the content of acid detergent lignin and increased the hemicellulose. The lowest level of acid detergent lignin was reported in the forage mass of grassland treated with Fertileader Axis, at a dose of 1000 ml/da (88.9 g.kg^{-1}DM), which was lower than the untreated control by 28.0 g.kg^{-1}DM. An opposite trend was found for hemicellulose. Organic fertilizing increased the content of hemicellulose in the forage. The highest effect is distinguished by the biomass from the grassland with applying Blago 5 at a dose of 300 ml/da (111.2 g.kg^{-1}DM), as the excess of polysaccharide compared to the control is 15.1%. The rest of the organic fertilizers and their doses had similar values from 100.6 g.kg^{-1}DM to 106.8 g.kg^{-1}DM. Bozhanska (2017) reported similar results for bird's-foot-trefoil after treatment with biostimu-

Table 3. Structural fiber components of the cell walls of bird's-foot-trefoil after applying organic fertilizers in 2022 (g.kg^{-1}DM)

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification
Control (C)	477.7	381.6	116.9	96.1	264.7	24.47
Blago 5 300 ml/da	461.6	350.4	89.4	111.2	261.0	19.37
Blago 5 600 ml/da	498.9	392.1	105.4	106.8	286.6	21.13
Fertileader Axis – 500 ml/da	471.8	368.4	100.4	103.5	268.0	21.28
Fertileader Axis – 1000 ml/da	442.2	341.6	88.9	100.6	252.8	20.10
X	470.4	366.8	100.2	103.6	266.6	21.27
SD	20.9	21.0	11.7	58.0	1.25	1.95

*NDF – Neutral–detergent fiber; ADF–Acid–detergent fiber; ADL – Acid–detergent lignin; Hemicellulose; Cellulose; Degree of lignification

lators, that showed increased levels of hemicellulose in all treated variants compared to the untreated control. Cellulose is a component that significantly affects the dry matter digestibility, on which the nutritional value of the forage depends. The forage mass of the grassland treated with Blago 5 at a dose of 600 ml/da showed the highest values compared to the control, respectively, 286.6 g.kg^{-1}DM. The calculated amount of cellulose in the other variants with fertilizing has values close to the control. Cellulose averaged 170.6 g.kg^{-1}DM compared to 221.1 g.kg^{-1}DM for the control variant. The obtained data show that fertilizing with Fertileader Axis at a dose of 1000 ml/da is important for the forage quality. The degree of lignification at all applied doses of fertilizing was lower than that of the control variant. The development phase in legume forage crops affects the composition of plant cell walls (Kicheva & Angelova, 2006). As the age of the plant increases, changes occur in the content of detergent fibers, which accompany the growth intensity of the stem in height and the accumulation of mechanical sclerenchyma tissue (Guertin, 1987). The fiber content increased with the height of the plant. Bird's-foot-trefoil has more intensive growth in the second year of its development, which is associated with taller stems. They, in turn, are a prerequisite for increasing the concentration of neutral and acid detergent fibers. This is confirmed by the results obtained, which are proven by the higher fiber values. They are also the result of the agroecological conditions of the area. Higher relative air humidity determines lower ADF and NDF values associated with higher *in vitro* digestibility of organic matter (IVOMD), which is in agreement with those obtained by Larbi et al. (2010a) results.

In the third year (Table 4), the concentration of neutral detergent fibers was lower only in applying Fertileader Axis at a dose of 1000 ml/da by 31.3 g.kg^{-1}DM, as at all other fertilizing doses, the values exceeded the control. Acidic detergent fibers registered a significant decrease in all variants, as the highest value was reported in the control, respectively, 323.0 g.kg^{-1}DM, and the lowest after applying Fertileader Axis at a dose of 500 ml/da (208.2 g.kg^{-1}DM). The values of acid detergent lignin in the fertilizing variants exceeded the control, as the average value was 106.8 g.kg^{-1}DM, and the control was 84.2 g.kg^{-1}DM. A minimum value of the acid detergent lignin was registered in the forage mass of bird's-foot-trefoil fertilized by Fertileader Axis at a dose of 1000 ml/da, which slightly exceeded the control by 5.9 g.kg^{-1}DM. The hemicellulose had the lowest values in the control (79.2 g.kg^{-1}DM), and the highest in fertilization with Fertileader Axis in a dose of 500 ml/da (126.0 g.kg^{-1}DM), with an average value of 98.0 g.kg^{-1}DM. Fertilizing had a positive effect on the cellulose content, lowering the values in all fertilizing options. The data confirm those obtained by Pavlov and Naydenova (2000), for the reduction of cellulose with advancing vegetation. This is evident from the obtained values, which for the fertilized variants are from 82.9 g.kg^{-1}DM to 192.3 g.kg^{-1}DM with a control value of 221.1 g.kg^{-1}DM. The obtained values for this indicator predetermine a higher nutritional value of the forage. The degree of lignification in the fertilized variants was superior to the control in terms of values. The reported maximum value of lignification of 331.8 g.kg^{-1}DM and a minimum of 261.3 g.kg^{-1}DM.

The average for the period (Table 5), with the highest positive effect in terms of neutral detergent fibers, was the forage mass fertilized with Fertileader Axis at a dose of 1000 ml/da (393.7 g.kg^{-1}DM). The highest effect in acid detergent fibers was found after applying Fertileader Axis at a dose of 500 ml/da (265.1 g.kg^{-1}DM). Fertilizing with both organic fertilizers had a higher positive effect on acid detergent fiber levels than on neutral detergent fiber. This is evident from the reduced values of acid detergent fibers in all fertilizing variants compared to the control. The content of acid detergent lignin after applying Fertileader Axis at a dose of 1000 ml/da is close to the control, while its amount is higher in the other fertilizing variants. The reported average value of ADF was 308.8 g.kg^{-1}DM compared to 339.2 g.kg^{-1}DM for the control.

Table 4. Structural fiber components of the cell walls of bird's-foot-trefoil after applying organic fertilizers in 2023 (g.kg^{-1}DM)

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification
Control (C)	394.1	323.0	84.2	79.2	221.1	261.9
Blago 5 300 ml/da	423.0	312.3	123.0	112.8	182.4	314.0
Blago 5 600 ml/da	398.1	282.3	111.5	89.2	174.2	331.8
Fertileader Axis – 500 ml/da	425.9	208.2	125.2	126.0	82.9	271.9
Fertileader Axis – 1000 ml/da	362.8	274.0	90.1	82.9	192.3	261.3
X	400.8	280.0	106.8	98.0	170.6	288.2
SD	25.6	45.0	18.8	20.4	52.1	32.6

*NDF – Neutral–detergent fiber; ADF – Acid–detergent fiber; ADL – Acid–detergent lignin; Hemicellulose; Cellulose; Degree of lignification

The low degree of this indicator is the result of the small range of variation between the minimum (265.1 g.kg^{-1}DM) and maximum value (339.2 g.kg^{-1}DM). Except for fertilizing with Fertileader Axis at a dose of 1000 ml/da, which has a value close to that of the unfertilized variant, in all other variants the acid detergent lignin is in a greater amount than the control. Hemicellulose at all fertilizer rates is in greater quantity than the untreated variant with an average value of 106.0 g.kg^{-1}DM. Fertilizing increased hemicellulose levels from 91.7 g.kg^{-1}DM to 151.9 g.kg^{-1}DM against 84.2 g.kg^{-1}DM for the untreated variant. Cellulose, which is a criterion for the digestibility and nutritional value of the forage, is an indicator of the positive effect of the applied fertilizing. This is proven by the reduced values for the fertilized variants with Fertileader Axis at a dose of 500 ml/da. The degree of lignification showed an increase in most of the variants. The average value is 260.6 g.kg^{-1}DM, and the change in this indicator is weak. From the statistical processing of the data, we found that fertilization over the years has been proven to influence the values of neutral detergent and acid detergent fibers, cellulose and the degree of lignification. Fertilization did not affect the other chemical indicators.

The period of the research experiment conducted over the years was characterized by a rarity in temperatures and precipitation. In years with higher relative air humidity, ADF and NDF values are lower. Prolonged droughts cause lignification and the accumulation of cellulose in plant cells, which increases the percentage of fiber components and reduces digestibility (Larbi et al., 2010b).

During the years with higher temperatures and lower relative air humidity, factors that influence the effect of the organic fertilizers and the amount of fiber fractions, it exceeded the untreated variant in the harvested grasslands after applying Blago 5 and Fertileader Axis. The obtained data confirm the results of Buxton and Fales (1994), that higher temperatures influence the fiber composition of the plant organism by increasing the concentration of neutral-detergent fibers included in the composition of the plant cell.

Pavlov and Naydenova (2000) also reported increased levels of NDF, ADF and ADL in bird's-foot-trefoil. Badrzadeh et al. (2008) found that neutral and acid-detergent fiber and *in vitro* organic matter digestibility (IVOMD) determined forage quality and this was due to agroecological conditions, botanical characteristics of plants and leaf:stem ratio.

Potential energy nutritive value of grass biomass from bird's-foot-trefoil with applying organic fertilizers

On average for the period 2021–2023 (Table 6), we found a slight increase in the values of gross energy in the forage mass of bird's-foot-trefoil, fertilized with Blago 5 at a dose of 600 ml/da and Fertileader Axis at a dose of 1000 ml/da. The excess compared to the control is insignificant, by 0.31 and 0.37%, respectively. The obtained results correlate with the analysis of the fiber components of the cell walls, and their higher content determines the source of dietary energy for ruminants (Fahey and Hussein, 1999). The highest value of neutral detergent fibers was found in fertilizing with Fertileader Axis at a dose of 500 ml/da (447.5 g.kg^{-1}DM), whereas the lowest level of acid detergent fibers (265.1 g.kg^{-1}DM) realized the highest dry matter digestibility (687.7 g.kg^{-1}DM).

Foliar application of the tested fertilizers did not affect the amount of exchangeable energy in the biomass of bird's-foot-trefoil. Blago 5 (300 ml/da) has a slightly pronounced effect on the value of the indicator, where the control (8.59%) is exceeded by 0.5%. The content of feed units gives an idea of the energy value or nutritional value of the forage, but obtaining animal production also depends on the amount of forage intake or the amount of net energy intake. The results of the experiment showed an increased number of feed units for milk in the grassland with bird's-foot-trefoil treated with Blago 5 at a dose of 300 ml/da (0.86), an equal

Table 5. Structural fiber components of the cell walls of bird's-foot-trefoil after applying organic fertilizers in 2021 – 2023 (g.kg^{-1}DM)

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification
Control (C)	420.7	339.2	99.9	84.2	233.4	253.2
Blago 5 300 ml/da	430.3	321.3	111.9	109.7	207.1	270.5
Blago 5 600 ml/da	417.1	315.8	108.6	92.5	208.3	283.5
Fertileader Axis – 500 ml/da	447.5	265.1	118.1	151.9	147.0	258.0
Fertileader Axis – 1000 ml/da	393.7	300.0	91.2	91.7	211.6	237.9
X	421.8	308.3	105.9	106.0	201.5	260.6
SD	19.7	27.9	10.5	27.3	32.3	17.3
LSD 0.05	34.0	39.4	20.1	47.2	45.5	27.6

*NDF – Neutral–detergent fiber; ADF – Acid–detergent fiber; ADL –Acid–detergent lignin; Hemicellulose; Cellulose; Degree of lignification

Table 6. Energy nutritional value and digestibility of dry matter of bird's-foot-trefoil treated with organic fertilizers on average for the period 2021-2023

Variants	GE	EE	FUM	FUG	VDMD by the method of Aufrere
Control (C)	18.81	8.59	0.80	0.74	63.18
Blago 5 300 ml/da	18.28	9.09	0.86	0.82	64.09
Blago 5 600 ml/da	19.12	8.60	0.80	0.73	64.85
Fertileader Axis – 500 ml/da	18.50	8.22	0.76	0.69	68.77
Fertileader Axis – 1000 ml/da	19.18	8.59	0.79	0.73	66.13

*GE – gross energy; EE – exchangeable energy, FUM – feed units for milk; FUG – feed units for growth; IVDMD – in vitro dry matter digestibility (%)

number of feed units when fertilized with Blago 5 at a dose of 600 ml/da (0.80), and a reduced number in the other variants (0.76 and 0.79). The feed units for milk in the dry matter of the bird's-foot-trefoil are more compared to feed units for growth, which allows the forage to be used for the needs of lactating animals.

A greater difference was found by variants in the forage units for growth, as the highest values were found after fertilizing with Blago 5 at a dose of 600 ml/da (0.82), whereas in the other variants, their values were below that of the control, as a significant difference was not established. Their variation is from 0.69 to 0.73 pieces.

The obtained results shows that the quality of the forage is related to its nutritional value, which is directly dependent on the concentration of the chemical constituents in the plant tissue, which confirms the statements of García and Cozzolino (2006). It has been found that as grass species progress in growth and development, the fiber components of cell walls increase and their nutritional value decreases (Naydenova et al., 2013). This trend according to Safari et al. (2011), is affected by climatic changes during the year.

The dry matter digestibility was the highest in the forage mass of bird's-foot-trefoil fertilized with Fertileader Axis in both doses of 500 (687.7 $g.kg^{-1}$DM) and 1000 ml/da (661.3 $g.kg^{-1}$DM). The control values of these two variants were exceeded by 55.9 $g.kg^{-1}$DM in variant 4, and 29.5 $g.kg^{-1}$DM in variant 5. The digestibility of the forage fertilized with Blago 5 is, respectively, 640.9 $g.kg^{-1}$DM at a dose of 300 ml/da and 648.5 $g.kg^{-1}$DM at a dose of 600 ml/da, which shows that no difference was found in the values of this indicator concerning the dose. All applied doses of both organic fertilizers realized a higher digestibility than the control, which proves the effect of biofertilization on forage quality. The degree of lignification is estimated to be a major factor limiting the nutritional value of forages and suppressing digestibility (Casler and Jung, 2006). Naydenova et al. (2013) found in a study with bird's-foot-trefoil, that as the developmental phase progressed, forage digestibility decreased.

Conclusions

Fertilization with organic fertilizers Blago 5 and Fertileader Axis showed a statistically significant positive effect in terms of acid and neutral detergent fibers, cellulose, and the degree of lignification in the fodder mass of bird's-foot-trefoil.

The biofertilizer Fertileader Axis applied in a dose of 1000 ml/da, reduces the amount of neutral detergent fibers, acid detergent lignin, hemicellulose and the degree of lignification.

The same biofertilizer applied at the lower dose of 500 ml/da, reduced the acid detergent fibers (by 74.1 $g.kg^{-1}$DM) and the cellulose content (by 86.4), to the highest degree compared to the control variant.

The included organic fertilizers showed that bird's-foot-trefoil responded positively to fertilizing, and increased the amount of hemicellulose maximally in all variants. The fertilizing lowered the cellulose levels, as after fertilizing with Fertileader Axis at a dose of 500 ml/da it reached 147.0 $g.kg^{-1}$DM.

Imported bioproducts had a negligible effect on the amount of gross and exchangeable energy, and feed units for milk were higher than feed units for growth. The highest nutritional value was registered after fertilizing with Blago 5 at a dose of 300 ml/da, whose forage had feed units for milk of 0.86% and for growth of 0.82%. Fertilizing with Fertileader Axis was more favorable in terms of digestibility, which was 68.77% in the forage fertilized with 500 ml/da, whereas the digestibility was 66.13% at a dose of 1000 ml/da.

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