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Botanical Composition and Quality Analysis of Grassland of Red Fescue (Festuca rubra L.) Treated with Lumbrical and Lumbrex Biofertilizers

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Abstract. The impact of biofertilizers (Lumbrical and Lumbrex), produced by red earthworm (*Lumbricus rubellus*), was studied on the botanical composition and forage quality of an artificial grassland of *Festuca rubra* L. 'Ryder' cultivar, expressed by the fiber composition of dried biomass. The studied bioproducts provide a higher (by 2%) presence of the main crop in the grassland and significantly reduce the weeds. The level of dry forage matter in the fiber structural components is influenced by the biofertilizers. The grasslands treated with Lumbrex 200 ml / da showed the lowest values of NDF (563.02 g kg^{-1} DM) and ADL (21.57 g kg^{-1} DM). The reduction of the lignin fraction was from 23.1% (Lumbrical 150 ml/m^2) to 63.6% (Lumbrex 200 ml/da). The foliar treatment with organic fertilizer decreased to the highest degree the lignification process and provided optimal conditions for obtaining ecologically pure agricultural products with improved quality indicators. Treatment of *Festuca rubra* L. with a granulated substance (Lumbrical 200 ml/m^2) decreased to the highest degree ADF amount (by 22.3%) in comparison to the control (367.82 g kg^{-1} DM). The highest concentration of hemicellulose (284.22 g kg^{-1} DM) and the highest *in vitro* dry matter digestibility (674.10 g kg^{-1} DM) was found in the forage mass of that variant. Indicator values exceeded the control by 32.7% and 13.1%, respectively. Bioproducts also reduced the cellulose content by 3.6% (Lumbrex 200 ml/da) to 20.6% (Lumbrical 150 ml/m^2). The highest energy nutritional value was found in the forage biomass in the variant with Lumbrex 150 ml/da. The compositions of the bioproduct tested increase the amount of gross and exchange energy, respectively, by 0.8 and 1.4% relative to the control.

Key words: bio-fertilization, *Festuca rubra* L., Lumbrical, Lumbrex.

Introduction

Technologies for enhancing the biological and productive potential of crop plants include agro-ecological methods developed on the basis of the optimal use of natural resources. The implementation of measures to preserve and improve the quality of grasslands allows the production of high quality plant and animal products.

The distribution of biological products and bio-fertilizers enriched with minerals and easily digestible micro and macroelements favours the biometric performance of forage grass crops (POPOV & YORDANOV, 2012) and the quality of the established grasslands (MASHEVA & MIHOV, 2008; MARINOVA *et al.*, 2019) and is common practice in agriculture in Bulgaria.

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Festuca rubra L. is a major component of grassland associations typical of the Central Balkan Mountain region. According to our research, the application of red earthworm products increases the productivity of hay-making grassland of red fescue in a foothill area and balances the mineral composition in dry feed. The soil application of Lumbrical (150 ml/m^2) in swards of red fescue increased their yield of fresh and dry mass by 34.5 and 37.0% respectively (BOZHANSKA, 2019). The biological activity of humic acids and their ability to form complexes with micro and macroelements, improves soil fertility (ANSARI & JAIKISHUN, 2011; CHAUHAN & SINGH, 2013), supports the development of beneficial soil microflora and facilitates the conversion and movement of organogenic elements in plant-accessible form (ANSARI & ISMAIL, 2012; MAHDI *et al.*, 2010; JAIKISHUN *et al.*, 2014). The granular fraction of *Lumbricus rubellus* activates the course of biochemical and physical processes in the soil, stimulates plant growth (LALITHA *et al.*, 2000; MAKULEC, 2002; ARANCON *et al.*, 2004; ANSARI & SUKHRAJ, 2010) and improves forage quality, reducing the fiber concentration of grassland by 7.5% and increasing the crude protein content by 20.8% (BOZHANSKA, 2019).

The production of forage with high nutritional and commercial value ensures their full utilization by farm animals. This is the purpose of the present study: to determine the influence of Lumbrical and Lumbrex biotopes on the botanical composition and nutritional value of red fescue sward in the Middle Balkan Mountains of Bulgaria.

Materials and Methods

The survey was conducted during the period 2014-2016 in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture – Troyan, Bulgaria.

Biofertilizers Lumbrical and Lumbrex, intended for soil and foliar application, were applied on artificial fly grassland of (*Festuca rubra* L.) 'Ryder' cultivar. The soil substance Lumbrical is an ecologically pure product resulting from the processing of organic waste through red earthworm (*Lumbricus rubellis*) and applied modern biotechnology. The product is high in nitrogen content (MARKOV, 2015) containing useful microorganisms, macro and micro elements in high concentrations. Organic matter is loose, dark brown and odourless. Biofertilizer is approved for application in organic production with Regulations: №15/03.08.1999 and №22/04.07.2001 of the Ministry of Agriculture and Forests, Bulgaria. Lumbrex liquid fertilizer (foliar application) contains humic and fulvic acids, macro and micro elements in certain concentrations.

We have applied commonly accepted grass meadow cultivation technology, which includes: 1. Soil processing - deep ploughing (23-25 cm) in autumn, immediately after harvesting the preculture and harrowing (in spring) to crush the lumps and level the terrain. 2. Pre-sowing rolling for the creation of a hard bed and better contact of the seeds with the soil. 3. Spring sowing (in the foothills the soil is better moisture preserved) done manually, scattered by the blocking method in 4 replication, with a plot size of 5 m^2 and a sowing rate of 2.5 kg da at 100% purity and seed germination rate. 4. Rolling of sown areas immediately after sowing.

In the year of creation of the swards (2014) the rainfall during the vegetation (March-October) was significantly higher (939.7 mm) compared to the average (514.4 mm) for a 20-year period. In 2015 and 2016, the amount of vegetation rainfall was 683.2 and 536.1 mm respectively. Average monthly air temperatures in the first (15.8°C), second (17.4°C) and third (16.3°C) vegetation were higher at 0.5 to 1.6°C relative to the average multi-year rate. The spring moisture in the years of the experiment created optimal

conditions for the formation of the first crop (Fig. 1).

Experimental variants are: 1. Control /nontreated/; 2. Lumbrical - 150 ml/m^2 (1 ml = 0.58 g); 3. Lumbrical - 200 ml/m^2 (1 ml = 0.58 g); 4. Lumbrex - 150 ml/da; 5. Lumbrex - 200 ml/da. The treatment of grasslands by granulated fraction of Lumbrical was performed immediately after the mowing, and foliar application by Lumbrex in tasseling stage.

The soils are light grey pseudopodzolic, low humus content (0.96-1.44%). The stockpile of soil with assimilable phosphorus (1.2-2.4 mg/100 g soil) according to KACHINSKI classification (1958) is very low, and assimilable potassium (5.9-9.9 mg/100 g soil) and mobile forms of nitrogen (8.6-20.2 mg/100 g of soil) very slight. The soil reaction is slightly acidic (pH_{H2O}=5.2-5.5; pH_{KCL}=4.3-4.4).

We observed the following indicators:

Botanical composition of grassland (%) - determined by weight analysis of grass green mass samples taken at each mowing of each variation. Their weighing is carried out in an air-dry state, by weighing the percentage of red fescue and weed species (in total).

Fiber components in plant cell were analyzed at the labaratory: Neutral Detergent Fibers (NDF, g kg^{-1} DM); Acid detergent fiber (ADF, g kg^{-1} DM) and Acid detergent lignin (ADL, g kg^{-1} DM) according to detergent analysis of VAN SOEST and ROBERTSON (1979), and *in vitro* dry matter digestibility (IVDMD, g kg^{-1} DM) by the two-step pepsin-cellulose method of AUFRERE (1982), which is carried out in two steps: I - Pre-treatment with pepsin (200 FIB-U g^{-1}), Merck 7190 in 1 N hydrochloric acid for 24 hours (for protein digestion) and II - Treatment in acid medium by cellulase enzyme "Onozuka R-10" that was isolated from *Trihoderma viride* /Endo-1.4-β-glucanase; 1.4 - (1.3:1.4)-β-D-glucan-4-glucan hydrolase/ with enzyme activity of 1.2 U g^{-1}.1 g of 1L in 0.05 M acetate buffer with pH 4.6 for 24 hours at 40°C (for cellulose digestibility). The polyosides were empirically calculated: Hemicellulose (g kg^{-1} DM) = NDF - ADF and Cellulose (g kg^{-1} DM) = ADF - ADL. The lignification degree is expressed through a coefficient as a percentage of ADL and NDF (AKIN & CHESSON, 1990).

The nutritional value of forage was estimated by the Bulgarian system as Feed unit for milk (FUM, in kg DM) and Feed units for growth (FUG, in kg DM) and calculated on the basis of equations according to the experimental values of CP, CFr, CF and NFE, recalculated by TODOROV (2010) digestibility ratios: Gross energy (GE, MJ/kg DM) = 0,0242*CP + 0.0366*CF + 0.0209*CFr + 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.

The swards were manually harvested, 20 days after a single foliar application of Lumbrex.

A variance analysis (ANOVA) was used for statistical data processing.

Results and Discussion

Botanical composition of an artificial grassland treated by Lumbrical and Lumbrex biofertilizers.

The biological features of the species, the qualities and adaptability of the variety used, as well as climate conditions in the seasons (BARSZCZEWSKI *et al.*, 2007), determine the growth and development of the crop component in the grassland and affect the botanical composition of regrowths and years. Red fescue is characterized by a slow initial growth rate, which allows a higher weed infestation (68-78%) of grasslands in first vegetation (Fig. 2).

In the year of sowing, under conditions of increased soil and air humidity, the share of that grass species exceeded the control by 2.0%, only in the variants of soil application with Lumbrical 200 ml/m^2. These results can be related to the conclusions of some authors (VLAHOVA *et al.*, 2015), according to which the nutrients imported by biofertilizers favour soil microflora activity, increase soil fertility and stimulate plant growth (TOPRE *et al.*, 2011; VLAHOVA & POPOV, 2018).

In a second vegetation, *Festuca rubra* L. was characterized by a stronger and more stable turf. The percentage of weeds in the soil treatment variants (Lumbrical 200 ml/ m^2) was lower reduced (by 3.4% compared to the control) compared to the foliar application (Lumbrex 150 and 200 ml/da) of biofertilizer, where the amount of weeds was reduced by 4.2% and 5.8% respectively, compared to the control. Foliar biofertilizers had a stronger positive effect on the botanical composition of red fescue. The impact of their effect is increased by enhancing the volume of the above-ground matter. The presence of the main crop in the treated grasslands ranged from 82.4% (Lumbrical 150 ml/m^2) to 93.3% (Lumbrex 200 ml/da).

Red fescue is a typical and persistent meadow grass, with a maximum growth rate in the third, fourth year of the growth cycle. In 2016 (third experimental year), the relative share of *Festuca rubra* L. in the forage mass of treated grasslands was 96.0-97.1%. The highest share of that sown grass was found in the variants of lower dose of Lumbrical and Lumbrex. The excess was 1.3-1.2%, respectively compared to the control.

Data from the analysis above suggests that fertilization (foliar and soil) of red fescue with Lumbrical and Lumbrex provided 2% higher share of main crop in the grassland and significantly reduced weeds. The share of this species is of fundamental importance for the formation and optimum accumulation of fodder biomass. The observed result of the effects of the fertilizers studied can be explained by the higher content of humic acids (2.28 times over fulvoacids - 7%), which stimulate the growth of the root system of the plants and increase the coefficient of nutrient utilization (TANG *et al.*, 2001). The quality and composition of applied organics improves the growth of other organs (ATIYEH *et al.*, 2002), which probably favors the adaptability of the red fescue to the growing conditions. *Fiber structural components of cell walls and in vitro digestibility of dry matter of Festuca rubra L. treated with biotope.*

Organic fertilizer introduced in the form of soil and leaf nutrition influences the nutritional and economic qualities of the feed culture and requires detailed consideration of the variation in the content of each structural component individually. The changes in the composition of the structural fiber components of the cell walls and *in vitro* digestibility of dry matter are a major factor for the quality and nutritional value of the feed (NAYDENOVA *et al.*, 2005).

The decrease in the amount of neutral and acid detergent fibers (related to the amount of forage taken), acid detergent lignin and cellulose and the increased content of fully digestible polyoside hemicellulose is an assessment of the effect of the studied biopreparations (Table 1). The positive result of the effect of the root and foliar application with the bioproduct is expressed in reducing the percentage concentration of lignin, neutral and acid detergent fibers, and increasing the amount of hemicellulose compared to the control variant.

On average, for the period of 2014-2016, the dry biomass of the foliar application variant with Lumbrex 200 ml/da had the lowest neutral detergent fiber content (563.02 g kg^{-1} DM) and the lowest values of indigestible acid detergent lignin by the animals (21.57 g kg^{-1} DM). Decreasing the concentration of both components is essential for the forage quality and the resulting animal production (KRÄMER *et al.*, 2010; MERTENS, 2016).

The comparison of laboratory biochemical data (at a fertilizer dosage of 150 and 200 ml/da) shows that Lumbrex 200 ml/da liquid fertilizer reduced the amount of biochemical characteristics associated with the nutritional value of the forage. The concentration of neutral and acidic detergent fibers and the concentration of the lignin fraction in the forage of *Festuca rubra* L. were lower than the control by 3.3%, 21.6% and 76.4% respectively.

Fig. 1. *Festuca rubra* L. – spring growing, first cut.

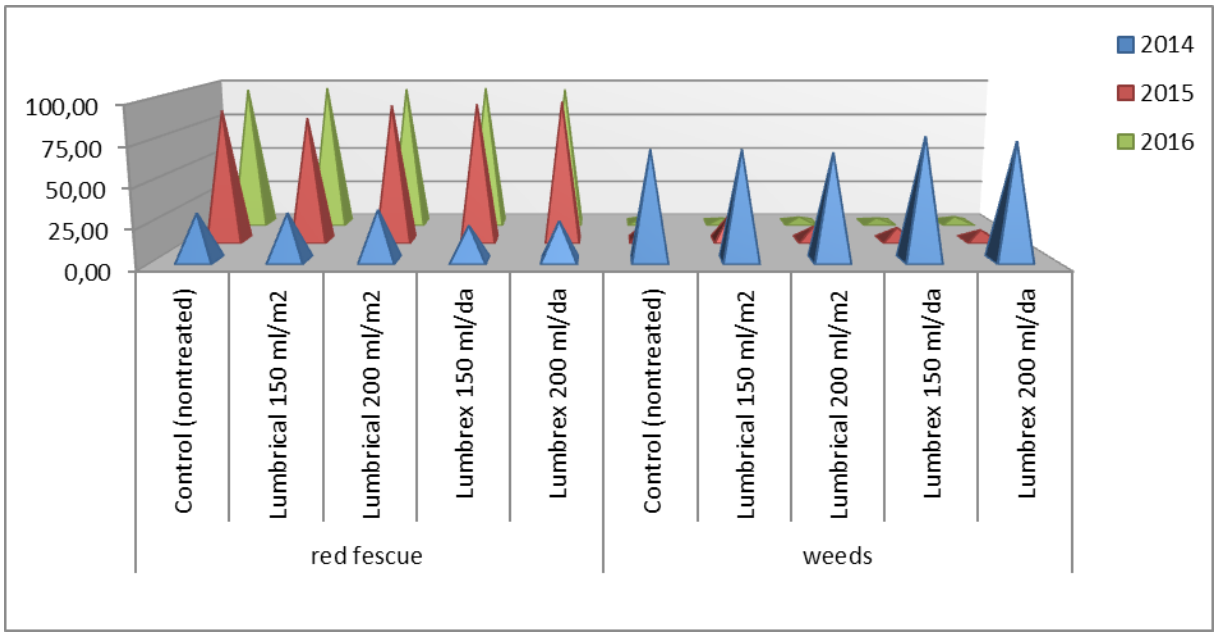

Fig. 2. Botanical composition of grassland with *Festuca rubra* L. treated by Lumbrical and Lumbrex (%).

Table 1. Main fiber cell structure components and *in vitro* digestibility of dry matter of *Festuca rubra* L. treated with Lumbrical and Lumbrex biofertilizers on average for the period 2014-2016 (g kg^{-1} DM).

Variants	NDF	ADL	ADL	Hemicellulosa	Cellulosa	IVDMD
Control	581.98	367.82	91.23	214.16	276.59	596.00
Lumbrical 150 ml/m^2	564.36	289.64	70.12	274.73	219.51	664.83
Lumbrical 200 ml/m^2	569.88	**285.66**	33.24	**284.22**	252.42	**674.10**
Lumbrex 150 ml/da	582.78	299.62	59.80	283.17	239.81	659.37
Lumbrex 200 ml/da	**563.02**	288.32	**21.57**	274.70	266.76	668.03
Average	***572.41***	***306.21***	***55.19***	***266.20***	***251.02***	***652.47***
SD	***9.5***	***34.8***	***28.1***	***29.4***	***22.5***	***32.0***

In grasslands enriched with granulated fraction of Lumbrical 150 and 200 ml/m^2, the detergent fiber decreased by 2.1-3.0% (for NDF), 21.3-22.3% (for ADF) and 23.1-63.6% (for ADL) compared to the control variant.

For the experimental period, the cellulose content of forage treated with the tested bioproducts was reduced by 3.6% (Lumbrex 200 ml/da) to 20.6% (Lumbrical 150 ml/m^2).

The fiber composition of forage plants is a major source of energy for ruminants (Hussain & Durrani, 2009) and a factor influencing the nutritional value of forage. The biological matter derived from the activity of red earthworm (*Lumbricus rubellis* L.) and applied to the grasslands of red fescue in a liquid and solid form can be considered as a specialized product that reduces the fiber content of the plant cell and increases the enzyme digestibility of forage biomass. The forage biomass treated with the granular substance of Lumbrical 200 ml/m^2 had the highest presence (73% - average for the experimental period) of the main crop in the grassland and with the highest *in vitro* dry matter digestibility (674.10 g kg^{-1} DM). The excess over the basic option and the average of the indicator is 13.1 and 3.3%. The harvested grassland of that variant is characterized by the lowest content of acidic detergent fiber (285.66 g kg^{-1} DM) in dry matter, and the amount of acidic detergent lignin was significantly lower (by 63.6%) than the soil application of Lumbrical 150 ml/m^2. Our results coincide with that found by Akin & Chesson (1990) regarding the content of the lignin fraction as a limiting factor for the digestibility of forage plants.

Hemicellulose is one of the main parameters determining the quality of forage and its weight occupies 20-40% of the composition of fiber components in the plant cell structure (McKendry, 2002). The soil nutrition of red fescue with Lumbrical 200 ml/m^2 affected the amount of hemicellulose at the highest degree (284.22 kg^{-1} DM). Indicator values exceed the control by 32.7%. In the other bio-fertilization variants, the increase in the fully digestible heteropolymer by animals was 28.3% (Lumbrex 200 ml/da) to 32.2% (Lumbrex 150 ml/da). In grasslands with a single foliar application, humic acids increase the permeability of cell membranes (Kaya *et al.*, 2005) and stimulate the biological and physiological processes in plants. Under the influence of the compositions of the studied lumbrical product (lumbri-culture), we observe significant changes in the content of the main fiber components as an energy source for the feeding of ruminants.

Percentage ratio between neutral detergent fibers and acidic detergent lignin determines the lignification degree of the biomass in each of the tested variants. Data analysis shows differences in lignification degree in the forage of grasslands with the application of bio-fertilization (Fig. 3).

On average, for the experiment period, the values in grasslands with reduced

dosage of Lumbrical (150 ml/m^2) and Lumbrex (150 ml/da) were higher than those treated at higher fertilization rates. The lignification degree is a biological process that influences the economic and nutritional value of the forage enriched. The foliar application of organic matter (Lumbrex 200 ml/da) reduced the lignification process to the highest degree and provided optimum conditions for obtaining environmentally friendly agricultural production with improved quality indicators.

Potential energy nutrition value of forage of Festuca rubra L., treated with Lumbrical and Lumbrex biofertilizers.

The precise identification of the energy value of forage has an important role to play in meeting the animal's food needs and realizing its productive potential. It is a key criterion for the contemporary assessment of the quality of biomass and is determined by the feed units for milk and growth (TODOROV & DARZANOV, 1995). The total energy value of the bio-fertilization variants varied from 17.96 MJ/kg DM (Lumbrical 200 ml/m^2) to 18.20 MJ/kg DM (Lumbrex 150 ml/da) - Table 2.

In the experimental years, the grassland with a lower dosage of foliar fertilizer had the most pronounced effect on the amount of gross energy. The excess over the control was 0.8%. The forage biomass of this variant had the highest content of physiologically exchange energy that is beneficial for animals (8.21 MJ/kg DM) and the number feed unit for milk (0.76 in kg DM) and growth (0.70 in kg DM) compared to the base and average indicator values. The data correlate with the analysis of the cell wall fiber components and the higher content of neutral (582.78 g kg^{-1} DM) and acidic (299.62 g kg^{-1} DM) detergent fibers (versus other treated variants) in the dry matter of forage.

Spraying with Lumbrex 200 ml/da and nourishing the grasslands with Lumbrical 150 ml/m^2 result in a higher energy value biomass in terms of the amount of gross energy. The values of the variants exceeded the net control and the mean for the experimental period respectively by 0.5-0.6 MJ/kg DM and 0.2-0.3 MJ/kg DM. The application of the tested preparations did not significantly affect the content of the exchange and net energy (FUM and FUG) in the dry feed mass of the variants with soil and leaf fertilization (150 ml/m^2 and 200 ml/da).

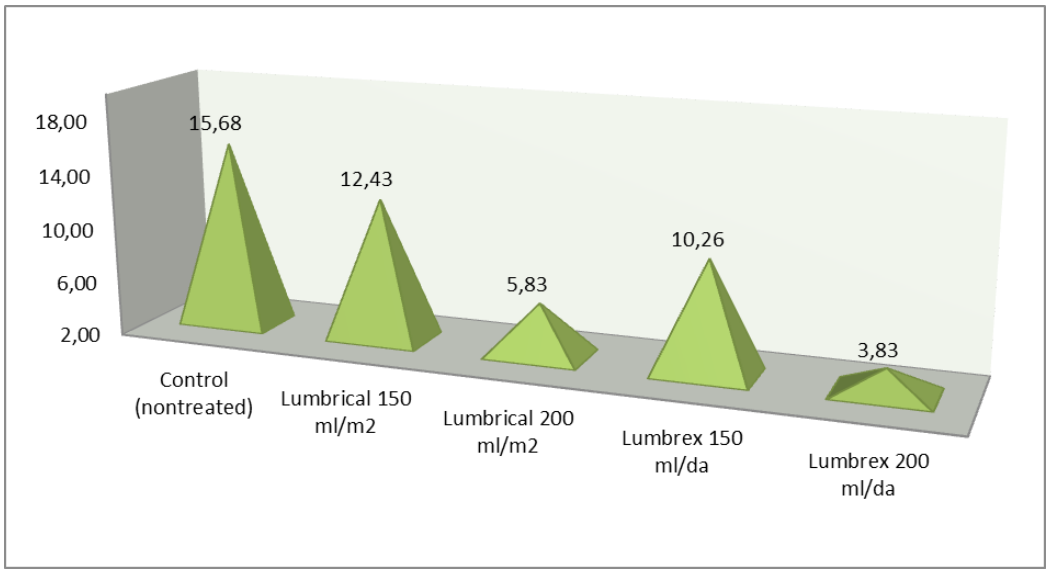

Fig. 3. Lignification degree of forage of *Festuca rubra* L. Treated with Lumbrical and Lumbrex biofertilizers (%).

Table 2. Potential energy nutrition value of grass biomass from *Festuca rubra* L. treated with Lumbrical and Lumbrex biofertilizers on average for 2014-2016. *Legend:* GE - gross energy - MJ/kg DM; EE - exchangeable energy (MJ/kg DM); FUM - feed unit for milk (in kg of dry matter); FUG - feed units for growth (in kg of dry matter).

Variants	GE	EE	FUM	FUG
Control	18.06	8.10	0.75	0.69
Lumbrical 150 ml/m^2	18.17	8.13	0.75	0.69
Lumbrical 200 ml/m^2	17.96	8.11	0.75	0.69
Lumbrex 150 ml/da	**18.20**	**8.21**	**0.76**	**0.70**
Lumbrex 200 ml/da	18.15	8.11	0.75	0.69
Averageo	***18.11***	***8.13***	***0.75***	***0.69***
SD	***0.099***	***0.045***	***0.004***	***0.005***

Conclusions

Swards treated with Lumbrex 200 ml/da had the lowest neutral detergent fiber and acid detergent lignin content. The imported foliar organic fertilizer reduced the lignification process to the highest degree by providing optimal conditions for obtaining environmentally friendly agricultural products with improved quality indicators.

Treating red fescue with Lumbrical 200 ml/m^2 lowered the acidic detergent fiber by 22.3%. Forage mass of the variant is characterized by the highest concentration of hemicellulose (284.22 g kg^{-1} DM) and in vitro dry digestibility (674.10 g kg^{-1} DM). The values of the indicators exceeded the nontreated control by 32.7% and 13.1%, respectively.

The studied biopreparations affected the content of the partially digestible and animal digestible polymer - cellulose in the dry matter of forage. The decrease in the values of the indicator is 3.6% (Lumbrex 200 ml/da) to 20.6% (Lumbrical 150 ml/m^2).

The highest energy nutritional value is found in the forage biomass of the lower foliar dosage (Lumbrex 150 ml/da). Compositions of the bioproduct tested increased the amount of gross and exchangeable energy, respectively, by 0.8 and 1.4% relative to the control.

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