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Application of Lumbrical and Lumbrex biofertilizers and their influence on the nutritional value and quality indicators in artificial grassland of bird's-foot-trefoil (*Lotus corniculatus* L.)

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Abstract

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The experiment was carried out during the period of 2014-2016 in the experimental field of the Department of Mountain Grass Associations and Maintenance of Their Biological Diversity at RIMSA – Troyan. The influence of Lumbrical and Lumbrex biofertilizers on the artificial grassland of *Lotus corniculatus* L., Leo cultivar, was studied. The consistency and dose of tested bioproducts have been found to influence the effectiveness of their effect and the quality of production. For the three-year study period, data suggests a higher amount of neutral and acid detergent fibers, acid detergent lignin, and cellulose in the dry feed mass of the soil treatment variants versus foliar application.

The treatment of bird's-foot-trefoil by a liquid fraction of Lumbrex 150 ml/da, in bud-formation period, decreased the values of the basic structural components of the cell walls respectively by 32.4% (ADL), 14.7% (ADF), 8.4% (Cellulose) and 8.2% (NDF) compared to the control. The biomass of the variant is characterized by the highest *in vitro* dry matter digestibility – 703.36 g kg^{-1} DM (6.7% above the control variant) and with the lowest lignification degree (the coefficient of lignification degree is 26.8% lower compared to the control).

Biological products imported had an insignificant effect on the amount of exchange energy (EE) and feed unit for milk (FUM) and growth (FUG). The total energy value (GE – gross energy) of bird's-foot-trefoil in treated grasslands exceeded the basic variant (19.05 MJ/kg DM) with 0.3% (Lumbrex 150 ml/da) to 2.4% (Lumbrical 150 ml/m^2).

Keywords: bio-fertilization; nutritional value; *Lotus corniculatus* L.

Introduction

The European systems for stock-breeding development skillfully combine the balance between the reality in the sector and the need to increase the production of environmentally friendly animal products by using local resources with minimal cost. The meadow and pasture complexes in Bulgaria are the main source of feed for livestock. The application of biological products in the sector is a good prospect to improve the quality of harvested biomass, with lower amounts of harmful emissions to the environment and soil (Godfray et al., 2010; Enchev & Kikindonov, 2015; Marinov-Serafimov et al., 2018; Marinova & Ivanova, 2018; Marinova et al., 2019).

Lumbrical and Lumbrex contain biologically active components (growth regulators, humic acids) that stimulate the development of soil microflora, facilitate the absorption of organogenic elements (Atanasova & Nencheva, 2012) and improve plant quality parameters (Vlahova & Popov, 2013; Marinova & Ivanova, 2018). Organic matter is a rich source of humus and all 16 elements (in concentrated form) necessary for the optimal growth and development of plants. Grasslands that are fertilized by bio-humus fertilizers produce ecologically pure products with high taste and nutritional qualities.

Perennial legumes, grown in Bulgaria under different ecological conditions, due to their specific biological features, are important forage for ruminants. The precise analysis of the basic chemical composition of the structural fiber components and dry matter digestibility for legumes is a significant factor in assessing the forage quality (Badrzadeh et al., 2008; Vasileva, 2008; Alizadeh & Teixeira da Silva, 2013; Naydenova et al., 2013; Bozhanska et al., 2015). The introduction of some environmentally friendly bioproducts in *Lotus corniculatus* L. grasslands, during the bud-formation period, reduces the cellulose content, neutral and acid detergent fibers, positively affects the amount and crude protein digestibility in the dry matter composition (Bozhanska et al., 2017). The fiber composition of the plant cell is a major energy source for ruminants, an indicator of the quality and consumption of forage (Moore, 1995).

The contemporary assessment of the grass biomass quality is related to the precise determination of its energy nutritional value, determined by the feed units of milk and growth (Todorov, 2010), whose numerical value influences the animal production amount.

The purpose of the study is to assess the forage quality of bird's-foot-trefoil treated with Lumbrical and Lumbrex, through fiber content in plant cell walls, *in vitro* digestibility of dry matter and estimation of potential energy nutritional value.

Material and Methods

The survey was conducted at the Research Institute of Mountain Stockbreeding and Agriculture – Troyan, in the period 2014-2016. Lumbrex and Lumbrical biofertilizers were tested on grassland with bird's-foot-trefoil of 'Leo' cultivar. These biofertilizers are ecological and pure substances with a high nitrogen content (Markov, 2015), resulting from the processing of organic waste by red earthworm (*Lumbricus rubellis*) and the application of modern biotechnology. They contain an organic substance of 45-50%; humic acids up to 14%; fulvoacids – 7%; ammonium nitrogen (NH_4-N) – 33.0 ppm; nitric nitrogen (NO_3-N) – 30.5 ppm; P_2O_5 – 1410 ppm; K_2O – 1910 ppm; a useful microflora of 2 x 1012 in numbers/g and a large number of NFE. The acidity of the product is 6.5–7.0 (pH in H_2O). The commercial products are a produced in the organic farm in the village of Kostievo (Plovdiv, Bulgaria), intended for soil and foliar fertilizing of plants in organic farming, according to EU Regulation 889/2008.

Agrochemical soil analysis

The soils in the area of experience are one of the poor in the country, making them unfavorable for agricultural crops because of their acidic (pH_{H2O} 5.2–5.5; pH_{KCL} 4.3–4.4) reaction and insufficient water-air regime (Table 1). Penkov et al. (1992) found them suitable for growing forage grasses because they provide high productivity.

Table 1. Agrochemical soil analysis (0–40 cm)

Soil characteristics	pH		Σ N – NH_4 + NO_3	P_2O_5	K_2O	Humus
Soil layer	H_2O	KCL	mg/kg^{-1}	mg/100		%
0–20	5.3	4.4	20.2	2.4	9.9	1.44
20–40	5.2	4.3	8.6	1.2	5.9	0.96

The total and assimilable phosphorus reserve in the soil of the experimental area (1.2–2.4 mg/100 g soil) was very low, and the assimilable potassium (5.9–9.9 mg/100 g of soil) and the mobile forms of nitrogen were poor (8.6–20.2 mg/100 g of soil). The humus content (0.96–1.44%) according to Kachinski classification (1958) was low. In such conditions, the application of foliar and soil fertilizers has a very positive influence on the processes of foliar and root feeding of the plants in order to increase the yield and quality of forage (Churkova, 2001; Churkova & Lingorski, 2010; Chourkova, 2013).

Experimental variants are: 1. Control (nontreated); 2. Lumbrical – 150 ml/m^2 (1 ml = 0.58 g Lumbrical); 3. Lumbrical – 200 ml/m^2 (1 ml = 0.58 g Lumbrical); 4. Lumbrex – 150 ml/da; 5. Lumbrex – 200 ml/da. Prior to sowing, we applied the necessary soil cultivation to create artificial grasslands. The sowing of the crop grown alone was carried out in spring (March), manually scattered using the blocking method in 4 replications, with a plot size of 5 m^2 and a seeding rate of 1.2 kg/da at 100% purity and germination rate. After sowing, the areas were rolled. During vegetation, Lumbrical fertilizer was introduced into the soil (after mowing, the dark brown granular fraction was evenly dispersed and mixed with the top soil layer, then watered abundantly). Spraying by Lumbrex was carried out in the phase of budding – beginning of flowering. The conventional technology for growing of grass meadows for forage was applied.

An average sample of dry biomass, taken from the first regrowth, was analyzed. The preparation of the plant material includes a ventilation drying at 65°C (to inactivate a large portion of the enzymes to terminate their activity without inducing denaturation of the protein fraction), followed by fixation for 20 min at 105°C and grinding the sample in a laboratory mill with a particle size of 1.0 mm.

The fibrous structural elements in the plant cell are analyzed in laboratory: 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) by the Van Soest and Robertson (1979) detergent assay and *in vitro* dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides are empirically calculated: Hemicellulose (g kg^{-1} DM) = NDF – ADF and Cellulose (g kg^{-1} DM) = ADF – ADL. The lignification degree is expressed as the percentage of ADL and NDF.

The nutritional value of the feed has been 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, CF, Cft and NFE, recalculated by Todorov digestibility ratios (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.

Experimental data were averaged and statistically processed using a variance analysis ANOVA.

Results and Discussion

Structural fiber components of cell walls and *in vitro* digestibility of dry matter of *Lotus corniculatus* L., treated with Lumbrical and Lumbrex

The biological value of forage depends to a large extent on fiber composition, digestibility and some other characteristics affecting animal productivity. Analyzed data indicated a higher amount of neutral and acid detergent fibers, acid detergent lignin, and cellulose in the dry feed mass in the variants with soil treatment compared to foliar. Average for the period of 2014–2016, the lowest values of the basic structural components of the cell walls (NDF – 366.68 g kg^{-1} DM; ADF – 248.76 g kg^{-1} DM; ADL – 52.25 g kg^{-1} DM, Cellulose – 196.51 g kg^{-1} DM) and the highest dry matter digestibility (703.36 g kg^{-1} DM) were found in the variant of a foliar application of Lumbrex in the bud-formation period (Table 2).

Liquid manure, applied at a dose of 150 ml/ da, reduced the amount of neutral and acid detergent fibers by 8.2 and 14.7%, respectively, compared to the control. There are analogous and clearly outlined results from the concentration of lignin and cellulose in the dry matter of the fodder crop. The treated vegetable mass had a lower ADL and cellulose content of 32.4% and 8.4%, respectively, compared to the base variant.

The quality of the vegetable mass is closely related to the biological process of lignification – a major factor limiting the nutritional value of forage and decreasing digestibility (Casler & Jung, 2006). Foliar treatment with Lumbrex 150 ml/da showed a positive effect on lignification coefficient values. Compositions (organic nitrogen, humic and fulvoacids, microelements and other organic substances) of the liquid fraction decreased the lignification degree in the bird‘s-foot-trefoil grassland with 26.8% compared to the control (Figure 1). In our other surveys conducted in the same agroecological area, the application of liquid biofertilizers into grasslands of *Lotus corniculatus* L. grown alone, increased the percentage of foliage of bird‘s-foot-trefoil and positively influenced the quality of the harvested grassland (Bozhanska et al., 2017). The results of this study suggest that the liquid form of Lumbrex stimulates the physiological activity of legume culture (through the gradual absorption of nutrients) and increases biological and productive potential.

The higher concentration of the test liquid formulation (200 ml/da) registered a lower efficiency with respect to the amount of cellulose (225.64 g kg^{-1} DM), neutral (412.71 g kg^{-1} DM) and acidic (298.53 g kg^{-1} DM) detergent fibers. The values for the dry matter of the variant were higher than those of the control (by 5.2%, 3.7% and 2.3%) and the average (3.7%, 2.1% and 3.1%) over the experimental period.

Table 2. Basic structural fiber components of cell walls and *in vitro* digestibility of dry matter of *Lotus corniculatus* L. treated with Lumbrical and Lumbrex on average for the period 2014–2016

Variant	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD
Control	399.27	291.80	77.30	107.47	214.50	659.38
Lumbrical 150 ml/m^2	419.86	305.92	81.52	113.94	224.40	647.60
Lumbrical 200 ml/m^2	422.02	300.89	73.85	121.13	227.04	658.27
Lumbrex 150 ml/da	<u>366.68</u>	<u>248.76</u>	<u>52.25</u>	117.91	<u>196.51</u>	<u>703.36</u>
Lumbrex 200 ml/da	412.71	298.53	72.89	114.18	225.64	672.16
Average	404.11	289.18	71.56	114.93	217.62	668.16
SD	22.7	23.2	11.3	5.1	12.8	21.5
$LSD_{0.05}$	24.6	28.4	19.5	8.1	26.5	24.6

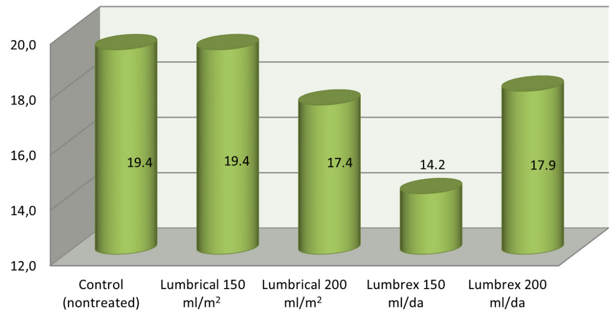

Fig. 1. Lignification degree of forage biomass from *Lotus corniculatus* L., treated with Lumbrical and Lumbrex biofertilizers (coefficient)

The digestibility of the harvested grassland of the variant is 1.9% higher than the control (659.38 g kg^{-1} DM).

The results can be related to the character and the dose of imported biological products. The solid consistency of Lumbrical is highly hygroscopic. The decomposition of organic matter into the soil requires optimal agro-climatic conditions (moisture and temperature) to stimulate the activity of soil microflora and the complete absorption from the root system of bird's-foot-trefoil. Dry forage matter of the variants that were fertilized by finely granulated fraction (150 ml/m^2 and 200 ml/m^2) is characterized by a higher content of cellulose, hemicellulose, neutral and acidic detergent fibers compared to the amount of structural fiber components in the control biomass (Cellulose – 214.50 g kg^{-1} DM; Hemicellulose – 107.47 g kg^{-1} DM; NDF – 399.27 kg kg^{-1} DM and ADF – 291.80 g kg^{-1} DM) and the average values for the three-year period (Cellulose – 217.62 g kg^{-1} DM; Hemicellulose – 114.93 g kg^{-1} DM; NDF – 404.11 g kg^{-1} DM and ADF – 289.18 g kg^{-1} DM).

Acid-detergent lignin is hard to digest and assimilate by animals. According to the data analysis, the foliar spraying with Lumbrex 200 ml/da decreased the indicator values by 5.7% compared to the control. A similar trend is also observed in the soil treatment with Lumbrical 200 ml/m^2, where the amount of lignocellulosic fraction in the dry matter was reduced by 4.5% compared to the basic variant.

The results obtained after the data analysis are indicative of the lignification degree of the plants and imply the provision of a forage mass of higher economic value.

Potential energy nutritional value of grassland of *Lotus corniculatus* L., treated with Lumbrical and Lumbrex

Dry forage feed enriched with Lumbrical and Lumbrex biofertilizers is more concentrated and of higher nutritional value. On average, for the three-year study period, the total energy value (GE – Gross Energy) of treated grasslands of bird's-foot-trefoil exceeded the basic variant (19.05 MJ/kg DM) with 0.3% (Lumbrex 150 ml/da) up to 2.4% (Lumbrical 150 ml/m^2) (Table 3).

Table 3. Potential energy nutritional value of grass biomass of *Lotus corniculatus* L. treated with Lumbrical and Lumbrex biofertilizers on average for the period 2014–2016

Variants	GE	EE	FUM	FUG
Control	19.05	8.29	0.76	0.69
Lumbrical 150 ml/m^2	19.51	8.17	0.75	0.67
Lumbrical 200 ml/m^2	19.13	8.28	0.76	0.69
Lumbrex 150 ml/da	19.12	8.18	0.75	0.68
Lumbrex 200 ml/da	19.30	8.20	0.75	0.68
Average	19.22	8.22	0.75	0.68
SD	0.2	0.1	0.0	0.0

Gross energy (GE, MJ/kg DM); Exchange Energy (EE, MJ/kg DM); feed unit for milk (FUM, number in kg DM) and feed units for growth (FUG, number in kg DM)

At the same time, the bioproducts studied did not have a positive effect on the amount of the exchange energy and the number of the feed unit for milk and feed unit for growth. The values of the indicators were lower than controls.

In the forage with soil nutrition of Lumbrical 200 ml/m^2 there was a slight prevalence in the amount of energy regulating the exchange processes (EE) in the animal organism and the net energy (FUM and FUG) compared to the other treated variants. The data correlate with the analysis of the cellular fiber components of *Lotus corniculatus* L. (in the variants enriched by granulated fraction), which make up 30-80% of the dry matter of plant biomass (Fahey and Hussein, 1999) and during their degradation they are the main energy source for ruminants. The values of the exchange energy were lower than the control (8.29 MJ/kg DM), but exceeded the average for the period (8.22 MJ/kg DM) with the minimum 0.7%, while the number of feed unit in the variant is higher than the rest of the treated grasslands and completely identical to the control (FUM – 0.76 in kg DM and FUG – 0.69 in kg DM).

For the study period, the number of milk feed units (0.75 in kg CB) in the dry matter of the treated legume culture was higher than the feed unit for growth (0.68 in kg DM), which makes the grassland with foliar nutrition more suited to the needs of lactating animals.

Conclusion

The consistency and the dose of tested bioproducts influence the effectiveness of their effect and the quality of the resulting production. For the three-year study period, the most significant effect in reducing the amount of fiber components of plant cell walls in *Lotus corniculatus* L. was found in the effect by Lumbrex, in its foliar application in the bud-formation period at a dose of 150 ml/da. Indicator values: Acid detergent lignin, acid detergent fibers, cellulose and neutral detergent fibers are lower than the control by: 32.4%, 14.7%, 8.4% and 8.2%, respectively. Treated grassland of the variant is also with the lowest degree of lignification.

Dry forage mass enriched with Lumbrical and Lumbrex biofertilizers is more concentrated and of higher nutritional value. The amount of gross energy (GE) in the variants exceeds the nontreated control by 0.3% (Lumbrex 150 ml/da) to 2.4% (Lumbrical 150 ml/m^2).

The results obtained give us reason to recommend the event as an addition to the cultivation technology for bird's-foot-trefoil.

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