

## Correlation and regression relationships between quantity and quality indicators of forage of *Lotus corniculatus* L. and *Festuca rubra* L., treated by bio-fertilizers

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### Abstract

Bozhanska, T. (2021). Correlation and regression relationships between quantity and quality indicators of forage of *Lotus corniculatus* L. and *Festuca rubra* L., treated by bio-fertilizers. *Bulg. J. Agric. Sci.*, 27 (1), 97–106

Correlation and regression dependences between the main chemical indicators of dry biomass of *Lotus corniculatus* L. (cv. ‚Leo‘) and *Festuca rubra* L. (cv. ‚Ryder‘), treated with Lumbrical and Lumbrex biofertilizers. The survey was conducted in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture – Troyan to assess the quality and nutritional value of legume and grass species, under no irrigated conditions and vegetative introduction of biological products. They are a result of the processing of organic waste through the red earthworm (*Lumbricus rubellis*) and the application of modern biotechnology. High correlation dependence was found between the height of treated grasslands and the crude protein content, hemicellulose and cellulose. The correlation coefficients between the indicators are:  $r = 0.91$ ,  $r = 0.64$  and  $r = 0.69$  (in bird’s-foot-trefoil) and  $r = 0.74$ ,  $r = 0.96$  and  $r = 0.73$  (in red fescue). The imported biological products increased dry matter digestibility for both forage crops. The regression dependence is statistically proven both for the percentage share of leaves ( $R^2 = 0.354$ ) and the mineral content ( $R^2 = 0.777$ ) for bird’s-foot-trefoil, and the height of the grassland ( $R^2 = 0.897$ ), crude protein ( $R^2 = 0.820$ ), crude fats ( $R^2 = 0.696$ ) and hemicellulose ( $R^2 = 0.955$ ) in the dry forage matter of red fescue. Analyzed data indicates a positive correlation between the percentage share of *Festuca rubra* L. in the grassland and the values of phosphorus ( $r = 0.98$ ), mineral substances ( $r = 0.79$ ) and cellulose ( $r = 0.59$ ). Regression equations were developed to predict the calcium and phosphorus content by the amount of stem fraction of *Lotus corniculatus* L. at a high determination coefficient:  $R^2 = 0.939$  (Ca) and  $R^2 = 0.907$  (P). The correlations between empirically calculated values of nitrogen-free extractable substances were statistically proven with a concentration of: neutral detergent fibers ( $r = 0.74$ ), acid detergent fibers ( $r = 0.74$ ), acid detergent lignin ( $r = 0.59$ ) and partially digestible polyside cellulose ( $r = 0.82$ ) in the dry matter composition of bird’s-foot-trefoil.

*Keywords:* *Lotus corniculatus* L.; *Festuca rubra* L.; bio-fertilization; correlation and regression dependencies

### Introduction

*Lotus corniculatus* L. and *Festuca rubra* L. are the main species in the natural grass associations for the conditions of the Central Balkan Mountains. They are characterized by high productive potential, ecological plasticity, sustainability and adaptability (Mitev & Naydenova, 2008; Churkova, 2011; Mitev & Naydenova, 2014). Bird’s-foot-trefoil and

red fescue are a valuable forage source for farm animals, and each farmer’s aim is to provide high-quality and quality biomass (Sanderson, 2010; Reheul et al., 2011; Butnariu et al., 2016; Churkova, 2020) closely related to animal health and animal health production. The application of biotechnologies that increase soil fertility (Wu et al., 2004; Ahmad et al., 2009; Ahmad et al., 2014; Radovich et al., 2014; Butnariu, 2018), improve the biological and qualitative characteristics

of meadow grasses (Georgieva & Nikolova, 2011; Bozhanska et al., 2017a,b; Bozhanska, 2018; Ivanova & Marinova, 2018; Marinova & Ivanova, 2018) and have a significant social and ecological effect. The application of biofertilizers, such as Lumbrical and Lumbrex (a result of the activity of red earthworm) into the agrotechnical characteristics of *Lotus corniculatus* L. and *Festuca rubra* L. offers high digestible forage rich in protein and mineral substances (Bozhanska, 2018; Bozhanska, 2019). When they are introduced as vegetative feeding (directly soil or leaf application), the biofertilizers provide the necessary nutrients and improve the physiological status of the plants (Vlahova et al., 2013). Their action can also be defined as a growth stimulant that enhances the metabolic processes in the plant, improves the rooting process, increases the volume of soil microflora and increases the resistance of plants to water and temperature stress (Nickell, 1994). The application of biological products balances the nutritional regime of plants and allows for the formation of a dynamic complex between them and the set of environmental conditions (Fageria et al., 2009; Haytova, 2013).

Data analysis on the growth and development, composition and nutritional value of forage grasses are predictors of the most accurate assessment of forage quality.

The aim of the present study is to determine the correlation and regression relationships between key nutrient values and the quality of forage biomass of bird's-foot-trefoil and red fescue with soil and foliar application with Lumbrical and Lumbrex biofertilizers.

## Material 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 on the impact of Lumbrex and Lumbrical biofertilizers on artificial grassland of bird's-foot-trefoil (cv. „Leo“) and red fescue (cv. „Ryder“). The experiment was carried out in nonirrigated conditions on light gray /pseudopodzolic/ soils with low organic content, low water permeability and surface over-wetting.

Lumbrical and Lumbrex are produced by the organic farm in the village of Kostievo (Plovdiv, Bulgaria), as a result from the processing of organic waste through red earthworm (*Lumbricus rubellis*) and applied modern biotechnology. They are used as soil and foliar application to supply plants in organic farming, according to EU Regulation 889/2008. They contain an organic substance of 45-50%; humic acids up to 14%; fulvoacids – 7%; ammonium nitrogen (NH<sub>4</sub>-N) – 33.0 ppm; nitric nitrogen (NO<sub>3</sub>-N) – 30.5 ppm; P<sub>2</sub>O<sub>5</sub> – 1410 ppm; K<sub>2</sub>O – 1910 ppm; a useful microflora of 2 x 10<sup>12</sup> pcs./g

and a large number of biological active substances. The acidity of the product is 6.5-7.0 (pH in H<sub>2</sub>O). Lumbrical is an organic matter of dark brown colour and a high degree of biochemical active substances from the group of growth regulators. Lumbrex is a foliar application extract, extremely effective and high in organic nitrogen.

Experimental variants are: 1. Control /nontreated/; 2. Lumbrical – 150 ml/m<sup>2</sup> (1 ml = 0.58 g Lumbrical); 3. Lumbrical – 200 ml/m<sup>2</sup> (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 grassland. The manual sowing of the single crops was carried out in the second half of March. The experimental design was a block with 4 replications. The plot size was 5 m<sub>2</sub> at a seeding rate of 1.2 kg/da (for bird's-foot-trefoil) and 2.5 kg/da (for red fescue) at 100% purity and germination rate. After sowing, the areas were rolled. The granulated fraction of Lumbrical was applied immediately after the mowing. The liquid form of Lumbrex was sprayed in the bud-formation period (for bird's-foot-trefoil) and spindle phase (for red fescue). The harvesting was conducted 20 days after the treatment of grasslands.

The following indicators were followed:

- Botanical composition of grassland (%) – determined by weight analysis of samples of green mass taken at each mowing of each variation. Their weighing was carried out in an air-dry state, by weighing the percentage of grass species.
- Morphological composition of grasslands (%) – carried out by weight of 40 plants taken from each variant (and repetition) during the harvesting period of the grassland. The amount of stems and leaves is determined by the weighting method, on the basis of which is calculated their percentage of biomass.
- Height (cm) – measured according to mowing during the harvest period of the grassland. The plants were measured according to the diagonals of each plot (at 4 points) from the soil surface to the top of the highest stems, and on the basis of the data obtained the mean values were calculated.
- The chemical composition of dry feed was analyzed according to Weende analysis: Crude protein (CP, g kg<sup>-1</sup> DM) according to *Kjeldahl* (according to BDS/ISO-5983); Crude fiber (CFr, g kg<sup>-1</sup> DM); Crude fat (CF, g kg<sup>-1</sup> DM) (according to BDS/ISO-6492) – by extraction into a *Soxhlet* extractor; Minerals substances – Ash (g kg<sup>-1</sup> DM) – (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 %

Table 1. Correlation dependences between the main qualitative and quantitative indicators of forage of *Lotus corniculatus* L. treated with Lumbribral and Lumbrex biofertilizers

|           | Leaves<br>%  | Stems<br>%   | Heights<br>cm | CP<br>g kg <sup>-1</sup> | CFr<br>g kg <sup>-1</sup> | CF<br>g kg <sup>-1</sup> | Ash<br>g kg <sup>-1</sup> | NFE<br>g kg <sup>-1</sup> | Ca<br>g kg <sup>-1</sup> | P<br>g kg <sup>-1</sup> | NDF<br>g kg <sup>-1</sup> | ADF<br>g kg <sup>-1</sup> | ADL<br>g kg <sup>-1</sup> | Hemi-<br>cell,<br>g kg <sup>-1</sup> | Cellu-<br>lose<br>g kg <sup>-1</sup> | DMD<br>g kg <sup>-1</sup> | GE<br>MJ/kg | EE<br>MJ/kg  | FUM<br>in kg<br>DM | FUG<br>in kg<br>DM |  |
|-----------|--------------|--------------|---------------|--------------------------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|-------------------------|---------------------------|---------------------------|---------------------------|--------------------------------------|--------------------------------------|---------------------------|-------------|--------------|--------------------|--------------------|--|
| Leaves    | 1            |              |               |                          |                           |                          |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| Stems     | -1.00        | 1            |               |                          |                           |                          |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| Heights   | 0.33         | -0.33        | 1             |                          |                           |                          |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| CP        | 0.28         | -0.28        | <u>0.91*</u>  | 1                        |                           |                          |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| CFr       | 0.27         | -0.27        | <u>0.61*</u>  | <u>0.67*</u>             | 1                         |                          |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| CF        | -0.12        | 0.12         | 0.01          | -0.03                    | <u>0.69*</u>              | 1                        |                           |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| Ash       | 0.17         | -0.17        | -0.72         | -0.45                    | -0.15                     | 0.03                     | 1                         |                           |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| NFE       | 0.39         | -0.39        | <u>0.64*</u>  | 0.28                     | 0.27                      | 0.16                     | -0.75                     | 1                         |                          |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| Ca        | -0.97        | <u>0.97*</u> | -0.10         | -0.05                    | -0.16                     | 0.06                     | -0.33                     | -0.30                     | 1                        |                         |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| P         | -0.95        | <u>0.95*</u> | -0.38         | -0.45                    | -0.36                     | 0.17                     | -0.25                     | -0.19                     | <u>0.89*</u>             | 1                       |                           |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| NDF       | -0.19        | 0.19         | <u>0.69*</u>  | 0.42                     | 0.10                      | -0.07                    | -1.00                     | <u>0.74*</u>              | 0.35                     | 0.27                    | 1                         |                           |                           |                                      |                                      |                           |             |              |                    |                    |  |
| ADF       | -0.28        | 0.28         | <u>0.55*</u>  | 0.24                     | 0.05                      | 0.06                     | -0.97                     | <u>0.74*</u>              | 0.40                     | 0.41                    | <u>0.98*</u>              | 1                         |                           |                                      |                                      |                           |             |              |                    |                    |  |
| ADL       | -0.51        | <u>0.51*</u> | 0.34          | 0.05                     | 0.01                      | 0.21                     | -0.87                     | <u>0.59*</u>              | <u>0.58*</u>             | <u>0.64*</u>            | <u>0.88*</u>              | <u>0.96*</u>              | 1                         |                                      |                                      |                           |             |              |                    |                    |  |
| Hemicell  | 0.62         | -0.62        | <u>0.64*</u>  | <u>0.77*</u>             | 0.24                      | -0.52                    | -0.09                     | 0.11                      | -0.44                    | -0.78                   | 0.08                      | -0.14                     | -0.40                     | 1                                    |                                      |                           |             |              |                    |                    |  |
| Cellulose | -0.06        | 0.06         | <u>0.69*</u>  | 0.39                     | 0.09                      | -0.08                    | -0.99                     | <u>0.82*</u>              | 0.21                     | 0.18                    | <u>0.99*</u>              | <u>0.97*</u>              | <u>0.85*</u>              | 0.10                                 | 1                                    |                           |             |              |                    |                    |  |
| DMD       | <u>0.59*</u> | -0.59        | -0.38         | -0.15                    | 0.00                      | -0.11                    | <u>0.88*</u>              | -0.47                     | -0.69                    | -0.67                   | -0.89                     | -0.94                     | -0.98                     | 0.31                                 | -0.83                                | 1                         |             |              |                    |                    |  |
| GE        | 0.06         | -0.06        | <u>0.72*</u>  | <u>0.65*</u>             | <u>0.90*</u>              | <u>0.69*</u>             | -0.53                     | 0.53                      | 0.08                     | -0.08                   | 0.48                      | 0.47                      | 0.44                      | 0.08                                 | 0.46                                 | -0.42                     | 1           |              |                    |                    |  |
| EE        | -0.42        | 0.42         | -0.35         | -0.47                    | -0.93                     | -0.71                    | -0.20                     | -0.10                     | 0.38                     | 0.50                    | 0.25                      | 0.28                      | 0.30                      | -0.20                                | 0.24                                 | -0.34                     | -0.70       | 1            |                    |                    |  |
| FUM       | -0.38        | 0.38         | -0.44         | -0.52                    | -0.97                     | -0.74                    | -0.07                     | -0.19                     | 0.31                     | 0.45                    | 0.12                      | 0.15                      | 0.17                      | -0.19                                | 0.12                                 | -0.21                     | -0.79       | <u>0.99*</u> | 1                  |                    |  |
| FUG       | -0.33        | 0.33         | -0.51         | -0.57                    | -0.99                     | -0.75                    | 0.04                      | -0.26                     | 0.25                     | 0.40                    | 0.01                      | 0.04                      | 0.06                      | -0.18                                | 0.01                                 | -0.10                     | -0.86       | <u>0.97*</u> | <u>0.99*</u>       | 1                  |  |

\*P &lt; 0.05

**Table 2. Correlation dependences among main qualitative and quantitative indicators of forage of *Festuca rubra* L. treated with Lumbriol and Lumbrex biofertilizers**

|           | Grasses %    | Heights cm   | CP g kg <sup>-1</sup> | CFr g kg <sup>-1</sup> | CF g kg <sup>-1</sup> | Ash g kg <sup>-1</sup> | NFE g kg <sup>-1</sup> | Ca g kg <sup>-1</sup> | P g kg <sup>-1</sup> | NDF g kg <sup>-1</sup> | ADF g kg <sup>-1</sup> | ADL g kg <sup>-1</sup> | Hemicellulose g kg <sup>-1</sup> | Cellulose g kg <sup>-1</sup> | DMD g kg <sup>-1</sup> | GE MJ/kg     | EE MJ/kg     | FUM in kg DM | FUG in kg DM |
|-----------|--------------|--------------|-----------------------|------------------------|-----------------------|------------------------|------------------------|-----------------------|----------------------|------------------------|------------------------|------------------------|----------------------------------|------------------------------|------------------------|--------------|--------------|--------------|--------------|
| Grasses   | 1            |              |                       |                        |                       |                        |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| Heights   | 0.29         | 1            |                       |                        |                       |                        |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| CP        | 0.02         | <u>0.74*</u> | 1                     |                        |                       |                        |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| CFr       | -0.63        | -0.74        | -0.57                 | 1                      |                       |                        |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| CF        | 0.21         | <u>0.88*</u> | <u>0.73*</u>          | -0.87                  | 1                     |                        |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| Ash       | <u>0.79*</u> | 0.38         | 0.51                  | -0.75                  | 0.42                  | 1                      |                        |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| NFE       | -0.15        | -0.54        | -0.80                 | 0.22                   | -0.28                 | -0.47                  | 1                      |                       |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| Ca        | -0.19        | -0.35        | 0.22                  | 0.47                   | -0.50                 | 0.13                   | -0.60                  | 1                     |                      |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| P         | <u>0.98*</u> | 0.14         | 0.00                  | -0.55                  | 0.09                  | <u>0.83*</u>           | -0.15                  | -0.04                 | 1                    |                        |                        |                        |                                  |                              |                        |              |              |              |              |
| NDF       | -0.14        | -0.44        | -0.69                 | 0.09                   | -0.13                 | -0.41                  | <u>0.99*</u>           | -0.68                 | -0.16                | 1                      |                        |                        |                                  |                              |                        |              |              |              |              |
| ADF       | -0.11        | -0.93        | -0.92                 | 0.61                   | -0.81                 | -0.39                  | <u>0.76*</u>           | 0.01                  | -0.01                | <u>0.66*</u>           | 1                      |                        |                                  |                              |                        |              |              |              |              |
| ADL       | -0.44        | -0.95        | -0.55                 | 0.66                   | -0.74                 | -0.35                  | 0.50                   | 0.37                  | -0.27                | 0.43                   | <u>0.83*</u>           | 1                      |                                  |                              |                        |              |              |              |              |
| Hemicell  | 0.09         | <u>0.96*</u> | <u>0.86*</u>          | -0.69                  | <u>0.91*</u>          | 0.33                   | -0.58                  | -0.23                 | -0.04                | -0.46                  | -0.97                  | -0.84                  | 1                                |                              |                        |              |              |              |              |
| Cellulose | 0.59         | <u>0.73*</u> | 0.12                  | -0.54                  | 0.50                  | 0.22                   | -0.16                  | -0.57                 | 0.41                 | -0.13                  | -0.47                  | -0.89                  | 0.52                             | 1                            |                        |              |              |              |              |
| DMD       | 0.16         | <u>0.95*</u> | <u>0.91*</u>          | -0.66                  | <u>0.83*</u>          | 0.42                   | -0.74                  | -0.06                 | 0.05                 | -0.63                  | -1.00                  | -0.85                  | <u>0.98*</u>                     | 0.51                         | 1                      |              |              |              |              |
| GE        | -0.89        | 0.07         | 0.08                  | 0.48                   | 0.01                  | -0.81                  | 0.00                   | -0.01                 | -0.96                | 0.01                   | -0.17                  | 0.01                   | 0.21                             | -0.15                        | 0.13                   | 1            |              |              |              |
| EE        | -0.50        | 0.36         | 0.13                  | -0.19                  | 0.56                  | -0.47                  | 0.37                   | -0.69                 | -0.63                | 0.47                   | -0.25                  | -0.22                  | 0.45                             | 0.14                         | 0.25                   | <u>0.63*</u> | 1            |              |              |
| FUM       | -0.42        | 0.36         | 0.13                  | -0.28                  | 0.60                  | -0.38                  | 0.40                   | -0.74                 | -0.54                | 0.52                   | -0.23                  | -0.22                  | 0.44                             | 0.15                         | 0.25                   | 0.53         | <u>0.99*</u> | 1            |              |
| FUG       | -0.30        | 0.36         | 0.13                  | -0.40                  | 0.65                  | -0.25                  | 0.45                   | -0.79                 | -0.41                | 0.57                   | -0.21                  | -0.21                  | 0.43                             | 0.15                         | 0.23                   | 0.38         | <u>0.95*</u> | <u>0.98*</u> | 1            |

\*P < 0.05

moisture; Nitrogen-free extractable substances (NFE, %) =  $100 - (\text{CP, \%} + \text{CFr, \%} + \text{CF, \%} + \text{Ash, \%} + \text{Moisture, \%})$  converted to  $\text{g kg}^{-1}$  DM; calcium (Ca,  $\text{g kg}^{-1}$  DM) – Stotz (complexometric) and phosphorus (P,  $\text{g kg}^{-1}$  DM) – with vanadate-molybdate reagent according to Gerike and Kurmis method- spectrophotometer (*Agilent 8453 UV – visible Spectroscopy System*) that measure in the sphere of 425 nm.

- Feed nutritional value was assessed by the Bulgarian system as feed unit for milk (FUM) and feed units for growth (FUG) and calculated on the basis of equations according to the experimental values of CP, CFr, CF and NFE, recalculated by the coefficients for digestibility (Todorov, 2010): Gross energy (GE, MJ/kg DM) =  $0.0242 \cdot \text{CP} + 0.0366 \cdot \text{CF} + 0.0209 \cdot \text{CFr} + 0.017 \cdot \text{NFE} - 0.0007 \cdot \text{Zx}$  and Exchangeable energy (EE, MJ/kg DM) =  $0.0152 \cdot \text{DP}$  (Digestible protein) +  $0.0342 \cdot \text{Dft}$  (Digestible fat) +  $0.0128 \cdot \text{DF}$  (Digestible fibers) +  $0.0159 \cdot \text{DNFE}$  (Digestible Nitrogen-free extractable) –  $0.0007 \cdot \text{Zx}$ .
- The fiber structural elements in plant cell were analyzed in a laboratory: Neutral Detergent Fibers (NDF,  $\text{g kg}^{-1}$  DM); Acid detergent fiber (ADF,  $\text{g kg}^{-1}$  DM) and Acid detergent lignin (ADL,  $\text{g kg}^{-1}$  DM) accord-

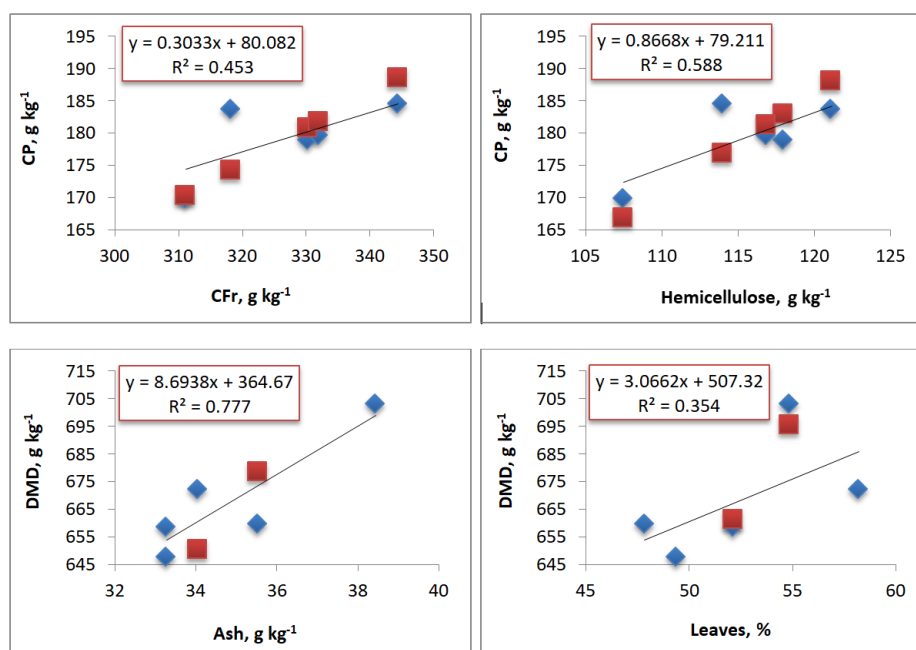
ing to detergent analysis (Van Soest & Robertson, 1979), and *in vitro* dry matter digestibility (DMD,  $\text{g kg}^{-1}$  DM) according to two-step pepsin-cellulose method (Aufreere, 1982). The polyoses were empirically calculated: Hemicellulose ( $\text{g kg}^{-1}$  DM) = NDF – ADF and Cellulose ( $\text{g kg}^{-1}$  DM) = ADF – ADL.

Data were processed by software products *Analysis Toolpak for Microsoft Excel 2010* and *STATSOFT Statistics for Windows 10*.

## Results and Discussion

### *The correlation and regression dependences between key qualitative and quantitative indicators*

Data for a three-year study period indicate a high correlation dependence between the height of treated grasslands and the crude protein content, hemicellulose and cellulose (Tables 1 and 2) in the dry matter of both forage crops. The correlation coefficients between the indicators are:  $r = 0.91$ ;  $r = 0.64$  and  $r = 0.69$  (for bird's-foot-trefoil) and  $r = 0.74$ ;  $r = 0.96$  and  $r = 0.73$  (for red fescue). For legume crops, plant height is in a strong correlation dependence with the amount of raw fiber ( $r = 0.61$ ), nitrogen-free extractable substances ( $r = 0.64$ ), neutral ( $r = 0.69$ ) and acidic ( $r = 0.55$ ) detergent fibers and the content



**Fig. 1.** Regression dependences between dry matter digestibility and percentage share of leaves and the ash content in *Lotus corniculatus* L. biomass treated with Lumbrical and Lumbrex, and between the amount of crude protein with crude fiber content and hemicellulose

of the exchangeable energy ( $r = 0.72$ ) in the forage. The height of grassland in grass species shows a positive correlation with the amount of crude fats ( $r = 0.88$ ) and a strong negative correlation with the concentration of acidic detergent fibers ( $r = -0.93$ ) and acid-detergent lignin ( $r = -0.95$ ).

The analysis of the main chemical composition (Mihovski & Goranova, 2006), the composition of cell wall components and digestibility give a clear assessment of the quality and nutritional value of forage (Pavlov, 1996; Naydenova et al., 2013).

A number of researchers (Georgieva & Nikolova, 2010; Terzic et al., 2012; Enchev, 2013; Pachev, 2013; Vasileva, 2015; Bozhanska et al., 2017a; Marinova & Ivanova, 2018) study the impact of foliar fertilizers, growth regulators and biostimulators to seek new active environmental factors to protect the environment and to influence the quantitative and qualitative characteristics of crops. The applied bioproducts put dry matter digestibility of bird's-foot-trefoil in high correlation dependence with the percentage of leaves and the content of mineral substances. The theoretical regression lines and the equations of regression dependence (Figure 1) between the indicators are:

- $Y = 3.0662x + 507.32$  at determination coefficient  $R^2 = 0.354$ ;

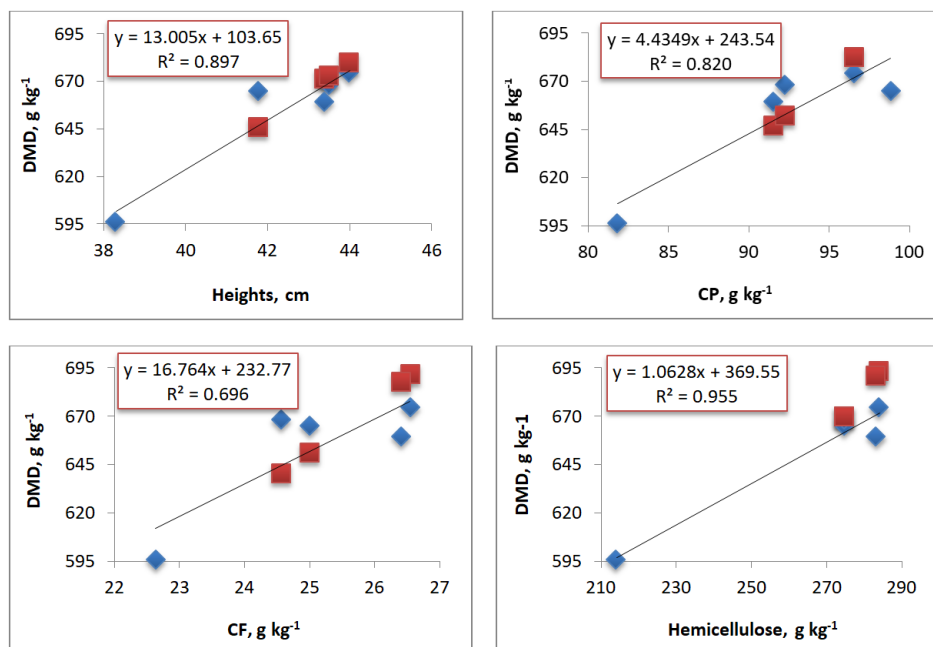
- $Y = 8.6938x + 364.67$  at the determination coefficient  $R^2 = 0.777$ .

Dry matter digestibility of red fescue strongly correlates with the biometric indicator height of grassland with some basic chemical indicators (crude protein and crude fats) and fiber components of cell walls (hemicellulose) in the dry forage mass. Their statistical correlation coefficients are true for the inequality  $1.0 \geq |r_{XY}| > 0.6$ , as a result of which we consider that there is strong correlation dependence between the analyzed indicators. In Figure 2 the corresponding regression equations are given:

- $Y = 13.005x + 103.65$  at determination coefficient  $R^2 = 0.897$ ;
- $Y = 4.4349x + 243.54$  at determination coefficient  $R^2 = 0.820$ ;
- $Y = 16.764x + 232.77$  at determination coefficient  $R^2 = 0.696$ ;
- $Y = 1.0628x + 369.55$  for the determination coefficient  $R^2 = 0.955$ ;

with a level of significance  $P < 0.05$ , through which it is possible to predict dry matter digestibility of forage of *Festuca rubra* L. treated with Lumbrical and Lumbrex biofertilizers.

The amount of protein fraction is a major factor influencing the quality, nutritional value and absorption of forage by



**Fig. 2. Regressional Dependences between dry matter digestibility in biomass of *Festuca rubra* L., treated with biofertilizers Lumbrical and Lumbrex and the height of grassland, the amount of crude protein, protein, hemicellulose and crude fat**

animals. The crude protein content of the treated grasslands with bird's-foot-trefoil is in good regression dependence with the fully digestible and absorbed polyose hemicellulose ( $R^2 = 0.588$ ) and the crude fiber concentration ( $R^2 = 0.453$ ) – Figure 1.

In red fescue forage, crude protein values increased from 11.9% to 20.8% after foliar and soil introduction of the studied bioproducts (Bozhanska, 2019). There is a high correlation dependence between this qualitative index and the amount of crude fat ( $r = 0.73$ ) and hemicellulose ( $r = 0.86$ ). Crude protein shows the highest degree of negative correlation with acid detergent fibers ( $r = -0.92$ ) and nitrogen-free extractable substances ( $r = -0.80$ ). The percentage of grass crops in the grassland affected the change in the average values of phosphorus, minerals and cellulose. The results of the analysis show a positive correlation between the indicators:  $r = 0.98$  (P);  $r = 0.79$  (Ash) and  $r = 0.59$  (Cellulose) – Table 2. The mineral composition of dry biomass is in good correlation with the amount of phosphorus ( $r = 0.83$ ) and completely opposite to the energy value of the forage ( $r = -0.81$ ). Crude fats influencing the taste qualities of the forage strongly correlate with fully digestible hemicellulose ( $r = 0.91$ ) and feed unit for growth ( $r = 0.65$ ) and milk ( $r = 0.60$ ). Regression dependence of neutral detergent fibers with nitrogen-free extractable substances was found ( $R^2 = 0.974$ ) and hemicellulose with red fescue height ( $R^2 = 0.921$ ) – Table 3.

The applied bio-fertilization significantly affected the morphological composition of grassland of bird's-foot-trefoil (Bozhanska, 2018) and the concentration of phosphorus and calcium in the dry matter of the harvested biomass. Calcium plays an important role in the growth of the root system and the need for it is manifested by seed germination (Nikolova et al., 2018). In this regard, the percentage share of the stems in bird's-foot-trefoil has shown a high positive correlation with the macroelements Ca ( $r = 0.97$ ) and P ( $r = 0.95$ ). The equations by which the content of the two elements by the weight of the stem fraction can be predicted are:  $Y = 0.8986x - 22.612$  at the determination coefficient  $R^2 = 0.939$  and  $Y = 0.2348x - 8.2219$  at determination coefficient  $R^2 = 0.907$  (Table 4).

The correlation dependence between the amount of leaf mass in treated grasslands of *Lotus corniculatus* L. with the content of calcium ( $r = -0.97$ ) and phosphorus ( $r = -0.95$ ) – is completely opposite (Table 1). The correlations are statistically proven between the empirically calculated values of nitrogen-free extractable substances with the concentration of: neutral detergent fibers ( $r = 0.74$ ), acid detergent fibers ( $r = 0.74$ ), acid detergent lignin ( $r = 0.59$ ) and partially digestible polyose cellulose ( $r = 0.82$ ).

Regression equations have been established:

- $Y = 3.5897x - 701.94$  at determination coefficient  $R^2 = 0.542$ ;
- $Y = 3.6824x - 845.46$  at determination coefficient  $R^2 = 0.550$ ;

**Table 3. Regression dependencies between indicators of chemical composition, structural fiber components, energy nutrition, height and botanical composition of grassland of *Festuca rubra* L., treated with Lumbrical and Lumbrex**

| Dependent indicator (Y) | Independent indicator (X) | Equation               | R <sup>2</sup> |
|-------------------------|---------------------------|------------------------|----------------|
| Ash                     | Grasses, weight %         | $Y = 3.3008x - 153.77$ | 0.622          |
| P                       | Grasses, weight %         | $Y = 0.2073x - 13.03$  | 0.956          |
| CP                      | Heights                   | $Y = 2.0664x + 5.005$  | 0.543          |
| CF                      | Heights                   | $Y = 0.6045x - 0.4764$ | 0.782          |
| Cellulose               | Heights                   | $Y = 13.459x - 345.08$ | 0.537          |
| CF                      | CP                        | $Y = 0.1776x + 8.6553$ | 0.531          |
| P                       | Ash                       | $Y = 0.0421x - 1.6952$ | 0.690          |
| ADF                     | NFE                       | $Y = 3.2589x - 1022.6$ | 0.578          |
| ADF                     | NDF                       | $Y = 2.4395x - 1090.2$ | 0.439          |
| ADL                     | ADF                       | $Y = 1.5808 - 400.74$  | 0.681          |
| EE                      | NFE                       | $Y = 0.2845x + 2.9777$ | 0.401          |
| NDF                     | NFE                       | $Y = 1.1493x + 103.79$ | 0.974          |
| Hemicellulosea          | Heights                   | $Y = 12.115x - 245.04$ | 0.921          |
| Hemicellulose           | CP                        | $Y = 3.883x - 91.848$  | 0.744          |
| Hemicellulose           | CF                        | $Y = 16.874x - 156.26$ | 0.835          |
| FUM                     | EE                        | $Y = 0.0995x - 0.0564$ | 0.984          |
| FUG                     | EE                        | $Y = 0.1028x - 0.1446$ | 0.908          |
| FUG                     | FUM                       | $Y = 1.0579x - 0.105$  | 0.968          |

P < 0.05 – statistical significance of the equation; R<sup>2</sup>- determination coefficient

**Table 4. Regressional dependencies between the chemical composition, structural fiber components, energy nutrition, height and botanical composition of grassland of *Lotus corniculatus* L., treated with Lumbrical and Lumbrex**

| Dependent indicator (Y) | Independent indicator (X) | Equation               | R <sup>2</sup> |
|-------------------------|---------------------------|------------------------|----------------|
| Ca                      | Stems, weight %           | $Y = 0.8986x - 22.612$ | 0.939          |
| P                       | Stems, weight %           | $Y = 0.2348x - 8.2219$ | 0.907          |
| ADL                     | Stems, weight %           | $Y = 1.3775x + 6.0692$ | 0.259          |
| CP                      | Heights                   | $Y = 6.6687x - 159.75$ | 0.835          |
| CFr                     | Heights                   | $Y = 9.8817x - 175.24$ | 0.372          |
| NFE                     | Heights                   | $Y = 3.7189x + 119.05$ | 0.406          |
| NDF                     | Heights                   | $Y = 19.636x - 594.17$ | 0.476          |
| ADF                     | Heights                   | $Y = 15.964x - 522.45$ | 0.303          |
| Hemicellulose           | Heights                   | $Y = 4.1575x - 95.899$ | 0.415          |
| Cellulose               | Heights                   | $Y = 11.127x - 348.06$ | 0.483          |
| GE                      | Heights                   | $Y = 0.1683x + 10.665$ | 0.519          |
| CP                      | CFr                       | $Y = 0.3033x + 80.082$ | 0.453          |
| CP                      | Hemicellulose             | $Y = 0.8668x + 79.211$ | 0.588          |
| CP                      | GE                        | $Y = 20.157x - 208.14$ | 0.416          |
| CF                      | CFr                       | $Y = 0.1832x - 27.352$ | 0.480          |
| GE                      | CFr                       | $Y = 0.013x + 14.963$  | 0.814          |
| GE                      | CF                        | $Y = 0.0376x + 17.993$ | 0.476          |
| NDF                     | NFE                       | $Y = 3.5897x - 701.94$ | 0.542          |
| ADF                     | NFE                       | $Y = 3.6824x - 845.46$ | 0.550          |
| ADL                     | NFE                       | $Y = 1.4314x - 369.48$ | 0.348          |
| Cellulose               | NFE                       | $Y = 2.2511x - 475.99$ | 0.673          |
| P                       | Ca                        | $Y = 0.2359x - 1.805$  | 0.787          |
| ADL                     | Ca                        | $Y = 1.6868x + 37.635$ | 0.333          |
| ADL                     | P                         | $Y = 7.0609x + 50.804$ | 0.413          |
| ADF                     | NDF                       | $Y = 0.9935x - 112.29$ | 0.951          |
| ADL                     | NDF                       | $Y = 0.4373x - 105.17$ | 0.773          |
| Cellulose               | NDF                       | $Y = 0.5561x - 7.112$  | 0.977          |
| Cellulose               | ADF                       | $Y = 0.5332x + 63.431$ | 0.932          |
| Cellulose               | ADL                       | $Y = 0.9561x + 149.2$  | 0.715          |
| ADL                     | ADF                       | $Y = 0.4668x - 63.421$ | 0.913          |
| FUM                     | EE                        | $Y = 0.1251x - 0.2752$ | 0.983          |
| FUG                     | EE                        | $Y = 0.1686x - 0.7055$ | 0.940          |
| FUM                     | FUG                       | $Y = 1.3682x - 0.3508$ | 0.987          |

$P < 0.05$  – statistical significance of the equation;  $R^2$  – determination coefficient

to predict the values of key indicators (NDF and ADF) involved in the plant cell composition and the assessment of nutritional value of bird's-foot-trefoil forage. The correlation coefficient between the crude fiber content and the gross energy is high absolute value ( $r = 0.90$ ), which corresponds to a strongly expressed empirical linear dependence. The established regression model:  $Y = 0.013x + 14.963$  at determination coefficient  $R^2 = 0.814$  ( $P < 0.05$ ) discloses a good opportunity for indicative estimation of the total energy value of forage by the amount of crude fiber.

## Conclusions

Biofertilizers Lumbrical and Lumbrex increase dry matter digestibility in both forage crops. Regression dependence of that indicator with the percentage share of leaves ( $R^2 = 0.354$ ) and the mineral content ( $R^2 = 0.777$ ) – for bird's-foot-trefoil as well as the biometric indicator for height of the grassland ( $R^2 = 0.897$ ), crude protein ( $R^2 = 0.821$ ), crude fat ( $R^2 = 0.696$ ) and hemicellulose ( $R^2 = 0.955$ ) in the dry forage mass of red fescue is statistically proven ( $P < 0.05$ ).



The content of crude protein, hemicellulose and cellulose in the dry matter of both forage crops is highly correlated with the height of treated grasslands. The correlation coefficients between the indicators are:  $r = 0.91$ ;  $r = 0.64$  and  $r = 0.69$  (for bird's-foot-trefoil) and  $r = 0.74$ ;  $r = 0.96$  and  $r = 0.73$  (for red fescue).

The data for a three-year study period shows a positive correlation between the percentage share of *Festuca rubra* L. in grass and the values of phosphorus ( $r = 0.98$ ), mineral substances ( $r = 0.79$ ) and cellulose ( $r = 0.59$ ). The developed regression equations allow for the prediction of calcium and phosphorus content by the amount of stem fraction in *Lotus corniculatus* L. with high determinant:  $R^2 = 0.939$  (Ca) and  $R^2 = 0.907$  (P).

The correlations were statistically proven between empirically calculated values of nitrogen-free extractable substances with the concentration of: neutral detergent fibers ( $r = 0.74$ ), acid detergent fibers ( $r = 0.74$ ), acid detergent lignin ( $r = 0.59$ ) and partially digestible polyose cellulose ( $r = 0.82$ ) in the dry matter composition of bird's-foot-trefoil.

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*Received:* November, 25, 2019; *Accepted:* January, 17, 2020; *Published:* February, 28, 2021