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# CORRELATION AND REGRESSION DEPENDENCES BETWEEN PRODUCTIVITY, COMPOSITION AND ENERGY-NUTRITIVE VALUE OF GRASSLANDS OF PERENNIAL RYEGRASS AND LEGUME FODDER CROP

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***Abstract***

*The study was conducted in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan, with the aim of determining the correlation and regression dependences between some basic chemical indicators and the energy nutritional value of perennial ryegrass and legume fodder crop grown in monoculture and mixed grasslands. Data analysis shows that the amount of crude protein has a statistically significant impact on the in vitro digestibility of dry matter (r = 0.82) and the number of feed units (r = 0.88 - for FUM and r = 0.87 - for FUG). The yield of fresh matter was positively correlated with the dry matter yield (r = 0.83), the amount of NDF (r = 0.74) and hemicellulose (r = 0.80). The high correlation and dependence between fiber components of perennial grasses and legume fodder crops gives a clear assessment for forage quality. The coefficients of determination between structural fiber components and crude fiber concentration ranged from $R^2 = 0.5176$ to $R^2 = 0.9186$, with very well-proven statistical significance of the equations.*

**Key words:** *Lolium perenne L., legume fodder crops, energy value of fodder.*

## INTRODUCTION

The forage production from perennial forage legumes and grasses favors and improves soil fertility, and contributes to obtaining quality animal production (Porqueddu et al., 2016). The percentage share of the components in the mixed grasslands, as well as their competitive ability, are important factors for maintaining the dynamic stability in the grassland (Bozhanska et al., 2017). Grass species determine the productivity and quality of the obtained biomass and contribute to the provision of forage during the grazing period (Wilkinson et al., 2020). Complex interactions among species, age, phase of development, as well as the application of some agrotechnical events have an impact on the changes in the composition and nutritional value of the formed grasslands (Bozhanska, 2018; 2019). The increased interest in organic agriculture leads to the search (establishment) of crops with high adaptability to the agro-ecological characteristics of the area (Kusvuran et al., 2014; Luscher et al., 2014; Vasileva, 2015; Vasileva et al., 2016). Cultivation of forage species typical of mountain conditions in mixed crops is a good alternative for obtaining quality grass mass that satisfies the animal's need for food (Bacchi et al., 2021). The biological cultivation of grasses as monocultures is a prerequisite for vigorous weed infestation of crops and a limited nitrogen supply of plants. The inclusion of legume components increases the nitrogen amount in the grass mass and decreases the weed infestation level (Arlauskienė et al., 2021). In several agroeco-systems, nitrogen has an impact on the provision of high yields in the formation of fodder mass (Thilakarathna, 2016), whereas mixed crops have a lower weed infestation level (8%) compared to monocultures (43%) (Finn et al., 2012). The purpose of the present study is to determine the correlation and regression dependences among the main indicators determining the nutritional value of monoculture and mixed grasslands for quick prediction of the digestibility and quality of forage through their chemical composition.

## MATERIALS AND METHODS

The experiment was conducted in the period 2020-2022 in the Department of Mountain Meadow Farming and Forage Production at the

Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria). For sowing the monoculture and two-component mixed grasslands, the following varieties were used, respectively: perennial ryegrass (Nira variety), red clover (Altaswede variety), white clover (Apolo) and bird's-foot-trefoil (Leo). The experimental variants included:

**Monoculture grasslands**:
- Perennial ryegrass (*Lolium perenne* L.);
- Red clover (*Trifolium pratense* L.);
- White clover (*Trifolium repens* L.);
- Bird's-foot-trefoil (*Lotus corniculatus* L.).

**Mixed grasslands**:
- Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.);
- Perennial ryegrass (*Lolium perenne* L.) + White clover (*Trifolium repens* L.);
- Perennial ryegrass (*Lolium perenne* L.) + bird's-foot-trefoil (*Lotus corniculatus* L.).

**Studied indicators**

Dry matter yield (kg/da) was determined by regrowths and years through mowing each replication of every harvest plot. After that, the plant samples were dried in laboratory conditions at 105°C and they were recalculated per 1 da based on the dry matter content.

The botanical composition of grasslands from forage legumes and grasses (weight %) was determined according to weight by analysis of samples of green mass, which were taken at each mowing of each variant. The weighting was conducted in an air-dry state, and the percentage share of the sown grass species was determined by weight.

Crude protein (CP, g/kg$^{-1}$ DM) was determined according to the Kjeldahl method (according to BDS - ISO-5983), for decomposition of the organic matter the sample was boiled with sulphuric acid in the presence of a catalyst. The acidic solution was alkalified with sodium hydroxide solution. The ammonia was distilled and collected in a certain amount of sulphuric acid, the excess of which was titrated with a standard solution of sodium hydroxide. Alternatively, the separated ammonia was distilled in a surplus of boric acid solution and then titrated with hydrochloric or sulphuric acid solution.

Crude fiber (CFr, g/kg$^{-1}$ DM) was determined according to the Weende analysis as the sample was treated sequentially with solutions of 1.25% (w/v) $H_2SO_4$ and 1.25% (w/v) NaOH. The residue was dried, ashed, and weighed.

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) were determined according to the detergent analysis of Van Soest & Robertson (1979); Degree of lignification (coefficient) was determined through the percentage ratio of ADL and NDF; Hemicellulose (g kg$^{-1}$ DM) = NDF – ADF; Cellulose (g kg$^{-1}$ DM) = ADF – ADL. *In vitro* enzymatic dry matter digestibility (DMD, g kg$^{-1}$ DM) according to Aufrere (1982).

The forage nutritional value was estimated according to the Bulgarian system, as feed units for milk (FUM) and feed units for growth (FUG) according to Todorov (2010): Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*CF + 0.0209*CFr + 0.017*Nitrogen free extract (NFE) – 0.0007*Zx.; Exchange energy (EE, MJ/kg DM) = 0.0152*DP + 0.0342*CF + 0.0128*DF + 0.0159*DNFE (Digestible nitrogen free extract) 0.0007*Zx.; Feed units for milk (FUM in kg DM) = EE* (0.075 + 0.039*q); Feed units for growth (FUG in kg DM) = EE*(0.04 + 0.1*q).

One-way analysis of variance (ANOVA), multiple comparisons of means by least statistically significant difference ($LSD_{0.05}$), correlation, and regression analysis were used for data analysis.

## RESULTS AND DISCUSSIONS

### Correlation and regression dependences between quantitative and qualitative parameters of forage from monoculture and mixed grass stands of perennial ryegrass and forage legumes

The correlation (positive or negative) between the studied indicators proves the statistical dependence between them.

Data analysis shows that the yield of fresh mass in the monoculture and mixed grasslands of forage legumes and grasses is in a strong positive correlation with the dry matter yield (r = 0.83), the amount of neutral detergent fibers (r = 0.74) and hemicellulose (r = 0.80) (Table 1).

Table 1. Correlational dependences among indicators of composition, nutritional value, and digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

| | Fresh matter yield, kg/da | Dry matter yield, kg/da | Weight % grasses | Weight % legumes | CP g kg$^{-1}$ | CFr g kg$^{-1}$ | NDF g kg$^{-1}$ | ADF g kg$^{-1}$ | ADL g kg$^{-1}$ | Hemicell g kg$^{-1}$ | Cellulose g kg$^{-1}$ | IVDMD g kg$^{-1}$ | FUM | FUG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fresh matter, yield | 1 | | | | | | | | | | | | | |
| Dry matter, yield | **0.83** | 1 | | | | | | | | | | | | |
| Grasses | 0.20 | 0,36 | 1 | | | | | | | | | | | |
| Legumes | -0.32 | -0.61 | -0.14 | 1 | | | | | | | | | | |
| CP | -0.77 | -0.58 | -0.22 | -0.01 | 1 | | | | | | | | | |
| CFr | 0.63 | 0.54 | 0.44 | -0.22 | -0.86 | 1 | | | | | | | | |
| NDF | **0.74** | 0.53 | 0.17 | 0.04 | -0.98 | **0.88** | 1 | | | | | | | |
| ADF | 0.57 | 0.48 | 0.36 | -0.02 | -0.87 | **0.96** | **0.92** | 1 | | | | | | |
| ADL | 0.05 | -0.19 | 0.19 | 0.43 | -0.43 | 0.61 | 0.55 | **0.73** | 1 | | | | | |
| Hemicellulose | **0.80** | 0.51 | -0.01 | 0.08 | -0.96 | **0.72** | **0.95** | **0.75** | 0.35 | 1 | | | | |
| Cellulose | 0.68 | 0.65 | 0.37 | -0.17 | -0.92 | **0.96** | **0.93** | **0.97** | 0.54 | 0.79 | 1 | | | |
| IVDMD | -0.84 | -0.62 | -0.18 | 0.41 | **0.82** | -0.85 | -0.83 | -0.73 | -0.30 | -0.80 | -0.79 | 1 | | |
| FUM | -0.74 | -0.59 | -0.53 | 0.11 | **0.88** | -0.96 | -0.90 | -0.93 | -0.60 | -0.76 | -0.93 | **0.84** | 1.00 | |
| FUG | -0.70 | -0.57 | -0.53 | 0.15 | **0.87** | -0.98 | -0.89 | -0.94 | -0.61 | -0.74 | -0.94 | **0.84** | 1.00 | 1 |

$P < 0.05$

The theoretical regression lines and the equations of regression dependence between the indicators are: shown in Figures 1, 2, 3, 4, 5, 6 and 7 where:

- y = 0.1766x - 166.1 with coefficient of determination – $R^2$ = 0.5535 ($P < 0.05$) – (Figure 1).
- y = 0.1118x – 224.95 with coefficient of determination - $R^2$ = 0.6347 ($P < 0.05$) (Figure 2).

In contrast, the connection of the quantitative indicator with crude protein content (r = -0.77), feed units for milk (r = -0.74), and feed units for growth (r = -0.70) and dry matter digestibility (r = -0.84) is negative.

The results of the correlation analysis (at a confidence level of 95%) show a statistically significant influence of the amount of crude protein on the *in vitro* digestibility of dry matter (r = 0.82) and the number of feed units (r = 0.88 for FUM and r = 0.87 for FUG) in the forage mass**.** The developed regression models (Figures 3 and 4) are statistically proven at $P < 0.05$ and are to be used for tentative prediction of the feed units of biomass from monoculture and mixed grasslands of forage legumes and grasses.

A strong negative correlation dependence was found between the concentration of crude protein (as the main quality indicator in the composition of the dry matter) with the content of:

- Crude fibers (r = -0.86);
- Neutral detergent fibers (r = -0.98);
- Acid detergent fiber (r = -0.87);
- Hemicellulose (r = -0.96);
- Cellulose (r = -0.92).

and a strong positive correlation dependence between the amount of crude fiber and the structural fiber components of the cell walls (Figure 5), namely:

- Neutral detergent fibers (r = 0.88);
- Acid detergent fiber (r = 0.96);
- Hemicellulose (r = 0.72);
- Cellulose (r = 0.96).

The coefficients of determination between structural fiber components and crude fiber concentration were from $R^2$ = 0.5176 to $R^2$ = 0.9186, with very well-proven statistical significance of the equations. A high negative correlation dependence also exists between crude fiber content with *in vitro* digestibility of dry matter (r = -0.85) and feed value, such as the feed units for milk (r = -0.96) and feed units for growth (r =-0.98) in dry matter. These indicators determine the nutritional value of forages and allow us to determine it with relatively high accuracy, through the content of crude fiber.

The correlation coefficients of neutral detergent fibers with acid detergent fibers (r = 0.92), hemicellulose (r = 0.95) and cellulose (r = 0.93), as well as those of acid detergent fibers with acid detergent lignin (r = 0.73), hemicellulose (r = 0.75) and cellulose (r = 0.97) were absolute values that corresponded to strong linear dependencies (Figures 6 and 7).

Proving a high correlation and dependence between the fiber components of perennial forage grasses and legumes in monoculture and mixed grasslands is essential to assess forage quality.

Proving a high correlation and dependence between the fiber components of perennial forage grasses and legumes in monoculture and mixed grasslands is essential to assess forage quality.

Dry matter digestibility showed a positive correlation with feed units for milk and growth (r = 0.84) and a strongly negative correlation with fresh matter yield (r = -0.84), crude fiber (r = -0.85), neutral detergent fibers (r = -0.83), acid detergent fibers (r = -0.73), hemicellulose (r = -0.80) and cellulose (r = -0.79).

Negative correlation dependences were obtained for FUM and FUG with NDF, ADF, lignin and cellulose. This indicates that for predicting the energy nutritional value of forage, it is better to use crude fiber content as an independent variable. The reason is that in grass mixtures, grasses and legumes are characterized by a different morphological structure and the variation in crude fiber content is less compared to structural fiber components. On the other hand, it is much easier (and economically advantageous) to determine the feed value by the amount of crude fiber.

No statistically significant interconnections were observed regarding the weight percentage of grasses and legumes in the composition of the grassland according to quantitative and qualitative parameters.

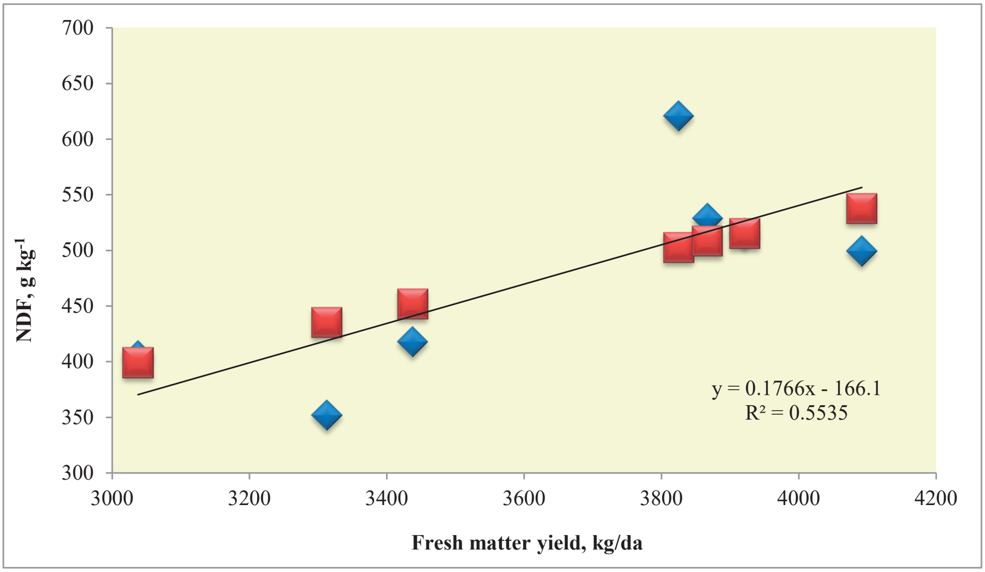


Figure 1. Regression dependence between the yield of fresh matter and the content of neutral detergent fibers in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

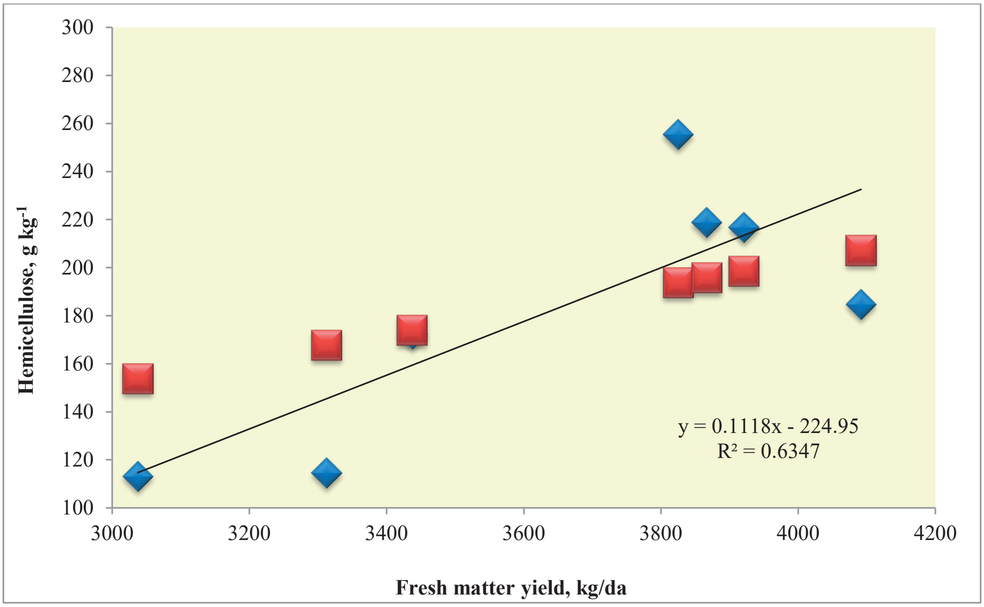


Figure 2. Regression dependence between fresh matter yield and hemicellulose content in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

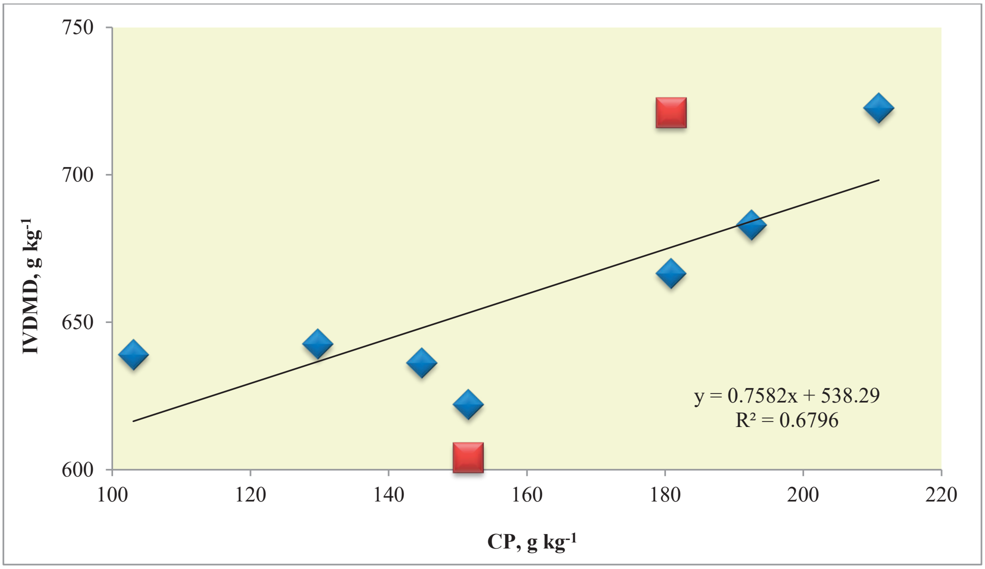


Figure 3. Regression dependence between crude protein content and *in vitro* dry matter digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

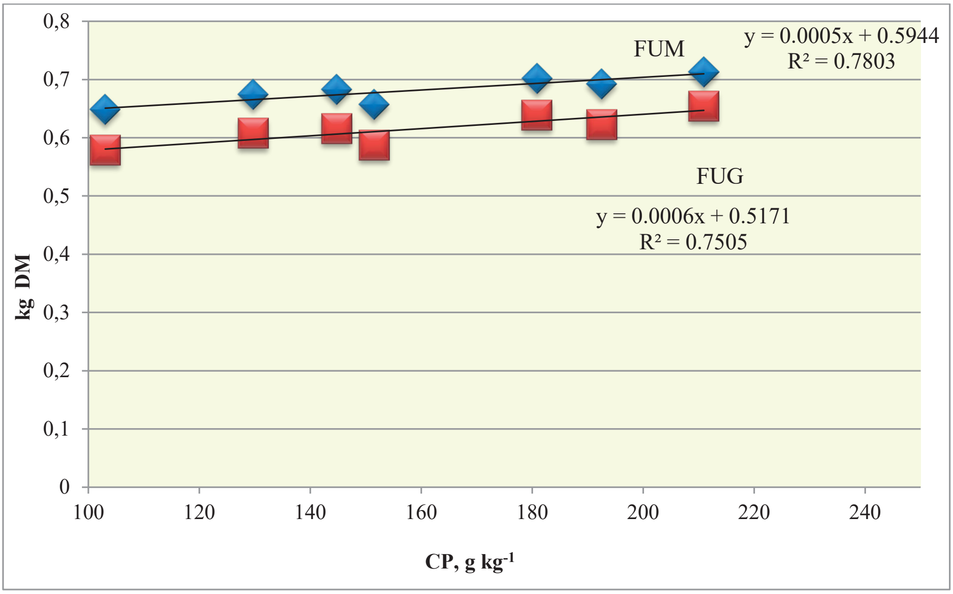


Figure 4. Regression dependence between the content of crude protein and the amount of feed units in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

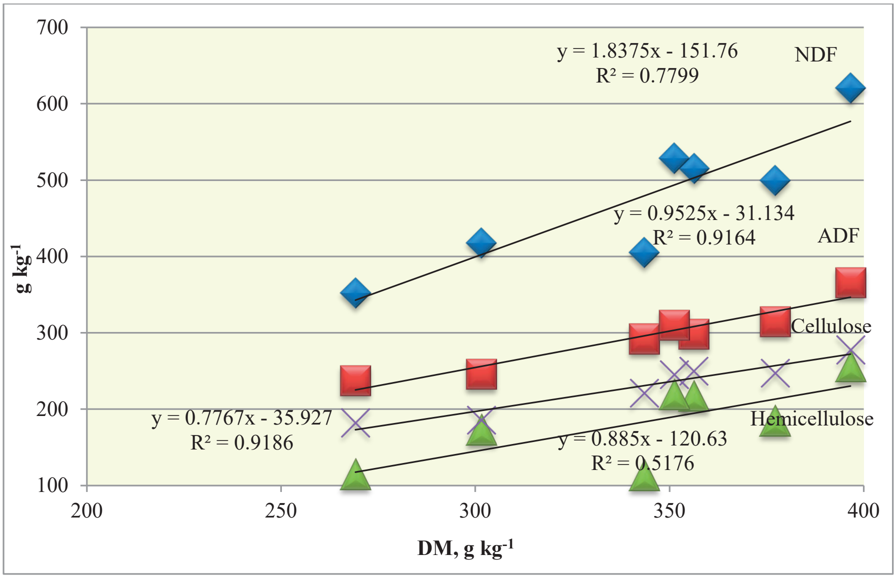


Figure 5. Regression dependence between the content of dry matter and the amount of NDF, ADF, cellulose, and hemicellulose of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

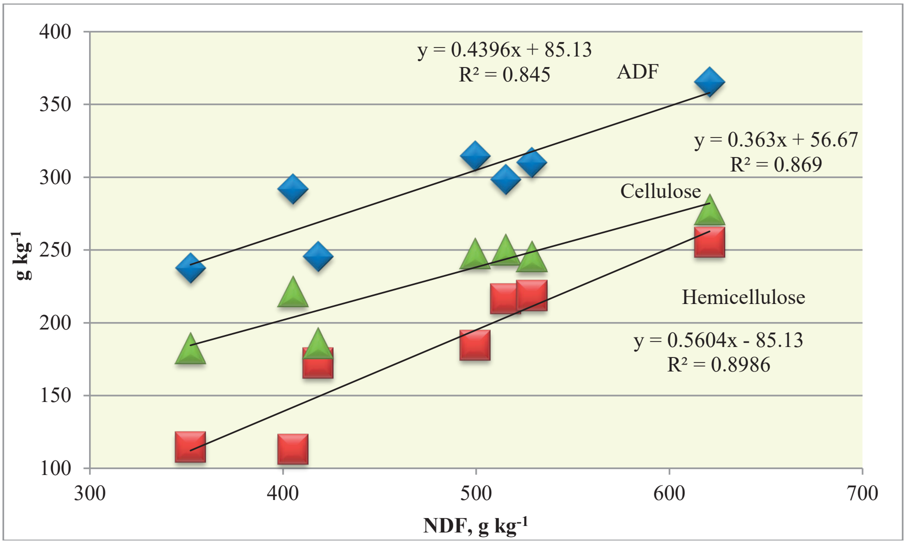


Figure 6. Regression dependence between the content of NDF with the amount of ADF, hemicellulose, and cellulose in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

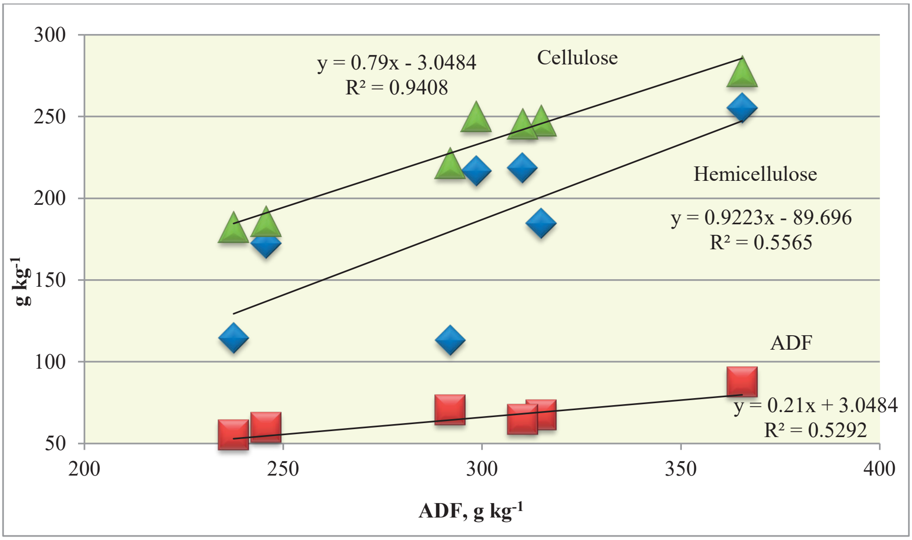


Figure 7. Regression dependence between the content of ADF with the amount of ADL, hemicellulose, and cellulose in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

## CONCLUSIONS

Data analysis shows that the amount of crude protein had a statistically significant impact on the *in vitro* digestibility of dry matter (r = 0.82) and the feed units (r = 0.88 - for FUM and r = 0.87 - for FUG). The yield of fresh matter was positively correlated with the dry matter yield (r = 0.83), the content of NDF (r = 0.74) and hemicellulose (r = 0.80).

A statistical dependence was established between the structural fiber components and the crude fiber concentration. The coefficients of determination in the derived regression equations ranged from $R^2$ = 0.5176 to $R^2$ = 0.9186.

The correlation coefficients of neutral detergent fibers with acid detergent fibers (r = 0.92), hemicellulose (r = 0.95), and cellulose (r = 0.93), as well as those of acid detergent fibers with acid detergent lignin (r = 0.73), hemicellulose (r = 0.75) and cellulose (r = 0.97) were absolute values that corresponded to strong linear dependencies.

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