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Correlation and regression relationships between quantitative and qualitative indicators of perennial grass and legume mixtures

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Abstract

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The growth and development of the following six double mixtures of legumes and grasses were examined: bird's-foot-trefoil + red fescue; white clover + perennial ryegrass; white clover + kenthucky bluegrass; red clover + timothy-grass; blue hybrid alfalfa + cock's foot and red clover + meadow fescue, to establish correlation and regression relationships between their quantitative and qualitative indicators determining their nutritional value.

High correlation relationships were found in the chemical composition among some main indicators. The amount of crude protein with the weight percentage of legume crops had a high correlation coefficient (r = 0.870) and the crude fiber content correlated positively with the height of grasses (r = 0.820) and legumes (r = 0.806).

There was a high positive correlation between crude fiber content with ADF (r = 0.827) and the cellulose (r = 0.814) and a high negative correlation between the mean values of crude fiber and those of the feed unit for milk – (r = -0.920) and growth (r = -0.860).

The graphical regression models allow determining the crude protein content by the weight percentage of legumes and the crude fiber content by the height of grass plants in the mixture.

The feed unite (FUM and FUG) can be predicted and determined by the graphical models based on crude fiber content with sufficient accuracy for practical purposes.

Key words: grass mixtures; correlations; regression relationships; chemical composition

Introduction

The forage quality depends largely on the nutritional value (Larbi et al., 2010) of grass mixtures, respectively on the species and botanical specificity of grass and legume component (Naydenova, 2008). The forage production of perennial grasslands favors the environment preservation, improves soil fertility and promotes the production of quality livestock in accordance with the requirements of the European Union.

The establishment of mixed crops of legume and grass meadow species requires a real soil and climate feature of the region of growing (Beierkuhnlein et al., 2011; Ficher et al., 2001; Mitev & Naydenova, 2008), with the aim to tolerate and preserve the basic morphological characteristics of crops that have a positive effect on the yield and nutritional value of the forage. The selection of grass components determines the yield and quality of grass biomass, as well as the forage provision throughout the grazing season (Sleugh et al., 2000). Complex relationships exist among species in mixed crops that determine the productivity and quality of forage (Sheaffer et al., 1984). The percentage share of legume and grass forage crops in mixtures influences the quality and nutritional value of forage. Their ability to combine and the competitiveness are important factors in maintaining dynamic stability in the grassland (Bozhanska, 2017).

The development phase in legume forage crops influences the content of components in the content of plant cell wall (Kicheva & Angelova, 2006). There are changes in the crude protein and crude fiber content that accompany the stem growth intensity and the accumulation of mechanical sclerenhyma cells as the age of the plant grows (Guertin, 1987). The crude protein decreases from the beginning of the vegetation to the end, while the crude fiber content increases as the plant height increases.

Pavlov & Naydenova (2000) reported increased levels of NDF, ADF and ADL in red and white clover as the age of plants increases.

Forage quality is related to its nutritional value, which is directly related to climatic conditions and plant maturity as well as to the concentration of chemical components in plant tissue determined by chemical or physical methods (Givens & Deaville, 1999; García & Cozzolino, 2006; Bozhanska et al., 2016).

During vegetation, herbaceous vegetation undergoes some changes related to the meteorological and hydrometeorological conditions of the environment. Thus the choice of main grass species has to be consistent with their resistance to abiotic and biotic factors, ecological stability and adaptability. The choice of suitable grasses and grass mixtures is one of the most important points in the technology for creating perennial grasslands. Bird's-foot-trefoil, white and red clover, timothy grass, cock‘s foot and red fescue are forage crops suitable for the region of the Central Balkan Mountain.

The aim of present study is to determine the correlation relationships among main indicators that determine the nutrient values of mixed grasslands and to develop regression graphical models to predict quickly the digestibility and quality of perennial two-component mixtures through their chemical composition.

Materials and Methods

The present study was conducted in the experimental field of the Department of ‘Mountain Grass Associations and Maintenance of Their Biological Diversity’ at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan.

The plant samples include legume and grass species in double mixtures typical for the region: bird‘s-foot-trefoil (cv. *Leo*) + red fescue (cv. *Ryder*); white clover (cv. *Huia*) + perennial ryegrass (cv. *Belida*); white clover (cv. *Huia*) + kenthucky bluegrass (cv. *Sobra*); red clover (cv. *Altaswede*) + timothy grass (cv. *Erecta*); blue hybrid alfalfa (local population originated from Troyan) + cock‘s foot (cv. *Loke*) and red clover (cv. *Altaswede*) + meadow fescue (cv. *Laura*). The experiment is set up according to the block method, in four replications with a size of the experimental plot of 5 m^2. The sowing is performed manually in a scattered manner with a sowing rate consistent to that of the species in pure state at a ratio of 50:50. Mixtures are cultivated without fertilization and under nonirrigated conditions. The grasslands are cut in the beginning of the flowering phase of legumes and tasseling/ear formation of grass species. Six regrowths are harvested for the trial period.

The following indicators are observed: dry matter yield (kg/da); plant height (cm); botanical and morphological composition of grassland (%); chemical composition of dry forage mass: Crude protein (CP) by *Kjeldahl* (according to BDS-ISO 5983), crude fibers (CF) according to *Weende* method (AOAC, 2000); NDF (Neutral Detergent Fibers), ADF (Acid Detergent Fibers) and ADL (Acid detergent lignin) by the Van Soest and Robertson (1979); Hemicellulose = NDF-ADF and Cellulose = ADF-ADL; Energy value (FUM and FUG) calculated by the coefficients indicated by Todorov (2010) and *in vitro* dry matter digestibility (IVDMD) by Aufrere (1982) according to two-stage pepsin-cellulose method.

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

Results and Discussion

The crude protein content of mixtures (Table 1) in the composition of harvested biomass showed the highest positive correlation with the indicator – weight percentage of legume crops ($r = 0.870$) and in contrast to the highest negative correlation relationship with the indicator – weight percentage of grass crops ($r = -0.836$).

The theoretical regression line and the equation of the regression relationship between the crude protein content and the weight percentage of legume crops in the dry biomass of perennial grass and legume mixtures are showed in Figure 1, where $y = 114.4818 + 0.5094x$ at a relatively high determination coefficient – $R = 0.536$ ($P < 0.05$).

The coefficient of intensity of protein change according to the percentage share of legumes ($I = 3.5$) shows that with a 1% increase of legumes in the grassland the crude protein content increases by 3.5 g.

The effective use of mixed grasslands and their nutritional value is closely related to the analysis of the basic chemical composition and the composition of cell wall components. The amount of crude fiber is in a very good regression relationship with the height of grass plants $R = 0.770$ (Figure 3) and considerably weaker with the height of the legume plants $R = 0.366$ (Figure 2).

Table 1. Correlation relationships among the indicators for the composition, nutritional value and digestibility of perennial grass and legume mixtures

	Dry matter yield	CP	CF	Height legumes	Height grasses	Legumes	Grasses	NDF	ADF	ADL	Hemi-cell.	Cellu-lose	FUM	FUG	IVD-MD
Dry matter yield, kg/da	1														
CP, g kg^{-1}	0.042	1.000													
CF, g kg^{-1}	-0.076	-0.709	1												
Height, legumes, cm	0.452	-0.758	0.806	1.000											
Height, grasses, cm	-0.132	-0.617	0.820	0.716	1.000										
Weight %. legumes	0.418	0.870	-0.571	-0.426	-0.452	1.000									
Weight %. grasses	-0.487	-0.836	0.540	0.384	0.529	-0.982	1.000								
NDF, g kg^{-1}	-0.163	-0.246	0.595	0.445	0.802	-0.279	0.382	1.000							
ADF, g kg^{-1}	-0.259	-0.437	0.827	0.569	0.748	-0.446	0.508	0.933	1.000						
ADL, g kg^{-1}	0.063	-0.001	0.567	0.447	0.748	0.132	-0.047	0.860	0.818	1.000					
Hemicellulose, g kg^{-1}	-0.025	0.015	0.230	0.229	0.573	-0.039	0.173	0.910	0.699	0.764	1.000				
Cellulose, g kg^{-1}	-0.460	-0.683	0.814	0.512	0.750	-0.811	0.835	0.732	0.873	0.433	0.448	1.000			
FUM in kg DM	0.205	0.460	-0.920	-0.604	-0.779	0.324	-0.312	-0.536	-0.781	-0.653	-0.163	-0.670	1.000		
FUG in kg DM	0.219	0.279	-0.860	-0.486	-0.672	0.208	-0.190	-0.556	-0.781	-0.696	-0.205	-0.633	0.969	1.000	
IVDMD, g kg^{-1}	0.259	0.437	-0.827	-0.569	-0.884	0.446	-0.508	-0.933	-1.000	-0.818	-0.700	-0.873	0.781	0.780	1.000

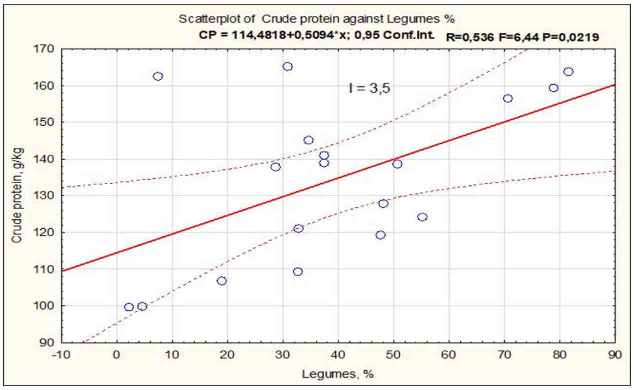

Fig. 1. Regression relationship between crude protein content and weight percentage of legumes in dry biomass of perennial grass and legume mixtures

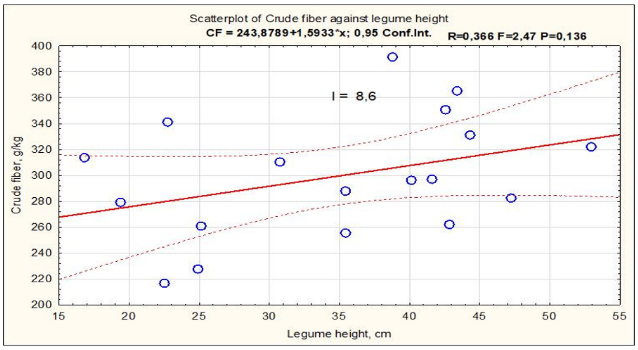

Fig. 2. Regression relationships between crude protein content and the height of legume crops in dry biomass of perennial grass and legume mixtures

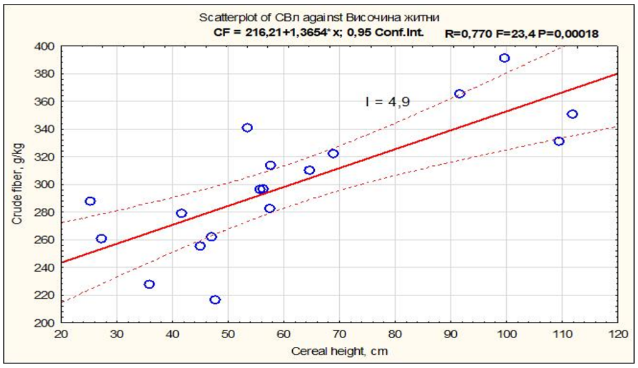

Fig. 3. Regression relationship between crude fiber content and the height of grasses in dry biomass of perennial grass and legume mixtures

Acid-detergent fibers and cellulose have in a good regression relationship with crude fibers R = 0.738 (Figure 4) and R = 0.691 (Figure 5), which confirms the good correlation between the crude fibers and the structural fiber components.

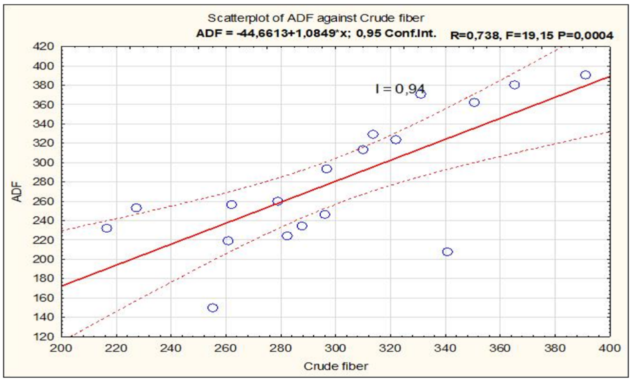

Fig. 4. Regression relationship between content of acid detergent fiber and crude fibers in dry biomass of perennial grass and legume mixtures

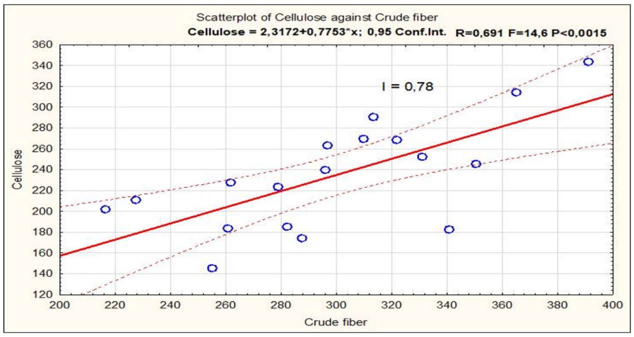

Fig. 5. Regression relationships between cellulose content and crude fiber in dry biomass of perennial grass and legume mixtures

The weight percentage of grasses in mixed grasslands has the greatest influence on the change in the average values of cellulose (Table 1). The results of the analysis show a positive correlation (r = 0.835) between both indicators. Negative correlation relationship is observed between weight percentage of legumes in comparison with weight percentage of grasses (r = -0.982) and the cellulose amount (r = -0.811).

The correlation coefficient between neutral and acid-detergent fiber content has a high (r = 0.933) absolute value that corresponds to a strong empirical linear relationship.

There is a high correlation between the values of crude fibers with structural fiber components: ADF (r = 0.827), NDF (r = 0.595), ADL (r = 0.567) and between crude fibers with cellulose (r = 0.814). The demonstration of a high correlation relation and relationship among the fiber components in forage biomass is essential for the assessment of mixed grasslands in natural grass associations such as meadows and pastures.

There is a very high negative correlation relationship between crude fiber with feed unit – the number of feed units for milk (r = -0.920) and the feed units for growth (r = -0.860) in the dry matter, allowing both parameters (FUM and FUG), which determine the nutritional value of forage, to be determined with relatively high accuracy through the crude fiber content.

The relationship between hemicellulose as a structural component and the feed value is significantly more insufficient (FUM – r = -0.163; FUG – r = -0.205). A higher negative correlation is registered between the cellulose content and FUM – (r = -0.670) and FUG – (r = -0.633). The relationship between FUM and FUG is expressed by a high correlation coefficient (r = 0.969 – the highest among all other indicators).

Dry matter digestibility shows a positive correlation with the average values of the feed unit for milk (r = 0.781) and growth (r = 0.780) followed by: weight percentage of legumes (r = 0.446), crude protein (r=0.437) and dry matter yield (r = 0.259). Negative correlation of this parameter is observed with the content of: acid-detergent fibers (r = -1.000), neutral detergent fibers (r = -0.933), the average height of grass plant components (r = -0.884), cellulose (r = 0.873), crude fiber content (r = -0.827), acid-detergent lignin (r = -0.818), hemicellulose (r = -0.700), height of legume plants (r = -0.569) and weight percentage of grass (r = -0.508) component.

The results are similar to the established regression equations by other authors (Todorov et al., 2007) for determining FUM and FUG content in meadow hays obtained from the study of many more samples of different origins and accepted for practical use in the development of rations for feeding of ruminants.

Table 2 shows the regression relationships of FUM and FUG with the height of grass crops. Determination coefficients are very low and the equations are not statistically proven, regardless of which function of description and analysis is used – linear or polynomial.

Grass and legume components involved in mixed grasslands are characterized by specific morphological structure and growth opportunities. Therefore, the problem of predicting the value of the feed unit in relationship to the height of plants is that the height of the plants varies depending on the competition among different species (legumes and grasses) of meadow grasses (Pavlov et al., 1997).

Table 2. Regression relationship among some indicators of the chemical composition (cellulose. hemicellulose). structural fiber components (NDF, ADF, ADL), energy nutrition (FUM, FUG), weight percentage and height of the components for perennial grass and legume mixtures

Independent indicator **(У)**	Dependent indicator (X)	Equation	R	SEE	F	P<
FUM	Height grasses	У = 0.7012 – 0.000156x	0.173	0.015	0.49	0.492
FUM	Height grasses	У = 0.6765 + 0.000667x – 0.000006x2	0.239	0.04	0.45	0.644
FUG	Height grasses	У = 0.6456 – 0.00022x	0.209	0.016	0.73	0.405
FUG	Height grasses	У = 0.6212 + 0.000597x – 0.000006x2	0.251	0.047	0.505	0.613
Cellulose	Weight % grasses	У = 166.2331 + 1.4886x	0.778	0.3	24.49	0.00015
ADF	NDF	У = 29.276 + 0.543x	0.883	0.072	56.4	0.00001
ADL	NDF	У = -40.7277 + 0.187x	0.682	0.05	13.9	0.0018
Hemicellulose	NDF	У = -29.348 + 0.4574x	0.845	0.072	40.08	0.00001
Cellulose	NDF	У = -69.994 + 0.356x	0.767	72	21.53	0.00027
FUM	NDF	У = 0.7081 – 0.000036x	0.168	0.024	0.464	0.5055
FUG	NDF	У = 0.6520 – 0.000043x	0.172	0.029	0.487	0.495
ADL	ADF	У = -40.4836 + 0.3077x	0.69	0.08	14.55	0.00152
Hemicellulose	ADF	У = 60.00 + 0.436x	0.495	0.19	5.19	0.036
Cellulose	ADF	У = 40.466 + 0.692x	0.906	0.08	73.7	0.000001
FUM	ADF	У = 0.7055 – 0.000049x	0.143	0.025	0.335	0.571
FUM	ADF	У = 0.6111 + 0.000655x – 0.000001x2	0.285	0.098	0.665	0.529
FUG	ADF	У = 0.6513 – 0.000068x	0.168	0.029	0.468	0.504
FUG	ADF	У = 0.5232 + 0.00088x – 0.000002x2	0.332	0.112	0.931	0.415
Hemicellulose	ADL	У = 139.0548 + 0.941x	0.477	0.43	4.7	0.0455
FUM	ADL	У = 0.6982 – 0.000143x	0.185	0.01	0.568	0.462
FUM	ADL	У = 0.7114 – 0.000722x + 0.000004x2	0.287	0.018	0.673	0.525
FUG	ADL	У = 0.6399 – 0.000168x	0.186	0.012	0.572	0.46
FUG	ADL	У = 0.6556 – 0.000859x + 0.000005x2	0.291	0.021	0.693	0.512
FUM	Cellulose	У = 0.7001 – 0.000036x	0.079	0.027	0.101	0.754
FUM	Cellulose	У = 0.5614 + 0.0016x – 0.000002x2	0.335	0.107	0.95	0.409
FUG	Cellulose	У = 0.6461 + 0.000059x	0.112	0.031	0.204	0.658
FUG	Cellulose	У = 0.4601 + 0.00155x – 0.000003x2	0.390	0.122	1.35	0.289
FUG	FUM	У = -01680 + 1.15696x	0.990	0.029	773.6	0.000001

R – coefficient of determination; SEE – standard error of the estimate; F – relationship among quantities; P – statistical significance of the equation

Growth in grass species depends on the type of development (winter or winter-spring), as well as on the formation of vegetative and generative stems over the years. In the assessment of mixed grasslands, the variability of plants over the years is important due to their biological durability and resistance to unfavorable soil and climatic conditions.

The regression relationships of FUM and FUG with the structural fiber components (NDF, ADF, lignin and cellulose) are low. This indicates that for predicting the total nutritional value, FUM and FUG, it is better to use the crude fiber content as an independent variable. The main reason for this is that in grass mixtures the variation in crude fiber content among different components is much less compared to the structural fiber components due to the specific morphological structure of grass and legume crops involved in the mixtures.

From the point of view of practice, it is significantly easier to determine the feed value through the crude fibers than with the structural fiber components.

The basic component analysis for the distribution of grass mixtures in the average data in the course of three years for all 18 productive and qualitative indicators sets out the variants and determines the following mixtures as positive for both factors: red clover + timothy grass and white clover + perennial ryegrass (Table 3).

Table 3. Numeric values for distribution of grass mixtures by 18 indicators Factor scores. based on correlations

Variants	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Bird's-foot-trefoil + Red fescue	-0.178	-0.432	0.782	-0.013	-1.827
White clover + Perennial ryegrass	0.540	0.195	-1.364	1.386	-0.236
White clover + Kenthucky bluegrass	1.055	-1.014	-0.484	-1.279	0.395
Red clover + Timothy grass	0.903	1.301	1.158	0.099	0.556
Blue hybrid alfalfa + Cock's foot	-1.330	0.989	-0.734	-0.938	0.044
Red clover + Meadow fescue	-0.990	-1.038	0.643	0.745	1.068

The mixture of white clover and kenthucky bluegrass (Figure 6) is positive according to the first factor describing 58.2% of the variations but it is negative according to the second factor – 19.51%. The mixture of blue hybrid alfalfa and cock's foot is positive for the second factor, and the mixtures of bird's-foot-trefoil + red fescue and red clover + meadow fescue are negative for both factors.

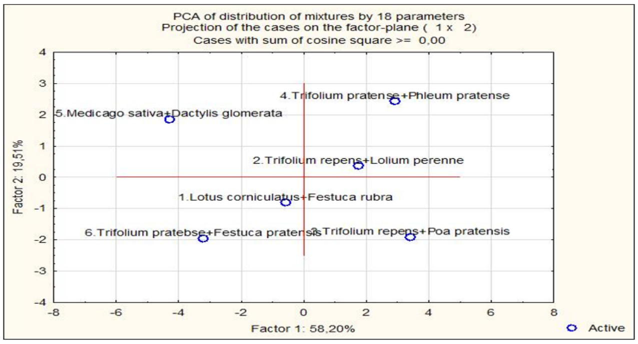

Fig. 6. Principal component analyses for the distribution of grass mixtures from the averaged data for the three years for all 18 productive and qualitative indicators

Conclusion

In the study of some basic indicators of chemical composition, nutritional value, growth indicators and percentage share of mixtures in the grasslands, there are high positive correlations among the crude protein content and weight percentage of legumes (r = 0.870), the quantity of crude fibers with the height of grass plants (r = 0.820) and legumes (r = 0.806).

A high positive correlation was found between: crude fiber with ADF (r = 0.827) and cellulose (r = 0.814) and high negative correlation between crude fiber and feed unit for milk – (r = -0.920) and growth (r = -0.860).

The developed graphical regression models allow determining the content of crude protein by the weight percentage of legumes and the amount of crude fiber by the height of grass plants in the mixture.

Feed value (FUM and FUG) can be predicted and determined by the developed graphical models based on the crude fiber content with accuracy sufficient for practical purposes.

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