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Statistical dependencies of fertilizing with humate fertilizers on productive and qualitative indicators of forage from meadow legume crops

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Abstract

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On the basis of the data received from the research experiment conducted with bird's-foot-trefoil, red clover and sainfoin, fertilized with humate fertilizers on the territory of the experimental field of Research Institute of Mountain Stockbreeding and Agriculture – Troyan during the period 2013–2015, correlation dependencies and regression equations were constructed. A positive correlation dependence was established between: the phosphorus content and the height of fertilizer meadows with legumes ($r = 0.90$); the content of nitrogen-free extractable substances and the relative share of the stems ($r = 0.86$); and the crude protein and the percentage share of leaves ($r = 0.80$). This proves the positive effect of fertilizing with boron and molybdenum humate on growth and development of legumes, the accumulation of nutrients in the forage. The regression equations derived prove the possibility of predicting the crude protein content from the share of leaves in the grass stand ($R^2 = 0.64$), the phosphorus content from the height of the stems ($R^2 = 0.81$) and of the nitrogen-free extractable substances from the relative share of the stems ($R^2 = 0.75$), in the grass stands. Two-factor analysis of variance showed the high impact of the conditions of the year ($\eta = 68.69$) on the dry matter yield.

Keywords: meadow legumes; fertilizing with biofertilizers; correlations; regressions; yield; quality

Introduction

The role of legumes, in this regard, is determined by the development of forage production in connection with animal feeding, the increased requirements related to environmental protection, water and soil. The legumes belong to the botanical family *Fabaceae*, and number about 18 000 species (Schneider et al., 2015). Both in Europe and Bulgaria, the representatives of forage legumes are alfalfa, white and red clover, sainfoin, bird's-foot-trefoil (Kirilov et al., 2005). They are characterized by the high content of crude protein (16–24%), calcium (0.9–1.5%) and essential amino acids, such as methionine and lysine, and the low sugar content.

Their ability to fix nitrogen from the air and reduce fertilizing with mineral nitrogen makes them desirable for the development of biological and sustainable farming. In the last two years, the area with legumes has increased in Bulgaria since the European Union's decision to further subsidize farmers for the cultivation of legumes during the 2014–2020 programming period (Kirilov, 2016). Legumes have qualities, meet the requirements for sustainable farming and are related to environmental studies (Nikolova & Georgieva, 2015).

A number of authors (Collomb et al., 2002; Roukos et al., 2011), in research experiments, determine the impact of botanical composition on the grass stands (Bozhanska et al., 2022) on the main chemical composition, nutritional value and digestibility, which affects the quality of the milk from ruminants (Chion et al., 2010).

The bird's-foot-trefoil is widespread in the natural and artificial grass stands (Phelan et al., 2015), moreover it is rich in high quality protein content and has high forage digestibility (Kaplan et al., 2009; Graves et al., 2012).

The digestibility of forage legume crops varies widely and depends on the type of crop, cultivation technology, climate conditions, the harvesting phase (Bozhanska et al., 2022; Iliev et al., 2022).

The red clover (*Trifolium pratense* L.) is key species of maintaining forage resources in the ecosystems of the foothill and mountain regions of Bulgaria. In artificial sowing, it is used as a major component in creating medium-long term grass stands for hay making, grazing or combined use (Naydenova & Bojanska, 2014).

Sainfoin is a forage legume widespread and well adapted to the high mountain farming system under dry conditions (Turk & Celik, 2006; Turk et al., 2011). It is useful in crop rotation in agriculture for erosion control because of the opportunity of growing in mixed crops with forage grasses. Forage quality plays an important role in animal feeding, and the factors affecting nutritional value are many and the extent to which they are interconnected can vary significantly (Bell et al., 2001).

Fertilizing affects the dry matter yield and the chemical composition of the legume forage (Bell et al., 2001; Bozhanska, 2019; Bozhanska et al., 2022), and nutritional levels are not the only criterion for assessing the nutritional value of plants. The growth stage is the most important factor affecting the chemical composition and digestibility of the forage, as plants reduce the composition of nutrients when the ripening progresses (Rebole et al., 2004).

The application of biofertilizers in the cultivation of vegetable crops improves the nutrition of plants (Vlahova et al., 2013), stimulates their growth and development, increases resistance to diseases, enemies and adverse climatic conditions (Haytova, 2013). Treatment with organic fertilizers has a positive effect on the nutritional value of the feed (Bozhanska, 2021).

The purpose of the present study is to establish correlation dependencies and regression connections between the yield and the quality of the forage, as well as the impact of the factors, such as the conditions of the year and the way of fertilizing with humate fertilizers the grass stands with bird's-foot-trefoil, red fescue and sainfoin.

Material and Methods

During the period 2013–2015, a field experiment was conducted in the experimental site of Research Institute of Mountain Stockbreeding and Agriculture – Troyan with legumes, such as bird's-foot-trefoil, sainfoin and red clover. The grass stands were treated with boron and molybdenum humate at a dose of 1600 ml/ha. The experimental design was a block method with 4 replications, with plot size of 5 m^2.

The technology of placing the experience, the method of fertilization and the harvesting of the grass stand are described in a published article (Churkova, 2019).

On the basis of reported indicators, such as dry matter yield (t ha^{-1}), botanical composition of the grass stand, determined in weight percentage (%), plant height (cm) and basic chemical composition expressed in: crude protein content (CP), crude fiber (CFr), crude fat (CF) and nitrogen-free extractable substances (NFE) measured in g kg^{-1} and was determined by the classic Weende method (AOAC, 2000). Crude protein values were reported on a nitrogen available basis by converting to N x 6.25 using the Kjeldahl method (AOAC, 1995). Crude fibers were determined by the method of Heteron and Jensen based on the solubility of non-cellulosic components in sulphuric acid and potassium hydroxide solutions.

The following indicators were reported: dry matter yield, determined by mowing, years and average for the period, by drying average samples to a constant weight at 105°C and recalculated according to the percentage of dry matter in the green matter (t ha^{-1}); botanical composition of the grass stand, determined immediately before harvest of first regrowth in weight percent (%), by taking average samples from each replicate; morphological composition – determined by weight of an average sample of each variant and each replication of stems, leaves and generative organs; plant height (cm) – measured in bud-formation period-beginning of flowering of 40 plants of each variant, taken from each replication (Churkova, 2019).

Based on the obtained data of the above-mentioned indicators, published in the two publications, statistical processing was done. For this purpose, the software products Analysis Toolpak for Microsoft Excel 2010 and Statgraphics Plus v.2.1 were used, and correlation dependences were determined and regression equations were derived. To determine the interconnection between the factors, such as yield with conditions of the year and method of fertilizing alone and the interconnection between them, a two-factor ANOVA analysis of variance was applied.

Results and Discussion

Correlation and regression dependences between dry matter yield, relative share of legume grasses in the grass stands, percentage share of stems and leaves and plant height with main qualitative indicators (Table 1).

The data on the interconnection between basic quantitative and qualitative indicators in Table 1, show a high correlation dependence between the phosphorus content and the height of fertilized legume terrains (r = 0.90) and the content of the nitrogen-free extractable substances and the relative share of the stems (r = 0.86). The content of crude protein and the percentage share of the leaves are in a very strong positive correlation (r = 0.80), whereas the crude protein with the stems in a very strong negative one (r = – 0.80). Dry matter yield is in a relatively strong correlation with the ash content (r = 0.58) and a strong negative dependence on the contents of crude fiber (r = – 0.66). This determines the higher forage quality, the better dry matter digestibility and the absorption by animals.

The theoretical regression line and the equation of the regression dependence between the content of crude protein and the percentage share of the leaves in the dry biomass of meadow legume crops is depicted in Figure 1, where Y = 0.0653 x + 14.5666 at a relatively high determination coefficient – R^2 = 0.64. The relationship obtained confirms the thesis that the greater the presence of leaves in the grass stand, the higher the protein content of the forage. Similar results were obtained by Bozhanska et al. (2022). Since the amount of crude protein is a determining factor for forage quality, and the leaves are the main elements of the plant accumulating protein, so the resulting correlation and regression dependence prove the resulting claim. In this way, the positive impact of boron and molybdenum humate on the quantitative increase of the leaves in the grass stands of bird's-foot-trefoil, red clover and sainfoin and on the content of protein in the forage is also evident.

The strong correlation dependence between the stems and the nitrogen-free extractable substances is represented by the equation Y = 0.0935 x + 32.029 (Fig. 2), at a high de-

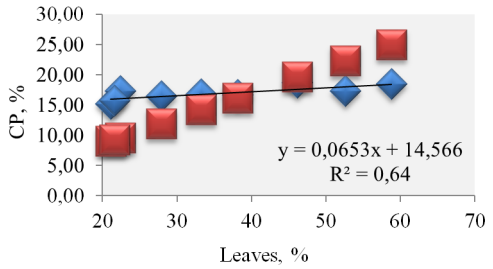

Fig. 1. Regression dependencies between the content of crude protein and the percentage of leaves in grass stands of meadow legume crops fertilized with humate fertilizers

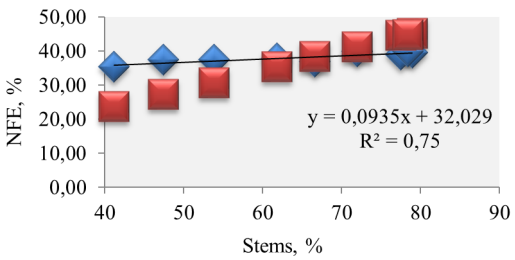

Fig. 2. Regression dependencies between the stems and the nitrogen-free extractable in grass stands of meadow legume crops fertilized with humate fertilizers

Table 1. Correlation dependences between dry matter yield, relative share of legumes in the grass stands, percentage share of stems and leaves and plant height with main qualitative indicators

	Dry matter yield	Legumes	Leaves	Stems	Height	Crude protein	Crude fat	Crude fiber	ash	NFE	*Ca*	*P*
Dry matter yield	1											
Legumes	-0.03	1										
Leaves	-0.17	-0.24	1									
Stems	0.17	0.24	-1	1								
Height	0.12	0.45	-0.75	0.75	1							
Crude protein	0.21	-0.12	0.80	-0.80	-0.59	1						
Crude fat	-0.34	0.41	0.55	-0.55	-0.27	0.46	1					
Crude fiber	-0.66	-0.47	0.19	-0.19	-0.10	-0.18	0.09	1				
ash	0.58	0.11	0.16	-0.16	-0.45	0.27	-0.02	-0.66	1			
NFE	0.26	0.37	-0.86	0.86	0.70	-0.74	-0.53	-0.48	0.02	1		
Ca	0.04	-0.32	0.53	-0.53	-0.77	0.72	0.26	0.00	0.28	-0.68	1	
P	0.03	0.30	-0.51	0.51	0.90	-0.42	-0.08	0.22	-0.63	0.35	-0.63	1

termination coefficient – $R^2 = 0.75$. In inequality $1.0 \geq (rxy) > 0.6$, the correlation coefficient is statistically proven, which confirms the established correlation between these two indicators.

Humate fertilizers has a positive effect on the change in the average values of the relative share of the stems and their height. The analyzed data indicate too strong correlation dependence (r = 0.75) between these indicators (Table 1). The theoretical regression line and the regression dependency equation (Figure 3) between the indicators are: $Y = 0.9588x + 5.3996$ at determination ratio $R^2 = 0.57$. Negative correlation dependence is observed between the heights of the stems and the percentage of the leaves (r = -0.75).

The treatment of grass stands with meadow legume crops with boron and molybdenum humate shows a tendency to increase the content of biologically active substances with the height of the plants (Churkova, 2019). This is confirmed by the high correlation between both tested indicators, corresponding (r = 0.70). Regression dependence and the regression equation derived (Figure 4) are: $Y = 0.0591x + 34.069$ at the determination factor $R^2 = 0.48$.

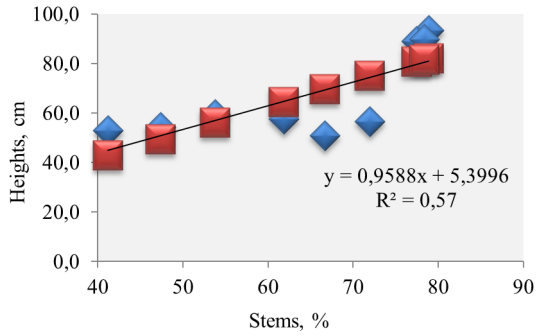

Fig. 3. Regression dependencies between the percentage of stems and plant height in grass stands with meadow legume crops fertilized with humate fertilizers

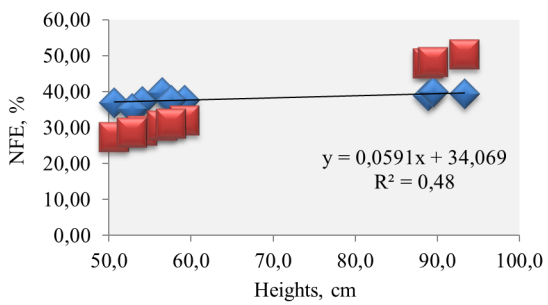

Fig. 4. Regression dependencies between stem height and nitrogen free-extractable substances in grass stands of meadow legume crops fertilized with humate fertilizers

Phosphorus and calcium macronutrients are the main factors that determine the growth and development of grass species. They stimulate the development of the root system and contribute to the movement of chemical elements to the vegetative parts of the plants. Humate fertilizers such as biostimulants accumulate phosphorus accumulation in the forage with the height of the plants. Especially the red clover and the sainfoin in the second year of their development reach high values of the stems and explain the too high correlation dependence (Table 1) between the content of the phosphorus and the height of the plants (r = 0.90). The equation by which it is possible to predict the amount of phosphorus by the height of the stems is: $Y = 0.0012x + 0.1354$ at the determination factor – $R^2 = 0.81$ (Figure 5).

Correlations are at a statistically proven level of significance between calcium content and that of crude protein (r = 0.72) and can be predicted by the equation y= 0.1771x – 0.2617 (Figure 6). The amount of calcium in the forage of the tested meadow legume crops fertilized with humate fertilizers was not affected by the heights of the stems, which was also confirmed by the strong correlation (Table 1) between the content of calcium and the heights (r = 0.77).

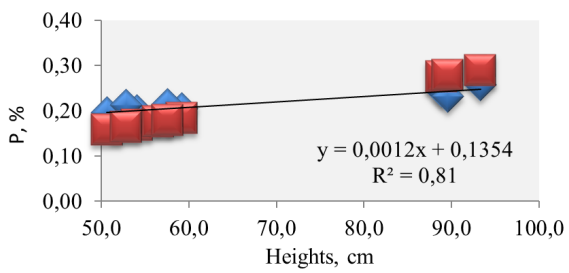

Fig. 5. Regression dependencies between the content of phosphorus and the heights in grass stands with meadow legume crops fertilized with humate fertilizers

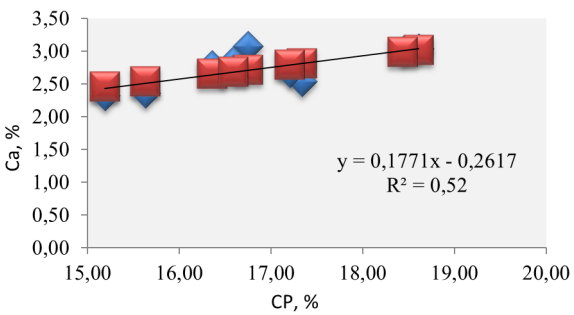

Fig. 6. Regression dependencies between the content of crude protein and calcium in grass stands of meadow legume crops fertilized with humate fertilizers

Table 2. Two-factor analysis of variance between dry matter yield, conditions of the year, method of fertilizing alone and interconnection between them

Source of Variation	SS	df	MS	F	P-value	F crit	*Ŋ*
Conditions of the year	61.74991	2	30.87496	153.1937	2.97682E-28	3.109311	68.69
Fertilizing method	5.271141	8	0.658893	3.269257	0.002803037	2.054882	5.86
Interaction of Conditions of the year x Method of fertilizing	6.54702	16	0.409189	2.030291	0.020371826	1.769953	7.28
Within	16.3249	81	0.201542				
Total	89.89297	107					

The established values of the F criterion show that there are reliable differences, both between the studied conditions during the years of the study, as well as the independent factors and in the interaction fertilizing method – conditions of the year. According to the results of the two-factor analysis of variance (Table 2), the studied factors, such as conditions of the year and method of fertilizing alone and in interaction, reliably affect the variance in dry matter yield and are statistically proven at a significance level of $P < 0.01$; $P < 0.001$ and $P < 0.05$.

Regarding the forage yield, the conditions of the year have the largest relative share in the variance of the trait (61.74991%) The fertilizing method accounts for 5.27%, and the interaction between the two factors accounts for 6.54% of its variation. The degree of impact of the conditions of the year is best expressed by $\eta = 68.69$, and the fertilizing method and the interaction of the factors have an insignificant impact, respectively 5.86 and 7.28. The intensity of the impact of soil and climatic conditions exceeds many times the independent impact of the fertilizing method and the interaction of both factors on the dispersion of the trait. In this regard, the conditions of the year is an important factor for the stability in terms of productivity and in the application of boron and molybdenum humate for grass stands of bird's-foot-trefoil, red clover and sainfoin.

Conclusions

The statistical processing of the data for quantitative and qualitative indicators, as a result of fertilizing with humate fertilizers grass stands with bird's-foot-trefoil, red clover and sainfoin shows their positive impact on the content of crude protein, calcium and phosphorus, as well as on the relative share of leaves in the stand.

The high correlation dependence found between the phosphorus content and the height of the fertilized meadow legume crops ($r = 0.90$), the content of nitrogen-free extractable substances and the relative share of stems ($r = 0.86$), as well as the content of crude protein and the percentage of leaves ($r = 0.80$) prove, the role of humate fertilizers on growth and the development of legume crops and their impact on the accumulation of nutrients in the forage. The regression equations derived prove the opportunity to predict the contents of crude protein from the share of the leaves in the grass stand ($R^2 = 0.64$), the phosphorus content from the height of the stems ($R^2 = 0.81$) and of the nitrogen-free extractable substances from the relative share of the stems ($R^2 = 0.75$), in anxiety. Two-factor analysis of variance showed a high impact of the conditions of the year ($\eta = 68.69$) on the dry matter yield.

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