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Evaluation of the effect of Reni preparations application on some essential amino acids in alfalfa (*Medicago sativa* L.) biomass by correlation and factor analysis

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Abstract

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The present study aims to evaluate the influence of different Reni preparations on essential amino acids in biomass of Mnogolistna 1 variety, using a mathematical approach (correlation and factor analysis). Three-year data, based on a field experiment, conducted at the Agricultural University – Plovdiv experimental field in the period 2017-2019 was analysed. The study is part of University project for establishing the influence of Reni preparation on the yield and quality as well as the relations between the researched indicators.

The proposed mathematical approach allows increasing the objectivity when evaluating the complex effect of Reni preparations on the main chemical components in alfalfa Mnogolistna 1. Reni treatment improves the biological value of proteins – increases the total amount of essential amino acids and changes the ratio of essential amino acids to other proteinogenic amino acids in favour of the essential ones. As a result of the conducted analysis, correlations between the studied indicators were established. The strongest positive correlation was found between the amino acids lysine and leucine (r = 0.960), threonine and phenylalanine (r = 0.980) and valine and isoleucine (r = 0.981), respectively. By using Factor analysis according to the method of Principal Component Analysis, the amino acids lysine, threonine and leucine correlated themselves only were combined in two new factors that explain 73% of the total variance of the variables.

Keywords: alfalfa; correlations; factor analysis

Introduction

Alfalfa green mass and hay are characterized with high protein content and essential amino acids – main indicators that determine the quality of resulting biomass. A number of studies have shown that the application of various growth regulators has a positive effect on the quality of alfalfa feed (Buck et al., 1988; Wang et al., 2003; Radu et al., 2010). The proteinogenic amino acids in the vegetative mass of alfalfa are related to the biological value and balance of proteins. Their amount in the individual protein fractions is under genetic control and difficult to change, but the ratio of protein fractions, and through them the quality of the protein can change under external influences.

The treatment with Reni preparations has a positive effect on the total content of protein-genic amino acids in Mnogolistna 1variety. The variety is a representative of alfalfa latest generations. It was created by an author team from Research insti-

tute "Obraztsov Chiflik" – Ruse, Higher Agricultural Institute – Plovdiv and Institute of Animal Science in Kostinbrod. Over 50% of plant leaves have 5 to 7 petals in a single petiole. This feature distinguishes it from all Bulgarian and foreign alfalfa varieties listed in the National List of the country. The rich leaf mass guarantees 1.5% higher crude protein content compared to standard varieties. The plants are strongly branched, dark green, with predominant purple inflorescences. In terms of yield of dry vegetative mass, the variety is as good as the best Bulgarian varieties. The plants are resistant to leaf diseases and root rot.

Reni preparations have been created at the Agricultural University – Plovdiv (Popov, 1995), as a means of regulating nitrogen metabolism. They are combinations of the microelements molybdenum, manganese, magnesium in different concentrations and ratios. Reni preparations are used for foliar treatment on alfalfa with a solution containing the components. Methods of the experiment conduction along with the studied indicators are available in our other works (Popov et al., 2007; 2010). The content of some nutrients in plants and the activities of the enzymes nitrogenase, nitrate reductase, glutamine synthetase and asparagine synthetase serve as a diagnostic sign for determining the exact concentration and ratio of the individual components in the various formulations.

Reni preparations increase the activity of the main enzyme systems, related to the metabolism of nitrate ions. As a result of Reni application, the production quality is improved, the yield has increased, and after their application the production is harmless to humans and animals. The treatment with Reni is performed once a year in the budding phase of each swath with a dose of 200 ml/da. The use of different Reni preparations on the variety increases the content of essential amino acids in only two of all options – Reni + Co by 14.52% and Reni by 4.8%, while Reni + B and ½ Reni + variant (½ Reni + B) it is lower (Sevov, 2011).

Reni application helps to study more detailed the mechanisms, influencing the biological nitrogen fixation in alfalfa.

The aim of the study is to assess the Reni application and its influence on some essential amino acids in alfalfa biomass, using database for this parameters by mathematical and statistical methods.

Material and Methods

The data from a field experiment, conducted at the Agricultural University – Plovdiv experimental field in the period 2017-2019 was analysed. The research was conducted in order to establish the influence of Reni preparation, applied alone and in combination with added microelements, on enzymes activity, multifoliate leaves expression, productivity and quality of multifolium alfalfa varieties. The experiment is based on the fractional plots method, in 4 repetitions with the size of the experimental plot – 10 m^2.

The evaluation of tested options was performed by comparing the following indicators – determining the quality of alfalfa: x_1 – lysine, x_2 – threonine, x_3 – valine, x_4 – methionine, x_5 – isoleucine, x_6 – leucine, x_7 – phenylalanine.

Correlation analysis show the presence of statistically significant correlations between the studied indicators, followed by factor analysis technique (Kline, 1994) in order to reduce the number of the seven initially included indicators. The factor analysis is performed by the principal component method (PCA). The number of principal components is determined by the number of eigenvalues of the correlation matrix that are greater than 1 (Kaiser's criterion). Eigenvalues show the contribution of the own vectors in explaining the total dispersion in the variables.

The factor model is usually determined by the factor weights, which represent the correlation coefficients between the respective observed indicators and the factors. Thus, a smaller number of generalized factors are determined, without their own meaning, but combining the properties of several indicators.

Adequacy assessment of the factor analysis was performed by using the Kaiser – Mayer – Olkin (CMO – test) and Bartlett tests.

The data processing was performed with the statistical program SPSS.

Results and Discussion

The correlation coefficients, expressing the relationship between the studied indicators are indicated in the correlation matrix (Table 1). A strong positive correlation was found between the amino acids lysine and leucine, threonine and phenylalanine; valine and isoleucine, respectively, with correlation coefficients – r = 0.960; r = 0.980 and r = 0.981. The correlation between the indicators was less expressed in lysine and phenylalanine (r = 0.920). All these correlation coefficients were statistically proven at the level of significance.

Table 1. A correlation matrix for Mnogolistna 1 variety

	x_1	x_2	x_3	x_4	x_5	x_6	x_7
x_1	1.00						
x_2	0.828	1.00					
x_3	0.633	0.872	1.00				
x_4	0.565	0.034	-0.246	1.00			
x_5	0.726	0.864	0.981**	-0.095	1.00		
x_6	0.960**	0.691	0.588	0.624	0.716	1.00	
x_7	0.920*	0.980**	0.810	0.230	0.833	0.804	1.00

The indicators x_1 – lysine, x_2 – threonine, x_3 – valine, x_4- methionine, x_5- isoleucine, x_6- leucine, x_7- phenylalanine are determining the quality of alfalfa.

The performed correlation analysis and the established high, statistically proven, and values of r gave us grounds to apply the methodology of the factor analysis. The Factor analysis was used as an appropriate method to reduce the dimensionality of the initial n-dimensional space by establishing a new basis of factors (Gorsuch, 1983; Milev et al., 2015).). The application of the factor analysis in the present study reduces the number of the seven included indicators by grouping in a common factor those that correlate with each other and dividing the non-correlated ones into different factors. A similar approach has been used in other studies (Ivanova et al., 2010; Petrovska, et al, 2012; Ilchovska et al., 2014).

Based on the results, accuracy in assessing, the effect of preparations on some amino acids can be increased, which is a prerequisite for an increased accuracy in making specific decisions in practice and in the actual treatment of alfalfa.

When performing the factor analysis, applying the method of the principal components, it was registered that two factors have eigenvector values greater than 1, which was decisive in the selection of two principal components (Figure 1).

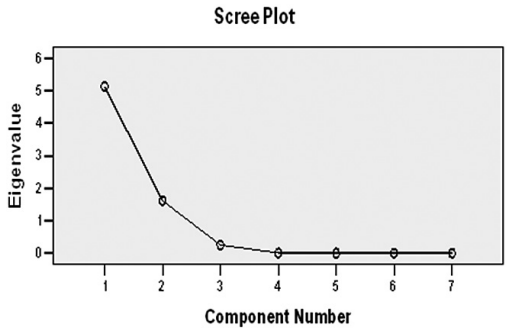

Fig. 1. The values of the own vectors

According to the first main component, explains 73.23% of the total variation, the traits lysine, threonine, isoleucine, leucine and phenylalanine have the greatest influence on the reflected clustering. The second main component explains 23.06% of the total variation, which determines the significance of the methionine trait.

Table 2 shows the factor weights and how the variation is distributed among the main components. They explain 96.23% of the total variance of the sample. The variables x_1 – lysine, x_2 – threonine, x_5 – isoleucine, x_6 – leucine and x_7 – phenylalanine have high factor weights in the first component.

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Data show that this factor should be associated primarily with the amino acids lysine, threonine, isoleucine, leucine and phenylalanine. This factor could be defined as a summary for the indicators that have the greatest relative weight in alfalfa grouping. The second component is mainly related to methionine. Results, obtained through the applied factor analysis are synchronized with the results of the correlation analysis, based on the same indicators.

Conclusion

As a result of the analysis, is found correlations between the studied indicators. The strongest positive correlation is found between amino acids lysine and leucine (r = 0.960), threonine and phenylalanine (r = 0.980) and valine and isoleucine (r = 0.981), respectively. The main indicators, having strongest influence on the separation of variants are amino acids lysine, threonine, isoleucine, leucine and phenylalanine, titratable organic acids, total dyes, lycopene and beta carotene combined in the first factor, and explaining 73.23% of the total variance of changes.

Table 2. Factor matrix obtained by the principal component method

N	Indicators	Main components		Symbols
		1	2	
1.	Lysine	0.938	0.342	x_1
2.	Threonine	0.942	-0.204	x_2
3.	Valine	0.861	-0.485	x_3
4.	Methionine	0.252	0.966	x_4
5.	Isoleucine	0.911	-0.340	x_5
6.	Levcine	0.884	0.413	x_6
7.	Phenylalanine	0.972	-0.011	x_7
Percentage of the total variation ,%		73.23	23.06	
Cumulative percentage of the total variation ,%		73.23	96.23	

Principal Component Analysis 2 component extracted

These results could be basis for determining the most promising varieties and their rational use.

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