FRAP CAPACITY EVALUATION OF PERENNIAL FORAGES IN THE MIDDLE OF JUNE BY PRINCIPAL COMPONENTS & CLASSIFICATION ANALYSIS

Monica Harmanescu[1]

[1]*Faculty of Agriculture, Banat's University of Agricultural Sciences and Veterinary Medicine "Mihai I al Romaniei" from Timisoara, Calea Aradului, no.119, Romania*
e-mail: monica.harmanescu@yahoo.com

Abstract
This main goal was to test Principal Components & Classification Analysis (PC&CA), an exploratory multivariate technique, for FRAP capacity evaluation of Romanian permanent grassland forages depending on fertilization: organic or mineral. The vegetal samples were harvested in the middle of June from hill grassland of Caras-Severin. Seven cases and fifteen variables were chosen for the multivariate data matrix. PC&CA offered the possibility to differentiate between organic fertilized cases from those fertilized exclusive mineral or unfertilized. The highest positive correlation coefficients for FRAP capacity were found for the two species from others botanical family, *Inula britannica* and *Rosa canina*. PC&CA can be considered a useful and flexible statistic instrument to evaluate the FRAP capacity of the grassland forage in the middle of June, depending on fertilization, in the soil-clime conditions of Caras-Severin.

Introduction
Principal components analysis is a multivariate technique used to obtain few artificial variables as principal components based on a high number of correlated characteristics, for extract and make accessible important information about the complex process [1, 2, 3, 4, 5, 6, 7]. In this study it was chosen a real-case for test the facilities offered by PC&CA: the data of the perennial forages FRAP capacity, soil, fertilization and the presence of some plants species in grassland covering from Caras-Severin. Why FRAP capacity of the plants from grassland? Because perennial forages offer the opportunity to obtain functional feed for animals with minimum investment, cheaper then others sources, with high benefic impact on animal's health.
FRAP capacity of plants is related to antioxidant compounds which can reduce the free radicals, responsible for many chronic diseases in human's organism [8, 9, 10]. The food safety and quality are influenced by the feed safety and quality. That's way it is important to identify parameters easy to monitor, rapid and with low cost, which can give relevant information on the quality of agricultural products used as feed and raw matter in food industry or for direct human consume. FRAP capacity of perennial forages can be in the future a parameter of feed quality, determined as routine.

Experimental
The quantitative characteristics of fifteen variables of the seven cases (one unfertilized and six fertilized) were used as PC&CA data matrix fitted by StatSoft - STATISTICA version 10. The vegetal (perennial grassland forages) and soil samples were harvested in the middle of June 2009. The location was N: 45°12' latitude; E: 21°60' longitude. Calcic Luvisol and temperate continental with Mediterranean influences clime characterized the studied permanent grassland ecosystem. The grassland was fertilized since 2003 as experimental field with five

replications for each case. One case was kept in unfertilized ecosystem conditions (M) and six cases were fertilized. Fermented sheep manure was chosen for organic fertilization cases (O1, O2, O3) with doses varied between 20-60 t/ha, being applied at each two years. The mineral nitrogen fertilizer varied between 100-200kg/ha in M1, M2 and M3 cases, on a constant doses of potassium and phosphorus (50kg/ha), applied yearly. The 200 kg/ha of mineral nitrogen were applied in two steps.

The active variables of PC&CA matrix were fourteen: soil pH, soil humus (%), soil total nitrogen content (%), organic and mineral nitrogen fertilization data, gravimetric percents of grassland forages for *Calamagrostis epigejos* (Ce), *Festuca rupicola* (Fr), *Poa pratensis* (Pp), *Lathyrus pratensis* (Lp), *Trifolium repens* (Tr), *Inula britannica* (Ib), *Filipendula vulgaris* (Fv), *Rosa canina* (Rc) and *Galium verum* (Gv). One supplementary variable was used: the FRAP capacity of perennial forages of the seven grassland cases (*FRAP).

Soil pH was quantified respecting the SR ISO 10390/1999 [11]. The soil humus was determined by Walklay – Black – Gogoaşă method [12]. The soil total nitrogen content was measured by Kjeldahl method [13]. The grassland floristic composition was established gravimetric (%). FRAP capacity of perennial forages was estimated quantitatively by FRAP method [14], using ethanol extracts (70%) diluted 1/20 and TPTZ reagent (tripiridil-triazine). The absorbance was read at 593 nm.

Results and discussion

The data acquired for the Calcic Luvisol pH varied in 5.4 – 6.0 range. The humus values were between 6.2% and 9.0%. Total nitrogen content of hill grassland soil was in 0.23 – 0.31% range. The recorded data for gravimetric percent of *Calamagrostis epigejos* (Ce) in grassland covering were between 5% and 13%, higher in mineral nitrogen fertilization cases (M1, M2, M3). *Festuca rupicola* (Fr) was present in 16 – 46% range, also with the highest values in the mineral nitrogen fertilization cases. *Poa pratensis* (Pp) gravimetric percents were under 5%. *Lathyrus pratensis* (Lp) was present in grassland forages under 6%, higher in organic fertilization cases (O2, O3), while *Trifolium repens* (Tr) was under 38%, the highest in O3 (the fertilization case with the highest dose of fermented sheep manure). The others two organic fertilization cases (O1 and O2) had also high quantity of *Trifolium repens*, varied in 25-27% range. The species from others diverse species as *Inula britannica* (Ib), *Filipendula vulgaris* (Fv), *Rosa canina* (Rc) and *Galium verum* (Gv) were under 5%, 9%, 18%, and respectively 7%. All these recorded data were introduced in multivariate data matrix as active variables. The FRAP capacity of the perennial grassland forages of the seven studied cases (supplementary variable) was in 122 – 278 μM Fe^{2+}/g range. The projections on the PC1xPC2 plan of the seven studied cases and of the fifteen variables are presented in Figure 1, respectively in Figure 2:

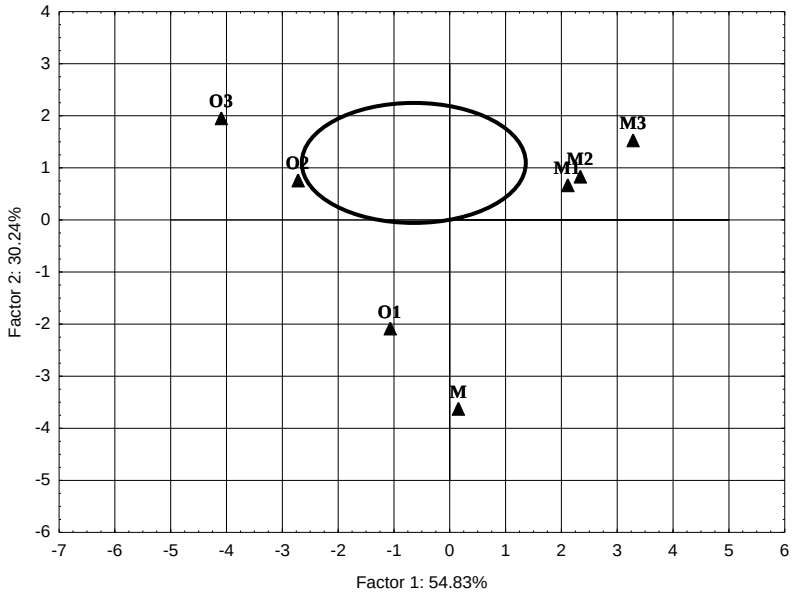

Figure 1. The projection of the seven studied cases on the PC1xPC2 plan

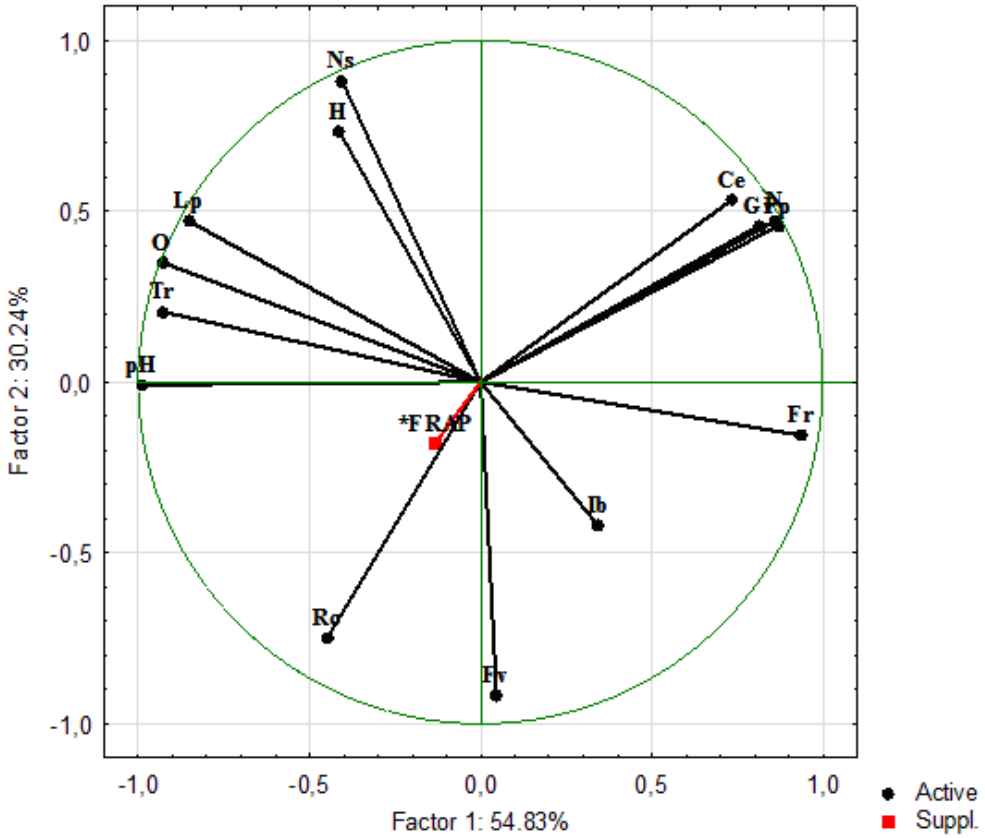

Figure 2. The projection of the fifteen variables on the PC1xPC2 plan

The PC&CA model was described 100% by six principal components. First three principal components had the eigenvalues higher than 1: 7.7 (PC1), 4.2 (PC2) and 1.3 (PC3). PC1 and PC2 described more than 85% of the PC&CA variance.

There were some variables which had a high positive influence on first principal component (PC1): the presence in permanent grassland forages of *Calamagrostis epigejos* (0.74), *Festuca*

rupicola (0.94), *Poa pratensis* (0.87), *Galium verum* (0.81) and mineral nitrogen fertilization data (0.86). A high negative influence on PC1 had soil pH (-0.99), *Lathyrus pratensis* (-0.85), *Trifolium repens* (-0.93), and the organic fertilization data (-0.93). In the second principal component (PC2) the highest positive influence had humus (0.73) and total nitrogen content (0.88) soil parameters. *Filipendula vulgaris* and *Rosa canina* had the highest negative impact on PC2: -0.91, respectively -0.75.

Projecting the seven cases of perennial forages harvested from the hill grassland on PC1xPC2 plan (Figure 1), it can be observed that mineral nitrogen fertilization trials were grouped distinctively (M1, M2 and M3). On the opposite side were grouped the forages from the trials fertilized with the highest doses of fermented sheep manure (O2 and O3). The smallest dose of fermented sheep manure (20t/ha) application differentiate the grassland ecosystem parameters of O2 from the others.

Figure 2 and the correlations of data matrix have shown that in the experimental field conditions of studied permanent grassland, *Lathyrus pratensis* and *Trifolium repens* had the highest presence in the fermented sheep manure application cases, the coefficients being 0.95, respectively 0.96, while in mineral nitrogen fertilization cases the coefficients were negatively: -0.50, respectively -0.68. The correlation between organic fertilization data and the *Galium verum* presence (%) had also a negative coefficient (-0.57). In mineral fertilization cases the *Poaceae* species were dominants, heaving high positive correlation coefficients: 0.88 for *Calamagrostis epigejos*, 0.68 for *Festuca rupicola* and 0.94 for *Poa pratensis*. *Galium verum* was also influenced significant positively by mineral fertilization, the correlation coefficient being 0.96. *Trifolium repens* was correlated negatively with mineral fertilization, the coefficient was -0.68. Between supplementary variable *FRAP capacity of perennial forages and the gravimetric percents of *Inula britannica* and *Rosa canina* the correlation coefficients were positive in this period of the year: 0.35, respectively 0.31.

Conclusion

PC&CA can be a flexible statistic tool to monitor the FRAP capacity of the grassland forage in the middle of June, depending on fertilization. Using this exploratory multivariate technique and the selected active and supplementary variables, it was possible to discriminate between organic fertilized trials (cases) from those fertilized exclusive mineral or unfertilized. It was possible also to highlight the correlations between fertilization data and the presence of different plants species responsible for the FRAP capacity in the middle of June, in soil-climatic conditions of experimental field from Caras-Severin hill. Organic fertilizer influenced the presence of *Lathyrus pratensis* (0.95) and *Trifolium repens* (0.96). Mineral fertilization had positive influence on the distribution of *Calamagrostis* epigejos (0.88), *Festuca rupicola* (0.68), *Poa pratensis* (0.94) and *Galium verum* (0.96). FRAP capacity of permanent grassland forages had positive correlation coefficients for *Inula britannica* (0.35) and *Rosa canina* (0.31).

Acknowledgements

The quantitative results, used as data matrix to test the PC&CA multivariate technique facilities for FRAP capacity of permanent grassland forages depending on fertilization, were aquisited with the financial support of Grant BD/CNCSIS Romania: "*On the influence of substances flows on the quality of forage from grassland*", 2007 – 2009 (www.cncsis.ro).

References

[1] F.K. Wanga, T.C.T. Dub, Omega, 28 (2000) 185-194.
[2] A.B.W. Manage, S.M. Scariano, Journal of Statistics Education, vol.21, no.3 (2013) 1-22.
[3] S.C. Crespo, A.L. Moreno-Chacón, A. Rojas. L.M. Melgarejo, Braz. Chem. Soc., vol.22, no.12 (2011) 2275-2280.
[4] G. Evgenidis, E. Traka-Mavrona, M. Koutsika-Sotiriou, International Journal of Agronomy, article ID 697879 (2011), 7 pages.
[5] K. Rymuza, E. Turska, G. Wielogórska, A. Bombik, Acta Sci. Pol., Agricultura, vol. 11, no.1 (2012) 79-90.
[6] P. Singh, V. Bajpai, S. Kumar, M. Srivastava, B. Kumar, Journal of Medicinal Plants Studies, vol.5, no.3 (2017) 384-391.
[7] G. Verma, P.K. Mahajan, A. Chandel, International Journal of Applied Research, vol.2, no.7 (2016) 857-859.
[8] C. Henriquez, S. Almonacid, I. Chiffelle, T. Valenzuela, M. Araya, L. Cabezas, R. Simpson, H. Speisky, Chilean Journal of Agricultural Research, vol.70, no.4 (2010) 523-536.
[9] M.B. Hossain, N.P. Brunton, C. Barry-Ryan, A.B. Martin-Diana, M. Wilkinson, Rasayan J. Chem., vol.1, no.4 (2008)751-756.
[10] R.L. Prior, X. Wu, K. Schaich, J. Agric. Food Chem., 53 (2005) 4290−4302.
[11] SR ISO 10390 (1999), *Calitatea solului. Determinarea pH-ului.*
[12] E. Stoica coord., C. Răuţă, N. Florea, Metode de analiză chimică a solului, Bucureşti, 1986.
[13] I. Gergen, *Analiza produselor agroalimentare*, Eurostampa Ed., Timişoara, 2004, pp.40.
[14] I.F.F. Benzie, J.J. Strain, Analytical Biochemistry, 239 (1996) 70-76.