DPPH SCAVENGE CAPACITY OF GRASSLAND FEED AT THE END OF MAY, DEPENDING ON FERTILIZATION, BY PC&CA

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Abstract

The main topic of this study was to use Principal Components & Classification Analysis (PC&CA) for statistic evaluations of DPPH scavenge capacity of perennial feed from hill grassland related to fertilization cases. It was harvested the plants at the end of May. StatSoft – STATISTICA, Version 10, performed PC&CA. DPPH values of perennial feed were included as supplementary variable in the PC&CA complex matrix. Active variables of the matrix were: soil humus, organic fertilization data with fermented sheep manure, mineral fertilization data based on nitrogen, the gravimetric distribution in grassland covering of *Trifolium* sp. (Tsp.%), *Calamagrostis epigejos* (Ce%), *Festuca rupicola* (Fr%), *Inula britannica* (Ib%), *Filipendula vulgaris* (Fv%). It was selected seven cases (with five replications): three fertilized organic, three mineral and one unfertilized. The correlation coefficient between *Filipendula vulgaris* (%) and DPPH scavenge capacity of perennial feed was 0.55. *Calamagrostis epigejos* (%) was influenced positively by the mineral nitrogen fertilization, correlation coefficient being 0.91, but this perennial plant did not increase the DPPH scavenge capacity of grassland feed (correlation coefficient was -0.47). Fermented sheep manure influenced positively the *Trifolium* sp. (%) in permanent grassland covering, correlation coefficient being 0.88, but it did not improve significantly the DPPH scavenge capacity of grassland feed in this period of the year (correlation coefficient was 0.01). PC&CA can represent a promising statistic tool for monitoring the DPPH scavenges capacity of perennial grassland forages as functional feed, depending on fertilization management.

Key words: multivariate analyses, grassland, scavenge capacity, perennial forage, functional feed.

Principal components analysis can be used for statistical interpretation of high number of data, based on the reduction of all variables number by creating artificial principal components (Rymuza *et al*, 2012). Nowadays there are many applications of principal components analysis in agronomic field. It was used with success, for example, to differentiate the population genetic diversity of *Ononis* spp. depending on ploidy levels, considering diploid and tetraploid plants (Kloda *et al*, 2008). Rymuza *et al.* (2012) used it to discriminate between the wheat growths in plough tillage system from those growths in direct sowing, concluded that the best discriminates variables for the studied cases were both the yield and the mass of 1000 grains. Choosing this multivariate technique Paduret *et al.* (2016) differentiated between the plant parts of the dandelion (*Taraxacum officinale*) based on the minerals contents, while Singh *et al.* (2017) discriminated some *Cymbopogon* species based on the marker peaks of volatile essential oils, phenolics and flavonoids. Verma *et al.* (2016) found that analysis of principal components is a facile statistic tool for explanatory work to optimize the kinnow productivity depending on reproductive and morphological characters. Tarko *et al.* (2017) considered also in their study that principal components analysis is a very important tool to define which of the studied variables characterize better the complex phenomena.

The present issue proposes to test the chemometric technique PC&CA for a real case: evaluations of the DPPH scavenge capacity of perennial forages as functional feed from hill grassland, related to fertilization management. Why the DPPH scavenge capacity of the grassland feed as variable for PC&CA chemometric matrix? Because in the last two decades it was an increased interest of many researchers to determine the DPPH scavenge capacity of plants (Ramakrishna *et al*, 2012; Mathangi *and* Prabhakaran, 2013; Paduret *et al*, 2016), especially for those recognized traditionally for their medicinal properties, or even of different parts of plants (Motlhanka *et al*, 2008; Rana *et al*, 2010).

DPPH reagent is a stable free radical with violet color, which has the capacity to accept an atom of hydrogen from compounds capable to donate it (antioxidants), becoming yellow (Molyneux, 2004). What are the free radicals? In nature free radicals are represented by hydroxyl radical, nitric oxide, superoxide anions, hydrogen peroxide, etc. (Ramakrishna *et al*, 2012; Sahu *et al*,

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2013). The state of art highlights in many studies that high concentration of free radicals in human's organism are responsible for oxidative stress and chronic diseases (Rana *et al*, 2010; Ramakrishna *et al*, 2012; Mathangi *and* Prabhakaran, 2013) as aging, cancer, cardiovascular diseases, etc. (Sahu *et al*, 2013).

It was observed that one of the most important factors, which influence significantly the action of antioxidants extracts from plants on DPPH free radical, is the hydroxyl group position (Motlhanka *et al*, 2008; Todorova *et al*, 2011). The phenolic group position, for example, influence the scavenge capacity as following: 3,4-di-OH >> 4-OH > 3-OH > 2-OH (Todorova *et al*, 2011). Another important factor is the extraction medium. Tarko *et al.* (2017) concluded that water extraction is not recommended for polyphenols. Sahu *et al*. (2013) considered that ethanol extracts of some leafy vegetables had higher antioxidant activity than hexane extracts. Tarko *et al.* (2017) found that methanol extracts had better results for antioxidants activity than ethanol extracts. But nowadays the methanol can not be used for human consumption, so it is recommended to use ethanol solution for antioxidant extraction for vegetal samples case (Motlhanka *et al*, 2008; Tarko *et al*, 2017). That's way in the present issue it was used ethanol extracts (70%) for the quantification of DPPH scavenge capacity of the grassland perennial forages as functional feed, related to fertilization management, by PC&CA.

MATERIAL AND METHOD

StatSoft – Statistica, Version 10, computed the PC&CA chemometric matrix. It was performed the chemometric model based on a real case: the numerical data obtained for vegetal and soil samples collected at the end of May 2008. A hill region was the permanent grassland location, in Caras-Severin, Romania, with the following coordinates: 45º12' latitude (N); 21º60' longitude (E). The region is characterized by a temperate continental with Mediterranean influences climate and Calcic Luvisol.

The PC&CA supplementary variable was the scavenge capacity data of perennial grassland feed, measured by DPPH method in triplicate, for the seven studied cases (25m^2/trial), with five replications for each case. Three cases (OI, OII and OIII) were fertilized at each two years with fermented sheep manure in 20–60 t/ha range. Three cases (MI, MII and MIII) were fertilized exclusively mineral yearly, with constant doses of phosphorus (50kg/ha) and potassium (50kg/ha) and different nitrogen doses varied between 100 - 200 kg/ha (in one or two steps). The last case was not fertilized, representing the normal growth conditions of plants in hill grassland ecosystem (noted U). The complete randomized blocks design was the grassland field organization. The permanent grassland was first fertilized in 2003.

The PC&CA active variables were soil humus, mineral fertilization data based on nitrogen, organic fertilization data with fermented sheep manure, the gravimetric distribution of *Calamagrostis epigejos* (Ce%), *Festuca rupicola* (Fr%), *Trifolium* sp. (Tsp.%), *Inula britannica* (Ib%) and *Filipendula vulgaris* (Fv%) in grassland perennial covering.

The DPPH measurements were made using 2,2-diphenyl-1-picrylhydrazyl reagent (Cuendet *et al,* 1997; Burits and Bucar, 2000). The ethanol extracts (70%) of perennial forages were diluted 1/10 before the addition of DPPH reagent. The absorbance was read at 517 nm at UV/VIS Specord-205 spectrophotometer of AnalyticJena. The soil humus content was quantified by Walklay – Black – Gogoasa method (Stoica *et al,* 1986).

RESULTS AND DISCUSSIONS

The soil humus (Hs) active variable of PC&CA chemometric matrix varied between 5.6% and 7.2%. The gravimetric (%) parameters of the perennial floristic matrix, introduced also as active variables, were the following: Ce(%), Fr(%), Tsp.(%), Ib(%) and Fv(%). *Calamagrostis epigejos* had the highest distribution in mineral fertilized cases (MI, MII, MIII), in 13 – 67% range, while in organic fertilized cases (OI, OII, OIII) was under 6%. *Festuca rupicola* percent was around 50%, the highest being in mineral fertilized case. The *Trifolium* sp. had the highest distribution in organic fertilized cases (more than 50%), while in unfertilized and mineral fertilization cases were around 16%, respectively under 5%. *Inula britannica* and *Filipendula vulgaris* gravimetric percents were generally under 15%.

The average values for DPPH scavenge capacity of perennial functional feed, as supplementary variable of PC&CA chemometric matrix, were around 6% in organic fertilization cases, 3% in exclusive mineral fertilization cases and 30% in unfertilized conditions.

The cases projection on PC1xPC2 plane of PC&CA chemometric model is shown in *figure 1*. The mineral and organic fertilized cases with the highest doses (MII, MIII, OII, and OIII) were distinct separated by chemometric PC&CA from the cases with minimum doses of fertilizers (MI and OI) and unfertilized case (*figure 1*). The projection of the cases based on the matrix data was computed considering the first two principal components. PC1 and PC2 summarized around 72% of total variance (*figure 2*).

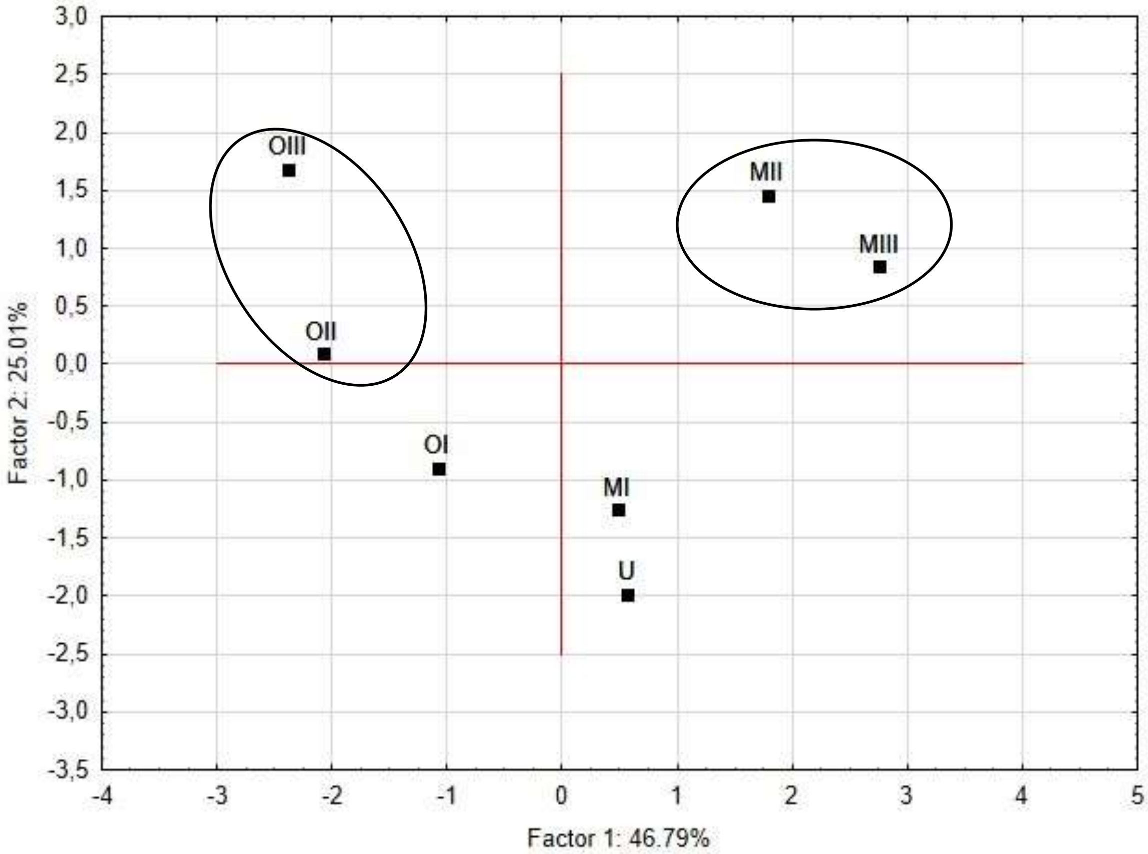

Figure 1 **The PC&CA cases projection on PC1xPC2 plane**

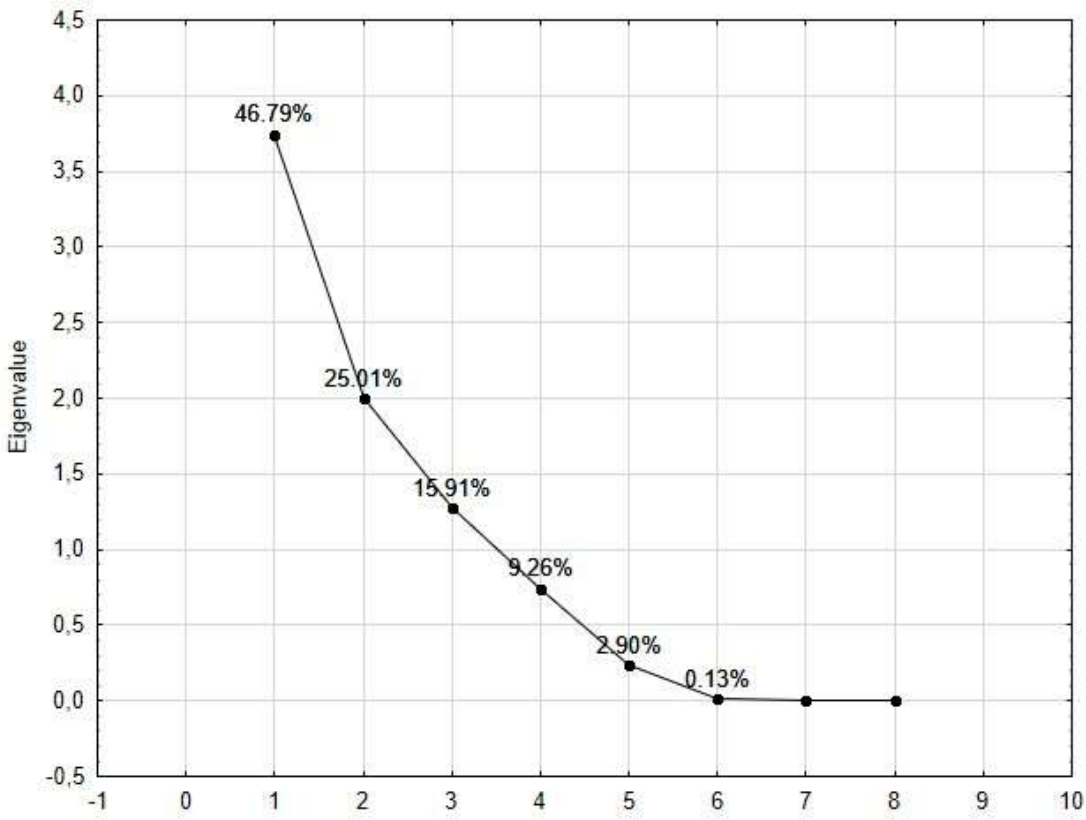

Figure 2 **The eigenvalues of PC&CA principal components**

Verma *et al.* (2016) sustain that it is important the Gutman's lower bound. In the studied case of the present issue, regarding the DPPH scavenge capacity of functional perennial forages, first three from the six principal components had the eigenvalue higher than 1.

The highest positive impact on the first principal component (PC1) had the active variables *Calamagrostis epigejos* (0.78) and mineral nitrogen fertilization data (0.87). The highest negativ impact on PC1 had *Trifolium* sp. (-0.95), *Inula Britannica* (-0.65), and the fermented sheep manure fertilization data (-0.88). The second principal component (PC2) was characterised positively mainly by the active variables *Calamagrostis epigejos* (0.58) and soil humus (0.60), and negatively by the active variables *Festuca rupicola* (-0.76), *Filipendula vulgaris* (-0.57) and DPPH scavenge capacity of perennial forages from grassland (-0.74).

Considering the first two principal components (PC1 and PC2), it was computed the chemometric projection of supplementary and active variables (*figure 3*).

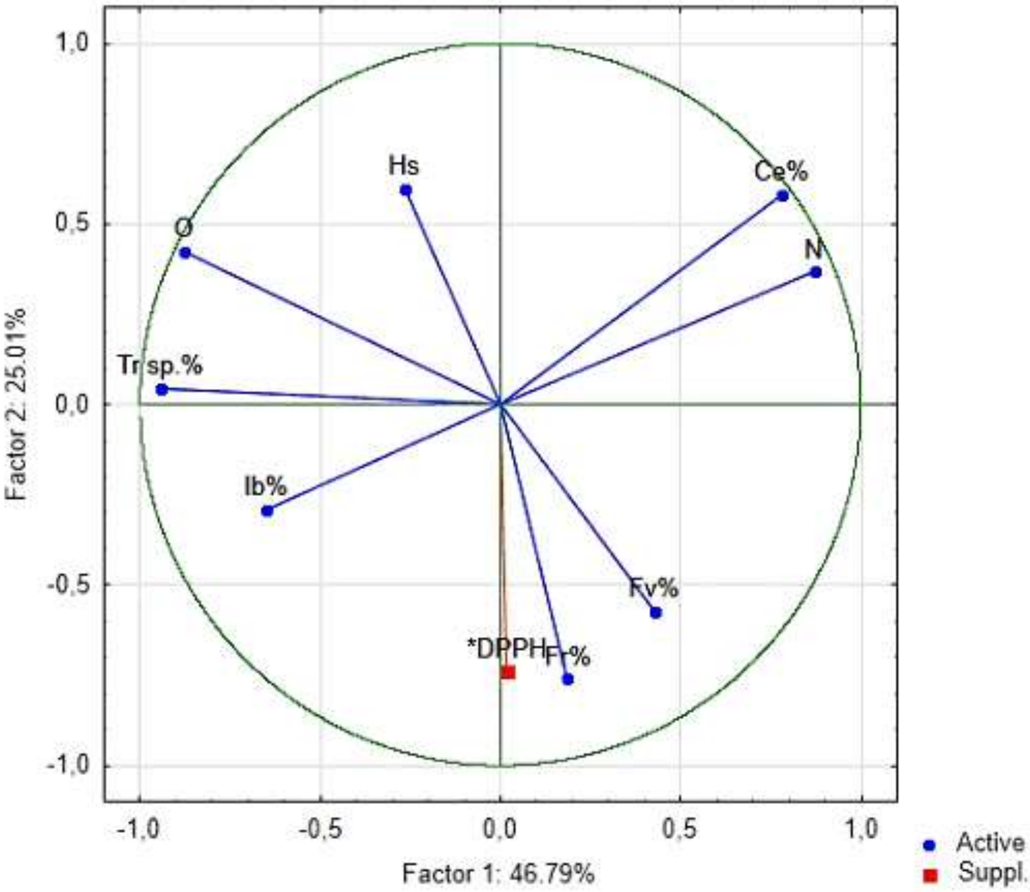

Figure 3 **The PC&CA supplementary and active variables projection on PC1xPC2 plane**

At the first sight, it is obviously that in this period of the year the distribution of *Calamagrostis epigejos* (%) and *Trifolium* sp. (%) in perennial grassland covering did not influence significantly the DPPH scavenge capacity of the grassland feed. Taking into consideration the correlation coefficients, it was possible to highlighted different characteristics of the chemometric data matrix. The mineral nitrogen fertilization influenced positively the perennial *Calamagrostis epigejos* (%) presence in grassland forages at the end of May, correlation coefficient being 0.91. The coefficient for the correlation between feed DPPH scavenge capacity and *Calamagrostis epigejos* (%) distribution was negatively (-0.47).

In *Trifolium* sp. (%) case, the fermented sheep manure influenced positively its presence in permanent grassland covering (correlation coefficient was 0.88), while the fertilization with different mineral nitrogen doses had significant negative impact (correlation coefficient was -0.84). In the studied period, in soil and climate conditions of hill region from Caras-Severin, the correlation between *Trifolium* sp. (%) and feed DPPH scavenge capacity had the coefficient 0.01.

With regard to the others spontaneous plants of permanent grassland, found in the opposite half of the PC&CA variables projection on PC1xPC2 plane, a positive coefficient higher than 0.5 was obtained for the correlation between *Filipendula vulgaris* (%) and feed DPPH scavenge capacity (0.55), predicted that this spontaneous plant can improve the feed DPPH scavenge capacity.

Selecting the PC&CA multivariate analysis technique for statistic discussions, it was possible to use a high number of experimental results to characterize better the DPPH scavenge capacity of the perennial forages from hill grassland at the end of May, depending on mineral or organic fertilization management. It was extracted relevant information regarding the facilities of Principal Component & Classification Analysis in grassland management decisions of organic and non-organic (conventional) farms, to improve the quality of perennial forages in terms of DPPH scavenge capacity.

CONCLUSIONS

Principal Components & Classification Analysis can represent a very helpful flexible statistic tool to extract and highlight relevant information about the DPPH scavenge capacity of perennial forages, depending on fertilization management and plants distribution in grassland covering. The highest feed DPPH scavenge capacity was identified for the forages harvested from unfertilized grassland trial (30%). Fertilization decreased the feed DPPH scavenge capacity, for fermented sheep manure cases the average value was 6%, while for exclusive mineral fertilization cases was the smallest (3%).

It was identified a positive coefficient for the correlation of feed DPPH scavenges capacity and *Filipendula vulgaris* (%): 0.55. *Calamagrostis epigejos* (%) was correlated positively with the mineral nitrogen fertilization management (0.91), but it did not increase the feed DPPH scavenge capacity (-0.47). Organic fertilizer management stimulated the presence of *Trifolium* sp. (%) in perennial grassland covering (0.88), but it did not improved significantly the DPPH scavenge capacity of grassland forages (0.01) in this period of the year.

DPPH scavenge capacity of perennial feed and Principal Components & Classification Analysis can be used to differentiate between the organic and conventional (non-organic) management systems in grassland case.

Perhaps in the future the feed DPPH scavenge capacity will be monitor quantitatively as routine parameter to characterize the quality of perennial grassland forages as functional feed for animals.

ACKNOWLEGMENTS

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