# EFFECTS OF FERTILIZATION ON POLYPHENOLS CONTENT IN SPRING OF FORAGES FROM PERMANENT GRASSLAND BY PC&CA

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**Abstract**

In this paper the aim was to try that the PC&CA (Principal Components and Classification Analysis) can be an important tool to study the effects of fertilization on polyphenols content of forages. The experimental field (permanent grassland) was fertilized organic (fermented sheep manure) and/or NPK mineral since 2003, near Grădinari (Caraş-Severin District), in a hill region of Banat County, with 10.4°C average temperature and Calcic Luvisol. The samples were harvested in spring and gravimetrically was established the matrix of floristic composition. The determination of polyphenols contents was made in conformity to Folin and Ciocalteu method, using UV-VIS SPECORD 205 spectrophotometer. The highest polyphenols content was identified in forages from unfertilized variant (136 μM gallic acid/g). The PC&CA distribution of forages samples was performed with Statistica-6 software and shows that the polyphenols content depends by the substances flows generated by applying organic and/or mineral fertilizers.

**Key words**: feed, Principal Components and Classification Analysis, animal's nutrition, antioxidant activity

Forages from grassland are of special importance because are the main source of feed for wildlife and is the cheapest source of forages for domestic animals. With the harvest and transport of forages from grassland to others areas, it is removed a large amount of nutrients from the soil, being necessary the application of chemical and/or organic fertilizers to assure an optimum quantitative and qualitative equilibrium between the output and input of substances in agro-ecosystem (Moisuc and Dukic, 2002).

On the flow of substances generated by applying the fertilizers will depend the floristic matrix and at the end the quality of produced forages. A special role in defining this quality is occupied by polyphenols content of the forages.

Polyphenols compounds are a large class of secondary metabolites present in all plants from grasslands, especially in permanent grasslands, at the same time being the main compounds that contribute to antioxidant capacity of the forages. Secondary metabolites are substances synthesised in small quantity by plants, are non-essential for plants nutrition, but with an important role on their viability and protection against the pathogens from environment and stress (Issa et al., 2006; Saviranta et al., 2009).

In animal's nutrition polyphenols present importance for many aspects. First of all the antifungal, antibacterial, antimutagenic activity, oxidative damage reduction and enzyme inhibiting properties of polyphenols were tested and confirmed (Dick et al., 1985; Tomas-Lorente et al, 1992; Constantinou et al., 1995; Apak et al., 2007; He et al., 2010), with important implications in forages storage, digestion and protection against the nutritional diseases of animals. For example the lipid peroxidation is an important factor of row matter deterioration during the processing and storage (Goli et al., 2005; Goupy et al., 2007; Morrissey et al., 2008). This negative effect is present also in the case of forages from permanent grassland. The resulted products from lipids oxidation reactions are potentially toxic and can occur in the gastric compartment during the digestion. The bioactive polyphenolic compounds from plants can reduce or inhibit the formation of lipid hydroperoxides (Goupy et al., 2007). This aspect has positive effects on shelf-life of forages and on the animal's health.

Another important aspect is regarding the ability of these bioactive compounds to protect protein molecules from degradation in rumen, improving considerable the efficiency of vegetal protein conversion to animal protein (Zhu et al., 1999). This can decrease the loss of energy during anaerobic fermentation in the rumen and animal's methane production in the atmosphere (Moss, 1993). But when are in high concentration in plants, polyphenols can become anti-nutritive factors and are responsible for astringency taste (Tanrioven and Eksi, 2005) both of forages, and also of the products with animal origin used directly / processed in human consume.

It is known that different types of fertilizers change the floristic matrix composition of

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grassland, influencing directly the polyphenols content of forages. In this study the aim was to try that the PC&CA (Principal Components and Classification Analysis) can be an important tool to study the effects of fertilization on polyphenols content of forages harvested in spring from hill permanent grassland.

## MATERIAL AND METHOD

The forages samples were harvested in March from hill permanent grassland (Grădinari, Caraș-Severin District), where the experimental filed were organized since 2003 in ten randomized plots, in multiple stage blocks: V1-unfertilized trial; V2, V3, V4 – fermented sheep manure (20 to 60 t/ha), V5, V6, V7 – organic and mineral fertilizers (20 t/ha fermented sheep manure and combination of 50 Kg/ha of $P_2O_5$, $K_2O$, N); V8, V9, V10 – only mineral fertilizers (50 kg/ha $P_2O_5$, 50 kg/ha $K_2O$ and different N doses: 100, 150, 200 kg/ha). The mineral fertilizers were applied yearly, while the fermented sheep manure at each two years in late winter. For each trial was made five replications.

The soil of permanent grassland was Calcic Luvisol. The annual average temperature was around 10.4°C.

The complexity of floristic matrix was determined gravimetrically in laboratory. From Poaceae familly dominant was *Festuca rupicola*, followed by *Calamagrostis epigejos*. *Trifolium repens* were presented from Fabaceae family. From other botanical family were present: *Rosa canina*, *Filipendula vulgaris*, *Galium verum* and *Inula britanica*. After harvesting the samples were oven dried at 70°C and grounded.

The determination of polyphenols was made in conformity to Folin and Ciocalteu method (1927), and the results were calculated in gallic acid equivalents. All reagents were analytical grade or purest quality. Deionised water was used. Absorption was measured using UV-VIS SPECORD 205 spectrophotometer by Analytik Jena. $R^2$ correlation coefficient for calibration curve was 0.9939. All analyses were made in triplicate and the mean values were reported.

Statistical analysis PC&CA was performed with Statistica-6 software.

## RESULTS AND DISCUSSIONS

The polyphenols contents obtained for the forages samples harvested in spring from experimental field (Caras-Severin), depending on the flow substances modified by fertilizers applied, varied between 81 and 136 µM gallic acid/g. In this period of year the highest polyphenols content was identified in forages from unfertilized variant (136 µM gallic acid / g) and smaller when nitrogen mineral fertilizer was applied (81µM gallic acid/g).

To find that the fertilization affect the polyphenols content of forages harvested in spring from permanent grassland it was performed Principal Components & Classification Analysis. The number of factors, based on correlations between polyphenols content and fertilizers, which characterize the phenomena in 85.72%, is two (tab.1 and fig.1).

Table 1

**The factors loadings, based on correlations between polyphenols content and fertilizers**

| Variables | Factor 1 | Factor 2 |
|---|---|---|
| Polyphenols | 0.842 | 0.493 |
| Manure | 0.663 | -0.726 |
| N mineral ferilizer | -0.894 | -0.072 |
| P mineral ferilizer | -0.854 | 0.034 |
| K mineral ferilizer | -0.911 | -0.035 |

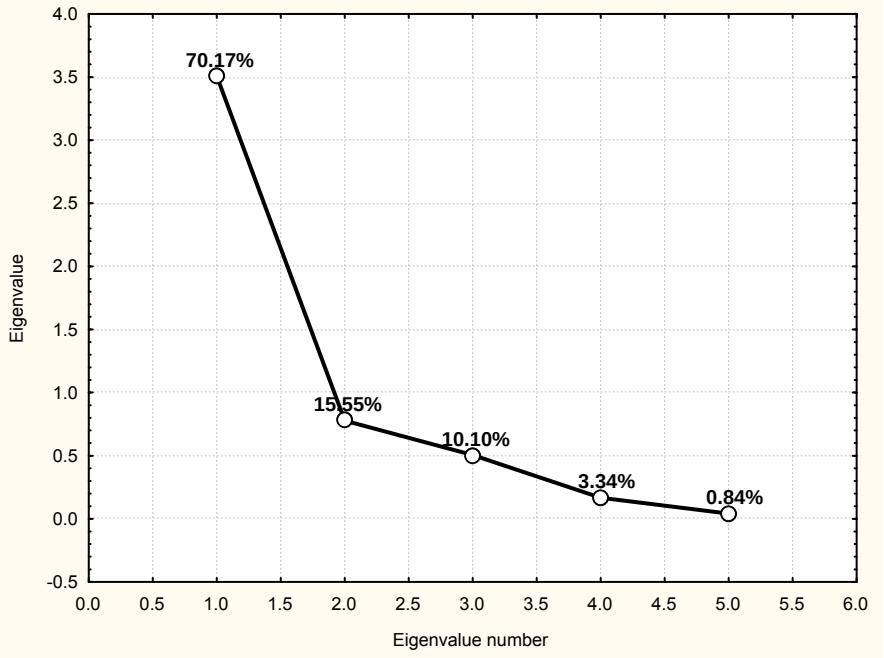


Figure 1 **The eigenvalue numbers of PC&CA based on correlations between polyphenols content and fertilizers**

It can be observed that the majority was assured by polyphenols content both in Factor 1 (0.842) and in Factor 2 (0.493). Reported to the vector Polyphenols, in the Factor 1 all the others fertilizers-variables have determined a decreasing on the content of these bioactive compounds in forages. The most significant decreasing was for mineral fertilizers, especially with nitrogen, followed by potassium and phosphorus (fig. 2).

The PC&CA distribution of forages samples in correlations with polyphenols content and applied organic and/or mineral fertilizers is shown in *fig. 3*.

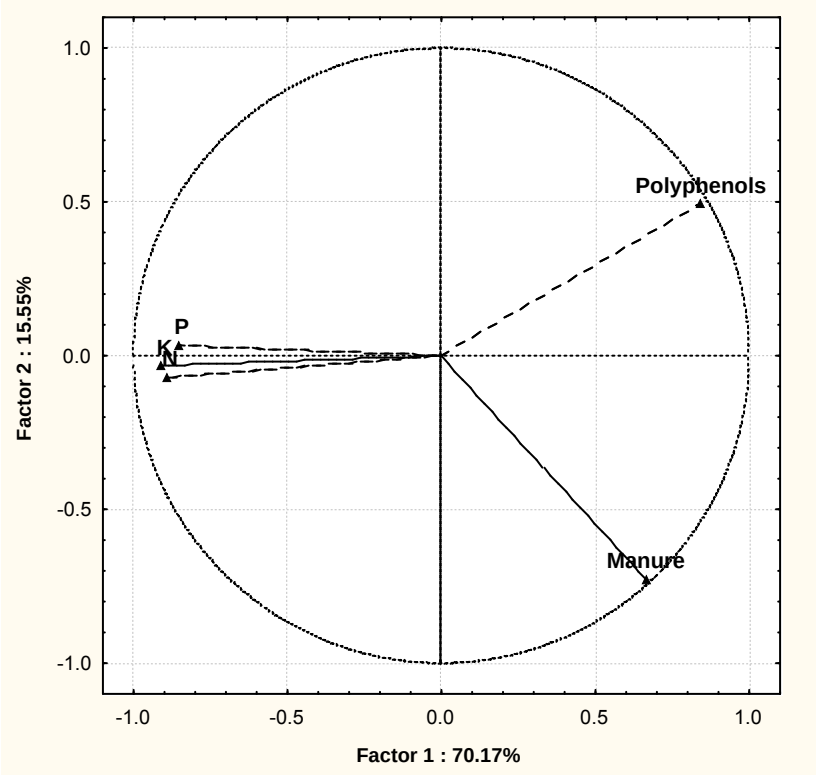


Figure 2 **The PC&CA distribution of fertilizers variables in correlations with polyphenols content of forages**

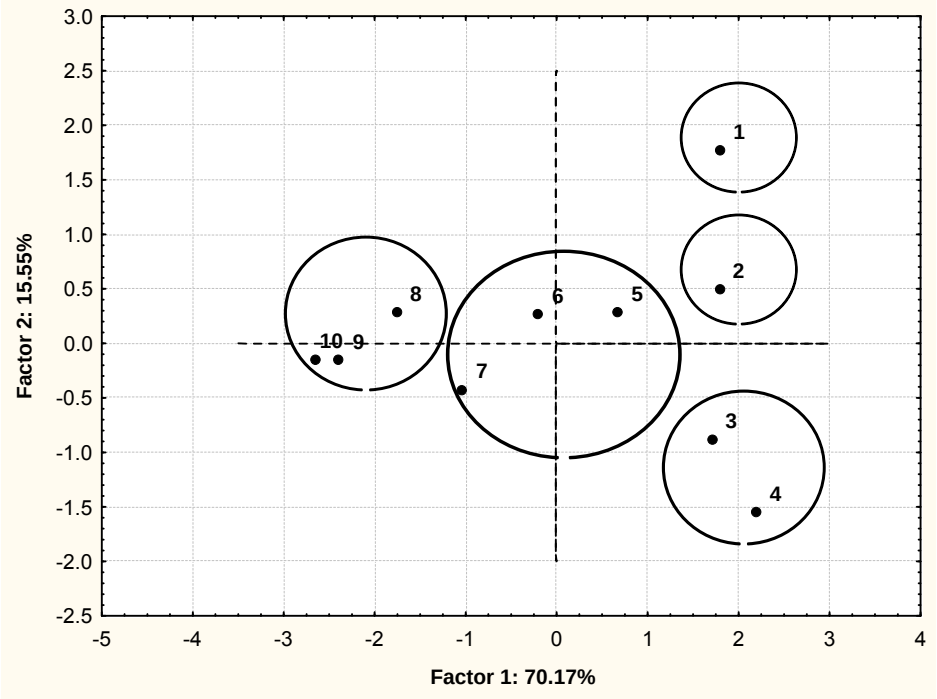


Figure 3 **The PC&CA distribution of forages samples in correlations with polyphenols content and applied fertilizers (1-10 forages samples are correspondent to 1-10 trials)**

It is obviously that the higher polyphenols concentration was determinate when the substances flow of experimental trial was no modified by applying the fertilizers (sample 1). The fermented sheep manure in the smaller dose (20 t/ha) decrease the content of this qualitative parameter (sample 2), but not so pregnant that the higher doses (samples 3 and 4).

The samples 8, 9 and 10, which represent the forages harvested from trials fertilized only mineral, were characterized by the smaller polyphenols contents.

The forage samples 5, 6 and 7 were influenced by substances flows generated of organic and mineral fertilizers and had the medium polyphenols concentrations.

The explanation consist that the contents of these bioactive secondary products in forages harvested in spring from permanent grassland, at the beginning at vegetation, after many years of fertilization, is strongly influenced by the generated substances flows from agro-ecosystem, directly depending by the floristic matrix composition. That's because the polyphenols content of various plant families is very different (Apak et al., 2007; Saviranta et al., 2009).

## CONCLUSIONS

In this paper the Principal Component and Classification Analysis was tested that it can be used to observe the effects of fertilisation on polyphenols content of forages harvested at the beginning of spring from hill permanent grassland. The final conclusion was that obtained PC&CA distribution of analysed forages samples can be a useful tool to shows that the polyphenols content depends by the substances flows generated by applying organic and/or mineral fertilizers. The highest polyphenols content was identified in forages from trial 1, where the agro-ecosystem flow substances were no modified by applying the fertilizers.

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