# POLYPHENOLIC COMPOSITION OF A PERMANENT PASTURE UNDER INFLUENCE OF ORGANIC FERTILIZATION

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**Abstract**

*Generally, tannins have a negative effect in animal feed intake, feed digestibility, and efficiency of production. These effects are depending on the content and type of tannin ingested and on the animal's tolerance, which in turn is dependent on characteristics such as type of digestive tract, feeding behavior, body size, and detoxification mechanisms. The study aimed to assess the influence of organic fertilization on the total polyphenols and tannins content in forages obtained from permanent grassland of Dorna Depression. The experience was monofactorial in three repetitions, located on grassland of Nardus stricta L. and Festuca rubra L., at an altitude of 940 m and 10% slope, to investigate the effect of fertilization with 20-50 t ha$^{-1}$ manure, applied annually or every 2 years. Samples were collected in autumn 2010-2011 and were analyzed for total polyphenols and tannins content by Folin Ciocalteu method. It was observed that the fertilization with manure increase the total polyphenols and tannins content compared with the control variant. Concentration of the total polyphenols increased from 22.4 mg/g for the unfertilized variant to 40.3 mg/g for the variant fertilized with 50+0+40+0 t ha$^{-1}$ manure. The tannins content also increased from 3.9 mg/g for the unfertilized variant to 13.1 mg/g for the variant fertilized with 50+0+40+0 t ha$^{-1}$ manure. Phenolics contribute to the micronutrient composition of forages and assessment of these compounds is important because they could affect the quality of animal products.*

**Key words**: tannins, polyphenols, forages, *Nardus Stricta* L, *Festuca rubra* L.

## INTRODUCTION

Polyphenols represent a large group of structurally related compounds present in many natural products, mainly in fruits and vegetables, contributing to their flavour and colour [9]. Phenolics also contribute to the micronutrient composition of forages, which in turn may affect animal product composition [4]. Natural polyphenols can range from simple molecules such as phenolic acid to large highly polymerized compounds such as tannins. Tannins are phenolic compounds commonly found in plants that play a role in protection from predation, as well as growth regulation; they are digested by herbivores. Found in the leaf, bud, seed, root, and stem tissues, tannins are widely distributed in many different species of plants and can have a large influence on the nutritive value of forages [8]. Tannins are separated into two classes: hydrolysable tannins and condensed tannins. Depending on their concentration and nature either class can have adverse or beneficial effects. When ruminants digest some plants, they acquire a surplus of tannins and rumen microbes do not have the enzymatic ability for degrading condensed tannins. In fact, digestion of tannins by ruminants in large amounts can reduce the activity and the proliferation of ruminal microorganisms reducing ruminal biohydrogenation [7]. Tannins can also precipitate proteins and inhibit the absorption of nutrients [1]. Very high levels of tannin intake can produce toxicity that can even cause death. Animals normally consuming tannin-rich plants can develop defensive mechanisms against tannins, such as the strategic deployment of lipids and extracellular polysaccharides that have a high affinity to binding to tannins. These mechanisms prevent tannins from causing adverse effects on rumen microbes.

The main purpose of the present study was to investigate the content of the total polyphenols and tannins of the forages and

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the effect of organic inputs on the polyphenolic compositions of the forages obtained from a permanent grassland of *Nardus stricta* L. and *Festuca rubra* L from Dorna Depression, Romania.

## MATERIAL AND METHOD

The research was conducted during 2010 - 2011 on permanent grassland of *Nardus stricta* L. and *Festuca rubra* L., located in Dorna Depression, Saru-Dornei village at an altitude of 940 m and slope of 10 degrees. In this experience was evaluated the influence of five different organic fertilizing variants: V1 - unfertilized control, V2 - 20 t $ha^{-1}$ manure applied every year, V3-30 t $ha^{-1}$ manure every year, V4-30 t $ha^{-1}$ every two years and V5-50+0+40+0 t $ha^{-1}$ manure on the quality of forages harvested from such areas.

The experience was monofactorial in 3 repetitions. The surface of each variant of fertilization was 20 $m^2$ (4 × 5 m). The repetition block area was 100 $m^2$ and the surface of the experience was 300 $m^2$. Freshly harvested hay samples were collected in September of each year. All plant samples were dried at room temperature and ground to obtain a homogeneous matrix and analyzed to determine the crude chemical composition: dry matter (DM), crude fat (EE), crude protein (CP), crude ash (CA), fiber content by treatment with neutral detergent (NDF), fiber content by treatment with acid detergent (ADF), sulphuric lignin content (ADL), total phosphorus (P). The chemical composition was determined by standard methods and was presented in detail in a previous paper [10].

*Reagents and solution*

All reagents used throughout the work were of analytical purity. A solution of 30% methanol (Merck, Germany) was used for hay samples extraction and a solution of 33% sodium carbonate decahydrate (Chemical Company, Romania) was used for sample preparation. Acetate buffer (pH 5.0) was prepared by mixing 1.92 g sodium acetate trihydrate (Chemical Company, Romania) and 0.34 mL acetic acid (Merck, Germany) and made up to 100.0 mL with double distilled water. Casein (Merck, Germany) as a precipitation agent and Folin–Ciocalteu's reagent (FCR, Merck, Germany) as a chromogenic agent were used. The prepared sample solutions were filtered by using 0.20 μm Minisart-plus membrane filters (Sartorius AG, Germany).

Extraction and determination of total phenols and tannins from the hay samples was carried out according to Jurisic Grubesic et al. [6] and are briefly presented below. The powdered hay samples (0.25 g) were extracted with 80 mL of 30% methanol (70°C, water bath, 15 min). After cooling and filtration, each extract was made up to 100.0 mL with 30% methanol (basic sample solution, BSS). Two milliliters of BSS were mixed with 8 mL of water and 10 mL of acetate buffer (solution 1, S1). Ten milliliters of S1 were shaken with 50 mg of casein during 45 min (adsorption of tannins) and then filtrated (solution 2, S2). One milliliter of S1 was mixed with 0.5 mL of FCR and made up to 10.0 ml with 33% $Na_2CO_3$ $10H_2O$. The same procedure was performed with S2. After filtration, the absorbance at 720 nm of the final blue solution was measured. Blank solutions without analyte were prepared and measured following the same procedure. The content of total polyphenols and tannins was evaluated upon three independent analyses. The absorbance values obtained for S1 correspond to the total polyphenol content. The differences between absorbencies of S1 and S2 correspond to concentration of casein-adsorbed tannins in hay samples.

Calibration curve was created for quantification between 5 – 50 μg of tannins and a good linearity ($r^2 = 0.998$) was observed. Procedural blanks were performed with each set of five samples, and the obtained values were subtracted from the values found in the samples. The content of total polyphenols and tannins was expressed as gram per kilogram toward the mass of dry forages.

*Statistical analysis*

Means and standard deviations were calculated for total polyphenols and tannins concentrations. Separate one-way analyses of variance (ANOVA) were used to determine whether there were significant differences among the unfertilized variant and the variants fertilized with different manure quantities. A value of $p < 0.05$ was considered significant. Clustering of variants from a multivariate analysis of all data recorded (CP, CA, EE, NDF, ADF, ADL, P, PFt – total polyphenols and tannins) and assessment of the relative influence of each variable on that clustering was established by principal components analysis (PCA). All statistical analyses were performed using STATISTICA 8 software.

## RESULTS AND DISCUSSIONS

With respect to synthetic pesticides and fertilizers, organic agriculture in general is characterized by the absence of these products throughout the cultivation period [3]. The literature suggests that organic agriculture could result in foods with higher polyphenol quantity, mainly for two reasons. First, the use of synthetic fertilizers could offer more bioavailable sources of nitrogen, accelerating plant development and plant resources from production of secondary metabolites to growth. Second, the absence of synthetic pesticides could result in higher exposure of the plant to stressful situations leading to an enhancement of natural defense substances such as phenolic compounds [11, 12].

Total phenolic compounds and tannins concentrations in forage samples were expressed in weight as tannic acid. The values are shown in Table 1. The total polyphenols concentration increased from the 22.35 g $kg^{-1}$ dry matter for the unfertilized variant to 31.97 g $kg^{-1}$ dry matter for variant 3, where 30 t $ha^{-1}$ every year was applied and to 40.28 g $kg^{-1}$ dry matter for the variant where 50+0+40+0 t $ha^{-1}$ manure was applied in 2010.

Table 1 Content of total polyphenols and tannins (g $kg^{-1}$ ± SD) in forage samples collected in 2010 and 2011

| Variant | Total polyphenols | Total polyphenols after casein adsorbtion | Tannins |
|---|---|---|---|
| 2010 | | | |
| V1-unfertilized control | 22.35 ± 0.15 | 18.45 ± 0.21 | 3.90 ± 0.11 |
| V2-20 t $ha^{-1}$ manure every year | 25.90 ± 0.17*** | 19.30 ± 0.16 | 6.60 ± 0.08*** |
| V3-30 t $ha^{-1}$ manure every year | 31.97 ± 0.12*** | 22.90 ± 0.19 | 9.07 ± 0.18*** |
| V4-30 t $ha^{-1}$ manure every two years | 29.26 ± 0.15*** | 19.97 ± 0.22 | 9.30 ± 0.14*** |
| V5-50+0+40+0 t $ha^{-1}$ manure | 40.28 ± 0.20*** | 27.17 ± 0.25 | 13.11 ± 0.08*** |
| 2011 | | | |
| V1-unfertilized control | 23.74 ± 0.13 | 18.69 ± 0.31 | 5.05 ± 0.14 |
| V2-20 t $ha^{-1}$ manure every year | 19.65 ± 0.18*** | 19.17 ± 0.27 | 0.48 ± 0.25*** |
| V3-30 t $ha^{-1}$ manure every year | 16.77 ± 0.21*** | 12.80 ± 0.17 | 3.91 ± 0.19*** |
| V4-30 t $ha^{-1}$ manure every two years | 20.09 ± 0.25*** | 15.70 ± 0.14 | 4.35 ± 0.22*** |
| V5-50+0+40+0 t $ha^{-1}$ manure | 21.12 ± 0.22*** | 14.59 ± 0.11 | 6.53 ± 0.12*** |

SD – standard deviation for n = 3, n - number of replicates, *** - $p < 0.001$

However, in 2011 it was observed a difference compared with 2010, when total polyphenol content decreased from the 23.74 g $kg^{-1}$ dry matter for the unfertilized variant to 20 g $kg^{-1}$ for variants fertilized with 20 t $ha^{-1}$ and 30 t $ha^{-1}$, respectively. A decrease of the polyphenolic content was also observed for the last variant (50+0+40+0 t $ha^{-1}$ manure) to 21.12 g $kg^{-1}$ dry matter.

Organic fertilization influenced the tannins content in forages obtained from the permanent grassland (Table 1). In 2010 tannins content significantly increased from 3.9 g $kg^{-1}$ dry matter to 9 g $kg^{-1}$ dry matter for the variants V3 and V4 where were applied 30 t $ha^{-1}$ manure every year and 30 t $ha^{-1}$ manure every two years, respectively. The highest increase was observed for the last variant to a total content of tannins of 13.11 g $kg^{-1}$, where 50+0+40+0 t $ha^{-1}$ manure was applied. In 2011 tannins concentration shows a different distribution in the analyzed samples. It was observed an increase from 5.05 g $kg^{-1}$ dry matter (unfertilized variant) to 6.53 5.05 g $kg^{-1}$ for only the last variant where was applied 50+0+40+0 t $ha^{-1}$ manure. Instead, for the variants V3 and V4 where was applied 30 t $ha^{-1}$ manure every year and 30 t $ha^{-1}$ manure every two years, respectively, the tannins concentration decreased to 3.91 g $kg^{-1}$ dry matter and 4.35 g $kg^{-1}$ dry matter, respectively. It was also observed a higher decrease of the tannins content for the variant V2 (0.48 g $kg^{-1}$ dry matter) where was applied 20 t $ha^{-1}$ manure every year. The tendency of total polyphenols content for 2011 is in accord with another study on polyphenols composition in forages collected from permanent grassland under the influence of manure fertilization [5].

A considerable variation between variants and years in the polyphenol and tannin concentrations in the collected forages was

observed. The analysis of specific tannins gives an indication of the presence of some anti-nutritive factors [2]. Except for the variant 5 in 2010, the forages investigated in this study had low tannin contents, that would be of little significance in their effects on digestion of nutrients by ruminants. With high protein content and low fibre and tannin contents, these forages could be considered with a potentially high nutritive value [2]

The PCA based on chemical composition of forages [10] including polyphenols and tannins concentrations for 2010 and 2011 are presented in Fig. 1.

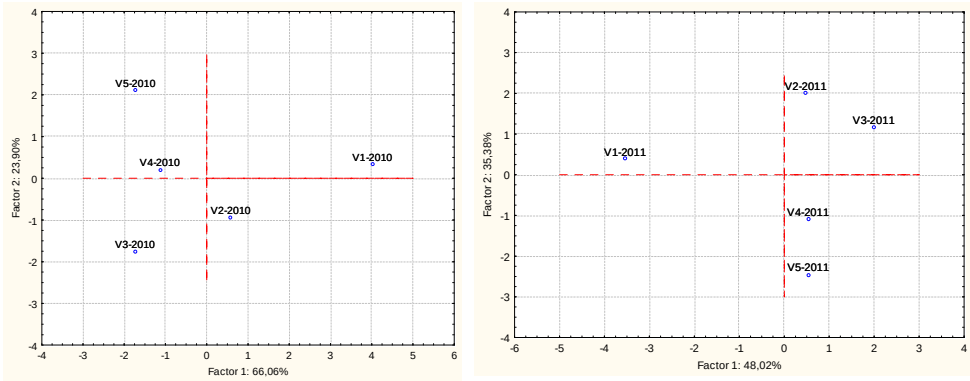


Fig. 1 Variants discrimination on the basis of principal component analysis performed on data recorded on chemical compositions

The same analysis was used to examine the most influential variables on the observed separation for 2010 and 2011 (Fig. 2). All variables were highly influential. In 2010 NDF, ADF and ADL were the main variables determining the ranking of variants on factor 1 (66.06% of variance accounted for), whereas the second factor (23.90% of variance accounted for) would be explained mainly by the content of total polyphenols, tannins and crude protein. In 2011 ADL, CP and P were the main variable determining the ranking of variants on factor 1(48.02% of variance accounted for) whereas the second factor (35.38% variance accounted for) would be explained mainly by the NDF and tannins content.

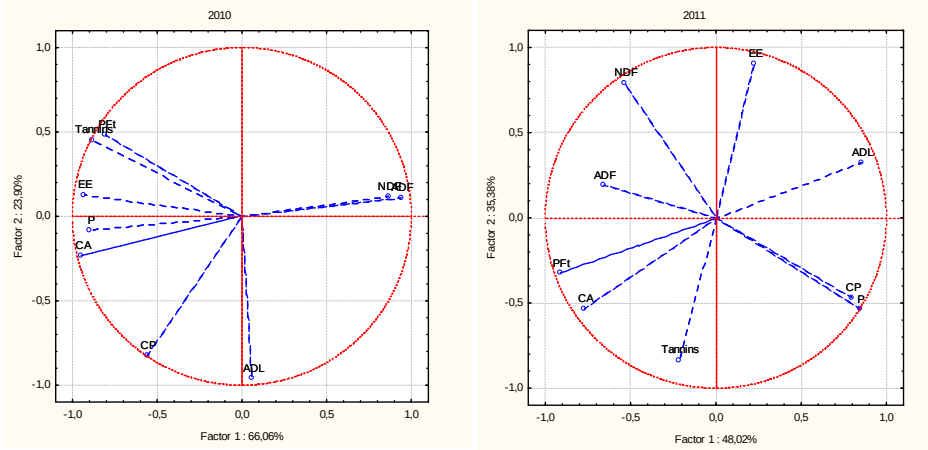


Fig. 2 Discrimination of the most influential variables on the observed separations on the basis of principal component analysis performed on data recorded on chemical compositions [10] polyphenols and tannin composition, (CP - crude protein, CA - crude ash, EE - crude fat, NDF- fiber content by treatment with neutral detergent, ADF - fiber content by treatment with acid detergent, ADL - sulphuric lignin content, P - total phosphorus, PFt – total polyphenols and tannins)

## CONCLUSIONS

The use of organic fertilization on the permanent grassland has an effect on the polyphenolic compositions. Different results were observed between the experiments carried out in 2010 and 2011. Because of these differences in total polyphenols and tannins concentrations it is necessary to develop a more complete understanding of the influence of organic fertilizations on phenolic compunds and tannins in forages obtained from a permanent grassland of *Nardus stricta* L. and *Festuca rubra*. Additional studies on the influence of another factors such as environmental conditions, maturity of plants and biodiversity could be useful. Also, information on the distribution of the polyphenols and tannins for each plant species collected from the grassland and on specific classes of polyphenols is still needed.

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