FERTILISATION IMPACT ON TOTAL FIBRE CONTENT OF A BANAT HILL GRASSLAND SPONTANEOUS FLORA IN SPRING

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Abstract

This study was conducted to estimate that organic fertilisation with sheep manure or mineral fertilisers had impact on the total fibre content in spring of spontaneous flora which covered a hill permanent grassland situated on a Calcic Luvisol in Romanian Banat County. The experimental field was organised respecting the complete randomised block design with five replications for each trial. The trial surface was 25 m^2. The total fibre content was determined using Weende method (AOAC method 962.09, Edition 15/1990). For these research it was selected a number of seven trials with different fertilisation (mineral or organic) and it was computed a multivariate analysis technique model based on Principal Components & Classification Analysis (PC&CA), using as active variables: fertilisation data; soil ecological parameters; and total fibre content of grassland spontaneous plants harvested in the middle and at the end of May 2008. As supplementary variables were used data of individual contribution (%) to the hill grassland spontaneous covering biomass of: *Festuca rupicola*, *Calamagrostis epigejos*, *Trifolium repens*, *Inula britanica* and *Filipendula vulgaris*. The percent of individual participation of selected spontaneous plants species in hill permanent grassland flora was determined gravimetrically. The first two principal components, PC1 and PC2, explained around 85% of total variance of PC&CA model. The analysed data have shown that in clime and soil conditions of May 2008 from Banat County, the mineral fertilisation had a high positive impact on total fibre content of spontaneous dominant grass from hill permanent grassland in both cases: in the middle and at the end of month.

Key words: fertilisation, total fibre, grassland, multivariate analysis, quality.

For a healthy life consumers need for their daily diet also food with animal origin (Socaciu C., 2003). The quality of agricultural products with animal origin is dependent by the nutritive and energetic value of feed. One of the most important qualitative parameter of feed which must be monitored in ruminant case is the fibre content.

The generally rules for forage quality values relating to the fibre content components are "**30-40**", meaning at least 30% ADF (acid detergent fibre) and 40% NDF (neutral detergent fibre). When ADF is less than 25% or NDF less than 35%, many nutrients pass through the rumen without being absorbed by animal organism (Ball D.M. *et al*, 2001), meaning lower forage inputs utilisable for animal performance and productivity.

For an equilibrate feed ratio, depending on animal age, sex, weight, breed, etc., the fibre content requirements are different (Crainiceanu E. *et al*, 2006). For example, the NDF value should increase to 55% when the animal body weight is higher that half of the normal weight of a mature animal; in the last part of pregnancy is important to increase the NDF in daily ratio of female with approximately 50%; for a high production of quality milk, the cattle, sheep and goats requires a feed with 25-30% NDF (Georgescu *et al*, 2007).

Forage from Romanian grasslands represents an important and cheap resource for ruminants feeding (Moisuc Al., Dukic D., 2002). The farmer problematic is to obtain the forage quantity and quality necessary for animal feeding, but respecting the legislation regarding the food safety and environmentally friendly practices. The inputs versus outputs equilibrium management plays an important role in grassland ecosystem health and forage quality, that's way, according to Marusca T. (2008) and Rotar I. *et al* (2004), the amendments and fertilisers application is necessary.

The present scientific study analysed, using PC&CA, that total fibre content of hill grassland forages in spring is influenced by mineral or organic fertilisation, in soil and climatic conditions of Romanian Banat of May 2008.

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MATERIAL AND METHOD

Total fibre content determination. The total fibre content was determined by Weende method (AOAC method 962.09, Edition 15/1990). It was two periods for forages samples harvesting, both in May 2008, for total fibre content determination. First time the grassland spontaneous plants were cut in the middle of May 2008. Second time the harvesting was made at the end of May 2008.

Ecological soil parameters quantified. Humus content was quantified using Walklay – Black – Gogoasa method and available phosphorus and potassium (mobile form) contents of grassland soil were determinate by Egner-Riehm-Domingo method (Stoica E. *et al*, 1986).

Grassland plants harvesting. The spontaneous plants samples were cut from 1 m^2 surface of each trial of the studied hill permanent grassland, at 3 cm above soil. The individual participation (%) of some spontaneous plants in hill permanent grassland covering flora was determined gravimetrically. The selected individual participation (%) of spontaneous plants species for this study were: *Festuca rupicola*, *Calamagrostis epigejos*, *Trifolium repens*, *Inula britanica* and *Filipendula vulgaris*.

Localisation and soil / climatic conditions of hill permanent grassland. The localisation of the studied hill permanent grassland was in Romanian Banat County, near Gradinari village (45°12'N; 21°60'E), on Calcic Luvisol with pH (H_2O) values from 5.66 to 6.27. The unfertilised trial (A) soil had pH (H_2O) = 6.09, the organic fertilised trials with sheep manure between 6.08 - 6.27, while in exclusive mineral fertilised trials cases the soil was acidified (5.66 - 5.88) reported to unfertilised trial.

In this region, situated in the south – west part of Romania, under the Carpathian Mountain protection, the clime is temperate continental with Mediterranean influences (Ianoş Gh., 2005). The average annual temperature in 2007 was around 12.5°C and in 2008 around 12.3°C. In spring of 2008 the average temperature increased from 7.6°C in March, to 12.2°C in April and 17.2°C in May. The annual precipitation was around 883 mm in 2007 and around 802 mm in 2008. In the 2008 spring the rainfall was around 118 mm in March, 98 mm in April and 38 mm in May. The climatic data were collected by the Meteorological Station Archive, Oravita (Caras-Severin).

Experimental field design. The hill permanent grassland was organised respecting complete randomised block design with five replications for each trial. The experimental trial surface was 25 m^2. For these research it was studied a number of seven trials with different fertilisation: unfertilised trial – **A**; exclusive organic fertilised trials with fermented sheep manure: **S1, S2, S3** with 20t/ha, 40t/ha and respectively 60t/ha; exclusive mineral fertilised trials: **M1** - 100N(Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha); **M2** - 150N(Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha); **M3** - (100+100) N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50K_2O(Kg/ha). The organic and mineral fertilisers application on hill permanent grassland was made first time in 2003. The organic fertilisation was made at each two years. The mineral fertilisers were applied yearly. NPK complex, ammonium nitrate, superphosphate, and potassium salt were used to assure the mineral nutrients for the grassland soil.

Statistical analysis of the experimental results using PC&CA. Multivariate analysis technique Principal Components & Classification Analysis (PC&CA) was computed with StatSoft - STATISTICA VERSION 10. The cases were the selected seven trials of the studied hill permanent grassland. As supplementary variables were used the data sets of gravimetric individual participation (%) of some spontaneous plants species in the hill grassland flora: *Festuca rupicola*, *Calamagrostis epigejos*, *Trifolium repens*, *Inula britanica* and *Filipendula vulgaris.* The total fibre content of grassland forages (cutting in two steps in May 2008), fertilisation doses, and soil ecological parameters were used as active variables of PC&CA. The experimental data were analysed via the correlation matrix.

RESULTS AND DISCUSSIONS

Total fibre content of spontaneous flora samples of hill permanent grassland was quantified in two steps for each of the seven experimental trials. First time the grassland forages samples for total fibre content determination were cut in the middle of May 2008 (TFCa), and the second time at the end of May 2008 (TFCb). The obtained results for total fibre content in both periods are shown in *table 1*.

Table 1

Total fibre content (%) of grassland forages samples cut in two steps in May 2008

Trials/TFC	TFCa (%)	TFCb (%)
A	20.85	24.18
S1	23.16	29.15
S2	25.06	27.85
S3	25.27	26.84
M1	28.82	28.79
M2	29.20	29.98
M3	30.19	34.07
where: TFCa - total fibre content of forages samples cut in the middle of May 2008, and TFCb - total fibre content of forages samples cut at the end of May 2008		

The forage of unfertilised trial had the smallest total fibre content: 20.85% in the middle of month, and 24.18% at the end of month. The highest values for total fibre content were obtained in M3 case, fertilised with the highest mineral nitrogen dose (100+100 kg/ha) for both cutting

periods, 30.19%, respectively 34.07%. Generally, at the end of May 2008 the total fibre content was higher than in the middle of the month.

The analysed ecological parameters of the hill grassland trials soil (A, S1, S2, S3, M1, M2, M3), used as active variables, were humus (%), mobile form of P (ppm) and K (ppm). The soil humus content of the selected seven grassland trials was: 5.78% (A), 5.59% (S1), 6.08% (S2), 7.15% (S3), 6.51% (M1), 6.16% (M2), and 6.24%(M3). The experimental results for phosphorus (mobile form) content of grassland soil in trials fertilised with sheep manure was 53.59ppm (S1), 61.64 ppm (S2), 87.56 ppm (S3), in mineral fertilised trials was 61.68 ppm (M1), 49.23 ppm (M2), 70.70 ppm (M3), reported to unfertilised trial with 58.30 ppm (A). The potassium content (mobile form) for fertilised trials soil was 203 ppm (S1), 232 ppm (S2), 223ppm(S3), 215 ppm (M1), 216 ppm (M2), 225ppm (M3), reported to A (unfertilised trial) with 209 ppm.

The percent of individual participation of *Festuca rupicola* in hill grassland spontaneous flora in May 2008 was 37% in unfertilised trial A; 14%, 9% and 6% for organic fertilised trials (S1, S2, respectively S3); 51%, 14% and 6% in mineral fertilisation cases (M1, M2, respectively M3). *Calamagrostis epigejos* individual participation at grassland covering varied between 0 – 67%; while in unfertilised trial (A) was not present, also as for S1 trial, for the other two organic fertilised trials (S2 and S3) was 6% and 5%. In mineral fertilised trials the percent of *Calamagrostis epigejos* was 13%, 67%, 56% (M1, M2, and M3).

The forages samples from unfertilised trial (A) had small quantity of *Trifolium repens*, under 0.5%. In mineral fertilised trials (M1, M2 and M3) *Trifolium repens* was present under 0.5%, or not, while in organic fertilised trials case (S1, S2, and S3) was quantified 38%, 46%, respectively 44%.

From other botanical families *Inula britanica* and *Filipendula vulgaris* had the following percent depending on fertilisation: 3% (A), 8% (S1), 10%(S2), 6% (S3), 11% (M1), 4% (M2), under 0.5% (M3); and respectively 10% (A), 7% (S1), 6% (S2), 5% (S3), 7% (M1), 4% (M2), and 10% (M3). All the data of plants individual participation (%) of grassland covering spontaneous flora were introduced in PC&CA model as supplementary variables.

For statistical analysis of the obtained results relating to the impact of fertilisers (organic or mineral) on total fibre content of permanent grassland forages samples harvested twice in May 2008 was used multivariate analysis technique PC&CA. For a 100% quality representation of PC&CA model it was obtained 6 principal components. The Eigenvalues of the PC&CA model are shown in *figure 1*.

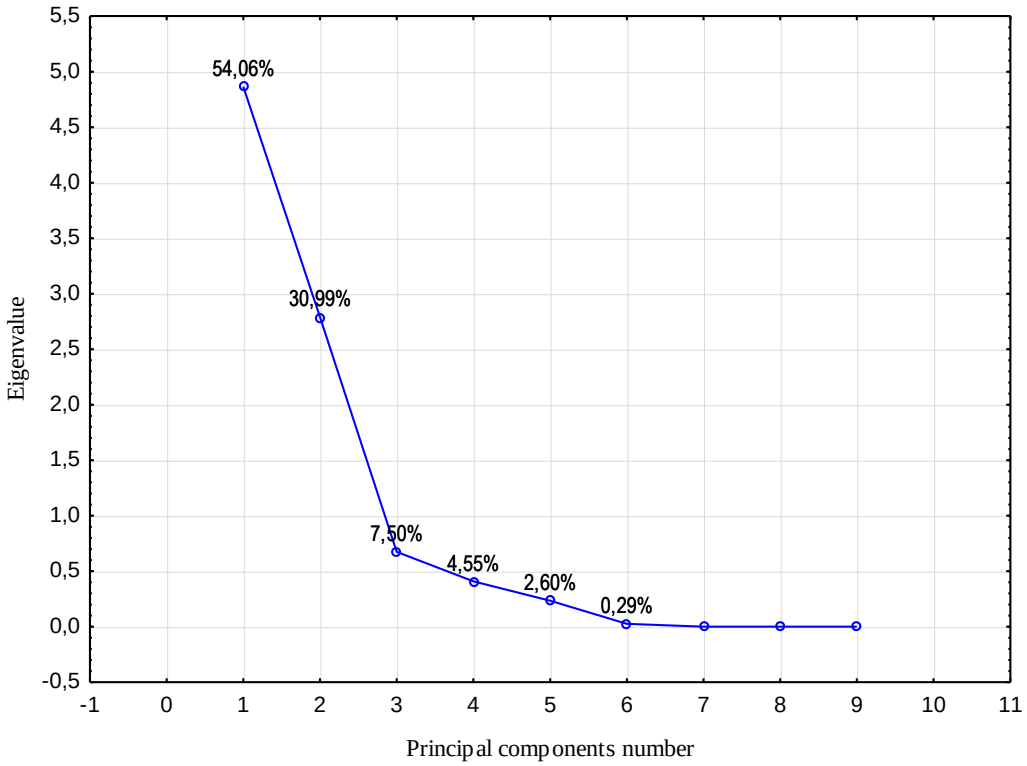

Figure 1 **The Eigenvalues of the PC&CA model**

The first two principal components, PC1 and PC2, explained around 85% of total variance, heaving eigenvalues 4.87, respectively 2.79. The third principal component (PC3) had eigenvalue 0.67 and explained 7.5 % of total variance. The fourth principal component (PC4) explained 4.55% of total variance, with an eigenvalue 0.41. PC5 and PC 6 had eigenvalues 0.23 and 0.03, and explained only 2.60% and 0.29% of total variance.

The correlations of PC1 and PC2 against variables data (factor loadings) of PC&CA model are shown in *table 2*. It is obviously that for active variables in the first principal component (PC1) case had a high positive impact the mineral fertilisation data and total fibre content of forages samples harvested in May 2008 from the hill permanent grassland, in both of cases (cut in the middle and at the end of month). The sheep manure applied on grassland trials had a negative impact as PC1 component. In PC2 structure the highest impact was positively for active variables sheep manure and ecological soil parameters.

Table 2

The correlations of PC1 and PC2 against variables data (factor loadings)

Variables/PC	PC1	PC 2
TFCa (%)	0.927	0,329
TFCb (%)	0.817	0,122
N (kg/ha)	0.980	0,015
P (kg/ha)	0.979	-0,032
K (kg/ha)	0.979	-0,032
Sheep manure (t/ha)	-0.621	0,703
Humus (%)	0.110	0,893
P mobile form (ppm)	-0.175	0,890
K mobile form (ppm)	0.183	0,761
***Fr (%)**	**0.052**	**-0,428**
***Cal (%)**	**0.861**	**0,010**
***Tr (%)**	**-0.676**	**0,420**
***Inb (%)**	**-0.314**	**0,047**
***Fv (%)**	**0.043**	**-0,310**
where *Fr – *Festuca rupicola*; *Cal - *Calamagrostis epigejos*; *Tr - *Trifolium repens*; *Inb - *Inula britanica* and *Fv - *Filipendula vulgaris*; supplementary variables.		

The projection of the seven cases of fertilised trials from hill permanent grassland on the PC1xPC2 plane is shown in *figure 2*.

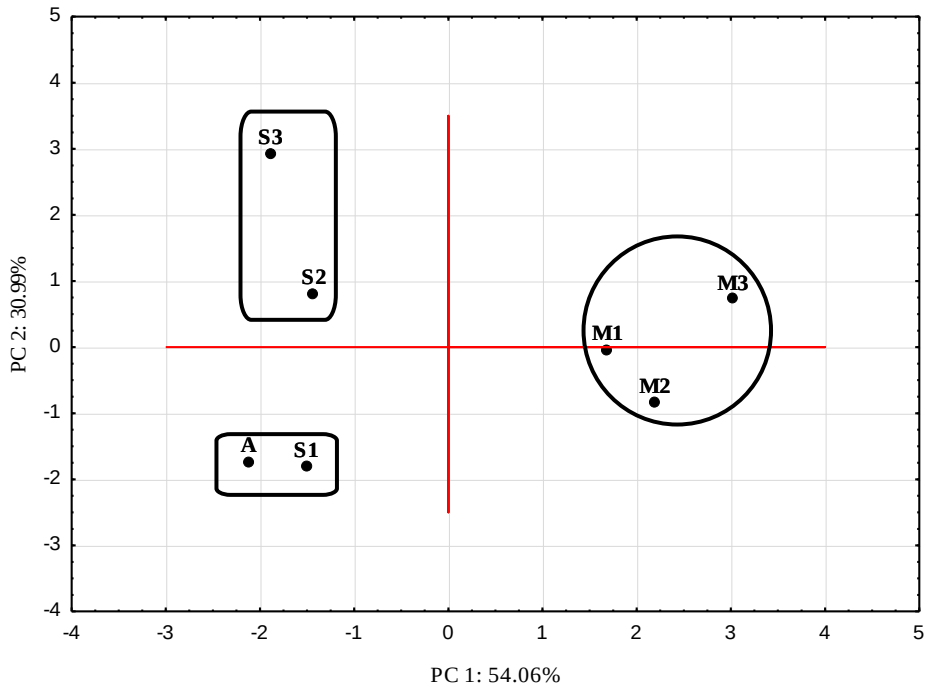

Figure 2 **The projection of the seven cases of hill permanent grassland on PC1xPC2 plane**

The selected seven trials of hill permanent grassland, as active cases for PC&CA model, were discriminated in three main categories: first group included the exclusive mineral fertilised cases M1, M2 and M3 with NPK complex, ammonium nitrate, superphosphate, and potassium salt; second group was formed by unfertilised trial (A) and the organic fertilised trial with the smallest dose of fermented sheep manure (S1, 20t/ha); and the third group contained the other two trials cases, fertilised organic with 40t/ha (S2) and 60t/ha (S3) sheep manure. Considering the grouping of different substances flows of the studied seven grassland trials, modified anthropic reported to unfertilised trial by fertilisers application (organic or mineral), it was analysed the spatial distribution of individual contribution of some plants species in grassland covering spontaneous flora by PC&CA.

The projection of the supplementary and active variables on the PC1xPC2 plane is shown in *figure3*.

The obtained correlation coefficients of the active against the supplementary variables of PC&CA model can be consulted in *table 3*.

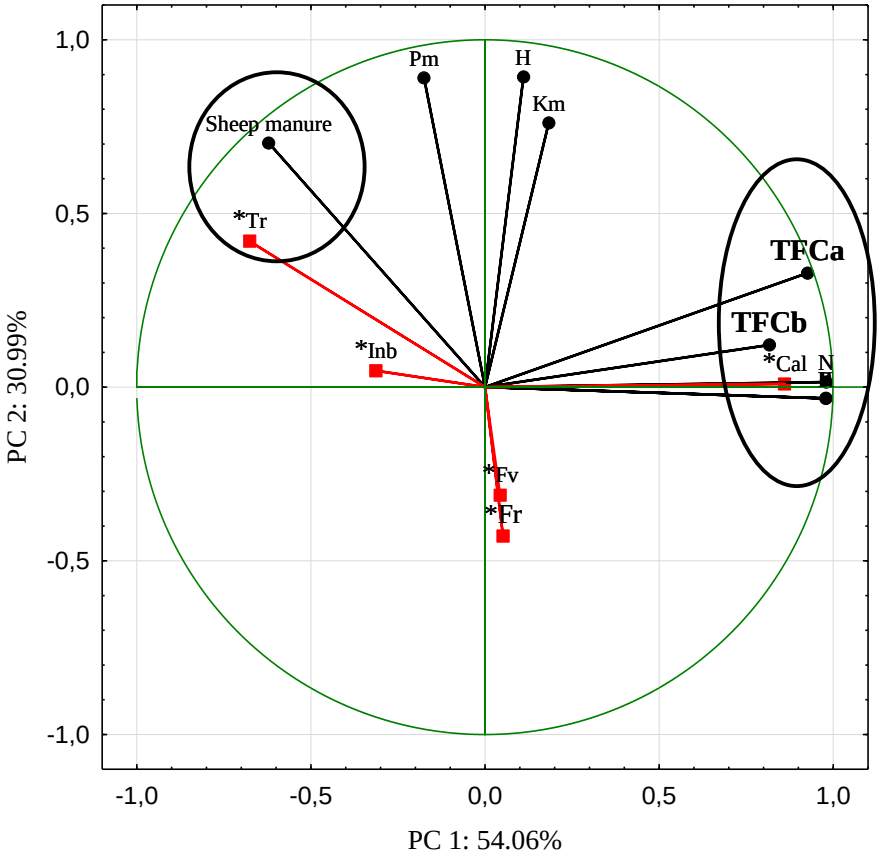

Figure 3 **The projection of the supplementary and active variables on the PC1xPC2 plane**

Table 3

The correlation coefficients of the active against the supplementary variables of PC&CA model

Variables	*Fr (%)	*Cal (%)	*Tr (%)	*Inb (%)	*Fv (%)
TFCa (%)	-0,110	0,795	-0,406	-0,139	-0,210
TFCb (%)	-0,390	0,729	-0,247	-0,323	0,078
N (kg/ha)	-0,046	0,909	-0,702	-0,494	0,127
P (kg/ha)	0,219	0,807	-0,746	-0,236	0,000
K (kg/ha)	0,219	0,807	-0,746	-0,236	0,000
Sheep manure (t/ha)	-0,529	-0,482	0,911	0,347	-0,475
Humus (%)	-0,114	0,062	0,158	0,078	-0,396
P mobile form (ppm)	-0,314	-0,200	0,361	-0,118	0,015
K mobile form (ppm)	-0,450	0,234	0,256	0,000	-0,175
where: *Fr – *Festuca rupicola*; *Cal - *Calamagrostis epigejos*; *Tr - *Trifolium repens*; *Inb - *Inula britanica* and *Fv - *Filipendula vulgaris*; supplementary variables.					

The correlation coefficient between individual contributions (%) of *Calamagrostis epigejos* in grassland covering biomass against total fibre content (%) of forages in the middle of May 2008 was 0.795, while at the end of May 2008 was 0.729. Considering all five studied supplementary variables, *Calamagrostis epigejos* individual contribution at total fibre content of permanent grassland covering flora was the highest for mineral fertilised trials. Holub P. *et al* (2012) find also that fertilisation with nitrogen stimulate the *Calamagrostis epigejos* aggressive expansion, in conditions of a dry acidophilus grassland from Czech Republic. According to Marusca T. (2008) and Jendrisakova S. *et al* (2011) *Calamagrostis* spp. has a high productivity, but decrease unfortunately the feeding value and palatability of forage because of rough leafs. Dengler J. *et al.* (2009) consider that the presence of *Calamagrostis epigejos* have a significant negative impact on the diversity of dry grasslands plants.

The presence of *Calamagrostis epigejos* in grassland forage of large areas recommends an adequate harvesting and preservation management. According to Dengler J. *et al* (2009), mowing twice yearly the *Calamagrostis epigejos* had significant effect for reducing the participation of this grass in covering flora of grassland. To assure the necessary quantity and a qualitative feed for animals during the period without grazing in Romania (in winter), *Calamagrostis epigejos* can be mechanical harvest and preserve for example in mixture with other plants richness for foraging as silage or semi-silage. Marusca T. (2008) affirms

that semi-silage (40-60% water content) improve the digestibility of feed and is better accepted by animals because of a smaller acidity and acetic acid and ammonium concentrations than silage (70-80% water content).

The correlation of sheep manure application against grass plants species individual contribution had negative coefficients, -0.529 in *Festuca rupicola* case, and -0.482 for *Calamagrostis epigejos*. That's indicate the grass species were not stimulated in organic farming system, in clime and soil conditions of May 2008, in studied hill permanent grassland from Romanian Banat region.

Analysing *figure 3* and *table 3* data, it can be observed that in May 2008 the organic fertilisation with sheep manure had a high positive impact on *Trifolium repens* contribution (%) in grassland covering spontaneous flora (correlation coefficient 0.911), while mineral fertilisers had a high negative influence (correlation coefficients from -0.702 to -0.746). The correlation of *Trifolium repens* individual contribution (%) against active variables for total fibre content of forages (TFCa and TFCb) had negative coefficients (-0.406, respectively -0.247).

CONCLUSIONS

Resuming the obtained data using PC&CA model for the seven trials of the hill permanent grassland, in soil and clime conditions of Romanian Banat in May 2008, the mineral fertilisation had a high positive impact on total fibre content of forages, stimulating the *Calamagrostis epigejos* contribution in covering spontaneous flora in both cases: in the middle and at the end of month.

For use the forages with high individual contribution of *Calamagrostis epigejos*, a plant with a low palatability, but with a high production capacity and fibre content, to equilibrate the animal feeding budget, it is recommendable to adopt different preservation methods to improve the digestibility (silage, semi-silage), in mixture with other richness fodder plants.

For the hill grassland with *Calamagrostis epigejos* native seeds from Romanian Banat it is recommendable the organic farming system, where no mineral nitrogen is applied, or the application of low mineral nitrogen quantity (50kg/ha), M1 case, when the percent of *Calamagrostis epigejos* was 13%. Also, an extensive regime management (minimum 2 cuts yearly) can reduce the individual participation of *Calamagrostis epigejos* in grassland forage.

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