

IMPACT OF ORGANO-MINERAL FERTILIZATION UPON PHYTOCOENOSIS AND FEED QUALITY OF THE GRASSLANDS IN THE REGION OF TRANSYLVANIA

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ABSTRACT

The paper aims to present the analysis of the qualitative and quantitative changes in the feed obtained as a result of the *Festuca rubra* - *Agrostis capillaris* organic and mineral fertilization. The experiment was conducted in 2014, in Băișoara village (Cluj county), at an altitude of 1240 m above sea level. The experiment included four variants in three repetitions, as follows: first variant (V1) – control variant, (unfertilized); second variant (V2) fertilized with 10 t manure/ha; V3 – fertilized with 10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg/ha active substance/ha; V4 – fertilized with 20 t manure/ha; V5 – fertilized with 10 t manure/ha + 100 N + 50 P₂O₅ + 50 K₂O kg/ha active substance/ha; V6 – fertilized with 100 N + 50 P₂O₅ + 50 K₂O kg active substance/ha. The grass was cut once in 2014, in 2015 and in 2016 and the botanical composition was determined, along with the feed quality. The *Festuca rubra* L. and *Agrostis capillaris* L. grassland responded very well to the application of the organic and mineral fertilization.

Keywords: grassland, organic fertilizer, mineral fertilizer, *Festuca rubra* L., *Agrostis capillaris* L., yield, feed quality.

INTRODUCTION

Globally, the natural grasslands cover 3.362 mil. ha, which represents approximately 23% of the world's land surface, while the arable land covers about 10% of it. Consequently, the reasons for which large grassland areas are still being grubbed up can be seen, leading to a negative impact on the environment (Sima and Popa, 2014).

The grasslands cover from a third up to a half of the agricultural land in the countries in South-Eastern and Central Europe. They extend from the lowlands to the uplands and are a part of the agricultural landscape. The combination of different soils, altitudes and other ecological conditions provide a special importance to the grasslands from the ecological stability and biodiversity point of view (Zimkova et al., 2007). Romania has one of the largest grassland areas in Europe, still managed with traditional methods (Dahlström et al., 2013).

The grasslands are closely related to the way in which they are used, and they can be

easily “directed” from one extreme to the other: abandon-intensive exploitation, (Marușca et al., 2010). The grassland floristic composition and productivity yield is an essential, ecological and demographic phenomenon, representing the net result of a complex set of physiological, ecological and evolutionary interactions in the demographic and physical processes. In most cases, an increase in the plant productivity, due to fertilization, leads to a decrease of the plant species number coexisting in a certain area. It is a well-known fact that the soil fertilization factors have a deep impact upon the grassland floristic composition, mainly in the acid pH, the calcareous pH and the salty habitats. Following the fertilizer application, the semi-natural grasslands have been gradually turned into intensively managed grasslands, (Nösberger and Messerli, 1998).

The experiments conducted so far, nationally and internationally, show that the species richness is reduced along with the intensification of the grassland systems, which leads to the installation of the species with higher forage value and better

productivity (Briemle, 1991; Bogdan, 2012; Briemle and Opperman, 2003; Cirebea, 2017; Cristea, 2004; Păcurar, 2005; Rotar, 2003, Rotar et al., 2016). The floristic composition is established based on scientific criteria, depending on the climate conditions, the exploitation manner and the agro-technique used, leading to a higher quality feed, both qualitatively and quantitatively, on a temporary grassland, as compared to the feed obtained on a permanent grassland (Naie et al., 2017).

The reaction of the grassland types is different (Rotar, 1997). The optimum NPK fertilizer doses recommended vary within close limits from one type of grassland to another, as follows: 150-200 N kg/ha, 50-100 kg P₂O₅/ha and 0-50 kg K₂O/ha (Cardaşol and Daniliuc, 1979).

Hejcmána et al. (2007) published results of a long-term fertilization experiment, which was implemented in 1941, in the Eifel Mountains, in Germany (on the Rengen grasslands, RGE). The grasslands did not provide, naturally, a good yield, being dominated by *Calluna vulgaris* and *Nardus stricta*. Six treatments were applied, Ca, N, P and K combinations, the fertilizers being applied yearly: the unfertilized variant, Ca, KAN, CANP, CANP-KCI and CANP-K₂SO₄ (fertilizer trade names used in Germany). In the middle of June 2005, the plant coverage was estimated visually, and the height of the grassland was measured, with the purpose of detecting floristic composition modifications, caused by the long-term fertilization.

The manure is a complex fertilizer: by means of an average dose of 20 t/ha, approximately 100 kg N reach the soil, 40 kg P₂O₅, 120 kg K₂O, 80 kg Ca, but also other micro-elements, according to Gueydon and Perrollaz Drieu (1994). According to Rotar and Carlier (2005), the manure is considered a very good fertilizer, not only from the chemical composition point of view, but also from the point of view of the effects obtained after its application. Vintu et al. (2011) showed that the *Nardus stricta* L. grassland fertilization with 20-50 t/ha manure led to obtaining yield increases ranging from 40 to 212%. Following the fertilization, the

percentage of the *Nardus stricta* L. species decreased and the leguminous plant percentage increased, but the chemical composition was also improved, by the increased content of crude protein and by the decreased content of crude fiber.

Rotar et al. (2011) analyzed the organic fertilization effect upon a *Festuca rubra* L. grassland's biodiversity and productivity. When fertilizing the grassland, yearly, with 20-30 t manure/ha, significant yield increases were recorded, but the application of a high 20 t/ha dose of manure led to a significant increase of the livestock value. It was shown that the floristic diversity decreased along with the increase of the dose applied.

MATERIAL AND METHODS

The experimental field was placed in the middle of Transylvania, in Băișoara village, Cluj County, at an altitude of 1230 m above sea level. The experiment was designed according to the randomized block method, in four repetitions (blocks), with six experimental variants. This experiment included mineral and organic fertilization (manure).

The surface of an experimental plot was 20 m² and the variants were: first variant (V1) – control (unfertilized); second variant (V2) – fertilized with 10 t manure/ha; V3 – fertilized with 10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg active substance/ha; V4 – fertilized with 20 t manure/ha; V5 – fertilized with 10 t manure/ha + 100 N + 50 P₂O₅ + 50 K₂O kg active substance/ha; V6 – fertilized with 100 N + 50 P₂O₅ + 50 K₂O kg active substance/ha; the experimental variants were established based on the experiments conducted earlier on the natural grasslands in Romania.

The experiment was placed on the *Festuca rubra* - *Agrostis capillaris* grassland type, which is specific to the nemoral zone, according to Țucra et al. (1987).

The annual average temperature was 7°C (in 2016). The annual average precipitation: 1181.0 mm/m² (in 2016). The soil type is Litosol Skeletal.

The floristic studies were described based on the Braun-Blanquet method, according to Păcurar and Rotar (2014), before mowing, when the Poaceae family plants were in the flowering stage. The crop was harvested once every year, in 2014, 2015 and in 2016, and the DM yield were determined.

The statistical interpretation of the results for dry matter yield was conducted using the variance analysis and the Duncan test program for polyfactorial experiments. For the interpretation of the floristic data, the PC-ORD program Version 6 was used, with Nonmetric Multidimensional Scaling (NMS). The program performing the multivariate analysis of the ecological data was included in the spreadsheets (McCune and Mefford 2011). The determination of the feed chemical composition was carried out in the Laboratory for Fodder Quality Determination, within the University of Agricultural Sciences

and Veterinary Medicine Cluj-Napoca, Department of Forage Crops.

RESULTS AND DISCUSSION

After applying the treatments, significant changes were obtained within the phytocoenosis. In the third experimental year (2016), as expected, all treatments applied had a positive impact upon the dry matter harvest and therefore, all variants showed statistical significance with positive increases, very distinctly significant. The variant with 10 t manure/ha + 100 N + 50 P₂O₅ + 50 K₂O kg/ha (V5), in the above-mentioned year, feature the highest yield, of 7.28 t/ha DM, with a 4.86 t/ha DM difference, as compared to the control variant (without fertilization). The smallest dry matter yield (DM) was recorded in variant (V2), with 4.17 t/ha DM (Table 1).

Table 1. The organic and mineral fertilizer impact upon dry matter yield (DM) in 2016

Fertilization	Yield (t/ha)	Percent (%)	Differences (t/ha)	Significance
V1 - 0 t/ha	2.41	100.0	0.00	Control variant
V2 - 10 t organic fertilizer/ha	4.17	172.7	1.76	***
V3 - 10 t organic fertilizer/ha + 50 N + 25 P ₂ O ₅ + 25 K ₂ O kg/ha	4.78	198.1	2.37	***
V4 - 20 t organic fertilizer/ha	4.81	199.2	2.39	***
V5 - 10 t organic fertilizer/ha + 100 N + 50 P ₂ O ₅ + 50 K ₂ O kg/ha	7.28	301.6	4.86	***
V6 - 100 N + 50 P ₂ O ₅ + 50 K ₂ O kg/ha	4.86	201.6	2.45	***

As opposed to our results, Morea Adriana (2008) obtained in the third year, for the same type of fertilization (10 t manure/ha + 100 N + 50 P₂O₅ + 50 K₂O), only 2.63 t/ha DM, without statistical significance.

At the level of 2016, the comparative

analysis, using the Duncan test showed the favourable effect of the organo-mineral fertilization (Cirebea et al., 2016).

The differences found were statistically significant (Table 2).

Table 2. The yield differences among variants and their significance in 2016

Fertilization	Yield (t/ha)	Variants in order of increasing yield				
		V2	V3	V4	V5	V6
		Yield D.M., t/ha				
		4.17	4.78	4.80	4.86	7.28
V1 - 0 t/ha	2,41	1.76	2.37	2.39	2.45	4.86
V2 - 10 t organic fertilizer/ha	4.17	0.61	0.64	0.69		3.11
V3 - 10 t organic fertilizer/ha + 50 N + 25 P ₂ O ₅ + 25 K ₂ O kg/ha	4.78		0.02	0.08		2.50
V4 - 20 t organic fertilizer/ha	4.80			0.06		2.47
V5 - 10 t organic fertilizer/ha + 100 N + 50 P ₂ O ₅ + 50 K ₂ O kg/ha	4.86					2.41
V6 - 100 N + 50 P ₂ O ₅ + 50 K ₂ O kg/ha	7.28					
Significance (***) = significant)		***	***	***	***	***

Following treatment application, in the third year, changes at the grass level were noted. The 2-dimension (2D) graphic representation allows us to explain 80.6% of the resulted floristic phenomenon. The highest importance is that of axis 1 (44.7%) and a smaller explanation percentage can be attributed to axis 2 (35.9%) (data presented in Table 3).

Table 3. Importance of axes and recommended ordinal space in 2016

Axis	Axis importance (r)	Cumulative	Recommended solution
1	44.7	44.7	2D
2	35.9	80.6	

(r- the determination coefficient for the correlations between the ordinal distances and the original distances in dimensional space)

The effect of applying organic fertilizers is represented on axis 1 and therefore, in the positive field, the effect of applying 10 and 20 t manure/ha ($p \leq 0,001$) is felt. Applying 10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg/ha only shows a tendency to explain the floristic phenomenon on the same axis 1 in the positive field, the effect not being statistically assured ($p \geq 0,05$), as it results from Table 4.

The effect of applying mineral fertilizers in larger quantities (V6 – 100 N + 50 P₂O₅ + 50 K₂O kg/ha) is represented on axis 1, in the negative field ($p \leq 0,01$), data shown by Figure 1, but also the organo-mineral fertilization (100 N + 50 P₂O₅ + 50 K₂O kg/ha + 10 t manure/ha), as it results from Table 5.

Table 4. Ordinal axis significance in 2016

Experimental factors	Axis 1		Axis 2	
	R	Significance	r	Significance
Organic fertilizer	0.741	***	-0.313	n.s.
Mineral fertilizer	-0.546	**	-0.251	n.s.
Organic and mineral fertilizer	-0.291	n.s.	-0.151	n.s.
n.s. = not statistically significant				

Table 5. The correlation between yields and biodiversity in 2016

Vectors	Axis 1		Axis 1	
	r	Significance	r	Significance
Harvest	-0.374	*	-0.570	**
Number of species	0.139	n.s.	0.216	n.s.
Shannon	0.523	**	0.246	n.s.
Poaceae	-0.591	**	-0.494	**
Cyperaceae	0.347	*	-0.007	n.s.
Fabaceae	0.765	***	-0.551	**
Plants from other botanical families	-0.297	n.s.	0.561	**
n.s. = not statistically significant				

The control variant (no treatment), is represented on axis 2, results shown by Figure 1.

Within the *Festuca rubra* L. grassland phytocoenosis - with *Agrostis capillaris* L., following the treatments, dominance and co-dominance changes were recorded. The

Agrostis capillaris L. species became dominant in the variants where large quantities of mineral and organo-mineral fertilizers were applied, V5 and V6 (35.25% and 37.00%), the same as the previous year (results shown above), as shown by Figure 1.

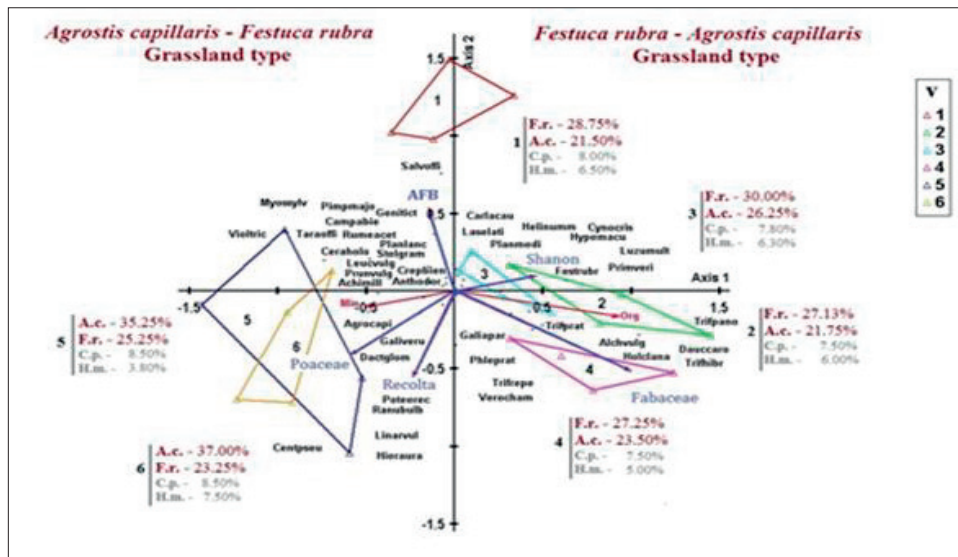


Figure 1. Ordering of floristic composition by applied treatments in 2016

(V1 – control variant, unfertilized; V2 – 10 t/ha Organic fertilizer; V3 – 10 t/ha Organic fertilizer + 50N+25P₂O₅+25K₂O/ha;

V4 – 20 t/ha Organic fertilizer; V5 – 10 t/ha Organic fertilizer + 100N+50P₂O₅+50K₂O/ha; V6 – 100N+50P₂O₅+50K₂O/ha;

F.r. - *Festuca rubra* L., A.c. - *Agrostis capillaris* L., C.p. - *Centaurea pseudophrygia* C.A. Mey., H.m. - *Hypericum maculatum* Crantz.)

After three years of treatment application, there were modifications at floristic level; thus, all treatments feature statistical significance ($p \leq 0.05$, $p \leq 0.01$), according to the values in Table 6.

When comparing the variants, we note that

the differences are not very big (T ranging between -1.9571 and -4.0747 with significant statistical significance, with a reduced homogeneity between the variants, $A = 0.3959$), these results being shown by Table 6.

Table 6. Comparison of the floristic composition of experimental variants in 2016 (MRPP)

Treatments	T	A	p-value	Significance
V1 vs. V2	-3.74215908	0.32940599	0.00436845	**
V1 vs. V3	-3.60777200	0.32674251	0.00373934	**
V1 vs. V4	-4.07474533	0.39592966	0.00437059	**
V1 vs. V5	-3.78520860	0.34752318	0.00410817	**
V1 vs. V6	-3.63181783	0.33434954	0.00398784	**
V2 vs. V3	-2.17710291	0.17327022	0.02204316	*
V2 vs. V4	-2.27961455	0.15563041	0.01258155	*
V2 vs. V5	-3.64715170	0.3119104	0.0043556	**
V2 vs. V6	-3.75697430	0.34498277	0.00430599	**
V3 vs. V4	-3.03202003	0.26082904	0.00701509	**
V3 vs. V5	-3.33900108	0.28074557	0.00293197	**
V3 vs. V6	-3.53292557	0.33688496	0.00403358	**
V4 vs. V5	-3.69540217	0.32478416	0.00409772	**
V4 vs. V6	-3.97681438	0.40207586	0.0044902	**
V5 vs. V6	-1.95716518	0.15494484	0.03411565	**

The Poaceae were favoured by the mineral fertilizer treatments applied. The increase of the Poaceae participation percentage, in this phytocoenosis was directly proportional to the increase of the mineral fertilizer dose. The dry matter harvest and the Poaceae percentage increased along with the high quantities of mineral fertilizers applied 100 N + 50 P₂O₅ + 50 K₂O kg/ha ($p \leq 0.001$), but also of the organo-mineral fertilizers applied 10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg/ha.

The Fabaceae families increased their percentage as the organic fertilizers were applied, but they decreased significantly when mineral fertilizers were applied. Dragomir et al. (1997) showed that the Fabaceae families had a special importance in the plant layer, contributing to the nitrogen content of the soils, and they had an impact upon its physical-chemical characteristics, as a consequence of the nitrogen biological fixation process in symbiosis with the *Rhizobium* bacteria.

The plants from other botanical families, in the year mentioned, were favored by the lack of fertilizers, featuring the highest percentage in the control variant.

After applying the treatments, the floristic diversity was diminished, the greatest diversity being recorded within the control phytocoenosis ($p \leq 0.05$).

Most of the species, such as: *Trifolium hybridum* L., *Trifolium pratense* L., *Trifolium repens* L., *Alchemilla vulgaris* L. emend. Fröhner, *Leucanthemum vulgare* Lam., *Plantago lanceolata* L., *Festuca rubra* L., etc., preferred small fertilizer dose fertilization (Table 7).

According to the values shown in Table 7 applying large fertilizer quantities, both mineral and organic, favoured only certain plants, such as: *Dactylis glomerata*, *Agrostis capillaris* L., *Centaurea pseudophrygia* C.A. Mey., *Ranunculus bulbosus* L., *Hypericum maculatum* Crantz., etc.

Table 7. The correlation of (significant) species reflected on the ordinal axes in 2016

Species	Axis 1			Axis 2		
	r	p-value	Significance	r	p-value	Significance
<i>Agrostis capillaris</i> L.	-0.854	0.001	***	-0.455	0.01	**
<i>Dactylis glomerata</i>	-0.304	n.s.	n.s.	-0.581	0.01	**
<i>Festuca rubra</i> L.	0.462	0.01	**	0.443	0.05	*
<i>Holcus lanatus</i>	0.351	0.05	*	-0.190	n.s.	n.s.
<i>Phleum pratense</i>	0.218	n.s.	n.s.	-0.334	0.05	*
<i>Luzula multiflora</i>	0.347	0.05	*	-0.007	n.s.	n.s.
<i>Lotus corniculatus</i> L.	0.559	0.01	**	-0.211	n.s.	n.s.
<i>Trifolium hybridum</i> L.	0.760	0.001	***	-0.337	0.05	*
<i>Trifolium pratense</i> L.	0.642	0.001	***	-0.655	0.001	***
<i>Trifolium repens</i> L.	0.703	0.001	***	-0.399	0.05	*
<i>Alchemilla vulgaris</i> L.	0.339	0.05	*	-0.232	n.s.	n.s.
<i>Achillea millefolium</i> L.	-0.690	0.001	***	-0.028	n.s.	n.s.
<i>Campanula abietina</i> Griseb.	-0.190	n.s.	n.s.	0.717	0.001	***
<i>Carlina acaulis</i> L.	0.218	n.s.	n.s.	0.380	0.05	*
<i>Centaurea pseudophrygia</i> C.A.M.	-0.419	0.05	*	-0.338	0.05	*
<i>Daucus carota</i> L.	0.486	0.01	**	-0.162	n.s.	n.s.
<i>Galium aparine</i>	0.009	n.s.	n.s.	-0.382	0.05	*
<i>Genista tictoria</i>	-0.023	n.s.	n.s.	0.439	0.05	*
<i>Helianthemum nummularium</i>	0.288	n.s.	n.s.	0.334	0.05	*
<i>Hypericum maculatum</i> Crantz.	0.402	0.05	*	0.045	n.s.	n.s.
<i>Laserpitium latifolium</i> L.	0.026	n.s.	n.s.	0.388	0.05	*
<i>Leucanthemum vulgare</i> Lam.	-0.292	n.s.	n.s.	0.364	0.05	*
<i>Pimpinella major</i> (L.) Huds.	-0.262	n.s.	n.s.	0.585	0.01	**

Legend: >0.61 → p=0.001; 0.45-0.60 → p=0.01; 0.33-0.44 → p=0.05; <0.33 → n.s.= not statistically significant

The results regarding the quality of the feed obtained in 2016, after the mineral and organic fertilization also underlined that the crude protein content varied depending on the applied fertilization. In our experiment, after three years of fertilization, the protein quantity decreased following mineral fertilization from 8.64% for the control, to 6.62% (V6). For the manure organic fertilization, increases up to 11.33% were recorded, when applying 20 t manure/ha (V4). As for the variants fertilized with organo-mineral fertilizers (V3 and V5), the protein content also increased slightly up to 9.91%, as shown by Figure 2. Similar results were also presented by other researchers: (Avaravei and Chelariu, 2010); Dale, 2011; Ionescu et al., 2010; Ionel et al., 1997; Samuil et al., 2013).

The crude fat content varied, as well, slightly increasing in the case of organic

fertilization. In the year mentioned, the highest content of crude fat was 3.87%, for variant V5 (10 t manure/ha + 100 N + 50 P₂O₅ + 50 K₂O kg/ha), and the smallest content of 2.82% was recorded for variant V3 (10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg/ha), according to the values in Figure 2.

The crude ash content featured variations, with an increase in the case of the organic fertilization, of up to 8.38% (V4 – 20 t manure/ha), from 5.58% (control). The results concerning the ash content in the variants fertilized with organo-mineral fertilizers (V3 and V5), but also in the variant fertilized only with mineral fertilizer (V6), recorded a decrease, down to 4.61% (Figure 2).

The crude fiber significantly increased after the applied treatments, both mineral and organic treatments, with values ranging between 35.38% in the control variant and 38.37% in variant V4 (Figure 2).

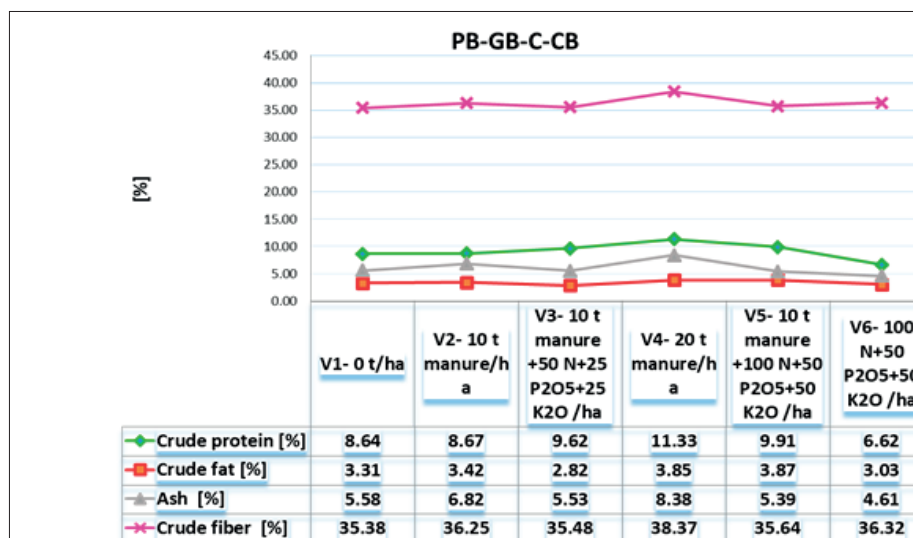


Figure 2. The impact of organic and mineral fertilization upon certain quality indices

The NDF content of the feed obtained after mineral and organic fertilization in 2016 increased significantly, the only slight decrease tendency being recorded in the case of 10 t manure fertilization (Figure 3).

The values ranged between 50.21% (the control variant) and 66.08% (V6). Other researchers obtained values similar to ours: Ammar et al., 2008; Mandaluniz et al., 2009.

The ADF content in the year under discussion, regarding the organic fertilization,

showed a decrease tendency, from 40.68% (the control variant), to 38.68% (V4), while in the other variants, increases up to 47.55% were recorded (V6).

The Gramineae and the Leguminous plants feature similar digestibility, with ADF values that are very close (Collins and Fritz, 2003).

Similar values were also presented by other researchers: 26.2-38.9%; (Dohme et al., 2007; Mandaluniz et al., 2009).

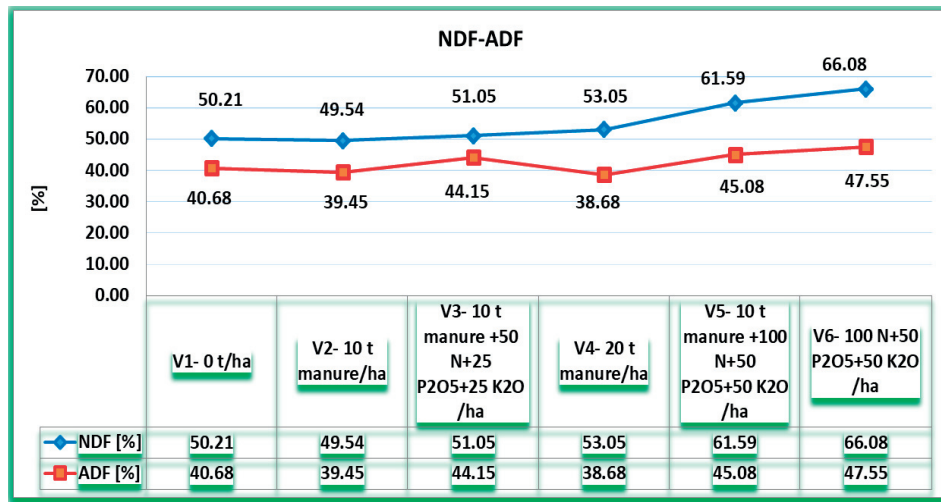


Figure 3. The impact of organic and mineral fertilization upon the NDF and ADF content (NDF-Neutral fiber determination; ADF- Acid fiber determination)

CONCLUSIONS

The dry matter harvest obtained following the organic and mineral fertilization was positively influenced by all applied fertilizer doses. The highest yield of 7.28 t/ha DM was obtained in 2016, the third fertilization year and a more favorable year, from the climate point of view, by the variant fertilized with 10 t manure/ ha + 100 N + 50 P₂O₅ + 50 K₂O kg/ha (V5). Applying organic and mineral fertilizers also lead to changes in the ratio between the dominant and co-dominant species in the studied grassland. Thus, starting with the second year of treatments, within the phytocoenosis of the *Festuca rubra* L. grassland - with *Agrostis capillaris* L., the *Agrostis capillaris* L. species became dominant within the variants with higher quantities of mineral and organo-mineral fertilizers, V5 and V6. *Agrostis capillaris* L., showed a percentage increase related to the treatments applied, ranging between 35.25% and 37.00%, in the second and third fertilization year. The Poaceae families and the plants in other botanical families were favored by the mineral and organo-mineral fertilization in big quantities (V6 – 100 N + 50 P₂O₅ + 50 K₂O kg/ha and V5 – 10 t manure/ha + 50 N + 25 P₂O₅ + 25 K₂O kg/ha), while the Fabaceae families showed an increase of the floristic composition, matching the manure fertilization (V2 – 10 t/ha and V4 – 20 t/ha). The results

obtained regarding feed quality outlined the fact that the crude protein content varied depending on the fertilization applied. The crude protein decreased following mineral fertilization, but it increased when the organic and organo-mineral fertilization was applied, in all the years of study. The largest protein quantity was obtained within variant V4, of 11.33%, as compared to the control variant, of 8.64 %.

As for the soil reaction to the application of the organic fertilizers, these fertilizers supplemented the nitrogen mineral form reserves, but only under the circumstances where a maximum organic fertilizer dose was applied (V4 - 20 t manure/ha), without NPK application. The manure's favorable effect was relevant also when it comes to the soil calcium improvement tendency, but only in those variants where fertilization was based on manure application (V2 and V4). Manure application favorably modified also the soil reaction, which expressed an acidification when applying NPK mineral fertilization, which can accentuate humidity, even with minimum organic substance.

We recommend NPK-based mineral fertilizer application only together with manure. This association led to very high harvest increases, of 7.28 t/ha DS (the highest yield being recorded in V5). The feed quality also improved, not only by increasing the protein quantity, but also by increasing the leguminous plant coverage degree.

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