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Posted Date: 4 April 2024

doi: 10.20944/preprints202404.0382.v1

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Fertilizers Impact on Grassland in North-Eastern Romania Studied by Nuclear and Related Analytical Techniques

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Abstract: To get more data concerning the influence of fertilizers (organic and mineral) on different forage plants existing in North-eastern Romanian grassland, the mass fractions of 14 essential, enzymatic, or considerd toxic elements was determined by Instrumental Neutron Activation Analysis together with the amount of crude proteins, ash, fibres as well as ether fact extract. Final results showed a significant variance of the content of analysed elements on organic as well as on mineral fertilized experimental plots, i.e. the content increased in 24% of plots, decreased in 23%, and remained unchanged in 45% while in 8% of plots their content was below the detection limits, as the case of Se. At the same time, an increase content of crude protein and ether extract was evidenced in fertilized grasses for all applied fertilizers while other global indicators such as neutral and acid fibre of sufuric lignin content decreased, suggesting significant higher nutritional values of fertilized forage plant.

Keywords: organic fertilizers; mineral fertilizers; grassland; dietary cation-anion difference; instrumental neutron activation analysis

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1. Introduction

Romania is a medium size country located in Southeastern Europe. Bordering on the Black Sea, the country is halfway between the equator and the North Pole and equidistant from the westernmost part of Europe–the Atlantic Coast and the most easterly–the Ural Mountains. At the same time, Romania has a well balanced relief so that about 1/3 of the country surface is represented by planes, 1/3 by hills and 1/3 by mountains with a maximum height of 2544 m [1,2].

Both geographical position and relief diversity determines a variety of climatic zone beginning with humid subtropical in the south to subarctic and tundra at altitudes higher than 1800 m, and with a medium temperature varying between + 11 ^{o}C in the South and + 8 ^{o}C in the North. Under these conditions, the average precipitations are of 589 mm for a relative humidity around of 70 % [3].

These factors significantly influenced the distribution of agricultural surface so that from a total of 14.8 Mil ha agriculture classes, the exploitable grassland (pasture and hay-field) covers about 4.9 Mil ha [4]. On the other hand, due to an improper management, a significant proportion of pastures, which cover about 60% of the mountain area (18% of entire country) are in many places overgrazed.

Besides a better management, the administration of both natural and mineral fertilizer could be a long term solution to prevent the consequences of overgrazing. It has been proved that the use of fertilizer could significantly increase the forage biomass per ha, and, at the same time, it could affect not only the nutritional value but also the content of a great diversity of elements. Among them there are Na, K, or Cl of which concentration define the Dietary Cation-Anion Difference (DCAD) [5], more enzymatic transition metals elements i.e., Mn, Fe, Co, Zn, Mo and Se [6] or even harmful such as Al and As [7,8]. To this list we could add Mg, and Ca of which role in plant metabolism or in the cell wall and membrane structure is essential [9,10] as well as Sr, due to its role in the process of osteosynthesis by stimulating bone formation together with the inhibition of bone resorption [11,12]. On the other hand, acording to [13], Sr could causes harmful effects do its mobility within plants.

For a better characterization of the fertilizer influence on the most common type of grasses, we have used the Instrumental Neutron Activation Analysis (INAA) in its both Thermal and Epithermal variants [14] to determine the mass fractions of about 14 major and traces elements in three types of perennial grasses in correlation with some of the most important biochemical descriptors of their nutritional values. It is worth mention that considered forage grasses are commonly used for cattle food.

2. Hypothesis and Research Objectives

Given the diversity of forage grasses as well as of their habitat, the main goals of this study are:

(i) to evidence to what extent the use of natural as well as of mineral fertilizer influence the content of some essential as well as harmful elements in three types of forage grasses common for Romanian medium to high altitude grasslands
(ii) to quantify the influence of fertilizer as it is reflected by the parameters that characterize the nutritional value of considered forages, i.e., the dietary requirements.

The results of our study performed under these circumstances will be further presented and discussed.

3. Materials and Methods

3.1. Sampling and Sample Preparation

To obtain a rather average information concerning the influence of fertilizer treatment on the mineral content of the most common types of forage grasses, the same kind of experiments were organized in four different places, all of them in the Suceava County, North-eastern Romania, at altitudes between 611 and 840 m, i.e. typical for Romania grasslands (Figure 1). For our study we have chosen, as mentioned before, three types of perennial grasses, all of them currently populating the temperate climate grassland, e.g. *Agrotis capillaris* L, *Festuca rubra* L and *Nardus stricta* L.

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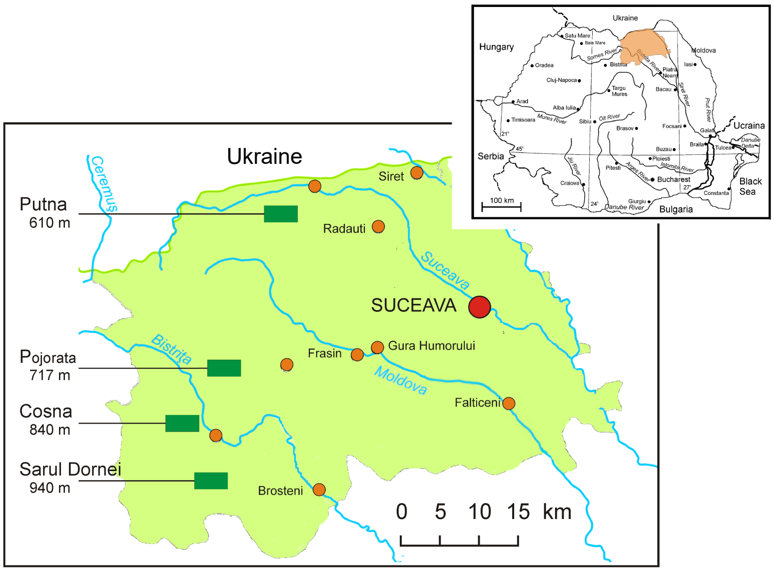

Figure 1. The location of experimental areas within the Suceava County with respect to Romanian territory (inset).

All experiments consisted of applying for five consecutive years a mixture of natural and mineral fertilizer following different schemes, as illustrated in Table 1. For this, 15 to 27 plots of land of 2 x 3 m randomly disposed to form compact blocks of 90, 126 and 162 m^2 respectively were kept unfertilized as reference, or were fertilized as previously mentioned (Fig.1SM Supplementary Material). For each experimental location, a detailed description of different procedure used in fertilizer administration are reproduced in Tables 5–7 (see Appendix).

After four years of controlled fertilization, for two consecutive years, the grass was harvested, cleaned for foreign vegetation, dried, ground and analysed for major and trace elements, and, as well as for the nutritional quality specific parameters

Table 1. General data concerning the location of experimental parcel, grass species involved in experiment as well as the type and amount of administrated fertilizers.

Locality **Grass Species**	**Natural Fertilizer** **(t/ha)**	**Chemical Fertilizer** **(t/ha)**
Cosna (840 m altitude) *Nardus stricta* L	20 to 50 t of manure applied annually/bi-annually (1 – 4 y)	$N_{100}P_{100}$; $N_{140}P_{140}$; $N_{200}P_{200}$; $N_{100}P_{100}$+$N_{40}P_{40}$; $N_{100}P_{100}$+$N_{100}P_{100}$; $N_{80}P_{80}$+$N_{640}P_{60}$
Pojorata 717 m altitude *Agrostis capillaris* L *Festuca rubra* L *Nardus stricta* L	10 to 50 t of manure applied annually/bi-annually (1 – 4 y)	30 kg -50 kg mineral nitrogen + 10 to 30 t of manure applied annually, bi-annually or every three years
Putna 611 m alt. *Agrotis capillata* L, *Festuca rubra* L	20 to 50 t of partially or totally fermented manure	—
Sarul Dornei 940 m alt. *Festuca rubra* L, *Nardus stricta* L	20 t to 30 t of manure applied annually and bi-annually following the schedule : 50+0+40+0 t during four years	—

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3.2. Analytical Techniques

The INAA was used to determine the mass fractions of following elements: Na, Mg, Al, Cl, K, Ca, Mn, Fe, Co, Zn, Se, As, Br, Sr, and Mo. Among them, Mn, Fe, Co, Cu, Zn, Mo and Se enter, according to [6], into composition of some enzymes, indispensable for plants vital processes. Important are also the alkali elements Na and especial K of which average mass fractions can reach up to 1.5% wt. together with the structural element Cl and Ca, considered one of the main constituent of plant cells [6]. At their turn, as mentioned before, Al, as As are considered toxic for animals and especialy for human consumption while Sr are could be harmful for plants [13]. For this reason, their presence needs a special attention.

On this, it is worth mentioning that the mass fractions of Na, K and Cl defines the Dietary Cation-Anion Difference (DCAD) [5] as:

$$\mathsf{DCAD} = (\mathsf{Na}^{+} + \mathsf{K}^{+}) - \mathsf{Cl}^{-} \quad (1)$$

where Na^{+}, K^{+} and Cl^{-} represent the concentrations of mentioned elements expressed in milli-equivalents per 100 g of dietary dry matter.

The DCAD represents a valuable proxy in estimating the influence of the grass major elements (Na, K and Cl) on cattle lactation and meat productivity. Accordingly, in dry cows, a negative DCAD can help prevent metabolic problems while in the case of lactating cows, a positive DCAD help increasing milk production and nutritional potential.

All INAA measurements were performed by using the fast pulsed reactor IBR 2. To determine the mass fraction of the above-mentioned elements, both short and long-time irradiation were used. More details on the experimental setup and quality control are provided in [15–18].

Special attention was paid to quality control, done by a simultaneous use of more Standard Reference Materials (SRM), assembled to compose the so-called Group of Standard Sample (GSS) proprietary software [15–17]. The aim of this procedure consisted of selecting the most suitable SRM lines to maximize, for all considered elements, both accuracy and precision of INAA determinations. This permitted to attain for each INAA measurements an accuracy between 3 and 15%, as expressed by means of Combined Statistical Uncertainty (CSU) [19]. See also Table A2 of ref. [18].

Simultaneously with the INAA measurements, a set of five chemical parameters, essential to characterize nutritional qualities of grasses, i.e., the content of Ash (A), Crude Protein (CP), Fat Ether Extract (FEE), Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF), were determined too.

The ash content was gravimetrically determined by muffle furnace ignition at 550 ^{o}C [20], CP and FAT were determined by Kjeldahl [21] and Soxhlet extraction [22] methods respectively while Van Sosest method was used to determine both ADF and NDF as well as the Sulfuric Lignin Content (*SLC*) [23]. For these methods, the CSU was no greater than 7 %. All these parameters were experimentaly determined by the Chemical Analyse Laboratory of the Iasi University of Life Sciences.

4. Results

The final results concerning the mass fractions of the most important elements in both unfertilized (for reference) and fertilized soil grasses are reproduced in Table 2, while a complet set of experimental data can be found in Table 1SM Supplementary Material.

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Table 2. Final results concerning the effect of fertilization on the mass fractions (in mg/kg) of the metabolic and structural elements (black ink), enzymatic (blue ink), as well as potential toxic elements (red ink) in foragin grasses involved in experiment. For a better comparison, the Refrence Plant (RP) [6], Maximum Tolerable Level (MTL) [21], and Recommended Dietary Mineral Reserve (RDMR) [23] are reproduced too. NB The complet set of experimental data is provided in Table 1SM Supplementary Material.

Element	Unfertilized	Fertilized	RP	MTL	RDMR
Na	50 - 90	50 - 110	150	40000	1000 - 2200
Cl	1960 - 4630	1480 - 3310	2000	40000	1300 – 2900
K	9500 - 16500	12200 - 21700	19000	30000	4700–10000
Mg	2100 – 5700	1940 – 5080	2000	6000	1100 – 2100
Ca	5160 – 14230	7150 – 11100	10000	7000	2200- 3800
Mn	80 – 550	70 – 590	200	1000	13 – 24
Fe	210 – 300	160 – 360	150	500	12 – 40
Co	0.16 – 0.23	0.16 – 0.27	0.2	25	0.10 – 0.15
Zn	40 – 70	33 – 71	50	2	1.1 – 0.3
Mo	0.2 – 1.0	0.2 – 0.5	0.5	5	0.2
Se	nd – 0.29	0.22 – 0.29	0.02	1000	13 – 14
Al	180 – 510	200 – 640	80	1000	—
As	1.0 – 1.5	1.4 – 1.7	0.1	30	—
Sr	14 – 40	13 – 42	50	2000	—

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To highlight the influence of fertilizers, in this table, we have provided not only the mass fractions of investigated elements, but also the corresponding values of the Refrence Plant [6], the Recommended Dietary Mineral Requirements (RDMR) [24] as well as the Maximum Tolerable Level (MTL) [24], while the totality of data can be found in Table 3SM Supplementary material. In this regard it should be mentioned that, for a better statistical analysis, as well as to be closer to real grasslands, all presented results referee to the average values obtained by combining the corresponding parameters for all three types of grasses considered in this study.

A first remark concerning global data provided in Table 2 points toward a great spread of final mass fractions of almost all elements, although the CSU, as mentioned before never exceeded 7 %.

In our opinion, this fact could be attributed on one hand, to the reduced experimental surfaces which determined a significant variability of numerical data, and, to the diversity of pedologic condition on the other. On this subject it should be taken into account that the experiment took place in four different places at height varied between 610 m for Putna and 940 m for Sarul Dornei (Figure 1) with different local climate and soil types.

For a better illustration of influence of the fertilizing procedures investigated in this study, in the Table 3 we have illustrated at which extent the presence of fertilizer inluenced the mass fractions of considered elements in fertilized grasses, while, a complet set of corresponing data is provided in Table 2SM Supplementary material. All values, are significant at $p < 0.05$ (95 % probability) according to t – Students's test. In this regard it should be remarked that the provided valued regards average results concerning all three grass species, i.e. *Agrotis capillaris* L, *Festuca rubra* L and *Nardus stricta* L.

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Table 3. The influence of fertilizer type on the content of metabolic and structural elements (black ink), enzymatic (blue ink), as well as potential toxic elements (red ink) in foragin grasses involved in experiment. All results were significant at p < 0.05 according to Student's t test. NB The complet set of experimental data is provided in Table 2SM Supplementary Material. nd stays for below detected limit.

Element	Organic Fertilizers			Mineral Fertilizers		
	Increased (%)	Unchanged (%)	Decreased (%)	Increased (%)	Unchanged (%)	Decreased (%)
Na	50	37.5	12.5	50	50	nd
Cl	87.5	12.5	nd	25	25	50
K	50	37.5	nd	nd	75	25
Mg	50	12.5	37.5	25	25	50
Ca	62.5	12.5	25	nd	25	75
Mn	nd	12.5	87.5	50	25	25
Fe	62.5	12.5	25	50	25	25
Co	25	75	nd	50	25	25
Zn	25	50	25	25	25	50
Se	nd	nd	nd	nd	nd	nds
Mo	75	nd	25	25	nd	50
Al	25	12.5	62.5	25	25	50
As	nd	62.5	37.5	50	25	25
Sr	75	nd	25	nd	50	50

5. Discussion

As mentioned before, our study has two main objectives: -i. to evidence at which extent the organic and mineral fertilization influence the intake of DCAD, enzymatic, structural or harmful elements into forages; -ii. to estimate at which extent the fertilized forages fulfil the dietary requirements.

Regarding the first objective, we have compared the content of the above-mentioned category of elements in the foraging grasses cultivated on fertilized grassland with the content of same elements in grasses collected from unfertilized plots.

These results reproduced in Tables 2 and 3 show with clarity that in all cases, Na present a chronic deficit, its mass fraction being significantly lower than RDMR. Also, it should be remarked that in Cosna, Pojorata and Sarul Dornei the Al mass fraction locally exceeded the MTL, which should be a concern for local authorities. In all other cases, the content of investigated elements corresponded in a lower or greater measure to the RDMR, never exceeding the MTL.

The same results confirmed a relative disperse reaction to fertilizer administration, which most probable, as mentioned before, could be attributed to local variability of soil and micro-climate. From this point of view, the most positive results were observed at Cosna experimental plots for organic fertilizers, where the presence of almost all elements showed a statistically proved growth at $p<0.05$.

An opposite tendency we have also noted in regard to Cosna experimental plots, but this time for mineral fertilizers, where the content of almost considered elements decrease with respect to non-fertilized areas.

The extreme variation we have noticed for Na and Ca of which content in fertilized grasses increased in six locations, mainly as a result of applying organic fertilizer as well as in the case of Mn, of which mass fraction decreased in six locations, mostly fertilized with manure.

Although V and Cr can play a significant role in plant metabolism, their content was in many cases below detection limit so we no more considered them in this report.

A more detailed information concerning the influence of different fertilizing procedures is provided in Table 3. Here we have illustrated at which extent the presence of considered elements increased, decreased or remained unchanged after fertilizer administrations. In all cases, the probability was more than 95 % ($p < 0.05$). Accordingly, the contents increased in 24% of plots, decreased in 23

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%, remained unchanged in 45 % while in 8 %, their content was below the detection limits, as the case of Se. It should be remarked, that according to Table 3 data, Fe remains the single elements of which content, excepting Sarul Dornei (organic fertilizer) and Pojorate (mineral fertilizer) experimental areas (Table 2SM Suplementary Material), increased in all other locations.

The best results were observed for Cosna plots (organic fertilizers, 2010) where the mass fraction of eight elements increased. On the opposite side was Pojorata (organic fertilizer, 2011) where the mass fractions of all elements either remained unchanged or even decreased.

In what concerns the second objective, we have calculated, by using the results obtained by both INAA and chemical analysis, the more representative descriptors such as Dietary Cation-Anion Difference (DCAC), Crude Protein (CP), Ash Content (AC); Ether Extract (EE); Neutral Detergent and Acid Detergent Fibre (NDF) and (ADF) respectively as well as Sulphuric Lignin Content (SLC).

The obtained results reproduced in Table 4 showed that DCAC, of which values are in fact determined by the content of Na, K, and Cl, as well as by the fertilizers, either organic or mineral, seem to be more evident for of mineral fertilizers. In our opinion, this fact can be due to a reduced content of Na, which the use of organic as well as mineral fertilizer could not increase significantly. Despite this fact, everywhere, DCAC presented positive values.

At the same time, both organic and mineral fertilizers seem to have a positive role in increasing the quality of foraging grasses as, according to results illustrated in Table 4, CP and EE, the last one consisting chiefly of fats and fatty acids, increased in almost all cases. A similar behaviour could be noticed with regard to AC which represents a measure of the total amount of minerals present within plants. The higher the AC, the more developed aerial part of the plant, and this characteristic could be well correlated with an increased content of the protein and fats, these results regarding all three grass species, i.e., *Agrotis capillaris* L, *Festuca rubra* L and *Nardus stricta* L.

Table 4. The influence of organic fertilizer on the parameters characterizing the nutritional qualities of grasses for both organic and mineral fertilizer. All results were significant at $p < 0.05$ according to Student's t test. NB The complet set of experimental data is provided in Table 2SM Supplementary Material.

Fertilization variant	DACD	CP	A	EE	NDF	ADF	ADL
			Organic fertilizers				
V1 Unfertilized control	3100	6.4	5.5	1.6	74.7	47.1	11
V2 20 t/ha every year;	3130	9.3	9.8	2	60.2	38.4	10.4
V3 30 t/ha every year;	3720	11.5	9	1.6	55.1	37.5	10.4
V4 40 t/ha every year;	3170	9.7	10.5	1.9	50.1	38.5	10.4
V5 50 t/ha every year;	3940	10.8	10.2	2.2	53.8	40.7	9.9
V6 20 t/ha every 2 years;	2100	11.7	8.8	1.7	57.5	37.7	9.9
V7 30 t/ha every 2 years;	2150	12	9	1.3	57.8	39.4	9.5
V8 40 t/ha every 2 years;	2590	12.8	9.4	1.3	55.9	38.6	9.6
V9 50 t/ha every 2 years;	2620	13.1	10	1.5	54.8	43.1	9.2
			Mineral fertilizers				
V1 Unfertilized control	3200	6.5	7.6	2.7	72	46	10
V2 N100P100	3420	6.6	6.3	3	59	34	9.6
V3 N140P140	4430	8.1	6.2	3.1	59	33	9.8
V4 N200P200	3180	10	7.1	3.2	52	33	9.5
V5 N100P100 + N40P40	3370	11	6	3	55	34	9.7
V6 N100P100 + N100P100	3070	14	7.3	3.3	54	32	10.5
V7 N80P80 + N60P60	2670	11	6.2	2.6	59	40	9.6

On this subject it is worth mentioning that the ash content increased only in the case of organic fertilizers, showing an opposite tendency for mineral ones. In our opinion, this fact, if future experiments will confirm, could play a significant role in increasing the use of organic fertilizer, more environmentally friendly.

 doi:10.20944/preprints202404.0382.v1

At the same time, the values of the other nutritional parameters such as NDF, ADF and ADL significantly decreased in the presence of bot mineral and organic fertilizers, which, in our opinion confirms the positive role of fertilizer in regeneration process of the overgrazed grasslands.

6. Conclusions

To evidence the possible influence of different fertilizing procedure, Instrumental Neutron Activation Analysis as well as other analytical techniques were used to determine the mass fractions of 14 elements as well as the numerical values of seven nutritional descriptors in three species of Gramineae, currently fond on Romanian grasslands. The grass samples were collected from unfertilized as well as from fertilized experimental plots, all of them located in the Suceava county, North-eastern Romania.

The analysis of the elemental content the most important 14 structural, metabolic, enzymatic or potential toxic elements showed first of all a significant Na deficit for all plants harvested from both fertilized and unfertilized experimental plots.

Also, we have noticed a significant variance of the content of analysed elements on both type of experimental plots e.g. the elemental content increased in 24 % of plots, decreased in 23 %, remained unchanged in 45 % while in 8 % it was below the detection limits, as the case of Se.

A better influence was noticed for crude protein and ether extract of which content increased in almost all fertilized grasses, both indicators pointing towards an increased nutritional value.

The values of other global indicators such as neutral and acid fiber of sulphuric lignin content decreased in almost all cases, this finding suggesting again a beneficial influence of both organic and mineral fertilization.

The great variability observed for all descriptors points towards the use, in future studies, of significant greater experimental plots to reduce as possible as the statistic uncertainties associated with small amounts of analysed material.

Author Contributions: Conceptualization, Otilia Culicov, Doina Tarcau, Mihai Stavarache and Vasile Vintu; Data curation, Otilia Culicov, Doina Tarcau, Inga Zinicovscaia, Octavian Duliu, Mihai Stavarache and Vasile Vintu; Formal analysis, Doina Tarcau, Inga Zinicovscaia, Octavian Duliu, Mihai Stavarache and Vasile Vintu; Investigation, Otilia Culicov and Inga Zinicovscaia; Methodology, Otilia Culicov, Doina Tarcau, Inga Zinicovscaia, Octavian Duliu, Mihai Stavarache and Vasile Vintu; Project administration, Otilia Culicov and Doina Tarcau; Resources, Otilia Culicov and Doina Tarcau; Software, Otilia Culicov and Octavian Duliu; Supervision, Inga Zinicovscaia; Validation, Doina Tarcau, Inga Zinicovscaia and Vasile Vintu; Visualization, Otilia Culicov, Inga Zinicovscaia, Octavian Duliu, Mihai Stavarache and Vasile Vintu; Writing – original draft, Otilia Culicov; Writing – review & editing, Otilia Culicov, Doina Tarcau, Inga Zinicovscaia and Octavian Duliu.

Acknowledgments: The project was realized within the Cooperation Programs between the Iasi University of Life Sciences, University Alexandru Ioan Cuza, Iasi, University of Bucharest and the JINR International Intergovernmental Organization, Dubna, Russian Federation.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

A	Ash
ADC	Acid Detergent Fibre
CP	Crude Protein
DCAD	Dietary Cation-Anion Difference
FEE	Fat Ether Extract
INAA	Instrumental Neutron Activation Analysis
MTL	Maximum Tolerable Level
NDF	Neutral detergent Fibre
RDMR	Recommended Dietary Mineral Reserve
SLC	Sulphuric Lignin Content
SRM	Standrd Reference material

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Table 5. Detailed description of the fertilizing procedure for *Nardus stricta* L at Cosna grassland.

Experience 1: Organic fertilizer		**Experience 2: Mineral fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	20 t/h manure applied every year	V2	$N_{100}P_{100}$
V3	30 t/h manure applied every year	V3	$N_{140}P_{140}$
V4	40 t/h manure applied every year	V4	$N_{200}P_{200}$
V5	50 t/h manure applied every year	V5	$N_{100}P_{100} + N_{100}P_{100}$
V6	20 t/h manure applied every 2 years	V6	$N_{100}P_{100} + N_{100}P_{100}$
V7	30 t/h manure applied every 2 years	V7	$N_{80}P_{80} + N_{60}P_{60}$
V8	40 t/h manure applied every 2 years		
V9	50 t/h manure applied every 2 years		

Table 6. Detailed description of the fertilizing procedure for Pojortata permanent grassland of *Agrostis capillaris* L and *Festuca rubra* L (Experience 1) as well as *Nardus stricta* L. (Experience 2).

Experience 1: Organic fertilizer		**Experience 2: Mineral fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	10 t/h manure applied every year	V2	30 kg/h mineral nitrogen+10 t/h manure applied every year
V3	20 t/h manure applied every 2 years	V3	50 kg/h mineral nitrogen+10 t/h manure applied every year
V4	30 t/h manure applied every 3 years	V4	30 kg/h mineral nitrogen+20 t/h manure applied every 2 years
V5	20 t/h manure annually +10 t/h manure every 2 years +no manure	V5	50 kg/h mineral nitrogen+20 t/h manure applied every 2 years
V6	20 t/h manure annually +no manure +10 t/h manure every 3 years	V6	30 kg/h mineral nitrogen+30 t/h manure applied every 3 years
V7	20 t/h manure annually +10 t/h manure every 2 years + 10 t/h manure every 2 years	V7	50 kg/h mineral nitrogen+30 t/h manure applied every 3 years

Table 7. Detailed description of the fertilizing procedure for *Nardus stricta* L at Cosna grassland.

Sarul Dornei: Organic fertilizer		**Putna: Mineral fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	20 t/h manure applied every year	V2	$N_{100}P_{100}$
V3	30 t/h manure applied every year	V3	$N_{140}P_{140}$
V4	30 t/h manure applied every 2 years	V4	$N_{200}P_{200}$
V5	50+0+40+0 t/h manure applied	V5	$N_{100}P_{100} + N_{40}P_{40}$

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