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Fertilization Effects on the Chemical Composition and *In Vitro* Organic Matter Digestibility of Semi-natural Meadows as Predicted by NIR Spectrometry

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Abstract

Management of livestock grazing in highly-productive mountain meadows is an important aspect for the economic viability and the environmental impact of a grassland-based farm. The main aim of this study was to build near infrared models to determine the chemical composition and in vitro organic matter digestibility of Romanian meadow forages. The treatments were organic and mineral fertilizer combinations, and forage samples were obtained from three fertilization experiments conducted in the Apuseni Mountains; these samples were analysed using classical and NIR methods. The samples were scanned in the NIR wavelength band. The CRA-W Gembloux 'local' calibration models were validated with Romanian meadow forages and then used in order to predict the forage quality of samples. A second objective of the study was to determine the effects of fertilization on forage quality. The results showed a decrease in crude protein content from the NPK treatment (150:75:75), which can be explained by a reduction of *Fabaceae* plants with this treatment from 17.25% of the populations in the control (semi-natural meadow not fertilized) to 6.25% in the fertilized plots. The decrease in protein content and *in vitro* organic matter digestibility was related to a reduced *Fabaceae* presence. Our recommendation is to use mineral fertilization with NPK doses less than 100:50:50 to improve meadow productivity; meanwhile organic fertilization can also be used to complement and maintain biodiversity and forage quality.

Keywords: Apuseni Mountains, chemical composition, forage quality, NIR, semi-natural meadows

Introduction

In the Romanian Apuseni Mountains, specifically in the Gârda area, more than 34% of grasslands are used as a source of hay to provide fodder for livestock (Gârda, 2010). Meadow management is an important aspect for the economic viability and the environmental impact of grassland-based farms in this region. Good meadow management reduces the need to buy expensive concentrate feeds and limits the environmental impact by using appropriate levels of fertilizers and choosing optimal grazing periods.

A semi-natural meadow is defined as one that may be mowed for fodder or provide grazing for livestock. The determination of forage quality from semi-natural meadows is important for both nutritional and economic reasons (Rotar *et al.*, 2010). Generally, forage quality is influenced by many factors, including soil, climatic conditions, floristic composition of the meadow, stage of maturity of plants when harvested, and forage preservation methods. The forage nutritive value depends on its chemical composition including: crude protein [CP], crude ash [ash], ether extract [EE], crude fibre [CF], neutral detergent fibre [aNDFom], acid detergent fibre [ADF], lignin [lignin (sa)] and *in vitro* organic matter digestibility [OMD].

Currently, new analytical tools for agriculture based on spectroscopic technologies are being developed to extend the work of Norris and coworkers in the 1960's (Hart *et al.*, 1962). Near Infrared (NIR) Spectroscopy is a well-known technology in the agricultural sector allowing the acquisition of chemical information from the samples with a large number of advantages, such as: an easy to use tool, fast and simultaneous analysis of several components, non-polluting, non-invasive and non destructive technology, and the possibility of online or field implementation (Dale *et al.*, 2012).

Although the development of the NIR technique has advanced the science of forage evaluation, wet chemistry analysis is still considered the "gold standard" for forage testing. Many certified feed analysis laboratories are capable of performing wet chemistry, NIR analyses, or both (Weiss and Pell, 2007). Certified feed analysis laboratories around the world use complex mathematical and statistical methods for predicting forage quality in all types of forages. The accuracy of the NIR method to determinate forage quality is determined by the differences between NIR predictions and results of reference methods that have to be less than the sampling error (Weiss and Pell, 2007).

In some developed countries several investigations on forages were initiated, which aimed to: develop non-destructive methods; assess the possibilities to use the NIR spectrometry technique in order to build a spectral database; build models of NIR spectrometry calibrations and validation for various parameters (protein, dry matter, ash, fiber, fat, NDF, ADF, lignin, digestibility, crude energy, etc).

In some countries of the European Community, NIR spectrometry has been used since 1980, although in many other countries it is still in an early stage of development. NIR spectrometry is widely used for rapidly determining the concentration of nutrients and feeding value in dried and fresh crop materials (Decruyenaere *et al.*, 2009; Rotar *et al.*, 2009), or in grassland products (De Boever *et al.*, 1999; Bovolenta *et al.*, 2008; Mahipala *et al.*, 2009). In Romania, NIR spectrometry was used first in 2000 (Vidican *et al.*, 2000).

Various researchers in Central and Eastern Europe conducted long-term mineral fertilizer experiments (Hejcman *et al.*, 2007); all these experiments showed that NPK fertilizers changed meadow species richness, reduced soil fertility and increased meadow forage value. Păcurar (2005) pointed out that prior to 2001, only organic fertilizers were applied to Romanian Apuseni Mountains. Mineral fertilizers were unobtainable and forage quality was not analysed (Păcurar, 2005). To address the local fertility problems of the Romanian Apuseni Mountains, different levels of fertilization were applied to semi-natural meadows in the Gârda area (Păcurar *et al.*, 2005). It was shown that fertilization based on varying organic and mineral fertilizer levels contributed to higher production of forages from semi-natural meadows and to a modification of plant species frequency (Păcurar *et al.*, 2010).

As there is a paucity of information regarding forage quality of Apuseni Mountains grasslands in the published literature, the purpose of this study was to improve the forage quality database and to develop the use of NIR spectroscopy to evaluate forage quality. Our concerns were not related to monitoring forage quantity, but rather to determine the quality of forages from stationary experiments performed on the semi-natural grasslands in the Apuseni Mountains. The main objective of this study was to use Walloon Agricultural Research Centre (CRA-W) Gembloux (Belgium) 'local' NIR models for analysing the semi-natural meadow forages from the Apuseni Mountains. The CRA-W Gembloux (Belgium) 'local' NIR models were established using different mountain grassland samples from throughout Europe. Due to the presence of *Arnica montana* L. in the Apuseni Mountains, which apparently have similar characteristics with tropical plants, tropical data were also used in the calibration model. A second objective was to assess the effect of different fertilization regimes on the nutritive quality of these meadows.

Materials and methods

The study was conducted in the Romanian Apuseni Mountains, at an altitude of 1130 m in the Glacier Plateau-Poiana Călineasa area near Glacier village (GPS coordinates of Ghețari Research Centre: Latitude: 46.500 N-Longitude: 22.816 E). This area is in the Bihor Mountains, in the central and highest part of the Apuseni Mountains.

Three different fertilization trials were carried out simultaneously. All the experiments were conducted on a Terra Rossa soil and were established in spring 2001 using either manure (i.e., organic), mineral fertilizer, or a combination of the two. The experimental design for the first experiment used five combination treatments with four replicate plots per treatment: T1-semi-natural meadow (control); T2-20 t/ha cow and horse manure; T3-10 t/ha cow and horse manure + 50N $25P_2O_5$ $25K_2O$/ha; T4-100N $50P_2O_5$ $50K_2O$/ha; and T5-10 t/ha cow and horse manure + 100N $50P_2O_5$ $50K_2O$/ha. The experimental design for the second experiment was four organic treatments with four replicate plots per treatment: O1-control; O2-10 t/ha cow and horse manure; O3-20 t/ha cow and horse manure; O4-30 t/ha cow and horse manure. The experimental design for the third experiment was four mineral treatments with four replications plots/treatment: M1-control; M2-50N $25P_2O_5$ $25K_2O$/ha; M3-100N $50P_2O_5$ $50K_2O$/ha; and M4-150N $75P_2O_5$ $75K_2O$/ha.

Each plot measured 10 m^2 and the annual fertilization input was applied each spring from 2001. The organic fertilizer came from a local farm in the Gârda area. Cattle and horse manures were mixed (50%/50%), producing a fertilizer containing 0.40% N, 0.39% P and 0.45% K (on a fresh matter basis) (Păcurar *et al.*, 2012). The mineral fertilizer used was a NPK complex applied at 20:10:10 kg/ha, respectively.

The results presented in this paper refer to samples collected in 2010. The floristic composition studies were carried out following the Braun-Blanquét method (Braun-Blanquét, 1964) and the results were published by Păcurar *et al.* (2012).

The grass was cut in the field in July 2010. Using a drill, 500 g samples were collected on each replicate plot (5 x 100g samples collected randomly from the total grass collected) in duplicate. The 104 samples (52 plots x 2 samples) were air-dried for 1 week and then in a drying stove at 60ºC for 2 days. They were then milled first using a 5 mm sieve (Grindomix GM 200, Retsch, Haan, Germany) and then a 1 mm sieve (Cyclotec™ 1093, Tecator, Sweden) as required for the NIR technique.

The spectra of samples were acquired in duplicate on a NIR system 6500 (Foss NIRSystems, Silver Spring, MD, USA). The NIR spectra were collected at 2 nm intervals between 400 and 2500 nm, with two repetitions, using the WinISI® 1.50 software (Infrasoft International, Port Matilda (now State College), PA, USA). Each spectrum was a mean of 32 scans/sample.

Based on the CP and OMD predicted values by the CRA-W Gembloux (Belgium) 'local' NIR calibration model, 20 contrasted samples were selected to carry out the reference 'wet chemistry' analysis: for CP, the Kjeldhal method-AOAC (1990); for ash, method 942.05-AOAC (1990); for EE, method 920.39-AOAC (1990); for CF, method 73/46/CEE-Fibre Cap (FOSS, DK); for aNDFom, ADF and Lignin (sa), the Van Soest-Fibre Cap method (FOSS, DK); and for OMD, the De Boever method (De Boever *et al.*, 1986).

The 20 samples were used to develop an external validation of CRA-W Gembloux NIR 'local' calibration model. The WinISI® 1.50 software was used in the treatment of the spectral data obtained and for establishing the mathematical models. The spectra (trimmed to 1300-2400 nm) were treated as following: the standard normal variate and detrend scatter correction (SNVD) procedure was applied to the spectral data; the spectra were then transformed through a mathematical first order derivation (1, 4, 4, 1 [1st derivative, 4 nm gap, 4 points smoothing, and 1 point second smoothing]); a cross validation based on 'leave one out' was used; and 12 Partial Least Squares (PLS) factors were chosen. The algorithm used for the calibration model was the modified PLS algorithm. The validation statistics were performed according to ISO/FDIS 12099:2010(E).

The validated model was then used to predict the chemical composition of the 84 remaining samples. The predicted nutritional quality parameters were analyzed by the GLM procedure (version 9.2; SAS Institute Inc., Cary, NC), using fertilization treatment in a randomized block design with blocks containing all treatment combinations with 4 replicates. Factors in the model were block and treatments. Chemical analyses were determined from each replicate plot, with two sub-samples for each plot, and statistical analyses were performed on mean values for each plot. When GLM indicated a significant effect of treatment ($p<0.05$), the differences were compared by the Duncan multiple range test.

Results and discussions

The CRA-W Gembloux NIR 'local' calibration model (Sinnaeve *et al.*, 1994) characteristics are presented in Tab. 1. 'Local' means that each sample is predicted with its own model calculated on a subset of samples selected on the basis of the closest calibration samples (highest correlations between the spectrum to be predicted and the spectra of the library).

For the validation of the CRA-W Gembloux NIR 'local' calibration model, the analysed values for CP, ash, EE, CF, aNDFom, ADF, Lignin (sa) and OMD from 20 samples were used. The calibration models (Tab. 1) gave the best results for CP (RCV^2=0.98), followed by aNDFom, CF, OMD, ADF, ash, Lignin (sa) and finally EE. The best calibration models were obtained for CP, with high R^2 and r^2 coefficients and low SECV. Good results were also obtained for OMD, CF and aNDFom, with R^2 and $r^2 > 0.94$. The results obtained for the calibration models were similar to those published by other authors for the same type of biological material. The R^2 obtained in our experiment was similar to De Boever *et al.*, (1999) and Andueza *et al.*, (2011) for CP, which were R^2 of 0.92 and 0.98, respectively; for CF and NDF the R^2 was similar to De Boever *et*

Tab. 1. NIR calibration performances and statistical results of the external validation

Content	Mean	N	SD	SEC	R^2	RCV^2	SECV	Bias	SEP	SEP(C)	SEP(C)/ SECV ratio	TUE
CP	10.44	1036	5.65	0.84	0.98	0.98	0.87	-0.608	0.561	0.396	0.455	1.139
Ash	8.87	1096	3.98	1.39	0.88	0.87	1.45	0.196	0.843	0.594	0.410	1.885
EE	3.84	65	1.24	0.60	0.76	0.60	0.78	-1.003	0.552	0.389	0.499	0.852
CF	29.86	849	8.68	1.87	0.95	0.95	1.97	-0.411	1.176	0.829	0.421	2.538
ADF	60.32	746	15.21	3.16	0.96	0.95	3.36	0.008	1.949	1.374	0.409	4.291
Lignin (sa)	35.82	708	8.30	2.48	0.91	0.90	2.60	0.419	2.341	1.651	0.635	3.368
aNDFom	6.74	513	4.37	1.42	0.89	0.86	1.58	-0.038	2.328	1.642	1.039	1.931
OMD	47.81	139	14.21	3.01	0.96	0.94	3.48	1.659	4.168	2.939	0.845	4.156

Note: CP,-crude protein; EE-ether extract; CF-crude fiber; aNDFom-neutral detergent fiber; ADF-acid detergent fiber; Lignin (sa)-acid detergent lignin; OMD-in vitro organic matter digestibility; SD-standard deviation; SEC-standard error of calibration; R^2-coefficient of determinations; RCV^2-cross-validation coefficient of determination; SECV-standard error of cross validation; Bias-errors derived from predicted-actual component values; SEP-standard error of prediction; SEP(C)-standard error of prediction corrected for bias; SECV-standard error of cross validation *al*., (1999) and Koukolova *et al*., (2010), which obtained R^2 values of 0.98, 0.74, 0.84, and 0.87 respectively. Similarly for aNDFom and for OMD value the R^2 values we obtained were similar to that reported by De Boever *et al*., (1999) and Andueza *et al*., (2011) which were 0.84 and 0.88, respectively.

The poorest results were observed for the EE calibration model due to the low fat content in forages, the high variability in chemical analysis (Amari and Abe, 1997; Berardo *et al*., 1997; Park *et al*., 1998), and low the low sample size. Also another reason can be the method used for classical analysis, because EE determines not only the fat content (i.e., triglycerides), but also soluble plant pigments. For example, another method that could be used for plant tissue is the chloroform-methanol extraction method based upon the principle of Bligh and Dyer (Fishwick and Wright, 1977). Moreover, the low number of samples used in the calibration model (i.e., 65 vs. at least 1000) and also the narrow range in EE variability could be the reason for the poor results. In most cases, EE is not determined on forage samples. In conclusion, it can be said that more classical analyses for better correlations needed to be conducted, particularly for EE, ash, Lignin (sa) and the ADF calibration models.

Bias values are also important in the evaluation of equation performance (Stuth *et al*., 2003). A high bias indicates systematic errors between calibration and prediction datasets (Roggo *et al*., 2003). Concerning the validation test, based on 20 samples (Tab. 1), for CP, ash, CF, aNDFom, ADF, Lignin (sa) and OMD, the calibration models gave good results, with a bias less than 1.7.

The standard error of prediction (SEP) is used for evaluating calibration model accuracy by indicating the variability in deviation of the reference data from NIR spectral data (Manley *et al*., 2008). The standard error of prediction corrected for the bias (SEP[C]) is an average difference between predicted and reference values. In this study, SEP[C] values were lower than 1.651 for CP, ash, EE, CF, aNDFom, ADF and Lignin (sa) and 2.939 for OMD, indicating that the prediction potential was rather low in the case of OMD. The SEP(C)/SECV ratios were lower than one for all parameters measured (Tab. 1) indicating that the prediction models developed were robust. The unexplained error confidence limit (TUE) is a limit that a validation SEP must exceed in order to be significantly different from the SEC at the confidence limit specified ($p<0.05$). In our case the TUE was higher than SEP for all the parameters except for OMD and aNDFom.

The predicted NIR results for the chemical analyses (CP, ash, EE, CF, aNDFom, ADF, Lignin (sa)) and OMD values were improved when using the external validation set shown in Tab. 2.

In the first combination (T) experiment, CP content was relatively low content in both measured samples and in the predicted values (Tab. 2). The ash, EE and Lignin (sa) content of forages from the first fertilization trial were similar ($P>0.05$) for all the treatments, with mean values of 9.72%, 3.76% and 8.68%, respectively. The second experiment trial, fertilized by organic (cow and horse) manure, showed significant differences in all the treatments compared with the control without fertilization for CP ($p<0.001$), ash ($p<0.01$), CF ($p<0.01$), ADF ($p<0.01$),

Tab. 2. Effects of fertilization on chemical composition and *in vitro* organic matter digestibility of meadow predicted by NIR spectrometry

Treatment	CP	Ash	EE	CF	aNDFom	ADF	Lignin (sa)	OMD
T1	13.39b	10.55a	4.00a	22.18c	40.44c	29.59a	8.41a	65.68b
T2	14.88a	10.76a	3.75a	22.39bc	41.00c	31.65a	9.11a	66.79a
T3	15.23a	9.91ab	3.61a	23.71abc	54.70a	29.83a	8.30a	56.67d
T4	12.33c	8.79b	3.79a	26.21a	44.23b	32.44a	8.40a	64.61b
T5	13.01bc	8.62b	3.66a	25.51ab	55.17a	32.48a	9.18a	59.28c
MSE_T	0.72	0.94	0.49	1.92	1.35	1.92	1.21	1.31
O1	13.37c	9.06b	3.58a	24.89a	40.40a	30.67ab	7.85b	65.51c
O2	15.66b	9.80ab	3.56a	23.79b	38.68b	29.44b	7.32c	68.96b
O3	16.71a	10.06a	3.35a	24.00ab	36.33c	31.00a	8.62a	71.50ab
O4	15.42b	9.88a	3.29a	24.21ab	35.54c	31.68a	7.84b	73.22a
MSE_O	0.58	0.44	0.33	0.88	1.02	0.83	0.30	1.99
M1	14.29a	10.68a	3.65a	21.36b	35.66d	29.28b	8.19b	69.44a
M2	13.84ab	9.73ab	3.65a	23.18b	41.20c	30.29b	8.26b	67.17b
M3	12.39bc	8.82ab	3.22a	26.55a	50.68b	34.31a	9.41a	57.04c
M4	11.56c	8.17b	3.32a	27.71a	55.39a	34.23a	8.64b	56.27c
MSE_M	1.02	1.27	0.38	1.53	0.42	1.54	0.44	1.16

Note: Different letters between treatments indicate significant differences (Duncan test-$p<0.05$). CP-crude protein; EE-ether extract; CF-crude fiber; aNDFom-neutral detergent fiber; ADF-acid detergent fiber; Lignin (sa)-acid detergent lignin; OMD-in vitro organic matter digestibility; T1-semi-natural meadow (control); T2-20 t/ha cow and horse manure; T3-10 t/ha cow and horse manure + 50N $25P_2O_5$ $25K_2O$; T4-100N $50P_2O_5$ $50K_2O$; T5-10 t/ha cow and horse manure + 100N $50P_2O_5$ $50K_2O$; O1-control; O2-10 t/ha cow and horse manure; O3-20 t/ha cow and horse manure; O4-30 t/ha cow and horse manure; M1-control; M2-50N $25P_2O_5$ $25K_2O$; M3-100N $50P_2O_5$ $50K_2O$; M4-150N $75P_2O_5$ $75K_2O$; MSE-standard error of the mean

Lignin (sa) ($p<0.05$) and OMD ($p<0.01$), whereas no differences were recorded for EE. By contrast, the aNDFom content declined (35.54%) with organic fertilization treatment ($p<0.05$). In the third experiment, the main effect of mineral fertilization decreased CP ($p<0.05$), ash ($p<0.01$), Lignin (sa) ($p<0.01$) and OMD ($p<0.001$), whereas CF($p<0.01$), aNDFom ($p<0.001$) and ADF ($p<0.01$) all increased.

There were treatment effects on the botanical composition of the plots subjected to the T5 treatment (10 t/ha cow and horse manure + 100:50:50), as the *Fabaceae* species covered 9.25% of the plots, whereas in the T4 treatment (100:50:50) plants from this family covered only 4.75% (Păcurar *et al*., 2011). As species in the legume family are noted for their high nitrogen content, this reduction helps to explain the relatively low CP content (12.33%) that was measured and predicted in our samples. The results obtained by Păcurar *et al*. (2012) on samples collected in the same trials indicated that organic fertilization induced an increase in the presence of Fabaceae species, whereas mineral fertilization resulted in a decrease in these species. Similar studies of meadow fertilization approaches carried out by Lee and Lee (2000) showed that mineral (nitrogen) fertilization simulated grass growth and reduced legume growth. Using mineral (nitrogen) application, Aydin and Uzun (2005) noted that botanical composition was negatively affected, resulting in forage with lower CP content, but a higher dry matter yield.

Organic fertilization apparently regenerated the vegetation, providing an inexpensive and non-destructive approach to accelerating succession in biodiversity compared with mineral fertilization (Rowe *et al*., 2006). In addition, cow and horse manure fertilization leads, in the short term, to an increase in the cover of competitive dominant plant species favored for growth (Rowe *et al*., 2006). After the organic fertilization application, major changes were observed in botanical composition; with applications of the O3 and O4 treatments, the *Trisetum flavescens* L.-*Agrostis capillaris* L. meadow type changed to the *Festuca rubra* L.-*Agrostis capillaris* L. meadow type (Păcurar *et al*., 2011). After mineral fertilizer application on *Festuca rubra* L.-*Agrostis capillaris* L. there was a change to the *Agrostis capillaris* L.-*Trisetum flavescens* L. meadow subtype in the M3 and M4 treatments (100:50:50 and 150:75:75 respectively) (Păcurar *et al*., 2012).

Like in the other experiments, the EE was not affected by the treatments. Mineral fertilization is known to increase the nutrient content in leaves and improves fodder quality and productivity in the short term (Hudewenz *et al*., 2012) which was confirmed by our results. Hejcman *et al*., 2007 reported that typical meadow species disappear from plots receiving high NPK fertilizer applications. In this study, the species number was reduced from 36 species in the O1 treatment (semi-natural meadow-control) to 24.25 species in the O4 treatment (150:75:75) (Păcurar *et al*., 2012).

The chemical composition of meadow forages predicted by NIR for other regions of the world were similar to those reported in our experiments: De Boever *et al*., (1999; western Europe) obtained similar CP (5.60%-14.10%), ash (7.60%-22.80%), EE (1.40%-3.90%), CF (21.50%-34.90%), aNDFom (51.80%-73.60%), ADF (24.50%-42.10%), and Lignin(sa) (1.30%-5.60%) values. In the Italian mountain region, Bovolenta *et al*., (2008) obtained similar CP (6.70%-13.20%), ash (9.70%-23.20%), ADF (19.80%-34.70%), Lignin(sa) (2.60%-13.10%) and OMD (30.50%-77.80%) values. Finally, in Australia, Mahipala *et al*., (2009) obtained similar aNDFom (55.50%-63.50%), ADF (34.10%-48.20%), Lignin(sa) (3.90%-19.10%) and OMD (30.10%-62.80%) values.

Păcurar (2005) established and conducted three experimental studies in 2001-2003 in the same meadow as ours, with the same treatments. His study investigated the ecology and sustainable management of these meadows. CP, CF and ash were analyzed over 3 years (2001-2003) and the sward was studied extensively. The CP, ash and CF content were between 7.80% and 9.95%, 9.11% and 15.03% and 26.68% and 30.17%, respectively.

Higher CP and ash content and lower CF content were noted in the T5 treatment in the first experiment during the first year of the trial, as well as in the T2 treatment in the following 2 years. In our study, after 9 years of treatments, no differences in ash content across all treatments were recorded. Regarding CP content, positive differences were recorded in the T3 treatment ($p<0.01$); for CF content, positive differences were recorded in the T4 treatment ($p<0.01$). In conclusion, the differences obtained in 2010 were due to changes in the herbaceous layer. The proportions of *Poaceae* and *Fabaceae* species changed (Păcurar *et al*., 2012) substantially in response to the fertilization regimes. Organic fertilization induced an increase in *Fabaceae*, whereas mineral fertilization led to an increase in *Poaceae* (Aydin and Uzun, 2005).

In the second experiment, however, the results obtained in 2001 by Păcurar (2005) showed that in the O4 treatment, CP and ash content were highest and CF content was lowest compared to the other treatments. Results obtained in 2002 and 2003 were different from 2001. In 2002, CP content decreased in all treatments compared with the O1 treatment (semi-natural meadow-control) ($p<0.05$), but in 2003 it increased in the O2 treatment (10 t/ha cow and horse manure) ($p<0.01$). Compared with our results for CP content, significant differences were found in the O3 and O4 treatments ($p<0.05$, $p<0.001$ respectively) compared to the control.

After short-term mineral fertilization, the results obtained by Păcurar (2005) for the third experiment in 2001-2003 showed a slight increase in CP content in the M3 treatment ($p<0.05$) in 2001 and in the M4 treatment in 2002-2003 ($p>0.05$). In 2010, after 9 years of mineral fertilization, CP content was decreased ($p<0.01$) and the botanical composition also changed compared to the control. CF content decreased in 2001 ($p<0.05$), but in 2002-2003, it increased in the M4 treatment and reached its highest value in the M3 treatment ($p<0.01$); in 2010, the M4 treatment (150:75:75) showed the highest CF content ($p<0.001$).

Concerning fiber determination, the original CF analysis has been largely replaced with the aNDFom analysis. As in our trial, DeBoever *et al.* (1999) observed that aNDFom content was almost double of the CF content with mineral treatments. This is explained by lower aNDFom and lignin levels in *Poaceae* species compared to *Fabaceae* species (Merchen and Bourquin (1994), Collins and Fritz (2003) and Gosselink *et al.* (2004)). Bovolenta *et al.* (2008) pointed out that CF and aNDFom contents are indirectly proportional to CP content and OMD value. It should also be noted that the mineral treatments induced large differences in aNDFom content, with much higher increases than for ADF content. This suggests that, with mineral treatments, aNDFom, ADF and CF content increased rather than CP content and the OMD values decreased. For organic treatments, however, lower aNDFom content was recorded in all fertilization treatments compared to the control plots, whereas CP content and OMD were higher.

Conclusions

The originality of the present paper is due to the use and successful validation of the CRA-W Gembloux NIR 'local' calibration model for a specific area from Eastern Europe that was not involved in the development of the model. The 'local' calibration model successfully confirms and predicts results found through traditional wet chemistry (i.e, proximate analysis), and additionally for organic matter digestibility of Apuseni semi-natural meadow forages. Regarding the calibration statistics it is recommendable to perform more chemical analyses for OMD and aNDFom to improve the accuracy. For ash, EE, aNDFom, Lignin (sa) and ADF, the calibration model should be improved by increasing the number of samples for reduced variability and heightened sensitivity. In summary, mineral fertilization decreased species richness but increased the meadow productivity. Mineral fertilization reduced the CP content of the forages as a result of a decrease in the number of *Fabaceae* species in the herbaceous component. CF, aNDFom and ADF content showed a higher variability with mineral or organic-mineral fertilizers than with organic fertilizers only. The organic fertilization used alone induced an increase in OMD. Our recommendation is to use mineral fertilization with NPK doses less than 100:50:50 to improve meadow productivity; meanwhile organic fertilization can also be used as a way to complement and maintain biodiversity and forage quality.

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