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Effect of gypsum and potassium fertilisation on the nutritive value of legume-grass mixture

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Abstract: The four-year field trial was conducted at the Rolnicze Gospodarstwo Doświadczalne Brody (Brody Experimental Farm), Poznań University of Life Sciences, Poland. This study aimed to assess how different doses of gypsum and potassium (K) fertilisers influenced the nutritive value of the alfalfa-grass mixture. The following two experimental factors were duplicated: gypsum fertilisation – two levels (0 and 500 kg/ha) and K fertilisation – four levels (0, 30, 60, and 120 kg/ha). The sward was harvested three times at the full budding phase of alfalfa. The content of nutritive components: crude protein (CP), crude fibre (CF), crude ash (CA) and water-soluble sugars (WSC) by NIRS technique was assessed. The combined application of gypsum and K significantly increased the yields obtained only in the 1st and 3rd harvests of the sward. In the case of CP and WSC, the application of gypsum and K showed no significant effect on the content of these components in the sward. At the same time, it significantly influenced the higher content of CF and CA only in the case of the 2nd harvest. Analysing the influence of only the effect of K on the results obtained, a response of increasing CF content in the sward under the influence of increasing doses of this nutrient was noted. The average potassium content of the sward increased from a K0 fertilisation level to an application rate of K60. In the case of CA content, there was a successive increase with the application of successive fertilisation rates from K0 to a rate of K120. Based on the average yield results, a similar response was observed for the increase in yields obtained with increasing potassium fertilisation rates from K0 to K120. CP content increased due to gypsum fertilisation, as did the achieved sward yields of the alfalfa-grass mixture. The biomass of the alfalfa-grass mixture without gypsum fertilisation contained more WSC than the fertilised one.

Keywords: plant nutrition; multi-year application; alfalfa-grass sward; forage; water-soluble carbohydrate

In field crops, the nutritional value of legume-grass mixtures depends on the choice of plant species, fertilisation with NPK and other micro- and macroelements, as well as the method and intensity of use of the mixture (Bi et al. 2019). Cultivating alfalfa in mixtures with grasses requires proper soil preparation and a pH of 7–7.5. Adequate soil content of Ca and S is crucial for leguminous plant symbiosis with *Rhizobium* bacteria, atmospheric nitrogen fixation, and nitrogen-sulfur synergy in protein production by plants. Gypsum, containing essential macroelements for plant nutrition, is an excellent source of Ca and S (Chen et al. 2005, Dontsova et al. 2005). Many fertilisers containing gypsum, including FGDG (synthetic gypsum or flue gas desulfurisation gypsum), also provide plants with various necessary microelements such as B, Fe, Mo, and Zn. As a byproduct, FGDG may contain heavy metals, such as Pb and Cr, depending on the type of coal burned (Sloan et al. 1999). The levels of these metals in gypsum are

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generally much lower than the permissible limits set for fertilisers intended for soil and agricultural use (EPRI 2011). FGDG is produced by coal-fired power plants as a byproduct during the purification of SO_2 gases generated during coal combustion (ACAA 2016). The FGDG produced by power plants is used for road and highway repairs (Payette et al. 1997). In the construction of houses, FGDG is utilised to produce gypsum wallboards (Kairies et al. 2006). A small portion is employed in agricultural applications for soil fertilisation, while the remaining unused FGDG is treated as waste and stored in gypsum stacks. In agricultural applications, gypsum has been recognised for its potential to improve soil quality and enhance the productivity of crops (Dick et al. 2006, Favaretto et al. 2006). The application of gypsum in arable soils is particularly beneficial, especially in the case of heavy clay soils with poor structure. Gypsum on such soils improves water infiltration rates and reduces surface crusting (Dontsova and Norton 2002). Also, gypsum may assist in alleviating issues related to low subsoil pH due to its moderate solubility and deactivation of toxic Al^{3+} ions through complexation (Toma et al. 1999). Consequently, gypsum fertilisation strengthens deeper rooting and enhances plant access to deeper water and mineral resources during drought conditions (Chen and Dick 2011, Casby-Horton et al. 2015). In the cultivation of alfalfa and alfalfa-grass mixtures, the impact of fertilisation primarily focuses on the application of phosphorus (P) and/or potassium in various combinations and proportions of these elements in the fertilisation dose (Berg et al. 2007, Macolino et al. 2013). Potassium is a crucial macronutrient for alfalfa's metabolism and physiological processes (*Medicago sativa* L.) (Hawkesford et al. 2012). In plants, the role of K is diverse and includes the transport of sugars and the enhancement of plant tolerance to abiotic stresses (Johnson et al. 2022). Potassium deficiency in soil can significantly inhibit photosynthetic carbon assimilation and result in leaf discoloration (Tränkner et al. 2018). The application of higher doses of P fertiliser in soils with K deficiency can intensify losses, plant dieback, and lead to lower yields (Berg et al. 2018, Volenec et al. 2021). In soils with low K content, fertilisation with this element can enhance alfalfa yield and plant durability, especially in the case of older alfalfa crops (Berg et al. 2007). On the other hand, in soils with high K content, additional fertilisation with this element does not significantly increase plant yields (Jungers et al. 2019, Thinguldstad et al. 2020). Nitrogen (N) fertilisation is essential for alfalfa only immediately after seeding due to its substantial potential for atmospheric nitrogen fixation (Rasmussen et al. 2008). It has also been demonstrated that several other mineral elements, such as molybdenum or sulfur, influence alfalfa performance (Wang et al. 2003, Mao et al. 2018). The effects of N, P, and K fertilisation on alfalfa's yield and nutrient content were presented in the meta-analysis of Li and Liu (2024).

To date, studies on the use of gypsum in fertilising alfalfa-grass sward in combination with K fertilisation have focused on yield assessment (Zielewicz et al. 2022), nutrient productivity (Zielewicz et al. 2023a), and soil enzyme activity (Zielewicz et al. 2022). However, there is a lack of research regarding the impact of gypsum fertilisation in conjunction with K fertilisation on the nutritional value of cultivated plants.

The novelty of our study lies in investigating the influence of multi-year, annual gypsum application in combination with various K fertilisation doses on the nutritional value of alfalfa-grass sward in terms of its suitability for ruminant nutrition. The first research hypothesis posits that the application of FGDG gypsum containing Ca and S would increase the content of protein (CP) and crude ash (CA) while decreasing the levels of structural sugars in the alfalfa-grass mixture, thereby positively influencing the forage nutritional value. The second research hypothesis suggests that reducing the potassium fertilisation dose to 60 kg K/ha (referred to as the farmer's dose) would have a similar impact on the nutritional value of the alfalfa-grass mixture as using a higher fertilisation dose of 120 kg K/ha. The main objective of the research is to determine the impact of FGDG gypsum fertilisation and varied potassium doses on changes in the content of crude protein, water-soluble carbohydrate (WSC), crude fibre (CF), crude ash in the sward of the alfalfa-grass mixture over consecutive years of its use.

MATERIAL AND METHODS

Experimental conditions. The field experiment with the sowing of a mixture of alfalfa and grasses was conducted from 2012 to 2015 at the Rolnicze Gospodarstwo Doświadczalne Brody (Brody Experimental Farm) – Experimental Station of the Department of Grassland and Natural Landscape, University of Life Sciences in Poznań (52°44'N, 16°28'E) on soil derived from

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loamy sand, classified as Albic Luvisols (Neocambic) (Kabała et al. 2019). Soil pH and the content of primary nutrients were determined in 2011, shortly after the harvest of winter rapeseed, which served as a predecessor crop for the alfalfa-grass mixture cultivation. The soil was characterised by the following physicochemical parameters: organic matter content was 1.28%, and the soil had a slightly acidic pH (pH_{KCl} = 6.5). The content of the available macronutrients, measured before the experiment started in 2011, was very high (164.0 mg/kg soil) for magnesium (Mg) and high (158.1 mg/kg soil) for phosphorus (P) but low (88.2 mg/kg soil) for K and very low (690.4 mg/kg soil) for Ca. Analysis of macronutrient content was carried out using the Mehlich 3 method (Mehlich 1984).

Experiment design. The experiment was established in 2011 using a split-split-plot design with two experimental factors, replicated four times in the field on plots of 27.0 m^2 (5.4 × 5.0 m). In this way, 32 experimental plots (16 × 27 m^2 with and 16 × 27 m^2 without gypsum fertilisation) were established. The experiment was 864 m^2 in size. The harvested area was 12.5 m^2 (5 × 2.5 m). An area of 5.4 m × 5 = 27 m^2 was divided into 2 parts 5.4 × 2.5 m (gypsum fertilised 5.4 × 2.5 m (13.5 m^2 and non-fertilised 5.4 × 2.5 m 13.5 m^2 – total 27 m^2). From each of these plots, 0.2 m × 2.5 m (0.5 m^2) was excluded at the edges of the plots. From the area of the gypsum fertilised and non-fertilised plots together, 1 m^2 was excluded (as an edge effect) (13.5–1.0 m = 12.5 m^2).

The presented research covers four years of utilising the alfalfa-grass mixture: the first in 2012, the second in 2013, the third in 2014, and the fourth (final) in 2015.

The experiment was composed of two factors:

1. gypsum FGDG (flue gas desulfurisation) – two levels of fertilisation: 0 gypsum (0 kg gypsum/ha) and 500 gypsum (500 kg gypsum/ha);
2. potassium treatments – four levels of fertilisation: K0 (0 kg K/ha), K30 (30 kg K/ha), K60 (60 kg K/ha), and K120 (120 kg K/ha).

In the autumn of 2011, a legume-grass mixture was sown after harvesting rapeseed and conducting pre-sowing tillage. The seed mixture consisted of alfalfa (*Medicago sativa* L.) – 80%, meadow fescue (*Festuca pratensis* Huds.) – 15%, and timothy (*Phleum pratense* L.) – 5%, sown at a rate of 25 kg of seeds per hectare.

FGDG gypsum or synthetic gypsum contains calcium sulfate ($CaSO_4 \times 2\ H_2O$) with 21.3% calcium (Ca) and 17% sulfur (S) (own data). This fertiliser was approved for preliminary testing and use in agriculture based on heavy metal content tests conducted by an accredited laboratory. Based on the Minister of Agriculture and Rural Development Regulation of 18. 06. 2008, the requirement for the allowed content for heavy metals by this fertiliser was fulfilled. It contained below these values: chromium (Cr) – 100 mg, cadmium (Cd) – 8.0 mg, nickel (Ni) – 60 mg, lead (Pb) – 200 mg, mercury (Hg) – 2.0 mg/1 kg DM of fertiliser.

It was initially applied before the establishment of the experiment, in the summer of 2011, at a dose of 500 kg per hectare, and was repeated each spring before the start of the subsequent growing season. Each year, two weeks after the application of FGDG gypsum, potassium (K) in the form of KCl (60% K_2O) was applied at doses determined by the experimental design. Alongside potassium, uniform phosphorus fertilisation at 60 kg P per hectare (triple superphosphate 46% P_2O_5) was also applied.

Plant sampling and analysis of plant material. The sward of alfalfa-grass was mowed three times during the growing seasons (cuts), at the full budding phase of alfalfa development. Swaths were made between 11.00 in the morning and 13.00 in the afternoon on each occasion. A 500-gram sample of fresh plant material was collected from each plot. The material for analyses of the nutritional quality was dried at 70 °C to constant weight in a drying oven Binder FED 720 and then ground in a laboratory mill. To determine the chemical composition of the plant sample, including crude protein, water-soluble sugars, crude fibre, and crude ash, near-infrared reflectance spectroscopy (NIRS) was employed. The analyses were conducted using the NIRFlex N-500 device, manufactured by Büchi (Flawil, Switzerland), calibrated with pre-existing INGOT® models specifically developed for dried forage (hay). The calibration and prediction parameters for the chemical composition analysis are presented in Table 1.

Table 1. Calibration and prediction parameters for chemical composition analysis

Item	SEC	SEP	Q-value
Protein	0.903	0.99	0.75
Ash	1.000	0.98	0.71
Crude fibre	1.192	1.27	0.72
WSC	1.248	1.21	0.77

SEC – standard error of calibration; SEP – standard error of prediction; Q-value – qualitative calibration; WSC – water-soluble carbohydrate

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Table 2. Weather conditions during the vegetation period in Rolnicze Gospodarstwo Doświadczalne Brody (Brody Experimental Farm) in the years 2012–2015 and the means of 1967–2011

Month/year	Average air temperature (°C)					Total rainfall (mm)				
	2012	2013	2014	2015	mean from years 1961–2011	2012	2013	2014	2015	mean from years 1961–2011
April	8.8	8.0	10.5	10.4	8.0	22.9	15.4	46.3	46.4	37.6
May	14.9	14.4	13.1	13.0	13.2	77.2	69.8	73.5	25.6	56.9
June	16.0	17.3	16.1	15.5	16.6	163.0	125.3	42.0	85.3	61.6
July	19.2	20.1	21.5	19.2	18.2	197.6	67.3	83.1	84.9	79.4
August	18.7	19.1	17.3	22.1	17.5	60.1	51.5	137.2	15.1	66.9
September	14.3	12.9	15.4	14.7	13.3	30.0	33.7	64.8	40.6	49.7
October	8.2	10.3	10.9	7.9	8.8	10.9	10.9	39.8	21.7	40.8
Average temperature (April–October)	14.3	14.6	15.0	14.7	13.6	–	–	–	–	–
Total rainfall (April–October)	–	–	–	–	–	561.7	373.9	486.7	319.6	392.9
Annual average temperature	8.9	8.8	10.1	10.2	–	–	–	–	–	–
Annual rainfall	–	–	–	–	–	811.5	516.5	632.5	471.0	–

Weather conditions. The course of weather conditions during the growing seasons from 2012 to 2015 is presented in Table 2. In 2012, favourable weather conditions prevailed for plant growth and development. The average annual air temperature was 8.9 °C, with precipitation levels reaching 811.5 mm. In the second year of the study, the annual precipitation sum was only 373.9 mm, which was 295 mm lower than the previous year. The average annual temperature was 8.8 °C, similar to the average from the previous year. The summer months, especially July (67.3 mm) and August (51.5 mm) were particularly dry. Better growth conditions were observed in 2014. The precipitation sum was higher by 116.0 mm, and the average temperature increased by 1.3 °C compared to the previous research year. Precipitation in May was at a similar level as in previous years. However, significant water deficits were recorded in June. The limited rainfall during this period (only 42.0 mm) was characterised by an unfavourable distribution, further negatively affecting plant development. Water deficits and rising temperatures were also observed in the final year of the study. In 2015, the precipitation sum was the lowest, reaching only 471.0 mm, and the average temperature was 10.2 °C.

Statistical analysis. The effect of experimental factors (years, gypsum doses, and potassium dose) and their mutual interactions on the content of examining parameters were assessed by a three-way analysis of variance. Means were separated by honest significant difference (*HSD*) using the Tukey method if the *F*-test showed significant factor effects at $P < 0.05$. Statistical processing of the results was performed using Statistica v. 6.0 programs (Statsoft, Kraków, Poland).

RESULTS

Botanical composition. The analysis of the botanical composition of the sward indicates that on the plots without gypsum application, over the four years of study, the average proportion of alfalfa increased from 75.5% in the first year to 80.7% in the final year of the study (Figure 1). A similar increase was observed on the plots with gypsum application. The average proportion of alfalfa in the sward on these plots was 76.0% in the first year of the study and as much as 81.7% in the 4th year. The increase in alfalfa proportion was 5.7%. Interestingly, the difference in the proportion of alfalfa in the sward between plots not fertilised with gypsum and annually fertilised with gypsum at a rate of 500 kg/ha in the 1st year was 1.5%, and in the final year, it was 1%. The mean proportion of grasses decreased by

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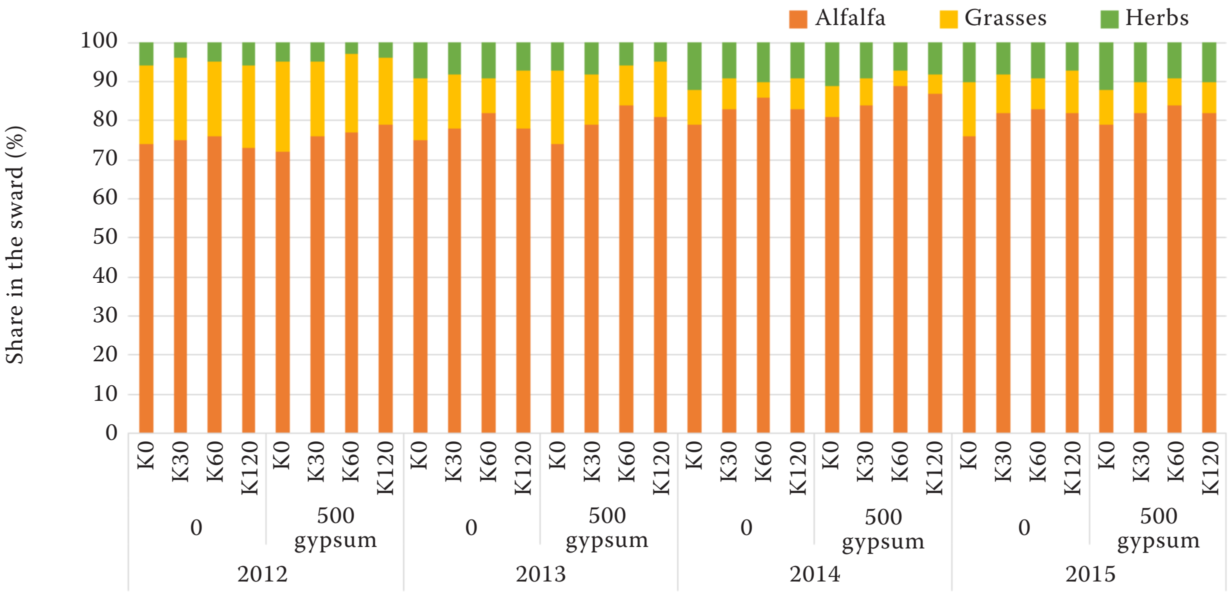

Figure 1. Percentage share in biomass of plant species in the sward. K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha, K60 – 60 kg K/ha; K120 – 120 kg K/ha; gypsum application: 0 kg/ha; 500 kg/ha

9.5% on the plots without gypsum application, from 20.2% in the first year of the study to 10.7% in the 4th year. On the plots with gypsum application, the average proportion of grasses in the 1st year of the study was 19.7%, and in the final year, it was 8%. The difference in the proportion of grasses on these plots between the first and last years was 11.7%. The average difference in the proportion of grasses between plots without gypsum application and with gypsum applied at a dose of 500 kg/ha in the 1st year was 1.5%, and in the final year, it was 2.7%.

Analysing the impact of K fertilisation on the botanical composition of the sward, it can be observed that the average proportion of alfalfa was highest on plots fertilised with K in an annual dose of 60 kg/ha in the variant without gypsum fertilisation (81.75%) as well as with its application (83.5%). The lowest proportions of alfalfa were recorded on plots not fertilised with K (K0). On plots without gypsum application, this proportion was 76%, while in variants with gypsum application, the proportion of alfalfa reached 76.5%. The highest proportion of grasses was observed on plots not fertilised with K (14.75%) in variants without fertilisation and with gypsum. Lack of K fertilisation increased the proportion of weeds in the sward. The average proportion of weeds on K0 plots without gypsum fertilisation was 9.25%; with its application, it was 8.75% (Figure 1). The average proportion of alfalfa, grasses and weeds in the sward varied between the years of the study and was: in year 1 – alfalfa 75.3%, grasses 19.9%, weeds 4.8%; in year 2 – alfalfa 78.8%, grasses 13.8%, weeds 7.4%; in year 3 – alfalfa 83.9%, grasses 6.8%, weeds 9.3%; and in the final year 4 – alfalfa 81.2%, grasses 9.3%, weeds 9.5%.

Yields of alfalfa-grass mixtures sward. The effect of gypsum application on yields was noticeable in all three harvests. The difference between the variant without gypsum and with gypsum application at 500 kg/ha was 0.63 t DM/ha for the 1st harvest, 0.29 t DM/ha for the 2nd harvest and 0.39 t DM/ha for the 3rd harvest, respectively. In total yield, the yield increase after gypsum application was 1.31 t DM/ha (10.9%). Another element of the experiment carried out was the effect of fertilisation with different doses of potassium. An increase in yield in response to applied K fertilisation was observed in all harvests. Yield increased progressively with the K application rates. The effect of the study year and gypsum fertilisation on DM yield was significant for the 1st and 3rd harvests, while the interaction of the study year and K application was significant in all three harvests. Potassium and gypsum application significantly affected yield only in 1st and 3rd harvests, as did test year × gypsum. The interaction effect of Y × gypsum × K was significant for DM yields only in 1st harvest (Table 3).

Crude protein content. The impact of gypsum fertilisation on CP content was significant in all harvests. The average CP content in the sward with-

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Table 3. Yields of alfalfa-grass mixtures sward in successive cuts

Factor	Factor level	Yield (t DM/ha)			Total yield
		1st harvest	2nd harvest	3rd harvest	
Year of study (Y)	2012	3.19^{d}	5.52^{a}	3.74^{b}	12.46^{c}
	2013	3.98^{c}	4.24^{c}	7.90^{a}	16.11^{a}
	2014	4.99^{a}	4.70^{b}	3.35^{c}	13.04^{b}
	2015	4.42^{b}	2.91^{d}	1.55^{d}	8.88^{d}
	P-value	< 0.001	< 0.001	< 0.001	< 0.001
Gypsum (kg/ha)	0	3.83^{b}	4.20^{b}	3.94^{b}	11.97^{b}
	500	4.46^{a}	4.49^{a}	4.33^{a}	13.28^{a}
	P-value	< 0.001	< 0.001	< 0.001	< 0.001
K fertilisation	0	3.65^{c}	4.01^{c}	3.84^{b}	11.50^{d}
	30	4.01^{b}	4.17^{c}	4.00^{b}	12.18^{c}
	60	4.38^{a}	4.43^{b}	4.30^{a}	13.12^{b}
	120	4.54^{a}	4.76^{a}	4.40^{a}	13.69^{a}
	P-value	< 0.001	< 0.001	< 0.001	< 0.001
			P-value		
Interactions	Y × gypsum	< 0.001	0.053	0.013	< 0.001
	Y × K	< 0.001	0.020	0.032	0.031
	gypsum × K	< 0.001	0.931	0.001	0.422
	Y × gypsum × K	0.017	0.569	0.723	0.562
Mean		4.15	4.34	4.13	12.62
SD		0.88	1.04	2.36	2.83
VC (%)		0.78	1.07	5.57	8.03

SD – standard deviation; VC – variation coefficient. Values in columns marked with the same letter do not differ significantly. DM – dry matter

out gypsum application was 169.7 g/kg DM; with its application, it was higher by 2.7%, averaging 174.3 g/kg DM. CP content increased as a result of gypsum fertilisation, as did the achieved sward yields of the alfalfa-grass mixture (Tables 3 and 4). There was no dilution effect of CP content with increasing yields of harvested sward biomass. K fertilisation significantly impacted CP content in the sward in all harvests and varied in each harvest (Table 4). In the first harvest, the highest CP content was recorded in the sward fertilised with K at doses of 60 and 120 kg/ha, while in the 2nd and 3rd harvests, the sward without K fertilisation (K0) had the highest CP content. However, considering the averages from all harvests, it can be concluded that K fertilisation did not significantly impact the CP content in the biomass of the alfalfa-grass mixture. The Y × K interaction was significant in all harvests, while the Y × gypsum × K interaction was significant only in the 2nd and 3rd harvests (Figure 2).

Water-soluble carbohydrate content. The WSC content in the sward mixture varied significantly, ranging from 62.7 g/kg DM (3rd harvest in 2014) to 125.7 g/kg DM (1st harvest in 2015).

On average, the sward without gypsum fertilisation contained 85.47 g of WSC per kg DM, while the fertilised one contained 83.57 g/kg DM. The difference between these gypsum fertilisation variants was statistically insignificant, equalling 1.97 g/kg DM (2.2%). Considering data from individual harvests, the impact of gypsum fertilisation on WSC content was significant only in the 1st and 2nd harvests. In both cases, the sward mixture without gypsum fertilisation contained more WSC than the one fertilised with gypsum. Potassium fertilisation had a significant impact on WSC content, which is evident in both individual harvest results and the average content of this component. The sward exhibited the highest WSC content from the 1st harvest at the K30 fertilisation level (103.1 g/kg DM) and in the 2nd and 3rd harvests without K application (85.1

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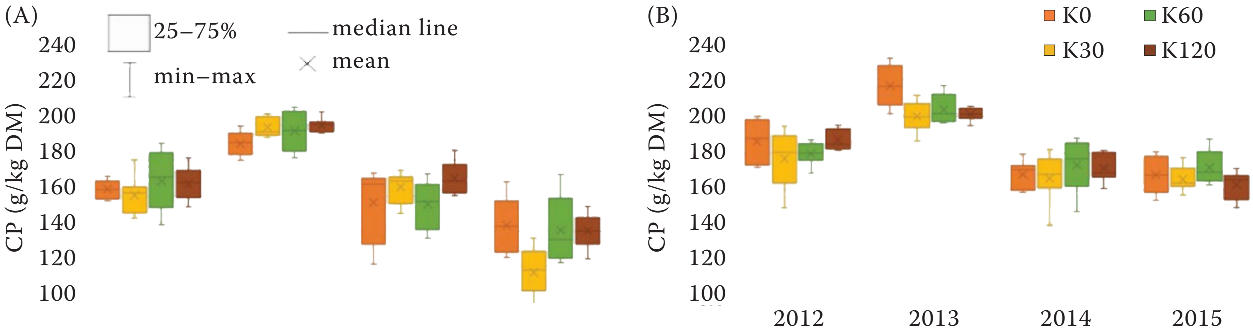
Table 4. The content of crude protein (CP) and water-soluble sugars (WSC) in the sward of the mixture of alfalfa-grass in successive cuts

Factor	Factor level	CP (g/kg DM)			Mean	WSC (g/kg DM)			Mean
		1st harvest	2nd harvest	3rd harvest		1st harvest	2nd harvest	3rd harvest	
Year of study (Y)	2012	164.9^{b}	182.4^{b}	161.5^{bc}	169.6^{b}	88.0^{bc}	95.6^{a}	93.3^{a}	92.3^{a}
	2013	196.2^{a}	205.3^{a}	167.4^{b}	189.6^{a}	88.9^{b}	84.4^{b}	67.7^{c}	80.3^{b}
	2014	161.7^{b}	169.8^{c}	205.5^{a}	182.7^{a}	83.3^{c}	75.1^{c}	62.7^{d}	72.1^{c}
	2015	135.4^{c}	167.0^{c}	158.5^{c}	153.6^{c}	125.7^{a}	74.1^{c}	75.1^{b}	91.6^{a}
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Gypsum (kg/ha)	0	157.9^{b}	177.6^{b}	173.2^{b}	169.7^{b}	101.6^{a}	82.6^{a}	73.5^{a}	85.4^{a}
	500	164.7^{a}	181.4^{a}	176.6^{a}	174.3^{a}	97.9^{b}	80.1^{b}	73.6^{a}	83.5^{a}
	P-value	< 0.001	0.005	0.028	0.011	0.020	0.009	0.646	0.243
K fertilisation	0	161.0^{ab}	182.8^{a}	180.0^{a}	174.8^{a}	100.2^{ab}	85.1^{a}	74.7^{ab}	86.3^{ab}
	30	155.5^{b}	175.8^{b}	176.4^{ab}	169.5^{a}	103.1^{a}	83.3^{a}	77.9^{a}	87.7^{a}
	60	162.6^{a}	181.0^{ab}	171.5^{b}	171.7^{a}	98.2^{ab}	79.4^{b}	70.2^{c}	82.2^{ab}
	120	166.1^{a}	178.6^{ab}	171.9^{b}	172.2^{a}	97.4^{b}	77.7^{b}	71.3^{bc}	81.8^{b}
	P-value	0.011	0.004	0.002	0.356	0.040	< 0.001	< 0.001	0.024
		P-value							
Interactions	Y × gypsum	0.397	0.328	0.286	0.902	0.386	0.013	0.253	0.986
	Y × K	< 0.001	0.008	0.008	0.779	0.003	0.006	< 0.001	0.551
	gypsum × K	0.120	0.550	0.392	0.698	0.022	0.028	0.664	0.793
	Y × gypsum × K	0.055	0.008	< 0.001	0.421	0.639	< 0.001	0.039	0.974
Mean		161.3	179.5	174.9	172.0	99.7	81.4	73.5	84.5
SD		26.3	17.9	23.7	24.2	20.7	11.8	13.6	19.2
VC (%)		691.9	320.7	563.5	585.1	429.5	139.0	183.6	369.2

K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha, K60 – 60 kg K/ha; K120 – 120 kg K/ha; gypsum application: 0 kg/ha; 500 kg/ha. SD – standard deviation; VC – variation coefficient. Values in columns marked with the same letter do not differ significantly. DM – dry matter



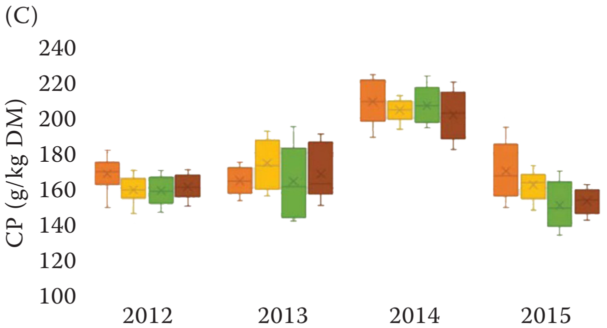


Figure 2. Average crude protein (CP) concentration in alfalfa-grass mixture for the combination of years (Y) and K fertilisation level (K) in (A) the 1st harvest; (B) the 2nd harvest, and (C) the 3rd harvest. K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha, K60 – 60 kg K/ha; K120 – 120 kg K/ha

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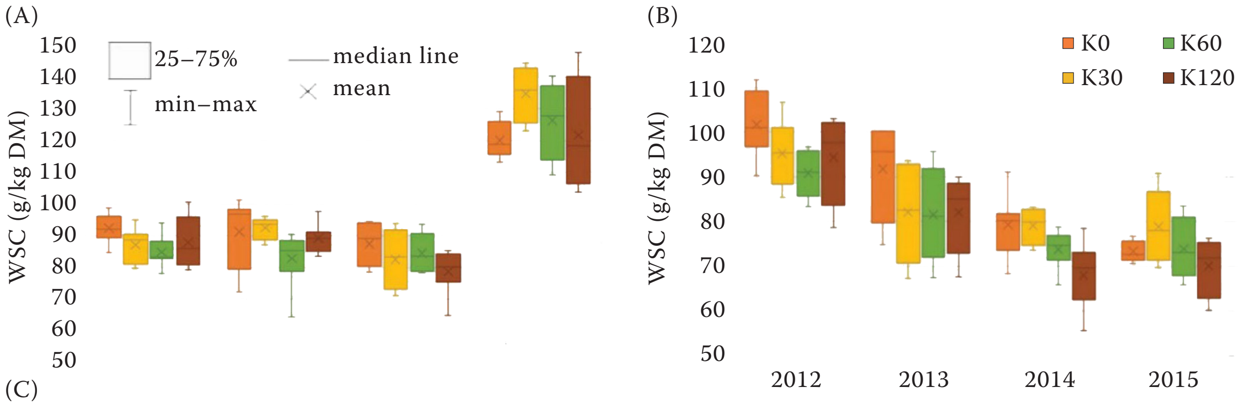


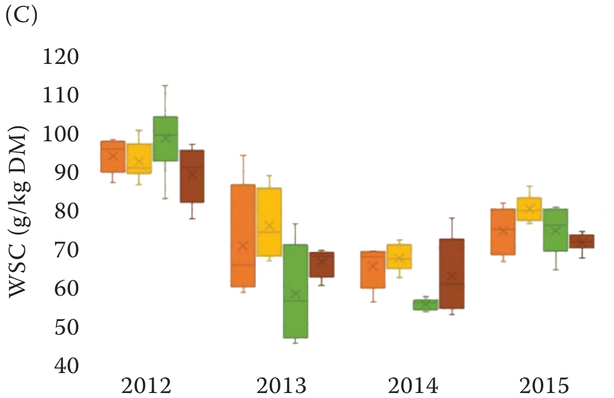
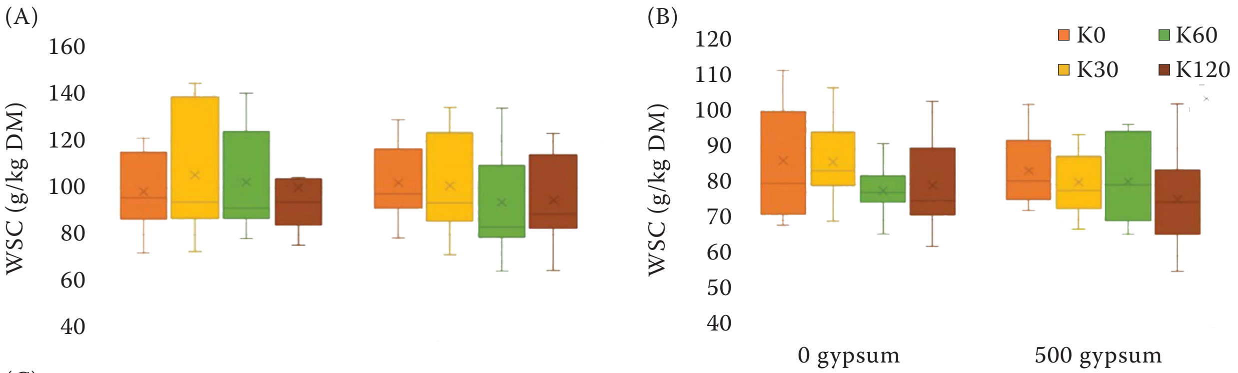


Figure 3. Average water-soluble sugars (WSC) concentration in alfalfa-grass mixture for the combination of years (Y) and K fertilisation level (K) in (A) the 1st harvest; (B) the 2nd harvest, and (C) the 3rd harvest. K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha, K60 – 60 kg K/ha; K120 – 120 kg K/ha

and 74.7 g/kg DM, respectively). The Y × gypsum and Y × gypsum × K interactions were significant only in the 2nd harvest (0.013), while the Y × K interaction was observed in all harvests (Figures 3 and 4). The effect of the combined application of gypsum × K had no significant effect on the WSC content of the sward.

Crude fibre content. Gypsum fertilisation did not have a significant impact on the content of this component in the biomass of the mixture. Sward fertilised with gypsum showed a tendency to have a slightly higher CF content (by 1.0 g/kg DM) than the unfertilised sward. However, K fertilisation had



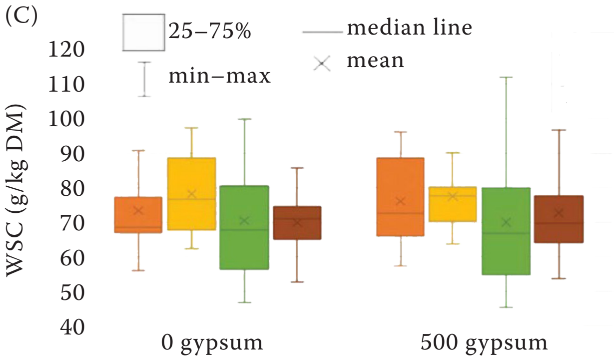


Figure 4. Average water-soluble sugars (WSC) concentration in alfalfa-grass mixture for the combination of gypsum and K fertilisation level (K) in (A) the 1st harvest; (B) the 2nd harvest, and (C) the 3rd harvest. K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha; K60 – 60 kg K/ha; K120 – 120 kg K/ha. 0 gypsum – 0 kg/ha gypsum; 500 gypsum – 500 kg/ha gypsum

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a significant impact on CF content. The lowest CF content (254.3 g/kg DM) was observed in the sward without K application (K0). The highest CF content was noted in the sward where 60 kg/ha (260.9 g/kg DM) of K was applied. The difference in CF content between these K fertilisation levels was 2.6%. The sward from the remaining K-fertilised plots (K30 and K120) also contained more of the component than the unfertilised sward (K0), by 4.0 and 4.3 g/kg DM, respectively (Table 5). The Y × K interaction was significant in all three harvests (Figure 5), while the Y × gypsum and Y × gypsum × K interactions were significant only in the 2nd harvest.

Crude ash content. Similarly, to the previous components, the study year had a significant impact on the level of CA accumulation in plants. On average, the highest CA content was observed in 2014 (103.4 g/kg DM), and the lowest in the following year (92.3 g/kg DM). The difference in CA content between these study years was 11.1 g/kg DM (12%). Gypsum fertilisation did not have a significant impact on CA accumulation in the biomass of the alfalfa-grass mixture (Table 5). Between variants fertilised and unfertilised with gypsum, the average difference in the CA content was only 0.5 g/kg DM (0.5%). Potassium fertilisation had a significant impact on the level of CA accumulation in the sward mixture. The highest CA content (101.1 g/kg DM) was found in the sward fertilised with K at a dose of 120 kg/ha, while the lowest in the sward from plots K0 (95.9 g/kg DM) and K30 (96.0 g/kg DM). The difference in CA content between the K0 and K120 fertilisation levels was 5.2 g/kg DM (5.4%). Interaction was observed in the 2nd harvest for Y × gypsum and gypsum × K, as well as in the 1st and 2nd harvests for Y × K. In the case of Y × gypsum × K, interaction was noticeable in the 2nd and 3rd harvests.

Correlations between crop yield, botanical composition and nutrients content. Significant corre-

Table 5. The content of crude fibre (CF) and crude ash (CA) in the sward of the mixture of alfalfa-grass in successive cuts

Factor	Factor level	CF (g/kg DM)			Mean	CA (g/kg DM)			Mean
		1st harvest	2nd harvest	3rd harvest		1st harvest	2nd harvest	3rd harvest	
Year of study (Y)	2012	274.3^{a}	253.6^{b}	262.4^{b}	263.5^{a}	97.8^{a}	106.8^{a}	94.9^{b}	99.8^{b}
	2013	254.2^{b}	247.9^{c}	286.4^{a}	262.8^{a}	100.4^{a}	103.8^{b}	94.6^{b}	99.6^{b}
	2014	276.1^{a}	274.4^{a}	237.3^{d}	259.0^{a}	97.9^{a}	94.7^{d}	112.8^{a}	103.4^{a}
	2015	250.8^{b}	249.1^{bc}	251.3^{c}	250.4^{b}	86.6^{b}	101.5^{c}	88.8^{c}	92.3^{c}
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Gypsum (kg/ha)	0	263.2^{a}	254.6^{a}	257.8^{a}	257.5^{a}	94.0^{a}	101.5^{a}	98.4^{a}	98.0^{a}
	500	261.7^{a}	256.3^{a}	255.0^{a}	258.5^{a}	95.3^{a}	101.8^{a}	98.3^{a}	98.5^{a}
	P-value	0.352	0.276	0.060	0.494	0.228	0.251	0.780	0.495
K fertilisation	0	261.1^{a}	249.8^{b}	252.2^{b}	254.3^{b}	93.1^{b}	98.3^{a}	96.2^{c}	95.9^{b}
	30	265.3^{a}	258.7^{a}	251.7^{b}	258.3^{ab}	90.1^{b}	100.4^{a}	97.4^{bc}	96.0^{b}
	60	262.4^{a}	255.9^{a}	264.0^{a}	260.9^{a}	97.6^{a}	102.9^{b}	99.4^{ab}	99.9^{a}
	120	260.8^{a}	257.4^{a}	257.6^{ab}	258.6^{ab}	97.9^{a}	105.0^{b}	100.5^{a}	101.1^{a}
	P-value	0.618	< 0.001	< 0.001	0.042	< 0.001	< 0.001	0.001	< 0.001
P-value									
Interactions	Y × gypsum	0.321	0.007	0.176	0.698	0.224	< 0.001	0.719	0.572
	Y × K	0.015	0.001	0.007	0.430	< 0.001	0.004	0.154	0.142
	gypsum × K	0.318	0.257	0.645	0.530	0.392	0.021	0.115	0.470
	Y × gypsum × K	0.482	0.024	< 0.001	0.135	0.319	0.006	0.047	0.598
Mean		262.4	255.5	256.4	258.1	94.7	101.7	98.4	98.3
SD		16.9	14.5	22.3	18.6	8.9	6.6	11.2	9.6
VC (%)		285.0	211.6	497.8	346.2	79.6	43.0	125.1	91.9

K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha; K60 – 60 kg K/ha; K120 – 120 kg K/ha; gypsum application: 0 kg/ha; 500 kg/ha. SD – standard deviation; VC – variation coefficient. Values in columns marked with the same letter do not differ significantly. DM – dry matter

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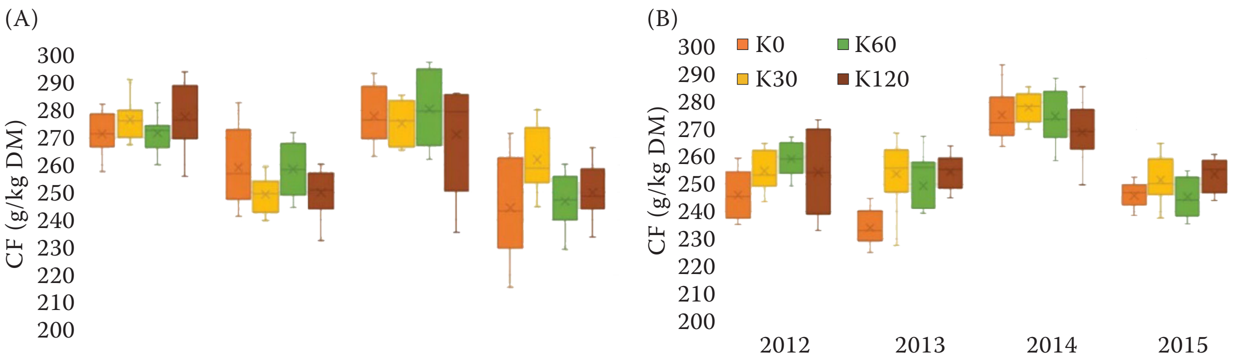

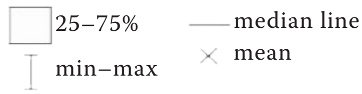

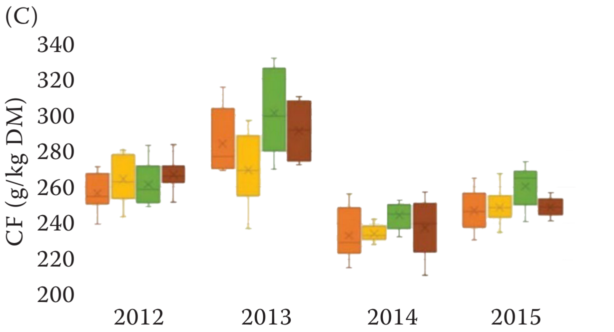

Figure 5. Average crude fibre (CF) concentration in alfalfa-grass mixture for the combination of years (Y) and K fertilisation level (K) in (A) the 1st harvest; (B) the 2nd harvest, and (C) the 3rd harvest. K fertilisation: K0 – 0 kg K/ha; K30 – 30 kg K/ha; K60 – 60 kg K/ha; K120 – 120 kg K/ha

lations were observed between DMY yields and the content of CP (r = 0.86), CF (r = 0.60) and CA (r = 0.64) in the sward. Also, positively correlated with yields was the proportion of weeds. The increasing proportion of weeds in the sward is not a cause for satisfaction due to the deteriorating quality of the forage. However, this group of plants, by covering free spaces in the thinned and ageing sward with each year of the study, also creates biomass and is part of the harvested crop. On the other hand, the proportion of weeds in the sward was negatively correlated with the proportion of grasses. In the nutrient group, the positive correlation of CA content with CP (r = 0.54) and CA with CF content in the sward (r = 0.59) is noteworthy (Table 6). Additionally, a negative correlation was observed between the proportion of alfalfa in the sward and the WSC content (r = –0.55). This situation is due to the fact that alfalfa, compared to grasses, contains only 5% sugars in DM. On the other hand, a positive correlation was noted between the proportion of grasses in the sward and WSC content (r = 0.57). Grasses such as: *Festuca pratensis* and *Phleum pratense*, which were present in the mixture sward contain several percent of sugars in DM improving the ensiling of biomass with a high proportion of alfalfa. Also, a negative WSC correlation was observed for the higher content in the CA sward (r = –0.68).

Table 6. Spearman's rank-order correlations removed pairwise for the examined parameters in 2012–2015

Variable	CP	CF	CA	WSC	A	G	H
CF	0.31						
CA	0.54*	0.59*					
WSC	–0.67*	–0.26	–0.68*				
A	0.05	–0.13	0.30	–0.55*			
G	–0.03	0.30	–0.15	0.57*	–0.92*		
H	–0.10	–0.50*	–0.28	–0.30	0.40*	–0.69*	
DMY	0.86*	0.60*	0.64*	0.04	0.09	–0.37	0.86*

CP – crude protein; CF – crude fat; CA – crude ash; WSC – water soluble carbohydrates; A – alfalfa; G – grasses; H – herbs: DMY – yield; *P < 0.05

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DISCUSSION

The impact of gypsum fertilisation. Gypsum contains both S and Ca as secondary nutrients essential for crops. It improves crop yield and is known as an excellent source of sulfur for plant nutrition (Rashmi et al. 2018). The impact of gypsum fertilisation on CP content was significant. CP content increased as a result of gypsum fertilisation, as did the achieved sward yields of the alfalfa-grass mixture (Tables 3 and 4). Here, there was no dilution effect of CP content with increasing yields of harvested sward biomass. The average CP content in the sward without gypsum application was 169.7 g/kg DM, while in the sward with gypsum application, it was 174.3 g/kg DM. The difference in CP content between the gypsum fertilisation variants was 4.6 g/kg DM (2.7%). In the study by Paulino et al. (2012), no significant effect of liming and gypsum fertilisation on CP content and biomass digestibility of *Brachiaria ruziziensis* was observed. Only a trend of accumulating higher CP contents in this grass species, used as cattle feed, was noted under the influence of higher levels of calcium (liming). In the study Matula and Pechová (2007), plants in all soils responded to the gypsum application to soils by a significantly higher formation of shoots. The yield of shoot biomass in the gypsum treatments was by 15% higher on average. A study by Helmy and Hassanien (2013) found that gypsum application increased significantly hay, pod and biological yields and insignificantly for seed yields. However, no significant differences could be detected between the different gypsum rates of 1.2 and 2.4 t/ha. Gypsum addition, show increased protein yield by gypsum, but no significant differences occurred between 1.2 t/ha and 2.4 t/ha gypsum. Venkatesh et al. (2002) found that protein and protein yield of peanut seeds increased by applying of gypsum.

In a study reported by Adkine et al. (2017), 18% to 20% increase in protein content of mustard seeds was observed with 54 kg/ha gypsum application. Protein content of mustard seeds was significantly influenced by the application of zinc sulfate and gypsum.

The influence of gypsum fertilisation on the WSC content in biomass was significant only for the 1st and 2nd harvests. The biomass of the alfalfa-grass mixture without gypsum fertilisation contained more WSC compared to the fertilised one. Based on the mean WSC contents, it can be observed that the unfertilised sward of the alfalfa-grass mixture contained 85.4 g/kg DM, while the fertilised contained 83.5 g/kg DM. The difference in WSC content between these gypsum fertilisation variants was 1.97 g/kg DM (2.2%).

Based on the obtained values of CF content in the variant fertilised with gypsum and the unfertilised one, it can be concluded that gypsum fertilisation did not have a significant effect on the content of this component in the biomass of the alfalfa-grass mixture. After comparing the mean values, it was observed that the grass fertilised with gypsum exhibited a slightly higher CF content (by 1.0 g/kg DM) compared to the unfertilised grass. However, the influence of gypsum was not significant regarding the higher CF content in the grass and was only a trend.

Effect of K fertilisation. As reported by Grzebisz and Diatta (2012), the main reason for low yields is not only the water supply but also the limited supply of nutrients, mainly potassium. Agriculture in Central Europe is orientated towards K mining and is the principal reason for low water-use efficiency, resulting in considerable seasonal variability in yields. Thus, the primary objective for farmers is to decrease the year-to-year variability of harvested yields. Potassium is a plant-growth factor that should be carefully considered on the basis of its physiological function in the plant, and as one of the most important amendments that can be applied by farmers to cope with water-stress deficiency (Cakmak 2005). Potassium fertilisation results in increased alfalfa persistence in grass mixtures, attributed to enhanced plant tolerance to drought stress and greater storage of WSC before the winter period (Teixeira et al. 2007, Wang et al. 2013). However, there is a viewpoint suggesting that high potassium fertilisation may reduce the nutritional value of alfalfa as forage (Berg et al. 2018). When analysing the mineral composition of the sward in this experiment, the level of K content was also determined (Zielewicz et al. 2023b). The K content, depending on the dose of fertilisation with this component and the year of the study, ranged from 15.5 to 28.6 g/kg DM. In this study, the effect of increasing K fertilisation rates on its content in the sward was noted. At the K0 dose, the average content of this nutrient in the biomass was 20.13 g/kg DM, K30 – 21.53 g/kg DM, K60 – 24.13 and at the applied K120 dose, 24.2 g/kg DM of potassium was observed in the sward.

In our research, it was observed that K fertilisation did not have a significant effect on the CP content in the biomass of the alfalfa-grass mixture. A study by Helmy and Hassanien (2013) found highly significant increased protein yield by K addition; the 50 kg

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K/ha achieved higher protein yield than the 100 kg K/ha. The effect of the combined application of gypsum × K had no significant effect on the WSC content of the sward.

The lack of response to K fertilisation is supported by the results of a meta-analysis conducted by Wan et al. (2022), which demonstrated that with excessive K application (> 150 kg/ha), the positive effect on CP content growth diminished to zero. As reported by Pant et al. (2004), excessive K concentration in tissues (> 3% DM) of alfalfa may have a negative impact on CP content and some essential nutrients for plants, especially Ca, Mg, and Na. In a study by Heuschele et al. (2023), the CP content in alfalfa stems decreased with increasing K fertilisation rate, but not to an extent that significantly influenced the CP content in the entire alfalfa biomass. This phenomenon could be related to increased dry matter content in stems compared to leaves, or the reduction of non-protein nitrogen in plant cells. Similar results indicating the lack of impact of K fertilisation on the nutritional value of alfalfa were obtained in other studies (Jungers et al. 2019), suggesting rather the influence of changes in plant physiology over the vegetation period and harvest time.

Fertilisation can also affect the content of WSC in plants. In our study, the effect of K fertilisation on WSC content in the biomass mixture was inconclusive. On the basis of the average WSC content, it can be observed that increasing doses of K fertilisation reduced the content of this nutrient in the sward. At K30, the sward contained 87.7 g/kg DM, at K60 it contained 82.2 g/kg DM and at K120 only 81.2 g/kg DM of WSC.

Based on the average values, K fertilisation had a significant impact on the CF content in the biomass of the alfalfa-grass mixture. Biomass from treatments fertilised with K30 and K120 also exhibited a higher content of this component, compared to the unfertilised treatment (K0). Opinions regarding the influence of K on CF content are ambiguous. The literature indicates that the use of K fertilisers, due to the stimulation of stem growth, leads to an increase in ADF and NDF content, as well as a decrease in digestibility and nutritional quality of alfalfa (Lissbrant et al. 2009, Jungers et al. 2019). However, the results of a study by Macolino et al. (2013) showed that the use of K fertilisers did not have a significant effect on the CF content in alfalfa. According to Jungers et al. (2019), higher K doses not only increase CF content but also increases crude ash content, while simultaneously reducing digestibility and CP content.

A significant effect on the accumulation level of CA in the biomass could be observed when using different K doses. Based on the averages, it was found that the biomass from the treatment fertilised with the highest K dose K120 had the highest CA content, while the lowest content was recorded in the biomass from the K0 and K30 treatments. The difference in CA content in the biomass between the K0 and K120 fertilisation levels was 5.4%.

Influence of gypsum and K fertilisation. The combined application of gypsum and K had a significant effect on increasing the yields obtained only in the 1st and 3rd sward harvests. Under the water deficit conditions encountered during the second harvest under our climatic conditions, the effect of gypsum and K on yield was not significant. Camas-Pereyra et al. (2022) indicate in their study that the corn grain yield was affected by gypsum, but not by potassium or gypsum × potassium interaction. For CP and WSC, the application of gypsum and K showed no significant effect on the content of these components in the sward. On the other hand, the application of gypsum and K had a significant effect on the higher content of CF and CA in the sward only for the 2nd harvest.

Influence of the study year and harvest date. Various pluvio-thermal conditions in consecutive years of research proved to be a significant factor shaping the content of most of the evaluated quality parameters of forage. It resulted from differences in the course of weather conditions in successive years of the study. According to literature, weather conditions during plant growth, especially temperature and precipitation, can significantly influence the quality of forage.

Plants from the legume-grass mixture were harvested three times during the season. As expected, the harvest time proved to be a significant factor shaping the chemical composition of the plants and their nutritional value. On average, the lowest CP content (161.3 g/kg DM) was characteristic of mixtures from the 1st harvest. In the subsequent harvests, a significant increase in the content of this component was observed. In contrast to our results, Taweel et al. (2005) demonstrated that the highest CP content in forage is typical for the 1st harvest, with a tendency for this component's content to decrease in subsequent harvests. Olszewska et al. (2019) found only minor differences in the CP content of successive harvests.

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In mixtures of alfalfa with *Festulolium braunii*, the CP content increased at successive harvests, whereas in pure-grown alfalfa, the opposite was observed. In the research conducted by Purwin et al. (2016), the CP content in the biomass of grasses from individual harvests differed significantly, with harvest 1 and 4 having higher values than harvests 2 and 3. Sun et al. (2010) reported that the CP content decreased with the elongation of regrowth time during the vegetative period. Delgarde et al. (2000) analysed the composition of forage collected in spring and summer at different regrowth stages and at different times of the day, noticing a reduction in CP content towards the end of the day.

Differences in WSC content in plants can be influenced by the season, plant development stage, and harvesting method (Sikora et al. 2019). Our research finds that the years of study and harvests had an impact on the WSC content in the biomass. The highest content was recorded in the 1st year of the study (92.3 g/kg DM), while the lowest in the 3rd year (72.1 g/kg DM). Analysing the influence of harvests, the highest amount of WSC was recorded in the biomass of the 1st harvest, and the lowest in the 3rd harvest. Differences in WSC content in successive cuts were also noted by Olszewska et al. (2019). The lowest WSC content were recorded in the 2nd harvest, particularly in the biomass with 50% and 70% of alfalfa in the mixture. In the 3rd harvest, the WSC content in the plants increased. Similar results were obtained by other authors who also observed significant fluctuations in WSC content during the vegetative period in other species. For example, Purwin et al. (2016) found the highest WSC concentration in *Lolium perenne* L. in the 1st harvest. In each subsequent cut, the WSC content significantly decreased. According to Watts (2008), the decrease may be associated with higher air temperatures in the second half of the growing season, which increases respiration and accelerates the depletion of accumulated sugars (WSC) in plants.

The content of structural carbohydrates depends on various independent factors, including the plant species and its developmental stage at harvest, as well as climatic conditions, primarily the amount of rainfall (Wróbel et al. 2022). In our research, the timing of mowing and the year of study significantly influenced the CF content. The highest content of this component (286.4 g/kg DM) was found in the third cut of 2013, while the lowest (237.3 g/kg DM) also in the last harvest in 2014. The difference between the CF contents equaled 49.1 g/kg DM (20.7%). Similarly, Vasileva and Naydenova (2017) observed the highest content of CF in a mixture of alfalfa with *Dactylis glomerata* L. in the 1st year of study, with a decrease in subsequent seasons. In the research by Purwin et al. (2016), the ADF content in the sward from cuts 2 and 3 was higher than in harvest 1.

The years of study significantly influenced the accumulation level of CA in plants. In the research, the CA content varied from 92.3 g/kg DM in 2015 to 103.4 g/kg DM in 2014. The harvest also had a significant impact on the CA content in the biomass of the mixture. The highest amount of this component was detected in the 3rd harvest of 2014, while the lowest in the 1st harvest of 2015. The difference between the contents was as high as 41.8%.

Our results can be used to develop K fertilisation guidelines for alfalfa-grass mixtures to improve their nutritional value (especially CP content), reduce fiber content, and improve digestibility.

Analysing the effect of yield on the content of individual components in the sward of the alfalfa-grass mixture, it can be seen that in the last year (2015), with the lowest yields of only 8.88 t DM/ha (Table 3), the biomass contained low contents of: CP (153.6 g/kg DM), CF (250.4 g/kg DM) and CA (92.3 g/kg DM) compared to the previous years of the study (Tables 4 and 5). With the highest yields (16.11 t DM/ha) that were achieved in the second year (2013), the highest content in the sward was recorded: CP (189.6 g/kg DM) and high content of CF (262.8 g/kg DM). However, it is worth noting that in 2012 and 2015, at lower yields, the sward contained the highest WSC contents, respectively: 92.3 and 91.6 g/kg DM. At the much higher biomass yields achieved in 2013 and 2014, the WSC content of the biomass was much lower at: 80.3 and 71.1 g/kg DM (Tables 4 and 5). The lower WSC content at the higher yields was due to the dilution effect of WSC in the higher amount of biomass harvested in these study years. This effect was pointed out by Holík et al. (2022) in their study.

While previous studies have examined the effects of gypsum or potassium fertilisation separately, our research uniquely investigates their combined impact on the nutritional value of alfalfa-grass mixtures. Specifically, the interaction between gypsum (Ca and S source) and potassium fertilisation and how they jointly affect CP, WSC, CF, and CA in the sward is a novel approach. The detailed analysis of how gypsum and potassium interact to affect different forage

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quality parameters (such as CF and CA) provides deeper insights into nutrient dynamics.

Due to the lack of clear results, there is a need for further research on other forage plant species, allowing for a better understanding of the impact of mineral fertilisation containing Ca and S. Particularly, research should focus on broader possibilities of using FGDG – gypsum in green areas and in the cultivation of forage crops on arable land, as well as its impact on changes in qualitative parameters of the nutritional value of forage plants.

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