JOURNAL OF WATER AND LAND DEVELOPMENT

e-ISSN 2083-4535

Polish Academy of Sciences (PAN) Institute of Technology and Life Sciences – National Research Institute (ITP – PIB)

JOURNAL OF WATER AND LAND DEVELOPMENT
DOI: 10.24425/jwld.2024.151796
2024, No. 63 (X-XII): 112–119

Influence of fertilisation type and harvest date on lignin content and structural carbohydrates in meadow plants

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RECEIVED 28.03.2024 ACCEPTED 20.09.2024 AVAILABLE ONLINE 02.12.2024

Highlights:
- Interaction between fertilisation type and harvest timing affects forage quality.
- Changes in ADL and structural carbohydrate content depend on the forb species.
- Fertilisation with FYM improves the nutritional value of late-mowed meadow sward.

Abstract: The nutritional value of green forage is a result of various pratotechnical practices, including fertilisation and harvest timing. Additionally, individual plant species present in natural grasslands may respond differently to those practices. The study aimed to investigate the effect of fertilisation type (farm yard manure (FYM) and NPK fertilisation) and the timing of the first regrowth harvest of meadow sward (ten harvest dates) on the lignin and structural carbohydrates of three dicotyledonous meadow plants: *Taraxacum officinale* F.H. Wigg., *Achillea millefolium* L. and *Lotus corniculatus* L. Plant material was harvested from 28 Apr until 26 Jun at 7-day intervals and analysed for cellulose (CL), hemicellulose (HCL), and acid detergent lignin (ADL). The levels of neutral detergent fibre (NDF), acid detergent fibre (ADF), and ADL were measured using near-infrared spectroscopy (NIRS) with a NIRFlex N-500 device. Cellulose was calculated as the difference between ADF and ADL, while HCL was calculated by subtracting ADF from NDF. *T. officinale* had the lowest HCL (average 65.5 $g \cdot kg^{-1}$ DM) and ADL content (47.1 $g \cdot kg^{-1}$ DM) while *A. millefolium* had the highest CL content (266.5 $g \cdot kg^{-1}$ DM) and ADL (52.3 $g \cdot kg^{-1}$ DM). In contrast, *L. corniculatus* accumulated the highest HCL (104.5 $g \cdot kg^{-1}$ DM) and the lowest CL content (246.1 $g \cdot kg^{-1}$ DM). An increase in all studied parameters content was observed with later harvest dates. On average, plants fertilised with FYM contained lower amounts of structural carbohydrates and ADL compared to those fertilised with NPK.

Keywords: *Achillea millefolium*, ADL, cellulose, hemicelluloses, *Lotus corniculatus*, meadow, organic and inorganic fertilisation, *Taraxacum officinale*

INTRODUCTION

Permanent grasslands are the primary source of forages for ruminants (Gabryszuk, Barszczewski and Wróbel, 2021; Wróbel, Zielewicz and Staniak, 2023). The nutritional value of these forages is determined by the content of structural carbohydrates, such as cellulose and hemicellulose, as well as lignin (Kuoppala *et al.*, 2009; Reiné, Ascaso and Barrantes, 2020). The levels of these plant components are influenced by various factors, including plant species, its growth stage at harvest, type and amount of fertiliser used, and weather conditions throughout the growing season (Rinne and Nykänen, 2000; Stopa *et al.*, 2022; Truba and

Sosnowski, 2022; Wróbel *et al.*, 2022; Paszkiewicz-Jasińska *et al.*, 2023; Sosnowski, Wróbel and Truba, 2023; Stopa *et al.*, 2023).

The maturity stage of plants present in the sward at the time of harvest, especially during the first spring regrowth, is the primary factor influencing the nutritional value of the harvested biomass (Vasiljević *et al.*, 2011; Fychan, Sanderson and Marley, 2016; Poetsch, Resch and Krautzer, 2016; Elgersma and Søegaard, 2018; Wróbel *et al.*, 2022). In practice, the first regrowth is typically harvested between mid-May (for intensive cattle forage production) and early July (for meadows under agro-environmental and climatic measures). The chemical composition and nutritional value of the sward change as the plants grow, generally declining as the season progresses (Niedbała *et al.*, 2022). The rate and extent of these changes also depend, among other factors, on the proportion of different plant species that make up the sward.

Permanent grasslands are multi-species communities with a dominance of perennial grass species. Dicotyledonous species, commonly known as forbs, are valuable components of these permanent grasslands. The presence of forbs in the sward, such as *Cichorium intybus* L., *Plantago lanceolata* L., *T. officinale*, and *A. millefolium*, enhances the nutritional value of the sward, contributing to a high and consistent yield. Additionally, forbs improve forage palatability, increase intake, promote digestive processes, and have preventive effects on animal health (Fraser and Rowarth, 1996; Distel *et al.*, 2020; Jordon *et al.*, 2022). The presence of forbs in a meadow sward is closely linked to the level of fertilisation, particularly with nitrogen. In intensively managed grasslands, the proportion of forbs is generally low but tends to increase as production becomes less intensive.

Effective management of permanent grasslands, aimed at ensuring high yields, relies on rational fertilisation practices using organic or inorganic fertilisers. Organic fertilisers, such as cattle manure, play a crucial role in plant production on many farms, particularly those that are organic. Manure is a valuable source of nutrients that enhances the species composition of the sward. It is particularly beneficial for the growth and development of high-value grasses and legumes, while also influencing their chemical composition (Wróbel, Zielińska and Fabiszewska, 2013).

Previous research has predominantly focused on evaluating the response of multispecies meadow sward to fertilisation and harvest timing as separate factors. However, there is a lack of studies examining how individual components of the sward, particularly dicotyledonous species, respond to the interaction between different types of fertilisers and the timing of biomass harvest. The novelty of our research lies in exploring the effects of these factors on changes in chemical composition, which are used as indicators of the lignification process, of selected dicotyledonous plant species commonly present in permanent grassland sward.

The aim of this study was to investigate the effect of fertilisation type (mineral NPK fertilisers and farm yard manure (FYM)) and harvest timing on the lignin content and structural carbohydrates in the biomass of three selected dicotyledonous plants: *T. officinale*, *A. millefolium*, and *L. corniculatus*.

MATERIALS AND METHODS

STUDY AREA CHARACTERISTICS

The research was conducted in 2016 on a three-cut permanent hay meadow located in Falenty, Central Poland (52°8'27.27" N, 20°55'39.426" E). The meadow was situated on mineral soils (with pH_{KCL} 6.32 and contents of P – 0.024%, K – 0.019%, Ca – 0.062%, and Mg – 0.013%), classified as leached brown soils and pseudopodzolic soils according to soil-agricultural maps. As per the Polish Soil Classification 2019 (Kabała *et al.*, 2019; Świtoniak *et al.*, 2019), these soils are specifically identified as eroded clay-illuvial soils and typical clay-illuvial soils, corresponding to Luvisols in the World Soil Classification System (IUSS Working Group WRB, 2014).

In 2014, two 0.5-ha patches were established within the meadow, one receiving annual fertilisation with farm yard cattle manure (N – 0.37%, P – 0.07%, K – 0.48% in fresh matter) applied every spring at a dose of 30 $Mg \cdot ha^{-1}$ (farm yard manure (FYM) treatment) and the second fertilised with inorganic fertilisers (NPK treatment). The mineral fertilisers NPK were applied at the following doses: 60 kg of N (as ammonium nitrate 34% N), 30 kg of P (as granulated triple superphosphate 46% P_2O_5), and 60 kg of K (as potassium salt 60% K_2O) per ha. Nitrogen and potassium were applied in three doses of 20 kg each – once in spring, and after the first and the second regrowth. Phosphorus was applied once in spring. Soil samples were collected from each of the meadows, from the topsoil layer (0–20 cm). The following parameters were analysed: phosphorus (P) content (total phosphorus) using the colourimetric method with ammonium heptamolybdate and sodium metabisulfite, potassium content using the emission method, magnesium (Mg) and calcium (Ca) contents using atomic absorption spectrometry (S series AA spectrometer, Thermo Fisher Scientific, Waltham, MA, USA), and soil pH in 1 mol KCl using the potentiometric method (Polish Committee for Standardizations, 2022).

COLLECTION AND ANALYSIS OF PLANT MATERIAL

The sward of the experimental meadow consisted of 65% grasses (*Dactylis glomerata* L., *Poa pratensis* L., *Festuca pratensis* Huds., *Festuca rubra* L., *Holcus lanatus* L.), 20% legumes (*L. corniculatus*, *Trifolium pratense* L., *Trifolium repens* L.), and the remainder was made up of dicotyledonous plants (*T. officinale*, *A. millefolium*, *Rumex acetosa* L., *P. lanceolata*, *Cerastium holosteoides* Fr. em. Hyl.). Three dicotyledonous plant species were selected for this experiment: *T. officinale* (TAROF), *A. millefolium* (ACHMI), and *L. corniculatus* (LOTCO), abbreviations were adopted according to the EPPO Global Database (EPPO, 2024). In the spring, herbage samples were collected at 7-day intervals from each part of the meadow. Sampling began on 28 Apr and was continued until 26 Jun, on the following dates: 28 Apr, 05 May, 11 May, 18 May, 25 May, 02 Jun, 08 Jun, 16 Jun, 23 Jun, and 30 Jun. The biomass yield of the experimental meadow ranged from 1.15 $Mg \cdot ha^{-1}$ on the first sampling date to 6.9 $Mg \cdot ha^{-1}$ on the final sampling date for the NPK-fertilised variant. For the FYM-fertilised variant, the yield ranged from 1.82 $Mg \cdot ha^{-1}$ on the first sampling date to 7.6 $Mg \cdot ha^{-1}$ on the final sampling date.

Three herbage samples, each weighing 100 grams, were manually cut with scissors at a height of 5 cm. The herbage samples were then dried, ground, and analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) using near-infrared spectroscopy (NIRS) on a NIRFlex N-500 instrument employing pre-existing INGOT® calibrations for meadow hay. Cellulose (CL) content was calculated as the difference between ADF and ADL, and hemicellulose (HCL) content was calculated as the difference between NDF and ADF.

The weather conditions during the growing season are shown in Figure 1. Meteorological data were obtained from a publicly available database (Tutiempo Network, S.L., no date).

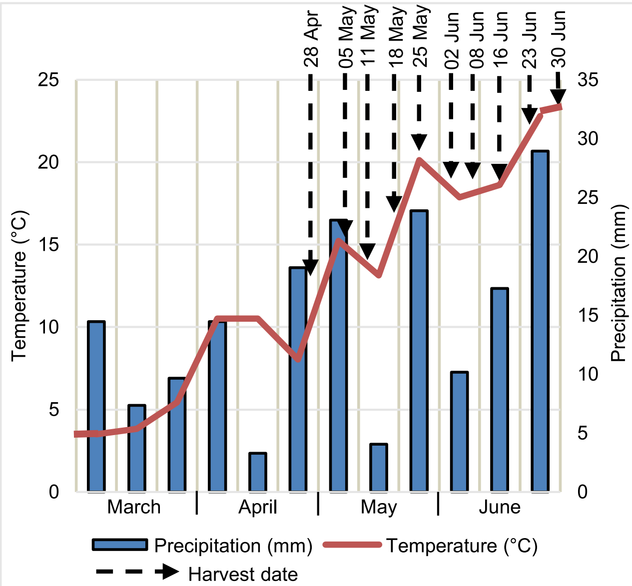

Fig. 1. Meteorological conditions on selected dates in 2016; source: own elaboration based on data by Tutiempo Network, S.L. (no date)

STATISTICAL ANALYSIS

The data were initially tested for normality using the Shapiro–Wilk test and homogeneity of variance was assessed with Levene's test. Subsequently, a three-way analysis of variance was conducted to examine variations between treatments. The model included fixed effects for species (S), harvesting term (HT), and fertilisation type (F), while random effects comprised interactions of S×HT, S×F, HT×F, and S×HT×F. Significant treatment effects were further analysed using Tukey's pairwise comparison at a 5% significance level. Statistical analyses were performed using Statistica v. 6.0.

RESULTS

CELLULOSE CONTENT

The average cellulose (CL) content varied significantly depending on plant species, harvest date, and type of fertilisation (Tab. 1).

L. corniculatus exhibited the lowest CL content (246.1 g·kg^{-1} DM), while *A. millefolium* showed the highest (266.5 g·kg^{-1} DM). The CL content consistently increased with each successive harvest date, ranging from 208.3 g·kg^{-1} DM on the first harvest date to 297.7 g·kg^{-1} DM on the last, indicating a difference of 89.4 g·kg^{-1} DM. Fertilisation type also significantly influenced CL content, with higher levels observed with inorganic fertilisation in *A. millefolium* and *L. corniculatus*. In contrast, the average CL content in *T. officinale* was higher in plants fertilised with farm yard manure (FYM) (Tab. 2). Additionally, CL content was significantly affected by interaction effects, including S×HT, S×F, HT×F, and S×HT×F (Tab. 1).

Table 1. Average values of the chemical composition of the examined plants

Factor	Specification	Concentration (g·kg^{-1} DM)		
		CL	HCL	ADL
S	TAROF	258.1b ±36.85	65.5a ±16.50	47.1a ±9.57
	ACHMI	266.5c ±40.60	86.2b ±14.08	52.3c ±8.59
	LOTCO	246.1a ±17.18	104.4c ±22.97	50.2b ±4.85
p		<0.001	<0.001	<0.001
HT	1st	208.3a ±12.20	87.3b ±11.24	40.8a ±1.96
	2nd	232.3b ±24.97	73.8a ±15.43	44.4bc ±3.14
	3rd	237.3c ±28.34	85.8b ±9.71	44.2b ±2.24
	4th	241.3c ±20.48	89.7bc ±10.45	43.1b ±1.63
	5th	251.3d ±14.72	87.7b ±18.83	46.4c ±1.43
	6th	266.7e ±8.66	76.6a ±21.45	52.4de ±3.05
	7th	264.5e ±18.16	93.2c ±24.10	51.2d ±3.74
	8th	276.3f ±15.49	88.1b ±25.89	54.4e ±5.25
	9th	292.9g ±26.31	94.1c ±37.38	59.2g ±7.23
	10th	297.7h ±33.39	77.5a ±40.36	62.6h ±8.27
p		<0.001	<0.001	<0.001
F	FYM	254.8a ±29.77	82.9a ±24.01	49.3a ±7.03
	NPK	259.0b ±37.88	87.8b ±24.15	50.5b ±9.18
p		<0.001	<0.001	<0.001
Interactions:		*p*		
– S×HT		<0.001	<0.001	<0.001
– S×F		<0.001	0.204	<0.001
– HT×F		<0.001	<0.001	0.002
– S×HT×F		<0.001	<0.001	<0.001

Explanations: DM = dry matter, S = species, HT = harvest time, F = fertilisation, TAROF = *Taraxacum officinale*, ACHMI = *Achillea millefolium*, LOTCO = *Lotus corniculatus*, FYM = farm yard manure, NPK = inorganic fertilisation, CL = cellulose, HCL = hemicellulose, ADL = acid detergent lignin, means in columns with the same letter do not differ significantly at p < 0.05 according to Tukey's HSD test.
Source: own study.

Table 2. Average values of the chemical composition for species (S) and fertilisation (F)

Species	Fertilisation	CL	HCL	ADL
ACHMI	FYM	261.5b	83.6c	50.7b
	NPK	271.4a	88.9b	53.9a
TAROF	FYM	261.8b	62.4e	48.0c
	NPK	254.3c	68.6d	46.2d
LOTCO	FYM	241.0d	102.8a	49.1c
	NPK	251.1c	106.0a	51.3b

Explanations: TAROF, ACHMI, LOTCO, FYM, NPK, CL, HCL, ADL as in Tab. 1, means in columns with the same letter do not differ significantly at p < 0.05 according to Tukey's HSD test.
Source: own study.

The CL content in *A. millefolium* fertilised with FYM ranged from 212.4 to 311.7 g·kg^{-1} DM, while with NPK fertilisation, it was significantly higher, ranging from 222.9 to 343.8 g·kg^{-1} DM. For both types of fertilisation, the CL content was the lowest on the first harvest date, increasing steadily with each subsequent date, and reaching the highest levels on the final date (Fig. 2).

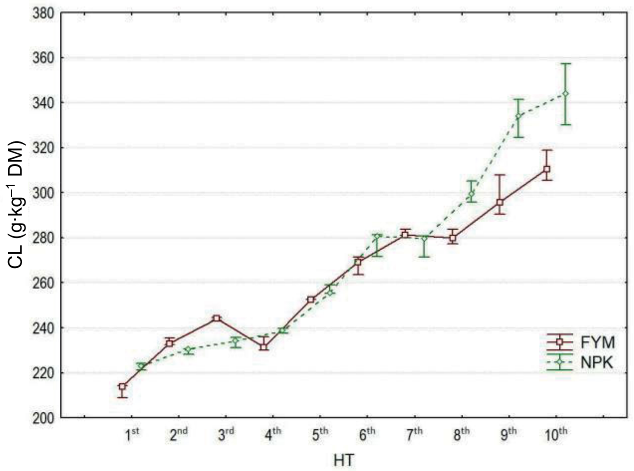

Fig. 2. Cellulose (CL) content in *Achillea millefolium* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

For the next species, *T. officinale*, the CL concentration was significantly higher in plants fertilised with FYM. It ranged from 217.8 to 279.6 g·kg^{-1} DM – with FYM fertilisation, compared to 211.1 to 274.5 g·kg^{-1} DM – with NPK fertilisation. The lowest CL content for both forms of fertilisation was recorded at the first harvest date, while the highest content occurred during the third harvest time for plants fertilised with FYM, and during the sixth harvest time for those fertilised with inorganic fertilisers (Fig. 3).

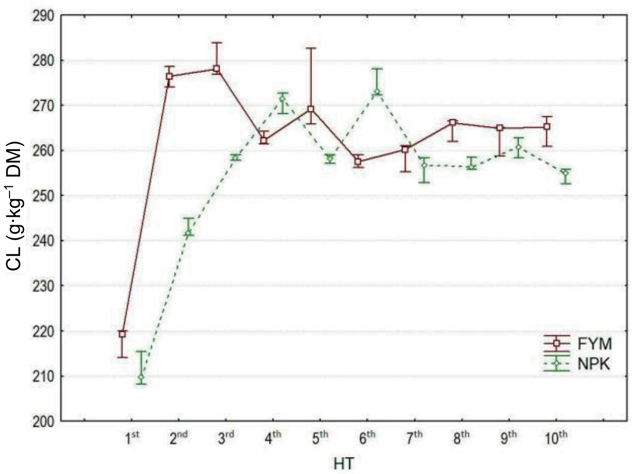

Fig. 3. Cellulose (CL) content in *Taraxacum officinale* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

The type of fertilisation also significantly influenced CL content in *L. corniculatus* which varied throughout the growing season. When fertilised with FYM, the CL content ranged from 192.6 to 296.3 g·kg^{-1} DM, while with NPK fertilisation it ranged from 193.1 to 324.8 g·kg^{-1} DM. Regardless of the fertilisation type, the lowest CL content was recorded in plants harvested on the first harvest date, and the highest content was observed in plants harvested on the last harvest date, particularly in those fertilised with inorganic fertilisation (NPK treatment) (Fig. 4).

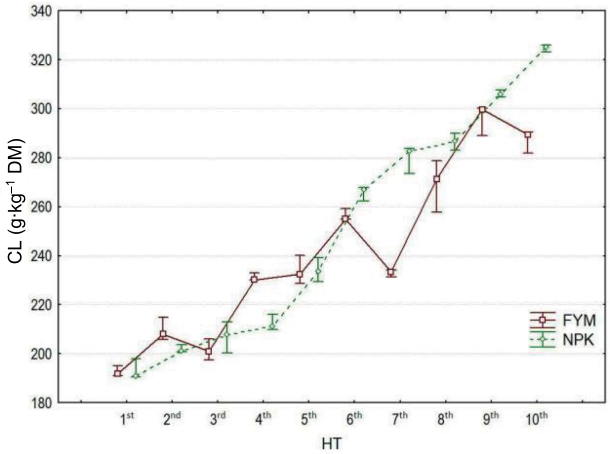

Fig. 4. Cellulose (CL) content in *Lotus corniculatus* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

HEMICELLULOSE CONTENT

The average hemicellulose (HCL) content differed significantly among the three plant species. *T. officinale* exhibited the lowest content (104.4 g·kg^{-1} DM), while *L. corniculatus* showed the highest. The harvest date also had a significant impact on the content of this parameter across the studied species. At the first harvest date, the HCL content was 87.3 g·kg^{-1} DM, decreasing to 77.5 g·kg^{-1} DM in the final harvest date. The lowest HCL content was recorded in plants harvested on the second, the sixth, and the last harvest dates, while the highest content was observed in the fourth, the seventh, and the ninth term. The HCL content in the tested plants was also significantly influenced by the type of fertilisation, with higher levels observed in plants that received mineral fertilisation. Additionally, HCL content was affected by interactions between species and harvest time (S×HT), harvest time and fertilisation (HT×F) and the combination of species, harvest time, and fertilisation (S×HT×F) (Tab. 1). The conducted analysis showed that the HCL content in *A. millefolium* L. plants treated with FYM was significantly lower, ranging from 62.3 g·kg^{-1} DM in the 2nd term to 100.8 g·kg^{-1} DM in the 8th harvest time. In plants fertilised with inorganic fertilisation, HCL ranged from 64.3 g·kg^{-1} DM in the 2nd term to 125.3 g·kg^{-1} DM in the 9th harvest time (Fig. 5).

The HCL content in the second herb tested, *T. officinale*, differed from that in *A. millefolium*. The lowest HCl content was recorded on the last harvest date, while the highest was on the 1st, regardless of the type of fertilisation. Plants fertilised with FYM contained significantly less HCL than those fertilised with NPK. The HCL content ranged from 20.4 to 94.9 g·kg^{-1} DM in FYM-fertilised plants, while those fertilised with inorganic fertilisers contained 26.5 to 100.3 g·kg^{-1} DM (Fig. 6).

The applied fertilisation had no effect on the HCL content in *L. corniculatus* (Tab. 2). In the biomass of *L. corniculatus* fertilised with FYM the HCL content ranged from 80.8 g·kg^{-1} DM (on the 2nd harvest date) to 125.3 g·kg^{-1} DM (on the 9th). In

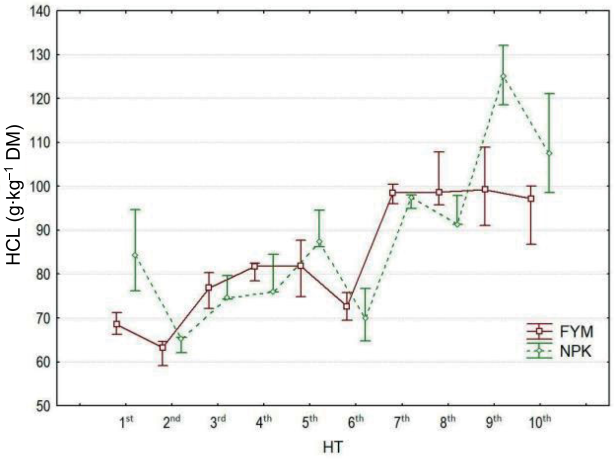

Fig. 5. Hemicellulose (HCL) content of *Achillea millefolium* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

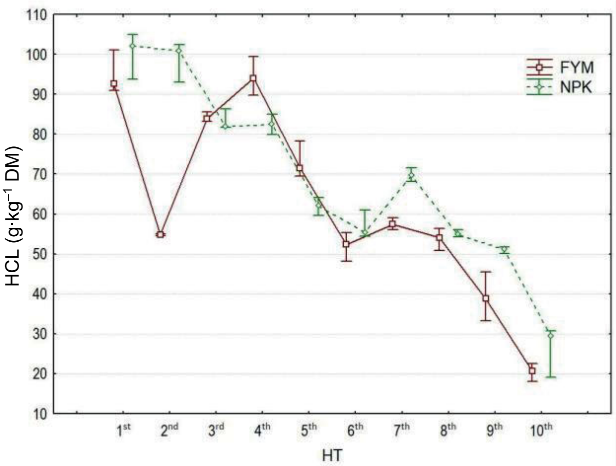

Fig. 6. Hemicellulose (HCL) content of *Taraxacum officinale* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

plants fertilised with mineral inorganic fertilisers, the HCL content ranged from 81.7 to 125.8 $g \cdot kg^{-1}$ DM. The lowest content was found in plants harvested on the 2nd harvest date, while the highest was observed on the 7th harvest date (Fig 7.).

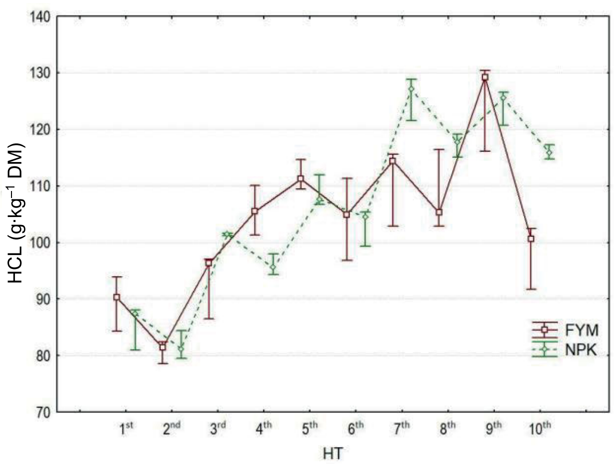

Fig. 7. Hemicellulose (HCL) content of *Lotus corniculatus* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

ACID DETERGENT LIGNIN CONTENT

The average content of acid detergent lignin (ADL) was influenced by all studied factors. *A. millefolium* had a significantly higher content of this parameter (52.3 $g \cdot kg^{-1}$ DM), compared to the other species, while *T. officinale* had the lowest ADL content (47.1 $g \cdot kg^{-1}$ DM). The ADL content also varied significantly with the harvest date, being the lowest at the 1st harvest date (40.8 $g \cdot kg^{-1}$ DM), and progressively increasing in subsequent terms to reach its highest level in the final harvest time (62.6 $g \cdot kg^{-1}$ DM). The type of fertilisation had a significant impact on ADL accumulation in the biomass of the studied species, with lower ADL content observed in plants fertilised with FYM. The analysis of the results revealed interactions between species and harvest time (S×HT), harvest time and fertilisation (HT×F), and the combined effects of species, harvest time, and fertilisation (S×HT×F) (Tab. 1).

The ADL content in the biomass of *A. millefolium* was significantly influenced by both the type of fertiliser and the harvest date. For plants fertilised with FYM, ADL content ranged from 42.5 $g \cdot kg^{-1}$ DM on the 1st date to 67.4 $g \cdot kg^{-1}$ DM on the 10th harvest date (Fig. 8). In contrast, plants fertilised with mineral inorganic fertilisers exhibited significantly higher ADL content compared to those fertilised with FYM (Tab. 2).

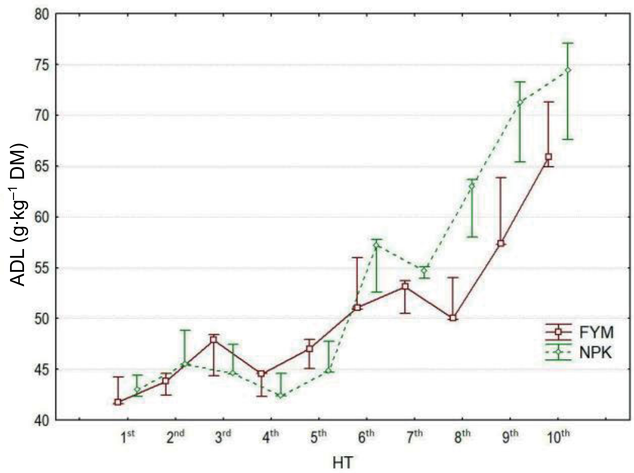

Fig. 8. Acid detergent lignin (ADL) content of *Achillea millefolium* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

During the studied growing season, the ADL content in *T. officinale* fertilised with FYM was the lowest in the 1st harvest and amounted to 38.2 $g \cdot kg^{-1}$ DM. In subsequent harvests, it was higher. The average ADL content in plants fertilised with inorganic fertilisation ranged from 39.6 $g \cdot kg^{-1}$ DM (the 1st harvest time) to 50.4 $g \cdot kg^{-1}$ DM (the 10th term). The average ADL content in *T. officinale* plants fertilised with FYM was significantly higher than in those fertilised with inorganic fertilisers, and similarly as in plants fertilised with NPK, with the lowest value observed in the 1st harvest time (38.2 $g \cdot kg^{-1}$ DM), and increasing in subsequent terms, reaching a maximum in the 10th harvest time (56.4 $g \cdot kg^{-1}$ DM) (Fig. 9).

The ADL content in the biomass of *L. corniculatus*, as well as in the other two species, was the lowest in the first harvest time, regardless of the form of fertilisation applied, and increased in subsequent terms. The type of fertilisation significantly influenced

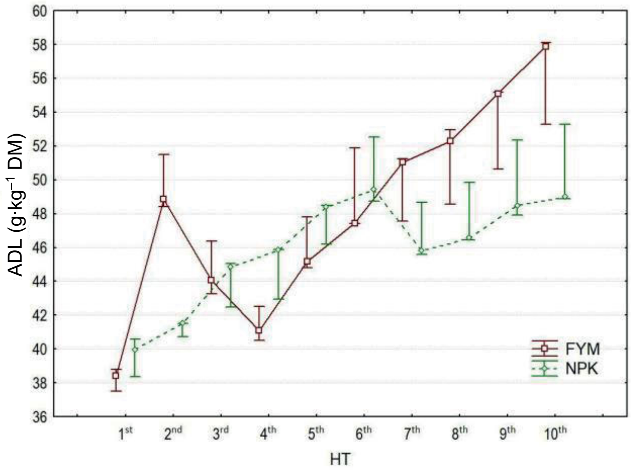

Fig. 9. Acid detergent lignin (ADL) content of *Taraxacum officinale* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

the accumulation of ADL in the plants. *L. corniculatus* fertilised with FYM exhibited significantly lower ADL content (Tab. 2), ranging from 41.1 to 60.9 g·kg^{-1} DM, compared to plants fertilised with mineral fertilisers, where ADL content ranged from 40.1 to 67.6 g·kg^{-1} DM (Fig. 10).

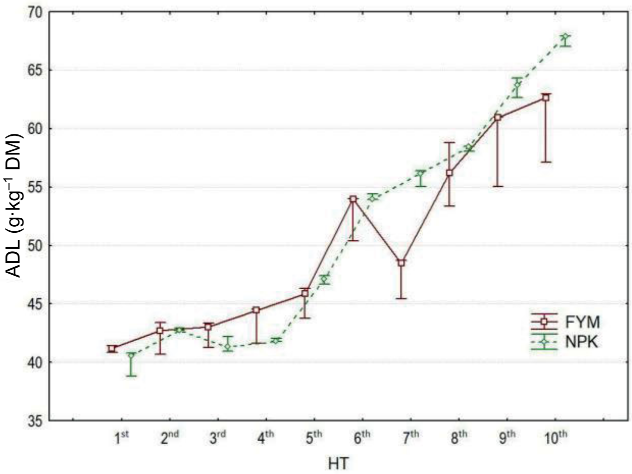

Fig. 10. Acid detergent lignin (ADL) content of *Lotus corniculatus* depending on the harvest time (HT) and fertilisation treatment; FYM, NPK, as in Tab. 1; source: own study

DISCUSSION

The dicotyledonous plant species selected for this study differ in morphology, biology (specifically the rate of generative development), and chemical composition, including the contents of structural carbohydrates and lignin. Among the tested species, *T. officinale* had both the lowest HCL content (average of 65.5 g·kg^{-1} DM) and the lowest ADL content (47.1 g·kg^{-1} DM). In contrast, *A. millefolium* exhibited the highest CL content (266.5 g·kg^{-1} DM) and ADL content (52.3 g·kg^{-1} DM). Meanwhile, *L. corniculatus*, the only legume species studied, accumulated the most HCL (104.5 g·kg^{-1} DM) while having the lowest CL content (246.1 g·kg^{-1} DM). Similar differences in structural carbohydrate content among these species were observed by Reiné, Ascaso and Barrantes (2020) in their study on the nutritional quality of plant species in Pyrenean hay meadows of high diversity, and by Vondrášková *et al.* (2012) in their evaluation of the nutritional quality of forbs from mountainous pastures in the Southwestern Bohemia Region.

According to our hypothesis, both the harvest date and the type of fertilisation applied influenced the content of the examined parameters. Changes in the content of cellulose, hemicellulose, and lignin were observed across successive harvest dates, regardless of the type of fertilisation. However, the direction of these changes varied depending on the plant species. In the case of two species, namely, *A. millefolium* and *L. corniculatus*, an increase in the content of all three parameters was observed with each successive harvest date. The observed changes in the chemical composition of these two species over different harvest dates are consistent with the results of studies on *L. corniculatus* (Karabulut, Canbolat and Kamalak, 2006), meadow sward (Wróbel *et al.*, 2022), species-rich grassland (Boob *et al.*, 2019), *Trifolium pratense* L. (Marković *et al.*, 2011; Vasiljević *et al.*, 2011; Elgersma *et al.* 2018), *Medicago sativa* L. (Karayilanli and Ayhan, 2016), and even warm-season grasses (Waramit, Moore and Fales, 2012).

In *T. officinale*, as well as in *A. millefolium* L. and *L. corniculatus*, an increase in ADL content was observed across successive harvest dates. However, the trends for cellulose and hemicellulose content differed. Specifically, *T. officinale* showed a decrease in HCL content over successive harvest dates, while CL content remained relatively constant. The observed differences in HCL and CL content among these species can be attributed to variations in their morphological structure and flowing date. Among the studied species, *T. officinale* is the fastest to produce flowers, doing so as early as April. *A. millefolium* is the latest, blooming from the end of June. The content of structural carbohydrates in plants typically increases during the growing season. Early in the season, simple sugars and starches dominate, but as the plant matures and forms flowers, the levels of complex carbohydrates like cellulose, hemicellulose, and lignin rise. *A. millefolium* forms an erect, hairy stem, with numerous flower heads gathered in an umbel-shaped inflorescence at the top. *L. corniculatus* forms a creeping or erect sparsely hairy shoot, with an inflorescence composed of corymb-shaped, clusters of 3–4 flowers. In contrast, *T. officinale* produces numerous leaves arranged in a ground-level rosette, from the centre of which grows an inflorescence, commonly called a capitulum, about 5 cm in diameter, consisting of approximately 150–200 ligulate flowers (Szweykowska and Szweykowski, 2012; Mirek *et al.*, 2020).

Our study revealed variations in the content of structural carbohydrates and lignin, which were influenced by both the harvest date and by the type of fertilisation applied. The response of the studied species to fertilisation was different. For *T. officinale*, the CL and ADL concentration was significantly higher in plants fertilised with FYM. However, *A. millefolium* and *L. corniculatus* fertilised with FYM contained lower amounts of structural carbohydrates and ADL compared to those fertilised with NPK. This finding is consistent with research by Sharada and Sujathamma (2018), which showed that organic fertilisers like FYM can reduce fibre and lignin content in rice straw (*Oryza sativa* L.). Additionally, Hakl *et al.* (2021) observed that long-term manure fertilisation enhanced the nutritional value of alfalfa. In their study, long-term manure application led to improved forage crude protein (CP) and neutral detergent fibre (NDF) digestibility. The authors attributed this positive effect to the enhanced root system development in manure-fertilised soil, which made the plants more resilient to drought stress.

CONCLUSIONS

The rate of accumulation of structural carbohydrates and lignin in the studied dicotyledonous plant species was influenced by their flowering date and morphological structure at the time of harvesting the first, spring swath. Among the species studied, *T. officinale* exhibited the least unfavourable changes in chemical composition. Despite this, the excessive presence of this species in the sward is disadvantageous due to its low yield potential. Another positive factor affecting forage quality was manure fertilisation. Plants fertilised with FYM contained lower amounts of structural carbohydrates and lignin compared to those fertilised with inorganic NPK fertilisers. The findings of our research can be applied to the management practices of extensive, late-mowed hay meadows, particularly to reduce the fibre fraction in plants and thereby improve quality forage. Further research is needed to better understand the interactions between harvest timing and manure fertilisation, especially in multi-species grasslands.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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