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Effect of harvest date on structural carbohydrates and lignin content in meadow sward in different pluvio-thermal conditions

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Abstract: The content of structural carbohydrates and lignin are important assessment criteria of the feed value of meadow plants. It is affected by many independent factors, including among others its development stage during the harvest as well as climatic conditions, especially the amount of rainfall. During the years 2014–2016, plant samples were harvested at weekly intervals, respectively five times from late April to late May. The effect of harvest date on cellulose, hemicelluloses and lignin contents was evaluated. The chemical composition of plants was varied, depending not only on harvest date but also on the year of study. Regardless of the course of meteorological conditions in subsequent growing seasons, the increase of cellulose (from 236.5 to 297.9 g·kg^{-1} DM), hemicelluloses (from 159.3 to 210.8 g·kg^{-1} DM), and lignin (from 31.5 to 43.1 g·kg^{-1} DM) in the following dates of harvest were observed. These parameters were also positively correlated with the total rainfall from the begging of vegetation season to the date of plants sampling (R^2 = 0.65, 0.12 and 0.44 for cellulose, hemicelluloses and lignin, respectively), and with the average daily air temperature in the moment of harvest (R^2 = 0.66, 0.32 and 0.52 for cellulose, hemicelluloses and lignin, respectively). The cellulose and lignin content, regardless of the harvest date, were significantly higher in the first year of the study (2014), when moisture conditions for plant development were optimal.

Keywords: air temperature, cellulose, digestibility of feed, hemicelluloses, lignin, nutritional value, sum of precipitation

INTRODUCTION

An important criterion for evaluating the nutritive value of forage is the content of structural carbohydrates and lignin [REINÉ *et al.* 2020]. Structural carbohydrates include cellulose, hemicelluloses, and pectin substrates. Structural carbohydrates together with lignin constitute a basic structural component of plant cell walls.

The content of structural carbohydrates, also known as crude fibre, depends on many independent factors, including among others, plant species, cultivar, and its development stage during the harvest as well as fertiliser doses and climatic conditions, mainly the amount of rainfall [BUXTON 1996; RINNE, NYKÄNEN 2000; TRUBA, SOSNOWSKI 2022]. The content of structural carbohydrates and lignin in forage determines its nutritional value, digestibility, and intake by animals [BUXTON, BRASCHE 1991; KUOPPALA *et al.* 2009]. With increased contents of lignin and structural polysaccharides (cellulose), a decline in digestibility is observed.

In recent years the trend of air temperatures increase as well as changes in the amount and distribution of rainfall in most European regions is observed, which is a visible proof of climate change [ČIMO *et al.* 2020; GOLIŃSKI *et al.* 2018]. Climate changes, which are also noticeable in Poland, lead to a systematic deterioration of humidity conditions [KASPERSKA-WOŁOWICZ *et al.* 2021]. According to ZIERNICKA-WOJTASZEK [2020], the country area with the optimum humidity conditions decreased to 47%. In future, the dry region area will increase to over 65%, while the optimum moisture region area will decrease to just over 30%.

Grasslands are more susceptible to long-term water shortages than other ecosystems [RAICH, TUFEKCIOGUL 2000].

Particularly grasses may be sensitive to rising temperatures and rainfall deficits because many of them are shallow-rooted and short-lived. Therefore, they quickly react to fluctuations in climatic conditions [Knapp, Smith 2001]. It will cause changes in the species composition of grasslands, their productivity and, consequently, the quality of obtained forages [Cantarel *et al.* 2013; Gabryszuk *et al.* 2021; Zielewicz *et al.* 2021].

Due to climatic changes that are causing soil water deficiency increase, slower meadow sward growth rates and changes in forage quality parameters will vary in individual growing seasons depending on average air temperatures and amount of precipitation. Therefore, it was assumed that variation in weather conditions in successive vegetation seasons may differently determine the development of meadow plants and have a significant effect on forage quality parameters.

The aim of the study was to evaluate the effect of the maturity stage at the time of harvest of the primary spring regrowth on changes in the content of structural carbohydrates and lignin in meadow sward depending on weather conditions.

STUDY MATERIALS AND METHODS

STUDY LOCATION

The study was conducted between 2014 and 2016 in the Institute of Technology and Life Sciences – National Research Institute in Falenty, central Poland (52°8'27.27" N 20°55'39.426" E) on a three-cut permanent hay-meadow located on mineral soils classified as leached brown soils and pseudopodzolic soils according to the soil-agricultural maps. In this specific case these units correspond to eroded clay-illuvial soils and typical clay-illuvial soils according to Polish Soil Classification 2019 [Kabała *et al.* 2019; Świtoniak *et al.* 2019] and to Luvisols according to the World Soil Classification system [IUSS Working Group WRB 2015].

Every year the experimental meadow was fertilised with mineral NPK fertilisers at the following doses: 60 kg N (ammonium nitrate 34% N), 30 kg P (granulated triple superphosphate 46% P_2O_5), and 60 kg K (potassium salt 60% K_2O) per ha.

BOTANICAL COMPOSITION AND NUTRITIVE VALUE ANALYSES

Every year in mid-May the botanical composition of the sward was evaluated with Klapp's method [Klapp 1962]. This method estimates the proportion of species in the sward to within 1%.

Every year ten plant samples were collected at seven-day intervals. Depending on the year sampling started at the end of April and was continued until the end of May (Tab. 1). Samples from an area of 1 m^2 were hand-cut with scissors at a height of 5 cm. Plant samples were intended for chemical analyses. After drying and grinding the plant samples the nutrient contents of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) were evaluated by the NIRS method [PN-EN ISO 12099:2017] with a NIRFlex N-500 apparatus using INGOT ready calibrations dedicated to meadow hay. Cellulose content was calculated as the difference between ADF and ADL, and hemicellulose content was calculated as the difference between NDF and ADF.

Table 1. Dates of meadow plants harvests in 2014–2016

Year	Dates of harvest				
	1st	2nd	3rd	4th	5th
2014	30th Apr	7th May	14th May	21st May	28th May
2015	29th Apr	7th May	14th May	21st May	28th May
2016	28th Apr	5th May	11th May	18th May	24th May

Source: own elaboration.

WEATHER CONDITIONS

The weather data came from the meteorological station from Falenty (Tab. 2). Using available meteorological data, average daily temperature and total precipitation were calculated for each period from the beginning of the growing season to the day of plant sampling. For the detailed assessment of rainfall and temperature in growing seasons the Selyaninov hydrothermal coefficient (k) [Selyaninov 1930] was calculated according to the following formula:

$$k = (10P)/\Sigma T \quad (1)$$

where: P = the total monthly rainfall (mm); ΣT = the monthly total of average daily air temperature >0°C.

Table 2. Values of the Selyaninov hydrothermal coefficient (k)

Year	Value in month		
	March	April	May
2014	1.82	1.45	2.35
2015	1.00	1.62	0.94
2016	2.37	1.27	0.65

Explanations: k > 3.00 – extremely humid; k ∈ 2.51–3.0 – very humid, k ∈ 2.01–2.50 – humid, k ∈ 1.61–2.00 – quite humid, k ∈ 1.31–1.60 – optimum, k ∈ 1.01–1.30 – quite dry, k ∈ 0.71–1.00 – dry, k ∈ 0.41–0.70 – very dry, k ≤ 0.40 – extreme dry.
Source: own study.

Characteristics of the weather conditions during the growing season in successive years of the study are shown in Figure 1. Mean air temperature for the period March–May

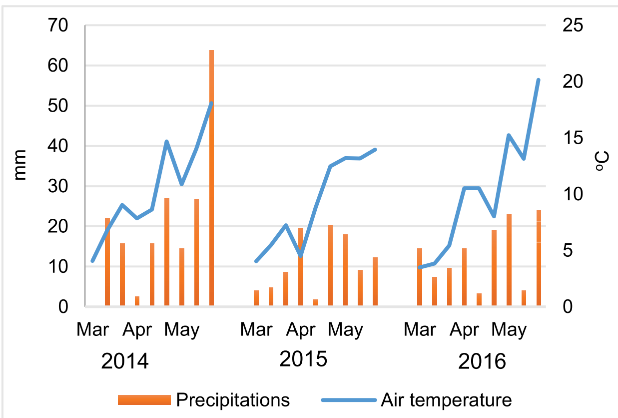

Fig. 1. Average decade air temperature and precipitations in period March–May in study years (2014–2016); source: own study

varied from 9.2°C in 2015 to 10.4°C in 2014. Greater differences between years occurred in total rainfall. In May 2015, the total rainfall was 39.4 and 32.8 mm in May 2016. By comparison, in May 2014 the rainfall was 104.9 mm. Based on the value of the hydrothermal coefficient k (Tab. 2) as a determinant of rainfall and temperature, April 2014 was optimum, and May 2014 was humid. In 2015, April was quite humid and May was dry. Weather conditions in the third year were the least favourable for plant development. April 2016 was quite dry, and May was very dry.

STATISTICAL ANALYSIS

The analyses of data involved the comparison of average values and standard deviations (*SD*) for the samples taken from each harvest date (*HD*). The results were presented graphically in the form of a scatter plot with marked deviation posts. In order to analyse possible linear relationships between meteorological data and evaluated parameters, the trends were visualised using Microsoft Excel. In addition, a two-way analysis of variance (ANOVA) was performed with harvest date (*HD*) and year of study (*Y*) as factors. The significance of differences was tested by Tuckey's HSD (honestly significant difference) test at $\alpha = 0.05$. All tests were performed using Statistica ver. 6 software (Statsoft, Poland).

RESULTS

BOTANICAL COMPOSITION

Grasses were the dominant group of plants in meadow sward (Tab. 3). In the first two years of study, their share was about 71%. In the third year, the share of grasses significantly decreased. *Dactylis glomerata* L., *Poa pretensis* L. and *Festuca rubra* L. were the dominant grass species in all years of study. In addition to grasses, dicotyledonous herbs and weeds were abundant. The most frequent species were: *Taraxacum officinale* F.H. Wigg., *Achillea millefolium* L. and *Trifolium pratense* L.

CELLULOSE CONCENTRATION

Cellulose is the basic structural polysaccharide of plant cell walls [COLLINS, FRITZ 2003]. The cellulose content in plants depended on the harvest date. The lowest content of cellulose (236.5 g kg^{-1} DM), irrespective of the study year, was observed in plants harvested on the first date. On the second and the third date the content of this parameter was significantly higher. An increase in its concentration was observed at subsequent harvest dates. The highest content of cellulose was stated in the plants harvested on the last date (297.9 g·kg^{-1} DM). The content of cellulose was influenced by the study year. In the first year (2014) it was significantly higher than in the second and the third year (Tab. 4). In 2014 it varied from 256 g·kg^{-1} DM on the second date to 325 g·kg^{-1} DM on the last date. In the second year (2015) and the third year (2016) it was the lowest on the first date. On the next dates, it systematically increased, reaching the highest value (over 280 g·kg^{-1} DM) on the fifth date, respectively the last one (Fig. 2).

Table 3. Botanical composition of meadow sward

Plant species	Percentage share in the year			Mean share
	2014	2015	2016	
Monocotyledones				
Dactylis glomerata L.	32	34	15	27.0
Poa pratensis L.	27	28	26	27.0
Festuca rubra L.	10	10	12	10.7
Festuca pratense Huds.	0	0	5	1.7
Agrostis stolonifera L.	0	0	2	0.7
Lolium perenne L.	1	1	1	1.0
Elymus repens (L.) Gould	1	1	1	1.0
Dicotyledones				
Taraxacum officinale F.H. Wigg.	15	13	20	16.0
Trifolium pratense L.	4	4	1	3.0
Achillea millefolium L.	6	5	5	5.3
Trifolium repens L.	0	1	1	0.7
Geranium pusillum L.	0	0	5	1.7
Heracleum sphondylium L.	0	0	1	0.3
Capsella bursa-pastoris L. Medik.	0	0	1	0.3
Ranunculus repens L.	0	0	1	0.3
Rumex acetosa L.	2	2	1	1.7
Rumex crispus L.	1	1	1	1.0
Sonchus arvensis L.	1	0	0	0.3
Plantago lanceolata L.	0	0	1	0.3

Source: own study.
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Table 4. Mean values of the traits for the harvest dates (*HD*) and years (*Y*)

Variable	The level	Content in plant (g·kg^{-1} DM)		
		cellulose	hemi-celluloses	lignin
HD	1st	236.5 ±34.2a	159.3 ±36.0a	31.5 ±6.4a
	2nd	241.7 ±14.1b	164.7 ±23.2ab	33.6 ±4.2ab
	3rd	259.8 ±10.8b	178.0 ±25.7ab	35.7 ±2.8bc
	4th	277.5 ±16.5c	188.6 ±19.0bc	38.7 ±2.7c
	5th	297.9 ±23.8d	210.8 ±21.1c	43.1 ±4.2d
Y	2014	285.2 ±26.1b	179.3 ±36.7ab	39.2 ±5.0b
	2015	251.3 ±27.2a	171.0 ±30.1a	36.0 ±4.6a
	2016	251.6 ±27.8a	190.6 ±23.6b	34.4 ±6.8a
SEM		3.65	3.67	0.69

Explanation: *SEM* = standard error of means.
Source: own study.

As can be seen in Figure 3, the cellulose concentration increased along with the total precipitation from the begging of the vegetation season to the date of plant sampling, and the average daily air temperature at the moment of harvest.

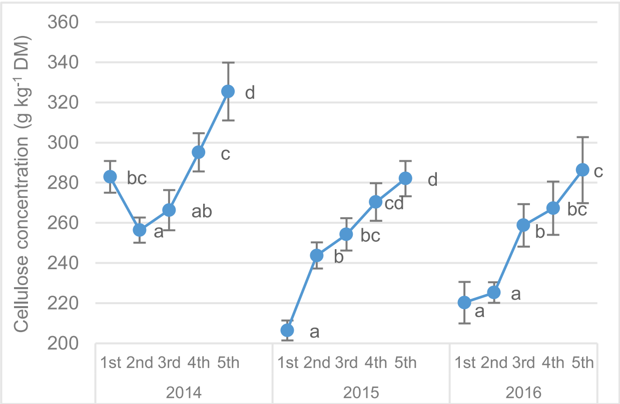

Fig. 2. Changes in cellulose concentrations ±*SD* in subsequent harvesting dates of meadow plants; source: own study

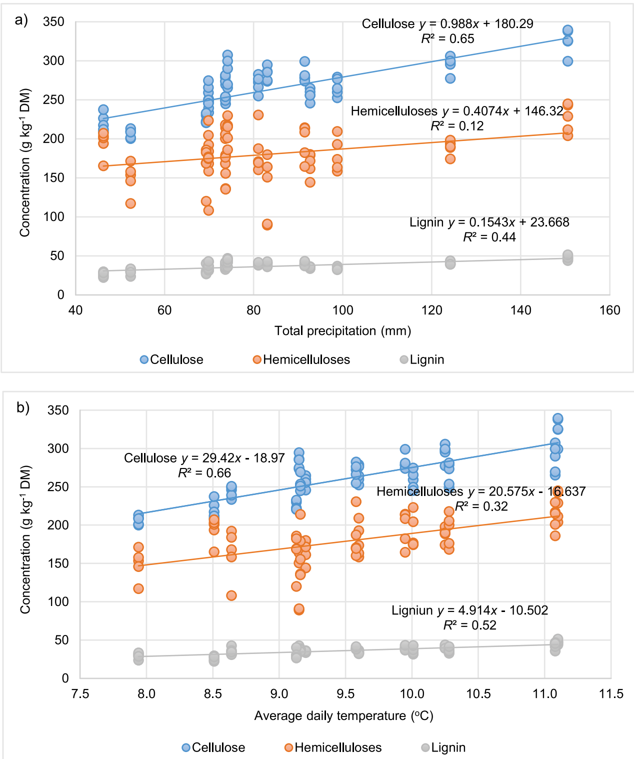

Fig. 3. Relationship between weather conditions and cellulose, hemicellulose, and lignin concentrations: a) total precipitation, b) average daily temperature; source: own study

HEMICELLULOSES CONCENTRATION

Hemicelluloses are the second most abundant plant biopolymer after cellulose [Collins, Fritz 2003]. The hemicellulose content was influenced by the harvest date. When analysing harvest dates (regardless of year) the lowest content of hemicelluloses (159.3 g·kg^{-1} DM) was observed in the plants harvested on the first date. In the next terms of harvest, a systematic increase in its concentrations was observed. On the last date, the content of hemicelluloses in plants reached the value of 210.8 g·kg^{-1} DM. The content of cellulose was also influenced by the year of study. On average, it was the lowest (171.0 g·kg^{-1} DM) in the second year of study and the highest (190.6 g·kg^{-1} DM) in the last year (Tab. 4).

In the first term of harvest in 2014 hemicellulose concentration was only 134.7 g·kg^{-1} DM. On the following dates it grew rapidly, reaching the value of 226 g·kg^{-1} DM on the last date of plant harvest. The growth rate of hemicellulose content in 2015 and 2016 was slower (Fig. 4). Hemicellulose content was less dependent on the amount of precipitation while average temperature proved to be a more significant factor (Fig. 3).

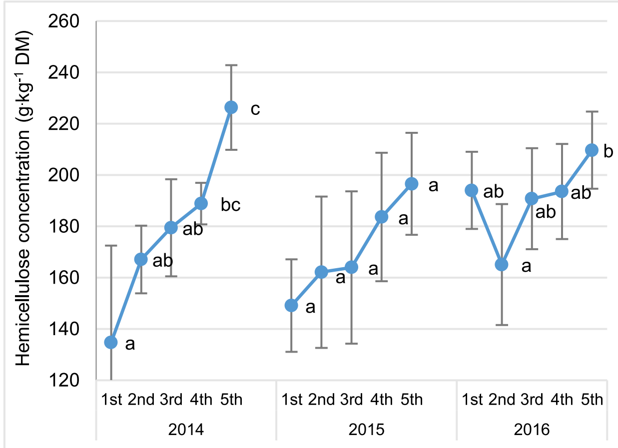

Fig. 4. Changes in the content of hemicelluloses in subsequent harvesting dates of meadow plants; error bars show standard deviation; source: own study

LIGNIN CONCENTRATION

Lignin, along with cellulose and hemicellulose, is one of the major components of the plant cell wall. It is a natural phenolic polymer of high molecular weight, complex composition and structure [Collins, Fritz 2003].

Mean concentrations of lignin content in plants depended on the harvest date and the year of study. Regardless of the year of study, on the first date of harvest mean concentration of lignin was 31.5 g·kg^{-1} DM. On the next dates of the harvest was observed slow increase in its content (Tab. 4). In the first year it was significantly higher than in the second and the third year.

In 2014 it in was the lowest on the second and third date of harvest, higher on the first and fourth and the highest on the fifth. In the second year (2015), it was the lowest on the first date. On the next dates, it grew insignificantly and hesitated from 35.16 to 39.78 g·kg^{-1} DM. In the third year (2016), it was the lowest on the first date and was systematically increasing on the next dates, reaching the highest value on the fifth date (Fig. 5).

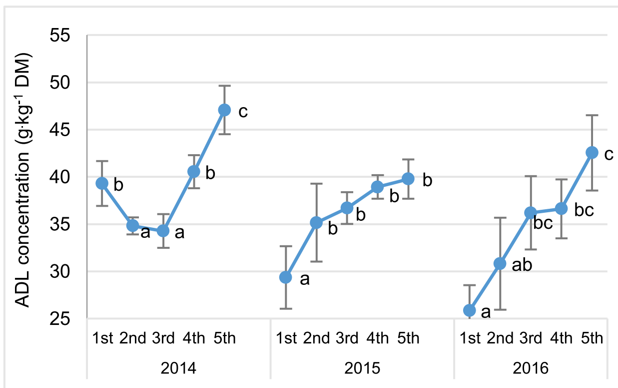

Fig. 5. Changes in lignin (ADL) concentrations in subsequent harvest dates of meadow plants; error bars show standard deviation; source: own study

The lignin concentration increased along with the total precipitation from the begging of vegetation season to the date of plant sampling, and the average daily air temperature at the moment of harvest (Fig. 3).

DISCUSSION

As hypothesised, the rates of changes in plants' chemical composition during harvest at different dates were observed. Regardless of the course of meteorological conditions in subsequent growing seasons, the increase of cellulose, hemicellulose and lignin in the subsequent dates of the harvest was observed. The findings of our experiment concerning the rates of changes in the chemical composition of meadow plants during harvest at different dates of harvest are in accordance with results regarding species-rich hay meadows [Boob *et al.* 2019], *Trifolium pratense* L. [Elgersma, Søegaard 2018; Marković *et. al.* 2011; Vasiljević *et al.* 2011], *Lotus corniculatus* L. [Karabulut *et al.* 2006], *Medicago sativa* L. [Karayilanli, Ayhan 2016] or even warm-season grasses [Waramit *et al.* 2012].

The observed changes in plant nutritional quality are due in part to changes occurring in stems and leaves. In the early spring, young plants have a high proportion of leaves. Leaves generally have a much higher digestibility, lower fibre content and twice as much crude protein as stem tissue from the same plant.

Ageing plants undergo morphological changes during the growing season. A rapid increase in stem proportion is observed. The stems at harvest have less nutritional value than the leaves, resulting in a drastic reduction in forage quality [Collins, Fritz 2003]. Summing up, the main determinant of whole plant nutritive value is an increase in the proportion of low quality stems in relation to leaves. Therefore, harvest delay reduces significantly forage quality, which is associated with poorer animal performance [Kuoppala *et al.* 2009; Rinne *et al.* 1999].

Although plant maturity affects forage quality more than any other single factor, environmental and agronomic factors also modify the forage quality.

Drought stress and high temperature are important environmental factors that limit plant growth and alter tissue chemistry [Xu, Zhou 2006]. It has been shown that temperature and drought applied separately can change the chemical composition of plants. In addition, some studies have shown that there is a significant interaction between drought and temperature in affecting plant chemical composition [Larsen *et al.* 2011]. In this context, water deficit and elevated temperature have modified chemical composition by affecting carbohydrate concentrations. For instance, *Medicago sativa* L. has accumulated starch but has decreased soluble sugars under drought conditions during vegetative growth [Erice *et al.* 2006].

The results of our study showed that the content of structural carbohydrates and lignin was varied, depending not only on harvest date but also on the year of study. In 2014, precipitation in May has the highest Selyaninov's hydrothermal coefficient, respectively 2.35, indicating optimal moisture conditions. In the next two years of study (in 2015 and 2016), weather conditions were less favourable due to low precipitations in the key periods of early plant development, particularly in May, which could have a negative impact on the development of plants (Tab. 2). In our study, the cellulose and lignin content, regardless of the harvest date, were significantly higher in the first year of the study (2014), as compared to other years. These parameters were positively correlated with the total rainfall from the begging of vegetation season to the date of plant sampling, and the average daily air temperature at the moment of harvest. According to the literature [Buxton 1996], the content of structural carbohydrates and lignin is influenced by many factors, including environmental conditions in which forages are grown and these cause year-to-year, seasonal, variation in forage quality even when plants are harvested at the same stage of maturity. High temperatures typically increase plant growth rates and reduce leaf/stem ratios and digestibility. Moderate water stress usually delays plant maturation and results in maintaining forage quality at a higher level [Halim *et al.* 1989]. Therefore, if drought-related leaf loss is not severe, water deficit may even improve forage digestibility. The results of our study are consistent with those of other forage crop studies. Under drought conditions, most authors reported a reduction of crude fibre and its fractions in grasses [Borawska-Jarmułowicz *et al.* 2022], as well as in legumes [Küchenmeister *et al.* 2013].

CONCLUSIONS

Changes in structural carbohydrate and lignin content in plants during the study period were influenced by the data of harvest and weather conditions. The increase of cellulose (from 236.5 to 297.9 g·kg^{-1} DM), hemicellulose (from 159.3 to 210.8 g·kg^{-1} DM), and lignin (from 31.5 to 43.1 g·kg^{-1} DM) in the following dates of harvest were observed. These parameters were also positively correlated with the total rainfall from the begging of vegetation season to the date of plants sampling (R^2 = 0.65, 0.12 and 0.44 for cellulose, hemicelluloses and lignin, respectively), and with the average daily air temperature in the moment of harvest (R^2 = 0.66, 0.32 and 0.52 for cellulose, hemicelluloses and lignin, respectively). The cellulose and lignin content, regardless of the harvest date, were significantly higher in the first year of the study (2014), when moisture conditions for plant development were optimal. These results may be used in the management practices of hay meadows for enhancing nutritional value, especially the reduction of fibre fractions in the plants. There is a need to continue experiments that allow for a better understanding of the interactions between harvest timing and weather conditions, especially in multi-species grasslands.

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