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NUTRITIONAL VALUE OF RED CLOVER (*Trifolium pratense* L.) AND BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) HARVESTED IN DIFFERENT MATURITY STAGES

Summary

During the years 2014-2015, herbage samples of red clover (Trifolium pratense L.*) variety Chlumecky and birdsfoot trefoil (Lotus corniculatus* L.*) variety Leo were harvested at weekly intervals on 9 occasions from late April to late June. Effects of legume plant species and date of harvest on crude protein (CP), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (ADL) and dry matter digestibility (DDM) were studied. Regardless of the date of harvest birdsfoot trefoil contained more crude protein, NDF and ADF fraction than red clover. Red clover contained more WSC. Crude protein concentration and DDM declined and WSC, NDF, ADF and ADL increased with advancing maturity of both legume species. But the rate of NDF increase with maturity in red clover was slower.*
***Keywords**: legumes, protein, structural carbohydrates, lignification, quality decline, growth rate*

WARTOŚĆ POKARMOWA KONICZYNY ŁĄKOWEJ (*Trifolium pratense* L.) I KOMONICY ZWYCZAJNEJ (*Lotus corniculatus* L.) ZBIERANYCH W RÓŻNYCH FAZACH DOJRZAŁOŚCI

Streszczenie

W latach 2014-2015, co tydzień, dziewięciokrotnie, od końca kwietnia do końca czerwca, pobierano próbki roślin dwóch gatunków: koniczyny łąkowej (Trifolium pratense L.*) odmiany Chlumecky i komonicy zwyczajnej (Lotus corniculatus* L.*) odmiany Leo. Badano wpływ gatunku rośliny bobowatej i terminu zbioru na zawartość białka ogólnego (CP), cukrów rozpuszczalnych (WSC), neutralnego włókna detergentowego (NDF), kwaśnego włókna detergentowego (ADF), lignin (ADL) i strawność suchej masy (DDM). Niezależnie od terminu zbioru komonica zwyczajna zawierała więcej białka ogólnego oraz frakcji włókna NDF i ADF niż koniczyna łąkowa. Koniczyna łąkowa zawierała więcej cukrów rozpuszczalnych. Wraz z postępem faz rozwojowych obu gatunków roślin zawartość białka ogólnego i strawność suchej masy malała, a zawartość WSC, NDF, ADF i ADL rosła. Jednak tempo wzrostu zawartości NDF w koniczynie łąkowej było mniejsze.*
***Słowa kluczowe:** rośliny bobowate, białko, węglowodany strukturalne, lignifikacja, spadek jakości*

1. Introduction

Forage quality is the key element of sustainable animal production. It is affected by many independent factors [2]. One of them the stage of plant development, which considerably affects chemical composition and forage quality [2, 4, 9, 14, 17, 21, 23, 25]. The fastest plant growth and development occurs in spring. Young plants have high proportion of leaves, high content of moisture, proteins and minerals and low content of fibers. During the growing season the aging plants undergo metabolic and morphological changes and forage quality decreases drastically, especially digestibility and the content of protein and minerals. For example the average decreases in crude protein concentration with advance in maturity for several forages averaged 1 g kg^{-1} d^{-1} in data reported by Minson [19].

To achieve high feed quality, harvesting should take place at the optimal stage of development of dominant sward species. But this is not always possible. Delayed harvest of meadow sward may occur in practice due to adverse weather conditions or limitations of harvesting machines.

Because plant growth and development is a dynamic process in which changes occur, an attempt to illustrate them in a graphical and mathematical form was made. These changes were determined for two legume species: red clover and birdsfoot trefoil. It was assumed that growth rate and change in quality parameters would differ among species. Different pace of development of this two species may determine their usefulness for harvest, and may also have a significant impact on the quantity and quality parameters of feed.

Red clover (*Trifolium pratense* L.) is one of the most abundant forage legume species present in most northern European grasslands. It's popular among farmers because of its high biomass production, high protein content and digestibility [6, 8, 13]. Red clover is recommended as a valuable meadow sward component, particularly useful for silage production [5, 20]. Widespread interest in this species stems from the fact that red clover contains high levels of polyphenol oxidase (PPO), an enzyme that has beneficial effects in improving nitrogen utilization in ruminants [4, 15] and in protecting lipids from degradation, both 'in silo' as well as in the rumen [28].

The birdsfoot trefoil (*Lotus corniculatus* L.) can be a potentially useful protein source in organic and low input production systems [3, 16]. Comparing to red clover it is a more persistent species. Due to its high quality, resilience to climate change, adaptability to various soil types, and for the ability to be consumed as fresh or conserved forage it's recognized as a very valuable forage plant. Birdsfoot trefoil is a legume with a high nutritive value, containing condensed tannins which, when fed to dairy cows, increases the efficiency of feed utilisation for milk production. The condensed tannins affect the rumen microflora, reduce feed

protein degradation and lower feed energy loss due to methane [30], without adversely affecting milk protein composition [27].

The aim of the study was to assess the effect of stage of maturity in the primary growth on the nutritive value of red clover (*Trifolium pratense* L,) and birdsfoot trefoil (*Lotus corniculatus* L).

2. Material and methods

2.1. Herbage sample collection

The research was carried out in 2014-2015 in the Institute of Technology and Life Sciences in Falenty. Two species of legume plants were used for this experiment: red clover (*Trifolium pratense* L.) variety Chlumecky and birdsfoot trefoil (*Lotus corniculatus* L.) variety Leo. The research material was collected from a three-cut experimental meadow located on mineral soil in Falenty. In spring 2012 the experimental meadow was split into two parts and renovated by undersowing with legume-grass seed mixtures. A half of the meadow was undersown with seeds mixture containing birdsfoot trefoil and the second – with red clover. The experimental meadow was fertilised every year with mineral fertilisers NPK in the following doses: 60 kg N, 30 kg P and 60 kg K per ha^{-1}.

During the years 2014-2015, in 7-days intervals, from each part of meadow six herbage samples from an area of 1 m^2 were taken. Samples were hand cut with scissors at 5 cm height. Depending on the year, sampling was started on April 23 (2014) and April 29 (2015) and was continued until June 18 (2014) and June 25 (2015).

2.2. Nutritive components analysis

Grass and legume components were hand-sorted. Legume samples were intended for chemical analyses. After drying and grinding the samples, the following nutrients were estimated: crude protein (CP), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL), using near-infrared spectroscopy (NIRS) [22] on a NIRFlex N-500 apparatus using ready-made INGOT ® calibrations for meadow hay.

Cellulose content was calculated as ADF-ADL and hemicelluloses as NDF-ADF. Lignin proportion i.e. lignification of cell wall, was calculated as 100 x ADL/NDF. Dry matter digestibility was calculated as: DDM = 88.9 - (0.779 x ADF).

2.3. Weather conditions

Both the average daily air temperature and the sum of precipitation in subsequent years of research differed significantly (Table 1). The year 2014 was more favorable for the growth and development of meadow vegetation. In 2015, lower temperatures in April and the shortage of precipitation in June were noted.

Table 1. Weather conditions during period of study
Tab. 1. Warunki pogodowe w okresie badań

	Month		
Year	April	May	June
Precipitation [mm]			
2014	45.21	104.89	60.71
2015	41.67	39.35	19.55
Temperature [°C]			
2014	10.4	14.4	16.4
2015	8.6	13.5	17.9

Source: own study / Źródło: opracowanie własne

2.4. Statistical analysis

Obtained data was compared using two-way analysis of variance (ANOVA) with legume species and year of study as factors. Significance of differences was checked with the Tuckey HSD test at $\alpha = 0.05$. In addition, the weekly change was analysed by linear regression models of each parameter. All tests were made using Statistica ver. 6 (Statsoft, Poland).

3. Results

3.1. Species effect

Birdsfoot trefoil, regardless of the date of harvest, contained more total protein and less sugar ($p \leq 0.05$) than red clover (Table 2). Differences in concentrations of cell wall compounds between red clover and birdsfoot trefoil were also significant.

Table 2. Mean concentration of nutritive components in red clover and birdsfoot trefoil (average from 9 harvest dates)
Tab. 2. Średnia zawartość składników pokarmowych w koniczynie łąkowej i komonicy zwyczajnej (średnia z 9 terminów zbioru)

Legume species	Year of study	CP [g·kg^{-1} DM]	NDF [g·kg^{-1} DM]	ADF [g·kg^{-1} DM]	ADL [g·kg^{-1} DM]	Cellulose [g·kg^{-1} DM]	Hemicelluloses [g·kg^{-1} DM]	Lignification [%]	WSC [g·kg^{-1} DM]	DM digestibility [%]
Red clover	2014	163.7a	380.2ab	287.7	44.7	243.0b	92.5ab	11.8a	130.8b	66,49
	2015	176.1ab	366.7a	278.4	46.4	232.0a	85.3a	12.8b	129.4b	67,21
Birdsfoot trefoil	2014	195.6b	388.0b	289.9	46.0	243.9b	98.2b	11.9a	87.1a	66,32
	2015	190.7b	385.1b	285.1	46.1	239.0b	99.9b	12.0a	96.5a	66,69
Mean for legume species										
Red clover		172.4a	368.6a	281.2a	45.9	235.3a	87.4a	12.5b	129.8b	67.00b
Birdsfoot trefoil		192.1b	385.9b	286.4b	46.1	240.4b	99.5b	12.0a	93.9a	66.59a
Mean for years										
2014		178.8	383.9	288.7b	45.3	243.4b	95.2	11.8a	110.0	66.41a
2015		183.5	374.5	281.8a	46.2	235.6a	92.7	12.4b	112.7	66.95b

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test $p \leq 0.05$)

Source: own study / Źródło: opracowanie własne

Red clover had a lower ($p \leq 0.05$) NDF, ADF, cellulose and hemicelluloses concentration than birdsfoot trefoil. But the lignification of cell wall in red clover was higher than in birdsfoot trefoil. There were no differences among legume species in ADL concentration. DDM was significantly higher in red clover. Red clover harvested in mid-May, which is considered to be the optimal term for the first cut, had an average concentration of crude protein at 181.7 g·kg^{-1} DM, NDF 371.9 g·kg^{-1} DM, ADF 268.8 g·kg^{-1} DM, ADL 43.4 g·kg^{-1} DM and WSC 129.3 g·kg^{-1} DM. Birdsfoot trefolil harvested in the same time contained 199.7 g·kg^{-1} DM crude protein, 381.3 g·kg^{-1} DM NDF, 269.4 g·kg^{-1} DM, 44.1 g·kg^{-1} DM ADL and 95.4 g·kg^{-1} DM WSC.

3.2. Weather condition effect

Some examined parameters were affected by the year of study. In 2014 when there was more rainfall ADF and cellulose content were significantly higher than in 2015 (Table 2). The next year of study was drier, particularly in May and June, and the lignification of cell walls was significantly higher

3.3. Harvest date effect

The highest concentration of crude protein was observed in the first term of harvest. Birdsfoot trefoil contained more crude protein (285 g·kg^{-1} DM) than red clover (243 g·kg^{-1} DM). In the next terms of harvest a decrease of crude protein content in both examined species was observed (Fig. 1). In the first four weeks of development the daily decline of crude protein concentration in red clover was about 3.0 g but in birdsfoot trefoil – 2.8 g. The highest decrease of protein content was observed between the fifth and the sixth term of harvest, before flowering stage. The concentration of crude protein in the fifth term of harvest was 161 g·kg^{-1} DM and 206 g·kg^{-1} DM in red clover and birdsfoot trefoil respectively. The daily decline of crude protein content was 6.1 g in red clover and 7.1 g in birdsfoot trefoil. In the next terms the changes of crude protein content were smaller. The differences in crude protein content between species were also less visible. In the last term, at the end of June, birdsfoot trefoil contained 124.7 g·kg^{-1} DM and red clover only 119.5 g·kg^{-1} DM.

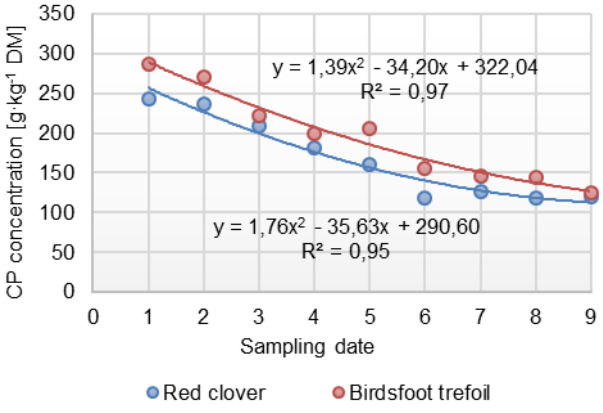

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Fig. 1. Changes in crude protein concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 1. Zmiany zawartości białka ogólnego w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

Red clover, regardless of the date of harvest, contained more WSC than birdsfoot trefoil. In the first date of harvest WSC concentration in red clover was 102.6 g·kg^{-1} DM but in birdsfoot trefoil only 86.6 g·kg^{-1} DM. In the next dates of harvest the increase of its concentrations was observed (Fig. 2).

With increasing concentration of WSC and a decline in crude protein an increase of the WSC/protein ratio was observed. For herbage of birdsfoot trefoil it was 0.3 (the first date) and 0.90 (the last date). In case of red clover WSC/protein ratio was higher and ranged from 0.42 (the first date) to 1.26 (the last date).

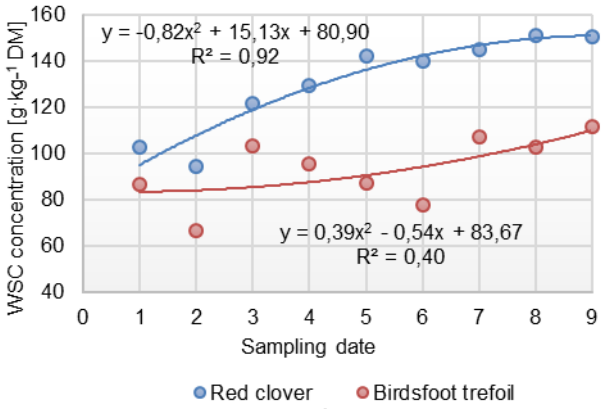

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Fig. 2. Changes in WSC concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 2. Zmiany zawartości cukrów prostych w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

Cell wall composition was shaped inversely to the total protein content. Regardless of the legume species, the lowest concentration of NDF and ADF fraction was observed in the first term of harvest. In this term red clover contained 328.3 g·kg^{-1} DM and 251.8 g·kg^{-1} DM of NDF and ADF fraction, respectively. The mean concentrations of both NDF and ADF as fractions in birdsfoot trefoil were lower. Birdsfoot trefoil contained 318.6 g·kg^{-1} DM and 246.1 g·kg^{-1} DM of NDF and ADF fraction respectively. In the next terms of harvest the increase of NDF and ADF concentrations was observed (Fig. 3 and 4). The highest dynamics of the NDF and ADF content in herbage of both species was observed between the fifth and the sixth term of harvest, in the same time when the decline of crude protein was noted. In this time, regardless of the species, mean daily increase was over 5.0 g and 4.0 g of NDF and ADF content respectively. From that moment the growth rate of NDF concentration in red clover clearly decreased, and in the last three terms of harvest it almost stopped. In the case of birdsfoot trefoil NDF concentration was increasing systematically, reaching the value of 432.8 g·kg^{-1} DM in the last term. The rate of ADF content increase in both species was similar.

In the first term of harvest hemicelluloses concentration in both species was similar and ranged from 72.5 to 76.5 g·kg^{-1} DM in birdsfoot trefoil and red clover respectively. Further changes in hemicelluloses content depended on the plant species. In the case of birdsfoot trefoil, the growth rate of hemicelluloses content was higher (Fig. 5). The highest hemicelluloses content (117.4 g·kg^{-1} DM) was found in herbage harvested in the 7th date of harvest. The growth rate of hemicelluloses content in red clover was

slower. It steadily increased until the fourth term of harvest reaching over 103.1 g·kg^{-1} DM. Starting from the sixth date of harvest a slow decrease in its concentration was observed.

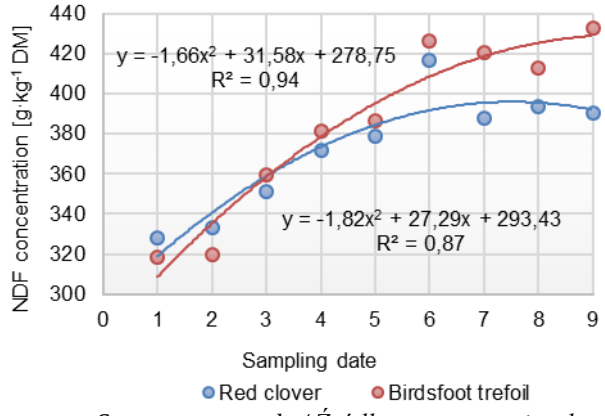

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Fig. 3. Changes in NDF concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 3. Zmiany zawartości NDF w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

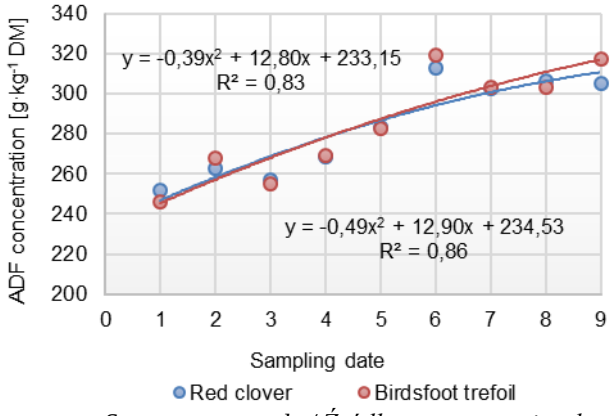

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Fig. 4. Changes in ADF concentrations in red clover and birdsfoot trefoil (mean from 2014-2015)
Rys. 4. Zmiany zawartości ADF w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

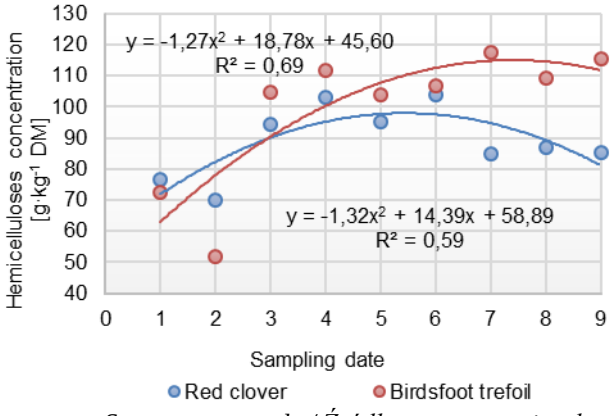

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Fig. 5. Changes in hemicelluloses concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 5. Zmiany zawartości hemicelulozy w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

In the case of changes in cellulose concentration there were no differences among legume species. At the beginning of the experiment the mean concentration of cellulose was 208.0 g·kg^{-1} DM. In the next terms of harvest the increase of its concentrations was observed (Fig. 6). In last term of harvest cellulose concentration was 255.9 g·kg^{-1} DM and 265.8 g·kg^{-1} DM in red clover and birdsfoot trefoil, respectively.

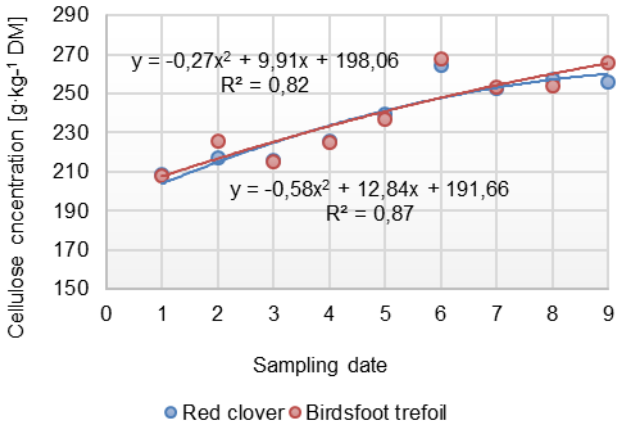

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Fig. 6. Changes in cellulose concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 6. Zmiany zawartości celulozy w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

Mean concentrations of ADL fraction in the first date of harvest was 38.0 and 43.2 g·kg^{-1} DM in birdsfoot trefoil and red clover respectively. In the next terms of harvest slow increase of its content was observed (Fig. 7). The daily increase of ADL concentration in red clover was 0.11 g and in birdsfoot trefoil was higher - 0.24 g.

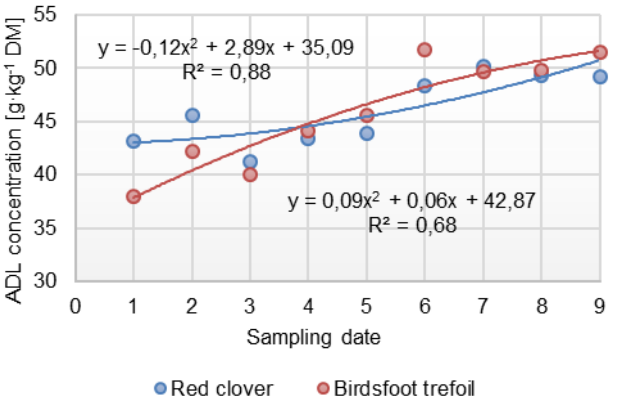

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Fig. 7. Changes in ADL concentrations in red clover and birdsfoot trefoil (mean from 2014-2015)
Rys. 7. Zmiany zawartości ADL w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

Significant correlation between the date of harvest and DM digestibility was found in both legume species. It was the highest in the first date of harvest when it was over 69% and in the next dates it systematically declined, at the same rate in both red clover and birdsfoot trefoil (Fig. 8). In the last date of harvest DM digestibility was 64.2% and 65.1% in birdsfoot trefoil and red clover respectively.

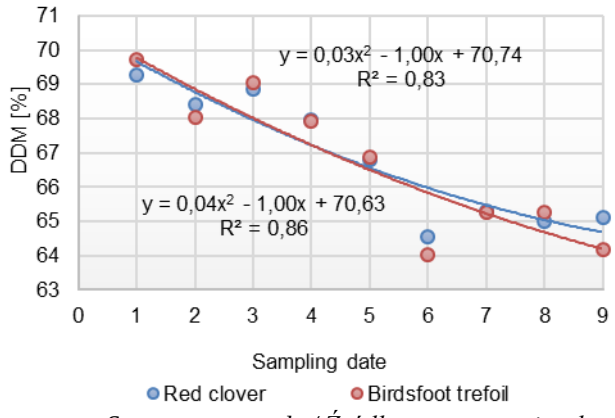

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Fig. 8. Changes in DDM concentrations in red clover and birdsfoot trefoil (means from 2014-2015)
Rys. 8. Zmiany strawności suchej masy w koniczynie łąkowej i komonicy zwyczajnej (średnie z lat 2014-2015)

4. Discussion

As hypothesized, changes in herbage chemical composition during harvest at different dates were observed. The content of crude protein and in vitro dry matter digestibility are two the most reliable quality parameters [26]. Their value decreases with age, as a result of a decreasing amount of leaves in relation to stems and the process of lignification, which are typical for all perennial legumes. The leaf contains more protein than the stem and that is why plants in earlier stages of growth contain more protein than those aging. As expected in this study the crude protein content was the highest in the first date of harvest (in the end of April and at the begging of May), declined in both species over prolonged growth and was the lowest in June.

Cell walls consist of cellulose, hemicelluloses, lignins and pectins. These compounds can be expressed as NDF. The potential intake of forage is closely related to NDF content. ADF is composed of cellulose and lignins, and there is an inverse relationship between ADF and digestibility. With increased contents of lignins and structural polysaccharides (cellulose) a decline in digestibility occurs. Cell wall content is higher in older herbage [19], as shown in our study.

The increase of ADF, ADF and ADL fractions in the following dates of harvest were observed. As expected, while contents of fiber compounds increased DM digestibility decreased in both species in the following terms of harvest.

Changes in the components shaping the herbage quality depended mainly on the leaf/stem ratio. The increase of NDF content with plant maturity occurs because of stem mass accumulation that exceeds leaf mass gain [2].

Our findings concerning the rate of changes in chemical composition of legumes herbage during harvest at different dates of harvest are in agreement with results of research on red clover [7, 10, 11, 18, 29], birdsfoot trefoil [12] and alfalfa [1].

Changes in quality parameters in relation to growth rate also differed among the legume species. Both species vary in morphology, resulting in widely different leaf-to-stem ratios which could affect different concentration of nutrients in examined dates of harvest. Birdsfoot trefoil herbage, regardless of its date of harvest, contained more protein than red clover. Moreover according to information given in literature [30] the protein in birdsfoot trefoil is more effectively utilised by ruminants than the protein in red clover. Thanks to the presence of condensed tannins in birdsfoot trefoil which may form stable complexes with dietary protein in the rumen, the proportion of bypass protein increases.

The differences in the speed of NDF increase between red clover and birdsfoot trefoil were observed. The rate of NDF increase with maturity is slower in red clover because it develops slower than birdsfoot trefoil in spring, resulting in a higher leaf/stream ratio in birdsfoot trefoil than in red clover after prolonged growth. When birdsfoot trefoil was in generative stage of growth red clover was still partly in vegetative stage. This is confirmed by other research on red clover [7] that showed that aging does not cause a drastic drop in quality unlike what is observed in other perennial legumes (such as alfalfa and yellow trefoil). This is due to a higher portion of leaves and lower contents of cell wall and lignin.

Birdsfoot trefoil had a higher rate of change of NDF with time than red clover, which implies that meadow sward with high proportion of birdsfoot trefoil would need to be harvested at the optimal moment to avoid loss in nutritive value during prolonged growth.

In our study some change rates were affected by year. It resulted from the fact that plants of the same species being in the same stage of growth can vary in nutrients content depending on daily temperature and availability of water.

5. Conclusions

Regardless of the date of harvest birdsfoot trefoil contained more crude protein, NDF and ADF fraction and less WSC than red clover.

Crude protein concentration and DDM declined while WSC, NDF, ADF and ADL increased with advancing maturity of both legume species.

The changes in nutritive value in red clover were smaller than those in birdsfoot trefoil.

6. References

[1] Adiyaman E., Ayhan V.: Investigation of feed value of alfalfa (*Medicago sativa* L.) harvested at different maturity stages. Legume Research - An International Journal, 2016, 39. 10.18805/lr.v0iOF.9292.

[2] Buxton D.R.: Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology, 1996, 59, 37-49.

[3] Collins R.P., Marshall A.H., Michaelson-Yeates T.P.T., Williams T.A., Olyott P., Latypova G., Fothergill M., Abberton M.T.: Developing the role of Lotus species in UK grasslands. M. Wachendorf, A. Helgadottir, G. Parente (Eds.), Proceedings of the 2nd COST 852 Workshop, Grado, Italy, 2006, 117-120.

[4] Copani G., Niderkorn V., Anglard F., Quereuil A., Ginane C.: Silages containing bioactive forage legumes: a promising protein-rich feed source for growing lambs. Grass and Forage Science, 2016, 71, 622-631.

[5] Dewhurst R.: Milk production from silage: Comparison of grass, legume and maize silages and their mixtures. Agricultural and Food Science, 2013, 22, 1, 57-69.

[6] Dewhurst R.J, Delaby L, Moloney A., Boland T., Lewis E.: Nutritive value of forage legumes used for grazing and silage. Irish Journal of Agricultural and Food Research, 2009, 48, 167-187.

[7] Elgersma A., Søegaard K.: Changes in nutritive value and herbage yield during extended growth intervals in grass-legume mixtures: effects of species, maturity at harvest, and relationships between productivity and components of feed quality. Grass and Forage Science, 2018, 73,78-93.
[8] Eriksen J., Askegaard M., Søegaard K.: Complementary effects of red clover inclusion in ryegrass-white clover swards for grazing and cutting. Grass and Forage Science, 2014, 69, 2, 241-250. https://doi.org/10.1111/gfs.12025.
[9] Falkowski M., Kukułka I., Kozłowski S.: Właściwości chemiczne roślin łąkowych. Wyd. AR Poznań, 2000, 132.
[10] Fychan R., Sanderson R., Marley C.: Effects of harvesting red clover/ryegrass at different stage of maturity on forage yield and quality. Grassland Science in Europe, 2016, Vol. 21, 323-325.
[11] Ignatovic S., Vucetic J., Lugic Z., Dinic B.: Effect of growth stage on macro and trace elements content in red and white Clover. Journal of Scientific Agricultural Research, 2001, 62, 220, 309-316.
[12] Karabulut A., Canbolat O., Kamalak A.: Effect of maturity stage on the nutritive value of birdsfoot trefoil (*Lotus corniculatus* L) hays. Lotus Newsletter, 2006, Vol. 36 (1), 11-21.
[13] Kleen, J., Taube F., Gierus M.: Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. The Journal of Agricultural Science, 2011, 149, 1, 73–84. https://doi.org/10.1017/S0021859610000456.
[14] Kuoppala K., Ahvenjärvi S., Rinne M., Vanhatalo A.: Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics, Journal of Dairy Science, 2009, Vol. 92, 11, 5634-5644.
[15] Lee M.R.F., Olmos Colmenero J.J., Winters A.L., Scollan N.D., Minchin F.: Polyphenol oxidase activity in grass and its effect on plant-mediated lipolysis and proteolysis of *Dactylis glomerata* (cocksfoot) in a simulated rumen environment. J. Sci. Food Agric., 2006, 86, 1503-1511.
[16] Lüscher A., Mueller Harvey I., Soussana J.F., Rees R.M., Peyraud J.L.: Potential of legume-based grassland-livestock systems in Europe: a review. Grass and Forage Science, 2014, 69, 206-228. doi:10.1111/gfs.12124.
[17] Łyszczarz R.: Wpływ terminu zbioru na wybrane cechy dwóch odmian kupkówki pospolitej. Biuletyn IHAR, 2003, 225. 139-149.
[18] Marković, J., Štrbanović, R., Terzic, D., Stanisavljević, R., Djokic, D., Vasic T. Andjelkovic B.: Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the stage of growth. Biotechnology in Animal Husbandry, 2011, 27. 1563-1569. 10.2298/BAH1104563M.
[19] Minson D.J.: Forage in ruminant nutrition. New York: 1990, Academic Press.
[20] Naadland, S. S., Steinshamn H., Krizsan S. J., Randby Å T.: Effect of organic grass-clover silage on fiber digestion in dairy cows. Animal, 2017, 11, 6, 1000-1007. https://doi.org/10.1017/S1751731116002421.
[21] Pelletier S., Tremblay G.F., Bélanger G., Bertrand A., Castonguay Y., Pageau D., Drapeau R.: Forage nonstructural carbohydrates and nutritive value as affected by time of cutting and species. Agron. J., 2010. 102:1388-1398. doi:10.2134/agronj2010.0158.
[22] PN-EN ISO 12099:2010. 2013: Pasze, ziarno zbóż i produkty przemiału - Wytyczne stosowania spektrometrii bliskiej podczerwieni, 38.
[23] Poetsch E.M., Resch R., Krautzer B.: Variability of forage quality between and within three maturity groups of *Lolium perenne* L. during the first growth. Grassland Science in Europe, 2016, Vol. 21, 293-295.
[24] Rinne M., Nykänen A.: Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. Agricultural and Food Science in Finland, 2000, 9 (2), 121-134.
[25] Szkutnik J., Kacorzyk P., Szewczyk W.: Zmiana zawartości białka ogólnego i włókna surowego w zależności od poziomu nawożenia i fazy rozwojowej traw. Grassland Science in Europe, 2012, 15, 185-191.
[26] Taylor N.L., Quesenberry K.H.: Red Clover Science. Current Plant Sciences and Biotechnology in Agriculture, 1996, 28, 65-67.
[27] Turner S-A., Waghorn G.C., Woodward S.L., Thomson N.A.: Condensed tannins in birdsfoot trefoil (Lotus corniculatus) affect the detailed composition of milk from dairy cows. Proceedings of the New Zealand Society of Animal Production, 2005, 65, 283-289.
[28] Van Ranst G., Lee M.R.F., Fievez V.: Red clover polyphenol oxidase and lipid metabolism. Animal, 2011, 5, 512-521.
[29] Vasiljević S., Ćupina B., Krstic D., Pataki I., Katanski S., Milošević B.: Seasonal changes of proteins, structural carbohydrates, fats and minerals in herbage dry matter of red clover (Trifolium pratense L.). Biotechnology in Animal Husbandry, 2011, 27. 10.2298/BAH1104543V.
[30] Woodward S.L., Waghorn G., Laboyrie P.G.: Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows. Proceedings of the New Zealand Society of Animal Production, 2004, 64, 160-164. doi:10.1002/9780470774793.notes.

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