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CHEMICAL COMPOSITION OF GREEN FORAGE IN RELATION TO LEGUME PLANT SPECIES AND ITS SHARE IN THE MEADOW SWARD

Summary

The aim of the study was to evaluate the impact of various percentage shares of legumes in the sward on the nutritional value of forage. Two species of legume plants were used in this experiment: red clover (Trifolium pratense L.) variety Chlumecky and birdsfoot trefoil (Lotus corniculatus L.) variety Leo. The research material consisted of green forage samples in which percentage shares of tested species of legume plant ranged from 0% to 100%. The content of organic and mineral components in green forage was evaluated. The relation between the percentage shares of red clover and birdsfoot trefoil and the content of nutritive components in green forage was calculated. Forages with birdsfoot trefoil in them, regardless of its percentage share, contained more total protein, crude ash and NDF and ADF fibers fraction. In contrast, forages with the participation of red clover contained more sugars, Ca, Mg and Mn, and were characterized by higher digestibility of organic matter. The proportions of both species also had a significant impact on the content of the majority of nutrients. Increasing the proportion of both red clover and birdsfoot trefoil in green forages resulted in a decrease in crude fiber content and its fractions: ADF and NDF. However, the share of both species was positively correlated with the total protein content, crude ash, ADL and organic matter digestibility. A positive correlation was found between the proportion of red clover in mixture and the Ca, Mg and Fe contents. The proportion of birdsfoot trefoil was positively correlated with the content of P, Ca, Mg and Fe.
***Key words**: red clover, birdsfoot trefoil, nutritive value, chemical composition*

ZALEŻNOŚĆ SKŁADU CHEMICZNEGO ZIELONKI OD GATUNKU ROŚLINY BOBOWATEJ I JEJ UDZIAŁU W RUNI ŁĄKOWEJ

Streszczenie

Celem badań była ocena wpływu zróżnicowanego udziału w runi roślin bobowatych na wartość pokarmową zielonki. Badaniami objęto dwa gatunki: koniczynę łąkową (Trifolium pratense L.) odmiany Chlumecky i komonicę zwyczajną (Lotus corniculatus L.) odmiany Leo. Materiał badawczy stanowiły próbki zielonki, w których procentowy udział badanego gatunku rośliny bobowatej wynosił od 0 do 100%. W zielonce oceniano zawartość składników organicznych i mineralnych. Obliczono zależność pomiędzy procentowym udziałem koniczyny łąkowej i komonicy zwyczajnej w zielonce a zawartością poszczególnych składników pokarmowych. Zielonka z udziałem komonicy zwyczajnej, niezależnie od jej procentowego udziału, zawierała więcej białka ogólnego, popiołu surowego oraz frakcji włókna NDF i ADF. Natomiast zielonka z udziałem koniczyny łąkowej zawierała więcej cukrów prostych, Ca, Mg i Mn oraz charakteryzowała się wyższą strawnością masy organicznej. Procentowy udział w zielonce obu gatunków miał też istotny wpływ na zawartość większości składników pokarmowych. Zwiększanie udziału w zielonce, zarówno koniczyny łąkowej jak i komonicy zwyczajnej, powodowało obniżenie zawartości włókna surowego oraz jego frakcji: ADF i NDF. Udział obu gatunków był natomiast dodatnio skorelowany z zawartością białka ogólnego, popiołu surowego, ADL i strawnością masy organicznej. Stwierdzono ponadto dodatnią korelację pomiędzy udziałem w zielonce koniczyny łąkowej a zwartością Ca, Mg i Fe. Udział komonicy zwyczajnej był dodatnio skorelowany z zawartością P, Ca, Mg i Fe.
***Słowa kluczowe**: koniczyna czerwona, komonica zwyczajna, wartość pokarmowa, skład chemiczny*

1. Introduction

Grassland legumes are important in organic livestock production because of their ability to fix atmospheric N_2, high yield without N fertilization and because of their high forage value [6, 10, 13].

Legume plants in comparison to grass species are more rich in mineral nutrients (mainly Ca and Mg), crude protein and contain less fiber, increasing the digestibility of feed. The presence of legumes in the sward improves the animal's performance [5, 3] and has a positive effect on animal products having higher concentration of components such as n-3 fatty acids, and a lower n-6/n-3 ratio, that are considered as beneficial to human health [9].

Therefore, symbiotic N_2 fixation of legumes is widely used to improve the N supply of the organic farming systems. Legumes in mixture with grasses can fix on average 150 kg N $ha\ yr^{-1}$ some of which subsequently becomes available to the accompanying grass [17].

Legume plants differ in terms of nutrition value, agronomic performance, persistence, soil requirements and suitability [11, 14].

Red clover (*Trifolium pratense* L.) is one of the main feed species in most northern European grasslands. Red clover is popular among farmers for its high protein content, high biomass production and good re-growth capability after mowing [11, 5, 7]. For this reason it is widely used for forage or cut and conserved as silage [15]. Widespread

interest in this species stems from the fact that red clover contains high levels of the enzyme polyphenol oxidase (PPO) in comparison with other forage legumes [27]. PPO has beneficial effects in improving nitrogen utilization in ruminants [12] and in protecting lipids from degradation, both 'in silo' as well as in the rumen, leading to a higher output of polyunsaturated fatty acids (PUFA) in ruminant products (meat and milk) [25].

The birdsfoot trefoil (*Lotus corniculatus* L.) is considered as a potentially useful protein source in low input production systems [2]. It is one of the most important species in the legume group of plants necessary to cultivate lawn legume pastures and mixtures. It can be maintained easier and longer, comparing to red clover. This plant is interesting due to its high productivity, quality, resilience to climate change, adaptability to various soil types, and for the ability of the plant to be consumed fresh, as hay and as silage. 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 [28], without adversely affecting milk protein composition [24].

Legume-grass mixtures generally provide more consistent forage yield across a wide range of environments than grass or legume monocultures [20, 23]. Results of many experiments have demonstrated that interseeding a legume into existing grass fields can increase forage DM yield and crude protein content [1], and improve forage nutritive value [23] with a lower decline of quality during plant maturation [16].

The legume proportion is an important aspect determining forage quality as well as agronomic performance of binary mixtures of legumes and grasses [11]. Studies have shown that a 30-50% share of legume plants provides good chemical composition and a high nutritional value of forage for ruminants [13].

The objective of this study was to investigate the effects of forage legumes (*Trifolium pratense* L. and *Lotus corniculatus* L) in binary mixtures with *Poa pratensis* L. on forage quality.

2. Material and methods

The research was carried out in 2014 at the Institute of Technology and Life Sciences in Falenty. The aim of the study was to evaluate the impact of various percentage shares of legume plants on the nutritional value of green forage. 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, belonging to Experimental Farm ITP in Falenty. In spring 2012 the experimental meadow was renovated by undersowing. One part of meadow was renovated with seeds mixture containing birdsfoot trefoil and the second - 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. On May 15th 2014, plant material for the study was prepared. From the growing meadow sward single specimens of red clover, birdsfoot trefoil and grass species (*Poa pratensis* L.) were chosen. Six bi-species mixtures of red clover and grass species and six bi-species mixtures of birdsfoot trefoil and grass species from the prepared plant material were made. The percentage share of legume plants in the mixture was as follows: 0%, 15%, 30%, 45%, 60% and 100%. From each mixture 3 100 gram samples of plant material were collected for chemical analysis.

After drying and grinding the samples, the following nutrients were estimated: total protein, crude fiber and its fractions: NDF, ADF and ADL, crude ash and water soluble sugars (WSC) using the NIRS method [18] on a NIR-Flex N-500 apparatus using ready-made INGOT ® calibrations for meadow hay.

The content of mineral elements (P, K, Ca, Mg, Na, Zn, Mn, Cu, Fe) was also evaluated. The content of minerals in the plant material was determined after its mineralization in a mixture of concentrated acids: nitric, perchloric and sulfuric. The content of potassium was determined by the emission method, the phosphorus content by the colorimetric method, and the content of Ca, Mg, Na, Zn, Mn, Cu and Fe by atomic absorption spectrometry (ASA).

The obtained data were subject to statistical evaluation. Significance of differences between means were verified with the T-Tukey test (HSD). The determined level of significance was 0.05. The correlations between the percentage share of red clover and birdsfoot trefoil in green forage and the content of the examined nutrients were calculated. The calculations were performed using Statistica software version 6 (Statsoft, Poland). In addition, regression curves between the share of legumes in mixture and the content of chosen nutrients were drawn.

3. Results and discussion

3.1. Content of organic nutrients

Protein is the basic nutrient supplied to animals in green forage. Green forage mixtures with birdsfoot trefoil, regardless of its percentage share, contained more total protein ($p \leq 0.05$) than green forage mixture with red clover (Table 1). Moreover, according to information given in literature [28] the protein in birdsfoot trefoil is less readily broken down by microbes in the rumen (a bypass protein), its protein is utilized more effectively by ruminants than the protein in red clover.

Table 1. Mean concentration of nutritive components in green forage with participation of legume plants: red clover and birdsfoot trefoil (average ± standard deviation)
Tab. 1. Średnia zawartość składników pokarmowych w zielonkach z udziałem roślin bobowatych: koniczyny łąkowej i komonicy zwyczajnej (średnia ±odchylenie standardowe)

Examined parameters	Green forage mixtures with	
	red clover	birdsfoot trefoil
Total protein [g kg^{-1} DM]	134.5± 27.6a	150.5±39.8b
Crude fibre [g kg^{-1} DM]	246.9±31.7a	252.8±35.4a
Crude ash [g kg^{-1} DM]	86.6±7.8a	93.5±8.6b
NDF [g kg^{-1} DM]	442.3±58.2a	454.1±60.3b
ADF [g kg^{-1} DM]	289.9±18.9a	295.4±22.5b
ADL [g kg^{-1} DM]	34.7±1.7a	35.6±2.3a
WSC [g kg^{-1} DM]	158.5±9.5b	132.4±19.5a
OM digestibility [%]	62.9±4.25b	61.4±4.49a
WSC/protein ratio	1.23±0.28b	0.96±0.36a

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

Source: own work / Źródło: opracowanie własne

The share of both legume species was positively correlated with protein content (Table 2), and increased linearly with increasing proportion of legume species (Fig. 1). In grass-red clover mixtures protein concentration ranged from 102.1 (±9.2) g·kg^{-1} DM to 182.1 (±12.5) g·kg^{-1} DM in mixtures with 0% and 100% share of red clover respectively. Total protein concentration in grass-birdsfoot trefoil mixtures increased linearly to a level of 218.8 (±16.0) g·kg^{-1} DM in mixtures with 100% share of this species.

Table 2. Matrix of correlation coefficients between percentage of red clover and birdsfoot trefoil in mixture with grasses and chemical composition of green forage

Tab. 2. Macierz współczynników korelacji pomiędzy procentowym udziałem koniczyny łąkowej i komonicy zwyczajnej w mieszance z trawami a składem chemicznym zielonki

Examined parameters	Green forage mixtures with	
	red clover	birdsfoot trefoil
Total protein [g kg^{-1} DM]	0.95*	0.97*
Crude fibre [g kg^{-1} DM]	-0.96*	-0.99*
Crude ash [g kg^{-1} DM]	0.73*	0.84*
NDF [g kg^{-1} DM]	-0.96*	-0.98*
ADF [g kg^{-1} DM]	-0.92*	-0.96*
ADL [g kg^{-1} DM]	0.85*	0.61*
WSC [g kg^{-1} DM]	-0.25	-0.69*
OM digestibility [%]	0.94*	0.94*
WSC/protein ratio	-0.86*	-0.91*

** significant at α=0.05*

Source: own work / Źródło: opracowanie własne

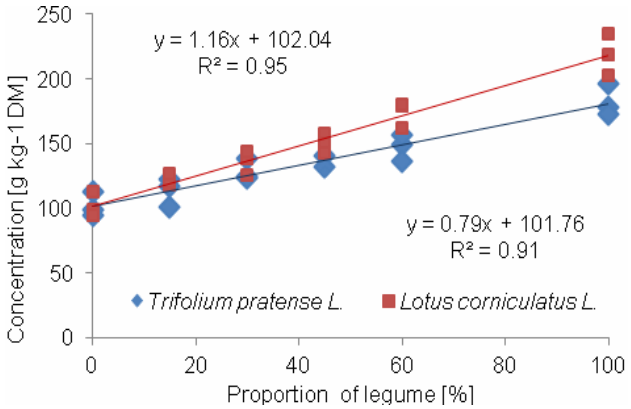

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Fig. 1. Total protein content in relation to a share of red clover and birdsfoot trefoil in mixture

Rys. 1. *Zawartość białka ogólnego w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

Minimum content of total protein in forage, which determines a relatively correct course of digestion in the digestive tract for high-yielding dairy cows should range from 150 to 170 g kg^{-1} DM of forage. Taking into account the ruminants needs in terms of protein content in forage, the optimal content of this nutrient was guaranteed by 30-40% share of birdsfoot trefoil and as much as a 60-80% share of red clover (Fig. 1).

The presence of structural carbohydrates, so-called crude fiber is the factor limiting the nutritional value of forage. Crude fiber content in examined forage mixtures was similar (p>0.05) and decreased linearly with increasing proportions of both legume species (Fig. 2). In mixtures with a 0% share of legumes the fiber concentration was 292.2 (±5,2) g·kg^{-1} DM. In grass-clover mixtures with a 100% share of legumes it reached 194.5 (±9.0) g·kg^{-1} DM and 196.0 (±22.2) g·kg^{-1} DM for red clover and birdsfoot trefoil respectively.

For a balanced level of nutrient digestibility and energy value of feeds, the concentration of fiber should not exceed 280-300 g·kg^{-1} DM of forage. In this aspect, all tested mixtures were characterized by optimal average concentration of crude fiber.

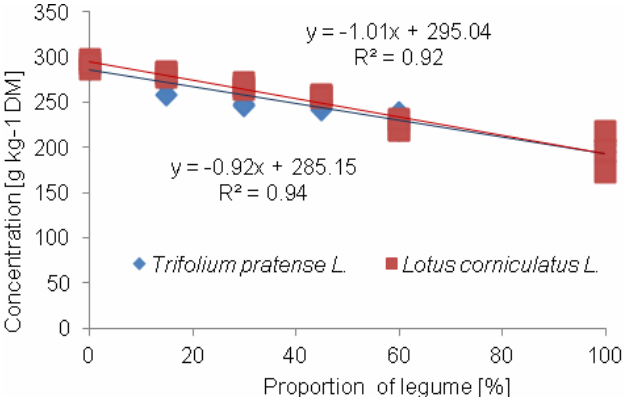

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Fig. 2. Crude fiber content in relation to a share of red clover and birdsfoot trefoil in mixture

Rys. 2. *Zawartość włókna surowego w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

Grass mixtures with birdsfoot trefoil were characterized by a higher concentration ($p \leq 0.05$) of NDF and ADF fractions than grass mixtures with red clover. In both cases NDF and ADF concentrations were negatively correlated with the percentage share of legumes in mixtures (Table 2). With increasing proportions of legumes in mixtures the NDF and ADF fractions concentration in forage decreased linearly (Fig. 3, 4). Mean concentrations of ADL fraction in examined forage mixtures were similar ($p \leq 0.05$) and were positively correlated with increasing proportions of both legume species (Table 2).

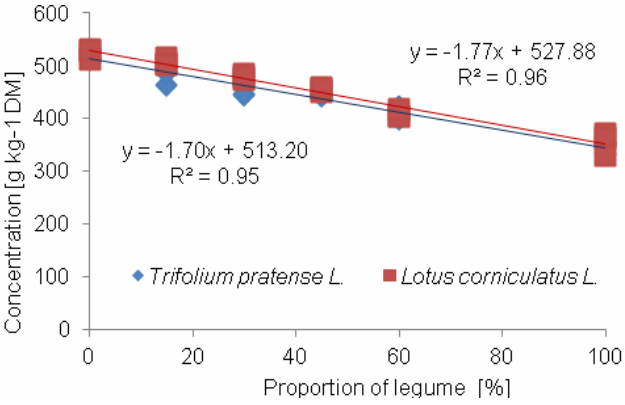

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Fig. 3. NDF fraction content in relation to a share of red clover and birdsfoot trefoil in mixture

Rys. 3. *Zawartość frakcji NDF w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

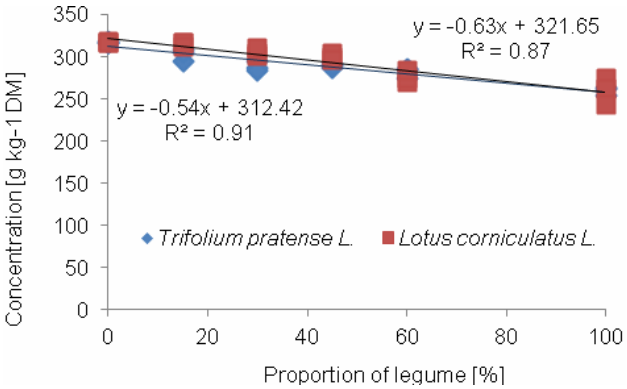

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Fig 4. ADF fraction content in relation to a share of red clover and birdsfoot trefoil in mixture
Rys. 4. *Zawartość frakcji ADF w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

Organic matter digestibility was significantly higher ($p \leq 0.05$) in grass-red clover mixtures (62.9% ±4.25) than in mixtures with birdsfoot trefoil (61.4% ±4.49). Significant correlation between the percentages of legumes in mixtures and OM digestibility was found ($p \leq 0.05$) in both mixtures with birdsfoot trefoil and mixtures with red clover. With increasing proportion of legume species the OM digestibility increased reaching in pure mixtures 65.4% (±1.68) and 65.8% (±1.38) for red clover and birdsfoot trefoil respectively.

The crude ash content was affected both by the legume species and its proportion in the mixture. Green forage mixtures with birdsfoot trefoil contained more crude fiber ($p \leq 0.05$) than green forage mixture with red clover (Table 1). The share of both legume species was positively correlated with the crude ash content (Table 2). Crude ash concentration in legume-grass mixtures increased linearly with increasing proportions of legume plants in mixtures (Fig. 5) reaching the concentration 97.6 g·kg^{-1} DM and 105.9 g·kg^{-1} DM for mixtures with 100% share of red clover and birdsfoot trefoil respectively.

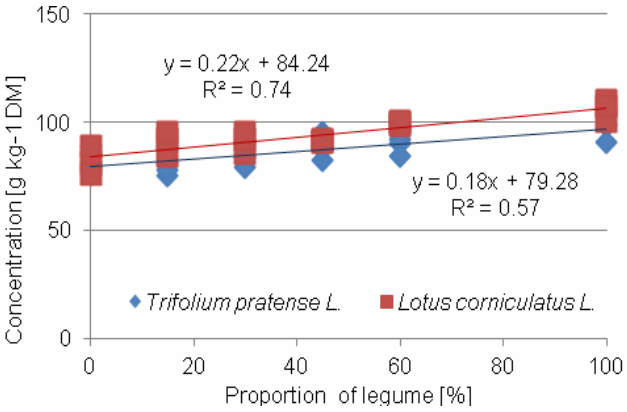

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Fig. 5. Crude ash in relation to a share of red clover and birdsfoot trefoil in mixture
Rys. 5. *Zawartość popiołu surowego w zależności od koniczyny łąkowej i komonicy zwyczajnej w mieszance*

Green forage mixtures with red clover, regardless of its percentage share, contained more WSC than green forage mixture with birdsfoot trefoil ($p \leq 0.05$, Table 1). In pure grass mixtures the WSC concentration was 155.8 (±13.3) g·kg^{-1} DM. In grass-clover mixtures with 100% share of legumes WSC its concentration dropped to 152.4 (±9.1) g·kg^{-1} DM and to 105.7 (±2.4) g·kg^{-1} DM for red clover and birdsfoot trefoil respectively. High content of total protein and low content of WSC caused significantly lower WSC/protein ratios in mixtures with birdsfoot trefoil than in mixtures with red clover ($p \leq 0.05$), (Table 1). A reduced ratio of WSC to total protein may indicate that this material is less suitable for ensiling.

3.2. Content of mineral nutrients

The legume plant species influenced the concentration of the majority of the assessed mineral nutrients (Table 3). The comparison of mean concentration of macro- and micronutrients showed that green forage mixture with red clover contained more ($p \leq 0.05$) Ca, Mg and Mn. The presence of birdsfoot trefoil in the mixture caused higher concentration ($p \leq 0.05$) of K, Zn and Cu. No differences in concentration of P, Na and Fe between both species were found.

Table 3. Mean concentration of mineral components in green forage with participation of legume plants: red clover and birdsfoot trefoil (average ± standard deviation)
Tab. 3. Średnia zawartość składników mineralnych w zielonkach z udziałem roślin bobowatych: koniczyny łąkowej i komonicy zwyczajnej (średnia ± odchylenie standardowe)

Examined parameters	Green forage mixtures with	
	red clover	birdsfoot trefoil
P [g kg^{-1} DM]	3.24±0.13a	3.31±0.28a
K [g kg^{-1} DM]	20.30±3.66a	27.4±2.23b
Ca [g kg^{-1} DM]	2.05±1.26b	1.47±0.81a
Mg [g kg^{-1} DM]	3.94±1.35b	2.86±0.63a
K:Mg	9.64±4.72a	15.91±2.74b
K:(Ca + Mg)	5.92±3.99a	9.24±2.88b
Na [g kg^{-1} DM]	0.15±0.07a	0.13±0.02a
Zn [ppm]	34.25±13.03a	46.32±14.99b
Mn [ppm]	56.44±16.20b	38.70±14.75a
Cu [ppm]	6.00±4.86a	14.62±7.57b
Fe [ppm]	54.81±27.05a	50.51±11.98a

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

Source: own work / Źródło: opracowanie własne

The proportion of both species in mixtures also had a significant impact on the content of the majority of mineral nutrients (Table 4). A positive correlation was found between the proportion of red clover in mixture and the Ca (r=0.98), Mg (r=0,96) and Fe (r=0.85) contents. The proportion of birdsfoot trefoil was positively correlated with the content of P (r=0.59), Ca (r=0.94), Mg (r=0.98) and Fe (r=0.81). A negative correlation was found between proportion of red clover and K, Na and Mn concentrations, and between proportion of birdsfoot trefoil and Mn concentration.

To cover the nutritional needs of dairy cows good quality forage should contain: 2.8–3.6 g·kg^{-1} P, 20.0 g·kg^{-1} K, 7.0 g·kg^{-1} Ca and 2.0 g·kg^{-1} Mg [8]. Taking into account the given above values it can be concluded, that forage mixtures, with red clover or birdsfoot trefoil, had optimal concentrations of P, K, and Mg, and to low Ca and Na.

The mean contents of microelements were similar to values obtained in red clover by Radkowski and Radkowska [19] and in most cases they were close to the optimal value.

Table 4. Matrix of correlation coefficients between percentage of red clover and birdsfoot trefoil in mixture with grasses and chemical composition of green forage
Tab. 4. Macierz współczynników korelacji pomiędzy procentowym udziałem koniczyny łąkowej i komonicy zwyczajnej w mieszance z trawami a składem chemicznym zielonki

Examined parameters	Green forage mixtures with	
	red clover	birdsfoot trefoil
P [g kg^{-1} DM]	0.19	0.59*
K [g kg^{-1} DM]	-0.73*	0.33
Ca [g kg^{-1} DM]	0.98*	0.94*
Mg [g kg^{-1} DM]	0.96*	0.98*
K:Mg	-0.95*	-0.72*
K:(Ca + Mg)	-0.96*	-0.96*
Na [g kg^{-1} DM]	-0.55*	-0.06
Zn [ppm]	0.25	0.33
Mn [ppm]	-0.61*	-0.91*
Cu [ppm]	-0.30	0.17
Fe [ppm]	0.85*	0.81*

* significant at α=0.05

Source: own work / Źródło: opracowanie własne

In addition to the mineral and organic composition of forage, the molar ratios: K:Mg and K:(Ca+Mg) are also important. The K:Mg ratio in the examined green forage mixtures with birdsfoot trefoil, was higher than the optimal recommended value 6-8.3 [4, 26]. In grass-red clover mixtures it was achieved in mixtures with a 45-60% share of red clover.

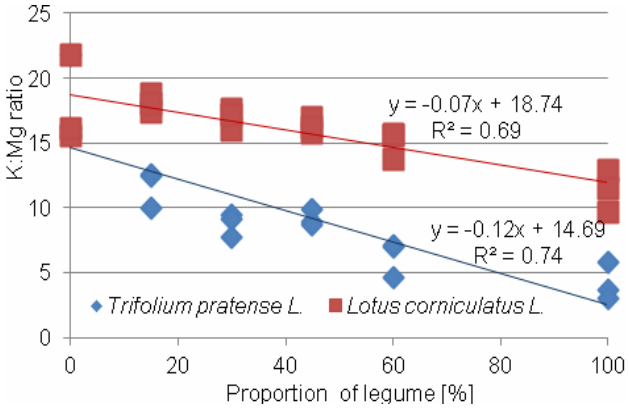

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Fig 6. Molar ration K:Mg in relation to a share of red clover and birdsfoot trefoil in mixture
Rys. 6. *Stosunek molowy K:Mg w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

Molar ratio of K:(Ca+Mg) in mixtures with red clover was lower ($p \leq 0.05$) and varied from 1.92 (100% of red clover) to 13.50 (100% of grass). In mixtures with birdsfoot trefoil this ratio was higher (Fig. 7) and in relation to species proportion in mixture ranged from 5.25 (100% of birdsfoot trefoil) to 13.50 (100% of grass). According to literature data [21], the optimal range of molar ratio K:(Ca+Mg) (1.9-2.2) can be obtained from grass-clover mixtures in which clover's share is above 45%. Due to the extremely deficient content of Ca in plants the range of molar ratios was close to optimal only in mixtures with a 100% share of red clover (Fig. 7).

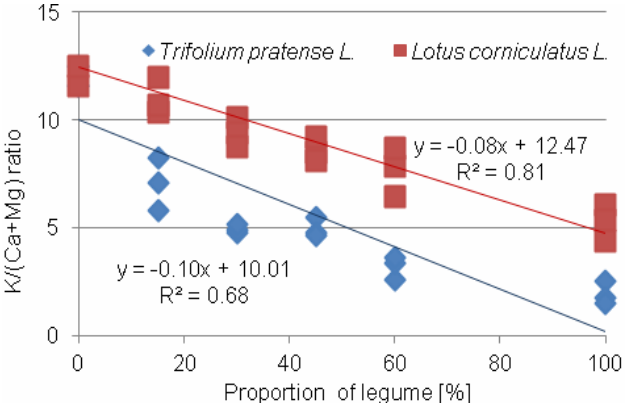

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Fig. 7. Molar ration K:(Ca+Mg) in relation to a share of red clover and birdsfoot trefoil in mixture
Rys. 7. *Stosunek molowy K:(Ca+Mg) w zależności od udziału koniczyny łąkowej i komonicy zwyczajnej w mieszance*

In this study, the effect of legume species on nutritive value of green forage mixtures was statistically proven. Significant differences ($p \leq 0.05$), between green forage with red clover and forage with birdsfoot trefoil, in most examined parameters were observed. Green forage mixtures with birdsfoot trefoil, regardless of its percentage share, contained more total protein, crude ash and were characterized by a higher concentration of NDF and ADF fraction. While green forage with red clover contained more WSC and was characterized by a higher digestibility of organic matter and higher WSC/protein ratio, considered as more favorable for the process of ensiling. Red clover also contained more Ca and Mg. Both species vary in morphology, resulting in widely different leaf-to-stem ratios which could affect different concentration of nutrients in examined mixtures.

The proportions of red clover and birdsfoot trefoil also had a significant impact on the content of the majority of nutrients. Increasing proportions in green forage of both species resulted in an increase in total protein, crude ash, and OM digestibility, and decrease in crude fiber content and its fractions: ADF and NDF, and WSC.

According to a previous study [21] the best chemical composition and the highest nutritional value of grass-clover mixtures is obtained when the share of red clover in the yield is 50%. According to Staniak [22] mixtures with 60 and 80% share of clover were best balanced in terms of protein and fiber content. In our study the optimal content of protein was guaranteed by a 30-40% share of birdsfoot trefoil and as much as a 60-80% share of red clover.

4. Conclusions

The nutritional value of green fodders depended both on the proportion of legumes in the sward and the legume species.

Birdsfoot trefoil contained more protein and only a 30-40% share of this species was needed to guarantee an optimal content of protein in forage.

Grass-clover mixtures, despite lower protein content, were characterized by higher OM digestibility, better suitability for ensiling and were better balanced in terms of mineral content.

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