# Fermentation and Proteolysis During the Ensilage of Wilted and Unwilted Diploid and Tetraploid Red Clover

## Fermentacja i proteoliza procesu kiszenia świeżej i podsuszonej zielonki z di- oraz tetraploidalnych form koniczyny czerwonej

Cezary PURWIN[1], Barbara PYSERA, Krystyna ŻUK-GOŁASZEWSKA, Zofia ANTOSZKIEWICZ, Janusz GOŁASZEWSKI, Maja FIJAŁKOWSKA, Krzysztof LIPIŃSKI

[1]Department of Animal Nutrition and Feed Management Uniwersity of Warmia and Mazury in Olsztyn Ul. Oczapowskiego 5 10-718 Olsztyn, Poland tel. 48 089 5233232 e-mail purwin@uwm.edu.pl

### Abstract

The effects of the following factors were analyzed in the study: wilting degree, genetic form of red clover (2n – 4n), cultivars within the genetic form: 2n (Krynia, Parada), 4n (Jubilatka, Bona) and DM x form, DM x 2n, DM x 4n. Fermentation and proteolysis during the ensilage of red clover were affected primarily by wilting, whereas genetic factors (genetic form, cultivar) exerted a lesser effect. However, the genetic form of red clover affected the true protein content of silage and the extent of proteolysis during the ensiling process. The effect of the genetic form of red clover on the extent of proteolysis in silage (at similar levels of water-soluble carbohydrates and buffering capacity) suggests that diploid and tetraploid red clover cultivars differ with respect to chemical properties (poliphenol oxidase activity, polyphenol content) affecting proteolysis.

**Keywords:** red clover, genetic forms, wilting, silage, biogenic amines.

### Streszczenie

Analizowano wpływ podsuszenia surowca, formy genetycznej koniczyny czerwonej (2n – 4n), odmiany w obrębie danej formy genetycznej: 2n (Krynia – Parada), 4n (Jubilatka – Bona) oraz SM x forma, SM x 2n, SM x 4n. Uzyskane wyniki wykazały dominujący wpływ czynnika podsuszenia nad czynnikami genetycznymi (forma genetyczna, odmiana) na przebieg fermentacji i zakres proteolizy podczas zakiszania koniczyny czerwonej. Stwierdzono jednak wpływ formy genetycznej koniczyny na zawartość białka właściwego w kiszonkach oraz stopień proteolizy w trakcie zakiszania. Stwierdzony wpływ formy genetycznej koniczyny na zakres proteolizy w kiszonkach (przy zbliżonym udziale cukrów rozpuszczalnych i pojemności buforowej zakiszanych zielonek) pozwala przypuszczać, że występuje

zróżnicowanie innych chemicznych cech zielonek wpływających na proteolizę (aktywność PPO, zawartość polifenoli) między formami genetycznymi i odmianami.

**Słowa kluczowe:** koniczyna czerwona, formy genetyczne, podsuszanie, kiszonki , aminy biogenne

## Streszczenie szczegółowe

Cechy morfologiczne odmian koniczyny (udział liści i łodyg w plonie zielonki) ze względu na zróżnicowaną zawartość białka i jego frakcji azotowych mogą wpływać na tempo i zakres hydrolizy w trakcie zakiszania, a także na zawartość cukrów rozpuszczalnych w wodzie. Przebieg fermentacji i tempo zakwaszenia środowiska może mieć wpływ na intensywność proteolizy. Celem badań była weryfikacja hipotezy roboczej zakładającej, że formy koniczyny czerwonej zróżnicowane pod względem ploidalności i odmian rolniczych, a także zawartością suchej masy w zielonce (podsuszanie) determinują przebieg procesu fermentacji i zakres zmian w składzie frakcji azotowych kiszonek. Zielonki badanych odmian zakiszano w 3 powtórzeniach w mikrosilosach (10 l) w stanie naturalnej wilgotności (kiszonka z surowca niepodsuszonego – U) oraz po 24 godzinach podsuszania na pokosach (kiszonka z surowca podsuszonego – W). Porównania między rodzajem surowca do kiszenia (stopień podsuszenia), formami genetycznymi di- i tetraploidalnymi oraz między odmianami w obrębie form genetycznych wykonano metodą kontrastów. Proces zakiszania zielonek wszystkich odmian koniczyny nie wpłynął na zawartość białka ogólnego, natomiast spowodował znaczne obniżenie zawartości białka właściwego. Podsuszenie zielonek z koniczyny istotnie różnicowało w kiszonkach zawartość wszystkich oznaczanych parametrów chemicznych z wyjątkiem kwasu mlekowego.

Uzyskane wyniki wykazały dominujący wpływ czynnika podsuszenia nad czynnikami genetycznymi (forma genetyczna, odmiana) na przebieg fermentacji i zakres proteolizy podczas zakiszania koniczyny czerwonej. Odnotowano częściowy wpływ formy genetycznej koniczyny na zawartość białka właściwego w kiszonkach oraz stopień proteolizy w trakcie zakiszania. Nie wykazano natomiast zróżnicowania między kiszonkami z form di- i tetraploidalnych dla pH, zawartości kwasów, WSC, ADF, N-ogólnego, tyraminy i putrescyny. Kiszonka z odmian diploidalnych miała większą ilość popiołu surowego, BAW, azotu amonowego, etanolu, i kadaweryny podczas gdy kiszonka z form tetraploidalnych większe zawartości SM, substancji organicznej, tłuszczu surowego i włókna surowego, NDF i histaminy. Zróznicowanie między odmianami w obrębie obu form dotyczyło zawartości kwasu masłowego, tyraminy i kadaweryny. Stwierdzono interakcje między stopniem podsuszenia zielonki (świeża, podsuszana) a formami genetycznymi koniczyny czerwonej. Istotne oceny efektów interakcji dla tłuszczu surowego, kwasu masłowego, NDF, histaminy, tyraminy, putrescyny i kadaweryny wskazują na zróżnicowanie tych cech między formami di- i tetraploidalnych w zależności od stopnia wilgotności (podsuszenia) zielonki. Stwierdzony wpływ formy genetycznej koniczyny na zakres proteolizy w kiszonkach (przy zbliżonym udziale cukrów rozpuszczalnych i pojemności buforowej zakiszanych zielonek) pozwala przypuszczać, że występuje zróżnicowanie innych

chemicznych cech zielonek wpływających na proteolizę (aktywność PPO, zawartość polifenoli) między formami genetycznymi i odmianami.

## Introduction

Red clover is one of the most widely used fodder legumes in the moderate climate zone. The crop is known for its high yield and ability to grow in a wide range of soil and environmental conditions, and to fix nitrogen from the air. Red clover is also a source of valuable nutrients for ruminants. This easy-to-root species is characterized by a fast growth rate, a beneficial influence on the soil, a high biomass yield, and an erect growth habit which facilitates handling [23, 25, 27]. However, due to the presence of anti-nutritional components and nutrient losses during haymaking, the use of red clover has decreased in recent years, and the species has largely been replaced by grasses and alfalfa. This trend was accompanied by a steady increase in the use of nitrogen fertilizers in fodder crop cultivation and protein concentrates in ruminant nutrition [9, 12]. The development of organic livestock production and attempts to reduce nitrogen emissions in intensive milk and beef production have again spurred interest in red clover. Another important consideration is progress in research into changes in the composition of nitrogen fractions in silage and their role in microbial protein synthesis in the rumen [3, 4, 13]. Intensive protein hydrolysis (breakdown intro amino acids) at the first stage of ensiling is one of the main reasons for low utilization of nitrogen in the rumen, including readily soluble nitrogen forms from ensiled roughage [18, 24]. Higher efficiency of microbial protein synthesis has been observed in ruminants fed red clover silage, compared with grass silage [10]. In a study by Givens and Rulquin [6], the efficiency of microbial nitrogen synthesis was 34% higher in red clover silage made without additives than in alfalfa silage, which resulted, among others, from differences in the true protein/total protein ratio. High activity of polyphenol oxidase has been shown to reduce protein hydrolysis during the ensilage of red clover [13]. Due to different concentrations of protein and its nitrogen fractions, the morphological traits of red clover cultivars (share of leaves and stems in total green matter yield) may affect the rate and extent of hydrolysis in the ensiling process, and the water-soluble carbohydrate content of silage [28]. The course of fermentation and the rate of acidification during ensiling can influence the extent of proteolysis.

The aim of this study was to verify the working hypothesis that the course of fermentation and changes in the composition of nitrogen fraction in silage may be affected by the genetic form, cultivar and wilting of red clover.

## Materials and Methods

The experimental plant material consisted of the green forage of two diploid and two tetraploid red clover cultivars harvested in the third year of an exact experiment [26, 27]. In the first year of the experiment, spring barley was used as cover crop for red clover. Cultivation measures typical of spring barley were applied. In the spring, at the beginning of the growing season, red clover was top-dressed with 30 kg N·ha$^{-1}$

(ammonium nitrate) 70 kg·ha$^{-1}$ superphosphate supplemented with boron (20% $P_2O_5$) and 60 kg $K_2O$ (potash salt). Red clover was harvested at the beginning of flowering. Red clover was ensiled in three replications: forage with a natural moisture content (unwilted silage – U) and forage wilted for 24 hours (wilted silage – W). The plant material was placed in 10 l microsilos permitting the escape of fermentation gases and percolating juice. Silage samples were collected for chemical analyses after 90 days of storage in silos.

Samples of green forage and silage were dried at 45°C using air-flow driers (BINDER) and ground using a Retsch mill for fibrous materials, with a mesh size of 1 mm. All samples were assayed for proximate chemical composition according to AOAC [1], water-soluble carbohydrate (WSC) content by the anthrone method [22], fiber fractions (NDF, ADF) by the method of Goering and Van Soest [7] using an ANKOM 220 fiber analyzer, and protein nitrogen content with the use of trichloroacetic acid [14]. The buffering capacity of herbage samples was determined before ensiling [15]. The pH of silage was measured with a HI 8314 pH-meter, and the concentrations of lactic, acetic and butyric acid were determined in water extracts by high-performance liquid chromatography using the Shimadzu HPLC system with a VARIAN MetaCarb 67H column. Biogenic amines were determined by high-performance liquid chromatography using the Shimadzu HPLC system with a UV–VIS detector, at a wavelength of 546 nm [11].

The results were verified statistically by an analysis of variance with orthogonal contrasts. In the applied model, the outcome variable were the above chemical properties of green forage and silage, and predictor variables were two diploid cultivars (Krynia and Parada), two tetraploid cultivars (Jubilatka and Bona), and wilting degree (unwilted, wilted). Contrasts were used to compare the effects of wilting degree, the genetic form of red clover (diploid and tetraploid) and cultivars within the genetic form (Diagram 1).

## Results

A decrease in the content of dry matter (DM), water-soluble carbohydrates (WSC) and NDF, and an increase in the content of crude ash, ether extract and ADF (Table 1) were noted during ensilage in all cultivars of uwilted (U) red clover. Such changes in the concentrations of DM, crude ash and ether extract were not observed in green forage and wilted (W) red clover silage. The ensiling process had no effect on total protein content, but it cased an insignificant decrease in true protein content in all red clover cultivars. Wilting had a significant effect on all chemical parameters in red clover silage except for ADF and lactic acid (Tables 2 and 3). The genetic form (ploidy) of red clover affected primarily the proximate chemical composition of silage and structural carbohydrate fractions (DM, crude ash, crude fiber, NDF), while it exerted a lesser effect on the concentrations of fermentation products (ethanol) and nitrogen compounds (N-$NH_3$/total nitrogen, histamine, cadaverine). Diploid red clover cultivars differed with respect to the crude ash content of silage, whereas tetraploid cultivars - with regard to the levels of DM, NDF and ADF. Among the parameters

Diagram 1. Orthogonal contrasts in the analysis of variance of silage made from unwilted and wilted diploid and tetraploid red clover

Diagram 1. Kontrasty ortogonalne w analizie wariancji cech kiszonek z niepodsuszonej i podsuszonej koniczyny czerwonej odmian di- i tetraploidalnych

| Contrasts - Kontrasty | | Unwilted red clover<br>Koniczyna niepodsuszona | | | Wilted red clover<br>Koniczyna podsuszona | | | |
|---|---|---|---|---|---|---|---|---|
| | 2n<br>Krynia | 4n<br>Parada | Jubilatka | Bona | 2n<br>Krynia | 4n<br>Parada | Jubilatka | Bona |
| #1 | wilting degree:<br>unwilted –wilted<br>stopień podsuszenia:<br>niepodsuszona-podsuszona | +1 | +1 | +1 | +1 | -1 | -1 | -1 | -1 |
| #2 | diploid cultivars – tetraploid cultivars<br>odmiany diploidalne-odmiany tetraploidalne | +1 | +1 | -1 | -1 | +1 | +1 | -1 | -1 |
| #3 | diploid cultivars: Krynia – Parada<br>odmiany diploidalne: Krynia – Parada | +1 | -1 | 0 | 0 | +1 | -1 | 0 | 0 |
| #4 | tetraploid cultivars: Jubilatka – Bona<br>odmiany tetraploidalne:<br>Jubilatka – Bona | 0 | 0 | +1 | -1 | 0 | 0 | +1 | -1 |
| #5 | wilting degree x genetic form<br>stopień podsuszenia x forma genetyczna odmian | +1 | +1 | -1 | -1 | -1 | -1 | +1 | +1 |
| #6 | wilting degree x diploid cultivars<br>stopień podsuszenia x odmiany diploidalne | +1 | -1 | 0 | 0 | -1 | +1 | 0 | 0 |
| #7 | wilting degree x tetraploid cultivars<br>stopień podsuszenia x odmiany tetraploidalne | 0 | 0 | +1 | -1 | 0 | 0 | -1 | +1 |

affected by ensiling, diploid cultivars differed with respect to butyric acid concentrations, the protein nitrogen/total nitrogen ratio, and the content of biogenic amines (histamine, tyramine, cadaverine), while tetraploid cultivars - with regard to the content of butyric acid, ethanol and biogenic amines (tyramine, putrescine and cadaverine), and the ammonium nitrogen/total nitrogen ratio. Interactions between DM content and ploidy were observed for the concentrations of crude fat, NDF, butyric acid and all biogenic amines. In diploid cultivars, the moisture content of plant material was significantly correlated with the content of total protein, NDF, histamine, putrescine and cadaverine, while in tetraploid cultivars – with the concentrations of crude ash, fermentation products (acetic acid, butyric acid, ethanol), tyramine, cadaverine and protein nitrogen, and the protein nitrogen/total nitrogen ratio.

The estimates of orthogonal contrast effects for all examined quality traits of silage are presented in Table 3. A comparison of the chemical composition of silage made from unwilted and wilted red clover showed that all estimates except butyric acid content were statistically significant (#1). A comparison of the genetic forms of red clover indicated that silage made from diploid and tetrapolid red clover did not differ with respect to such variables as protein content, pH, fatty acid concentrations, the levels of WSC, ADF, total nitrogen, tyramine and putrescine. Silage made from diploid red clover had a higher content of crude ash, N-free extracts, ammonium nitrogen, ethanol and cadaverine. Silage made from tetraploid red clover had a higher content of DM, organic matter, crude fat, crude fiber, NDF and histamine. Diploid and tetraploid red clover cultivars (#3 and #4) differed with regard to the concentrations of butyric acid, tyramine and cadaverine. Contrast effects #5 involved interactions between the wilting degree of green forage (unwilted, wilted) and the genetic form of red clover. Significant interactions were noted for the content of crude fat, butyric acid, NDF, histamine, tyramine, putrescine and cadaverine, thus pointing to differences in the values of the above parameters between diploid and tetraploid cultivars, determined by the wilting degree of plant material. The values of the analyzed traits were generally lower in wilted silage. The decrease in crude fat content was greater in diploid cultivars, while the decrease in the values of the other parameters was higher in tetraploid cultivars. The only exception was butyric acid content, which was higher in wilted silage made from diploid red clover than in unwilted silage. A reverse trend was observed in tetraploid cultivars. As shown by contrasts 6 and 7, the interaction between the wilting degree of green forage and cultivars within the genetic forms of red clover was also significant.

## Discussion

The green forage of all red clover cultivars was characterized by a comparable content of total protein and WSC, which indicates that fermentation conditions during ensiling were similar [5]. The WSC content determined in silage shows that the presence of WSC was not a limiting factor in fermentation [8]. Since the buffering capacity of green forage was similar, the course of fermentation in unwilted red clover could be affected by the dry matter content of plant material, which was higher in diploid cultivars. However, the increased dry matter content of cv. Parada and Krynia

had no limiting effect on the rate of fermentation (no significant effect on the concentrations of lactic acid, acetic acid, ethanol and WSC). A lower decrease in organic matter content during the ensilage of diploid red clover (a lower ash content of silage, compared with green forage), at similar lactic acid concentrations, could be indicative of more efficient lactic acid fermentation (greater contribution of homofermentative microflora) [16]. The genetic forms of red clover and cultivars within these forms had the strongest effect on butyric acid concentrations in silage. The differences between silage made from diploid and tetraploid red clover and between cultivars within the genetic forms were statistically significant. Butyric acid is a sensitive indicator of secondary fermentation in silage, particularly in silage made from high-protein components. This is due to the activation of *Clostridium sp.* (spore-forming rods) resulting from a high moisture content of plant material and a slow pH decline on the first days of ensiling. Butyric acid levels were relatively low [13], and the significant differences in its concentrations could be due to the different rate of acidification of the studied raw materials. The limiting effect of wilting on butyric acid fermentation was noted with respect to the vast majority of silage samples analyzed in the study, which is consistent with the findings of other authors [2, 21]. The inhibitory effect of wilting on butyric acid fermentation was not observed only in diploid red clover cultivars.

An analysis of nitrogen fractions revealed the effect of the genetic form of red clover and cultivar within the diploid form on the protein nitrogen/total nitrogen ratio. This ratio in silage is determined by its value in green forage and the dynamics of changes during ensilage. According to Messman et al. (17), the ratio of protein nitrogen in silage and protein nitrogen in green forage can be a measure of proteolysis extent during ensiling. This ratio, calculated based on the values in Tables 1 and 2, was comparable in the unwilted tetraploid cultivars Bona and Jubilatka, at 60.6% and 61.6%, respectively. Greater differences were reported between the diploid cultivars Krynia and Parada where the ratio reached 57.9% and 64.6%, respectively. Higher values, in the range of 67.9% to 75.6%, were noted in wilted red clover. The extent of proteolysis during ensiling is determined by the activity of enzymes in plant cells and the time of their action. Other factors that affect the rate of proteolysis include DM content, acidity (pH), temperature and the presence of proteolysis inhibitors typical of a given plant species [17, 20]. DM content and pH are considered the most important factors, while plant species has a profound effect on the rate of proteolysis at lower wilting degrees [10]. The quality of nitrogen compounds in silage is not always improved by wilting, due to a slower pH decline. In a previous study [19], a higher degree of wilting of grasses and alfalfa increased the extent of proteolysis in silage. In the present experiment, the genetic form (ploidy), cultivar and wilting of red clover had an inhibitory effect on the concentrations and proportions of biogenic amines in silage. The production of biogenic amines in silage is determined by the extent of proteolysis and/or the rate of decarboxylation [16]. In this study, the correlation between amine content and proteolysis was confirmed with respect to the degree of wilting, ploidy and the effect of diploid cultivars.

## Conclusions

Fermentation and proteolysis during the ensilage of red clover were affected primarily by wilting, whereas genetic factors (genetic form, cultivar) exerted a lesser effect. The genetic form of red clover affected the protein nitrogen/total nitrogen ratio in silage. The effect of the genetic form of red clover on the extent of proteolysis in silage (at similar levels of water-soluble carbohydrates and buffering capacity) suggests that diploid and tetraploid red clover cultivars differ with respect to chemical properties (poliphenol oxidase activity, polyphenol content) affecting proteolysis.

## References

[1] AOAC, Association of Official Analytical Chemists, Official Methods of Analysis, 18th Edition, Arlington, 2005.

[2] Arrigo Y., Influence du cycle, du state et du mode de conservation sur la teneur en acides amines des fourrages, Revue suisse Agric. (2006) 38(5): 247-255.

[3] Davies D.R., Theodorou M.K., Kingston-Smith A.H., Merry R.J., Advances in silage quality in the 21st Century, Proc. 14th Internat. Silage Conf., Belfast, Northern Ireland (2005) 121-133.

[4] Frank B., Persson M., Gustafsson G., Feeding dairy cows for decreased ammonia emission, Livest. Prod. Sci. (2002) 76: 171–179.

[5] Gąsior R., Brzóska F., The effects of wilting and additives on silage quality, protein degradation in the silo and in the rumen and dairy cattle productivity, Ann. Anim. Sci. (2000) 27(4): 129–141.

[6] Givens D.I., Rulquin H., Utilization by ruminants of nitrogen compounds in silage-based diets, Anim. Feed Sci. Technol. (2004) 114: 1–18.

[7] Goering H.K., Van Soest P.J., Forage fiber analyses (apparatus, reagents, procedures and some applications), Agriculture Handbook, ARS–USDA, Washington DC, (1970) 379.

[8] Han K.J., Collins M., Vanzant E.S., Dougherty C.T., Characteristics of baled silage made from first and second harvests of wilted and severely wilted forages, Grass Forage Sci. (2006) 61: 22–31.

[9] Huhtanen P., Shingfield K.J., Grass silage: factors affecting efficiency of N utilization in milk production, Proc. XIVth Internat. Silage Conf., Belfast, Northern Ireland (2005) 35-51.

[10] Jones R., Understanding the processes of protein degradation in forage crops provides opportunities for improved silage quality and enhanced animal

production, Proc. Alltech's Sixteenth Annual Symp. (Lyons T.P. and Jacques K.A., eds), Nottingham University Press (2000) 423–437.

[11] Joosten H.M.L.J., Olieman C., Determination of biogenic amines in cheese and some other food products by HPLC in combination with thermo-sensitized reaction, J. Chromatogr. (1986) 356: 311–319.

[12] Kingston-Smith A.H., Thomas H.M., Strategies of plant breeding for improvement of rumen function, Ann. Appl. Biol. (2003) 142: 13-24.

[13] Lee M. R. F., Scott M.B., Tweed J.K.S., Minchin F.R., Davies D.R., Effects of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant ( Lactobacillus plantarum L54 ), Anim. Feed Sci. Technol. (2008) 144: 125-136.

[14] Licitra G., Hernandez T.M., Van Soest P.J., Standardization of procedures for nitrogen fractionation of ruminant feed, Anim. Feed Sci. Technol. (1996) 57: 347-358.

[15] McDonald P., Henderson A.R., Buffering capacity of herbage samples as a factor in ensilage, J. Sci. Food Agric. (1962) 13: 395–400.

[16] McDonald P., Henderson A.R., Heron S.J.E., The Biochemistry of Ensilage, second ed. Chalcombe Publications, Marlow. UK, 1991.

[17] Messman M.A., Weiss W.P., Koch M.E., Changes in total and individual proteins during drying, ensiling, and ruminal fermentation of forages, J. Dairy Sci. (1994) 77: 492–500.

[18] Nadeau E., Englund J. E., Gustafsson A.H., Nitrogen efficiency of dairy cows as affected by diet and milk yield, Livestock Sci. (2007) 111: 45-46.

[19] Purwin C., Jakość kiszonek z mieszanek traw i motylkowatych produkowanych prasami zwijającymi [Quality of the grass and grass-legume silage made by baler technology], Rozprawy i monografie [Dissertations and Monographs], UWM Olsztyn, (2007) 127 (in Polish).

[20] Slottner D., Bertilsson J., Effect of ensiling on protein degradation during ensilage, Anim. Feed Sci. Technol. (2006) 127: 101–111.

[21] Sullivan M.L., Hatfield R.D., Polyphenol oxidase and o-diphenols inhibit postharvest proteolysis in red clover and alfalfa, Crop Sci. (2006) 46: 662-670.

[22] Thomas A.T., An automated procedure for the determination of soluble carbohydrates in herbage, J. Sci. Food Agric. (1977) 28: 639-642.

[23] Wilczek M., Ćwintal M., Andruszczyszyn K., Plonowanie i jakość tetraploidalnej koniczyny łąkowej (czerwonej) w zależności od niektórych czynników agrotechnicznych. Część III. Jakość [Yielding and quality of tetraploid red clover depending on some agrotechnical factors. Part III. Quality], Biul. IHAR (1999) 210: 119-129 (in Polish).

[24] Winters A.L., Fychan R., Jones R., Effect of formic acid and a bacterial inoculant on the amino acid composition of grass silage and on animal performance, Grass Forage Sci. (2001) 56: 181-192.

[25] Żuk-Gołaszewska K., Bieniaszewski T., Fordoński G., Olszewski J., Plonowanie di- i tetraploidalnych odmian koniczyny czerwonej w siewie mieszanym z tymotką łąkową, Zesz. Nauk. AR Kraków (1999) 62: 407- 414. [The yield of diploid and tetraploid red clover grown in a mixture with Timothy-grass] (in Polish)

[26] Żuk-Gołaszewska K., Bielski, S., Gołaszewski J., Productivity of spring barley grown with red clover as undersown crop, Pol. J. Natur. Sci. (2006a) 20(1): 121-133.

[27] Żuk-Gołaszewska K., Gołaszewski J., Sądej W., Bielski S., Seed yields of diploid and tetraploid varieties of red clover as dependent upon sowing rate, Pol. J. Natur. Sci. (2006 b) 20(2): 615-628.

[28] Żuk-Gołaszewska K., Purwin C., Pysera B., Wierzbowska J., Gołaszewski J., Yields and quality of green forage from red clover di- and tetraploid forms, J. Elementol. (2010) 15 (4): 757-770.

Table 1. Chemical composition of green forage and silage

Tabela 1. Skład chemiczny zielonek i kiszonek

| Variable/Trait | Materials | Cultivars - Odmiana | | | | |
|---|---|---|---|---|---|---|
| | | Krynia 2n | Parada 2n | Bona 4n | Jubilatka 4n | SEM |
| Dry matter g x kg-1 | forage | 178.4 | 176.7 | 156.4 | 138.4 | 5.71 |
| | zielonka | | | | | |
| Sucha masa g x kg-1 | silage U | 146.1 | 142.2 | 130.1 | 119.9 | 3.42 |
| | kiszonka U | | | | | |
| | silage W | 372.2 | 372.5 | 355.8 | 342.2 | 8.43 |
| | kiszonka W | | | | | |
| g x kg-1 DM/g x kg-1 SM | | | | | | |
| Crude ash | forage | 111.0 | 113.2 | 112.0 | 111.2 | 5.62 |
| Popiół surowy | zielonka | | | | | |
| | silage U | 112.8 | 123.5 | 121.0 | 126.7 | 2.12 |
| | kiszonka U | | | | | |
| | silage W | 106.3 | 109.2 | 122.8 | 108.5 | 2.49 |
| | kiszonka W | | | | | |
| Crude protein | forage | 186.8 | 185.4 | 186.1 | 188.1 | 9.32 |
| Białko ogólne | zielonka | | | | | |
| | silage U | 182.2 | 189.0 | 184.9 | 188.6 | 3.63 |
| | kiszonka U | | | | | |
| | silage W | 191.6 | 189.3 | 187.7 | 189.1 | 2.67 |
| | kiszonka W | | | | | |
| True protein | forage | 139.3 | 139.5 | 139.8 | 141.5 | 12.52 |
| | zielonka | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Białko właściwe | silage U | 78.8 | 91.9 | 83.9 | 87.4 | 2.10 |
| | kiszonka U | | | | | |
| | silage W | 102.5 | 102.8 | 103.9 | 107.6 | 2.45 |
| | kiszonka W | | | | | |
| Ether extract | forage | 21.9 | 21.0 | 21.8 | 20.3 | 4.21 |
| Tłuszcz surowy | zielonka | | | | | |
| WSC | forage | 103.7 | 99.8 | 94.9 | 99.0 | 16.65 |
| | zielonka | | | | | |
| | silage U | 14.1 | 8.1 | 12.3 | 10.6 | 1.57 |
| | kiszonka U | | | | | |
| | silage W | 29.9 | 30.0 | 27.2 | 26.0 | 5.75 |
| | kiszonka W | | | | | |
| NDF | forage | 453.6 | 500.0 | 453.7 | 498.6 | 31.47 |
| | zielonka | | | | | |
| | silage U | 426.6 | 421.7 | 428.2 | 385.6 | 8.63 |
| | kiszonka U | | | | | |
| | silage U | 43.2 | 42.5 | 34.3 | 34.1 | 3.43 |
| | kiszonka U | | | | | |
| | silage W | 23.9 | 26.2 | 25.6 | 26.6 | 2.67 |
| | kiszonka W | | | | | |
| | silage W | 382.3 | 405.9 | 406.6 | 383.5 | 11.57 |
| | kiszonka W | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ADF | forage zielonka | 321.2 | 335.9 | 325.0 | 331.9 | 14.61 |
| | silage U kiszonka U | 363.6 | 370.9 | 374.2 | 347.8 | 13.63 |
| | silage W kiszonka W | 324.5 | 340.4 | 338.5 | 345.1 | 8.04 |
| Buffering capacity mg lactic acid/1g DM Pojemność buforowa | forage zielonka | 46.2 | 49.14 | 50.01 | 48.17 | 3.23 |

Table 2. Fermentation products and nitrogen fractions in silage

Table 2. Produkty fermentacji i frakcje azotowe w kiszonkach

| Variable/Trait | Treatment Podsuszenie | Krynia | Parada | Bona | Jubilatka | SEM |
|---|---|---|---|---|---|---|
| pH | U | 4.19 | 4.25 | 4.20 | 4.32 | 0.13 |
| | W | 4.41 | 4.43 | 4.46 | 4.36 | 0.12 |
| g x kg -1 DM | | | | | | |
| Lactic acid Kwas mlekowy | U | 124.3 | 132.7 | 117.1 | 121.8 | 16.88 |
| | W | 64.5 | 68.0 | 56.6 | 68.6 | 3.68 |
| Acetic acid Kwas octowy | U | 45.9 | 47.8 | 37.8 | 52.6 | 4.31 |
| | W | 31.3 | 24.3 | 28.1 | 24.2 | 3.27 |
| Butyric acid Kwas masłowy | U | - | 0.04 | - | 0.17 | 0.05 |
| | W | 0.08 | 0.15 | 0.08 | 0.07 | 0.03 |
| Ethanol Etanol | U | 47.2 | 44.3 | 73.9 | 35.6 | 10.84 |
| | W | 24.9 | 19.1 | 26.9 | 26.5 | 4.27 |
| Total N | U | 29.2 | 30.2 | 29.6 | 30.2 | 0.58 |
| N ogólny | W | 30.7 | 30.3 | 30.0 | 30.3 | 0.43 |
| Protein N | U | 12.6 | 14.7 | 13.4 | 14.0 | 0.33 |
| N białkowy | W | 16.4 | 17.2 | 16.6 | 15.5 | 0.39 |
| N-NH3 | U | 1.74 | 2.13 | 3.27 | 2.96 | 0.47 |
| | W | 1.52 | 1.36 | 2.65 | 1.82 | 0.13 |
| Protein N/ total N | | | | | | |
| | U | 432.6 | 486.2 | 453.6 | 463.6 | 10.82 |
| N białkowy/ N ogólny | | | | | | |
| g x kg -1 N | | | | | | |
| | W | 536.9 | 568.7 | 553.7 | 511.3 | 24.91 |
| N-NH3/ total N | | | | | | |
| | U | 59.7 | 70.5 | 110.5 | 98.2 | 5.95 |
| N-NH3/ total N | | | | | | |
| g x kg -1 N | W | 49.7 | 44.8 | 88.1 | 60.2 | 4.70 |
| Histamine Histamina | U | 82.4 | 215.2 | 74.2 | 75.1 | 19.84 |

| | | | | | | |
|---|---|---|---|---|---|---|
| mg x kg -1 DM | W | 110.6 | 122.9 | 66.9 | 77.8 | 9.87 |
| Tyramine Tyramina | U | 274.1 | 355.8 | 327.7 | 332.2 | 24.50 |
| mg x kg -1 DM | W | 214.8 | 255.2 | 139.0 | 255.6 | 34.60 |
| Putrescine Putrescyna | U | 955.8 | 1173.6 | | 1186.6 | 55.46 |
| mg x kg -1 DM | W | 764.7 | 697.3 | 472.9 | 704.9 | 73.57 |
| Cadaverine Kadaweryna | U | 195.8 | 723.6 | 466.8 | 720.2 | 30.51 |
| mg x kg -1 DM | W | 255.4 | 445.1 | 331.1 | 443.6 | 49.52 |

Table 3. Estimates of orthogonal contrast effects for the quality traits of silage made from wilted and unwilted diploid and tetraploid red clover

Ocena efektów kontrastów ortogonalnych cech jakościowych kiszonki z podsuszonej i niepodsuszonej koniczyny czerwonej odmian di- i tetraploidalnych

| Variable/Trait<br>Zmienna/ Cecha | Contrasts (as described in Materials and Methods)<br>Kontrasty (opisane w materiałach i metodach) | | | | | | |
|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
| Dry matter - Sucha masa | -226.1* | -42.5* | 1.8 | -12.0* | 8.4 | 4.2 | 3.4 |
| Crude ash – Popiół surowy | 10.7* | 8.0* | -6.1* | 2.0 | 4.4 | -6.5 | 12.4* |
| Crude protein – Białko ogólne | -3.2** | -0.9 | -2.2 | 2.5 | 6.4 | -9.1* | 2.2 |
| True protein – Białko właściwe | -17.3* | -4.6 | -8.9 | -1.9 | 10.5 | -8.2 | 10.8* |
| Protein N/total N<br>N białkowy/ N ogólny | -83.6* | -21.2 | -42.7* | -16.2 | 39.1 | -21.8 | 52.4* |
| Ether extract – Tłuszcz surowy | 12.9* | -7.6* | -0.8 | 0.4 | -19.4* | 2.9 | -1.2 |
| pH | -0.17* | 0.05 | -0.04 | 0.05 | 0.11 | -0.04 | 0.30 |
| Acetic acid – Kwas octowy | 19.1* | -3.3 | 2.6 | 5.4 | -0.2 | -9.0 | 18.7* |
| Butyric acid – Kwas masłowy | -0.03 | 0.05 | -0.05 | 0.07 | 0.25 | 0.02 | 0.14 |
| Lactic acid – Kwas mlekowy | 109.2 | -23.2 | -6.0 | 7.9 | -11.9 | -4.9 | -28.4 |
| N-NH3 | 0.7* | 2.0* | -0.1 | -0.6* | 0.8 | -0.6 | 0.5 |
| N-NH3/total N<br>N-NH3/N ogólny | 24.0* | 66.2* | -3.0 | -20.1* | 24.8 | -15.8 | 15.6 |
| Ethanol - Etanol | 25.9* | 13.7* | 4.4 | -19.4* | 8.4 | -2.9 | -37.9* |
| WSC | -17.0* | -3.0 | 2.9 | -1.5 | 7.5 | 6.2 | -0.5 |
| NDF | 21.0* | -16.3* | -9.4 | -32.9* | -36.4* | 28.5* | -19.5 |
| ADF | 27.0* | -16.9 | -11.7 | -29.9* | -31.2 | 8.6 | -33.0 |
| Total N – N ogólny | -0.5* | -0.1 | -0.4 | 0.4 | 1.0 | -1.5 | 0.4 |
| Histamine – Histamina | 17.2* | -118.7* | -72.5* | 5.8 | -59.2* | -120.4* | -9.8 |
| Tyramine - Tyramina | 106.3* | -22.8 | -61.0* | 60.5* | 105.4* | -41.3 | -112.1* |
| Putrescine - Putrescyna | 447.3* | -57.8 | -75.2 | 152.6* | 454.7* | -285.0* | -157.0 |
| Cadaverine - Kadaweryna | 157.8* | 170.8* | -358.8* | 183.0* | 193.4* | -338.1* | 140.8* |

* significance at $p<0.05$ - * istotność $p<0.05$