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# CHANGES IN THE AMINO ACID COMPOSITION DURING THE ENSILING OF LUCERNE AND RED CLOVER IN ROUND BALES*

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**Abstract**

Changes in the amino acid profile of protein during ensiling depend on the proteolytic potential of plant species, the rate of the wilting and acidifying of ensiled herbage and, on the other hand, on the microbiological processes and fermentation of amino acids. The interaction of these factors determines the subsequent efficiency of nitrogen utilization in the rumen. The aim of this study has been to determine the influence of ensiling in round bales on changes in the amino acid content in lucerne and red clover silages. The amino acid composition was determined by analyzing the content of amino acids in herbages and silages. The lucerne silage protein was characterized by a higher contribution of all amino acids except Trp in comparison with red clover silage. Ensiling in bales of wilted lucerne and clover herbage lowered the content of Asp, His ($P < 0.01$), Ile, Lys ($P < 0.05$) in both plants. The ensiling process and plant species affected ($P < 0.05$) the total content of amino acids per 100 g of protein, where the range of changes in both raw materials was different. The loss of amino acids observed during the ensiling of wilted lucerne in round bales should be regarded as typical, whereas changes in the amino acid profile of red clover should be considered as atypical. A significantly higher ADIN content in red clover than in lucerne silage indicates that a rapid and significant increase of temperature occurred in experimental bales, which were characterized by a low degree of density, and this temperature rise strongly affected the availability of amino acids of the red clover protein also to the current microflora in the silage. Ensiling in round bales changed the amino acid profile of the lucerne and red clover protein. The ensiling process in this technology, however, severely deteriorated

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the quality of the red clover protein by lowering the content of all essential amino acids except Cys and Met.

**Keywords:** legumes, amino acids, protein fractions, silage, bales.

# INTRODUCTION

Two main measures of the quality of protein for ruminants are its rumen degradability and amino acid composition (Givens, Rulquin 2004). Analysis of the impact of feed preservation on feed's nutritional value is limited mostly to the energy and protein content and digestibility. However, more attention is drawn to the solubility of protein, which is the result of changes in the composition of protein nitrogen fraction (Fijałkowska et al. 2015). Less knowledge has been gained regarding the amino acid composition of feed protein for ruminants and the impact of a feed preservation process on the content of individual amino acids. Arrigo (2006) showed a decrease of amino acids in roughage during preservation and storage, and the impact of a herbage conservation method on the decrease of amino acids. Changes in the amino acid profile of protein during ensiling depend on the proteolytic potential of plant species, i.e. proteases and polyphenol oxidase activity, content of tannins and the rate of the wilting and acidifying of ensiled herbages (Coblentz et al. 1998). Other factors that modify the amino acid composition of silage protein are microbiological processes, i.e. decarboxylation, deamination and fermentation of amino acids related to the course of fermentation (Purwin et al. 2010).

Subsidies for legume crops have led to a significant increase in the acreage of red clover and lucerne. Differences in the chemical composition and proteolytic potential of red clover and lucerne are reflected in the different content of the fermentation products and protein degradation products in the silage (Brzóska et al. 1999, Purwin et al. 2006). Legume silages are a very good supplement to feed rations based on maize silage, but are at risk of being exposed to clostridial fermentation. It is easier to achieve good fermentational and hygienic quality of lucerne and red clover silage if balers are used for ensiling.

Some previous studies on changes in the amino acid composition of protein during the ensiling of lucerne and clover herbages were conducted under laboratory conditions (Djordević et al. 2005, Guo et al. 2008, Edmunds et al. 2012). There is lack of data regarding changes in the amino acid composition of protein in legumes being ensiled in round bales.

The aim of this study has been to determine the influence of ensiling in round bales on changes in the amino acid content of lucerne and red clover silages.

# MATERIAL AND METHODS

The study was conducted in 2009 at the Agricultural Experiment Station of the University of Warmia and Mazury in Olsztyn, located in Łężany. The experimental plant material comprised the first cut of lucerne (cv. Alba) and red clover (cv. Nike) herbage in the second year of growing. Phosphorus and potassium were applied at 80 kg $P_2O_5$ and 100 kg $K_2O$ per ha, respectively. Lucerne and red clover were harvested at the beginning of flowering. After wilting the herbage for 48 hours and one course of tedding, experimental silages were produced using balers SIPMA Z 230 (SIPMA Poland) set at the bale density of 122.5 kg $m^{-3}$ for lucerne and 123.1 kg $m^{-3}$ for red clover. The time between bale forming and wrapping with six layers of white 30 µm stretch film did not exceed 60 minutes. Wrapped silage bales were stored in vertical position.

Samples of herbages and silages were collected according to the procedures described by FIJAŁKOWSKA et al. (2015). Afterwards, they were assayed for the proximate chemical composition by standard methods (AOAC, 2005). The content of the nitrogen fractions during ensiling was determined (Table 1) as described by FIJAŁKOWSKA et al. (2015). Thermal monitoring was carried out

Table 1

Chemical composition(g $kg^{-1}$ DM) and the content of nitrogen fractions (g $kg^{-1}$ TN) in experimental herbages and silages

| Item | Lucerne | | Red clover | |
|---|---|---|---|---|
| | fresh | silage | fresh | silage |
| Dry matter (g $kg^{-1}$) | 188 | 455 | 204 | 419 |
| NDF | 443 | 477 | 438 | 506 |
| pH | nd | 4.70 | nd | 4.77 |
| Lactic acid | nd | 50.4 | nd | 46.4 |
| Acetic acid | nd | 21.3 | nd | 22.1 |
| Butyric acid | nd | 0.78 | nd | 1.27 |
| Total nitrogen (g $kg^{-1}$ DM) | 29.6 | 28.8 | 26.5 | 24.4 |
| Protein nitrogen | 702 | 573 | 765 | 716 |
| BSN | 273 | 322 | 180 | 199 |
| NPBSN | 109 | 226 | 123 | 138 |
| BSPN | 164 | 96.1 | 56.7 | 60.6 |
| Peptide N | 84.1 | 65.1 | 87.4 | 84.0 |
| Amino acid N | 24.9 | 160 | 35.6 | 54.5 |
| NDIN | 96.1 | 113 | 108 | 258 |
| ADIN | 68.6 | 95.9 | 76.8 | 177 |
| $N\text{-}NH_3$ | nd | 61.4 | nd | 62.4 |
| Temperature (°C) 6 hour of ensiling | 38 | | 40 | |
| 24 hour of ensiling | 55 | | 54 | |
| 48 hour of ensiling | 38 | | 44 | |

TN – total nitrogen; BSN – buffer soluble nitrogen; NPBSN – non protein buffer soluble nitrogen; BSPN – buffer soluble protein nitrogen; NDIN – neutral detergent insoluble nitrogen; ADIN – acid detergent insoluble nitrogen; nd – not determined

by measuring the temperature of bales using a thermistor electrode connected to a data logger Squirell (Grant 2020).

The amino acid composition was determined by analyzing the content of amino acids in hydrolizates with the use of an automatic amino acid analyzer with a sodium column (AAA 400 INGOS, the Czech Republic). Samples were hydrolized by hydrogen chloride acid (6MHCL) during 24 hours at 110°C under $N_2$ atmosphere. Methionine and cysteine were determined after oxidation by performic acid and hydrolized as described above, for 23 hours. The tryptophan content was determined according to Standard PN-EN ISO 13904: 2005.

The results were processed statistically by two-way Anova using Statistica, version 10 (StatSoft, Inc. 2011). The model describing changes in amino acid concentrations accounted for the effects of the legume species, ensiling and the species x ensiling interaction. The significance of differences between means (species and ensiling) was estimated with the Duncan's test.

## RESULTS AND DISCUSSION

The total content of amino acids (Table 2) found in fresh lucerne was similar to the results of other authors (HOMOLKA et al. 2008). However, in

Table 2

Amino acid composition of lucerne and red clover protein (g 100 $g^{-1}$ crude protein)

| Amino acid | Lucerne | | Red clover | | SEM | Treatment | | SxE |
|---|---|---|---|---|---|---|---|---|
| | fresh | silage | fresh | silage | | S | E | |
| Asp | 14.60 | 11.09 | 12.08 | 5.494 | 0.946 | * | ** | * |
| Thr | 1.712 | 2.530 | 3.610 | 1.283 | 0.329 | ns | ns | ** |
| Ser | 1.589 | 2.850 | 4.233 | 1.443 | 0.398 | ns | ns | ** |
| Glu | 10.16 | 7.504 | 8.033 | 4.118 | 0.569 | ns | ns | * |
| Pro | 5.712 | 6.222 | 5.700 | 4.311 | 0.244 | ** | ns | ** |
| Gly | 3.621 | 4.184 | 4.167 | 2.081 | 0.320 | ns | ns | * |
| Ala | 4.187 | 4.377 | 4.623 | 2.148 | 0.361 | ns | * | * |
| Val | 4.096 | 4.507 | 5.350 | 2.278 | 0.394 | ns | * | ** |
| Cys | 1.824 | 1.141 | 0.857 | 1.007 | 0.128 | ns | ns | ns |
| Met | 1.925 | 1.377 | 0.893 | 1.170 | 0.119 | ** | ns | ** |
| Ile | 4.835 | 3.811 | 3.597 | 1.897 | 0.278 | ** | * | * |
| Leu | 6.859 | 6.660 | 5.710 | 3.322 | 0.491 | ** | ns | ns |
| Tyr | 3.499 | 4.191 | 4.053 | 1.881 | 0.314 | * | ns | ** |
| Phe | 3.877 | 4.011 | 3.340 | 1.934 | 0.293 | ** | ns | ns |
| Trp | 1.333 | 0.955 | 0.943 | 1.037 | 0.056 | ns | ns | * |
| Lys | 4.747 | 4.165 | 3.713 | 2.216 | 0.319 | ** | * | ns |
| His | 3.056 | 2.539 | 3.223 | 1.571 | 0.225 | ns | ** | ns |
| Arg | 4.403 | 3.212 | 2.740 | 1.473 | 0.272 | ** | ns | ns |
| Total (g $kg^{-1}$ TN) | 820.4 | 753.3 | 768.6 | 406.7 | 53.00 | * | * | * |

S – species; E – ensiling; SEM – standard error of the mean; ns – not significant;
** – significant at $P < 0.01$; * – significant at $P < 0.05$

comparison with the data of Djordević et al. (2005), lower levels of Lys and Arg but higher of Met and His were found in the study on fresh lucerne. The red clover herbage had a lower total content of amino acids by about 47-92 g $kg^{-1}$ TN compared to the results obtained by Purwin et al. (2011), who conducted research on a few varieties of red clover.

The plant species influenced the content of Pro, Met, Ile, Leu, Phe, Lys, Arg ($P < 0.01$), and Asp, Tyr ($P < 0.05$) in the analyzed silages (Table 2). For example, the lucerne silage protein was characterized by a higher contribution of all amino acids except Trp in comparison with red clover silage. Ensiling of wilted lucerne and clover herbage in bales lowered the content of Asp, His ($P < 0.01$), Ile, Lys ($P < 0.05$) in both plants. The different impact of ensiling on the level of individual amino acids in the clover and lucerne protein was confirmed by the interaction ($P < 0.01$) for Thr, Ser, Pro, Val, Met, Tyr ($P < 0.01$), and Asp, Glu, Gly, Ala, Ile, Trp ($P < 0.05$). Numerous interactions regarding changes in the content of individual amino acids between red clover and lucerne and wilting herbages on swaths were found by Purwin et al. (2014).

Among essential amino acids, the contribution of Thr, Val and Phe increased during the ensiling of lucerne, whereas in red clover the content of these amino acids decreased (Figure 1). A reverse relationship occurred for Cys, Met, and Trp. Among non-essential amino acids (Figure 2), an increased content of Ser, Pro, Gly, Ala and Tyr was found along with decreased Asp, Glu during the ensiling of lucerne. In red clover, the content of all non-essential amino acids decreased.

A similar decline in the content of Arg and Glu during ensiling was detected by Winters et al. (2001). Purwin et al. (2011) found a decrease in Arg,

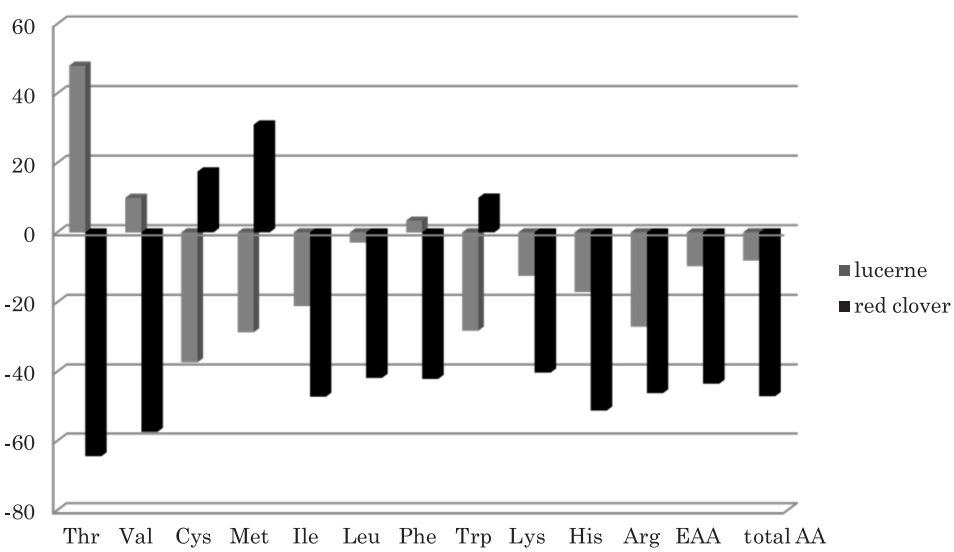


Fig. 1. Changes in the essential amino acid composition of the lucerne and red clover protein during ensiling (%)

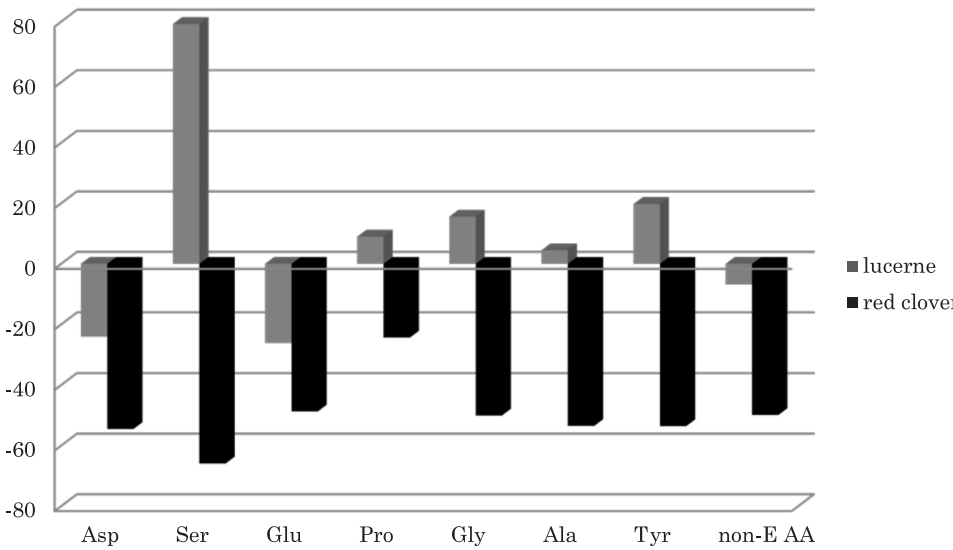


Fig. 2. Changes in the non-essential amino acid composition of the lucerne and red clover protein during ensiling (%)

His, Lys and Leu and an increase in Val, Met, Thr, Ile and Phe in red clover silages produced without wilting. The observed large losses of Lys and Arg may be explained by the fact that these amino acids are the subject of metabolism of many species of LAB and the proteolytic group of *Clostridium* (Rhodes et al. 1999).

The ensiling process and plant species affected ($P < 0.05$) the total content of amino acids per 100 g of protein (Table 2, Figure 1), where the range of changes in both raw materials was different, as confirmed by the interaction ($P < 0.05$). The red clover silage was found lose 47% of the total amino acids, while in lucerne there was a minimal decrease (8.2%) in the total content of amino acids. The loss of amino acids observed during the ensiling of wilted lucerne silage in round bales should be regarded as typical and similar to the results of other authors (Guo et al. 2008). Presumably, an increase in osmolarity in plant cells and a reduction in the buffering capacity limited the intensity of microbiological processes and, consequently, changes in the forms of nitrogen during ensiling (Arrigo 2006, Krzywiecki et al. 2008). On the one hand, an increased osmotic pressure of the environment inhibited the activity of plant enzymes and the release of amino acids affected by proteolysis and peptidolysis (Cavallarin et al. 2005). On the other hand, it inhibited the loss of free forms of amino acids by reducing the range of bacterial catabolism, decarboxylation and deamination by *Enterobacterium* and *Clostridium* and by limiting the lactic acid fermentation and simultaneous amino acid catabolism operated by lactic acid bacteria. This may be confirmed by the low level of $N\text{-}NH_3$ found in silages from both plants (Table 1).

Unlike in lucerne, a large decrease in the total amino acids appeared in red clover being ensiled in round bales. This effect might be associated with a high proportion of nitrogen in the insoluble fraction, permanently bound with the cell wall carbohydrates (ADIN; Table 1). It may be assumed that during thermal processes a large portion of red clover amino acid, both in the free and bound form in proteins and peptides, was bound in insoluble forms, which did not undergo acid hydrolysis.

In the earlier study of Purwin et al. (2011), where fresh red clover was ensiled in microsilos, a significantly lower decrease was found in the total content of amino acids and total non-essential amino acids (by 13.3%) as well as in total essential amino acids (by 4.6%). However, ensiling under laboratory conditions (in microsilos) eliminates the increase in temperature during silage-making and its effects on silage protein. The thermal cause of a decrease in the amino acid content in hydrolizate can be confirmed by the fact that the content of all amino acids was reduced except sulfur amino acids. Our comparison of the protein amino acid profile of red clover herbage and silage may indicate that microbial changes which occurred in amino acids, such as reducing the amount of Glu while increasing the content of Pro, or decreasing Asp while increasing Ala, were not typical (Seyfarth et al. 1989, Homolka et al. 2008, Michálková et al. 2009). The lowering of the content of Glu may be explained by the fact that it is the only amino acid which is decarboxylated to γ-amino butyric acid (GABA) by plant enzymes during ensiling (Seyfarth et al. 1989), which may substantiate the lack of any increase in the content of Pro in red clover silage.

In conclusion, the significant changes in the amino acid profiles during ensiling reported in this paper are consistent with the observations of Arrigo (2006), who compared the impact of herbage preserving methods on the protein amino acid profile and found the greatest deviation from the initial total content of silage amino acids of 30% dry matter. Changes in the amino acid composition of proteins have been confirmed in numerous studies (Winters et al. 2001, Givens, Rulquin 2004, Purwin et al. 2011).

However, changes in the amino acid profile of red clover should be considered as atypical. The significantly higher ADIN content in red clover than in lucerne silage indicates that experimental bales, which were characterized by low density (120-130 kg DM $m^{-3}$), were affected by a rapid and significant increase of temperature (Table 1), which strongly modified the availability of amino acids of the red clover protein also to the silage's current microflora. The deterioration of the protein quality found as a result of ensiling may support the results of Hoffman et al. (1997), Dewhurst et al. (2003), Hoffman, Bauman (2003), who showed that the beneficial composition of nitrogen fraction in red clover silage was not confirmed by the studies on animals. Sobiech et al. (2015) demonstrated better growth performance of ram lambs offered lucerne silage than red clover silage.

## CONCLUSIONS

Ensiling in round bales changed the amino acid profile of the lucerne and red clover protein. The ensiling process in this technology, however, severely deteriorated the quality of red clover protein by lowering the content of all essential amino acids except Cys and Met.

The initial rise in temperature during the ensiling of red clover in round bales, despite lowering the solubility of the protein, can also strongly modify the amino acid profile of the protein, while the increased content of ADIN in red clover silage may be accompanied by a very large loss of essential amino acids.

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