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THE EFFECT OF ENSILING IN ROUND BALES ON THE CONTENT OF NITROGEN FRACTIONS IN LUCERNE AND RED CLOVER PROTEIN*

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Abstract

The aim of the study was to determine changes in nitrogen fractions during the ensiling of lucerne and red clover herbage in bales. Protein nitrogen and non-protein nitrogen compounds in herbage and silage were distinguished. Furthermore, buffer soluble nitrogen compounds (BSN) were determined and divided into buffer soluble protein and non-protein nitrogen (BSPN and NPBSN, respectively). In addition, peptide nitrogen and amino acid nitrogen were distinguished within the NPBSN fraction, while nitrogen compounds were divided into those of low solubility (NDIN) and completely insoluble (ADIN).

The research findings confirmed the effect of a legume plant species on changes in the content of nitrogen fractions during the ensiling in round bales. The ensiling of lucerne herbage grossly decreased the protein nitrogen fraction while raising the total soluble fraction of nitrogen. Nitrogen in the form of soluble proteins, NDIN and ADIN was the least affected. The study demonstrated a different response of the two legume plant species to ensiling with respect to all fractions. A high proportion of protein nitrogen in round bale wilted red clover silage was accompanied by a large contribution of insoluble nitrogen fractions. A large share of the protein nitrogen fraction in red clover silage does not necessarily mean a higher nutritional value of the protein because it may contain a large amount of protein compounds that are non-hydrolysable by bacterial and intestinal enzymes.

Key words: lucerne, red clover, nitrogen fractions, ensiling, bales.

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INTRODUCTION

Crude protein contained in plant material is a heterogeneous mixture of different proteins and non-protein nitrogen compounds. During the ensiling process, certain proteolytic changes occur, as a result of which there is an increase of non-protein nitrogen compounds (NPN) and a decrease in the contribution of proteins. Losses of nitrogen compounds obserevd during the plant material preservation process do not stem from the loss of crude protein (total nitrogen-TN), but are due to the transformation of its form from protein into NPN compounds, which are far less efficiently used for the microbial protein synthesis in the rumen, cause a worse nitrogen balance in ruminants and consequently lead to a higher emission of nitrogen into the environment (Purwin et al. 2009, 2010). The determination of the NPN contribution does not provide enough knowledge about the nutritional value of this fraction, which is composed of compounds characterized by different molecular size and solubility. There are, for example, easily soluble compounds such as ammonium salts, nitrates, amides, amines and free amino acids, characterized by low usability in the rumen, as well as compounds like peptides, well utilized in the process of microbial protein synthesis. This fraction also includes low solubility (NDIN) and completely insoluble (ADIN) nitrogen compounds. Also, the protein nitrogen fraction may be varied by different solubility. Nitrogen in the form of soluble proteins is rapidly metabolized in the rumen and less easily soluble proteins undergo intestinal digestion, thus being used most effectively (Licitra et al. 1996). The inhibition of proteolysis during ensiling can improve nitrogen utilization by elevating the contribution of protein nitrogen (Lee et al. 2008) and peptide nitrogen in silage.

The parameters which characterize the degradation of nitrogen compounds in silage include the share of protein and non-protein nitrogen (hydrolysis) as well as ammonia nitrogen in total nitrogen (deamination) (Brzóska et al. 1999, Jones 2000, Purwin et al. 2006). Some accurate indicators of protein hydrolysis are the amino acid nitrogen content (N of free amino acids) in silage (Winters et al. 2001, Givens, Rulquin 2004) and buffer soluble nitrogen (Hedqvist, Udén 2006). Increasingly more attention is drawn to the necessity to separate soluble nitrogen fraction (nitrogen soluble in buffer) into protein nitrogen, peptide nitrogen and amino acid nitrogen, which – by having different degradability – can have various effects on the efficiency of the microbial protein synthesis.

The research hypothesis assumes that the different proteolytic potential of legumes can result in changes in the contribution of nitrogen fractions in herbage ensiled in round bales. The aim of the study was to determine changes in the nitrogen fractions during the ensiling of bales of lucerne and red clover herbage.

MATERIAL AND METHODS

The experiment 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 herbage of lucerne (cv. Alba) and red clover (cv. Nike) in the second year of growing. No nitrogen top-dressing was applied, but phosphorus and potassium were supplied at 80 kg P_2O_5 and 100 kg K_2O per ha. Lucerne and red clover were harvested at the onset of flowering. After 48-hour wilting of herbage and one course of tedding, experimental silages were made with SIPMA Z 230 balers (SIPMA S. A. Poland), setting the bale density at 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 was 60 minutes at the most. Wrapped silage bales were stored in the vertical position.

Primary samples of herbage were collected at four locations in each field, from the whole depth of the swath, along the length of 0.3 m. They were then combined to obtain pool samples. Average samples were taken from each location (n = 4). After 90 days of ensiling, silage samples were taken from bales with a probe (Ø 20 mm), inserted 40 cm deep on the diameter line and half the height of a bale (Hancock, Collins 2006). Samples of herbage and silage were dried at 40°C using Binder FED 115 drying ovens with forced convection, and were ground in a Retsch SK 100 cross beater mill to the particle size of 1 mm. Changes in the following nitrogen fractions during ensiling were determined: protein N, buffer-soluble N (BSN), including buffer-soluble protein N (BSPN), non-protein buffer-soluble N (NPBSN), peptide N, amino acid N, N-NH_3, neutral detergent-insoluble N (NDIN) and acid detergent-insoluble N (ADIN).

Samples of fresh herbage immediately after cutting and silage samples were assayed for the proximate chemical composition by standard methods (AOAC 2005), water soluble carbohydrate (WSC) by the anthrone method (Thomas 1977), NDF, ADF and ADL by the method proposed by Van Soest et al. (1991), protein nitrogen with the use of trichloroacetic acid (TCA), as described by Guo et al. (2008), BSN – using McDougall's buffer, according to the procedure of Hedqvist, Uden (2006). The BSN fraction was subdivided into BSPN, peptide N and amino acid N according to the procedure described by Purwin et al. (2014). Other determinations included silage pH (pH - meter HI 8314N - Hanna Instruments), the organic acid content in aqueous extract with the HPLC method on a Shimadzu MetaCarb 67H column (VARIAN) and N-NH_3 with the Conway method.

The results were processed statistically by two-way Anova. A model constructed to describe chemical composition, fermentation products and composition of nitrogen fractions accounted for the effects of legume species, ensiling and the species x ensiling interaction. The significance of differences between means (species and ensiling) was estimated by the Duncan's test.

RESULTS AND DISCUSSION

Ensiling caused an increase in DM, CA, NDF, ADF and ADL ($P < 0.01$) and a decrease in the WSC content ($P < 0.01$) – Table 1. Interactions were noted between the species and ensiling process with regard to the content of DM, WSC, ADF ($P < 0.05$) and ADL ($P < 0.01$). They resulted, respectively, from a higher degree of wilting of lucerne than red clover, bigger fermentation loss (WSC) in red clover and a relative increase in the structural carbohydrate fraction, which does not decompose during fermentation (McDonald et al. 1991). The TN content was higher ($P < 0.01$) in lucerne than in red clover (Table 2). Ensiling was responsible for a decrease in this component ($P < 0.05$), while the reason why the TN content declined could have been the reduced share of leaves due to mechanical damage during harvest (Nowak 2000). The species of ensiled plants strongly affected the composition of N fractions in silages, hence lucerne was characterized by the total nitrogen fraction having higher shares of soluble fractions BSN, NPBSN, BSPN and AA-N ($P < 0.01$) and lower proportions of protein N and peptide N ($P < 0.05$), sparingly soluble fraction NDIN ($P < 0.01$), and insoluble ADIN ($P < 0.01$) than red clover. Processes associated with the ensiling of lucerne and red clover herbage contributed to the reduction of protein N and peptide N ($P < 0.05$), increased participation of the soluble fraction: BSN ($P < 0.05$), NPBSN, AA-N ($P<0.01$) and a higher share of the fraction of sparingly soluble and insoluble NDIN and ADIN ($P < 0.01$). There was no effect of species on the share of N-NH_3. The interactions between species and ensiling ($P < 0.01$) concerned the contribution of NPBSN, BSPN and N-AA,

Table 1

Chemical composition of herbage and silage (g kg^{-1} d.m.)

Item	Lucerne		Red clover		SEM	Treatment		SxE
	fresh	silage	fresh	silage		S	E	
Dry matter g kg^{-1}	188	455	204	419	36.79	ns	**	*
Crude ash	70.8	86.3	65.6	84.2	2.99	ns	**	ns
WSC	70.3	29.7	120	37.1	11.10	**	**	*
NDF	443	477	438	506	9.25	ns	**	ns
ADF	376	397	350	415	8.19	ns	**	*
ADL	82.4	94.5	62.0	86.0	3.66	**	**	**
pH		4.71		4.77	0.024	ns	-	-
Lactic acid		50.4		46.5	1.508	ns	-	-
Acetic acid		21.3		22.1	1.967	ns	-	-
Butyric acid		0.780		1.27	0.341	ns	-	-
Total acids		81.7		84.4	2.584	ns	-	-

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

Table 2

Composition of nitrogen fractions in herbage and silage (g kg^{-1} TN)

Item	Lucerne		Red clover		SEM	Treatment		SxE
	fresh	silage	fresh	silage		S	E	
Total nitrogen (g kg^{-1} d.m.)	29.6	28.9	26.5	24.4	0.656	**	*	ns
Protein nitrogen	702	573	765	716	25.45	*	*	ns
BSN	273	322	180	199	18.12	**	*	ns
NPBSN	109	226	123	138	14.43	**	**	**
BSPN	164	96.1	56.7	60.6	13.04	**	**	**
Peptide N	84.1	65.1	87.4	84.0	3.32	*	*	ns
Amino acid N	24.9	160	35.6	54.5	17.12	**	**	**
NDIN	96.1	113	108	258	20.12	**	**	**
ADIN	68.6	95.9	76.8	177	13.15	**	**	**
$N\text{-}NH_3$		61.4		62.4	1.56	ns	-	-

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

NDIN and ADIN. As for BSPN, the interaction resulted from the opposite direction of changes in the contribution of this fraction, i.e. its decrease in ensiled lucerne and increase in ensiled red clover. With respect to N-AA and NPBSN, the species-specific interaction resulted from a larger increase in the share of these forms in ensiled lucerne than red clover. The interaction for NDIN and ADIN was caused by an over eight-fold higher increase in NDIN and over four-fold higher increase in ADIN in red clover silage compared to lucerne silage.

The significant differences in the protein N content between lucerne and clover herbage, identified in the current study, are consistent with the data of Kirchof et al. (2010). However, it should be noted that the content of this fraction in both plants was low. In the experiment reported by Owens et al. (1999), the contribution of protein N in TN was higher by about 150 g compared with the current results, reaching 863 in lucerne and 882 g kg^{-1} TN in red clover hernage. Papadopoulos and Mckersie (1983) determined the nitrogen content of protein at 915 and 959 g kg^{-1} TN in the first cut herbage of lucerne and red clover, respectively. Simultaneously, these authors confirmed a significant impact of the species on this nitrogen fraction in TN. Jones et al. (1995), who analyzed the crop of lucerne and red clover, obtained a higher proportion of protein N in TN than in our study: by about 137 g kg^{-1} (lucerne) and 100 g kg^{-1} (red clover).

While ensiling lucerne herbage in round bales, the share of the soluble forms of N (BSN) rose by about 18%, due to a high increase in NPBSN of BSN (*ca* 106%), mostly because of the increase of N-AA (*ca* 660%). Simulta-

neously, the share of peptides (*ca* 23%) and soluble proteins (*ca* 41%) in this fraction decreased. In red clover silage being made in bales, an increase of the soluble fraction was smaller than in lucerne. The percentage of of BSN, NPBSN, NAA, increased by 10, 12 and 15%, respectively. Meanwhile, in contrast to lucerne, the soluble protein fraction contribution increased (*ca* 11%), while the share of peptides decreased insignificantly (4%). While analyzing the soluble fractions of nitrogen in red clover herbage, HEDQVIST and UDEN (2006) obtained a significantly higher contribution of buffer soluble nitrogen – BSN (390 g kg^{-1} TN) compared to the present research. Likewise, its non-protein and protein part (316 and 73.7 g kg^{-1} TN, respectively) as well as peptide N (127 g kg^{-1} TN) and N-AA (41.3 g kg^{-1} TN) were higher. These observations prove that lucerne protein is more susceptible to hydrolysis during ensiling (SULLIVAN, HATFIELD 2006). The insoluble (ADIN) and sparingly soluble (NDIN) fractions in TN of lucerne silage were higher by 39% and 18%, respectively, than in raw materials. Regarding red clover, the process of ensiling in round bales increased the participation of NDIN by 140% and ADIN by 129%.

With the wilting and ensiling conditions of lucerne and red clover being similar (the degree of compaction in bales, number of film layers), in addition to their different susceptibility to proteolysis, these plants might respond differently to the rise in temperature that occurs during the first days of ensiling in bales.

CONCLUSION

The study revealed a different response of lucerne and red clover herbage to ensiling in round bales, manifested by changes in the all nitrogen fractions. The ensiling of lucerne herbage strongly reduced the contribution of protein nitrogen, while raising the total soluble fraction of nitrogen. The smallest changes affected nitrogen in the form of soluble proteins, NDIN and ADIN. A high proportion of protein nitrogen in round bale wilted red clover silage was accompanied by a large contribution of the insoluble nitrogen fractions. A large percentage of protein nitrogen in red clover silage does not necessarily mean a higher nutritional value of its protein it may contain a large amount of protein compounds that are non-hydrolysable by bacterial and intestinal enzymes.

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