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CHANGES OF NITROGEN COMPOUNDS DURING ENSILING OF HIGH PROTEIN HERBAGES – A REVIEW*

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Abstract

Losses of crude protein during ensiling of herbages, in contrast to carbohydrates, do not affect the reduction of its content; their form is changed into greater solubility non-protein compounds and also highly degraded forms, which lower the efficiency of the microbial protein synthesis in the rumen. These processes are accompanied by a change of amino acid composition of herbage protein and decrease in intestinal digestibility of protein from feeds as a result of the formation of indigestible complexes with carbohydrates (ADIN). Reduction of protein degradation in silages is achieved by accelerated acidity through addition of acids or dominance of homofermentative bacteria. The positive effects of fermentation inhibitors or sorbents use, as well as the wilting of raw material on the level and rate of protein degradation were demonstrated by many researchers. A greater contribution of protein nitrogen and reduction of deamination in silages can also be obtained by using bacteria inoculants. Increasing the proportion of protein nitrogen is accompanied by the improved efficiency of microbial protein synthesis.

Key words: silage, legumes, proteolysis, crude protein, nitrogen fractions

Current interest in the excessive nitrogen emission to the environment, in the form of ammonia to the atmosphere and nitrates into groundwater, in animal production primarily concerns ruminants, and results from low utilization of nitrogen (Huhtanen, 2010). The quantity of the nitrogen pool leaving the animal body depends on the efficiency of nitrogen conversion which in milk production is 18–30%, and in meat production 10–20%, which is far below the potential of cattle, which exceeds 40% (Dewhurst et al., 1996; Frank et al., 2002). These losses result from the low efficiency of microbial protein synthesis in the rumen, caused on the one hand by rapid and excessive degradation of plant protein, and on the other hand by slow release

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of energy from cell wall components in the rumen (Davies et al., 2005). For these reasons, many studies have focused on strategies to improve nitrogen balance in milk and meat production based on the grass and legume silages (Moorby et al., 2002; Nadeau et al., 2007). Improving the efficiency of nitrogen utilization can be achieved by improving the energy supply of rumen microorganisms and reducing the degree of protein degradation in the rumen. A common strategy aimed at improving nitrogen energy balance is the use of maize silage and high-starch feeds. However, the proportion of the latter is limited due to the risk of subclinical rumen acidosis (Moorby et al., 2002; Nadeau et al., 2007). Increasing the proportion of low degradability protein in the rumen is applied in practice through, for instance, supplementation with extracted meals, which increases protein synthesis, but impairs the utilization of total nitrogen (Huhtanen and Shingfield, 2005). In practice, little attention is paid to the improvement of the protein composition of roughage that can be achieved by restrictive fertilization of fodder, and above all, by reducing the processes of proteolysis during ensiling of high-protein herbages (Davies et al., 2005; Purwin et al., 2010).

One of the main causes of lower degree of nitrogen utilization in the rumen is extensive hydrolysis of protein during ensiling (Jones, 2000). This process proceeds through two pathways.

In the first phase of ensiling, protein degradation to non-protein nitrogen compounds (NPN) takes place under aerobic conditions, mainly due to the action of proteolytic plant enzymes (Gąsior and Brzóska, 2002). Proteases in lucerne exhibit a special activity. Ohshima and McDonald (1978) showed that the plant enzymes are known to decarboxylate aspartic acid to alanine and glutamic acid to γ-aminobutyric acid (GABA).

The second pathway of proteolysis is carried out by lactic acid bacteria, acetic acid bacteria, or butyric acid bacteria. Amino acids are broken down by deamination to form ammonia and suitable organic acids, and by the decarboxylation to form CO_2 and biogenic amines (Gąsior and Brzóska, 2002).

Another factor limiting the use of nitrogen in the rumen is the large decrease in the content of soluble carbohydrates in silages resulting in a deficiency of energy substrates for rumen microorganisms. These losses are visible in the low silage nitrogen utilization by ruminants and may range, by various authors, from 44 to 87% of crude protein (Papadopoulus and McKersie, 1983; Albrecht and Muck, 1991; Jones et al., 1995). Dewhurst et al. (2003) showed that the degradability of nitrogen compounds from red clover silage (65%) in rumen was lower than that from white clover (67%), grass (70%) and lucerne silage (72%). This may confirm an improved efficiency of microbial synthesis in dairy cows fed red clover silage compared with grass silage (Davies et al., 1999).

Characteristics of nitrogen fractions in herbages and silages at different stages of silage production

Changes in chemical composition and quality of herbages begins at the time of cutting plants and lasts until the intake of conserved forages by animals (Figure 1). Crude protein contained in the plant material is a heterogeneous mixture of several proteins and non-protein nitrogen compounds. In total nitrogen of herbage, true pro-

tein constitutes 75 to 90% (Jones, 2000; Givens and Rulquin, 2004; Slottner and Bertilsson, 2006; Martineau et al., 2007), and the rest are non-protein nitrogen compounds (NPN), among which one may find organic nitrogen as free amino acids, peptides of different chain length, amides, nucleotides, chlorophyll and mineral nitrogen as nitrates.

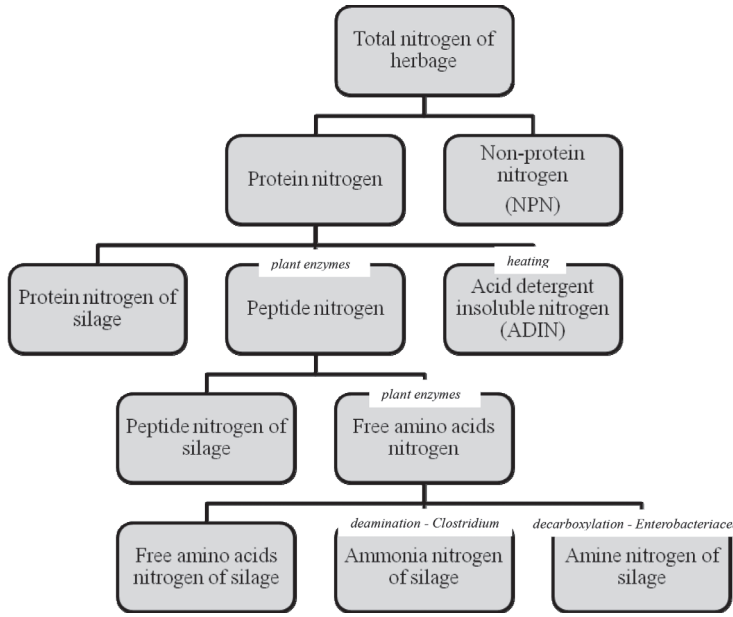

Figure 1. Composition of total nitrogen of silage

Non-protein nitrogen compounds of herbages contain a small amount of ammonia nitrogen, which is less than 1% of total nitrogen (Ohshima and McDonald, 1978; McDonald et al., 1991). Majority of the nitrogen absorbed by plants from the soil is reduced from NO_3 to NO_2 by nitrogen reductase and then to NH_4. Ammonia is not accumulated in plants, even though it may be absorbed by roots (Buxton and O'Kiely, 2003). The only form of inorganic nitrogen which can accumulate in plants are nitrates. This happens when the nitrogen supply exceeds the plant demand for growth.

The rate at which nitrates are converted to organic nitrogen in plants depends on the intensity of photosynthesis. Environmental factors limiting photosynthesis in leaves, such as shading, drought, disease, pesticides and others often lead to an increased concentration of NO_3. Grasses are more likely to accumulate NO_3, particularly in lower stem internodes (Buxton and O'Kiely, 2003).

Legumes accumulate less nitrate due to low fertilization and symbiotic nitrogen fixation in the form of NH_3. Nitrate toxicity in livestock production can occur at high N-fertilization (Buxton and O'Kiely, 2003).

Almost all true protein of plants has enzymatic character and its existence is connected with growth and biochemical functions of cells (Table 1). Chloroplasts con-

tain approx. 75% of the total amount of this component in leaves. The predominant enzyme (approx. 50% of soluble protein) is a ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo).

Table 1. Characteristics of herbage protein (Van Vuuren, 1993)

Protein solubility	Fractions	Location in cell	Amount
Soluble proteins	Fraction 1 – RuBisCo	chloroplasts	30–40%
	Fraction 2 – contains approx. 25% of leaf protein	chloroplasts, cytoplasm	15–20%
	Free amino acids		5–10%
Insoluble proteins	Chlorophyll-protein complexes	chloroplast membranes	≈30%
	Cell wall glycoproteins associated with cellulose	cell walls	≈10%

Crude protein content in herbages depends on the phase of the vegetation, fertilization and water supply. Plant maturity affects not only the content but also the proportion of particular protein fractions (soluble, degradable, indigestible; Elizalde et al., 1999). Plants in the earlier phase of vegetation have more NPN mainly composed of N-NH_3, nitrates, amines, amides and free amino acids. Fertilization, especially mineral, has influence on plant physiology and leads to changes in the content of particular nitrogen fractions. The increase of nitrogen in soil increases total nitrogen content, but the pool of NPN grows faster than the pool of protein nitrogen. Nitrogen fertilization reduces undegradable protein fraction in the rumen which flows bypass to the intestine (Jones, 2000).

Changes in the chemical composition and quality of herbages begin at the time of cutting plants (Table 2). During wilting first proteolytic changes take place. The scope of proteolysis during wilting depends on the rate and extent of water losses. Kemble and Macpherson (1954) observed that during a 3-day wilting more than 20% of plant protein degraded to NPN. Increasing the concentration of amino acid nitrogen, ammonia and amide nitrogen affected by 26.5 hours of wilting is convergent with the reduced content of true protein (Stallings et al., 1981).

Wilting on windrows of red clover and lucerne up to 40% DM affects the increase in buffer soluble nitrogen fraction (BSN), particularly its non-protein and free amino acids part. Differences between legume species were found. During wilting of lucerne, herbage contribution of soluble proteins decreased while the proportion of non-protein compounds increased. Contrary to lucerne, in red clover rapidly soluble protein fraction increased, and such a dynamic increase of free amino acid nitrogen was not found. Also, significant losses of peptides were observed (Purwin et al., 2014 a).

In the first phase of ensiling, at raised temperature conditions, the activity of plant proteases increases and extensive hydrolysis of proteins to amino acids occurs (Duniére et al., 2013). Furthermore, at this time enterobacteria affect decarboxylation and deamination of amino acids and the decay of nitrates. As a result of these changes nitrogen oxide and dioxide, ammonia, and biogenic amines are created (Lin et al., 1992). Rapid rise in temperature depends on the weight and density of ensiled material, differing in duration and the amplitude of temperatures (Guo et al., 2007).

Table 2. Composition of nitrogen fractions in fresh and wilted herbages and silages from high-protein plants (% total nitrogen)

Species		N protein	N-AA	NH_3	ADIN	References
Lucerne	Fresh	70.2–85.0	2.49–6.65	0.28	4.8–6.86	Guo et al., 2008; Purwin et al., 2014 a
	Wilted	64.2–82.9	6.01–6.83	0.43	6.69	
	Silage	29.8–47.9	24.0-51.9	4.4–10.9	1.71–10.5	Hristov and Sandev, 1998; Schwab et al., 2003; Guo et al., 2008; Grabber, 2009; Grabber and Coblentz, 2009; Hymes-Fecht et al., 2013
Red clover	Fresh	76,0–76.5	3.56–4.3	1.00	7.68	Ohshima and McDomald, 1978; Purwin et al., 2014 a
	Wilted	69.5	4.66		6.46	Purwin et al., 2014 a
	Silage	43.9–74.9	11.3–29.9	2.7–14.4	2.65–11.8	Ohshima and McDonald, 1978; Schwab et al., 2003; Grabber, 2009; Grabber and Coblentz, 2009; Hymes-Fecht et al., 2013
Grass	Silage	31.2–51.4	22.3–52.4	3.5–8.6	1.4–2.89	Nsereko et al., 1998; Krizsan and Randby, 2007; Purwin et al., 2010
Sainfoin	Fresh	78.9–87.8	2.4	0.5		Cavallarin et al., 2005; Lorenz and Udén, 2011
	Wilted	72.1	10.8	2.7		Cavallarin et al., 2005
	Silage	48.4–74.0	29.9–34.1	5.56–14.4	6.8–8.8	Lorentz et al., 2010; Lorenz and Udén, 2011
Birdsfoot trefoil	Silage	34.3–42.3	34.6–38.6	3.8–5.2	1.2–3.7	Grabber and Coblentz, 2009; Hymes-Fecht et al., 2013

During fermentation the proteases activity is rapidly reduced, and the activity of enterobacteria is maintained to achieve the pH of the silage mass below 5.0. In this phase, the activity of proteolytic enzymes ceases as a result of lowering pH. Plant proteases perform different functions and, depending on plant species, they have a different pH-activity subject to temperature (Jones, 2000). Optimum pH for the activity of these enzymes is, for lucerne and red clover, 5.5 (Jones et al., 1995) and 6.5 and 7.0 respectively (McKersie, 1985). However, the majority of plant enzymes retains 15–30% of its activity at pH 4.0. Cutting time affecting the increase in the WSC content causes an increase in acid production during fermentation, resulting in a faster rate of pH drop and inhibits protein degradation (Owens et al., 1999).

While the degree of protein hydrolysis in the plant material is the effect of plant enzymes action, decomposition of free amino acids is limited by enterobacterial activity, species composition of lactic acid bacteria and the growth of proteolytic bacteria (Winters et al., 2001).

The final products of the proteolytic transformation during ensiling are non-protein nitrogen compounds including free amino acids, amines and ammonia (Purwin et al., 2009). This fraction is greatest in lucerne silages and may constitute, according to different authors, from 44 to 87% of total nitrogen (Luchini et al., 1997; Kung and Muck, 2006), and from 7 to 40% of total nitrogen in red clover (Papadopoulos and McKersie, 1983).

After opening the silo before silage feed-out, reheating of the ensiled material may occur. In both these phases, under the influence of temperature and oxygen, Maillard reaction may occur which involves condensation of a carbonyl group of a reducing sugar with a free amino group of the amino acid, peptide or protein. The rate of this reaction may be nine thousand times faster at 70ºC than at 10ºC. Silage nitrogen is then bound in complexes with carbohydrates indigestible for animals, included in the acid detergent insoluble nitrogen (ADIN; Purwin et al., 2014 b). With mild effect of the temperature factor the lowering of lysine availability occurs. With the increased intensity of heating, the Maillard reaction can avoid the formation of 1-amino-1-deoxyketose, which results in the damage to other amino acids, such as arginine, tryptophan, cystine, histidine (Michalska and Zieliński, 2007).

The presence of the oxygen kept in the ensiled material extended the survival of enterobacteria in the storage period, which can start a re-growth and development at the moment of feed-out phase by re-exposure to oxygen and an increase in pH. At the same time nitrogen fractions are used by aerobic bacteria and mold.

Mutual proportions between particular forms of non-protein nitrogen (Table 2) depend on the rate of amino acids degradation (Givens and Rulquin, 2004). Breakdown of amino acids occurs in the fermentation process, in which the proteolytic clostridia (especially *C. sporogenes* and *C. bifermentans*) selectively degrade amino acids by deamination, decarboxylation and in Stickland reactions (McDonald et al., 1991; Jones, 2000). In the amino acids (AA) decarboxylation, a major role is played by *Enterobacteriaceae* which reduce nitrate to nitrite, fermented hexose to acetic acid, formic acid and ethanol, thereby inhibiting the development of clostridia (Krzywiecki et al., 2008), and lactic acid bacteria, which also have the ability to deaminate amino acids to serine and arginine. Qualitative changes in the amino acid compo-

sition of silage protein (Figure 2) occurring as a result of deamination and decarboxylation, involve an increased contribution of alanine (Jones, 2000), methionine and branched chain amino acids, and reduce levels of amino acids such as arginine, histidine (Givens and Rulquin, 2004), and the formation of biogenic amines in silage (histamine, tyramine, cadaverine and putrescine; Gąsior and Brzóska, 1999).

Amino acids in the feeds may thus be in the form of peptide bonds and in free forms. Contribution of the free forms in relation to the total quantity of individual amino acids can show differences in the hydrolytic properties of proteins.

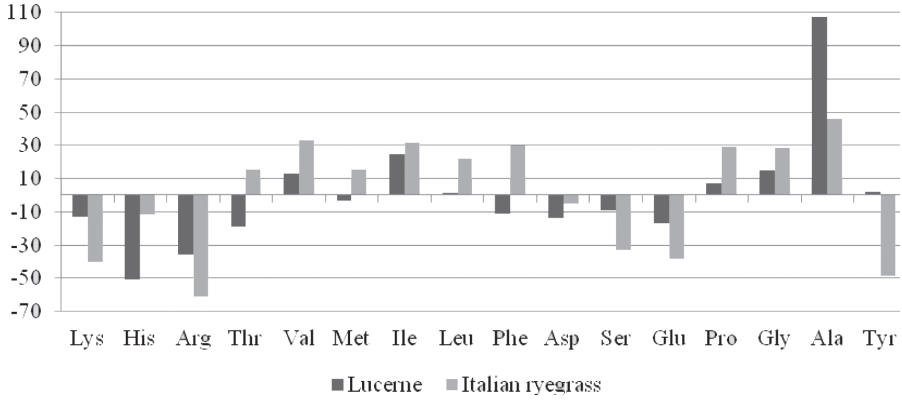

Figure 2. Change (%) in amino acid composition of wilted lucerne and Italian ryegrass silages as compared to herbage (Givens and Rulquin, 2004)

Participation of free amino acids is the result of their release in the process of proteolysis and peptydolysis, and losses as a consequence of deamination and decarboxylation. A significant impact on the proportion of free amino acids is exerted by the conversion of certain amino acids into others in Stickland reactions. During wilting and ensiling, the process of changing the amino acid composition of protein in hydrolysates occurs, which has been confirmed in numerous studies (Winters et al., 2001; Givens and Rulquin, 2004), but the change in the forms of individual amino acids also takes place. The use of amino acids introduced into the rumen in free form and in the form of peptides or proteins can vary considerably. The extent of these changes may affect the effectiveness of the utilization of particular amino acids and the utilization of nitrogen at all. Minimizing hydrolysis of protein and consequent catabolism of amino acids can potentially reduce the demand for energy necessary for the synthesis of amino acids *de novo*, and it can improve the efficiency of protein synthesis. Most studies concerning changes in amino acid composition were related to changes in the composition of protein hydrolysates of silages or changes in the contribution of total free amino acids during ensiling.

While comparing different methods of preserving herbages, Arrigo (2006) observed the largest deviation from the total initial content of AA in the amino acid composition of the protein of dried grass as hay on the swath and explained these losses of leaves during machining, cutting and tedding four times.

Research on the various species of *Lactobacillus* sp. indicate that proteolytic systems in these bacteria differ in activity. Very strong proteolytic action is characteristic of the strains *L. helveticus*, *L. paracasei* sp., *L. paracasei*, *L. acidophilus*, *L. casei* and *L. buchneri* subsp. *bulgaricus delbrueki* (Sasaki et al., 1995) and *Enterococcus faecalis* (Hegazi, 1987). Morishita et al. (1981) and Chopin (1993) observed that lactic acid bacteria are microorganisms with a high requirement for free AA and peptides. If the concentration of amino acids in the free form in herbage is too low, the hydrolysis of proteins to amino acids is required for the growth of lactic acid bacteria and for achieving a sufficient acidification of silage. Proteases of lactic acid bacteria hydrolyze the protein to oligopeptides consisting of 4–8 amino acids outside the cell, then they are received to the cell (Kunji et al., 1996) and hydrolyzed by intracellular aminopeptidases. Thus released amino acids are again incorporated in the bacterial proteins or they are catabolized by them, but they do not return in a free form to the silage environment (Van Boven and Konigs, 1988). In the study by Purwin et al. (2014 a), wilting of lucerne and red clover herbages increased the amount of amino acids linked by a peptide bond without deterioration in the amino acid profile. But losses in exogenous amino acids limiting milk protein synthesis, i.e. Lys, Met, Hys, Arg, were not observed.

Indicators of protein degradation in silage

Indicators which characterize degradation of nitrogen compounds in silage include contribution of protein and non-protein nitrogen (Licitra et al., 1996) and ammonia nitrogen in the total nitrogen (Davies et al., 1998; Brzóska et al., 1999; Jones, 2000; Purwin et al., 2006; Guo et al., 2008, 2011) as a result of hydrolysis and deamination, respectively. The extent of protein hydrolysis during ensiling is defined as the ratio of protein nitrogen in silage to the amount of protein nitrogen in herbage before ensiling. A precise indicator of protein hydrolysis is free amino acid nitrogen content in silage (Jones, 2000; Winters et al., 2001; Givens and Rulquin, 2004), as well as water-soluble nitrogen (Slottner and Bertilsson, 2006), or in buffer (Hedqvist and Udén, 2006). An indicator of changes in the quality of protein, as a result of oxygen and thermal processes in silage, is acid detergent (ADIN) and neutral detergent insoluble nitrogen (NDIN) content.

Currently used systems of feed evaluation take into consideration the specific character of nitrogen transformations in the digestive tract of ruminants, dividing protein into degradable and undegradable in the rumen (IZ PIB-INRA, 2009). American Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992) unifies diverse methods of separation of nitrogen fractions. It adopts solubility in borate-phosphate buffer and neutral and acid detergent as a criterion for the partition of nitrogen compounds. Simultaneously, the susceptibility of these compounds to enzymatic degradation in the gastrointestinal tract of ruminants is defined and shown in Figure 3. This system allows the assessment of changes in protein quality during preservation and storage of forages, however, it requires making the analytical separation of nitrogen fraction into protein nitrogen and non-protein nitrogen compounds, determination of buffer soluble nitrogen (BSN), buffer soluble protein nitrogen (BSPN), non-protein buffer soluble nitrogen (NPBSN) and an additional

separation of less soluble nitrogen and completely insoluble compounds (NDIN, ADIN).

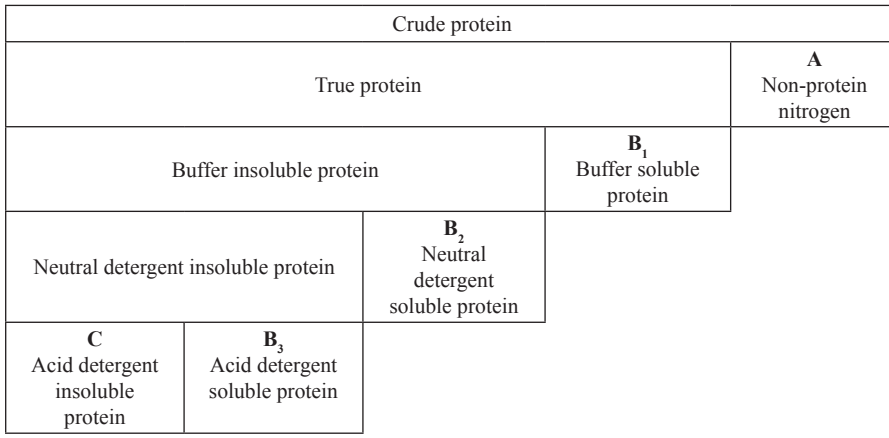

Figure 3. Protein fractions by Cornell Net Carbohydrate and Protein System (Nadeau et al., 2012)

For additional information on nitrogen transformations in silo, it is necessary to isolate the peptide nitrogen, amino acid nitrogen and ammonia nitrogen, as well as to evaluate changes in the status of individual amino acids during ensiling of peptide linkages to free amino acids and determine the content of biogenic amines in silage, as the resulting product in the process of decarboxylation of free amino acids.

Factors affecting the proteolysis and methods of restriction

The rate and extent of proteolysis during ensiling are influenced by many factors (Duniére et al., 2013). The most important of them are: species of ensiled plants (Papadopoulos and McKersie, 1983; Albrecht and Muck, 1991), dry matter content in ensiled forage (Macpherson and Slater, 1959), the rate of pH change (Scalet et al., 1984; McKersie, 1985) and temperature (Muck and Dickerson, 1988).

Dry matter content and pH in ensiling material are considered as the most important factors to proteolysis, and may be modified while species of ensiled plants becomes a factor that strongly influences the rate of proteolysis with a lower degree of wilting (Wilkins, 1984; Jones, 2000; Edmunds et al., 2012). During wilting of herbages, the range of proteolysis depends on the rate of water losses from the cells (McDonald et al., 1991). Pitt et al. (1985) showed that even a slight increase in dry matter content has a large effect on the rate of proteolysis. Wilting not always improves the quality of nitrogen compounds in silage. In good weather conditions it can improve fermentation quality and reduce silage effluent (Dawson et al., 1999), as well as improve the quality of silage protein by its protection against hydrolysis (Hristov and Sandew, 1998). However, wilting is the process during which there is a rapid increase in the pool of free amino acids, peptides, and amides.

Proteolytic potential is closely connected with the species of ensiled plants and depends on the overall activity of proteases contained in the cells and the availability

of susceptibility of protein substrates to proteolysis (Ohshima and McDonald, 1978; Guo et al., 2007). A species feature is also the content of water soluble carbohydrates (WSC), buffer capacity and the presence of proteolysis inhibitors (Slottner and Bertilsson, 2006; King et al., 2012; Lee et al., 2012). Buffer capacity and WSC content are key factors for the rate of change in pH during ensiling (McDonald et al., 1991). One factor strongly related to the species is the presence of natural mechanisms protecting the protein, i.e. the activity of polyphenol oxidase (PPO) and tannins which form complexes resistant to proteolysis (Min et al., 2003; Lee et al., 2006). Polyphenol oxidase converts the polyphenols present in the plant to quinones, which are highly reactive and readily form polymers, which are resistant to the activity of proteases and subsequently inactivate plant proteases (Lee et al., 2006; Sullivan and Hatfield, 2006). This is accompanied by the reduction of proteolysis size in the silage, and reduction of losses of the polyunsaturated fatty acids as PPO inhibits lipolysis by deactivating plant lipases (Lee et al., 2004). These processes are the most intense during the ensiling of red clover. Proteins protected in this way may account for 80% of true protein contained in the silage, resulting in an improvement in the utilization of nitrogen in the rumen (Winters and Minchin, 2001). Polyphenol oxidase in plants occurs in two forms: active (approx. 10%) and latent (approx. 90%). The first one exhibits activity within the cells at neutral pH, while the second one requires activation (Lee et al., 2008). In intact tissues, activation of PPO does not occur through compartmentation of enzyme (chloroplast) and the substrate (vacuole). To be activated, PPO requires a simultaneous damage of plant tissues (binding enzyme and substrates) and the presence of oxygen. These conditions are met to varying degrees during ensiling. Red clover silages with low PPO activity contained 25 and 20% respectively of less soluble nitrogen and free amino acid nitrogen than red clover silage with higher enzyme activity (Winters and Minchin, 2001). Significantly reduced PPO activity is characteristic of lucerne (Lee et al., 2008). In the research, Purwin et al. (2015) found different reaction of these two legume species to ensiling with regard to all fractions. Ensiling of lucerne had a stronger impact on reduction of the contribution of protein nitrogen and increased the soluble fraction as compared to red clover. The smallest changes occurred in the content of nitrogen in the soluble protein form and nitrogen in the NDIN and ADIN forms. High contribution of protein nitrogen in red clover ensiled in bales was accompanied by a large fraction of insoluble nitrogen and the low contribution of amino acids in the hydrolyzate. A large fraction of the protein nitrogen does not necessarily imply a greater nutritional value of the protein in red clover silage as it may contain a large number of fractions that are non-hydrolyzed in the intestine and by bacteria.

Apart from wilting, reduced degradation of protein in silage is obtained by proper direction of fermentation processes and acceleration of acidification by the addition of acids or bacterial inoculation (Weinberg and Muck, 1996; Pyś et al., 2000; Winters et al., 2001; Givens and Rulquin, 2004). Proteolysis is very often inhibited by additives used as inhibitors and stimulators of fermentation. A typical fermentation inhibitor, which is also an inhibitor of proteolysis, is formic acid. On the one hand, by acidification it acts restrictively on fermentation and limits the bacterial deamination and decarboxylation; on the other hand, it reduces the activity of plant proteases

(Woolford, 1984; Brzóska et al., 1994; 1995 a, b; Hertig and Potkański, 2001; Kung et al., 2003; Guo et al., 2007, 2008; Dorszewski, 2009). Formic acid, due to its low production costs and high acidification capacity, has become the basic component of additives which inhibit fermentation and proteolysis (McDonald et al., 1991; Lindgren, 1999; Randby, 2000; Johansson et al., 2002; Nagel and Broderick, 1992; Polan et al., 1998; Nadeau et al., 2000; Winters et al., 2001; Guo et al., 2008; Purwin et al., 2010; Lorenz and Udén, 2011).

Reduction of deamination in silage and increased contribution of protein nitrogen may also be obtained by using bacterial inoculants (Merry et al., 1997; Winters et al., 2001; Lee et al., 2008). The effectiveness of proteolysis inhibitors depends on the applied dose whereas the effectiveness of bacterial inoculants heavily relies on the number of colony forming units and their viability (Davies et al., 2005). Beneficial effect of fermentation inhibitors or sorbents, as well as wilting of raw materials on the level and rate of protein degradation was found by Brzóska et al. (1999) and Guo et al. (2008).

Protection of protein from degradation during ensiling is also achieved by the use of additives which form protein complexes resistant to enzymatic degradation, i.e. formaldehyde or tannins, or by the use of plants with high tannin content in mixtures, for example birdsfoot trefoil and white clover (Potkański et al., 2002; Burggraaf et al., 2008; Copani et al., 2014). After release from the cells, it binds plant protein, making it resistant to degradation by proteases, both in silo and in the rumen (Jones et al., 1995; Davies et al., 2005), thus reducing its solubility and concentration of ammonia in the rumen fluid (Chiquette et al., 1989), and increases the amount of non-ammonia nitrogen reaching the small intestine (Waghorn et al., 1987). It potentially improves the efficiency of nitrogen utilization in rations for cows (Coblentz and Grabber, 2013). Barry and McNabb (1999) showed that the content of tannins in the rumen reduces proteolysis by approx. 5% and increases the productivity of animals. These compounds influence the digestibility of feed ingredients and animal productivity, both negatively and positively, depending on the concentration in feeds and their biological activity. They can cause a reduction in protein digestibility *in vivo* and losses of nitrogen in the feces, a decrease in feed intake, the inhibition of the endogenous digestive and microbial enzymes activity, and reduce losses of endogenous proteins (Getachew et al., 2001; Frutos et al., 2004; Theodoridou et al., 2012). Efficiency of tannins and formaldehyde can be increased by combination with the addition of formic acid. As a result, two goals are achieved: a decrease in the pH outside the plant enzymes tolerance as well as binding of plant proteins in complexes (Guo et al., 2007).

Attempts at limiting proteolysis in the silage by treatment of herbages with gamma radiation (Heron et al., 1986; Charmley and Veira, 1991), and the addition of metal ions with formic acid to ensiled herbages (Brzóska et al., 1999) have also been carried out. However, no inhibitory effect of these factors on the proteolytic activity of hydrolases was obtained.

Using the Maillard reaction, Broderick et al. (1993) studied the effect of the thermal treatment on the protection of proteins from proteolysis in silo. Temperature 100ºC for 2 minutes before the lucerne ensiling decreased protease activity by 34%

and increased the efficiency of the bacterial protein synthesis by 28%. The effect of this is to increase the amount of amino acids reaching the small intestine by 42% (Charmley and Veira, 1990).

Conclusions

Composition of nitrogen fractions will vary depending on plant species. During ensiling numerous transformations in its chemical composition occur as a result of lowering pH caused by fermentation of carbohydrates and protein to organic acids. The degree and extent of these changes is the result of proteolytic activity of plant and microbial enzymes and protein substrates supply depending on the species of ensiled plants. These processes are accompanied by a change of amino acid composition of protein and a decrease in the intestinal digestibility of dietary protein as a result of the formation of indigestible complexes with carbohydrates (ADIN). Proteolytic processes during wilting and ensiling are accompanied by changes in the proportions of individual amino acids, as well as increase in the share of amino acids in free forms. These changes result from the supply of free amino acids created in the process of proteolysis and peptidolysis and due to losses from deamination and decarboxylation.

The extent of proteolysis processes during ensiling of high-protein herbages influencing the efficiency of nitrogen use in ruminants depends on selection of plant species and the technology of ensiling.

In practice, limiting of proteolysis is achieved by addition of acidifying fermentation inhibitors to the natural moisture herbage in one-phase system technology. The second way is wilting of herbages to 35–40% DM before ensiling in two-phase system technology. Regardless of the technology, a high density of plant material is one of the most significant factors limiting protein degradation during ensiling.

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