# Effect of harvest and ensiling on different protein fractions in three different legumes

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#### Abstract

Crude protein (CP) is an important nutrient in legumes. However, during harvest and ensiling proteolysis occurs which may affect the nutritive value of legumes. Thus, the objective of the present trial was to determine the relative amount of 5 feed nitrogen (N) fractions in the first and third cut of fresh, wilted and ensiled lucerne (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*) and red clover (*Trifolium pratense*). Generally, all silages were of good fermentation quality as evidenced by the high ranking according to the Deutsche Landwirtschafts-Gesellschaft (DLG). Regardless of the cuts, during wilting and ensiling the relative amount of the non-protein N (A), slowly degradable (B<sub>3</sub>) and undegradable fractions (C) increased, whereas that of the fast (B<sub>1</sub>) and variably degradable (B<sub>2</sub>) fractions decreased. Compared with red clover and sainfoin, the proportion of fraction A was greater and that of fractions B<sub>2</sub> and B<sub>3</sub> were lower in lucerne. These differences among legume species might be an effect of plant secondary compounds present in red clover and sainfoin.

Keywords: legumes, silage, wilting, protein fractions

#### Introduction

During harvest and ensiling the crude protein (CP) fraction undergoes a degradation thereby altering the CP availability and overall nutritive value of legume forages. As a result of proteolysis and desmolysis, undesired substances such as biogenic amines are formed, which ultimately might impair animal health status. Licitra *et al.* (1996) proposed a fractionation method for CP. The non-protein nitrogen (NPN) was denoted as the A fraction, while the true protein was divided into B<sub>1</sub> (buffer soluble CP = fast degradable), B<sub>2</sub> (buffer insoluble CP = variable degradable), B<sub>3</sub> (CP insoluble in neutral detergent = variable to slow degradable) and C (CP insoluble in acid detergent = indigestible) fractions based on decreasing solubility. As reviewed by Hoedtke *et al.* (2012) extrinsic and intrinsic factors influence the relative proportion of the different protein fractions. The aim of this study was therefore to monitor changes in the relative abundance of the five protein fractions in lucerne (LU), red clover (RC) and sainfoin (SF) just after cutting in the fresh state, after 1 d of wilting and after ensiling. This was repeated twice, in early summer and autumn (cuts 1 and 3, respectively).

### Materials and methods

The LU, RC and SF were cultivated as pure swards in Posieux (altitude 650 m a.s.l.). In July and September 2012, fresh and wilted samples were collected at three different locations on the field on the day of cutting and 1 d after cutting, respectively. In addition, from the same location wilted legume samples were collected and ensiled for 86 and 95 d in 1.5 L laboratory silos. In the forage samples, the relative amount of the 5 fractions were analysed according to Licitra *et al.* (1996). In addition, in the silages, dry matter (DM), pH, NH<sub>3</sub> content and fermentation products were determined. Data were analysed using analysis of variance with legume species (LU, RC, SF), cutting number (cut 1 and 3) and time of sample collection (fresh, wilted and ensiled) and the 2- and 3-way interactions as fixed factors (SYSTAT 13).

## Results and discussion

After mowing, the DM content of the legumes ranged from 14 to 18%. After 1 d of wilting, the DM content of the first cut was greatest in LU (48%) followed by SF (39%) and RC (34%). For

the third cut, the LU and SF DM content was similar (32% for both) but lower compared to RC (36%). The DM content of the silages remained comparable in order and magnitude as the wilted LU, SF and RC and were 49, 37 and 33% in the first cut and 31, 31, and 34% in the third cut, respectively. Based on the DLG evaluation scheme (Staudacher and Schenkel, 2007) one LU batch of the first cut was evaluated with only 73 points whereas all other legume silages ranked between 96 and 100 points, implying a very good fermentation quality. The main reason for the lower score of the LU batch was a higher acetic acid content, but the quality could still be regarded as good. Expressed per total N, the proportion of ammonia N in the LU, RC and SF amounted to 8.4, 7.3 and 5.5% in the first cut and 10.9, 7.0 and 4.6% in third cut, respectively (P < 0.01). Regardless of cutting number, CP content of the fresh LU were similar  $(\bar{P} = 1.00)$ . By contrast, CP content of the RC and especially of the SF were greater in the third than the first cut (P < 0.01) (Figure 1a). Due to the fermentation activity and degradation of sugars, CP content was generally greater in the silages as in the fresh grass (Figure 1a).

In Figure 1 b-f, the relative amounts of fraction A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and C expressed as percentage of total CP of fresh, wilted and ensiled LU, RC and SF harvested in early summer (first cut) or autumn (third cut) are presented. The relative amount of NPN increased during the wilting and especially during the ensiling process (Figure 1b). The greatest changes were observed in LU where the relative amount of fraction A in the silages was 60% greater compared to RC and SF (P < 0.01). A possible explanation that this shift was most evident in LU might be the fact that condensed tannins in the SF or products of the polyphenol oxidase of the RC could have hindered proteolysis of CP, resulting in lower amount of fraction A. Similarly, Tabacco et al. (2006) showed that the addition of chestnut-tannin to lucerne reduced proteolysis and consequently the amount of NPN in silages.

Regardless of cut number, the relative amount of fraction B1 and B2 decreased during the wilting and ensiling process (P < 0.01) (Figure 1c and d). The relative proportion of fraction B<sub>3</sub> was greater in wilted than fresh LU and RC, but again lower in the silages (P < 0.01). In the third cut, the same was observed also for SF whereas in the first cut the relative amount increased from fresh to the ensiled samples (Figure 1e). In LU and RC the relative amount of fraction C was low in both cuts and only minimal changes were observed between fresh, wilted and ensiled samples (Figure 1f). In the first cut the relative amount of fraction C was greater than in the third cut and was greater in wilted than fresh SF with intermediate values for ensiled SF (Figure 1f).

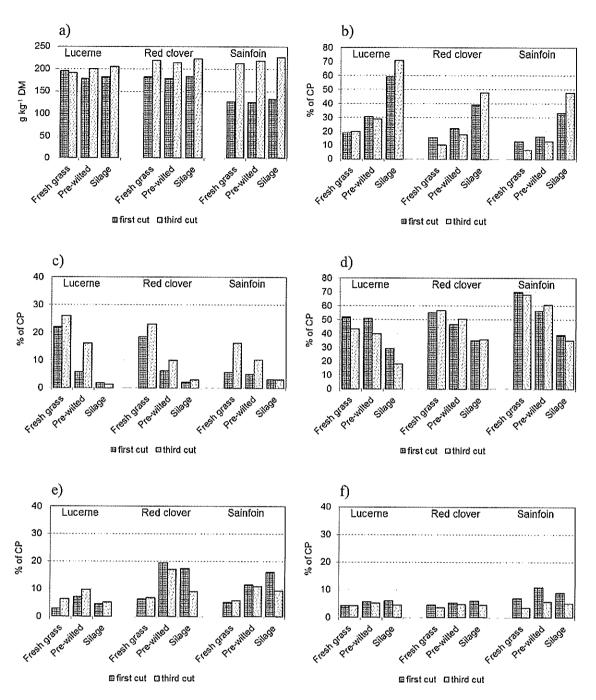


Figure 1: Crude protein content (a) and relative amount (expressed in percentage of total crude protein) of fraction A (b), B<sub>1</sub> (c), B<sub>2</sub> (d), B<sub>3</sub> (e) and C (f) in fresh, wilted and silages of lucerne, red clover and sainfoin harvested in early summer (first cut) and autumn (third cut). Values are least square means of 3 batches collected in the field at different locations.

#### **Conclusions**

The relative proportion of the CP fraction changed during the process of harvesting and conservation resulting in a marked shift towards a greater amount of NPN and a marked decrease of the fast and variable degradable true protein fraction.

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