Red Clover and the Future for Pasture Legumes as an Alternative Protein Source for Ruminants

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Current developments in UK agriculture, especially the ban on the use of animal protein in feeds as a consequence of the BSE crisis, demand reconsideration of alternative protein sources to meet ruminant livestock requirements. This is most relevant when considering winter feeding which relies heavily on low protein grass silage in combination with protein concentrates. Pasture legumes such as clovers and lucerne offer an alternative source of protein for animal feed. However, extensive protein breakdown can occur during the ensilage process and, in addition, conversion of grazed or ensiled legume protein to ruminant protein is often an inefficient process with high losses of potentially polluting nitrogen in animal wastes. At IGER we are developing strategies to minimise legume protein loss during both direct ingestion by ruminants and during the ensilage process.

**Protein loss in legume silage**

Our initial objective was to apply conservation methods developed at IGER to a range of legume crops and to assess the effects of these methods on protein composition of the silage. When red clover, lotus, sainfoin, lucerne and pea were conserved as baled silage over a season, we found that there was...
considerable crop-dependent variation in the amount of protein loss (Figure 5.1). Results showed higher residual protein in red clover, lotus and sainfoin silages compared with lucerne and pea silages. One reason for the relatively high protein contents observed in lotus and sainfoin silages could be the presence in these species of compounds called tannins (Robbins et al. IGER Innovations 4, 2000). It is well established that tannins bind with proteins, making them resistant to breakdown by protein-degrading enzymes (proteases). However, the high protein contents in red clover silage cannot be accounted for by tannins as this species does not contain these compounds. Nevertheless, we consistently find lower rates of protein breakdown during ensilage of red clover compared with other legumes.

**Other protein protection mechanisms**

These findings instigated further research into mechanisms of protein protection in this species. Protein breakdown during ensilage is mainly due to plant protease activity occurring during the early phase of the fermentation. Bacteria which proliferate during the fermentation produce acids which reduce the pH and inhibit protease activity. An experiment was carried out to establish whether reduced protein breakdown was a consequence of a very rapid fermentation occurring during ensilage of red clover or specific properties of the plant itself. We ensiled red clover in the presence of bacteria and after the elimination of micro-organisms by treatment with gamma-irradiation. Since no fermentation acids are produced with red clover ensiled under sterile conditions no inhibition of plant enzymes should occur. Protein breakdown was monitored over a 90 day ensilage by measuring free amino acid content (a protein breakdown product) (Figure 5.2). Both treatments with red clover showed low levels of protein breakdown as compared with changes in free amino acid content of white clover. In addition, the rate of protein breakdown in red clover in the presence of fermenting bacteria was slightly higher than that for sterile red clover in which plant protease activity was not inhibited. These findings show that protein protection observed during ensilage of red clover is not related to the rate of silage fermentation and must be due to other factors. It was recently reported that red clover, in contrast to most pasture legumes, has high levels of the enzyme polyphenol oxidase (PPO) and this may account for the low rates of protein loss observed during red clover ensilage. PPO reacts with phenols to produce quinones and these highly reactive compounds produce protein-quinone complexes in a similar manner to tannins.

**Figure 5.1** Protein nitrogen as a percentage of total nitrogen in baled silages

**Figure 5.2** Free amino acids released during ensilage of white clover, red clover and sterile red clover
This prompted further research into PPO and we have now isolated a red clover line which shows very low activity of this enzyme. This has enabled us to compare protein breakdown during ensiling in high and low PPO lines of red clover. Much higher levels of protein breakdown in leaf extracts were found in the low PPO red clover line compared with the normal, high PPO-line. In addition, when PPO activity in the high PPO line was inhibited by the presence of ascorbic acid, protein breakdown was as high as in the low PPO line (Figure 5.3). The conclusion from this work is that high levels of PPO activity reduce the amount of protein breakdown during ensiling. In additional work, we have shown that normal red clover rapidly forms high molecular weight, quinone containing, protein complexes, but such complexes are only formed very slowly by the low PPO plants (Figure 5.4). These findings confirm the role of PPO activity in the protection of protein during the ensilage of red clover whilst the rapid formation of protein complexes suggests that this phenomenon may also be relevant in a grazing context.

It now appears that wasteful protein breakdown in silage made from forage legumes can be reduced by the presence of either tannins or PPO activity. However, the two most important legumes, white clover and lucerne, do not contain tannins and also express very low levels of PPO activity, despite the almost ubiquitous distribution of this enzyme in plants. Therefore, it is important to consider ways in which protein protection traits could be introduced into these two species. Tannins are being investigated in one programme at IGER (Robbins et al. IGER Innovations 4, 2000) whilst, for red clover, the low PPO line may well mimic the underlying biochemical reasons for the low PPO activities found in white clover and lucerne. Thus, understanding the phenomenon in red clover could lead to strategies for enhancing the activity of this enzyme in other forage legumes. Analysis suggests that the enzyme in the low PPO line is mainly in a latent form which can be activated in the laboratory by acidic conditions or the presence of a detergent. We now need to determine the biochemical basis for the production of this abnormal form of the enzyme and understand the

![Figure 5.3 Protein breakdown in leaf extracts of high and low PPO red clover lines when incubated in conditions similar to those during silage making](image)

![Figure 5.4 Electrophoretic separation of proteins in extracts of high and low PPO lines following incubation over (a) 15 minutes and (b) 24 hours. This indicates protein/phenol complexes (arrows) are formed very rapidly in high lines but more slowly in low PPO lines.](image)
underlying genetic differences from the normal, high PPO, plants. We will then be able to analyse the low PPO phenomenon in white clover and lucerne and develop genetically based protocols for its enhancement.

The future for red clover
The demonstration of a protein protection mechanism within red clover also argues for the greater utilisation of this forage legume within UK agriculture. The species is already an important crop in Europe, Northern America and other temperate regions. It provides palatable fodder for grazing and its upright growth makes it easy to harvest for hay and silage. Its widespread acceptance can be attributed to its high yields, tolerance of a wide range of environmental conditions and soil types, capacity to fix nitrogen and high nutritive value to ruminants. In addition, the flower of red clover (Figure 5.5) has been traditionally used by many cultures for its medicinal properties, which may be due to the high content of secondary phenolic compounds such as coumarins and isoflavones. Despite these desirable characteristics, use of red clover in the UK has declined in recent times with increases in the use of fertiliser N and protein concentrates. This trend is already being reversed with the increasing use of more traceable and sustainable forms of livestock production. In anticipation of this need, the breeding work on red clover at IGER is aimed at producing varieties combining high yield, persistency, greater tolerance of grazing, reduction of anti-nutritional factors and improving seed yield. The first variety from this programme has been submitted for National List testing.

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Figure 5.5 Red clover flowers have traditionally been used for medicinal purposes