Effect of different conservation methods and maturity stages on peroxidase activity in sainfoin *(Onobrychis viciifolia)*

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Introduction

Forage is the major part of the diet for ruminant animals and provides energy, proteins and minerals. Sainfoin (*Onobrychis viciifolia*) is a perennial forage legume with a deep taproot which is often grown in conjunction with forage grasses to reduce bloat hazard. In addition, it shows anthelmintic properties and improves soil fertility by nitrogen fixing [1]. Sainfoin contains high amounts of secondary metabolites like polyphenols. Particularly proanthocyanidins have an effect on the protein metabolism by decreasing the concentration of ammonia in the rumen [2] that could increase the nitrogen value for the ruminants. Peroxidases are widely distributed enzymes in the plant kingdom and they have a variety of functions. They are involved in cell wall modifications, phytochrome metabolism, play a role as producers of reactive oxygen species, regulators of H_2O_2 signalling and defence mechanism [3]. Peroxidase (POD) and polyphenol oxidase are enzymes involved in the formation of quinoid structures and could therefore influence the interaction between polyphenols and protein. To investigate whether these enzymes contribute towards the beneficial effects of sainfoin, activity measurements and investigations into the isoenzyme patterns were performed.

Methods

Sainfoin cvs. Ambra and Sepial (Caussade Semences, France) were cultivated in 2007 at two different locations in France, Blars (Lot) and Réalville (Tarn et Garonne). The plant material was cut in May and June at four different harvesting times and either used as fresh forage or processed to silage and hay. The fresh forage was ensiled in experimental silos of 15 kg without additive. The hay was made from the fresh forage and was cured under good weather conditions. Sainfoin cv. Perly (commercial, France), was grown in Clermont Ferrand/Theix France and was harvested in summer 2008 at two stages in the first growth cycle (end of flowering and green seeds) and in the second cycle at 5 week regrowth after the end of flowering. Stems were separated manually, cut in order to mimic the mastication of the sheep, placed in nylon bags and were incubated for 4 hours in the rumen of fistulated sheep. Six sheep received 60 g/day of polyethylene glycol (PEG) which was infused 2 times per day by the ruminal cannula, six sheep were given 200 ml of water (without PEG). POD activity was determined as described [4] with *o*-dianisidine as artificial substrate by measuring the changes in the light extinction at 460 nm. Native acryl amide gel electrophoresis for 10 hours was used to separate the isoenzymes.

Results

In contrast to many other enzyme activities, POD was still active in freeze dried material. Sainfoin tissues showed a very low polyphenol oxidase activity, but a high POD activity. POD activity, however, was dependent on variety and harvesting time. Cv. Sepial generally showed lower POD activity compared to cv. Ambra. A comparison of fresh forage, hay and silage showed that POD activity is clearly present in all three plant materials but is affected by plant material processing. The activities in hay were drastically higher than in the fresh forage (Figure 1). In contrast, conservation as silage led to a decrease of POD activities. (Figure 1) Thus it maybe assumed that the polyphenol spectrum is modified during processing and storage.

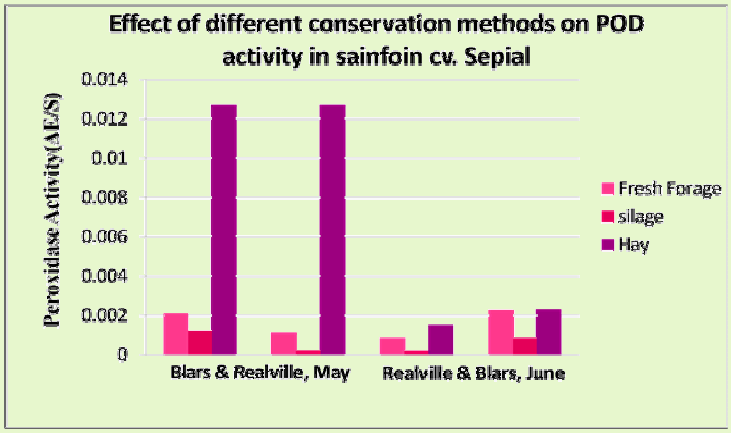

Figure 1: Comparison of the POD activity in fresh forage, silage and hay produced from *Onobrychis viciifolia* cv. Sepial grown in two different locations

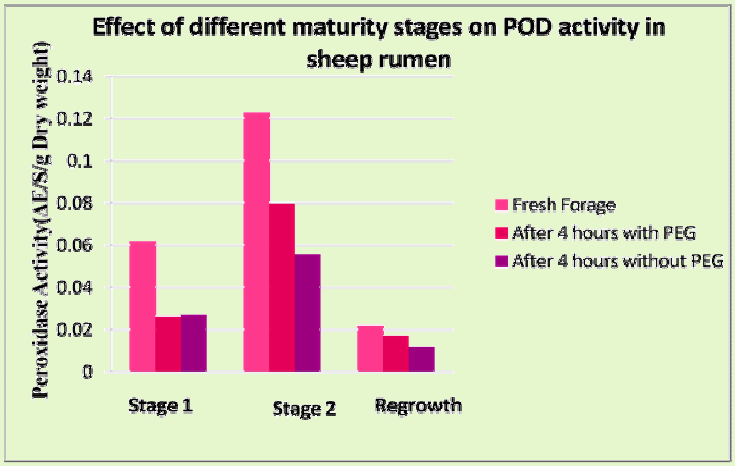

Figure 2: POD activity in sainfoin cv. Perly stem after 4 hours incubation in the sheep rumen in the absence and presence of PEG compared to the fresh forage stem.

Results observed by electrophoresis showed a quite similar pattern for all investigated samples. The only exceptions were the silage samples which showed additional bands. However up to now it is still unclear if these additional enzymes are derived from the plant or from microorganism involved in the silage process.
Work on this topic is in progress.

References

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Acknowledgements

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