

## Variation in the nutritive value of red clover (*Trifolium pratense* L.) with special reference to polyphenol oxidase activity

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Red clover has high polyphenol oxidase (PPO) activity among forage legumes. Several studies indicate that the specific PPO activity may lead to a decreased proteolysis in silo and in the rumen. However, in literature positive effects were often attributed to the PPO activity without measuring the enzyme activity or without quantifying the relationship between the PPO activity and the potentially improved forage quality. Therefore, effects of specific PPO activity in red clover grown under field conditions on forage quality as well as sources of variation influencing the PPO activity for a wide range of cultivars were investigated.

The present thesis consists of three studies. In the first one, effects of species and substrate, plant organ (leaves vs. stem), leaf age (youngest leaves versus a mixture of all leaves of a tiller) and sample preparation (freezing vs. cooling) on specific PPO activity over a range of forage plants were investigated. Results showed red clover having the highest PPO activity compared to birdsfoot trefoil, alfalfa and perennial ryegrass. Caffeic acid was the most effective substrate. Specific PPO activity was higher in red clover leaves compared to stems, while leaf age had no significant effect. No difference was found between plant material frozen or flash-frozen in liquid nitrogen for storage prior PPO analysis. Storing samples for 24 h or 48 h cooled at +4°C before freezing at -27°C enhanced the specific PPO activity compared to cooling for 1 h.

For the second and third study, 12 red clover cultivars with different geographical origin were grown under field conditions in pure stands in a 4-cut-system in different stress regimes (without/with mechanical stress) in two subsequent years. The relationships between agronomic traits, forage quality and specific PPO activity were investigated. Results of the second study showed that forage quality parameters were related to the agronomic traits, such as mean stage by count (MSC) and leaf weight ratio (LWR). A small variation in cultivars was achieved for forage quality parameters. The effect of year was more pronounced. In the first production year mechanical stress due to rolling induced a higher specific PPO activity, but cut (season) had a greater effect than cultivar and applied stress. However, specific PPO activity was poorly related to MSC, LWR, seasonal parameters and phasic acid.

In the third study, the focus was on the drying procedure (oven- (OD) vs. freeze-drying (FD)), its effect on crude protein (CP) fractions and their relationship with the specific PPO activity. OD resulted in higher amounts of CP fractions A and B3 and lower amounts of fraction B1. Multiple regression analysis indicated that specific PPO activity was of low importance for explaining the variation in protein fractions. Concerning the calculated contents of ruminally undegraded protein (RUP), variation among cultivars was detected. RUP was related to fraction B3. In this regard, irrespective of PPO activity, factors influencing the proportion of protein fraction B3 in red clover are of particular interest regarding its contribution to RUP in red clover.

Overall, specific PPO activity was of minor importance for the variation in CP fractions. For the investigated forage quality parameters a small variation among cultivars was achieved and the effect of year was more pronounced using MSC as co-variable. The results suggest that the tested genetic material was quite similar, despite the different origins.