

12. Effect of single and mixed-species legume silages on ruminal fermentation and methane production *in vitro*

*Einfluss von reinen Leguminosen und Mischungen von Leguminosen auf die *in vitro* Pansenfermentation und Methanproduktion*

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Feeding temperate legumes helps to meet the protein requirements of ruminants. However, excessive ruminal protein degradation is a limiting factor of these diets. Some legumes contain bioactive compounds like condensed tannins (CT) which are known to promote ruminal bypass of protein (1). Furthermore, evidence exists that CT from legumes can decrease ruminal methane production (1). In the present study two CT-containing legumes were compared with two CT-free legumes and mixed species thereof in their effect on *in vitro* ruminal fermentation characteristics using the Hohenheim Gas Test.

Methods: Five legumes [lucerne (LC, Sanditi), sainfoin (SF, Perly), red clover (RC, Milvus) and two birdsfoot trefoil cultivars (BTP, Polom; BTB, Bull)] were harvested at the early flowering stage from three different locations (batches) in the field. After 24 h of wilting, each batch was ensiled without additives in 30-l silos for 86 d. Silages were ground using a Moulinex food processor. Subsequently, single-species silages as well as 1:1 mixtures of the CT-containing legumes (SF, BTP, BTB) and either LC or RC (200 mg) were incubated with 30 ml of ruminal fluid/buffer mixture (1:2; v:v) for 24 h at 39°C in six runs. Ruminal fluid was collected from a ruminally fistulated cow consuming hay *ad libitum*. After incubation, total gas and methane production, and concentrations of volatile fatty acids (VFA) and ammonia (NH₃) were determined. Apart from proximate constituents, total CT were determined in the silages by the HCl-butanol method. Utilizable crude protein (uCP) content of single- and mixed-species legumes was calculated by taking the CP content of the silage and the NH₃ concentration of the incubation fluid after 24 h into account. Data were evaluated by analysis of variance with feed as fixed effect in the model. Comparisons between means were performed by the Fligner-Wolfe test.

Results: The CP content (g/kg dry matter) of LC (196), RC (197) BTP (198) and BTB (197) was similar whereas that of SF (139) was lower. Conversely, the CT content (g/kg dry matter) was higher in SF (136) and clearly lower in BTP (22) and BTB (36). Total gas production (ml) tended to be lower with SF (38.8) compared to RC (44.1, $P=0.06$), SF/RC (44.1, $P=0.02$) and SF/LC (44.6, $P=0.07$) but not compared to LC (41.8, $P=0.19$). Single- and mixed-species legume silages had no effect on absolute methane production ($P=0.48$) and methane in total gas ($P=0.17$). The concentration of total VFA in the incubation fluid (mmol/l) after 24 h was lower in SF (65.4) compared to LC (70.2, $P=0.01$), SF/LC (68.9, $P=0.03$), RC (71.8, $P<0.01$) and SF/RC (68.8, $P=0.04$). Furthermore, total VFA concentration was lower in BTP (68.4, $P=0.02$) and BTB (67.8, $P=0.01$) compared to RC (71.8). Mixtures of BT and RC did not differ ($P>0.1$) from the single legumes. Only VFA concentration of BTP (68.4) was lower ($P=0.05$) compared to BTP/RC (71.4). The NH₃ concentration (mmol/l) was lower in SF (12.9) compared to LC (17.9, $P<0.01$) and RC (16.1, $P<0.01$). The mixture of SF and LC (14.9) differed ($P=0.01$) in NH₃ from both single legumes whereas the mixture SF/RC only differed from SF. Furthermore, NH₃ in BTP (16.9, $P=0.08$) and BTP/RC (17.0, $P=0.07$) tended to be higher compared to RC (16.1). The calculated content of uCP of the legume silages reflected differences in CP content. However, when relating uCP to CP content, values for SF increased or tended to increase compared to LC ($P<0.01$) and RC ($P=0.06$).

Conclusion: Due to the lower CP content of the SF silage, ruminal fermentation was less intensive compared to the other legumes. The replacement of half of SF by LC or RC mostly improved the fermentation activity. Although the absolute amount of uCP was lower in SF compared to LC and RC, its ratio to CP content indicates an increase in ruminal bypass protein caused by the action of the CT.

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