Tannin extracts from legumes change ruminal biohydrogenation of polyunsaturated fatty acids and decrease methane production *in vitro*

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Introduction

Bioactive compounds like condensed tannins (CT) build complexes with protein (Hagerman and Butler, 1981), cellulose, hemicellulose and pectin (McSweeney et al., 2001) which protect the nutrients from ruminal degradation. Furthermore, in vitro studies showed that CT bind to cell coat polymers of bacteria. The reaction of CT with nutrients can cause both positive and negative effects. Beside reduced ruminal ammonia and blood urea concentration (Carulla et al., 2005), lower ruminal biohydrogenation (BH) of polyunsaturated fatty acids (PUFA) (Khiaosa-Ard et al., 2009) and better live weight gain of lambs (Waghorn et al., 1987) were reported. However, Scharenberg et al. (2007) found higher fecal N excretion and reduced apparent digestibility of fiber and minerals when lambs were fed sainfoin (SF). These opposing effects could be due to variations in dietary intake and chemical structure of the CT which can vary among species (Mueller-Harvey, 2006) and within species (Azuhnwi et al., 2013) and therefore influence the activity of the CT (Frazier et al., 2010). Sainfoin and birdsfoot trefoil (BT) are CT-containing legumes, which seem to show great promise for ruminant nutrition, as they can decrease ruminal protein degradability and increase available N in the duodenum in sheep (Theodoridou et al., 2010) as well as increase milk production in dairy cows (Hymes-Fecht et al., 2013). Therefore, the objectives of the in vitro study were to compare the effects of CT extracts from SF and BT at the same inclusion level and of two different inclusion levels of SF CT extracts on ruminal fermentation and BH of PUFA.

Material and Methods

The in vitro study was carried out using a batch culture technique (Sterk et al., 2010). Either 0 mg (CON), 2.5 mg BT extracts (BT5), 2.5 mg SF extracts (SF5) or 14 mg SF extracts (SF28) were weighted into glass flasks. Subsequently, 0.5 g of a basal diet (60% hay, 33% maize silage, 7% linseed; ground to 1 mm) together with 50 mL of a ruminal fluid-phosphate buffer-mixture (1:4, v/v) were added to each flask. The ruminal fluid was taken from 2 Holstein Friesian cows 2 h after the morning feeding, mixed and strained through a double layer of cheese cloth. The incubation of the

flask was carried out at 39°C and was stopped by putting the flasks in iced water after 3, 6, 12, and 24 h. Methane and total gas production were only analyzed in the 24 h flasks, ammonia concentration, concentration of volatile fatty acids and fatty acid composition were analyzed in all flasks as previously described by Sterk et al. (2010). All treatment time point combinations were incubated in triplicate in 2 consecutive runs (n = 6). Data were evaluated by analysis of variance, separately for each time point, using treatment as fixed effect in the model.

Results and Discussion

Independent of the treatment, the concentration of acetate and butyrate decreased while the concentration of propionate, iso-butyrate, valerate and iso-valerate increased with increasing incubation time. After 24 h of incubation, the total amount of volatile fatty acids was similar among treatments. However the composition changed. The concentration of acetate (P=0.01), butyrate (P<0.01), iso-butyrate (P=0.03), valerate (P<0.01) and iso-valerate (P<0.01) after 24 h of incubation, were lower while the concentration of propionate (P<0.01) was greater in SF28 compared to CON. Additionally, BT5 decreased the concentration of iso-valerate (P<0.01) after 24 h of incubation compared to CON. At the same time, the concentration of NH_3 (P<0.01) was decreased in both BT5 and SF28 compared to CON. The total gas (P<0.01) and methane (P=0.05) production and the amount of methane per total gas production (P<0.01) after 24 h of incubation were lower in SF28 compared to CON. In addition, methane per total gas production was decreased (P<0.01) with BT5 compared to CON. These results suggest a decrease in ruminal protein and fiber degradation and concomitantly a decrease in methane production with SF28. Similar effects with acacia tannin extracts were reported by Khiaosa-Ard et al. (2009). In contrast to SF28, no effect of SF5 on ruminal fermentation was observed, suggesting that the concentration in SF5 was too low to cause any effects. This is in line with Hervas et al. (2003), who determined a concentration effect with quebracho tannin extracts in ewes. In contrast to SF5, BT5 caused little effects on some fermentation traits which indicates differences in the activity of CT from the two legumes.

There were no differences in the PUFA profile after 3 h of incubation among treatments. Independent of the treatment, the concentration of 18:2-n6 and 18:3-n3 decreased while the concentration of 18:0 increased with increasing incubation time. The concentration of 18:2-n6 and 18:3-n3 were greater (P<0.01) in SF28 and BT5 compared to CON after 6, 12 and 24 h of incubation. In addition, the concentration of 18:3-n3 after 6 and 24 h of incubation and the concentration of 18:2-n6 after 12 and 24 h of incubation were greater (P<0.01) in SF5 compared to CON. The concentration of the *c9t11* CLA isomer tended to be lower (P=0.06) in SF28 compared CON after 3 h of incubation, but continuously increased with increasing incubation time and finally tended to be greater (P=0.06) after 24 h of incubation in SF28 compared to CON. After 6 h of incubation the concentration of 18:1 *t*11 was decreased ($P<0.01$) in SF28 and BT5 compared to CON, after 12 h of incubation was only decreased ($P<0.01$) in SF28 compared to CON and after 24 h of incubation became similar among treatments. From 6 h of incubation onwards, the concentration of 18:0 was lower ($P\leq0.01$) in SF28 and BT5 (not significant after 12 h of incubation) compared to CON. The current findings indicate that CT extracts from SF and BT lower ruminal BH of PUFA. Vasta et al. (2009) and Khiaosa-Ard et al. (2009) concluded, that the last step of BH was influenced by tannin extracts due to an increase of C18:1 *t*11 and an decrease in C18:0. Correspondingly, in the present study the concentration of C18:0 was also reduced in both, SF28 and BT5. However, this was not in favor of a greater C18:1 *t*11 concentration but in favor of a greater 18:3-n3 and C18:2-n6 concentrations.

Conclusion

Tannin extracts from SF and BT have an impact on ruminal fermentation and BH and a clear concentration effect was observed with SF extracts. Furthermore, it seems that the mode of action differs between the 2 legumes, but the differences were not very pronounced at the low inclusion level. Further research is needed to compare the CT extracts at higher inclusion levels.

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