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Effect of condensed tannin supplementation on *in vivo* nutrient digestibilities and energy values of concentrates in sheep

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ABSTRACT

The objective of this study was to evaluate the effect of supplemented condensed tannins (CT) from the bark of the Black Wattle tree (*Acacia mearnsii)* on *in vivo* nutrient digestibility and energy values measured with sheep under standardized conditions. A commercial *A. mearnsii* extract (containing 0.203 g CT/g dry matter (DM)) was mixed into a pelleted concentrate. Four treatments consisting of grass hay and concentrate were investigated, containing different concentrations of the CT-rich extract (CON, without CT; CT1, CT3 and CT5, with 1, 3 and 5% CT-rich extract in ration DM), resulting in CT concentrations of 0, 2.03, 6.19 and 10.2 g/kg DM, respectively. In a 21-day period, nutrient digestibility of the concentrates was determined by difference with wethers ($n = 4$ per treatment, German Blackheaded Mutton) in metabolism crates following a standardized procedure. The organic matter digestibility of the concentrates was unaffected by CT1 and decreased strongly with CT3 (−21%) and CT5 (−28%; $P < .05$). Digestibility of fibre fractions was already reduced with CT1 ($P < .05$), representing a very low level of CT supplementation. The concentration of metabolizable energy of the concentrates estimated from digestible nutrients decreased strongly (-25%) from 12.9 (CON) to 9.7 MJ/kg DM (CT5) ($P < .05$). In conclusion, CT supplementation from *A. mearnsii* to rations of sheep reduced nutrient digestibilities at much lower levels than previously reported for CT from other sources (*e.g.*, forage legumes).

1. Introduction

In ruminant nutrition, the use of secondary plant compounds like condensed tannins (CT) obtained from mostly tropical trees and shrubs as well as from forage legumes has gained some importance in research. The literature about CT with both beneficial and adverse function in ruminants according to their concentration and chemical structure, is vast and often conflicting (Piluzza et al., 2014). Increasing CT concentrations in ruminant rations elevated the amount of undegraded feed protein leaving the rumen, most likely caused by decreased rates of degradation by rumen microorganisms and reduced growth rate of proteolytic bacterial species (Min et al., 2003). Furthermore, CT have improved bodyweight gain, wool production and reproductive efficiency in sheep and reduced the impact of gastro-intestinal parasitism, as reviewed by Waghorn (2008). However, CT in ruminant rations have also been reported to cause a reduction in nutrient digestibility (Frutos et al., 2004a; Silanikove et al., 1994), create negative post-ingestive feedback (Silanikove et al., 1996) and impair animal performance (Grainger et al., 2009).

For temperate forages, CT concentrations of 20–45 g/kg dry matter (DM) are generally seen as having beneficial effects on ruminant production (Min et al., 2003). However, a more specific contemplation seems necessary. When forages are fed as a sole diet, the CT in birdsfoot trefoil (*Lotus corniculatus*) have been beneficial for ruminant production, but the CT in sainfoin (*Onobrychis* ssp.), sulla (*Hedysarum coronarium*) and big trefoil (*L. pedunculatus*) do not appear to benefit productivity except for mitigating the impact of parasites (Waghorn, 2008). There is also a considerable variability in concentration and chemical structure of CT especially in forage legumes such that the mode of action and clear effects are difficult to predict (Mueller-Harvey, 2006). As an alternative to forage legumes, industrial products containing defined amounts of tannins (*e.g.*, made from the bark of the Black Wattle tree (*Acacia mearnsii*)) have been used for supplementation of ruminant rations. However, also with those more standardized products the effects on ruminant performance are equivocal such that there is still a lack of knowledge on clear dose-effect results of CT extracts on ruminants.

The aim of this study was to investigate the influence of different levels of a supplemented commercial product rich in CT from *A. mearnsii* on *in vivo* nutrient digestibility and energy value of

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concentrates under standardized conditions in sheep. We hypothesized that addition of CT in moderate concentrations of up to 30 g/kg DM would not negatively affect nutrient digestibility in sheep.

2. Material and methods

2.1. Animals and experimental design

In June 2013, a digestibility trial with sheep was conducted at the Experimental and Educational Centre for Agriculture 'Haus Riswick', Chamber of Agriculture of North Rhine Westphalia, Kleve, Germany (51° 47′ 18N, 6° 8′ 19E) to study the effect of CT supplementation on nutrient digestibility and energy value of concentrates supplemented with CT.

The digestibility trial was accompanied by a long-term feeding trial (169 days) with dairy cows that was conducted in a free-stall dairy barn at the same centre. Here, the same CT product was used to study the effect of CT supplementation on performance and N use efficiency in dairy cows (Gerlach et al., 2018) and the effects on gaseous emissions (CH_4 and NH_3) on dairy barn level (Schmithausen, 2017). The CT product (commercially available) used in both studies was an extract rich in CT made from the bark of *A. mearnsii* (declared concentration of CT of 0.725 g/g DM, Weibull Black, TANAC S.A., Montenegro, Brazil) and was mixed into the commercially produced pelleted concentrates during processing.

The digestibility of the concentrates supplemented with different amounts of CT-rich extract was measured with four male wethers (German Blackheaded Mutton) per treatment according to GfE (1991). As it is not possible to feed concentrates as a single feed, their digestibility was determined by difference by feeding them together with a forage of known digestibility and deducting the estimated effect of the latter in the calculations as described by Schneider and Flatt (1975) and GfE (1991).

Rations consisted of chopped grass hay and the different concentrates. The proportions were chosen to achieve concentrations of the CT-rich extract of 0 (CON), 1% (CT1), 3% (CT3) of ration DM in accordance with the dairy cow trial; additionally, a treatment with 5% CT-rich extract (CT5) of ration DM was tested. According to GfE (1991) one group received a ration consisting of grass hay only to determine the digestibility and feeding value of the hay. Composition of the different concentrates is given in Table 1 and the composition of the grass hay as well as of rations including the CT concentration is presented in Table 2. Lower concentrations of crude protein (CP), ether extract (EE), starch and fibre fractions in the supplemented concentrates were caused by the addition of the CT-rich extract (dilution effect). Each ration was offered to four wethers in two meals per day. A 14-d adaptation period was followed by a 7-d collection period where animals were kept in metabolism crates and all faeces and feed refusals were collected on a daily basis and aliquots (20% of daily amount) were stored at −18 °C. Samples of the hay and the concentrates were collected daily and a cumulative sample was added up for analyses. Samples were stored at −20 °C until analysis for chemical composition and estimation of energy value. At the end of each collection period composite samples of ration ingredients as well as of faeces spanning the entire 7-d period were prepared, freeze dried and analysed chemically.

2.2. Chemical analyses and calculations

Analysis of the chemical composition of the ration components and faeces samples was done by the Landwirtschaftliche Kommunikations- und Service GmbH (Lichtenwalde, Germany). The DM concentration of faeces was determined daily using a two-step procedure involving predrying samples at 60 °C, followed by oven-drying at 105 °C. Proximate analyses were done according to VDLUFA (2012) and method numbers are given below. Ash, EE and CP were analysed using methods 8.1, 5.1 and 4.1.1. The concentrations of crude fibre (CF), neutral detergent fibre assayed with heat-stable amylase and expressed exclusive residual ash (aNDFom) and acid detergent fibre expressed exclusive residual ash (ADFom) were determined following methods 6.1.1, 6.5.1 and 6.5.2., respectively. Starch was analysed following method 7.2.1 (VDLUFA, 2012). The determination of enzyme-soluble organic matter (ESOM) was done according to method 6.6.1. The Hohenheim gas test (HGT; method 25.1) was conducted for measuring the 24 h *in vitro* gas production (GP, ml/200 mg DM). The analysis of total phenol and total tannin concentration in the Weibull Black product was conducted using the Folin method (Makkar, 2000) and the concentration of CT was determined with the HCl-butanol method (Terrill et al., 1992).

The concentration of metabolizable energy (ME) of the grass hay and the concentrates was calculated according to following equations:

For grass hay (GfE, 2008): $\text{ME (MJ/kg DM)} = 5.51 + 0.0828 \times \text{ESOM} - 0.00522 \times \text{ash} + 0.02507 \times \text{EE} - 0.00392 \times \text{ADFom}$; all expressed as g/kg DM;

For concentrate (GfE, 2009): $\text{ME (MJ/kg DM)} = 7.17 - 0.01171 \times \text{ash} + 0.00712 \times \text{CP} + 0.01657 \times \text{EE} + 0.00200 \times \text{starch} - 0.00202 \times \text{ADFom} + 0.06463 \times \text{GP}$ (ml/200 mg DM); all expressed as g/kg DM unless stated.

Using the amounts and chemical compositions of feed and faeces, nutrient digestibilities were calculated. The digestibilties of the concentrates were calculated as difference between digestibility of hay and digestibility of hay + concentrate. The digestibilities of the proximate constituents were then taken to calculate ME concentration of the concentrate according to GfE (2001):

$\text{ME (MJ/kg DM)} = 0.0312 \times \text{DEE} + 0.0136 \times \text{DCF} + 0.0147 \times (\text{DOM} - \text{DCL} - \text{DCF}) + 0.00234 \times \text{CP}$, where DEE is digestible ether extract, DCF is digestible crude fibre and DOM is digestible organic matter (all expressed as g/kg DM). The NEL values were estimated from ME according to Weißbach et al. (1996): $\text{NEL} = \text{ME} [0.46 + 12.38 \times \text{ME}/(1000 - \text{ash})]$.

2.3. Statistical analyses

Statistical analyses were conducted using SAS 9.3. Data of the digestibility trial with sheep were analysed using one-way ANOVA. The Tukeys Honestly Significant Difference test (Tukey-HSD) was applied for *post hoc* comparisons. For all analyses, differences were considered significant with $P \leq .05$.

3. Results

When measured with the Folin method (Makkar, 2000), the total phenol concentration in the Weibull Black product was 0.630 g/g DM and the total tannin concentration was 0.568 g/g DM. Therefore, the total tannin concentrations in rations were 5.68, 17.3 and 28.6 g/kg DM for CT1, CT3 and CT5, respectively. The concentration of CT determined with the HCl-butanol method (Terrill et al., 1992) was 0.203 g/g DM. The low CT concentration in the extract resulted in dietary CT concentrations of 2.03, 6.19 and 10.2 g/kg DM for CT1, CT3 and CT5, respectively (Table 2).

The digestibility trial with wethers could be conducted without health problems, decreases in feed intake or changes in texture of faeces. Supplementation of the CT-rich extract did not cause feed avoidance or other irregularities in feeding behaviour. In Table 3, the nutrient digestibilities and energy values of the concentrates estimated from digestible nutrients are presented. The *in vivo* digestibility of organic matter of the concentrates was unaffected by CT1 and decreased strongly with CT3 (−21%) and CT5 (-28%; $P < .05$). The decrease was even more pronounced for digestibility of aNDFom, but with a large variation among animals. For aNDFom and ADFom, the digestibility was already reduced with CT1 ($P < .05$), whereas other constituents were only affected with CT3 and CT5. The ME concentration of the concentrates estimated from digestibility of proximate constituents decreased markedly (−25%) from 12.9 to 9.7 MJ/kg DM ($P < .05$), for