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Intake, digestibility and nutrients supply to wethers fed ryegrass and intraruminally infused with levels of *Acacia mearnsii* tannin extract

Gilberto Vilmar Kozloski*, Carla Joice Härter, Fernanda Hentz, Suélen Capa de Ávila, Tiago Orlandi, Cristiano Miguel Stefanello

Departamento de Zootecnia (Animal Science Department), Universidade Federal de Santa Maria, Cidade Universitária, Bairro Camobi, Santa Maria, RS 97105-900, Brazil

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ABSTRACT

Four Polwarth × Texel wethers (30 ± 1.8 kg body weight), fitted with a chronic rumen catheter and duodenal cannulae, housed in metabolic cages and offered ryegrass (*Lolium multiflorum* Lam) ad libitum were used in a 4 × 4 Latin Square experiment to evaluate the effects of infusing *Acacia mearnsii* tannin extract on intake, digestion and nutrients supply. Treatments consisted of no tannin (0) or intraruminal infusion of a tannin extract solution in a final volume that allowed for a tannin extract concentration of 20, 40 or 60 g/kg of dry matter (DM) intake. Intake and total digestibility of organic matter (OM), neutral detergent fiber (NDF) and N compounds linearly reduced ($P \leq 0.05$) at increased levels of tannin infusion. Urinary N excretion linearly decreased ($P \leq 0.05$) whereas the proportion of ingested N, which was retained was not affected by treatments. Duodenal flow of α-amino N was not affected by treatments whereas rumen microbial N entering the duodenum tended to linearly decreased ($P = 0.15$) at increased levels of tannin infusion. Ruminal degradability of both OM and dietary N compounds linearly decreased ($P < 0.05$) at increased levels of tannins. The efficiency of rumen microbial protein synthesis was not affected by treatments. In conclusion, the use of *Acacia mearnsii* tannin extract as feed supplement for wethers fed ryegrass, at rates from 20 to 60 g/kg DM intake, does not affect the α-amino N supply at the duodenum whereas it shows a negative impact on energy intake.

1. Introduction

Temperate grasses have usually high levels of total and soluble N, which may result in large losses of N as ammonia absorbed from rumen and excreted as urea in urine (Klopfeinstein, 1996). Tannins are polyphenolic compounds that complex with dietary proteins and microbial enzymes showing the potential to reduce ruminal ammonia concentrations and to improve the flow of non-ammonia N to the small intestine (Min et al., 2003; Waghorn and McNabb, 2003). However, tannins can also reduce feed intake and digestibility (Reed, 1995; Barry and McNabb, 1999). Moreover, their effects vary with tannin type (i.e. hydrolysable or condensed and extractable or bound tannins), concentration and molecular weight (Reed, 1995; Min et al., 2003).

Most research regarding the effect of tannins on rumen bacterial growth and protein degradation were conducted with legumes or forage trees, which have tannins as natural plant compounds. In turn, Carulla et al. (2005) observed that supplementing *Acacia mearnsii* tannin extract for lambs fed ryegrass increased forage intake whereas it depressed organic matter (OM) digestibility. However, because methane emission and urinary N excretion also decreased, energy and N retention were not affected. Although the study above indicated a positive potential of

* Corresponding author. Tel.: +55 3220 8355.
E-mail address: kozloski@smail.ufsm.br (G.V. Kozloski).

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using this extract as a dietary supplement, only one level of supplementation (41 g/kg dry matter (DM)) was tested and duodenal flow of digesta was not measured.

The present study was carried out to evaluate the effects of intraruminal infusion of levels of *Acacia* tannin extract on intake, ruminal and total digestion, duodenal microbial and α-amino N supply, and N retention in wethers fed ryegrass.

2. Material and methods

2.1. Feedstuffs, animals, housing and experimental design

Research protocols followed the guidelines recommended by the Animal Care and Ethical Committee of the Universidade Federal de Santa Maria. Four Polwarth × Texel wethers (30 ± 1.8 kg body weight (BW)), housed in metabolic cages and offered fresh ryegrass (*Lolium multiflorum* Lam, containing (g/kg DM): OM, 877 ± 9.0; total N, 35 ± 5.2; soluble N, 8.6 ± 2.0; neutral detergent fiber (NDF), 518 ± 20) ad libitum were used in a 4 × 4 Latin Square experiment. The wethers were fitted with a chronic rumen catheter (siliconized PVC, 35 cm length, 10 mm e.d. × 7 mm i.d.) and a duodenal cannulae. Treatments were no tannin (0) or intraruminal infusion of *Acacia mearnsii* tannin extract (Weibull Black, Tanac S.A., Montenegro, Brazil, reported to have 720 g/kg of total tannins) at a rate of 20, 40 or 60 g/kg of ingested DM, based on DM intake of the previous day. Wethers had free access to water and to a mineral salt containing (g/kg): Ca: 100, P: 45, S: 4.12, Na: 205, Co: 0.025, Cu: 0.450, Fe: 1.5, I: 0.05, Mn: 1.0, Se: 0.009, Zn: 2.52 and F: 0.45.

Ryegrass pasture was fertilized monthly with 25 kg N/ha. The pasture area was divided into plots of 25 m^2 and managed as a rotational system, with forage being harvested when the swards reached a height of 20–25 cm, at intervals that varied from 30 to 45 days. Ryegrass was harvested (cut 5 cm above the ground level) daily between 1600 h and 1700 h and stored at −10 °C until feeding to reduce plant tissue metabolism. Forage was offered at 0800 h and 1700 h ad libitum (100–200 g orts/kg). Tannin extract was diluted in distilled water (100 g/l) and intraruminally infused twice daily along with the feeding. Experimental periods lasted for 15 days (10 day adaptation and 5 day collection period). Feed was weighed and sampled daily from day 10 to 15 of each experimental period. Total feed refusals and feces were taken daily throughout the collection period and stored at −20 °C. At the end of each experimental period they were weighed and a sample (10% of wet weight from each sheep) was collected. All samples were oven-dried at 55 °C for at least 72 h, ground through a 1 mm screen (Willey mill, Arthur H. Thomas, Philadelphia, PA, USA) and pooled by animal and period for subsequent chemical analysis. Total urine was collected daily during the collection period in buckets containing 100 ml of 3.6 M H_2SO_4. Volume was measured and a sample of 10 ml/l was diluted to 50 ml with distilled water and stored frozen (−20 °C) until analysis. On day 15, duodenal digesta samples (100 ml) were collected at 3 h intervals over a 24 h period, composited within animal and period and stored frozen (−20 °C). For analysis, these samples were thawed, dried in a forced-air oven (55 °C) for 7 days and ground to pass through a 1 mm screen. Before drying an aliquot of the duodenal fluid (10 ml) was taken and stored at −20 °C for ammonia N analysis. On day 15 of each period, feed was offered only at 0800 h and rumen fluid samples (approximately 100 ml) were collected at 0, 1, 2, 3, 4, 6 and 8 h after feeding. These samples were filtered through a 50 μm nylon filter, the pH was immediately measured with a digital pH meter (Marte Ltda, MG, Brazil) and two aliquots (18 ml) of filtered fluid were removed. In one aliquot, 2 ml of 3.6 M sulphuric acid and, in the other, 2 ml of 500 g/l trichloroacetic acid (TCA) was added. Samples were centrifuged (4000 × *g* for 20 min) at room temperature and the supernatants collected and stored frozen at −20 °C. Pellets were discarded. The supernatant of TCA acidified samples were assumed to contain free amino acids and short chain peptides, while the pellet comprised protein and long chain peptides (Greenberg and Shipe, 1979).

2.2. Chemical analysis

Dry matter content was determined by drying at 105 °C for at least 8 h. Ash was determined by combusting at 550 °C for 3 h and OM by mass difference. Total N was assayed by a Kjeldahl method (Method 984.13; AOAC, 1997). The NDF analysis was based on the procedures described by Mertens (2002) with use of heat-stable α-amylase, except that the samples were weighed into polyester filter bags (porosity of 25 μm) and treated with neutral detergent in an autoclave at 110 °C for 40 min. (Senger et al., 2008). Concentrations of acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Method 973.18 of AOAC (AOAC 1997). Analysis of neutral detergent insoluble N (NDIN) was performed according to Licitra et al. (1996). Total N in urine samples was assayed by the Kjeldahl method and allantoin and uric acid concentrations were determined according to Chen and Gomes (1992). Uric acid was determined using a commercial kit (LABTEST, Lagoa Santa MG, Brazil) after xanthine and hypoxanthine were converted to uric acid with xanthine oxidase. Thus, the uric acid values were the sum of uric acid, xanthine and hypoxanthine and the total purine derivatives (PD) were the sum of uric acid and allantoin.

Rumen fluid samples acidified with H_2SO_4 were analyzed for ammonia N (Weatherburn, 1967) and reducing sugars (Dubois et al., 1956). The TCA-acidified samples were treated with 6 M hydrochloric acid (2 ml of sample and 2 ml of 6 M hydrochloric acid) at 110 °C for 24 h, neutralized by adding 16 ml of 0.75 M sodium hydroxide, filtered and analyzed for α-amino N. This analysis was also performed in duodenal samples. Approximately 0.1 g of dried duodenal samples were weighed into screw-cap tubes and treated with 2 ml of HCl 6 N at 110 °C for 24 h. After hydrolysis, 8 ml of 1.5 N NaOH was added into the tubes, the content diluted to 50 ml with distilled water and then filtered through a filter paper. Concentration of α-amino N in rumen fluid and duodenal filtrates was analyzed by using a non-automated method adapted from Palmer and Peters (1969) as follows: 100 μL of filtrate was mixed into a test tube with 400 μL of distilled water, 1 ml of borate buffer (19.1 g/l of $Na_2B_4O_7{\cdot}10H_2O$, 0.05 M, pH 9.2) and 250 μL of 1 g/l of 2,4,6-trinitrobenzeno sulfonate (TNBS). The mixture was incubated in a water bath at 37 °C for 20 min. Thereafter, 500 μL of 1 N HCl was added to each test tube and absorbance was read at 420 nm (UV BEL Photonics 2000) against a blank that included distilled water instead sample. L-Serine (100 mg/l) was used to make a standard curve that included test tubes containing 0.33, 0.66, 1.33 and 2.00 μg of α-amino N.

Total phenols and total tannins (Folin–Ciocalteau method), as well as the condensed tannins (CT, HCl–butanol method) concentration, were quantified in tannin extract after aqueous acetone (70%, v/v) extraction following the procedures of Makkar (2000). Ten replicates of two sets of the commercial tannin extract were analyzed. For total phenols and total tannins analysis, tannic acid was used as standard whereas CT concentration was calculated as leucocyanidin equivalent through the equation: [Abs 550 nm × 78.26 × dilution factor]/% DM (Makkar, 2000).

2.3. Calculations

2.3.1. True digestibility of OM and N compounds

True digestibility of OM (OMTD) was estimated considering that neutral detergent soluble fractions of the feces are from endogenous origin and only the NDF fraction of feces is originated from feed (Van Soest, 1994) as follows: [OM intake (g/d) − fecal NDF (g/d)]/OM intake (g/d). True digestibility of N compounds was calculated as: [N intake (g/d) − fecal NDIN (g/d)]/N intake (g/d).

2.3.2. Duodenal flux of DM

Duodenal flux of DM was estimated based on ADL concentration in duodenal digesta and feces as follows: Duodenal DM (g/d) = [fecal DM (g/d) × fecal ADL (g/kg DM)]/duodenal ADL (g/kg DM) (Porter and Singleton, 1971).

2.3.3. Duodenal flux of non-ammonia non-microbial N (NANMN)

Duodenal flux of NANMN was calculated as: duodenal N (g/d) − [microbial N (g/d) + ammonia N (g/d)].

2.3.4. Ruminal degradability of OM and N compounds

Ruminal degradability of OM was calculated as: 1 − [duodenal flux of OM (g/d)/OM intake (g/d)], and ruminal degradability of N compounds was calculated as: 1 − [NANMN (g/d)/N intake (g/d)]. However, different from calculating total digestibilities, where data of intake and fecal excretion from day 10 to 15 of each experimental period was included, only the intake observed from day 12 to 14 was accounted for calculating these ruminal variables. We assumed that it is more representative of duodenal digesta samples taken at the last experimental day.

2.3.5. Microbial N supply

The amount of absorbed purines (*X*, mmol/d) corresponding to the amount of PD excreted (*Y*, mmol/d, considering 158 mg/mmol