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# Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract

T. Orlandi, G.V. Kozloski*, T.P. Alves, F.R. Mesquita, S.C. Ávila

*Departamento de Zootecnia (Animal Science Department), Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil*

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ABSTRACT

Four Holstein steers (156 ± 33 kg of body weight (BW)) fitted with duodenal cannula and rumen catheter, were housed in metabolism pens and used in a 4 × 4 Latin square trial to evaluate the effect of dietary inclusion of levels of *Acacia mearnsii* tannin extract on rumen fermentation, digestion and duodenal flux of amino acids. The diet was offered at a restricted amount of 20 g dry matter (DM)/kg BW and consisted of oat (*Avena strigosa*) plus concentrate, in a proportion of 0.55:0.45 (DM basis) respectively. Treatments were no tannin (Control) or inclusion of tannin extract in the concentrate at a rate of 20, 40 or 60 g/kg DM (i.e. 9, 18 or 27 g/kg of total dietary DM). The apparent total-tract organic matter (OM) digestibility was not affected whereas the neutral detergent fiber (aNDF) digestibility and the OM true total-tract digestibility tended to linearly decrease ($P < 0.10$) at increased levels of tannin extract inclusion. Tannin extract linearly reduced ($P < 0.05$) the total-tract digestibility of N compounds, as well as the urinary N excretion, and linearly increased ($P < 0.05$) the fecal N excretion, N retention and the efficiency of N utilization by steers. The ruminal pH was similar for all treatments whereas the concentration of ammonia N and reducing sugars in ruminal fluid linearly decreased ($P < 0.05$) with tannin extract inclusion. Linear positive responses ($P < 0.05$) to tannin treatments were observed for duodenal flux of total N, α-amino N and non-ammonia non-microbial N. The microbial N supply tended to be negatively affected ($P < 0.10$) whereas both the ruminal OM digestibility and ruminal degradability of feed N compounds linearly decreased ($P < 0.05$) with increasing tannin extract levels. The efficiency of ruminal microbial protein synthesis tended to increase quadratically ($P < 0.10$) at increased levels of tannin extract. The dietary inclusion of tannin extract positively impacted ($P < 0.05$) or tended to impact ($P < 0.10$) the duodenal flux of all amino acid groups, as well as of most individual amino acids. The amino acid profile in duodenal digesta was more closely related to the profile in feed at increased tannin extract inclusion. In conclusion, dietary inclusion of *Acacia mearnsii* tannin extract up to a level of 18 g/kg DM decreased the urinary N excretion and improved the amino acid supply in steers fed fresh-frozen oat forage plus concentrate without significantly affecting the total OM digestibility.



*Abbreviations:* ADF, acid detergent fiber; BW, body weight; DM, dry matter; EMPS, efficiency of microbial protein synthesis; EE, ether extract; EAA, essential amino acids; GAA, glucogenic amino acids; INDF, indigestible NDF; NAN, non-ammonia N; aNDF, neutral detergent fiber; NANMN, non-ammonia non-microbial N; NEAA, non-essential amino acids; NFC, non-fiber carbohydrate; NDIN, neutral detergent insoluble N; OM, organic matter; PD, purine derivatives; RDP, ruminal degradability of feed N compounds.

* Corresponding author.
*E-mail address:* gilberto.kozloski@ufsm.br (G.V. Kozloski).

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## 1. Introduction

Animal performance depends, among other factors, on the metabolizable energy and protein supply. Metabolizable protein is defined as the amino acids absorbed from the small intestine, which originate from rumen microbial, undegraded feed and endogenous true protein (NRC, 2001). When there is no lack of N for rumen bacteria, the supply of rumen microbial protein is directly related to the amount of organic matter (OM) fermented in the rumen (Van Soest, 1994). In turn, when N availability is above rumen bacteria requirements, the excess is absorbed as ammonia, metabolized to urea in liver and partially excreted in urine. Ingestion of excessive amounts of rumen degradable protein increases urinary N losses, which in turn negatively impacts the environment, decreases the efficiency of feed N use and, may limit the metabolizable protein supply and performance of high productive animals (Rossi et al., 2007). This undesired nutritional condition is common in animals fed N-rich high digestible forages and/or concentrate containing oil-seed meals as protein sources, where ruminal degradability is usually higher than 0.65 (NRC, 2001). To reduce the ruminal degradability of these protein sources without affecting its intestinal digestibility is among the current research challenges in ruminant nutrition.

Tannins are plant polyphenols with the capacity to form complexes mainly with proteins reducing their degradation in the rumen (Waghorn et al., 1987). However, the chemical structure, concentration and the biological effects of tannins in forage legumes, and consequently their nutritional impact, show large variability (McSweeney et al., 2001). Alternatively, industrial tannin extracts, as from *Acacia mearnsii*, show the potential to be used as a feed additive for ruminants. Carulla et al. (2005) observed that supplementing this extract at a rate of 41 g/kg of diet dry matter (DM) to lambs fed ryegrass increased forage intake but depressed the OM digestibility. However, because methane emission and urinary N excretion also decreased, energy and N retention were not affected. Grainger et al. (2009) reported that offering this extract at a rate of 19 g/kg DM also reduced methane emissions and urinary N excretion, however, tannins also reduced feed intake, OM digestibility and milk production by dairy cows grazing a ryegrass pasture. Griffiths et al. (2013), in turn, reported the positive potential of the tannin extract in reducing urinary N losses without affecting milk production in dairy cows grazing high-N ryegrass pastures when supplemented, as an oral drench, with no more than 185 g/day of a black wattle powder containing 601 g/kg of condensed tannins.

All of the above studies, however, were conducted with animals fed forage based diets without or with small amounts of supplementary concentrate feedstuffs. Tannins reduce not only protein degradation but also carbohydrate degradation by rumen bacteria (Frutos et al., 2004). Most OM in forages is comprised of fibrous carbohydrates, with limited digestibility in the lower gastrointestinal tract, thus the total OM digestibility is usually decreased by tannins in ruminants fed forage based diets (Kozloski et al., 2012). In turn, assuming that most starch and true protein that escapes ruminal degradation and flows to the duodenum is digested in the lower gastrointestinal tract, it was hypothesized that there is a level of tannin extract inclusion in diets containing medium to high levels of starch and true protein sources, offered to cattle, where the metabolizable protein supply is improved without or with a minor impact on the total OM digestibility. Moreover, none of previous studies tested the effect of the tannin extract on duodenal flux of individual amino acids.

The present study was conducted to evaluate the impact of increased dietary inclusion of tannin extract from *Acacia mearnsii* on digestibility, ruminal fermentation, rumen microbial protein synthesis, N utilization and duodenal flux of individual amino acids in steers fed a temperate grass forage plus concentrate containing soybean meal as the main protein source.

## 2. Materials 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 Holstein steers (156 ± 33 kg of body weight (BW)) housed in metabolism pens were used in a 4 × 4 Latin square experiment. The steers were fitted with a chronic rumen catheter (siliconized PVC, 35 cm length, 10 mm o.d. × 7 mm i.d.) and a duodenal silicone rubber T-type cannula, and the experiment started after approximately 30 days after surgeries. To minimize the effects of feed intake level on digestion processes, the experimental diet was offered at restricted amount of 20 g dry matter (DM)/kg BW and consisted of vegetative oat (*Avena strigosa*) plus concentrate, in a proportion of 0.55:0.45 (DM basis) respectively. To ensure that the same forage type was offered throughout the experimental periods, all forage was harvested from a pasture fertilized with urea at a rate of 100 kg/ha, when the grass sward reached a height of approximately 30 cm. It was then cut 10 cm above the ground level and immediately stored in a cooling chamber at −20 °C until offered in each experimental period. The concentrate was a mixture of soybean meal (0.30), cracked corn grain (0.35) and rice bran (0.35). The chemical composition of forage and concentrate is shown in Table 1. With the exception of the cracked corn grain, all other dietary components were reported to have protein with rumen degradability above 0.60 (Valadares Filho et al., 2010). Diets were formulated to contain high levels of crude protein, above steer requirement, as to simulate the diet usually offered for dairy cows grazing temperate grass pastures and given concentrate supplementation in Southern of Brazil. Treatments were zero tannin (Control) or inclusion of *Acacia mearnsii* tannin extract (Weibull Black, Tanac S. A., Montenegro, RS, Brazil) in the concentrate at a rate of 20, 40 or 60 g/kg DM (i.e. 9, 18 or 27 g/kg of total dietary DM). The extract was the same previously used by Kozloski et al. (2012) and contained 694 g/kg DM of total tannins, which was analyzed using the Folin-Ciocalteu method after aqueous acetone (70%, v/v) extraction following the procedures of Makkar