NUTRITION, FEEDING, AND CALVES

Short Communication: Effect of Tannic Acid on Composition and Ruminal Degradability of Bermudagrass and Alfalfa Silages

G. T. Santos*,[1] R. L. Oliveira,* H. V. Petit,†,[2] U. Cecato,* L. M. Zeoula,*,[1] L. P. Rigolon,* J. C. Damasceno,* A. F. Branco,*,[1] and V. Bett*

*Departamento de Zootecnia, Universidade Estadual de Maringá, Av. Colombo, 5790, CEP 87020-900 Maringa, Parana, Brazil

†Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, PO Box 90, Lennoxville, QC J1M 1Z3, Canada

ABSTRACT

We measured the effects of the addition of tannic acid on chemical composition and crude protein (CP) ruminal degradability of bermudagrass, cv. coast cross (*Cynodon dactylon* L.) and alfalfa (*Medicago sativa* L.) silages with nylon bags incubated in the rumen of two fistulated lactating cows. Silage pH was greater for alfalfa than for bermudagrass. The addition of tannic acid had no effect on silage pH. Ammonia N was greater in alfalfa than in bermudagrass silage. Treated and control silages had similar ammonia N concentrations. The percentage of CP that was solubilized at time 0 was lower for alfalfa than for bermudagrass silage. The addition of tannic acid decreased the portion of CP solubilized at time 0 and increased the potentially degradable fraction of CP. The potentially degradable fraction of CP was greater for alfalfa than for bermudagrass. There was an interaction of species and treatment; the disappearance rate of CP increased with tannic acid treatment of alfalfa but decreased with acid treatment of bermudagrass. The effective degradability of CP was similar for control and treated alfalfa but lower for treated than for control bermudagrass. Tannic acid treatment is effective in decreasing the rapidly soluble fraction of alfalfa and bermudagrass silages, which could be beneficial to the animal because it would decrease the excess of N in the rumen after feeding. Tannic acid treatment decreased CP degradability of bermudagrass silage but had no effect on alfalfa silage, suggesting that tannic acid concentration required to effectively decrease CP degradability differs among forages.

(**Key words:** dairy cattle, tannin, degradability, silage)

Abbreviation key: EDCP = effective degradability of CP.

The rate of degradability of protein during ensiling is high as a result of extensive proteolysis, which occurs during wilting and ensiling (9). However, greater tannin concentration in trefoil (*Lotus corniculutus* L.) than in alfalfa (*Medicago sativa* L.) silage is related to less intense hydrolyzation of proteins into soluble NPN (4). Tannins decrease protein deamination (3) and are negatively correlated with concentration of NPN in legume silage (1). Birdsfoot trefoil cultivars with greater tannin concentrations have lower proteolysis during ensiling, which results in greater proportions of protein N after silage fermentation (16). Decreases in the soluble and the potentially soluble fractions of CP were also observed for cultivars that have greater rather than lower tannin concentrations (16).

Many plant species have naturally low tannin concentrations, suggesting that tannin addition could be a strategy to decrease protein degradability of forages. The negative relationship between NPN and tannic acid concentrations (1) suggests that tannic acid inhibits several animal and plant enzymes. Mühlbach et al. (11) used tannins to inhibit ruminal proteolysis of soybean meal; complex reversion was carried out with pepsin and pancreatic-pepsin with a significant decrease in ruminal ammonia production after incubating soybean meal treated with tannins. Our objectives were to determine the effects of adding tannic acid at ensiling on ruminal degradability and fermentation characteristics of bermudagrass and alfalfa.

Bermudagrass (*Cynodon dactylon* L.) and alfalfa (*Medicago sativa* L.) were obtained from the experimental field of the Iguatemi Experimental Farm, Maringa State University, Brazil. Bermudagrass was cut in November, 1999, 30 d after the first growth, and alfalfa

Received December 13, 1999.
Accepted March 16, 2000.
Corresponding author: H. J. Petit; e-mail: petith@em.agr.ca.
[1]Sponsored by Bolsista do Conselho Nacional de Pesenvolmiento Científico e Tecnológia, Brasilia, Brazil.
[2]Contribution Number 631 from the Dairy and Swine Research and Development Centre.

was cut in December at 10% flower stage. Both forages had similar maturities. They were cut at 5-cm height, grossly chopped into 2.5-cm pieces with a forage crusher (model EN-10, Nogueira Itapira sp. S.A.), and scattered over a plastic cover for partial wilting and drying. All the material was mixed with acetic acid at the ratio of 4 ml/kg of green ensiled matter to help silage conservation (10). Later, each forage was divided in two parts, one was used as control, and the other was treated with 21% (vol/wt) tannic acid solution (Labsynth Productos para Laboratorio Ltda, Sao Paulo, S.P., Brazil). The tannic acid solution was sprayed over the chopped forage in a 5-cm layer with a final tannic acid solution to protein ratio of 1:10. The material was homogenized, allotted to six replicates, and ensiled in PVC experimental silos with a 4-kg capacity. The same homogenization and ensiling procedures were carried out with the nontreated forage. We used four different treatments: bermudagrass with tannic acid (**TB**), control bermudagrass (**CB**), alfalfa with tannic acid (**TA**), and control alfalfa (**CA**) silages. Silos were kept at room temperature (21°C) for 180 d; thereafter they were emptied. Juice was squeezed from a portion of the silages, filtered, and used for determination of pH and ammonia N. Subsequently, silage from each silo was frozen (–20°C) in plastic bags until analyzed.

Ruminal CP degradabilities were estimated for the four silages. Equal portions of each of the six silos within treatment were mixed together to determine degradability. Two lactating Holstein multiparous cows, fitted with a ruminal cannula, were fed a TMR containing 30 kg of corn silage, and 8 kg of concentrate, and mineralized salt to meet NRC requirements (12). Rations were fed three times daily (0800, 1300, and 1630 h), and cows had access to pasture (*Cynodon* sp.) between 0900 and 1300 and between 1800 and 0600 h. The four silages were incubated in the rumen of each of the two cows in a complete randomized design, and cows served as the replicates. Ankom rumen in situ nylon bags (Ankom Technology Corporation, Fairport, NY) was used for incubation. Bags were 10 × 20 cm with a pore size of 53 ± 15 μm. Seven-gram silage samples were placed in nylon bags and anchored with a 30-cm length of braided fishing line. All bags were clamped to a 540-g weight, which was tied to a 60-cm long mainline tied outside the fistula. Duplicate bags were introduced over time to have incubation periods of 6, 9, 24, 28, 72, and 96 h. All bags were removed at once and washed in cold water until the rinse water was clear. Time 0 disappearances were obtained by washing unincubated bags as described. All washed bags were dried in a forced-air oven at 55°C until constant weight was reached. The percentages of disappearance of CP at each incubation time were calculated from the proportions remaining after incubation in the rumen.

Degradation of CP was calculated using the following equation of Ørskov and McDonald (14):

$$p = a + b(1 - e^{-ct})$$

where p = percentage disappearance at time t, a = the intercept representing the portion of CP solubilized at time 0, b = the fraction of CP that potentially is degradable in the rumen, c = the constant rate of disappearance of fraction b, and t = time of incubation. The nonlinear parameters a, b, and c were estimated by an iterative least-squares procedure (17), and best-fit values were chosen with the Secant method (DUD) using the convergence criterion (10^{-8}) of SAS (17). The effective degradability of CP (**EDCP**) is the amount that actually will be degraded in the rumen and was calculated with the following equation (14):

$$EDCP = a + [(b - c)/(c + k)],$$

where k is the estimated rate of solid outflow from the rumen and the others are the same as described above. Effective degradability of CP was estimated for each ingredient assuming rumen solid outflow rates of 5%/h.

Silage DM was determined by toluene distillation (5). Liquid was squeezed from samples of silage at the opening of the silos and used to determine pH and ammonia N. Silage pH was measured with a potentiometer (model HI 931000, Hanna Instruments, Mauritius) and ammonia N was determined in silage juice by distillation over MgO (2). Total N in silage was measured by the Kjeldahl method (2). Acid detergent fiber was determined according to Goering and Van Soest (6).

Data on silage composition were subjected to ANOVA using the general linear models procedure of SAS (17) according to a two-way analysis of variance with treatment and type of silage as effects; there were six repetitions (silos) per treatment. Degradability data for predicted a, b, c, and t_0 values, and for the effective degradabilities of CP were analyzed by the general linear models procedure of SAS (17) as a two-way analysis of variance with treatment and type of silage as effects with cows as the replicates. Significant was declared at $P < 0.05$ unless otherwise noted.

The percentage of DM was significantly greater for tannic acid-treated bermudagrass silage than for the other silages, which resulted in an interaction between species and treatment (Table 1). Silage DM content was similar for control alfalfa silage, tannic acid alfalfa silage, and control bermudagrass silage. In earlier studies (8), direct acidifiers increased silage DM in third- but not in second-cut alfalfa, while biological additives