JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Effects of supplementing endophyte-infected tall fescue with sainfoin and polyethylene glycol on the physiology and ingestive behavior of sheep

F. Catanese, R. A. Distel and J. J. Villalba

J ANIM SCI 2014, 92:744-757.
doi: 10.2527/jas.2013-6713 originally published online January 14, 2014

Effects of supplementing endophyte-infected tall fescue with sainfoin and polyethylene glycol on the physiology and ingestive behavior of sheep[1,2]

F. Catanese,* R. A. Distel,* and J. J. Villalba†[3]

*Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Centro CientíficoTecnológico CONICET Bahía Blanca, and Departamento de Agronomía, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina; †Department of Wildland Resources, Utah State University, Logan 84322-5230

ABSTRACT: Tannins in sainfoin (*Onobrychis viciifolia*) may bind to alkaloids in endophyte-infected tall fescue [E+; *Lolium arundinaceum* (Schreb.) Darbysh.] and attenuate toxicosis. If so, supplementing E+ with sainfoin will increase use of E+ by sheep, and polyethylene glycol (PEG)—a polymer that selectively binds to tannins—will reduce such response. To test these predictions, thirty-six 2-mo-old lambs were randomly assigned to 3 treatments (12 lambs/treatment). During exposure, all lambs were individually penned and fed E+ supplemented with beet pulp (CTRL), fresh-cut sainfoin and beet pulp (SAIN), or fresh-cut sainfoin plus PEG mixed in beet pulp (SAIN+PEG). Feed intake was measured daily. Rectal temperatures and jugular blood samples were taken at the beginning and end of exposure. After exposure, all lambs were offered choices between endophyte-free tall fescue (E–) and orchardgrass, and preference for E– was assessed. Then, all lambs were allowed to graze a choice of E+ and sainfoin or a monoculture of E+. The foraging behavior of lambs was recorded. When sainfoin was in mid-vegetative stage, lambs in SAIN ingested more E+ than lambs in CTRL ($P = 0.05$), but no differences were detected between lambs in SAIN+PEG and CTRL ($P = 0.12$). Sainfoin supplementation improved some physiological parameters indicative of fescue toxicosis. Lambs in SAIN had lower rectal temperatures ($P = 0.02$), greater numbers of leukocytes ($P < 0.001$) and lymphocytes ($P = 0.03$), and greater plasmatic concentrations of globulin ($P = 0.009$) and prolactin ($P = 0.019$) than lambs in CTRL. Some of these differences were offset by the SAIN+PEG treatment. When lambs were offered choices between E– and orchardgrass, only lambs in SAIN had greater intake of E– than lambs in CTRL ($P < 0.001$). When lambs were allowed to graze a choice of E+ and sainfoin, all treatments used E+ to the same extent ($P > 0.05$). On the other hand, when they grazed on a monoculture of E+, lambs in SAIN+PEG showed greater acceptance of E+ than lambs in SAIN or in CTRL ($P < 0.05$). In summary, sainfoin supplementation alleviated several of the classic signs of fescue toxicosis and increased intake of endophyte-infected tall fescue. Tannins in sainfoin partially accounted for this benefit since feeding a polymer that selectively binds to tannins (PEG) attenuated some these responses. However, sainfoin supplementation during initial exposure to E+ did not lead to an increased preference for E+ during grazing.

Key words: alkaloids, condensed tannins, fescue toxicosis, ingestive behavior, sainfoin, sheep

J. Anim. Sci. 2014.92:744–757
doi:10.2527/jas2013-6713

[1]We acknowledge R. Stott for veterinary services and T. Lisonbee and B. Maughan for technical support.

[2]This research was supported by grants from the Utah Agricultural Experiment Station, the National Institute of Food and Agriculture (NIFA-Managed Ecosystems) USDA (Award 2009-35101-05360), and from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) de la República Argentina, through the Fondo para la Investigación Científica y Tecnológica (FONCyT, PICT 170 Préstamo BID). This paper is published with the approval of the Director, Utah Agricultural Experiment Station, and Utah State University, as journal paper number 8544.

[3]Corresponding author: juan.villalba@usu.edu
Received May 16, 2013.
Accepted November 22, 2013.

INTRODUCTION

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] is a cool season perennial grass, key for livestock production in temperate regions of the world. Tall fescue pastures are commonly infected with the fungal endophyte *Neotyphodium coenophialum*, which confers the plant several agronomic advantages (Burns and Chamblee, 1979). However, the endophyte produces toxic ergopeptide alkaloids such as ergovaline (Bacon and Siegel, 1988), which negatively impacts animal production (Brown et al., 2009) due to its effects on food intake and digestion (Hannah et al., 1990), reproductive performance (Porter and Thompson, 1992), and thermoregulatory capacity (Gadberry et al., 2003). In the United States approximately 90% of tall fescue pastures are endophyte infected (**E+**; Popay et al., 2005, 2009), costing the U.S. beef industry between US$600 million to more than $1 billion per year (Hancock and Andrae, 2009).

The strong binding capacity of condensed tannins to nitrogen-based compounds (Charlton et al., 2000) may make alkaloids in E+ less available in the gastrointestinal tract, thus reducing their toxic effects. Tannin-containing legumes increase intake of E+ in sheep and cattle (Lyman et al., 2011; Owens et al., 2012). However, supplementing grasses with legumes also improves the balance of carbohydrates to protein in the rumen (Rutter, 2006) and increases grass intake (Bodine et al., 2000), thus providing an alternative explanation for this effect.

We hypothesized that condensed tannins in legumes such as sainfoin (*Onobrychis viciifolia*) attenuate fescue toxicosis. If so, we predicted that 1) lambs ingesting E+ and supplemented with sainfoin will improve some physiological parameters indicative of fescue toxicosis and increase intake of and preference for E+ relative to unsupplemented animals and 2) feeding polyethylene glycol (**PEG**), a polymer that binds to tannins and inactivates their effects in the digestive tract (Silanikove et al., 1994), will offset any benefit gained from feeding sainfoin.

MATERIALS AND METHODS

The study was conducted at the Green Canyon Ecology Center in Logan, UT (41°45′59″ N, 111°47′14″ W; pen trial), and at the Utah Agricultural Experiment Station Pasture Research Facility in Lewiston, UT (41°56′59″ N 111° 52′14″ W; grazing trial), according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (approval number 2116). The study occurred from the beginning of June to the end of August of 2012. Throughout the experimental period, lambs had free access to water and trace mineral salt blocks (mineral composition: minimum 96% NaCl, 320 mg/kg Zn, 380 mg/kg Cu, 2,400 mg/kg Mn, 2,400 mg/kg Fe, 70 mg/kg I, and 40 mg/kg Co).

Forages

Well-established stands of endophyte-free (**E–**) and endophyte-infected (E+) tall fescue (‘Kentucky 31’), sainfoin (‘Shoshone’), and orchardgrass (*Dactylis glomerata* L.; ‘Latar’) provided the forages for this study. Stands were seeded in spring of 2009 in 2 ha (orchardgrass and E–) and two 0.2 ha (E+ and sainfoin) paddocks at the Utah State University pasture research facility in Lewiston, UT. Nutritional and toxicological composition of the forage species is reported in Table 1.

Pen Trial

Animals and Preconditioning Period. Thirty-six 2-mo-old commercial Finn–Columbia–Polypay–Suffolk crossbred lambs of an average initial BW of 30 ± 4 kg (mean ± SD) were weaned, dewormed with a subcutaneous injection of ivermectin (0.2 mg/kg of BW; Ivomec, Merial, Duluth, GA), placed in a paddock measuring 20 by 20 m, and fed ad libitum amounts of alfalfa pellets and 200 g/(lamb·d) of rolled barley for 15 d. Following this period, lambs were individually penned outdoors under a protective roof in adjacent pens measuring 1.5 by 2.5 m and fed ad libitum amounts of alfalfa pellets and 300 g of beet pulp/(lamb·d) for the first 5 d. Thereafter, to familiarize lambs with the experimental protocol, all lambs received 250 g (DM basis) of beet pulp from 0800 to 0900 h, 200 g (DM basis) of fresh-cut sainfoin in full bloom stage from 0900 to 1000 h and ad libitum amounts of alfalfa pellets from 1000 to 1600 h. Familiarization lasted 10 d.

Conditioning Period. Lambs were blocked by BW and sex and randomly assigned to 1 of 3 treatments (12 lambs/treatment). From 0900 to 0930 h each lamb in the treatment (**SAIN**) received 250 g (DM basis) of beet pulp and from 0945 to 1045 h, 250 g (DM basis) of fresh-cut sainfoin. Sainfoin was harvested every day at 0730 h by hand clipping (15 cm particle size), transported to the Green Canyon Ecology Center, and fed to lambs during the same day. Lambs in the treatment (**SAIN+PEG**) received the same amounts of supplements and at the same times, except that beet pulp was mixed with 33 g (as fed basis) of PEG—PEG 3500 (Spectrum Chemicals, New Brunswick, NJ). It has been shown that PEG binds selectively to condensed tannins limiting their bioavailability, thus attenuating the effects of tannins on the consumer’s body (Silanikove et al., 1994; Priolo et al., 2000). The dose selected was expected to completely bind all the tannins provided by sainfoin supplementation (Waghorn et al., 1987). During the same period of time (0900 to 1045 h) lambs in the control treatment (**CTRL**) received 500 g beet pulp to compensate for the amount of supplement received in the other treatments.

Every day at 0750 h, E+ was harvested with a lawn mower (5 cm particle size), transported to the Green

Table 1. Crude protein, fiber, and plant secondary compound content (mean ± SD, DM basis) of the forages and supplements offered to lambs during the study

Item	CP, g/100 g	ADF, g/100 g	NDF, g/100 g	CT,[1] g/100 g	Ergovaline, µg/kg
Pen trial – conditioning period					
E+[2]	11.5 ± 1.17	40.8 ± 4.58	59.6 ± 6.40	–	100.2 ± 10.74
Sainfoin subperiod 1	13.2 ± 1.28	41.8 ± 3.91	48.7 ± 4.99	7.7 ± 0.53	–
Sainfoin subperiod 2	15.7 ± 1.87	29.9 ± 2.71	36.5 ± 4.68	3.0 ± 0.27	–
Beet pulp	9.8 ± 1.02	26.2 ± 3.20	38.1 ± 3.63	–	–
Alfalfa pellets	15.6 ± 1.61	30.1 ± 3.77	41.0 ± 3.66	–	–
Pen trial – preference between E–[3] and orchardgrass					
E–	9.4 ± 0.97	42.9 ± 5.31	61.7 ± 5.51	–	Nondetectable
Orchardgrass	12.4 ± 1.57	40.3 ± 4.29	55.3 ± 5.67	–	–
Grazing trial					
E+	12.0 ± 1.46	40.8 ± 4.01	60.2 ± 6.78	–	170.4 ± 19.15
Sainfoin	13.2 ± 1.34	41.2 ± 3.77	48.2 ± 4.48	7.6 ± 0.51	–

[1]CT = condensed tannins.

[2]E+ = endophyte-infected tall fescue.

[3]E– = endophyte-free tall fescue.

Canyon Ecology Center, and offered ad libitum to all lambs from 1100 to 1600 h. No other feed was offered until the next day. The conditioning period lasted for 23 d and involved 2 subperiods. In subperiod 1 (first 17 d of the conditioning period) sainfoin was harvested from a plot where plants were in the full bloom stage, with high concentration of fiber, which progressively constrained intake of the legume during the 1 h supplementation interval. Therefore, during subperiod 2 (last 6 d of the period) sainfoin was harvested from a different plot where plants were in the mid-vegetative stage and had lower concentrations of fiber and greater concentrations of CP than those in subperiod 1 (Table 1). Tall fescue was in the vegetative stage throughout the conditioning period.

Individual daily intake of each feed was calculated by the difference between the amount offered and the amount refused. Intake was expressed as grams DM per kilogram BW. Lambs were weighed at 0800 h after an overnight fast at the beginning and end of the conditioning period.

Rectal temperature was measured during the last 5 d of the conditioning period.

Measurements were performed using a rectal digital thermometer, which was held in the rectum until reaching a stable reading. Measurements were conducted at 1600 h, 6 h after animals started feeding on E+ and when environmental temperatures were peaking for the day.

Blood tests were performed on two 10-mL blood samples (with and without heparin added; Becton Dickinson Vacutainer System, Rutherford, NJ) collected via jugular venous puncture at 0800 h at the beginning and the end of the conditioning period. Samples with heparin were immediately submitted to the Utah Veterinary Diagnostic Laboratory (Logan, UT) for total blood cell count (Advia 120 Hematology Analyzer; Siemens Medical Solution Diagnostics, Tarrytown, NY). Samples with no heparin were allowed to clot for 45 min before centrifugation. Serum was separated by centrifugation (2,300 × g for 25 min at 4°C) and stored at –20°C until analysis. Serum analyses were performed at the Pathobiology and Diagnostic Investigation Laboratory (chemical analyses) and the Diagnostic Center for Population and Animal Health (cortisol and prolactin) of the Michigan State University (Lansing, MI). Serum samples were analyzed using an Olympus AU640e automated chemistry analyzer and Olympus system reagents (Center Valley, PA) for alkaline phosphatase (**ALP**), aspartate aminotransferase (**AST**), γ-glutamyl transpeptidase (**GGT**), creatine kinase, total bilirubin, bilirubin, blood urea nitrogen (**BUN**), albumin, and total protein (**TP**). Globulin was calculated as TP – albulmin. The analyzer was calibrated according to the manufacturer's instructions. Manufacturer instructions were followed by assays. Serum cortisol was determined by RIA procedures (Siemens, Los Angeles, CA). Minimum detectable concentration of cortisol in serum was 5 nmol/L. Interassay CV was 8%, and intra-assay CV was 3%. Prolactin was determined by ELISA (Blue Gene ELISA Kits; Life Sciences Advanced Technologies Inc., Saint Petersburg, FL). Minimum detectable concentration of prolactin in serum was 1.0 pg/mL. Interassay and intra-assay CV was <10%.

Feed fed to lambs during the conditioning period and in subsequent tests was sampled daily before feeding, composited for 7 d, and then prepared for chemical analyses. Feed samples were dried for 48 h at 60°C, ground using a Wiley Mill (1-mm mesh), and analyzed for CP (AOAC Int., 2002; method 990.03), NDF (without the addition of sodium sulfide; Van Soest et al., 1991), and ADF (using NDF residue; AOAC Int., 2002; method 973.18). Ergovaline concentrations in E+ and E– were determined according Rottinghaus et al. (1991), and condensed tannin concentrations in sainfoin were determined according to Terrill et al. (1992). Both analyses were done on freeze-dried samples.

Preference between Endophyte-Free Tall Fescue and Orchardgrass. The day after conditioning ended, all lambs received ad libitum amounts of alfalfa pellets for 5 d to minimize the differences that treatments could have promoted in the rumen environment. Aiken et al. (2001) observed that calves previously fed E+ for 66 d require 3 to 4 d to return to a normal physiological condition.

The day after the washout period ended, all lambs received a 2-choice test between E– and orchardgrass (novel forages for the lambs). The reason of feeding E– instead of E+ was to prevent lambs in SAIN and SAIN+PEG from associating tall fescue with the negative postingestive effects of alkaloids, which could have conditioned aversive responses (Aldrich et al., 1993). This in turn may have potentially affected lambs' acceptance and preference for E+ in the ensuing grazing trial, attenuating the effects of the conditioning period on lambs' foraging behavior.

Forages were harvested daily at 0730 h from the experimental pastures using a lawn mower (5 cm particle size), transported to the Green Canyon Ecology Center, and offered simultaneously to lambs from 0830 to 1030 h. Intake of each forage was calculated as described before. Testing lasted 4 d. Each day, all lambs were fed 800 g of alfalfa pellets immediately after the preference test.

Grazing Trial

The day after the pen trial ended all lambs were transported to the Utah State University pasture research facility in Lewiston, UT, and fed alfalfa pellets ad libitum for 5 d. The aim of this washout period was to ensure that differences in grazing responses could be attributed to the treatments animals experienced during conditioning and not to the short-term effects of forages consumed before testing.

Because lambs are reluctant to forage in isolation (Sibbald and Hooper, 2004), pairs of lambs—blocked by weight—were formed within each treatment (6 pairs for CTRL and SAIN+PEG and 5 pairs for SAIN). Pairs were considered the experimental unit. Treatment SAIN had 1 pair less than the other treatments because 1 lamb died during conditioning. Once formed, members of each pair (identified by a particular combination of colors painted on the lambs' head and back) were always tested together. Pairs of lambs from each treatment were randomly penned in 2 holding pens (4.5 by 6.5 m) built under 2 protective roofs adjacent to the experimental pastures. Each pair of lambs was released to graze into a fenced experimental plot with a choice of strips of sainfoin and E+ (preference tests) or a monoculture of E+ (E+ acceptance tests).

Two 0.13-ha (11 by 116 m) adjacent (by the longer side of the rectangular area) experimental pastures were used for the grazing trial. One pasture contained a pure stand of E+ in vegetative stage whereas the other pasture contained a pure stand of sainfoin in early blooming stage. The forage in these paddocks accumulated since the pastures were cut to a uniform sward surface height early in the year (end of May) using a rotary swather and allowed for ad libitum consumption of lambs during the grazing trial.

Preference Tests: Choice between Endophyte-Infected Tall Fescue and Sainfoin. Seventeen grazing plots were built using electric fence on half of the E+ pasture and on half of the adjacent sainfoin pasture such that each plot contained 1 strip of sainfoin and 1 strip of E+ of equal area. The size of plots ranged between 43.6 and 63.7 m^2, as the dimension of each plot was proportional to the average BW of the pair of lambs that grazed in the plot [0.825 m^2/(kg BW·lamb)]. Plot location was assigned randomly to pairs of lambs. A rising plate meter was used to estimate the initial standing biomass in each paddock (Scrivner et al., 1986). Dry matter availability was 4.95 and 2.54 tons DM/ha in sainfoin and E+ pastures, respectively.

Every day, each pair of lambs was released from a holding pen to its corresponding grazing plot. The sequence of release for each pair into its plot was constant across days. Release started at 0730 h and ended at 0745 h. Lambs were returned to their respective holding pens at 1130 h, following the same order of release. Then, all lambs were offered 1.3 kg/(lamb·d) of alfalfa pellets at noon. No other feed was offered until the next day. Testing lasted 7 d.

Forage samples of E+ and sainfoin were collected daily, combined, and analyzed for CP, NDF, ADF, ergovaline (E+), and condensed tannins (sainfoin).

The foraging behavior of each lamb in each pair was recorded at 2-min intervals (scan sampling; Altmann, 1974) throughout the first 2 h of grazing, discriminating between grazing events on either E+ or sanfoin and nongrazing events. Observations were performed by 2 observers from observation towers (4-m high) adjacent to one side of the experimental pastures. Preference for each species was calculated as the proportion of total number of scans in which lambs were grazing.

Daily foraging induced sward heterogeneity and biomass depletion, which was a function of lambs' foraging behavior. We assessed the intensity of E+ defoliation according to methods described by O'Reagain and Grau (1995). For each plot, a single transect (6 m) was laid out from one corner to the opposite corner of E+. Twenty plants per plot were identified at regular intervals (approximately 30 cm) along the transect using numbered and color-coded wire tags inserted into the ground. The day before each grazing test begun, the extended height of the tallest leaf of each marked plant was measured. Plants were thereafter measured on d 1, 3, 5, and 7 of each test at 1400 h. Negative differences between 2 successive measures of height in a given plant were taken as indication of defoliation, which was used to compute the probability of being defoliated 1, 2, and 3 times. The proportion of plant material removed was calculated according to the formula (Initial height – Final defoliation height)/Initial height.

Endophyte-Infected Tall Fescue Acceptance Tests. Seventeen grazing plots were built using electric fence in the remaining half of the E+ pasture that had not been previously grazed. Plot dimensions were proportional to the average BW of the lambs in each pair [0.413 m^2/(kg BW·lamb)]. Initial standing biomass of E+ was 3.1 tons DM/ha. The grazing protocol and measurements were the same as described for preference tests, except that observations discriminated between grazing on E+ and nongrazing events. Testing lasted 7 d.

Statistical Analyses

Statistical analyses were performed using the R environment (R Development Core Team, 2012). For every case, a matrix of orthogonal contrasts was specified for each of the variables involved. In the case for treatment effects, this matrix contrasted the effects of CTRL vs. SAIN and SAIN+PEG vs. SAIN, allowing us to test sensible hypotheses. Mixed effects models were evaluated during a selection process according to the procedure detailed in Zuur et al. (2009). Model diagnostics also included testing for normal distribution, homogeneity of variance, and linearity. Least square means and standard errors were obtained with the "lsmeans" package (Lenth, 2012). We used the Tukey-Kramer honestly significant difference test (Ruxton and Beauchamp, 2008) to evaluate unplanned comparisons (e.g., CTRL vs. SAIN+PEG). When necessary, an angular transformation was used for proportion data.

Conditioning Period. Intake of E+ during conditioning was analyzed using a mixed effects model (Pinheiro et al., 2012), which included treatment (CTRL, SAIN+PEG, and SAIN), day, subperiod (1 and 2), and treatment × subperiod interaction as fixed effects and lamb as random effect. The model was fitted with an autoregressive order-1 covariance structure (chi-squared test, $P < 0.001$) and variance heterogeneity was modeled with a potential function (chi-squared test, $P < 0.001$). Values for BW and ADG at the end of exposure were analyzed using a simple fixed-effects model in which initial BW was used as a covariate.

Rectal temperature was analyzed using a mixed effects model, which included treatment, day, and treatment × day interaction as fixed effects and lamb as a random effect. Endophyte-infected fescue intake was incorporated in the model as a covariate because differences in intake can influence body temperature (Osuji, 1974) in ways that may obscure interpretations of the model. Because E+ intake data were influenced by our treatments, before using E+ intake as a covariate, we checked for homogeneity of slopes, derived from the relationship between the response variable and the covariate at each level of the treatment factor (Logan, 2010). The model was fitted with an autoregressive order-1 covariance structure (chi-squared test, $P = 0.022$).

Blood parameters were analyzed with a fixed effects model with the initial observations used as covariates.

Preference between Endophyte-Free Tall Fescue and Orchardgrass. Intake and preference for endophyte-free fescue and orchardgrass intake were analyzed using a mixed effects model including treatment, day, and treatment × day interaction as fixed effects and lamb as a random effect.

Grazing Trial. Data obtained from scan sampling were expressed as proportions and analyzed using a mixed effects model including treatment, day, and treatment × day interaction as fixed effects and lambs nested within each pen as a random effect.

The probability of a plant being defoliated 1, 2, or 3 times was analyzed using a binomial mixed effects model (Bates et al., 2012) including treatment as the fixed effect and pen as a random effect. Separate analyses were performed for each sampling day because we observed a strong heterogeneity of variances between sampling days. Defoliation intensity was also evaluated using separate analyses for each sampling day. In this case, a mixed effect model was used, allowing for treatment as a fixed effect and pen as a random effect.

RESULTS

Chemical Composition of the Forages

The chemical composition of the forages and supplements is reported in Table 1. Tall fescue had greater concentration of fiber and lower concentration of CP than sainfoin. During subperiod 2, sainfoin had lower concentrations of fiber, greater concentrations of CP, and lower concentrations of condensed tannins than those in subperiod 1. No ergovaline was detected for E– and concentrations of this alkaloid were greater during the grazing trial than during the pen trial (Table 1).

Pen Trial – Conditioning Period

Endophyte-Infected Tall Fescue Intake. Ingestion of E+ showed a significant ($P < 0.001$) treatment × subperiod interaction (Fig. 1). No differences in

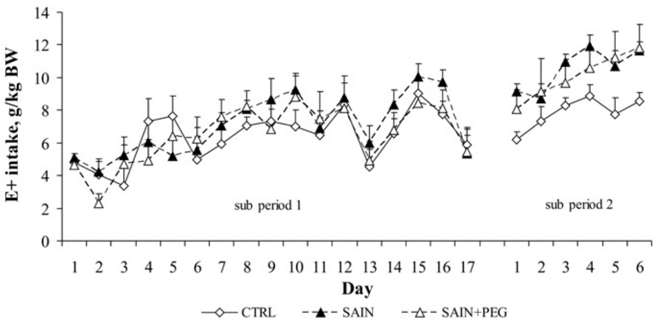

Figure 1. Endophyte-infected tall fescue (E+) intake by lambs supplemented with sainfoin (SAIN), sainfoin and polyethylene glycol (SAIN+PEG), or not by lambs supplemented with fresh-cut sainfoin and beet pulp (SAIN), fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG), or beet pulp (CTRL; DM basis). Sainfoin was in full bloom stage or mid-vegetative stage in subperiods 1 and 2, respectively. Each value represents the mean of 12 animals. Vertical bars represent SEM.

intake of E+ were detected between lambs in SAIN and those in CTRL (6.81 vs. 6.75 ± 0.93 g/kg BW, respectively; means ± SEM, $P = 0.967$) or between lambs in SAIN+PEG and those in CTRL ($P = 0.996$) during subperiod 1. However, E+ intake was greater for lambs in SAIN than for lambs in CTRL (9.37 vs. 6.50 ± 1.03 g/kg BW, respectively; means ± SEM, $P = 0.049$) during subperiod 2. No differences in E+ intake were observed between lambs in SAIN and those in SAIN+PEG (9.37 vs. 8.95 ± 1.05 g/kg BW, respectively; means ± SEM, $P = 0.854$) or between lambs in SAIN+PEG and those in CTRL ($P = 0.124$) during subperiod 2.

Body Weight. Lambs in CTRL, SAIN, and SAIN+PEG treatments had similar final BW (32, 31, and 31 ± 0.3 kg, respectively; means ± SEM, $P = 0.194$) and ADG (83, 61, and 59 ± 10 g/d, respectively; mean ± SEM, $P = 0.193$).

Rectal Temperature. Rectal temperature was affected by the treatments ($P = 0.041$). Lambs in CTRL showed higher rectal temperatures than lambs in SAIN (39.97 vs. 39.71 ± 0.07°C, respectively; means ± SEM, $P = 0.019$) but similar to lambs in SAIN+PEG (39.77 ± 0.07°C; mean ± SEM, $P = 0.128$). Lambs in SAIN and SAIN+PEG did not differ ($P = 0.601$) in rectal temperature. Average rectal temperature fluctuated among days ($P < 0.001$; data not shown), and it was positively correlated ($r^2 = 0.116$, $P < 0.001$) with mean air temperature (ranging from 31.7 to 36.7°C; Utah State University weather reports for North Logan, UT).

Complete Blood Cell Count. Results on blood cell counts are summarized in Table 2. No differences between groups regarding blood cell count were observed at the beginning of the conditioning period ($P > 0.05$; data not shown). After the conditioning period, hemoglobin concentration was greater in SAIN and CTRL than in SAIN+PEG lambs ($P = 0.006$) whereas packed cell volume (**PCV**) was greater in SAIN than in SAIN+PEG ($P = 0.046$). Lambs in SAIN showed reduced width of red blood cell distribution width when compared to lambs in SAIN+PEG and CTRL ($P = 0.035$). Lambs in SAIN had greater numbers of leukocytes than CTRL and SAIN+PEG lambs ($P < 0.001$) and greater numbers of lymphocytes than lambs in CTRL ($P = 0.033$). The ratio between neutrophils and lymphocytes was greater in CTRL lambs than in SAIN or SAIN+PEG lambs ($P = 0.007$).

Table 2. Complete blood cell count of lambs fed endophyte-infected tall fescue and supplemented with beet pulp (CTRL), fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG), or fresh-cut sainfoin and beet pulp (SAIN)

	Treatment					
Item	CTRL	SAIN+PEG	SAIN	SEM	*P*-value	Ref.[1]
RBC[2] (× 10^6/μL)	13.1	12.5	13.1	0.18	0.065	9–15
Hemoglobin, g/dL	12.6[a]	12.2[b]	12.9[a]	0.12	0.006	9–15
Packed cell volume, %	34.7[ab]	33.8[b]	35.3[a]	0.45	0.046	27–45
Mean cell volume, fL	26.6	26.4	26.5	0.16	0.710	28–40
MCHC,[3] g/dL	36.7	36.6	37.1	0.22	0.343	31–34
RDW[4]	18.4[a]	18.4[a]	17.9[b]	0.11	0.035	–
Leukocytes (× 10^3/μL)	9.0[c]	9.6[b]	10.3[a]	0.17	<0.001	4–12
Lymphocytes (× 10^3/μL)	6.1[b]	6.6[ab]	7.3[a]	0.22	0.033	2–9
Neutrophils (× 10^3/μL)	2.4	2.2	2.2	0.17	0.389	0.7–6
Monocytes (× 10^3/μL)	0.29	0.37	0.38	0.063	0.565	0–0.75
Basophils (× 10^3/μL)	0.09	0.07	0.06	0.015	0.255	0–0.3
Eosinophils (× 10^3/μL)	0.11	0.11	0.10	0.021	0.778	0–1
Platelets (× 10^3/μL)	461.3	522.0	481.1	24.74	0.186	250–750
Mean platelet volume	6.9	6.6	6.7	0.12	0.147	–
N:L[5]	0.39[a]	0.33[b]	0.30[b]	0.010	0.007	–

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).
[1]Ref. = reference; reference range values were taken from Merck (2012a).
[2]RBC = red blood cells.
[3]MCHC = mean corpuscular hemoglobin concentration.
[4]RDW = red blood cell distribution width.
[5]N:L = neutrophil:lymphocyte ratio.

Blood Serum Biochemistry and Hormones Profile. Results on blood serum biochemistry and hormones profile are summarized in Table 3. No differences between groups regarding these parameters were observed at the beginning of the conditioning period ($P > 0.05$; data not shown). Following the conditioning period, serum concentrations of the liver enzymes AST ($P = 0.015$) and GGT ($P = 0.017$) were greater in SAIN lambs than in CTRL lambs whereas the opposite pattern was observed for ALP ($P = 0.026$). There were no differences in these parameters between lambs in SAIN and those in SAIN+PEG ($P \geq 0.463$).

Blood urea nitrogen concentration was the highest for lambs in SAIN+PEG, intermediate for lambs in SAIN, and lowest for CTRL lambs ($P < 0.001$). Total serum protein was higher in SAIN than in CTRL lambs ($P = 0.006$), due mainly to the difference in globulin concentration between both treatments

Table 3. Blood serum biochemistry and hormonal profile of lambs fed endophyte-infected tall fescue and supplemented with beet pulp (CTRL), fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG), or fresh-cut sainfoin and beet pulp (SAIN)

	Treatment					
Item	CTRL	SAIN+PEG	SAIN	SEM	*P*-value	Ref.[1]
ALP,[2] U[3]/L	208.8[a]	168.3[b]	165.8[b]	11.55	0.026	27–156
AST,[4] U/L	57.0[b]	65.9[a]	69.0[a]	2.76	0.015	49–123
GGT,[5] U/L	43.2[b]	45.2[ab]	47.5[a]	0.95	0.017	20–44
Creatine kinase, U/L	93.5	121.7	123.0	9.65	0.054	7.7–101
BUN,[6] mg/100 mL	6.5[c]	12.7[a]	10.5[b]	0.80	<0.001	10–26
Creatinine, mg/100 mL	0.83	0.77	0.79	0.024	0.157	0.9–2.0
Total protein, g/100 mL	5.5[b]	5.7[ab]	5.9[a]	0.08	0.006	5.9–7.8
Albumin, g/100 mL	2.9	2.9	3.0	0.03	0.743	2.7–3.7
Globulin, g/100 mL	2.6[b]	2.8[ab]	2.9[a]	0.07	0.009	3.2–5.0
A:G[7]	1.1[a]	1.1[ab]	1.0[b]	0.02	0.010	–
Total bilirubin, mg/100 mL	0.13	0.12	0.13	0.012	0.755	0.0–0.5
Triglycerides, mg/100 mL	15.9	16.1	16.2	1.42	0.984	–
Cholesterol, mg/100 mL	36.4	41.5	37.7	1.53	0.052	44–90
Prolactin, pg/mL	139.3[b]	180.5[ab]	210.5[a]	17.33	0.019	–
Cortisol, nmol/L	22.8[a]	13.1[b]	19.3[ab]	2.56	0.008	–

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Reference range values were taken from Merck (2012c).

[2]ALP = alkaline phosphatase.

[3]U = Enzyme unit.

[4]AST = aspartate aminotransferase.

[5]GGT = gamma-glutamyl transpeptidase.

[6]BUN = blood urea nitrogen.

[7]A:G = albumin:globulin ratio.

($P = 0.009$), whereas no differences in these parameters were observed between lambs in SAIN and lambs in SAIN+PEG ($P \geq 0.149$). Serum prolactin was greater in SAIN than in CTRL lambs ($P = 0.019$) whereas cortisol was greater in CTRL lambs than in SAIN+PEG lambs ($P = 0.008$); no differences in both hormone concentrations were observed between SAIN and SAIN+PEG lambs ($P \geq 0.088$).

Pen Trial – Preference between Endophyte-Free Tall Fescue and Orchardgrass

When lambs were offered a choice between E– and orchardgrass, they all displayed strong preferences for E– (0.87 ± 0.17, mean ± SD). There was a treatment × day effect ($P = 0.027$) and preference for E– was greater for lambs in SAIN than for lambs in SAIN+PEG on the last day of trial (Fig. 2; $P = 0.014$).

Lambs in SAIN showed a greater intake of E– than lambs in CTRL (6.43 vs. 4.01 ± 0.51 g/kg BW, respectively; means ± SEM, $P < 0.001$; Fig. 2) whereas intake values displayed by lambs in SAIN+PEG (5.18 ± 0.51 g/kg BW) did not differ from those displayed by lambs in CTRL ($P = 0.685$) or in SAIN ($P = 0.946$).

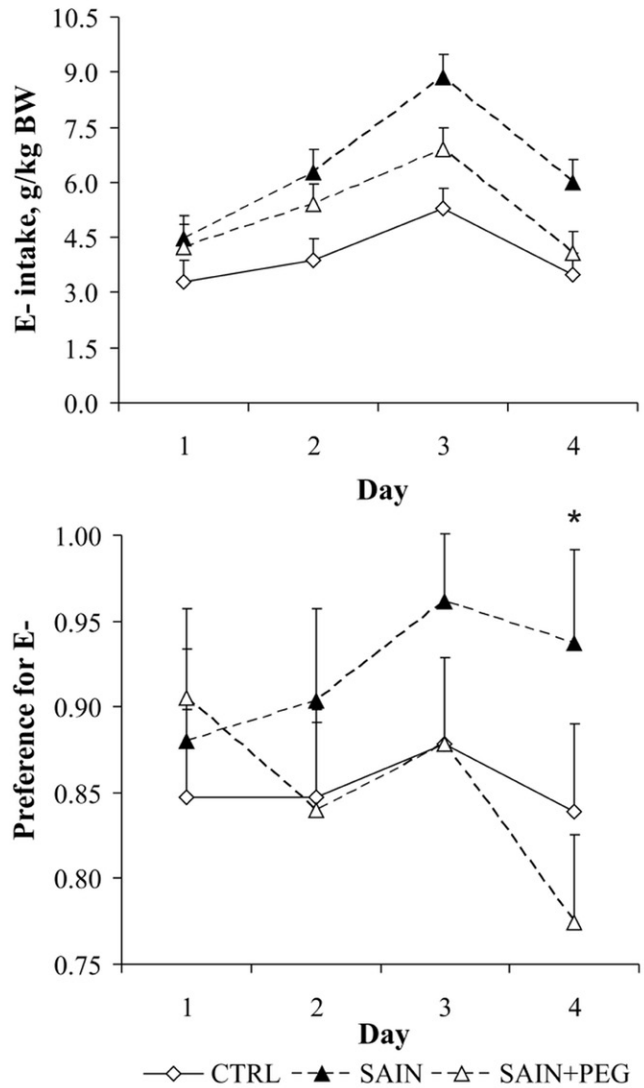

Figure 2. Intake of and preference for endophyte-free tall fescue (E–) by lambs offered a choice between E– and orchardgrass (DM basis). Lambs were previously conditioned with endophyte-infected tall fescue supplemented with beet pulp (CTRL) or with endophyte-infected tall fescue supplemented with fresh-cut sainfoin and beet pulp (SAIN) or fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG). Each value represents the mean of 12 animals. Vertical bars represent SEM. For each day, *$P < 0.05$.

Orchardgrass intake was not different among CTRL, SAIN, and SAIN+PEG treatments ($P = 0.572$).

Grazing Trial

Choice between Endophyte-Infected Tall Fescue and Sainfoin – Scans. Averaged across days, all lambs showed a greater proportion of scans on sainfoin than on E+ (0.87 ± 0.11 vs. 0.12 ± 0.11, respectively; means ± SD). Lambs exposed to CTRL, SAIN, and SAIN+PEG spent the same proportion of scans foraging on E+ (0.11, 0.13, and 0.10 ± 0.04, respectively; means ± SEM, $P = 0.757$) and on sainfoin (0.88, 0.86, and 0.89 ± 0.02, respectively; means ± SEM, $P =$

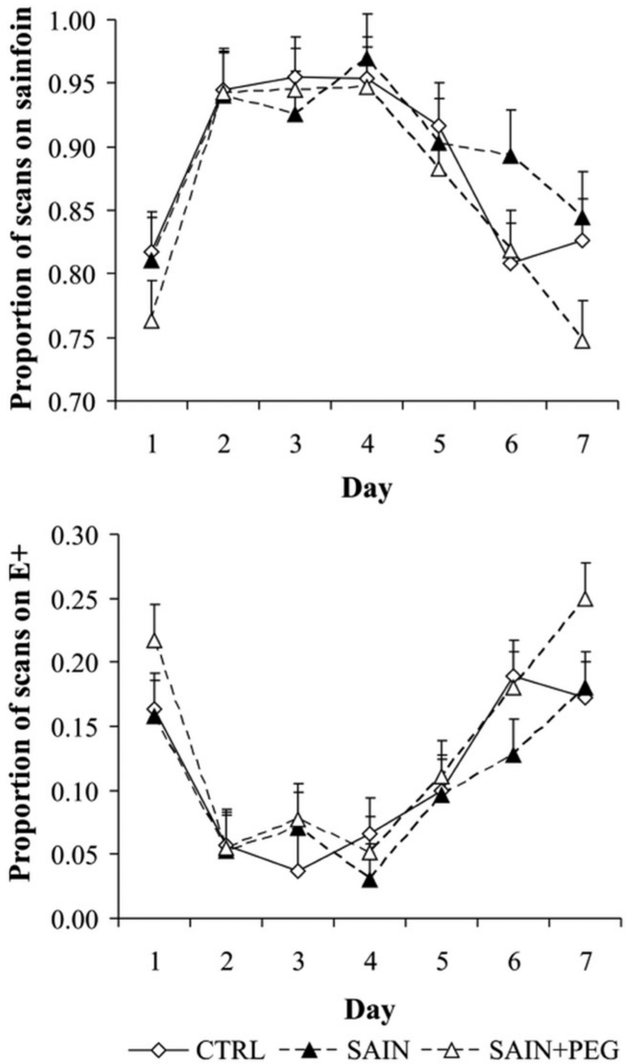

Figure 3. Average proportion of scans by grazing lambs when they had a choice of sainfoin and endophyte-infected tall fescue (E+). Lambs were previously conditioned with endophyte-infected tall fescue supplemented with beet pulp (CTRL) or with endophyte-infected tall fescue supplemented with fresh-cut sainfoin and beet pulp (SAIN) or fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG). Each value represents the mean of 6 (CTRL and SAIN+PEG) or 5 (SAIN) pairs of animals. Vertical bars represent SEM.

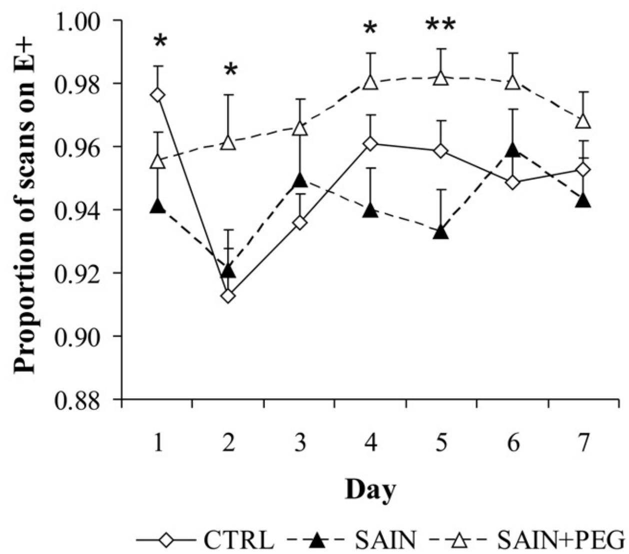

Figure 4. Average proportion of scans by lambs when they grazed a monoculture of endophyte-infected tall fescue (E+). Lambs were previously conditioned with endophyte-infected tall fescue supplemented with beet pulp (CTRL) or with endophyte-infected tall fescue supplemented with fresh-cut sainfoin and beet pulp (SAIN) or fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG). Each value represents the mean of 6 (CTRL and SAIN+PEG) or 5 (SAIN) pairs of animals. Vertical bars represent SEM. For each day, *$P < 0.05$ and **$P < 0.01$.

0.889). Scans on E+ dropped significantly ($P < 0.001$) on d 2 of testing, remained constant the following 2 d, and increased on the last 3 d ($P = 0.001$; Fig. 3). Scans on sainfoin also showed a significant day effect ($P = 0.002$). They increased on d 2 of testing ($P < 0.001$), remained constant the following 2 d, and decreased during the last 3 d ($P = 0.007$; Fig. 3).

Choice between Endophyte-Infected Tall Fescue and Sainfoin – Defoliation Intensity. The probability of a marked E+ plant being defoliated at least 1 time was similar among groups ($P = 0.955$) and was low at the end of testing (0.18, 0.21, and 0.18 ± 0.08, for CTRL, SAIN, and SAIN+PEG, respectively; means ± SEM). The cumulative proportion of E+ removal was also low and similar between CTRL, SAIN, and SAIN+PEG lambs (0.03, 0.05, and 0.04 ± 0.03, respectively; means ± SEM, $P = 0.830$).

Endophyte-Infected Tall Fescue Acceptance Grazing Tests – Scans. There was a treatment × day effect ($P < 0.001$). Lambs in SAIN+PEG had a greater proportion of scans on E+ than lambs in SAIN on d 2, 4, and 5 of testing ($P = 0.048$, $P = 0.014$, and $P = 0.001$, respectively; Fig. 4). A greater proportion of scans on E+ was also observed for CTRL lambs than for SAIN lambs on d 1 of testing ($P = 0.044$), and differences disappeared during the remaining days of the tests ($P \geq 0.310$; Fig. 4).

Endophyte-Infected Tall Fescue Acceptance Grazing Tests – Defoliation Intensity. During d 3, the probability of a marked E+ plant being defoliated at least 1 time was greater for lambs in SAIN+PEG than for lambs in SAIN ($P = 0.022$; Fig. 5). During d 5 and 7, lambs in SAIN+PEG were also more likely to defoliate at least 2 times the same E+ plant than lambs in SAIN ($P = 0.049$ and $P = 0.002$, respectively; Fig. 5). During d 7, lambs in SAIN+PEG were more likely to defoliate 3 times the same E+ plant than lambs in CTRL ($P = 0.030$; Fig. 5).

The cumulative proportion of E+ removal was similar between CTRL, SAIN, and SAIN+PEG lambs (0.61, 0.64, and 0.66 ± 0.02, respectively; means ± SEM, $P = 0.299$). However, lambs in SAIN made a more intense defoliation of E+ than lambs in CTRL ($P = 0.025$) on d 3 of the tests whereas no differences

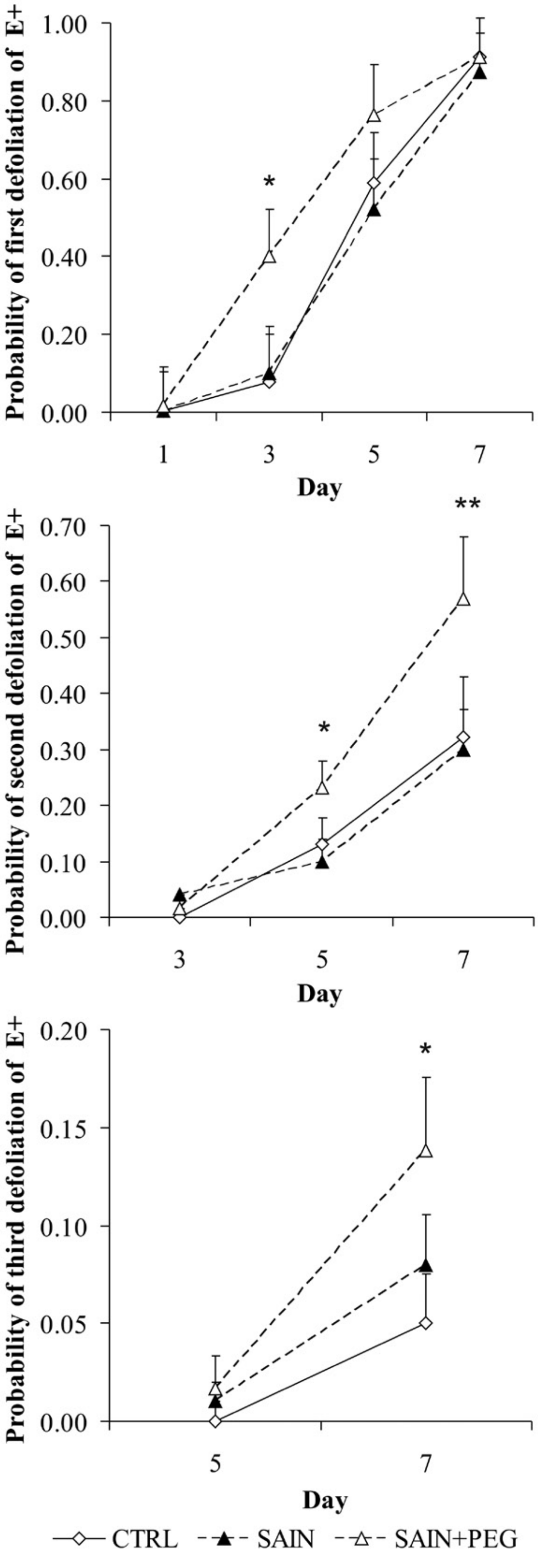

Figure 5. Probability for a given plant of endophyte-infected tall fescue (E+) being defoliated for first, second, and third time by lambs previously conditioned with E+ and supplemented with beet pulp (CTRL) or with E+ supplemented with either fresh-cut sainfoin and beet pulp (SAIN) or fresh-cut sainfoin plus polyethylene glycol mixed in beet pulp (SAIN+PEG). All lambs grazed a monoculture of E+. Sampling was initiated on the first day of the grazing trial and then it was repeated every 2 d. Each value represents the mean of 6 (CTRL and SAIN+PEG) or 5 (SAIN) pairs of animals. Vertical bars represent SEM. For each day, $^*P < 0.05$ and $^{**}P < 0.01$.

were observed between lambs in SAIN and lambs in SAIN+PEG ($P = 0.393$).

DISCUSSION

Intake of Endophyte-Infected Tall Fescue during the Conditioning Period

One of the predictions in the present study was that sainfoin supplementation would increase use of E+ by sheep. Results suggest that such positive effect of supplementation was associated with sainfoin phenology. When sainfoin was at the flowering stage (subperiod 1) intake of E+ was similar among treatments whereas when sainfoin was at the mid-vegetative stage (lower fiber and greater CP content; subperiod 2) lambs supplemented with the legume displayed greater consumption of E+ than controls. This response can be explained, at least in part, by the fact that increased ruminal distension in ruminants (e.g., due to the ingestion of high-fiber forages such as sainfoin at the flowering stage) depresses feed intake (Villalba et al., 2009). This depression could have influenced the ensuing consumption of E+ in sainfoin-supplemented animals relative to CTRL lambs that did not receive the legume. In addition, the greater concentration of tannins in sainfoin during subperiod 1 may have contributed to a reduction in E+ intake. Condensed tannins cause negative postingestive effects (Provenza et al., 1990) and greater concentrations of tannins in the supplement could have caused an anorectic response in sainfoin-supplemented lambs.

The supply of feeds or other forages to animals grazing E+ typically reduces E+ intake by livestock (Daura and Reid, 1991; Hess et al., 1996; Elizalde et al., 1998). However, Lyman et al. (2011, 2012) showed that cattle graze more E+ after a 30-min period of grazing birdsfoot trefoil—a tannin-containing legume—than when they first graze E+ and then the legume. Likewise, lambs supplemented with birdsfoot trefoil ate more E+ than unsupplemented animals (Owens et al., 2012). The increased use of E+ following supplementation with a tannin-containing legume has been partially attributed to the inactivation of alkaloids in E+ as a result of tannins binding with them in the gastrointestinal tract. The capacity of condensed tannins to precipitate alkaloids was originally suggested by Bate-Smith and Swain (1962). Ensuing research showed stable complexes form between alkaloids and tannins (Okuda et al., 1982; Charlton et al., 2000). Nevertheless, in addition to the benefits of tannins, ingestion of a legume (e.g., sainfoin) following a basal diet containing grasses (e.g., E+) may improve the balance of carbohydrates and protein in the diet (Rutter, 2006). A better nutrition has the potential to increase E+ intake because of an improvement in fiber digestion and an increased likelihood of detoxifying alkaloids (Provenza et al., 2003), which provides an alternative explanation. The present study separated the potential benefits of tannins from those of nutrients provided by sainfoin at enhancing the use of E+ by sheep. Polyethylene glycol binds to condensed tannins (Jones and Mangan, 1977) and has been used to selectively neutralize tannins in livestock diets (Silanikove et al., 1994; Priolo et al., 2000). Polyethylene glycol-condensed tannin ratios of 1:2 completely inactivate condensed tannins in sheep fed tannin-containing leaves of *Ceratonia siliqua* (Silanikove et al., 1994). Lambs in the SAIN+PEG treatment consumed enough PEG (33 g) to neutralize all the tannins ingested with the sainfoin supplement. The fact that PEG neutralized condensed tannins in sainfoin in the present study is also evident by the greater BUN values observed in SAIN+PEG than in SAIN lambs. Polyethylene glycol reduces the protective effects that tannins have on the ruminal degradation of proteins (Waghorn et al., 1987). Ruminal microorganisms brake down proteins into ammonia, among other components, and ammonia concentration in the ruminal environment is reflected by BUN (Lobley et al., 1995). When PEG was present in the supplement, no differences in E+ intake were observed between lambs in SAIN+PEG and lambs in CTRL. In contrast, E+ intake was greater for lambs in SAIN than for lambs in CTRL during the same feeding period. Thus, these results suggest that condensed tannins are at least partially involved in the positive effects of legume supplementation on E+ intake.

Physiological Parameters during the Conditioning Period

Sainfoin supplementation improved some physiological parameters in lambs that are negatively affected by ingestion of E+. Moreover, the benefits of sainfoin were in some instances attenuated by supplementation with PEG, which binds tannins in the rumen, and further supports the benefits of condensed tannins at reducing the negative postingestive effects of E+.

Lambs in SAIN+PEG showed lower hemoglobin concentration and PCV than lambs supplemented with only sainfoin. Negative impacts on erythrocyte morphology and composition (related to some forms of anemia) have been attributed to the low copper concentration typically found in E+ (Dennis et al., 1998). Copper is required for the proper formation of hemoglobin and its deficiency can affect red blood cell parameters (McDowell, 1992). However, lambs in SAIN and SAIN+PEG had similar intake of E+ and yet lambs in SAIN+PEG displayed a greater degree of red blood cell heterogeneity. Alkaloids were shown to induce hemolysis in sheep (Swick et al., 1983), which could contribute to the increased red blood cell heterogeneity commonly observed in animals suffering fescue toxicosis (Oliver et al., 2000). This may explain the greater degree of red blood cell heterogeneity in animals experiencing to a greater extent the negative impacts of E+ (CTRL and SAIN+PEG). However, results on hemoglobin and PCV related to fescue toxicosis are far from consistent among previous studies (Oliver et al., 2000) and further research is still needed to provide a more accurate explanation.

Sainfoin supplementation improved some immunological parameters such as the total number of leukocytes and lymphocytes relative to control lambs. Beef steers grazing E+ show reduced immunological response compared to steers grazing low-endophyte fescue (Saker et al., 1998). Lambs in SAIN also showed greater globulin concentrations and a reduced albumin:globulin ratio than control lambs, which can be interpreted as a protective action of sainfoin on the immune system (Oliver et al., 2000). Schultze et al. (1999) found that prolonged exposure to E+ specifically reduces α- and γ-globulin concentrations.

Reduced serum concentration of ALP is commonly observed in cattle fed E+ (Rice et al., 1997; Oliver et al., 2000). Unexpectedly, lambs in SAIN or SAIN+PEG showed decreased concentrations of ALP than lambs in CTRL. Nevertheless, ALP values of all lambs exceeded the normal range of reference values suggested for sheep. Sheep fed with the potent alkaloid swainsonine showed consistent increases in serum ALP (Taylor et al., 2000), which can be related to liver damage (Edrington et al., 1995). Therefore, if ergot alkaloids in E+ impacted lambs through the same mechanisms as swainsonine, then a reduction in serum ALP in lambs offered sainfoin would be expected. Aspartate aminotransferase increased with sainfoin supplementation. Consistent with this, AST is greater in cattle fed low-endophyte fescue than in animals ingesting E+ (Brown et al., 2009). Lambs grazing a grass–clover pasture had greater AST than lambs grazing a *Brassica* pasture with high concentration of glucosinolates (Cox-Ganser et al., 1994). Finally, GGT was greater in SAIN than in CTRL lambs, and this increment exceeded the range of normal values. High activity of GGT can be a response to glutathione depletion caused by the oxidative stress generated in the liver by the ingestion of ergot alkaloids (Whitfield, 2001; Settivari et al., 2008). Nevertheless, ingestion of tannins by lambs offered sainfoin can also account for high GGT values not necessarily linked to hepatic toxicity (Frutos et al., 2004).

A reduction in serum prolactin is normally observed in animals suffering fescue toxicosis (e.g., Porter and Thompson, 1992; Oliver et al., 2000). Lambs fed with sainfoin showed greater prolactin than those not supplemented, and supplementation with PEG reduced the magnitude of this effect. This suggests an interaction between alkaloids and tannins, mainly because prolactin concentrations are suppressed by the effects of alkaloids on the pituitary gland (Strickland et al., 1992). Prolactin concentrations correlate with lymphocyte production and regulate the humoral and cellular immune response (Freeman et al., 2000), which may explain the reduced immunological profile of lambs in CTRL. However, immunity can also be affected by chronic stress (Davis et al., 2008) such as the ingestion of a toxic plant (Kronberg et al., 1993). Stress induces the activation of the hypothalamic–pituitary axis resulting in increased secretion of cortisol from the adrenal gland (Harbuz and Lightman, 1992). Only lambs in SAIN+PEG showed a consistent reduction in cortisol when compared to lambs in CTRL. However, lambs in SAIN showed a lower neutrophil:lymphocyte ratio than lambs in CTRL, which can be interpreted as an indicator of lower levels of chronic stress for lambs in SAIN (Davis et al., 2008).

Ergot alkaloids affect animals' thermal homeostasis because they are strong vasoconstrictors that obstruct heat dissipation through the skin (Strickland et al., 1993; Browning and Leite-Browning, 1997). High environmental temperature induces hyperthermia in sheep fed endophyte-infected fescue seeds or noninfected seeds with added ergovaline (Gadberry et al., 2003). In the present study, according to the Livestock Conservation Institute (LCI, 1970) temperature–humidity index values during rectal temperature sampling were classified between danger and emergency (temperature–humidity index values ranging from 81 to 88; Utah State University weather reports for North Logan, UT). Supplementation of E+ with sainfoin reduced rectal temperatures (39.71°C for lambs in SAIN) while average rectal temperature in CTRL (39.97°C) exceed the normal range of values for sheep (Merck, 2012b) suggesting heat stress. Even when the numerical difference seems small it has biological relevance; for example, ewes showing rectal temperatures above 39.9°C produced lambs of lower birthweight than ewes showing rectal temperatures below 39.8°C (McCrabb et al., 1993). Moreover, the effect observed on rectal temperature was evident even when lambs in SAIN consumed similar or greater amounts of E+, the forage that induces hyperthermia.

Collectively, neutralization of tannins by PEG only attenuated some of the benefits induced by sainfoin supplementation. Therefore, even when tannins may be involved in ameliorating the negative physiological effects of ergot alkaloids, other variables such as the nutrients provided by the legume may contribute to that benefit. Nevertheless, legume supplementation did not affect ADG or final BW. It is likely that an extended exposure to the treatments or a greater inclusion of E+ in the diet may be needed to detect an effect.

Foraging Behavior of Sheep following the Conditioning Period

During preference tests between E– and orchardgrass, lambs in SAIN showed a greater intake of E– than CTRL lambs. In contrast, intake of E– did not differ between SAIN+PEG and CTRL treatments. This suggests that sainfoin supplementation improved lambs' experience with tall fescue, thus enhancing subsequent lambs' consumption of tall fescue in a choice test. Condensed tannins were involved in this process since ingestion of PEG, a polymer that selectively inactivates tannins reduced intake of tall fescue. Sheep show greater intake of low quality (Villalba and Provenza, 1997) or toxin-containing feeds (Villalba et al., 2004; Baraza et al., 2005) when ingestion of these feeds is reinforced by nutrient supplementation or by the interaction, and inactivation, of multiple toxins in the gastrointestinal tract. Lambs infused in the rumen with quebracho tannins selectively increase intake of and preference for E+ relative to control lambs, suggesting a reduced impact of alkaloids by tannin supplementation (Lisonbee et al., 2009; Villalba et al., 2011).

When lambs grazed E+ and sainfoin during choice tests, all lambs spent most of their feeding time grazing sainfoin and all treatments used E+ to a similar extent. Therefore, when a nutritious legume was available ad libitum, prior experience with the different supplementation treatments explored in the present study did not have an influence on preference for E+. It is likely that lambs did not learn about the benefits of mixing sainfoin with E+ during conditioning or that different preferences acquired for E+ during that period were not manifested as a consequence of the presence of a more nutritious, and safe, legume. Therefore, in an ensuing test lambs were just offered E+ and its acceptance was assessed as a function of the lambs' previous experiences during conditioning. Lambs previously supplemented with SAIN+PEG showed the greatest use of E+. This response may represent the ability of ruminants to adjust feed intake and preferences as a function of their previous experience with the concentration of toxins in feeds (Launchbaugh et al., 1993). For instance, lambs that were initially conditioned to consume oats with either a low, medium, or high concentration of lithium chloride (LiCl, a salty-tasting toxin) and then offered oats with the medium concentration of LiCl decreased, maintained, and increased intake, respectively (Launchbaugh et al., 1993). It is likely that due to the binding effects of condensed tannins on ergot alkaloids, lambs in SAIN experienced lower doses of ergovaline than those in the CTRL and SAIN+PEG treatments. However, when the protective effects of sainfoin were removed during the E+ acceptance tests, lambs in SAIN likely experienced an increased dose of toxins relative to the conditioning period. Consistent with the ingestive responses of lambs conditioned to a low dose of LiCl and then offered a feed with a greater dose (Launchbaugh et al., 1993), lambs in SAIN reduced their intake of E+. This phenomenon is known as "successive negative contrast" and it was observed in ruminants changed from high-quality to low-quality forages (Catanese et al., 2011) or from low-tannin to high-tannin feeds (Bergvall et al., 2007). This negative contrast may have been attenuated in SAIN+PEG lambs, given that PEG reduced the positive effects of sainfoin supplementation, which may explain the greater use of E+ by this group. Lambs in CTRL experienced the negative postingestive effects of E+ since the beginning of the study and this could have led to an aversion that remained throughout the grazing trial.

Supplemental feeds or forages often decrease E+ intake and thus dilute the concentration of alkaloids in the diet. In contrast to this approach, supplemental tannin-containing legumes such as sainfoin may contribute to increase the total amount of nutrients ingested while maintaining or enhancing the use of E+. Sainfoin supplementation also improved some physiological parameters indicative of fescue toxicosis. These effects were in part influenced by condensed tannins in sainfoin, as the addition of a polymer that binds to tannins attenuated some of these responses. Nevertheless, previous experience with sainfoin supplementation did not modify preference for E+ during grazing and it reduced acceptance when E+ was grazed in a monoculture.

LITERATURE CITED

Aiken, G. E., E. L. Piper, and C. R. Miesner. 2001. Influence of protein supplementation and implant status on alleviating fescue toxicosis. J. Anim. Sci. 79:827–832.

Aldrich, C. G., M. T. Rhodes, J. L. Miner, M. S. Kerley, and J. A. Paterson. 1993. The effects of endophyte infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. J. Anim. Sci. 71:158–163.

Altmann, J. 1974. Observational study of behaviour: Sampling methods. Behaviour 49:227–267.

AOAC Int. 2002. Official methods of analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Bacon, C. W., and M. R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1:45–55.

Baraza, E., J. J. Villalba, and F. D. Provenza. 2005. Nutritional context influences preferences of lambs for foods with different plant secondary metabolites. Appl. Anim. Behav. Sci. 92:293–305.

Bate-Smith, E. C., and T. Swain. 1962. Flavonoid compounds. In: M. Flori and H. S. Mason, editors, Comparative biochemistry. Academic Press, New York, NY. p. 755–809.

Bates, D., M. Maechler, and B. Bolker. 2012. lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. http://cran.r-project.org/web/packages/lme4/index.html. (Accessed February 2, 2012.)

Bergvall, U. A., P. Rautio, T. Luotola, and O. Leimar. 2007. A test of simultaneous and successive negative contrast in fallow deer foraging behaviour. Anim. Behav. 74:395–402.

Bodine, T. N., H. T. Purvis II, C. J. Ackerman, and C. L. Goad. 2000. Effects of supplementing prairie hay with corn and soybean meal on intake, digestion, and ruminal measurements by beef steers. J. Anim. Sci. 78:3144–3154.

Brown, K. R., G. A. Anderson, K. Son, G. Rentfrow, L. P. Bush, J. L. Klotz, J. R. Strickland, J. A. Boling, and J. C. Matthews. 2009. Growing steers grazing high versus low endophyte (*Neotyphodium coenophialum*)-infected tall fescue have reduced serum enzymes, increased glucogenic enzymes, and reduced liver and carcass mass. J. Anim. Sci. 87:748–760.

Browning, R., Jr., and M. L. Leite-Browning. 1997. Effect of ergotamine and ergonovine on thermal regulation and cardiovascular function in cattle. J. Anim. Sci. 75:176–181.

Burns, J. C., and D. S. Chamblee. 1979. Adaptation. In: R. C. Buckner and L. P. Bush, editors, Tall fescue. Monogr. 20. Am. Soc. Agron., Madison, WI. p. 9–30.

Catanese, F., E. Freidin, M. I. Cuello, and R. A. Distel. 2011. Devaluation of low-quality food during early experience by sheep. Animal 5:938–942.

Charlton, A. J., A. L. Davis, D. P. Jones, J. R. Lewis, A. P. Davies, E. Haslam, and M. P. Williamson. 2000. The self-association of the black tea polyphenol theaflavin and its complexation with caffeine. J. Chem. Soc., Perkin Trans. 2317–322.

Cox-Ganser, J. M., G. A. Jung, R. T. Pushkin, and R. L. Reid. 1994. Evaluation of Brassicas in grazing system for sheep: II. Blood composition and nutrient status. J. Anim. Sci. 72:1832–1841.

Daura, M. T., and R. L. Reid. 1991. Energy and protected protein supplements to lambs on endophyte-infected tall fescue pasture. J. Anim. Sci. 69:358–368.

Davis, A. K., D. L. Maney, and J. C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Funct. Ecol. 22:760–772.

Dennis, S. B., V. G. Allen, K. E. Saker, J. P. Fontenot, J. Y. M. Ayad, and C. P. Brown. 1998. Influence of *Neotyphodium coenophialum* on copper concentration in tall fescue. J. Anim. Sci. 76:2687–2693.

Edrington, T. S., C. A. Kamps-Holtzapple, R. B. Harvey, L. F. Kubena, M. H. Elissalde, and G. E. Rottinghaus. 1995. Acute hepatic and renal toxicity in lambs dosed with fumonisin-containing culture material. J. Anim. Sci. 73:508–515.

Elizalde, J. C., J. D. Cremin Jr., D. B. Faulkner, and N. R. Merchen. 1998. Performance and digestion by steers grazing tall fescue and supplemented with energy and protein. J. Anim. Sci. 76:1691–1701.

Freeman, M. E., B. Kanyicska, A. Lerant, and G. Nagy. 2000. Prolactin: Structure, function, and regulation of secretion. Physiol. Rev. 80:1523–1631.

Frutos, P., M. Raso, G. Hervás, A. R. Mantecón, V. Pérez, and F. Giráldez. 2004. Is there any detrimental effect when a chestnut hydrolysable tannin extract is included in the diet of finishing lambs? Anim. Res. 53:127–136.

Gadberry, M. S., T. M. Denard, D. E. Spiers, and E. L. Piper. 2003. Effects of feeding ergovaline on lamb performance in a heat stress environment. J. Anim. Sci. 81:1538–1545.

Hancock, D. W., and J. G. Andrae. 2009. Novel endophyte-infected tall fescue. Univ. of Georgia Coop. Ext. Circ. C861. Univ. of Georgia, Athens GA.

Hannah, S. M., J. A. Paterson, J. E. Williams, M. S. Kerley, and J. L. Miner. 1990. Effects of increasing dietary levels of endophyte infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. J. Anim. Sci. 68:1693–1701.

Harbuz, M. S., and S. L. Lightman. 1992. Stress and the hypothalamo-pituitary-adrenal axis: Acute, chronic and immunological activation. J. Endocrinol. 134:327–339.

Hess, B. W., L. J. Krysl, M. B. Judkins, D. W. Holcombe, J. D. Hess, D. R. Hanks, and S. A. Huber. 1996. Supplemental cracked corn or wheat bran for steers grazing endophyte-free fescue pasture: Effects on live weight gain, nutrient quality, forage intake, particulate and fluid kinetics, ruminal fermentation, and digestion. J. Anim. Sci. 74:1116–1125.

Jones, W. T., and J. L. Mangan. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28:126–136.

Kronberg, S. L., J. W. Walker, and J. A. Fitzgerald. 1993. Feeding behavior of grazing ruminants experiencing stress. Physiol. Behav. 54:1191–1194.

Launchbaugh, K. L., F. D. Provenza, and E. A. Burritt. 1993. How herbivores track variable environments: Response to variability of phytotoxins. J. Chem. Ecol. 19:1047–1056.

Lenth, R. V. 2012. lsmeans: Least-squares means. R package version 0.99-80. http://cran.r-project.org/web/packages/lsmeans/index.html. (Accessed February 2, 2012.)

Lisonbee, L. D., J. J. Villalba, and F. D. Provenza. 2009. Effects of tannins on selection by sheep of forages containing alkaloids, tannins and saponins. J. Sci. Food Agric. 89:2668–2677.

Livestock Conservation Institute (LCI). 1970. Patterns of transit losses. LCI, Omaha, NE.

Lobley, G. E., A. Connell, M. A. Lomax, D. S. Brown, E. Milne, A. G. Calder, and D. A. H. Farningham. 1995. Hepatic detoxification of ammonia in the ovine liver: Possible consequences for amino acid catabolism. Br. J. Nutr. 73:667–685.

Logan, M. 2010. Biostatistical design and analysis using R: A practical guide. Wiley-Blackwell, Hoboken, NJ.

Lyman, T. D., F. D. Provenza, J. J. Villalba, and R. D. Wiedmeier. 2011. Cattle preferences differ when endophyte-infected tall fescue, birdsfoot trefoil, and alfalfa are grazed in difference sequences. J. Anim. Sci. 89:1131–1137.

Lyman, T. D., F. D. Provenza, J. J. Villalba, and R. D. Wiedmeier. 2012. Phytochemical complementarities among endophyte-infected tall fescue, reed canarygrass, birdsfoot trefoil, and alfalfa affect cattle foraging. Animal 6:676–682.

McCrabb, G. J., B. J. McDonald, and L. M. Hennoste. 1993. Lamb birthweight in sheep differently acclimatized to a hot environment. Aust. J. Agric. Res. 44:933–943.

McDowell, L. R. 1992. Minerals in animal and human nutrition. Academic Press, New York, NY.

Merck. 2012a. The Merck veterinary manual: Hematologic reference ranges. Accessed Feb. 26, 2013. www.merckmanuals.com/vet/appendixes/reference_guides/hematologic_reference_ranges.html.

Merck. 2012b. The Merck veterinary manual: Normal rectal temperature ranges. Accessed Aug. 15, 2013. www.merckmanuals.com/vet/appendixes/reference_guides/normal_rectal_temperature_ranges.html.

Merck. 2012c. The Merck veterinary manual: Serum biochemical reference ranges. Accessed Feb. 26, 2013. www.merckmanuals.com/vet/appendixes/reference_guides/serum_biochemical_reference_ranges.html.

Okuda, T., K. Morie, and M. Shiota. 1982. Effects of interaction of tannins and coexisting substances. III. Formation and solubilization of precipitates with alkaloids. Yakugaku Zasshi: J. Pharm. Soc. Japan 102:854–858.

Oliver, J. W., A. E. Schultze, B. W. Rohrbach, H. A. Fribourg, T. Ingle, and J. C. Waller. 2000. Alterations in hemograms and serum biochemical analytes of steers after prolonged consumption of endophyte-infected tall fescue. J. Anim. Sci. 78:1029–1035.

O'Reagain, P. J., and C. A. Grau. 1995. Sequence of species selection by cattle and sheep on South African sourveld. J. Range Manage. 48:314–321.

Osuji, P. O. 1974. The physiology of eating and the energy expenditure of the ruminant at pasture. J. Range Manage. 27:437–443.

Owens, J., F. D. Provenza, R. D. Wiedmeier, and J. J. Villalba. 2012. Supplementing endophyte-infected tall fescue or reed canarygrass with alfalfa or birdsfoot trefoil increases forage intake and digestibility by sheep. J. Sci. Food Agric. 92:987–992.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and the R Development Core Team. 2012. nlme: Linear and nonlinear mixed effects models. R package version 3.1-104. http://cran.rproject.org/web/packages/nlme/nlme.pdf. (Accessed February 2, 2012)

Popay, A. J., J. G. Jensen, and B. M. Cooper. 2005. The effect of non-toxic endophytes in tall fescue on two major insect pests. Proc. N. Z. Grassl. Assoc. 67:169–173.

Popay, A. J., B. A. Tapper, and C. Podmore. 2009. Endophyte-infected meadow fescue and loline alkaloids affect argentine stem weevil larvae. N. Z. Plant Prot. 62:19–27.

Porter, J. K., and F. N. Thompson Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594–1603.

Priolo, A., G. C. Waghorn, M. Lanza, L. Biondi, and P. Pennisi. 2000. Polyethylene glycol as a means to reducing the impact of condensed tannins in carob pulp: Effect on lamb growth and meat quality. J. Anim. Sci. 78:810–816.

Provenza, F. D., E. A. Burritt, T. P. Clausen, J. P. Bryant, P. B. Reichardt, and R. A. Distel. 1990. Conditioned flavor aversion: A mechanism for goats to avoid condensed tannins in blackbrush. Am. Nat. 136:810–828.

Provenza, F. D., J. J. Villalba, L. E. Dziba, S. B. Atwood, and R. E. Banner. 2003. Linking herbivore experience, varied diets, and plant diversity. Small Rumin. Res. 49:257–274.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org/. (Accessed February 2, 2012.)

Rice, R. L., D. J. Blodgett, G. G. Schurig, W. S. Swecker, J. P. Fontenot, V. G. Allen, and R. M. Akers. 1997. Evaluation of humoral immune responses in cattle grazing endophyte-infected or endophyte-free fescue. Vet. Immunol. Immunopathol. 59:285–291.

Rottinghaus, G. E., G. B. Garner, N. C. Creighton, and J. L. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte infested tall fescue: Seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39:112–115.

Rutter, S. M. 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Appl. Anim. Behav. Sci. 97:17–35.

Ruxton, G. D., and G. Beauchamp. 2008. Time for some a priori thinking about post hoc testing. Behav. Ecol. 19:690–693.

Saker, K. E., V. G. Allen, J. Kalnitsky, C. D. Thatcher, W. S. Swecker Jr., and J. P. Fontenot. 1998. Monocyte immune cell response and copper status in beef steers that grazed endophyte-infected tall fescue. J. Anim. Sci. 76:2694–2700.

Schultze, A. E., B. W. Rohrbach, H. A. Fribourg, J. C. Waller, and J. W. Oliver. 1999. Alterations in bovine serum biochemistry profiles associated with prolonged consumption of endophyte-infected tall fescue. Vet. Hum. Toxicol. 41:133–139.

Scrivner, J. H., D. M. Center, and M. B. Jones. 1986. A rising plate meter for estimating production and utilization. J. Range Manage. 39:475–477.

Settivari, R. S., T. J. Evans, E. Rucker, G. E. Rottinghaus, and D. E. Spiers. 2008. Effect of ergot alkaloids associated with fescue toxicosis on hepatic cytochrome P450 and antioxidant proteins. Toxicol. Appl. Pharmacol. 227:347–356.

Sibbald, A. M., and R. J. Hooper. 2004. Sociability and the willingness of sheep to move away from their companions in order to graze. Appl. Anim. Behav. Sci. 86:51–62.

Silanikove, N., Z. Nitsan, and A. Perevolotsky. 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J. Agric. Food Chem. 42:2844–2847.

Strickland, J. R., D. L. Cross, T. C. Jenkins, R. J. Petroski, and R. G. Powell. 1992. The effect of alkaloids and seed extracts of endophyte-infected fescue on prolactin secretion in an in vitro rat pituitary perfusion system. J. Anim. Sci. 70:2779–2786.

Strickland, J. R., J. W. Oliver, and D. L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. Vet. Hum. Toxicol. 35:454–464.

Swick, R. A., C. L. Miranda, P. R. Cheeke, and D. R. Buhler. 1983. Effect of phenobarbital on toxicity of pyrrolizidine (Senecio) alkaloids in sheep. J. Anim. Sci. 56:887–894.

Taylor, J. B., J. R. Strickland, T. May, and D. E. Hawkins. 2000. Effect of subacute swainsonine (locoweed; *Oxytropis sericea*) consumption on immunocompetence and serum constituents in a nutrient restricted state. Vet. Hum. Toxicol. 42:199–204.

Terrill, T. H., A. M. Rowan, G. B. Douglas, and T. N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58:321–329.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Villalba, J. J., and F. D. Provenza. 1997. Preference for wheat straw by lambs conditioned with intraruminal infusions of starch. Br. J. Nutr. 77:287–297.

Villalba, J. J., F. D. Provenza, A. K. Clemensen, R. Larsen, and J. Juhnke. 2011. Preference for diverse pastures by sheep in response to intraruminal administrations of tannins, saponins, and alkaloids. Grass Forage Sci. 66:224–236.

Villalba, J. J., F. D. Provenza, and G. Han. 2004. Experience influences diet mixing by herbivores: Implications for plant biochemical diversity. Oikos 107:100–109.

Villalba, J. J., F. D. Provenza, and R. Stott. 2009. Rumen distension and contraction influence feed preference by sheep. J. Anim. Sci. 87:340–350.

Waghorn, G. C., M. J. Ulyatt, A. John, and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57:115–126.

Whitfield, J. B. 2001. Gamma glutamyl transferase. Crit. Rev. Clin. Lab. Sci. 38:263–355.

Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects modelling for nested data. Mixed effects models and extensions in ecology with R. Springer, New York, NY.