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Tasco-Forage: II. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract¹

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ABSTRACT: Effects of applying Tasco-Forage, an *Ascophyllum nodosum* seaweed-based product prepared by a proprietary process, to endophyte (*Neotyphodium coenophialum* [Morgan-Jones and Gams] Glenn, Bacon, and Hanlin)-infected and endophyte-free tall fescue (*Festuca arundinacea* Schreb.) were studied in each of 3 yr (1995, 1996, and 1997) in Virginia and in 1996 and 1997 in Mississippi. There were 48 steers at each location in each year (n = 240) in a 2 × 2 × 2 factorial arrangement with two replications at each location. Steers in Virginia were Angus and Angus × Hereford with initial weights of 245 kg (SD = 20), 234 kg (SD = 9), and 265 kg (SD = 5) in yr 1, 2, and 3, respectively. Steers in Mississippi were 3/4 Angus and 1/4 Brahman and weighed 230 kg (SD = 8) and 250 kg (SD = 2) in yr 2 and 3, respectively. Tasco (3.4 kg/ha) was dissolved in water and applied to pastures in April before grazing was begun and again in July at the same rate. The grazing period was from mid-April to late September or mid-October. Total gains were higher (*P*

< 0.05) for steers grazing uninfected than for those grazing endophyte-infected tall fescue. Rectal temperatures were increased (*P* < 0.05) due to endophyte infection at both locations; Tasco application decreased temperature of steers grazing infected fescue in Virginia (interaction, *P* < 0.07) but increased temperatures of steers grazing infected fescue in Mississippi (interaction, *P* < 0.05). Presence of the endophyte resulted in rough hair coats and loss of hair color, but the effect was partially offset (*P* < 0.05) by Tasco application in Virginia in 1995. Both monocyte phagocytic activity (all years and locations) and major histocompatibility complex class II expression (1995 only) were decreased (*P* < 0.05) in steers due to endophyte infection, but this effect was reversed (*P* < 0.05) by application of Tasco to pastures. Application of the extract from *A. nodosum* seems to have use in alleviating adverse effects of endophyte on immune function and may improve hair coat condition in cattle grazing infected fescue, but effects on rectal temperature varied due to location.

Key Words: *Festuca*, Forage, Grazing, Immune Response, Steers, Toxicity

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Introduction

Tall fescue (*Festuca arundinacea*) is one of the most important cool-season perennial grasses in the eastern United States but is frequently infected with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin; Glenn et al., 1996). Presence of the endophyte is associated with

tall fescue toxicosis (Stuedemann and Hoveland, 1988). Symptoms include heat intolerance, photosensitivity, rough hair coats, elevated internal body temperature, reduced weight gains, and reduced serum prolactin. Recently, lowered Cu status and depressed immunocompetence were reported in steers that grazed endophyte-infected tall fescue (Saker et al., 1998).

Tasco-Forage is a water-soluble extract of the brown seaweed *Ascophyllum nodosum* harvested off the coast of Nova Scotia and prepared by a proprietary process (Acadian Seaplants Ltd., Dartmouth, Nova Scotia, Canada). An increase in superoxide dismutase activity (Zhang, 1997; Ayad, 1998; Fike et al., 2001), glutathione reductase, and ascorbate peroxidase (Ayad, 1998) as well as increased concentrations of α -tocopherol, β -carotene, and ascorbic acid (Zhang, 1997) were reported in

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tall fescue treated with the extract. Fike et al. (2001) reported improved performance of lambs grazing endophyte-infected tall fescue treated with the extract. Wether lambs and yearling beef steers that grazed Tasco-treated fescue exhibited increased serum vitamin A and whole blood Se concentrations (Fike et al., 2001). In biological systems, ramifications of oxidative stress on tissues, including depression of immune function in mammals, can be offset by increasing antioxidant defenses. Dietary antioxidants can be supplied through select vitamin and trace mineral supplementation or through natural products with high antioxidant activity.

Thus, experiments were conducted in Virginia and Mississippi to determine effects of Tasco on expression of fescue toxicosis including immune function depression in grazing beef steers.

Materials and Methods

Experiments were conducted with beef steers grazing endophyte-infected and uninfected tall fescue during 1995, 1996, and 1997 at the Southwest Virginia Agricultural Research and Extension Center at Glade Spring (81°40' west longitude; 36°47' north latitude; 652 m elevation) and during 1996 and 1997 at the Prairie Research Unit, Prairie, Mississippi (88°40' west longitude; 33°50' north latitude; 984 m elevation). At each location, there were eight pastures, four each that had been established with infected and uninfected 'Kentucky-31' (**KY-31**) tall fescue. Pastures at Glade Spring were established during 1986 and a description of the soils and the field experimental design was reported in Dennis et al. (1998). The endophyte-infected pastures at the Prairie Unit were established in 1978 and the uninfected fescue was established in 1980 on a Houston Clay (very-fine, smectitic, thermic Oxyaquic Hapluderts) with a slope of 1 to 3%.

During 1995, a pilot study was conducted in Virginia with 48 weaned beef steers (Angus and Angus × Hereford) 9 to 10 mo of age, with an initial BW of 245 kg (SD = 20). Steers were blocked by breed and weight and were randomized within blocks to either infected or uninfected tall fescue pastures that were treated or untreated with Tasco-Forage. Chemical composition of the extract was provided by Fike et al. (2001). Treatments were arranged in a 2 × 2 factorial within a randomized block design. Tasco was applied in water solution on April 4 and July 6, 1995, at 3.4 kg dry extract/ha.

Pastures were fertilized with 60 kg N/ha prior to initiation of grazing in the spring. Other fertilizer was applied based on annual soil test recommendations. Pastures were clipped once in the late spring to remove the stem and inflorescence growth. Presence and absence of the endophyte was verified by the Tall Fescue Toxicity Diagnostic Center, Auburn, AL. Percentage of endophyte in Virginia in 1995 averaged 67 and < 5% for the infected and uninfected pastures, respectively. The infected pastures ranged from 59 to 76%. During

1996 and 1997, percentage of endophyte infection averaged 80 and < 1% in Virginia, and pastures in Mississippi averaged 70 and < 10% in infected and uninfected pastures, respectively. Concentrations of total ergot alkaloids and ergovaline averaged 585 and 107 µg/g dry weight in Virginia and 615 and 144 µg/g dry weight in Mississippi for the two respective years (Fike et al., 2001). Random samples from uninfected pasture were included in the analyses and were consistently below detection levels for both total ergot alkaloids and for ergovaline.

Each pasture was 1.5 ha; thus, the stocking rate was one steer per 0.25 ha. In 1995, grazing began on April 25 and ended on October 16. Steers were vaccinated with *Pasturella haemolytica* (One Shot; Pfizer Animal Health, New York) *Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens* Type C & D *Bacterin-Toxoid* (7 WAY), infectious bovine rhinotracheitis, parainfluenza-3 virus, bovine viral diarrhea, and bovine respiratory syncytial virus (BRSV-Vac4; Bayer, Shawnee Mission, KS) and were treated for internal parasites with Ivomectin (Merk AgVet Division, Rahway, NJ) prior to the initiation of grazing. Steers were also implanted with Ralgro (Schering-Plough Animal Health, Union, NJ) initially and at 90-d intervals. Steers had access to water and plain salt at all times. Steers were weighed on two consecutive days initially and the weights were averaged. Steers were then weighed at 28-d intervals throughout the grazing season. Hair coat scores were recorded at each weighing by two or three trained evaluators using a scale on which 1 = slick, shiny hair with no evidence of retention of old hair, 2 = < 25% of the body covered with old, unshed hair, 3 = 25 to 50% of the body covered with old, unshed hair, 4 = 50 to 75% of the body covered with old, unshed hair, and 5 = muddy, dirty hair coat, evidence of having deliberately lain in mud, and > 50% of the body covered with old, unshed hair. Scores were then averaged for each steer. Rectal temperatures were recorded in April, May, July, and September during the morning prior to 1100. Blood samples were collected initially and at 28-d intervals throughout the grazing season for determination of serum minerals (Fike et al., 2001), monocyte phagocytic activity, and major histocompatibility complex (MHC) class II expression. Samples were collected via jugular venipuncture into 10-mL Vacutainer (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) tubes with EDTA and sodium heparin and a 7-mL Trace Mineral Vacutainer tube. Monocyte phagocytic activity and MHC class II expression were determined as described by Saker et al. (1998). Briefly, phagocytic capability of monocytes was determined by measuring fluorescent polystyrene bead uptake (Steinkamp et al., 1982). Cells were adjusted to a concentration of 1×10^6 cells/mL in Krebs Ringer Bicarbonate (**KRH**)-gelatin solution. Cells were divided into two tubes labeled for 4 and 37°C, and 100 µL of autologous bovine serum and 20 µL of Fluoresbrite Beads (Polysciences, Warrington, PA) were added to each tube. Tubes were incubated for 1

h, and then a phosphate-buffered saline, gelatin, and EDTA solution was added to each tube to terminate the reaction. Determination of phagocytic activity was assessed as the mean channel fluorescence and the percentage of cells resulting from the subtraction of the 4°C control sample from the 37°C sample after being read on the flow cytometer set to emit at 525 nm. The MHC class II expression was detected by flow cytometry using a ruminant monoclonal antibody (**moab**; THI4B; VMRD, Pullman, WA), as described by Splitter and Everlith (1989). Cells were adjusted to a concentration of 1×10^6 cells/mL in Hank's Balanced Salt Solution and incubated for 1 h with a 1:2,500 dilution of the moab. Cells were then incubated with fluorescein-conjugated rabbit anti-mouse immunoglobulin G, 1:100 concentration (Jackson ImmunoResearch Laboratories, Avondale, PA) for 1 h. Cells were washed and examined by gating on the monocyte population using an Epics EXCEL flow cytometer (Coulter, Miami, FL).

Data for Virginia were analyzed as a complete randomized block design with a factorial arrangement of treatments using a model that tested effects of endophyte, Tasco, and their interactions. Each date within year was analyzed separately. Pasture was the experimental unit.

In 1996 and 1997, 96 Angus and Angus \times Hereford steers (48 each year, initial mean BW 234 kg [SD = 9] and 265 kg [SD = 5] for 1996 and 1997, respectively) were blocked by breed and weight and randomized to treatments at the Virginia site. At the Mississippi site, 48 3/4 Angus \times 1/4 Brahman steers (initial BW 230 kg [SD = 8] and 250 kg [SD = 2] for 1996 and 1997, respectively) were blocked by weight and randomized to treatments. Thus, a total of 192 steers were used in the experiment during these 2 yr. All steers were vaccinated and implanted as described above. Steers at both locations were handled under animal care and use protocols approved by the Animal Care and Use Committees for Virginia Tech and Mississippi State University. Each pasture in Virginia was 1.5 ha with a stocking rate of one steer per 0.25 ha as described previously. Each pasture in Mississippi was 2.4 ha, which gave a stocking rate of one steer per 0.40 ha.

At the beginning of the grazing season in 1996, three of the six steers within each of the eight pastures at each location were given a Cu oxide bolus to supply 25 g of Cu (Copasure; Schering-Plough, Kenilworth, NJ) with an expected payout period of 120 d. In 1996, grazing began April 16/17 and ended October 1/2 for Mississippi and Virginia, respectively. In 1997, grazing began April 23/22 and ended October 8 and September 30 for the respective locations. The 1-d difference in beginning and ending dates between locations allowed the large number of blood samples to be analyzed within the appropriate time after the samples were collected and transported to Virginia for analysis. Steers were weighed initially and at 28-d intervals. Visual hair coat scores and rectal temperatures were recorded each time steers were weighed. Steers were handled in the order

of field randomization. Weights and samples were collected during early morning and were generally completed before 1000. Blood samples were collected at the start of each study and then every 3 mo thereafter throughout the grazing season for determination of monocyte phagocytic activity and MHC class II expression. Blood samples collected in Mississippi for immune function analysis were packaged with ice on the day of sampling and shipped by overnight delivery to the Virginia/Maryland Regional College of Veterinary Medicine, Blacksburg.

For statistical analyses, pasture was the experimental unit. For data collected in 1996, a randomized block design was used with a factorial combination of location, endophyte, and Tasco as effects of interest. The effect of Cu was analyzed as a subplot effect in a split plot arrangement. For data collected in 1997, a randomized block design with a factorial arrangement of location, endophyte, and Tasco was used. To analyze 1996 and 1997 data together, the analysis of variance table was arranged following Kempthorne (1952) with year as a main plot factor and location, endophyte, and Tasco in a factorial combination in the subplot position of a split plot arrangement; the effect of Cu (which was investigated only in the 1996 experiment) was included as a sub-subplot effect. A Type I analysis (SAS Inst. Inc., Cary, NC) was used because we wished to compare treatment means only for those effects that were actually observed (i.e., a Type III analysis was not used because we did not wish to use 1996 data on Cu response to estimate 1997 data that were not included in the experiment). When Cu did not interact with any other effect (tests that were based on 1996 data only), we assumed that Cu would not have interacted with these other effects in 1997 and proceeded to interpret effects based on the upper two splits in the analysis of variance (i.e., averaged over Cu). When Cu interacted with any other effect, the interaction was interpreted using only 1996 data. Data collected at monthly intervals were analyzed as repeated measures (Kirk, 1995); when a repeated measurement interacted with a treatment factor, subsequent analyses were completed within a measurement period. Normality assumptions were assessed with the Shapiro-Wilk (1965) test. Tukey's (1949) test for non-additivity was used to test for block \times treatment interaction.

Results and Discussion

Performance of Steers. During the initial study in Virginia in 1995, steers gained more ($P < 0.05$) on uninfected than on infected tall fescue (Figure 1). These results have been well documented in the literature (Stuedemann and Hoveland, 1988; Hoveland, 1993). Tasco application did not affect steer gain. Ergot alkaloid concentrations in tall fescue were within the range known to cause clinical symptoms of fescue toxicosis (Garner et al., 1993). Furthermore, it is likely that concentrations were higher than measured because ergo-

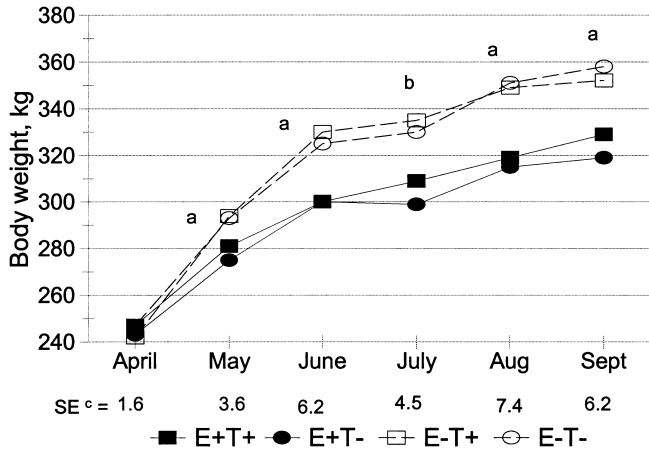


Figure 1. Performance of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia during 1995. ^aEffect of endophyte ($P < 0.05$); ^beffect of endophyte ($P < 0.01$). ^cSE = standard error of the mean, $n = 2$ for each data point where pasture is the experimental unit.

valine is quickly lost in samples that are not freeze-dried (Garner et al., 1993).

In 1996 and 1997, initial weights of steers in Mississippi were lighter ($P < 0.01$) than in Virginia when the grazing season began (236 vs 244 kg, respectively, SE = 3), but the difference was small. Steers gained more ($P < 0.001$) weight in Mississippi than in Virginia during 1996, but in 1997 gains were similar between the two locations (year \times location interaction; $P < 0.01$; data not shown). Steers grazing uninfected tall fescue gained more ($P < 0.001$) weight during the grazing season than

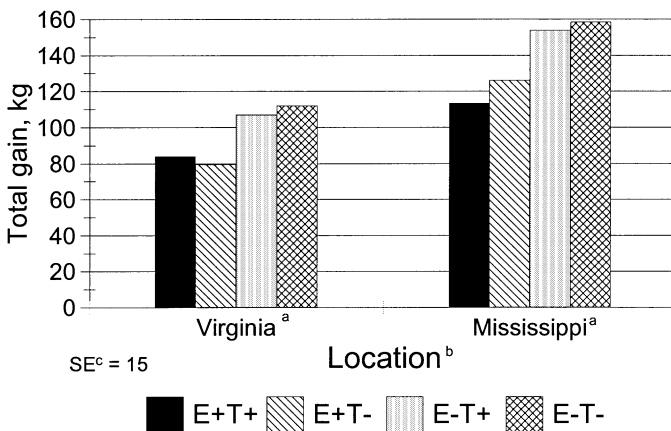


Figure 2. Total seasonal gains of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia and Mississippi. ^aEffect of endophyte ($P < 0.001$); ^beffect of location ($P < 0.001$). ^cSE = standard error of the mean, $n = 6$ for each bar where pasture is the experimental unit.

steers that grazed infected tall fescue; this result was consistent across years and locations (Figure 2). Application of Tasco and Cu supplementation (data not shown) had no effect on steer gains.

The effect of the endophyte on total seasonal gain agrees with numerous reports from other scientists (Stuedemann and Hoveland, 1988; Fribourg et al., 1991). Alkaloid concentrations in tall fescue have been related to poor animal performance. Application of Tasco had inconsistent effects on alkaloid levels in the plants and seemed unrelated to steer gains (Fike et al., 2001).

Hair Coat Condition. During the initial year in Virginia, steers grazing infected tall fescue had rougher ($P < 0.05$) hair coats than steers grazing uninfected tall fescue from d 28 until the trial ended (Figure 3). Application of Tasco to infected pastures alleviated some of the adverse effect on hair coat condition. Steers that grazed the Tasco-treated, infected fescue differed ($P < 0.05$) from those that grazed the non-Tasco-treated infected fescue in both May and June. Tasco had no effect on hair coat condition in steers that grazed the uninfected tall fescue (Tasco \times endophyte interaction; $P < 0.05$), except in May, when steers that grazed the untreated, uninfected fescue had lower ($P < 0.01$) coat scores than steers grazing the treated, uninfected fescue.

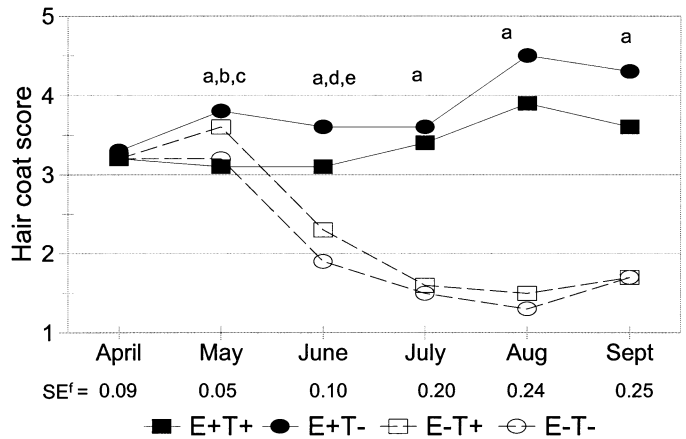


Figure 3. Hair coat condition of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia during 1995. 1 = slick, shiny hair with no evidence of retention of old hair; 2 = < 25% of the body covered with old, unshed hair; 3 = 25 to 50% of the body covered with old, unshed hair; 4 = 50 to 75% of the body covered with old, unshed hair; and 5 = muddy, dirty hair coat, evidence of having deliberately lain in mud, and > 50% of the body covered with old, unshed hair. ^aEffect of endophyte ($P < 0.05$); ^bendophyte \times Tasco interaction ($P < 0.01$); ^ceffect of Tasco within E+ and E- tall fescue ($P < 0.01$); ^dendophyte \times Tasco interaction ($P < 0.05$); ^eeffect of Tasco within E+ tall fescue ($P < 0.05$). ^fSE = standard error of the mean, $n = 2$ for each mean where pasture is the experimental unit.

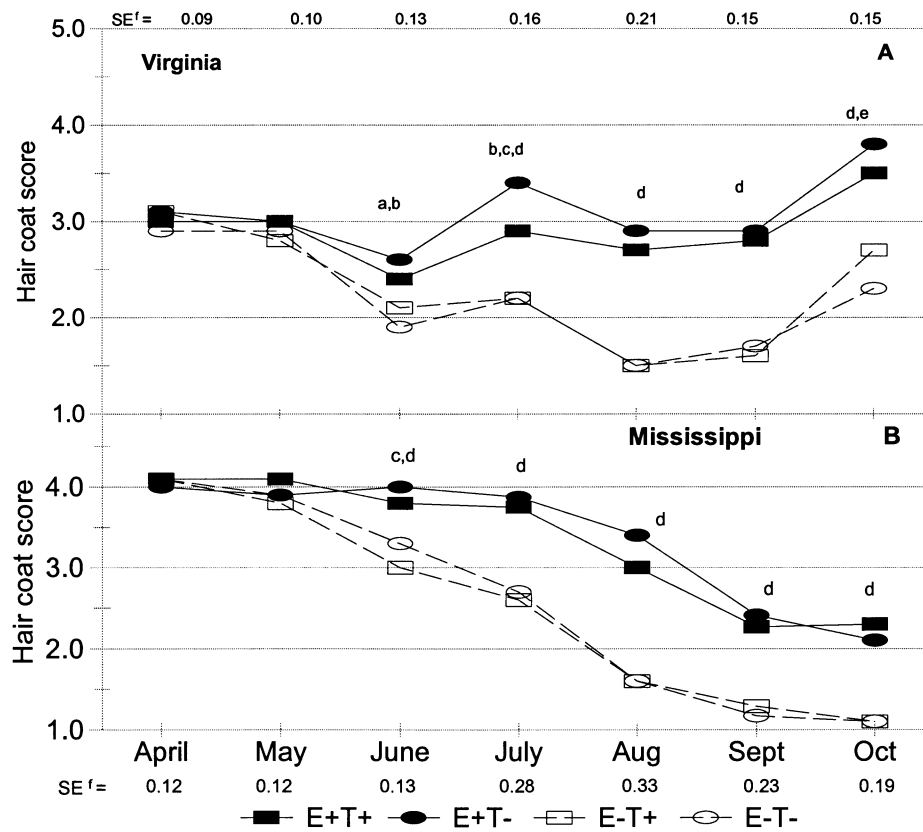


Figure 4. Hair coat condition of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia (A) and Mississippi (B) during 1996 and 1997. 1 = slick, shiny hair with no evidence of retention of old hair; 2 = < 25% of the body covered with old, unshed hair; 3 = 25 to 50% of the body covered with old, unshed hair; 4 = 50 to 75% of the body covered with old, unshed hair; and 5 = muddy, dirty hair coat, evidence of having deliberately lain in mud, and > 50% of the body covered with old, unshed hair. ^aEffect of endophyte ($P < 0.05$); ^bendophyte \times Tasco interaction ($P < 0.16$); ^ceffect of Tasco ($P < 0.14$); ^deffect of endophyte ($P < 0.01$); ^eTasco \times endophyte interaction ($P < 0.08$). ^fSE = standard error of the mean, $n = 6$ for each mean where pasture is the experimental unit.

By June during 1996 and 1997, 56 d after grazing began, and continuing throughout the grazing season, steers grazing uninfected tall fescue in both Mississippi and Virginia had a better ($P < 0.001$) hair coat condition than steers that grazed infected tall fescue (Figure 4). Application of Tasco to infected tall fescue did not alleviate the negative effect of the endophyte on hair coat condition in Virginia ($P < 0.16$) or in Mississippi. Applying Tasco to uninfected tall fescue did not influence hair coat condition at either location.

Copper supplementation to steers improved hair coat condition in May ($P < 0.09$), June ($P < 0.08$), and July ($P < 0.03$), but the effect disappeared by August (data not shown). Effects of Cu were consistent across locations, endophyte levels, and Tasco application but were small, and the difference never exceeded 0.3 on the 1-to-5 scale.

Rectal Temperatures. In 1995, steers that grazed infected tall fescue had higher rectal temperatures in July ($P < 0.01$) and September ($P < 0.06$) than steers that grazed uninfected fescue. However, steers grazing infected fescue treated with Tasco had lower ($P < 0.05$)

rectal temperatures in July (Figure 5; Tasco \times endophyte interaction; $P < 0.10$). By July in 1996 and 1997, steers that grazed infected tall fescue in Virginia again had higher ($P < 0.05$) rectal temperatures than steers that grazed uninfected tall fescue (Figure 6A). Steers on Cu and Tasco treatments had approximately 0.6°C lower rectal temperature compared with non-Cu-supplemented steers that grazed the untreated tall fescue, but the effects were not additive (Tasco \times Cu interaction, $P < 0.07$; data not shown). By September, rectal temperature was higher ($P < 0.05$) in steers grazing infected than in those grazing uninfected fescue, but neither Tasco nor Cu affected temperature. In October, rectal temperatures of steers that grazed infected fescue were higher ($P < 0.10$) in Tasco- than in non-Tasco-treated fescue but did not differ in the uninfected fescue pastures (Tasco \times endophyte interaction; $P < 0.07$). Tasco-treated, uninfected tall fescue had little effect on rectal temperature during the grazing season in Virginia.

In May, steers that grazed infected tall fescue in Mississippi had higher ($P < 0.05$) rectal temperatures than

steers that grazed uninfected fescue (Figure 6B), but the effect was less consistent than that observed in Virginia. In September and October, Tasco-treated, infected fescue increased rectal temperatures of steers in Mississippi, but, as was observed in Virginia, Tasco treatment had little effect on steers that grazed uninfected tall fescue (Tasco × endophyte interaction; $P < 0.13$ and $P < 0.05$, respectively).

There were interactions with Cu, Tasco, and endophyte in both Mississippi and Virginia, but these relationships were inconsistent between locations and sampling dates. Although Cu and Tasco seemed to interact with each other and with endophyte status, their combined effects will require further investigation.

Toxicosis from ingestion of infected fescue is generally most visible during hot weather (Hemken et al., 1981), but reduced gain by cattle grazing infected fescue has occurred during cool months as well (Beconi et al., 1995). High environmental temperatures stress both plants and animals; therefore, it is expected that clinical signs would be magnified during that period of the grazing season. The presence of alkaloids and lower Cu concentrations in infected fescue (Dennis et al., 1998) may have promoted oxidative stress and altered hormone-controlled temperature regulation in steers consuming the plants. Neuroendocrine-immune system interactions can help to explain temperature changes observed in animals consuming infected fescue. Hormones such as prolactin, growth hormone, and dopamine, whose concentrations have been shown to be influenced by alkaloids and stress, and cytokines produced by stimulated leukocytes interact to cause changes in animal

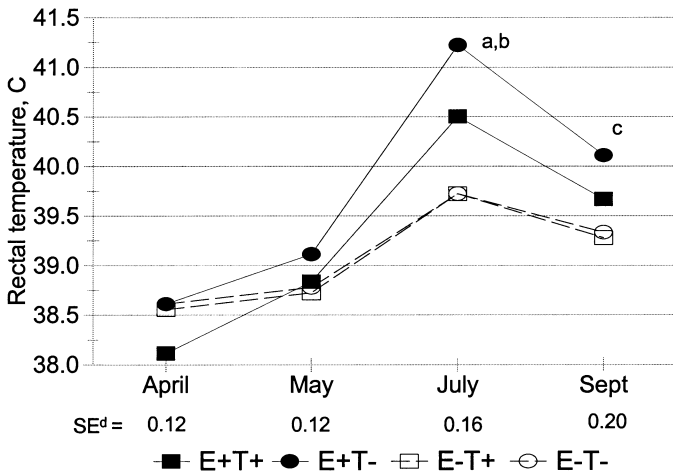


Figure 5. Rectal temperature of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia during 1995. ^aEffect of endophyte ($P < 0.01$); ^bendophyte × Tasco interaction ($P < 0.10$); ^ceffect of endophyte ($P < 0.06$). ^dSE = standard error of the mean, $n = 2$ for each mean where pasture is the experimental unit.

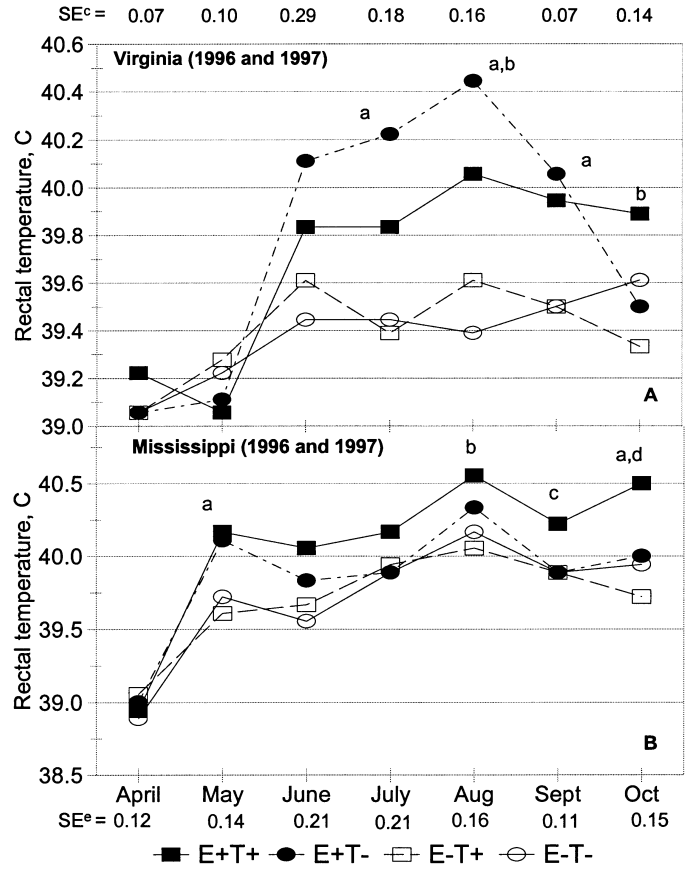


Figure 6. (A) Rectal temperature of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia in 1996 and 1997. ^aEffect of endophyte ($P < 0.05$); ^bTasco × endophyte interaction ($P < 0.07$). ^cSE = standard error of the mean, $n = 6$ for each mean where pasture is the experimental unit. (B) Rectal temperature of steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Mississippi in 1996 and 1997. ^aEffect of endophyte ($P < 0.05$); ^beffect of endophyte ($P < 0.08$); ^cTasco × endophyte interaction ($P < 0.13$); ^dTasco × endophyte interaction ($P < 0.05$). ^eSE = standard error of the mean, $n = 6$ for each mean where pasture is the experimental unit.

behaviors and thermoregulation (Kelly and Dantzer, 1990).

The Angus steers in Virginia showed the effect of infected tall fescue through increased rectal temperatures, whereas the Brahman × Angus steers in Mississippi were less affected by the endophyte. These results agree with McMurphy et al. (1990), who reported that under grazing conditions in Oklahoma, Angus steers demonstrated increased rectal temperatures while grazing infected tall fescue but Brahman-Angus steers did not. These authors also reported higher weight gains by the Brahman-Angus steers than by the Angus

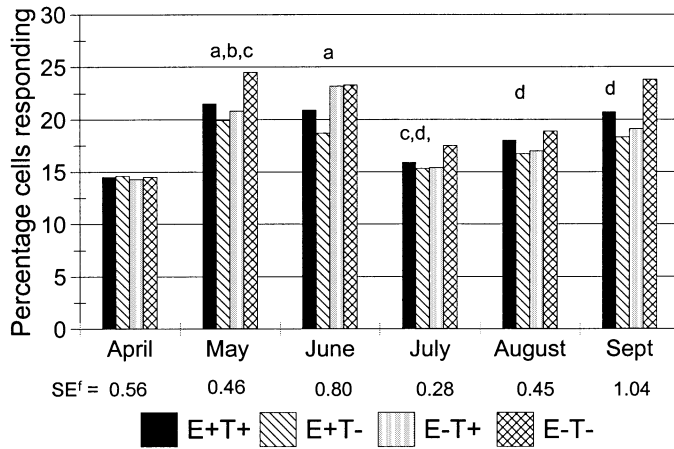


Figure 7. Monocyte phagocytic activity in steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia during 1995. ^aEffect of endophyte ($P < 0.05$); ^bendophyte \times Tasco interaction ($P < 0.01$); ^ceffect of Tasco ($P < 0.08$); ^dendophyte \times Tasco interaction ($P < 0.05$); ^eeffect of endophyte ($P < 0.06$). ^fSE = standard error of the mean, $n = 2$ for each mean where pasture is the experimental unit.

steers. Our research also shows similar results, although at different locations.

Immune Function. Presence of the endophyte fungus *Neotyphodium coenophialum* in tall fescue has been suggested to impair immune cell function of steers grazing the forages (Saker et al., 1998). In the 1995 pilot study, presence of the endophyte depressed phagocytic activity and MHC class II expression (Figures 7 and 8; Saker et al., 1998). The immune suppression associated with ingestion of endophyte-infected tall fescue may be fueled by excessive free radical production as a consequence of plant and/or environmental factors (Dew et al., 1990; Halliwell, 1997). Tasco treatment of infected fescue pastures reversed the effect of endophyte on both phagocytic activity (Figure 7) and MHC class II expression (Figure 8), but the opposite effect was observed for steers that grazed uninfected fescue (endophyte \times Tasco interaction; $P < 0.05$). Monocyte phagocytic activity ($P < 0.09$) and MHC class II expression ($P < 0.01$) were higher in steers that grazed Tasco-treated, infected fescue than in steers grazing untreated, infected fescue 28 d after steers began grazing in 1995. The difference in MHC class II expression was observed throughout the remainder of the grazing season, whereas the effect on phagocytic activity was less consistent.

During 1996 and 1997, the response of monocyte phagocytic activity to Tasco differed due to presence of the endophyte (endophyte \times Tasco interaction; $P < 0.05$; Table 1). In July and October, monocyte phagocytic activity was higher ($P < 0.05$) in steers that grazed Tasco-treated than in those that grazed untreated, infected tall fescue in Virginia, but in Mississippi this response was only observed in July (location \times endo-

phyte \times Tasco interaction; $P < 0.05$). In Virginia in October, steers had lower ($P < 0.05$) phagocytic activity if they had grazed infected rather than uninfected tall fescue in the absence of Tasco treatment.

In July, averaged over both years and locations, MHC class II expression was higher ($P < 0.05$) in steers that grazed infected than in those that grazed uninfected tall fescue when both were treated with Tasco (endophyte \times Tasco interaction; $P < 0.05$; Table 1). No other effects of these treatments were measurable during the grazing season.

Copper did not influence phagocytic activity and no interactions were present. Copper did interact ($P < 0.05$) with Tasco on MHC class II expression (data not shown). To examine this interaction, only data from 1996 were used. In July, a location \times Tasco \times Cu interaction was present. In Mississippi, Cu and Tasco did not interact. In Virginia, MHC class II expression was higher ($P < 0.05$) in Cu-supplemented steers that grazed the non-Tasco-treated pastures than in those that grazed treated pastures. In non-Cu-supplemented steers, Tasco treatment had no effect. In October, a Tasco \times Cu interaction was also present but did not interact with location. Averaged over Mississippi and Virginia in October, Tasco treatment had no effect in Cu-supplemented steers but MHC expression was higher ($P < 0.05$) in non-Cu-supplemented steers if they grazed Tasco-treated fescue.

Monocyte phagocytic activity and MHC class II molecule expression are components of the innate immune response, which is the first line of defense against dis-

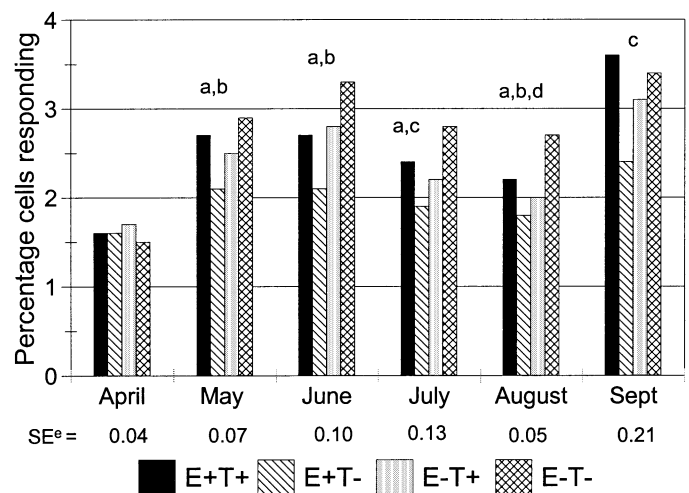


Figure 8. Monocyte major histocompatibility complex class II expression in steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract, in Virginia during 1995. ^aEffect of endophyte ($P < 0.05$); ^bendophyte \times Tasco interaction ($P < 0.01$); ^cendophyte \times Tasco interaction ($P < 0.05$); ^deffect of Tasco ($P < 0.08$). ^eSE = standard error of the mean, $n = 2$ for each mean where pasture is the experimental unit.

Table 1. Monocyte phagocytic activity and major histocompatibility complex (MHC) class II expression in steers that grazed endophyte-infected (E+) or endophyte-free (E-) tall fescue in Virginia and Mississippi during 1996 and 1997 that was either treated (T+) or not (T-) with Tasco, a brown seaweed extract

| Item | E+ | | E- | | SE ^a |
|-------------------------------|--------------------|---------------------|---------------------|---------------------|-----------------|
| | T+ | T- | T+ | T- | |
| Phagocytic activity | | | | | |
| April (initial) | 28.20 ^b | 27.04 ^b | 27.06 ^b | 28.98 ^b | 1.55 |
| July ^e | 23.86 ^b | 18.14 ^c | 19.49 ^{bc} | 21.47 ^{bc} | 1.63 |
| October (final) ^{ef} | | | | | |
| Mississippi | 18.48 ^c | 15.93 ^{bc} | 14.96 ^{bc} | 14.14 ^b | 1.43 |
| Virginia | 24.61 ^c | 19.28 ^b | 19.06 ^b | 26.27 ^c | 1.43 |
| MHC class II expression | | | | | |
| April (initial) | 26.37 ^b | 28.50 ^b | 26.85 ^b | 25.80 ^b | 1.20 |
| July ^e | 36.02 ^b | 32.94 ^{bc} | 31.86 ^c | 35.34 ^{bc} | 1.24 |
| October (final) | 23.63 ^b | 21.22 ^b | 24.09 ^b | 23.38 ^b | 1.19 |

^aStandard error of the mean; n = 12 for each mean; pasture was the experimental unit.

^{b,c,d}Means within a row followed by the same letter are not different ($P < 0.05$); protected LSD test.

^eEndophyte \times Tasco interaction ($P < 0.05$).

^fLocation \times endophyte \times Tasco interaction ($P < 0.05$).

ease challenge. The ability of the monocyte to phagocytize an invading organism and process and present a small portion of it via MHC class II molecules to the T cell is a necessary step in immunocompetence. The ability of an animal to respond appropriately to immune challenge will influence its health and productive capacity. Our findings suggest that application of Tasco is a mechanism by which to maintain adequate specific innate immune responses in steers grazing infected fescue. As was observed in the 1995 study in Virginia, treatment of uninfected fescue with Tasco did not seem to enhance monocyte immune cell response compared with steers grazing untreated, uninfected fescue. Tasco application to uninfected fescue seemed to have a dampening influence on monocyte cell function, suggesting that the presence of the endophyte may influence utilization of the Tasco in either a pro-oxidant or antioxidant fashion, possibly depending on the degree of oxidant stress present in the plant and/or animal. Toxins, including alkaloids associated with the endophyte fungus, cause oxidative stress in the plant (Ayad, 1998) and in the animal consuming the plant (Fike et al., 2001), resulting in an altered reduction/oxidation balance. Supplementation with antioxidant compounds can help to counteract that imbalance between free radical overproduction and consumption. A relationship between oxidative stress and the immune system has been identified (Halliwell, 1990). Stimulation or suppression of cell function seems to be influenced by alterations in enzyme and cytokine activities. Antioxidant therapy may protect the immune system in a situation of oxidizing stress; however, *in vitro* experiments suggest that antioxidants could exert an immunosuppressive effect in the absence of oxidative stress (Gougerot-Pocidallo and Revillard, 1993). The mechanism associated with the dampened monocyte cell response from steers consuming the Tasco-treated, uninfected fescue (Table 1;

Figures 7 and 8) warrants further investigation to identify the mechanism by which cell activation is regulated by exogenous antioxidants/immunomodulators.

Dawe et al. (1997) concluded that one of the effects of fescue toxicosis is impairment of immune function and suggested that decreased immune function in cattle might be due to effects of ergot alkaloids on serum prolactin levels. Prolactin has been reported to function as a regulatory factor in immune response (Reber, 1993). In our previous experiments with lambs (Fike et al., 2001), Tasco application had no effect on serum prolactin, but prolactin was clearly depressed in all lambs grazing infected fescue. Immune function was not measured in that experiment. In the present experiment with steers, the depression in immune function noted with infected tall fescue seemed to be lessened by application of Tasco. Prolactin was not measured, and thus we cannot rule out a relationship among prolactin, Tasco, and immune function.

Phagocytic cell function can be influenced by specific integrins, a superfamily of widely expressed cell surface adhesion molecules (Gao et al., 1995). Recently, integrins have been recognized as important transducers of signals that are vital to cell functions such as cell growth, differentiation, and oxidant production (Williams and Solokim, 1999). Individual heterodimer integrin subunits can be associated with specific cell types. Polymorphonuclear cell function is mediated mainly by β_2 and β_1 integrins, whereas a specific focal adhesion kinase signals cell functions in monocytes. Dean et al. (1998) reported on factors in bovine serum associated with receptor-mediated intracellular events in bovine alveolar macrophages. The array of host defense mechanisms mediated by activated monocytes are initiated by ligation of cell surface receptors (Williams and Solokim, 1999). Protection against common bovine-associated respiratory pathogens such as *Pasteurella haemolytica*

is influenced by integrin-mediated signaling pathways (Car et al., 1990). Phagocytic cells are the first line of defense against respiratory pathogens that can impair immune cell function. Previous studies have indicated that phagocytic cell function is influenced by enzymatic (superoxide dismutase, glutathione) and nutrient (Cu, Se, vitamin E) antioxidant activity. Our current studies indicate that *A. nodosum* extract alters monocyte cell function and serum antioxidant status in ruminants (Fike et al., 2001). Certain antioxidants help to maintain cell membrane integrity and may influence cell-specific integrin signaling, in which case monocyte-associated integrins, which have been shown to mediate cell functions, may be a likely mechanism in the enhanced monocyte activity exhibited by steers grazing Tasco-treated, infected fescue.

Recently, it has been suggested that vitamin E (α -tocopherol) may prevent inflammation associated with integrin-mediated phagocytic cell functions (Yoshida et al., 1999). These studies suggest another possible mechanism for enhanced immune function through supplementation with Tasco. Ruminants grazing Tasco-treated, infected fescue showed altered vitamin E and Se concentrations (Fike et al., 2001). Furthermore, an increase in α -tocopherol in tall fescue and Kentucky bluegrass (*Poa pratensis* L.) in response to *A. nodosum* extract applications has been demonstrated (Zhang, 1997; Zhang and Schmidt, 1999). Vitamin E is present in all cell membranes and acts as an antioxidant to prevent lipid peroxidative damage. It is also thought to be an immunomodulator, enhancing immunity (Tengerdy, 1989). Based on current studies with vitamin E and modulation of CD11b/CD18 expression on specific PMN function (Yoshida et al., 1999), vitamin E may have played an immunomodulatory role in our study as well. The enhanced monocyte activity exhibited in stressed steers grazing Tasco-treated, infected fescue may have been a consequence of multiple integrin-associated mechanisms of both monocytes and vitamin E.

Based on alterations in the antioxidants vitamin A, vitamin E, and Se reported in ruminants grazing Tasco-treated, infected fescue compared with untreated, infected fescue (Fike et al., 2001), the authors expected to see altered Cu-dependent parameters in the Tasco treatment-group steers as well. An increase in plant superoxide dismutase activity was reported in both the infected and uninfected fescue with Tasco (Fike et al., 2001). This enhancement in tall fescue superoxide dismutase activity agrees with field studies previously reported indicating enhanced superoxide dismutase, glutathione reductase, and ascorbate peroxidase activity in Tasco-treated tall fescue (Ayad, 1998). The blood Cu status of steers grazing Tasco-treated pastures did not differ from that of steers grazing untreated pasture; however, the effect of the endophyte on lowered plasma and serum Cu was observed (data not shown; Saker et al., 1998). Hepatic Cu would be a more sensitive indicator of absolute Cu status in the animal, but, unfortu-

nately, liver biopsy was neither economically nor logistically feasible with these steers. Although no effect of Tasco treatment on plant or animal tissue Cu levels was measured, a possible relationship with antioxidant activity and immune function should be investigated further.

Copper supplementation per se may have potential for improving hair coat condition, but effects observed in this research were small. Bioavailability of the Cu source and level of supplementation may be related to the magnitude of effect and should be further investigated. The indications of a negative Cu supplementation-Tasco interaction may suggest a Tasco effect on Cu metabolism, although this was not measurable in our experiments.

It is not clear whether the response of steers to Tasco was due to changes in the forage (Fike et al., 2001) or to direct ingestion of the Tasco, because the extract was sprayed on the plant canopy. However, Tasco is 100% water-soluble and was applied approximately 2 wk before grazing was begun in April. Precipitation likely occurred in these humid environments that would have washed most, if not all, of the extract from the plant onto the soil, minimizing any chance for direct ingestion. Subsequent studies with direct feeding of *A. nodosum*-based products to cattle, swine, and horses (K. Pond, V. Allen, H. Brady, J. Morrow-Tesch, and K. Saker, unpublished observations) have verified the effect on monocyte immune function, but the level of dosing has been higher than would have been possible under the field conditions of the current experiment. It seems likely that in these experiments the animal response was largely a reflection of altered plant metabolism. Further studies are needed to clarify this.

Implications

A depression in immunocompetence in cattle is a consequence of grazing tall fescue (*Festuca arundinacea* Schreb.) infected with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin). Treatment of infected tall fescue with Tasco-Forage, a proprietary extract of the seaweed *Ascophyllum nodosum*, enhanced monocyte cell function, thus improving innate immunity in grazing beef steers. Improved specific immune cell function may be closely linked with altered antioxidant status, including vitamin E, in both the plant and the animal. Enhanced innate immunity during the grazing period would help reduce transport- and early feedlot-related disease scenarios frequently associated with cattle from infected tall fescue pastures. Although Tasco, possibly through its antioxidant activity, has shown beneficial effects during the stocker phase of beef production with tall fescue pasture, other forage species and longer-term benefits require further investigation.

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