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Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants[1]

J. H. Fike*,[2], V. G. Allen*,[3,4], R. E. Schmidt*, X. Zhang*, J. P. Fontenot†, C. P. Bagley‡, R. L. Ivy§, R. R. Evans§, R. W. Coelho*,[5], and D. B. Wester¶

Departments of *Crop and Soil Environmental Sciences and †Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, 24061; †Mississippi State University, ‡Starkville 39762 and §Prairie 39761; and ¶Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Lubbock 79409-2125

ABSTRACT: Seaweed (*Ascophyllum nodosum*) is a known source of plant growth regulators, and application to turfgrasses has increased activity of the antioxidant superoxide dismutase (SOD) and specific vitamin precursors. Increased antioxidant activity in both plants and animals diminishes oxidative stress. Two pasture experiments investigated effects of Tasco-Forage (a proprietary seaweed-based product) applied to tall fescue (*Festuca arundinacea*) on antioxidant activity in plants and in ruminants that grazed the forage. In Exp. 1, fescue was 70 to 100% infected with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin). Twenty-four wether lambs (initial BW 41 kg; SD = 5) grazed fescue treated with 0, 1.7, or 3.4 kg Tasco/ha applied in April and July, 1994, with four replications per treatment. Grazing occurred for 26 d beginning April 21 and for 22 d beginning July 19. In July, there was a linear increase in daily gains ($P < 0.05$), and serum vitamin A ($P < 0.13$) and whole-blood Se ($P < 0.10$) tended to increase in lambs grazing Tasco-treated fescue. In Exp. 2, 48 Angus and Angus × Hereford steers (initial BW 245 kg; SD = 20) grazed infected or uninfected tall fescue in Virginia that was treated (3.4 kg/ha) or untreated with Tasco in April and July, 1995. Steers that grazed infected tall fescue had lower ($P < 0.02$) serum vitamin A and E and tended ($P < 0.07$) to have lower whole-blood Se in September compared with steers that grazed uninfected tall fescue. Tasco decreased ($P < 0.06$) serum vitamin E but tended to increase whole-blood Se ($P < 0.10$) in September and serum vitamin A in July ($P < 0.12$). During 1996 and 1997, the experiment was repeated in Virginia with Angus steers and was replicated at Prairie, MS, where 1/4 Brahman × 3/4 Angus steers were used. Forty-eight steers were included at each location in each year (n = 192 total steers for 1996 and 1997). Steers that grazed infected tall fescue in Mississippi had lower ($P < 0.05$) serum vitamin E by the end of the grazing season. At both locations Tasco increased ($P < 0.05$) activity of superoxide dismutase in both infected and uninfected fescue. The endophyte in tall fescue seemed to decrease antioxidant activity in grazing steers, whereas Tasco seemed to increase antioxidant activity in both the forage and the grazing ruminant. Tasco may provide opportunities to reduce oxidative stress in plants and animals.

Key Words: Antioxidants, Cattle, Festuca, Seaweeds, Sheep, Vitamins

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Introduction

Tall fescue (*Festuca arundinacea*), one of the most important forages for livestock production (Bacon and Siegel, 1988), is often infected with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin; Glenn et al., 1996), which shares a symbiotic relationship with the host plant (Bacon and Siegel, 1988). Several disorders occur in livestock grazing infected tall fescue (Stuedemann and Hoveland, 1988), including lowered immunocompetence in steers (Dawe et al., 1997; Saker et al., 1998).

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[2]Formerly the Henry Budd Graduate Research Assistant, Virginia Tech.

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[3]Correspondence: E-mail: felician@ttacs.ttu.edu.

[4]Present address: Dept. of Plant and Soil Sci., Texas Tech Univ., Lubbock 79409-2122.

[5]Present address: EMBRAPA/Clima Temperado, Caixa Postal 403, 96001-970, Pelotas/RS, Brazil.

Removal of the endophyte from fescue resolves the animal health problems but leaves the plant more vulnerable to biotic and abiotic stresses (Hoveland, 1993).

Plant hormones, including cytokinins, influence plant growth and alter antioxidant activity in plants (Hare and Van Staden, 1997). Plants under stress have shown a reduction in cytokinin levels (Hare and Van Staden, 1997), apparently in response to increased internal ethylene production, among other possible compounds, which lowers cytokinins and auxins, leading to alterations in tissue differentiation (Sanyal and Bangerth, 1998). Brown seaweed (*Ascophyllum nodosum*) is a commercially available source of cytokinin (Jameson, 1993). Turfgrass research demonstrated improved growth and stress tolerance (Yan, 1993; Nabati et al., 1994; Goatley and Schmidt, 1990) and increased antioxidant activity (Schmidt and Zhang, 1997) when treated with an extract from brown seaweed. Little information exists on effects of Tasco on forage grasses. No data were available on animal responses to infected and low-endophyte tall fescue when treated with Tasco.

Our hypotheses were that 1) application of Tasco-Forage to uninfected tall fescue could enhance plant stress tolerance and 2) increased antioxidant activity in tall fescue could improve performance and stress tolerance of the grazing animal, regardless of the endophyte.

Materials and Methods

Experiment 1. During 1994, 24 wether lambs (Dorset × Rambouillet × Finn; initial BW 41 kg; SD = 5) grazed an existing stand of endophyte-infected tall fescue at the Kentland Farm, Blacksburg, VA (81°5′ west longitude; 37°25′ north latitude; 650 m elevation). Tall fescue was treated with 0, 1.7, or 3.4 kg Tasco-Forage/ha, an extract from brown seaweed (Acadian Seaplants, Dartmouth, Nova Scotia, Canada) prepared by a proprietary process. Tasco is derived by an alkaline hydrolysis procedure from the fresh, intact *Ascophyllum nodosum* and is 100% soluble in cold water. Average chemical composition of the extract is given in Table 1. The biological activity of *A. nodosum*, processed by Acadian Seaplants to contain 9% solids, was equivalent to 50 ppm kinetin (dry basis; J. S. Craigie, National Research Council of Canada, personal communication, 1992). Tasco was dissolved in water and foliarly applied on April 5, 1994, and again on June 20, 1994, with four replications of each treatment in a completely randomized block design. On April 15, 1994, all paddocks were fertilized with 56 kg N, 56 kg P_2O_5, and 56 kg K_2O/ha as urea, triple super phosphate, and potassium chloride, respectively. Paddocks, 0.035 ha each, were blocked based on slope aspect and were located on a Shottower soil (Clayey, kaolinitic, mesic Typic Paleudults). Percentage of endophyte infection ranged from 70 to 100% within the paddocks and was determined based on 30 tillers/paddock collected in August 1994 and submitted to the Auburn University Fescue Diagnostic Laboratory, Auburn, AL.

Table 1. Composition of Tasco-Forage[a], an extract of *Ascophyllum nodosum* brown seaweed used to treat fescue pastures in Exp. 1 and 2

Item	Value
pH	10–10.5
Ash, %	45–55
Maximum moisture, %	6.5
Total nitrogen, %	1.0–2.0
Mineral	
Available P_2O_5, %	1.0–4.0
Soluble K_2O, %	18.0–22.0
Sulphur, %	1.0–2.0
Magnesium, %	0.2–0.5
Calcium, %	0.4–0.7
Sodium, %	3.0–5.0
Boron, ppm	75–150
Iron, ppm	75–250
Manganese, ppm	8–12
Copper, ppm	1–10
Zinc, ppm	25–75
Iodine, ppm	650–700
Selenium, ppm	<2.5
	g of AA/100 g of protein
Amino acid	
Alanine	3.81
Arginine	0.22
Aspartic acid	5.44
Cystine	trace
Glutamic acid	7.69
Glycine	3.16
Histidine	0.42
Isoleucine	1.94
Leucine	4.84
Lysine	1.33
Methionine	1.39
Phenylalanine	2.82
Proline	4.42
Serine	0.14
Threonine	1.27
Tyrosine	1.80
Valine	3.46

[a]Acadian Seaplants Limited, Dartmouth, Nova Scotia.

Two grazing periods were imposed: April 21 to May 17 and July 19 to August 10. In each period, lambs were blocked by weight and randomized within blocks to the three treatments with two lambs per paddock and eight lambs per treatment. Between grazing periods, all lambs grazed in a common area where forages were a mixture of alfalfa (*Medicago sativa*), red (*Trifolium pratense*) and white (*T. repens*) clovers, tall fescue, and orchardgrass (*Dactylis glomerata*). Lambs in Periods 1 and 2, selected for uniformity of weights, were blocked by weight and were randomized within blocks to the three Tasco application rates. Full weights of lambs were taken at the beginning and end of each grazing period. Lambs had access to H_2O and NaCl blocks at all times and were handled under a protocol approved by the Virginia Tech Animal Care and Use Committee.

Blood was collected by jugular puncture at the beginning and end of each grazing period for determination of serum prolactin. Prolactin was measured using a double-antibody radioimmunoassay (Koprowski and Tucker, 1971), except that each solution of prolactin was ionnated as described by Akers and Keys (1984). Additionally, at the end of the second grazing period, blood samples were collected via jugular puncture in nonadditive Vacutainers protected from sunlight for estimation of serum vitamins A and E. Blood was collected separately in heparinized tubes for analysis of Se in whole blood. Vitamin E samples were analyzed at 294 nm after extraction with cyclohexane and reconstitution with xylene (McMurray and Blanchflower, 1979). Vitamin A samples were analyzed at 325 nm after extraction with hexane and reconstitution with ethanol (Catignani and Bieri, 1991). Selenium was determined by atomic absorption spectroscopy using vapor hydride generation (AOAC, 1990).

Forage mass was estimated in each paddock at the beginning and end of each grazing period by cutting either two (Period 1) or four (Period 2) random strips (0.51 × 2.54 m at a 4-cm height) per paddock with a rotary mower and drying to a constant weight at 55°C. At the end of Period 1, after sampling was completed, paddocks were mowed to approximately 7.5 cm height to equilibrate forage mass per paddock before reapplying Tasco treatments for Period 2. Immediately before grazing began for each period, samples of tall fescue were collected randomly from within each paddock for chemical analysis. Plants were cut at approximately 2.5 cm height, frozen in liquid N, and stored in a standard freezer until they were freeze-dried for chemical analysis.

Forage was ground through a 1-mm screen with a stainless steel Wiley mill (Model ED-5, Thomas Scientific, Boston, MA). Concentrations of N-formylloline and N-acetylloline were determined by the University of Kentucky Department of Agriculture, following the procedures of Kennedy and Bush (1983) and Yates et al. (1990). Ergovaline was estimated by HPLC by the Auburn University Fescue Diagnostic Center using a modified ergot method of Fleiger et al. (1989) and Kren et al. (1990). Concentrations of minerals, including Se, Cu, Zn, Mn, Fe, P, K, Ca, Mg, S, and Al, were determined by atomic emission with an inductively coupled plasma spectrometer after digestion with 2:1 nitric:perchloric acid (Muchovej et al., 1986). For Se, a second stage digestion with 6 *N* HCl was performed and Se was determined by vapor hydride generation.

Data were analyzed as a randomized complete block design for each sampling date. Linear and quadratic effects of treatments were further tested by orthogonal contrasts.

Experiment 2. A grazing experiment with 48 Angus and Angus × Hereford steers (initial BW 245 kg, SD = 20) was conducted at the Southwest Virginia Agricultural Research and Extension Center at Glade Spring (81°40′ west longitude; 36°47′ north latitude; 652 m elevation) during 1995. A full description of the experimental site and conditions was reported by Dennis et al. (1998) and Saker et al. (2001). Steers were vaccinated, treated for internal parasites, and implanted as described by Saker et al. (2001). Steers had ad libitum access to water and NaCl. Soils were primarily Frederick (Fine, mixed, semiactive, mesic Typic Paleudults) and Hagerstown (Fine, mixed, semiactive, mesic Typic Hapludalfs). Four replications of endophyte-infected (67%) and four replications of uninfected (< 5%) tall fescue were treated (3.4 kg dry extract/ha) or not treated with Tasco in a randomized complete block design with a factorial arrangement of treatments. Each paddock replication was grazed by six steers that had been blocked by breeding and initial weight and randomized within blocks to the four treatments. Tall fescue was fertilized with 60 kg N/ha on April 15, 1995. Phosphorus and K were applied according to soil test recommendations. Tasco was dissolved in water to apply 3.4 kg dry extract/ha in approximately 190 L solution/ha on April 4, 1995, and on July 6, 1995. Grazing began on April 25 and ended on October 16, 1995. Blood samples were collected on May 23, July 18, and September 12 into 15-mL Vacutainer tubes without additives that were protected from light for determination of serum vitamin A and E. Serum vitamin A and E were analyzed as described in Exp. 1. Additionally, whole blood samples were collected at each date for determination of Se as described in Exp. 1.

Green leaves of tall fescue used for superoxide dismutase determination were collected monthly within each pasture beginning June 2 and ending December 21. Leaves were placed in cloth bags, frozen in liquid N in the field, and stored in a freezer at –20°C until they were analyzed using the procedures of Giannopolitis and Ries (1977). Forage samples for chemical analysis were collected on April 23, July 18, and September 12 by clipping approximately 30 samples at about 8 cm and compositing samples within each pasture. Concentrations of CP (AOAC, 1980), total nonstructural carbohydrates (**TNC**; Davis, 1976; Smith, 1981), and minerals (Muchovej et al., 1986) were determined. Total ergot alkaloids were determined by the Auburn University Fescue Diagnostic Center. Forage mass in each pasture replication was determined on these dates by cutting four randomly chosen strips (51 × 305 cm at a 2.5-cm cutting height) with a Gravely mower. Additionally, ungrazed forage mass was determined in April at the time grazing began and then at monthly intervals throughout the grazing season by cutting one strip (51 × 183 cm at a 2.5-cm cutting height) within a 3- × 3-m caged area in each pasture replicate.

During 1996 and 1997, experiments at Glade Spring were repeated. Tasco was applied (3.4 kg/ha) on April 25 and August 27, 1996, and April 9 and July 8 (block 1) and 14 (block 2), 1997. Additionally, the experiment was replicated at the Prairie Research Unit, Prairie, MS. In Mississippi, Tasco treatments were applied on April 12 and July 8, 1996, and April 21 and July 15,

1997. A full description of these experimental sites and conditions was provided by Saker et al. (2001). Percentage of endophyte infection averaged 80 and < 1% in Virginia, and pastures in Mississippi averaged 70 and < 10% in infected and uninfected pastures, respectively, during 1996 and 1997.

In Virginia, fescue samples were collected for determination of forage mass and forage mineral composition at 28-d intervals during both 1996 and 1997 as described for 1995. In Mississippi, fescue was sampled to determine forage mass and mineral composition as described for Virginia pastures on July 10, October 3, and November 25, 1996. In 1997, tall fescue was sampled at 28-d intervals beginning April 22 and ending September 10. Samples collected in Mississippi for superoxide dismutase analysis were shipped in liquid N by overnight delivery service to Virginia. In 1996 and 1997, fescue samples were collected at 28-d intervals at both locations. In the infected fescue, total ergot alkaloids were determined in 1996 (Auburn University Fescue Diagnostic Center, Auburn, AL) and ergovaline was measured in 1997 (Veterinary Medical Diagnostic Laboratory, Univ. of Missouri, Columbia, MO) by a modification of the procedures of Hill et al. (1993). Concentrations averaged 585 and 107 μg/g dry weight for Virginia and 615 and 144 μg/g dry weight for Mississippi for the two respective years. Random samples from uninfected pasture were included in the analyses and were consistently below detection levels for both total ergot alkaloids and for ergovaline.

During 1996, mineral composition was determined in both infected and uninfected fescue samples and in blood serum collected by jugular venipuncture at 28-d intervals. Additional blood samples were collected from steers in Mississippi on July 8 and September 30, 1996, and October 8, 1997, for determination of serum vitamins A and E as described for yr 1 in Virginia. During 1996 and 1997, forage mass was determined as in 1995. Ungrazed forage mass was not monitored.

Data from the 1995 study in Virginia were analyzed as a complete randomized block design with a factorial arrangement of treatments. The model tested effects of endophyte, Tasco, and date. 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. Pasture was the experimental unit.

Data from the 1996 and 1997 trials in Virginia and Mississippi were analyzed as a complete randomized block design using a model that tested effects of block, endophyte, Tasco, location, year, and all interactions. Year was the main plot factor in a split plot arrangement, with a factorial combination of location, endophyte, and Tasco in the subplot portion of the analysis (Kempthorne, 1952). Data collected at monthly intervals were analyzed as repeated measurements. Pasture was the experimental unit for all analyses. When factors interacted, simple effects were tested with a protected LSD test. Normality assumptions were assessed with the Shapiro-Wilk (1965) test. Tukey's (1949) test for nonadditivity was used to test for block × treatment interaction.

Results

Experiment 1

Forage Mass and Nutritive Value. Application of Tasco had no measurable effect on forage mass when measured prior to or at the end of the first grazing period in spring (Table 2). Likewise, Tasco had no effect on regrowth when measured immediately prior to grazing on July 15. At the end of the 21-d grazing period in summer there was a trend ($P < 0.11$) for decreased forage mass with increased rates of Tasco. However, in September, 1 mo after lambs were removed from the paddocks, there were no differences in forage mass among treatments.

Measures of forage nutritive value for this experiment were reported by Fike (1995). Tasco had inconsistent effects on fiber content, IVDMD, TNC, and CP. Tasco had no effect on mineral composition of the forage.

Alkaloids. Twenty-five days after the second Tasco application, the alkaloid formyl loline in the fescue responded quadratically ($P < 0.11$) to Tasco (Table 3). Ergovaline tended ($P < 0.15$) to decrease with increased rate of Tasco. Tasco had no effect ($P > 0.20$) on acetyl loline.

Lamb Performance. During the first grazing period, lamb performance was not influenced by Tasco application (Table 4). All lambs tended to lose weight during this period. During the summer grazing period (Period 2) there was a linear increase ($P < 0.05$) in lamb gain in response to Tasco treatment.

Blood Measurements. Serum prolactin in lambs decreased ($P < 0.01$) in Period 1 (initial 103 vs final 36 ng/mL; SE = 9) and Period 2 (initial 72 vs final 9 ng/mL; SE = 6) but was not influenced by Tasco treatments (Table 4). When measured at the end of the summer grazing period, serum vitamin A tended ($P < 0.13$) to increase linearly in response to Tasco. Tasco had no effect on serum vitamin E, but whole-blood Se increased linearly ($P < 0.10$) in response to increased rates of Tasco.

Experiment 2

Forage Mass and Nutritive Value. During 1995 in Virginia, forage mass tended ($P < 0.12$) to be lower in Tasco-treated than in untreated caged areas when averaged over the growing season (Figure 1). Forage mass differed by date, but no date × treatment interaction ($P > 0.60$) was present and effects of Tasco on forage mass were not significant ($P > 0.20$) at individual harvest dates. During 1995 to 1997, forage mass in grazed areas in Virginia differed ($P < 0.01$) by year and by

Table 2. Forage mass of endophyte-infected tall fescue treated with Tasco-Forage, an extract of brown seaweed, and grazed by wether lambs in Virginia

Item	Date	Tasco, kg/ha 0	1.7	3.4	SE[a]
		kg DM/ha			
Pregrazed	April 19	1,033	1,065	754	110
Postgrazed	May 18	733	696	620	92
Pregrazed	July 15	1,162	1,156	1,174	116
Postgrazed[b]	August 10	1,053	825	752	114
1 mo postgrazed	September 6	1,446	1,425	1,238	263

[a]Standard error, n = 4.
[b]Linear effect of Tasco ($P < 0.11$).

sampling date (data not shown). In midsummer, Tasco-treated pastures had less ($P < 0.07$) forage mass than non-Tasco-treated pastures and tended to have less ($P < 0.19$) forage mass in late summer than the non-Tasco-treated pastures (Figure 2A). In late summer, more ($P < 0.10$) forage mass was present in endophyte-infected than in uninfected pastures except in 1997, when no difference was observed (endophyte × year interaction; $P < 0.05$; data not shown).

In Mississippi, less ($P < 0.05$) forage mass was present in Tasco-treated fescue in midsummer for 1996, but no difference was present in 1997 (year × Tasco interaction; $P < 0.05$; data not shown). At the end of the grazing season, endophyte-infected paddocks had more ($P < 0.05$) forage mass than uninfected paddocks (Figure 2B).

During 1995 in Virginia, CP and TNC averaged 20.8% and 11.0%, respectively, during the grazing season (Coelho et al., 1997). The CP ($P < 0.15$) and TNC ($P < 0.11$) tended to increase slightly in fescue treated with Tasco averaged over the three sampling dates, but differences were small. No effects of Tasco application on mineral composition of tall fescue were observed in Virginia during 1995 or at either location during 1996 (data not shown).

Blood Measurements. In May 1995 in Virginia, 28 d after steers began grazing, no effect of treatments on serum vitamin A was observed (Table 5). By July 18, presence of the endophyte lowered ($P < 0.001$) serum vitamin A regardless of Tasco treatment. The effect ($P < 0.05$) of endophyte was observed in September, but differences were not as large. In July, serum vitamin A tended ($P < 0.12$) to be greater in steers that grazed Tasco-treated pastures (regardless of endophyte), but this response was not observed at the other sampling dates.

By May 1995, serum vitamin E tended to be higher ($P < 0.09$) in steers that grazed uninfected than in those that grazed infected tall fescue, but this response was negated by Tasco (Tasco × endophyte interaction, $P < 0.12$; Table 5). In July and September, serum vitamin E was higher ($P < 0.01$) in steers that grazed uninfected

Table 3. Alkaloid concentrations on July 15, 1994, in endophyte-infected tall fescue treated with Tasco-Forage, a brown seaweed extract

Item	Tasco, kg/ha 0	1.7	3.4	SE[a]
	μg/g DM			
Formyl loline[b]	1,550	1,704	1,412	96
Acetyl loline	768	872	788	82
Ergovaline[c]	4.3	3.8	3.6	0.29

[a]Standard error of the means, n = 4; pasture was the experimental unit.
[b]Quadratic effect of Tasco ($P < 0.11$).
[c]Linear effect of Tasco ($P < 0.15$).

Table 4. Performance, serum prolactin, vitamins A and E, and whole-blood Se in wether lambs grazing endophyte-infected fescue treated with Tasco-Forage, a brown seaweed extract

Item	Tasco, kg/ha 0	1.7	3.4	SE[e]
Period 1 (April–May)				
Initial weight, kg	41	41	40	2.5
Total gain, kg	–0.9	–1.3	0.1	0.8
Daily gain, kg	–0.03	–0.05	0.00	0.03
Serum prolactin, ng/mL[a]				
Initial	105	105	99	17
Final	48	45	13	4
Period 2 (July–August)				
Initial weight, kg	42	42	41	2.0
Total gain, kg[b]	0.6	2.7	2.4	0.6
Daily gain, kg[b]	0.03	0.13	0.11	0.03
Serum prolactin, ng/mL				
Initial	65	71	95	20
Final	4	13	8	4
Serum vitamins				
Vitamin A, ppb[c]	306	331	371	28
Vitamin E, ppm	1.9	1.9	1.9	0.2
Whole-blood Se, ng/mL[d]	241	264	274	13

[a]Effect of date ($P < 0.01$).
[b]Linear effect of Tasco ($P < 0.05$).
[c]Linear effect of Tasco ($P < 0.13$).
[d]Linear effect of Tasco ($P < 0.10$).
[e]Standard error of the mean, n = 4; pasture was the experimental unit.

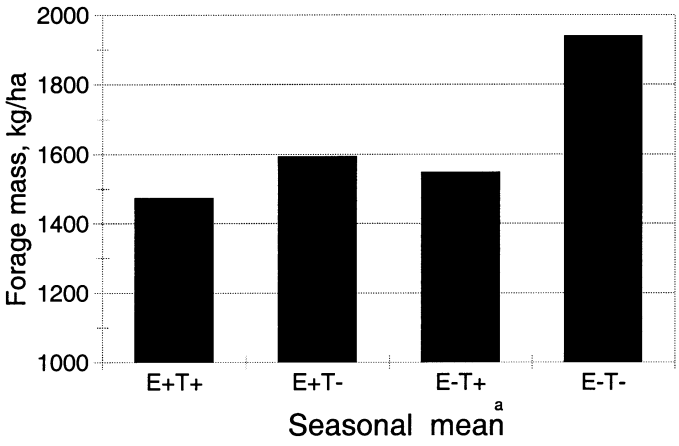

Figure 1. Mean seasonal forage mass of ungrazed endophyte-infected (E+) and uninfected (E–) tall fescue in Virginia (1995) that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, in April and July (n = 14 for each mean; SE = 160). [a]Effect of Tasco ($P < 0.12$).

than in those that grazed infected tall fescue. Tasco treatment tended ($P < 0.13$) to lower serum vitamin E. By September, Tasco treatment decreased ($P < 0.06$) serum vitamin E and the effect was consistent across endophyte status.

Whole blood Se did not differ among treatments in May. In July, the Tasco treatment of uninfected tall fescue tended to increase ($P < 0.12$) whole-blood Se (Table 5). Steers that grazed infected tall fescue seemed unaffected by Tasco treatment, but Se was higher ($P < 0.02$) in steers that grazed Tasco-treated than in those that grazed untreated, uninfected tall fescue (Tasco × endophyte interaction; $P < 0.07$). In September, presence of the endophyte resulted in lower ($P < 0.07$) whole-blood Se, and Tasco treatment of both infected and uninfected tall fescue tended ($P < 0.10$) to increase Se. Other than Se, no effects of Tasco application were observed on serum minerals in Virginia during 1995 or at either location during 1996 (data not shown).

In Mississippi during 1996, effects of the endophyte on serum vitamin E were similar to those observed in Virginia during the previous year. By July, steers grazing infected tall fescue tended ($P < 0.07$) to have lower serum vitamin E, and by the end of September, serum vitamin E was lower ($P < 0.01$) because of the endophyte (Table 6). Serum vitamin E was not influenced by Tasco application in 1996. By October in 1997, steers that grazed infected fescue had lower ($P < 0.05$) serum vitamin E than steers that grazed uninfected fescue when no Tasco was applied, but this response was not consistent across Tasco treatments (Tasco × endophyte interaction; $P < 0.05$). No effects of either Tasco or endophyte were observed on serum vitamin A in the Mississippi steers.

Ergot Alkaloids. Effects of Tasco on ergot alkaloids was inconclusive (data not shown). During 1995, ergot alkaloids were lower ($P < 0.05$) in July in infected tall fescue treated with Tasco. No effect of Tasco treatment was observed in either May or September. In 1996, averaged over the Virginia and Mississippi locations, ergot alkaloids were increased ($P < 0.01$) by Tasco application in July. No effects were observed at the other sampling dates in 1996. During 1997, no differences in ergovaline were observed on any date at either location.

Superoxide Dismutase. Superoxide dismutase differed due to year and sampling date, but no interaction with treatments was present (data not shown). Application of Tasco increased ($P < 0.01$) superoxide dismutase activity in tall fescue in Virginia in all 3 yr (Figure 3). Averaged across endophyte-infected and uninfected fescue, Tasco increased superoxide dismutase activity by approximately 30% (11,783 vs 7,363 units/g dry weight, SE = 479). The response of superoxide dismutase to Tasco application in Mississippi was less consistent. No date × treatment interactions were present. Averaged over the three sampling dates in 1996, no effects of

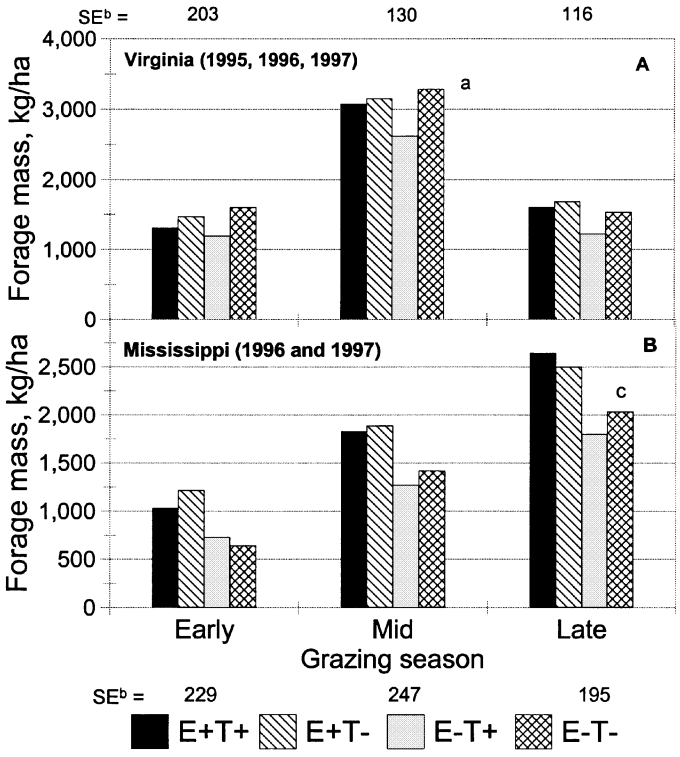

Figure 2. (A) Forage mass of grazed endophyte-infected (E+) and uninfected (E–) tall fescue in Virginia (1995, 1996, and 1997) that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, in April and July. Early = April/May; Mid = July; Late = September/October. [a]Effect of Tasco ($P < 0.07$); [b]SE = standard error of the mean; n = 6 for each mean. (B) Forage mass of grazed E+ and E– tall fescue in Mississippi (1996 and 1997) that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, in April and July. Early = April; Mid = July; Late = September; [b]SE = standard error of the mean; n = 4 for each mean. [c]Effect of endophyte ($P < 0.05$).

Table 5. Serum vitamins A and E and whole-blood selenium in steers grazing endophyte-infected (E+) or uninfected (E–) tall fescue that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, at Glade Spring, VA during 1995

	E+		E–		
Item	T+	T–	T+	T–	SE[a]
Vitamin A, ppb					
May 23	556	490	573	570	45
July 18[bc]	540	505	676	607	32
September 12[d]	534	539	562	596	17
Vitamin E, ppm					
May 23[e]	3.9	3.2	3.5	4.4	0.46
July 18[bf]	4.3	5.1	5.5	6.0	0.42
September 12[bg]	5.1	5.6	6.6	7.4	0.34
Selenium, ng/mL					
May 23	73	55	67	67	7
July 18[ch]	70	72	97	68	8
September 12[ij]	82	71	109	84	11

[a]Standard error of the mean, n = 2; pasture was the experimental unit.
[b]Effect of endophyte ($P < 0.01$).
[c]Effect of Tasco ($P < 0.12$).
[d]Effect of endophyte ($P < 0.02$).
[e]Endophyte × Tasco interaction ($P < 0.12$).
[f]Effect of Tasco ($P < 0.13$).
[g]Effect of Tasco ($P < 0.06$).
[h]Endophyte × Tasco interaction ($P < 0.07$).
[i]Effect of endophyte ($P < 0.07$).
[j]Effect of Tasco ($P < 0.10$).

either endophyte or Tasco were observed (Figure 4A). However, averaged over the six sampling dates in 1997, superoxide dismutase was increased by both application of Tasco ($P < 0.05$; 2,902 vs 2,193 units/g fresh weight; SE = 217) and presence of the endophyte ($P < 0.01$; 3,104 vs 1,991 units/g fresh weight; SE = 217; Figure 4B).

Table 6. Serum vitamins A and E in steers that grazed endophyte-infected (E+) or uninfected (E–) tall fescue that was either treated (T+) or not (T–) with Tasco-Forage at Prairie, MS

	E+		E–		
Item	T+	T–	T+	T–	SE[a]
Vitamin A, ppb					
July 8, 1996	581	549	534	579	32
September 30, 1996	700	673	669	727	36
October 8, 1997	669	663	710	677	32
Vitamin E, ppm					
July 8, 1996[b]	6.4	6.3	7.7	7.4	0.6
September 30, 1996[c]	5.9	6.3	8.0	7.0	0.5
October 8, 1997[def]	7.1	6.8	6.7	8.1	0.4

[a]Standard error of the mean, n = 2; pasture was the experimental unit.
[b]Effect of endophyte ($P < 0.07$).
[c]Effect of endophyte ($P < 0.01$).
[d]Endophyte × Tasco interaction ($P < 0.05$).
[e]E+ differed from E– when no Tasco was applied ($P < 0.03$).
[f]T+ differed from T– within E– tall fescue ($P < 0.02$).

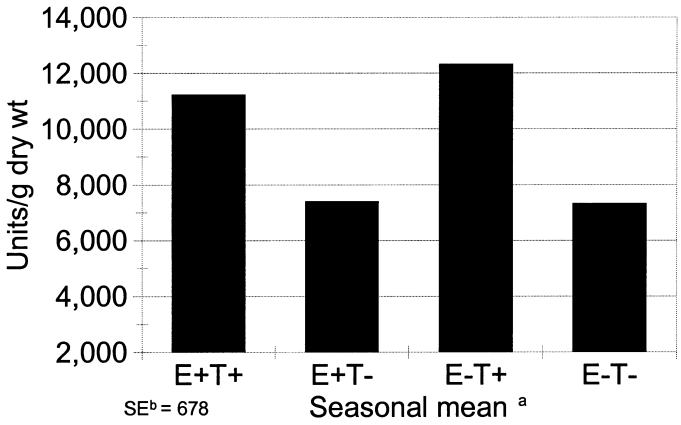

Figure 3. Superoxide dismutase in endophyte-infected (E+) and uninfected (E–) tall fescue in Virginia (1995, 1996, and 1997) that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, in April and July. Each bar represents a mean of monthly sampling intervals from June through October. [a]Effect of Tasco ($P < 0.01$). [b]SE = standard error of the mean; n = 30 for each mean.

Discussion

Seaweeds have been used as soil amendments and in animal feeds for many years, but historically much of the information has been anecdotal. Blunden and Woods (1969) suggested that seaweeds contained carbohydrates that could both stimulate and reduce plant growth. Seaweeds are known sources of natural plant growth regulators (Jameson, 1993), including cytokinins (Blunden, 1977), auxins (Sanderson et al., 1987), and gibberellins (Wildgoose et al., 1978). Vitamins and vitamin precursors contained in seaweeds include α-tocopherol (Jensen, 1969), β-carotene, niacin, thiamin, and ascorbic acid (Jensen, 1972). Seaweed is also known to contain a wide range of minerals, but mineral content has not explained changes in mineral concentrations of plants treated with seaweed (Blunden, 1977). *Ascophyllum nodosum* has been the most widely researched seaweed for agricultural applications (Senn, 1987).

The influence of various seaweeds and products derived from seaweeds on plant growth has been variable and has frequently appeared confounded with environmental or nutrient stresses (see review by Fike, 1995). *Ascophyllum nodosum* extract from the same source used in the current research and applied at 326 g/ha increased root and shoot dry weight of tall fescue and Kentucky bluegrass (*Poa pratensis*) in terrarium studies (Zhang, 1997). In that research, 326 g/ha seemed more effective than 163 g/ha for Kentucky bluegrass. Tall fescue was not tested at the lower rate. In the research reported here, application of 3.4 kg/ha seemed to decrease forage mass at the end of the second grazing period in Exp. 1 in August, but it was not clear whether the decline in forage mass during grazing was due to decreased growth or to an increase in intake by the

grazing lambs. In Exp. 2 in Virginia, forage mass was lower ($P < 0.07$) in July in Tasco-treated pastures grazed by cattle than in the untreated pastures. This effect was also observed in July in 1 of the 2 yr in Mississippi. Attempts to clarify this by ungrazed caged areas in Virginia indicated a trend ($P < 0.12$) for decreased forage mass due to Tasco treatment, but this was only observed when the data were tested across all sampling dates during the growing season. In our experiments, the rate of Tasco application was 10 times that used in the previous turfgrass research (Zhang, 1997) and may account for differences in effects on plant growth. A tendency for Tasco to decrease growth during the stressful midsummer period would agree with results of Ayad (1998) in Texas. Ayad (1998) demonstrated that application of 3.4 kg Tasco to infected and uninfected tall fescue had no influence on total seasonal yields under high moisture (100% replacement of potential evapotransporation, but at 50% of potential evapotransporation, yields of both infected and uninfected tall fescue were lower ($P < 0.06$) than those of the non-Tasco-treated plants. In the current experiment, increased intake of Tasco-treated forage by the grazing animal could at least partially explain the results and cannot be ruled out. Increased gains by lambs during midsummer in Exp. 1 could have reflected higher intake. In Exp. 2, weight gains of steers were not different, suggesting that forage intake was not affected (Saker et al., 2001).

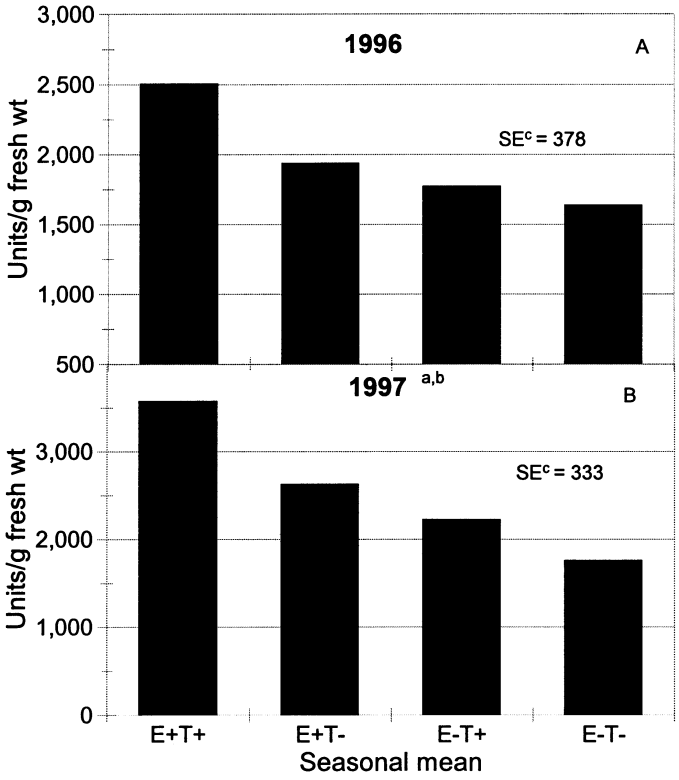

Figure 4. Superoxide dismutase in endophyte-infected (E+) and uninfected (E–) tall fescue in Mississippi (1996 and 1997) that was either treated (T+) or not (T–) with Tasco-Forage, a brown seaweed extract, in April and July. (A) For 1996, each bar represents a mean of three sampling dates; n = 6 for each mean. (B) For 1997, each bar represents a mean of monthly sampling intervals from April through September; n = 12 for each mean. [a]Effect of Tasco ($P < 0.05$); [b]effect of endophyte ($P < 0.01$); [c]SE = standard error of the mean.

Both tall fescue and the endophytic fungus of tall fescue are known to produce alkaloids, and the ergopeptide alkaloids are normally associated with many of the problems of fescue toxicosis (Porter and Thompson, 1992). Several ergot alkaloids have been shown to reduce serum prolactin in steers (Browing et al., 1997) and beef heifers (Browing and Leite-Browing, 1997). Results of initial studies with lambs and steers in Virginia suggested that Tasco might reduce concentrations of loline and ergot alkaloids, but data collected during 1996 and 1997 from Virginia and Mississippi did not substantiate this. Furthermore, the decline in serum prolactin in lambs on all treatments in Exp. 1 further suggests that this was not the mode of action.

Tasco does not seem to influence traditional measures of forage nutritive value, including fiber composition, CP, TNC, or IVDMD. Few effects were observed, and differences, when they occurred, were small and inconsistent. If effects of Tasco on mineral composition of tall fescue occur, they were not measurable under the field conditions of these experiments. A soil fertility gradient across the Mississippi pastures prevented use of mineral data at this location. Lowered Cu status in tall fescue (Dennis et al., 1998) and in steers that grazed the forage (Saker et al., 1998) were related to presence of the endophyte in tall fescue in the Virginia pastures. However, application of Tasco did not seem to alter Cu status in either the plant or the animal.

Effects of Tasco on Se in forage and animals needs further investigation. Lambs in Exp. 1 and steers in the Virginia experiment in 1995 strongly tended ($P < 0.10$) to show increased whole-blood Se. Supplementation with Se in human diets has led to an increase in activity of the antioxidant glutathione peroxidase (**GP**; Girodon et al., 1997). Both vitamin E and Se (through GP) act as antioxidants, and the two are complementary in their interactions and have been shown to enhance the immune system in dairy calves (Reddy et al., 1986). The increase in serum Se in our experiments may have indicated an increase in GP, although this was not measured. Anderson et al. (1979) found that the relationship between GP in erythrocytes and whole-blood Se justified the use of a GP analysis to estimate the animals' Se status.

The primary effect of Tasco application to tall fescue in our experiments seemed to be through antioxidant activity in both plants and animals. It is known that seaweeds contain substituted phenols and polyphenols, a class of compounds that include effective antioxidants (Le Tutour, 1990). Le Tutour (1990) found a synergistic effect between vitamin E and the marine algaes *Lami-*

naria digitata and *Himanthalia elongata* on preservation of sunflower oil.

Effects of the endophyte on vitamin E status in grazing steers may be a key to elucidating the etiology of fescue toxicosis and need further investigation. In Virginia and Mississippi, presence of the endophyte seemed to depress serum vitamin E, and differences became greater by the end of the grazing season. Whether Tasco application can modify this effect needs further investigation. Although not measured in the current experiment, α-tocopherol and ascorbic acid were increased in tall fescue and Kentucky bluegrass due to *A. nodosum* extract (Zhang, 1997; Zhang and Schmidt, 1999).

Sheep and cattle in Virginia tended to exhibit an increase in serum vitamin A in response to grazing Tasco-treated fescue, but no effect was observed in Mississippi. Increased vitamin A status in grazing animals is of questionable value because green plant material is an excellent source of β-carotene that is considered adequate to meet the requirement for vitamin A. However, increased serum vitamin A in both sheep and steers may be a useful indicator of a generalized enhancement of antioxidant status based on results observed in these experiments. Furthermore, Zhang and Schmidt (1999) found increased β-carotene in Kentucky bluegrass treated with *A. nodosum* extract plus humic acid.

Superoxide dismutase activity in forage was increased by application of Tasco to both infected and uninfected tall fescue in Virginia and Mississippi. Furthermore, the effect was both rapid and long-lasting. An increase in superoxide dismutase was observed by 28 d following application in April to pastures in Virginia, and the effect was measurable through November (Zhang, 1997), although Tasco was not reapplied after July. Zhang (1997) demonstrated increased superoxide dismutase activity in tall fescue, Kentucky bluegrass, and creeping bentgrass (*Agrostis palustris*) in response to the combination of 0.05 kg humic acid and 0.33 kg *A. nodosum* extract/ha in a terrarium study. *Ascophyllum nodosum* extract in that experiment was not tested alone. Ayad (1998), working with small plots in the southern High Plains of Texas, demonstrated increased superoxide dismutase in genetically similar endophyte-infected and uninfected tall fescue in response to 3.4 kg Tasco/ha. Additionally, activity of glutathione reductase and ascorbate peroxidase increased in the fescue in response to Tasco application (Ayad, 1998). An increase in antioxidant activity could lead to increased stress tolerance in the plant (Li and Van Staden, 1998). Superoxide dismutase is the most efficient scavenger of toxic superoxide anions and is essential to the ascorbate-glutathione cycle (Beyer et al., 1991). Cytokinin or cytokinin-like growth regulators can also inhibit activity of free radical groups, including hydrogen peroxide and superoxide (Sexton and Woolhouse, 1984).

Endophyte had no effect on superoxide dismutase in Virginia pastures but did increase superoxide dismutase activity in fescue grown in Mississippi. Ayad (1998) also found that presence of the endophyte increased superoxide dismutase activity by approximately 23% in genetically similar endophyte-infected and uninfected tall fescue grown in the southern High Plains of Texas. Possibly, differences in climatic stress interact with the endophyte to alter effects of superoxide dismutase. A higher concentration of superoxide dismutase in infected tall fescue under stress could be related to its greater drought tolerance. Li and Van Staden (1998) reported that maize plants with increased drought resistance had greater levels of superoxide dismutase.

Whether Tasco could improve stress tolerance of uninfected or infected tall fescue under field conditions remains unknown. Improved stress tolerance in turfgrasses has been documented (Goatley and Schmidt, 1990; Yan, 1993; Nabati et al., 1994). Further research is needed to determine whether rates and timing of application of Tasco could be defined to improve plant stress tolerance under field conditions while maintaining or enhancing effects on antioxidant activity in both the plant and the animal.

Implications

Tall fescue (*Festuca arundinacea*) is the predominant forage grass of the humid eastern United States and represents a major grazing resource. The presence of the endophyte *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin) in tall fescue, although it improves stress tolerance in the plant, often causes reduced performance and other negative effects on grazing livestock. One effect of fescue toxicity may be lowered vitamin E status in the grazing animal. Tasco-Forage, a seaweed (*Ascophyllum nodosum*)-based proprietary product, may be a tool in ameliorating some of the deleterious effects of the endophyte in tall fescue on grazing cattle and sheep through increased antioxidant function. Forage application of Tasco may increase the antioxidant activity in tall fescue and in the wether lambs and steers that graze the treated fescue. Increased antioxidant function could reduce damage from toxic oxygen species and improve stress tolerance in both plants and animals.

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