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# Monocyte Immune Cell Response and Copper Status in Beef Steers That Grazed Endophyte-Infected Tall Fescue<sup>1</sup>

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**ABSTRACT:** A 3-yr study was conducted to evaluate immune response and Cu status of yearling beef steers as a consequence of grazing tall fescue (*Festuca arundinacea* Schreb.) infected (E+) with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin). During a preliminary study in 1994, 24 weanling Angus and Angus × Hereford steers were blocked by breed and weight (initial BW 271 kg; SD 25) and were randomized to E+ and low endophyte (E-) fescue in pastures at Glade Spring, VA. Grazing began in April and was discontinued in July. In 1995 and 1996, 24 weanling Angus and Angus × Hereford steers (initial BW 249 kg, SD 20 and 240 kg, SD 15, respectively) were randomized to the E+ and E- pastures at Glade Spring during each year. Grazing began in April and continued until September in 1995 and October in 1996. In 1994, steers that grazed E+ fescue exhibited lower ( $P < .05$ ) phagocytic activity, major histocompatibility complex (MHC) class II expression,

ceruloplasmin, and serum Cu than steers that grazed E- tall fescue. During 1995, steers grazing E+ fescue had lower ( $P < .05$ ) phagocytic activity and MHC class II expression than steers that grazed E- fescue. In 1996, one-half of the steers within each paddock received a Cu oxide bolus at the beginning of the grazing season. During 1996, phagocytic activity was lower ( $P < .01$ ) and MHC class II expression tended ( $P < .07$ ) to be lower in steers that grazed E+ tall fescue than in steers that grazed E- tall fescue. Copper supplementation increased ( $P < .05$ ) MHC class II expression in July regardless of endophyte status over nonsupplemented steers. Steers that grazed E- tall fescue had higher ( $P < .05$ ) plasma or serum Cu concentrations than steers that grazed E+ tall fescue in each year of the study. These data indicate that the endophyte compromised the immune function of grazing steers, and the data suggest a relationship with depressed Cu status.

Key Words: Immune Response, Steers, Endophytes, Copper, Forage, Minerals

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## Introduction

Fescue toxicosis in beef cattle has been associated with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin; Glenn et al., 1996) and the presence of alkaloids in endophyte-infected (E+) tall fescue (*Festuca arundinacea* Schreb.; Steudemann and Hoveland, 1988; Garner et al., 1993). Economic losses associated with fescue toxicosis in the US exceed \$600

million annually (Fribourg et al., 1991). Clinical signs include heat intolerance; long, rough hair coats; elevated body temperature; reduced weight gains; decreased conception rates; reduced serum prolactin; and decreased immune response (Gay et al., 1988). Many of these clinical signs are similar to Cu deficiency in ruminants (McDowell, 1992).

Altered prolactin secretion, decreased Cu and ceruloplasmin concentrations, and reduced forage intake and weight gains have been reported in animals that graze E+ tall fescue (Gomm et al., 1982). Steers that consumed E+ fescue had deficient plasma and hepatic Cu concentrations (Coffey et al., 1992). Dennis et al. (1998) reported that Cu concentrations were lower in E+ than in endophyte-free (E-) tall fescue.

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Immune responses of calves that graze E+ tall fescue have not been clearly characterized. Adequate Cu status seems to be important in maintaining optimal immunocompetence and performance in stress-challenged animals. The effects of the lowered Cu concentration in E+ tall fescue on Cu status in the animal and its relationship to immune function have not been previously investigated to our knowledge. Thus, the objective of this experiment was to determine the effect of the endophyte on select immune responses in steers that grazed E+ and E- tall fescue and to investigate the relationship of these responses to plant and animal Cu status.

### Materials and Methods

Experiments were conducted with beef steers grazing E+ and E- tall fescue during 1994, 1995, and 1996 at the Southwest Virginia Agricultural Research and Extension Center at Glade Spring. There were eight pastures, four each that had been established with E+ and E- 'Kentucky-31' (KY-31) tall fescue during 1986. A description of the soils and the field experimental design is reported in Dennis et al. (1998).

During 1994, a pilot study was conducted with 24 weaned beef steers (Angus and Angus  $\times$  Hereford) 9 to 10 mo of age and with an average initial BW of 271 kg (SD 25). Steers were blocked by breed and weight and were randomized within blocks to either E+ or E- tall fescue pastures. During 1994, two field replications (four pastures) were used in this study. Each pasture was 1.5 ha; thus, the stocking rate was one stocker per .25 ha. In 1994, grazing began in April and ended in July.

In 1995 and 1996, 24 Angus and Angus  $\times$  Hereford steers (initial BW 249 kg, SD 20 and 240 kg, SD 15 for 1995 and 1996, respectively) were blocked by weight and randomized to treatments. Additionally, in 1996, three of the six steers within each of the eight paddocks were given a copper oxide bolus to supply 25 g of Cu (Copasure<sup>®</sup>; Schering-Plough, Kenilworth, NJ) at the beginning of the grazing season. As in 1994, two replications of E+ and two replications of E- tall fescue pastures were used with six steers per pasture replicate, with the exception that in 1996 all 48 steers in all four replications were included in the study for serum Cu. In 1995, grazing began on April 24 and ended on September 12. In 1996, grazing began on April 16 and the last samples were collected on October 3. In all 3 yr, steers were continuously stocked throughout the experimental period. Steers were provided free access to water and iodized salt. No trace minerals were provided because mineral supplementation could have confounded the results of this study. In each year, steers were vaccinated against *Pasturella hemolytica* (One Shot<sup>®</sup>; Pfizer Animal Health, NY), *Clostridium perfringens* C and D (7 WAY<sup>®</sup>; Bayer, Shawnee Mission, KS), infectious

bovine rhinotracheitis parainfluenza-3 virus, bovine viral diarrhea, and bovine respiratory syncytial virus (BRSV-Vac4; Bayer, Shawnee Mission, KS) before grazing began.

Tall fescue was tested (Fescue Toxicity Diagnostic Center, Auburn Univ., Auburn, AL) each year for the presence of the endophyte. Percentages of infection during 1994 through 1996 were >75% for E+ and < 5% for E- pastures with little variation among replications.

In 1994, blood samples were obtained for determination of plasma Cu and immune function testing in July at the end of the study. Blood samples were collected initially in April and then at 28-d intervals during 1995. For 1996, blood samples were collected initially in April, July, and October. For immune function and plasma Cu, blood samples were collected via jugular venipuncture into two, 10-mL Vacutainer<sup>®</sup> (Becton Dickinson, Cockeysville, MD) tubes with EDTA and heparin sodium. For serum Cu during 1996, blood samples were collected into 15-mL Vacutainer tubes without additives at 28-d intervals throughout the grazing season.

Tall fescue samples were collected from pastures for determination of Cu concentration on the same days that blood was collected from the steers. A description of the procedures and effects of the endophyte on Cu concentration are provided by Dennis et al. (1998). Mean Cu concentrations in tall fescue were 6.2, 6.0, and 5.6 ppm for 1994, 1995, and 1996, respectively.

Plasma Cu was analyzed by atomic absorption spectrophotometry following dilution (1:2; vol/vol) with deionized distilled water (AOAC, 1990) in 1994 and 1995. In 1996, serum Cu was measured as atomic emission by an inductively coupled plasma spectrometer following digestion with 2:1 (vol:vol) nitric: perchloric acid (Muchovej et al., 1986).

Ceruloplasmin activity was determined with a micro-method technique (Smith et al., 1983). Standards were prepared using bovine ceruloplasmin (Sigma Chemical Co., St. Louis, MO), and standard curves and ceruloplasmin oxidase values were calculated from a Softmax program (Molecular Devices, Menlo Park, CA) set up on a UV microplate reader at 550 nm.

Two milliliters of whole blood was removed from each sample collected in EDTA for determination of total leukocyte counts. Counts were made using a Coulter cell counter (Model ZM; Coulter Electronics, Hialeah, FL). One milliliter of each remaining EDTA-collected sample was diluted 1:45 with lysis buffer (8.43 g NH<sub>4</sub>Cl, 840 mg NaHCO<sub>3</sub>, 2.92 g EDTA-free acid, adjusted with 10% NaOH to a pH of 7.4) and centrifuged at 200  $\times$  g for 10 min. The cell pellets were washed twice in Hank's balanced salt solution (HBSS). Cells used for measurement of oxidative metabolism and major histocompatibility complex (MHC) class II surface antigen expression were resuspended to a concentration of 1  $\times$  10<sup>6</sup> cells/mL in

Table 1. Performance, select immune response, and copper status of steers that grazed endophyte-infected and endophyte-free tall fescue from April to July at Glade Spring, VA, during 1994<sup>a</sup>

Item	Endophyte status		SE
	Infected	Not infected	
Final body weight, kg	288	320**	8
Phagocytic activity, mcf <sup>b</sup>	10.5	23.0*	.87
Major histocompatibility complex class II, mcf <sup>b</sup>	1.34	2.76*	.20
Leukocyte count, $\times 10^3/\mu\text{L}$	8.4	9.4	.60
H <sub>2</sub> O <sub>2</sub> release, mcf <sup>b</sup>	1.26	1.46	.13
Plasma Cu, ppm	.62	.72**	.02
Ceruloplasmin, mg/mL	.31	.40*	.03

<sup>a</sup>n = 12 for each mean.

<sup>b</sup>mcf represents mean channel fluorescent emission of the percentage of cells responding as measured by flow cytometry.

\* and \*\* indicate a difference between E+ and E- within a row ( $P < .05$  and  $P < .01$ , respectively).

HBSS buffer. The remaining cells were resuspended to a concentration of  $1 \times 10^6$  cells/mL in Krebs-Ringer solution (KRH) with HEPES gelatin for measurement of phagocytic activity.

Oxidative metabolism was measured via the conversion of dichlorofluorescein diacetate (5 mM, Eastman Kodak, Rochester, NY) to 2',7'-dichlorofluorescein, which occurs in the presence of intracellular hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Cytochalasin B (12.5  $\mu\text{L}$  of a 50 ng/ $\mu\text{L}$  stock solution; Sigma Chemical) was used to stimulate monocyte production of H<sub>2</sub>O<sub>2</sub>. The protocol was modified from that reported by Bass et al. (1983) for neutrophil oxidative burst. Mean channel fluorescent emission for each sample was recorded on a Coulter Epics XL Flow Cytometer and used as the determinant of H<sub>2</sub>O<sub>2</sub> release.

Major histocompatibility complex class II expression was detected by flow cytometry using a ruminant monoclonal antibody (TH14B; VMRD, Pullman, WA), as described by Splitter and Everlith (1989). Briefly, HBSS-suspended cells were incubated on ice for 1 h with a 1:2,500 dilution of the ruminant monoclonal antibody and washed with PBS. Cells were then incubated with fluorescein-conjugated rabbit anti-mouse immunoglobulin G, 1:100 concentration (heavy and light chains; Jackson Immunoresearch Laboratories, Avondale, PA) for an additional hour. Cells were washed and examined by gating on the monocyte population using the flow cytometer.

The KRH gelatin-suspended cells were used to measure monocyte phagocytic activity by a method similar to that reported by Steinkamp et al. (1982). Phagocytosis of fluorescein isothiocyanate (FITC) conjugated polystyrene beads (Fluoresbrite Beads<sup>®</sup>; Polysciences, Warrington, PA) by monocytes was determined by fluorescent emission on the flow

cytometer.

Data were analyzed as a complete randomized block design using the GLM procedures of SAS (1985). Because of the differences in experimental procedures, duration of the grazing season, and sampling intervals among years, each year was analyzed separately. Data for 1996 were tested as a complete randomized block design with a split-plot arrangement of treatments for Cu supplementation.

## Results and Discussion

During all 3 yr, steers that grazed E+ tall fescue showed signs indicative of fescue toxicosis as evidenced by lower weight gains (Table 1, for 1994), elevated body temperatures, and long, and rough summer coats (Saker et al., 1996; our unpublished observations). These indicators were consistent with results from studies conducted from 1989 through 1993 using these paddocks in longer-term grazing trials (Tully et al., 1990; Fontenot and Allen, 1994). At the end of the preliminary study in 1994, possible signs of decreased immunocompetence were observed as suggested by increased incidence of nasal discharge in steers that grazed E+ vs E- tall fescue (44 and 12%, respectively).

The preliminary study conducted during 1994 indicated that phagocytic activity of monocytes from steers that grazed E- fescue was increased ( $P < .05$ ) compared with that of steers that grazed E+ fescue (Table 1). Steers that grazed E- tall fescue also had

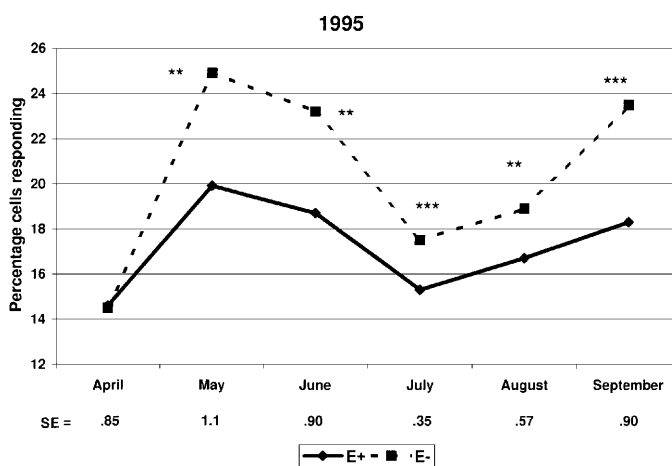


Figure 1. Monocyte phagocytic activity in steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue during 1995. Monocyte function is expressed as mean channel fluorescent emission of responding cells, where fluorescence is defined as channel number, with channel range 1 to 1,024. Each point represents the mean of 12 steers per treatment. \*\* and \*\*\* indicate a difference between means at each day ( $P < .01$  and  $.001$ , respectively).

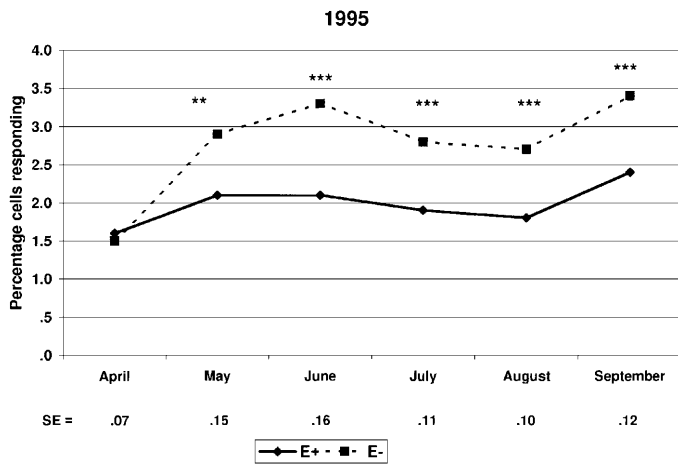


Figure 2. Monocyte major histocompatibility complex class II expression in steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue during 1995. Monocyte function is expressed as mean channel fluorescent emission of responding cells, where fluorescence is defined as channel number, with channel range 1 to 1,024. Each point represents the mean of 12 steers per treatment. \*\* and \*\*\* indicate a difference between means within a day ( $P < .01$  and  $.0001$ , respectively).

greater ( $P < .05$ ) monocyte MHC class II expression compared with steers that grazed E+ tall fescue. Total leukocyte counts and  $H_2O_2$  release did not differ between steers.

In 1995, total leukocyte counts (data not shown) as well as monocyte phagocytosis and MHC class II expression were higher ( $P < .05$ ) in steers that grazed E- tall fescue than in steers that grazed E+ tall fescue by d 28 and remained higher ( $P < .05$ ) throughout the study period (Figures 1 and 2). In 1996, phagocytic activity was higher in steers that grazed E- tall fescue at d 84 ( $P < .07$ ) and 170 ( $P < .01$ ; Figure 3), and MHC class II expression for steers that grazed E- tall fescue was higher ( $P < .07$ ) than for steers that grazed E+ tall fescue at d 170 (Figure 4). Steers supplemented with a Cu bolus had higher ( $P < .05$ ) MHC class II expression on d 84 in 1996 than did steers that were not supplemented (Figure 4), but the response had disappeared by the end of the grazing season (d 170). The effectiveness of the Cu bolus was expected to be approximately 120 d. More research is needed with additional animal numbers to clarify this relationship with MHC class II expression and with phagocytic activity.

Effects of the endophyte infection on immune function observed with steers in our research are consistent with those of previous studies with laboratory animals. Dew et al. (1990) investigated the relationship between *N. coenophialum* and the humoral and cellular immune systems using rat and mouse models. Rats fed E+ fescue had lower leukocyte counts, decreased spleen cell response to mitogen

stimulation, and increased T suppressor cell populations compared with rats fed E- fescue.

In 1994, plasma Cu concentration was higher ( $P < .01$ ) in steers grazing E- than in steers grazing E+ tall fescue (Table 1). Plasma ceruloplasmin activity was also higher ( $P < .05$ ) in steers grazing E- fescue than in steers grazing E+ tall fescue. In 1995, steers that grazed E- tall fescue maintained higher ( $P < .05$ ) plasma Cu from July through September than did steers that grazed E+ tall fescue (Figure 5). Plasma Cu in steers grazing E+ tall fescue generally declined in the latter part of the grazing season. In 1996, steers that grazed the E- tall fescue had higher ( $P < .05$ ) serum Cu than did steers that grazed E+ tall fescue from July through October (Figure 6). As was observed for the previous year, serum Cu generally declined through September. Serum Cu seemed to increase in both groups at the October, 1996 sampling date. Supplementing steers with a Cu bolus resulted in higher serum Cu by June, and this difference was sustained throughout the grazing season (Figure 7).

Copper deficiency in steers that grazed E+ tall fescue may have influenced immunocompetence in these steers as suggested by the decreased ( $P < .01$ ) leukocyte count compared with that of steers that grazed E- tall fescue. Leukocyte count is one of many hematologic variables that indicate immune system status. Differences were not observed among treatment groups in 1994, perhaps because of the shorter grazing period and subsequent shorter exposure to the

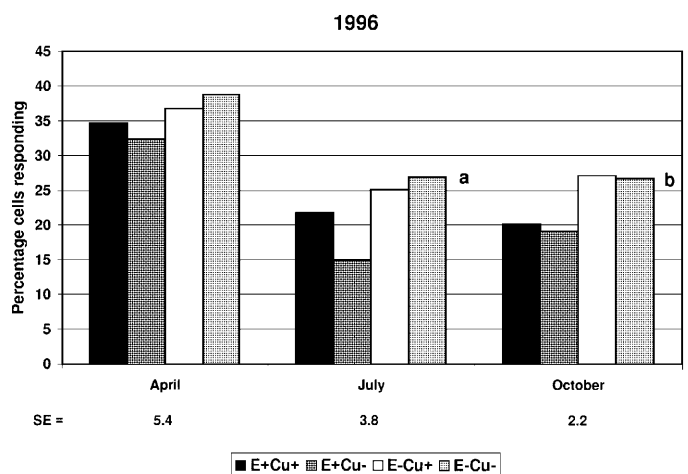


Figure 3. Monocyte phagocytic activity in steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue and either supplemented (Cu+) or not (Cu-) with a copper bolus during 1996. Monocyte function is expressed as mean channel fluorescent emission of responding cells, where fluorescence is defined as channel number, channel range 1 to 1,024. Each bar represents the mean of six steers per treatment. <sup>a</sup>Indicates an effect of endophyte ( $P < .07$ ). <sup>b</sup>Indicates an effect of endophyte ( $P < .01$ ).

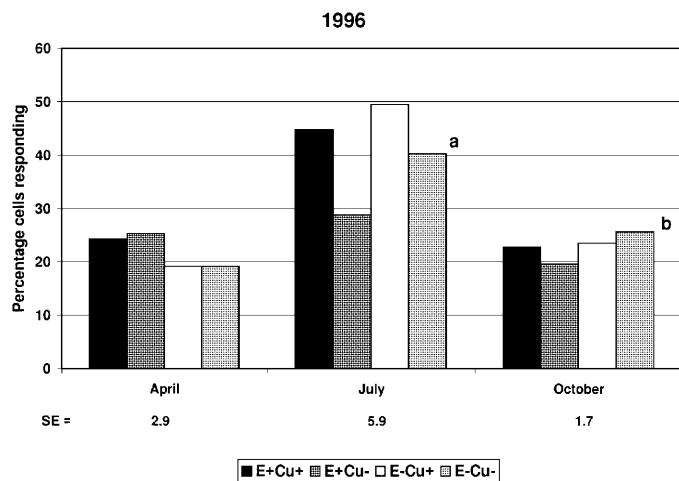


Figure 4. Monocyte major histocompatibility complex class II expression in steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue and supplemented (Cu+) or nonsupplemented (Cu-) with a copper bolus during 1996. Monocyte function is expressed as mean channel fluorescent emission of responding cells, where fluorescence is defined as channel number, with channel range 1 to 1,024. Each point represents the mean of six steers per treatment. <sup>a</sup>Indicates an effect of copper ( $P < .05$ ). <sup>b</sup>Indicates an effect of endophyte ( $P < .07$ ).

effects of E+ fescue. The link between Cu deficiency and altered immune response has not been clearly identified, but studies suggest involvement with brain catecholamines. Alterations in the concentration of dopamine-B-hydroxylase, a Cu-dependent enzyme involved in dopamine metabolism, resulted in lowered immune response in rodents (Prohaska and Lukasewycz, 1989; Prohaska and Bailey, 1993). More recent studies (Strickland et al., 1994) have suggested that alkaloids associated with tall fescue toxicosis, which have dopamine-like action, could be a link between Cu status and immune response.

Investigators have shown a reduction in neutrophil and lymphocyte function as a result of Cu deficiency in sheep and steers (Suttle and Jones, 1989; Xin et al., 1991). However, Ward et al. (1997) reported that humoral immunity was not enhanced in cattle fed a corn silage-based diet or fed milk replacer and supplemented with Cu and that cell-mediated immune response was decreased. Monocyte function, although less commonly measured, is an integral part of the immune response (Ho, 1989), and alterations in dietary Cu have been shown to influence monocyte function in Lewis rats (Babu and Failla, 1990). In the present research, Cu supplementation seemed to increase MHC class II expression in endophyte- and nonendophyte-challenged steers in July. Additionally, monocyte phagocytic activity and MHC class II expression in steers that grazed E- tall fescue was enhanced over steers that grazed E+ fescue.

In the preliminary study in 1994, results for monocyte  $H_2O_2$  release did not suggest the same relationship with endophyte infestation. This was not an unexpected finding because microbicidal activity is contingent on a free-radical process, and previously reported studies indicate that the loss of microbicidal function seems to be closely related to a failure to produce superoxide within the phagosome that serves to destroy or neutralize foreign antigens (Arthur and Boyne, 1985). Therefore, alterations in phagocytosis and antigen expression would precede changes in  $H_2O_2$  release in the stimulated monocyte as suggested by our test results for monocyte cell function. The shortness of the grazing period (84 d) may have provided insufficient time for this to occur. The findings in our study support those of Babu and Failla (1990), namely, a relationship between monocyte function and dietary Cu adequacy.

Copper deficiency in cattle has been associated with clinical signs that resemble those of fescue toxicosis (McDowell, 1992). Reduced growth performance is documented in cases of fescue toxicosis (Hoveland et al., 1983) as well as in cases of Cu deficiency (McDowell, 1992). In a 2-yr Virginia study, weaned beef heifers supplemented with Cu showed increased ( $P < .05$ ) weight gains and liver Cu concentrations ( $P < .001$ ) compared with heifers fed a nonsupplemented corn silage diet and E+ fescue hay (Saker, 1995).

Copper concentrations in tall fescue (5.9 ppm averaged over endophyte status and the 3 yr of this experiment) in these pastures did not meet Cu requirements for beef steers (NRC, 1996; Dennis et al., 1998). Even though recommended dietary Cu concentration for beef cattle (NRC, 1996) is 10  $\mu\text{g/g}$  DM, several factors, including dietary interactions

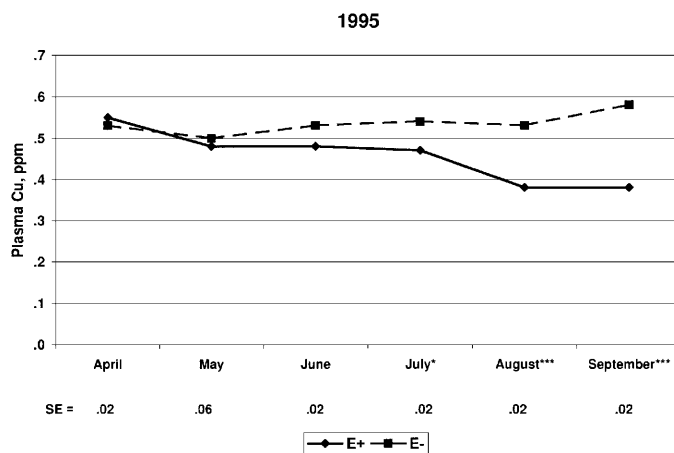


Figure 5. Plasma Cu concentrations of steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue during 1995. Each point represents the mean of 12 steers per treatment. \* and \*\*\* indicate a difference between means at each time ( $P < .05$  and  $.001$ , respectively).

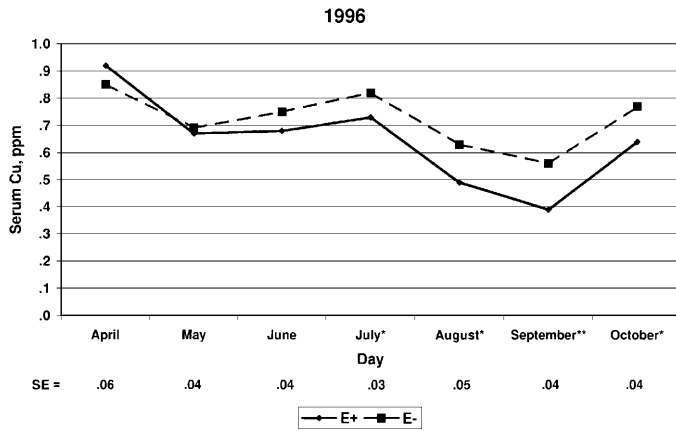


Figure 6. Serum Cu concentrations of steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue during 1996. Each point represents the mean of 24 steers per treatment. \* and \*\* indicate a difference between means at each time ( $P < .05$  and  $.01$ , respectively).

with Mo, S, Fe, and Zn and production-related stressors, can alter Cu availability to the animal (Underwood, 1981). Even though Mo was not analyzed in this study, high-Mo forage in this geographic area has not been reported to be a problem. Plant concentrations of S and Fe for samples in the present study (Dennis et al., 1998) did not seem high enough to interfere with Cu uptake or utilization by the steers. Data from the present study, in conjunction with findings of Dennis et al. (1998), suggest that a possible Cu-endophyte interaction exists in the plant and in the animal. Because the magnitude of the difference in Cu concentration in tall fescue that results from the endophyte is small (about  $1 \mu\text{g/g DM}$ ; Dennis et al., 1998) relative to the animal response, bioavailability of Cu to the animal may be altered as well. However, reduced forage intake is a well-known consequence of infection with the endophyte (Schmidt and Osborn, 1993) and may be the primary factor in reducing the Cu status of the grazing animal. Whatever the mechanism, infection with the endophyte in our study seemed to lower the Cu status of the animal as evidenced by the decreased plasma concentrations of Cu and an altered activity of the Cu-dependent enzyme, ceruloplasmin. A more complete picture of Cu status in steers that graze E+ fescue could be gained through liver biopsy and should be further investigated. However, our results support previous research by Stoszek et al. (1979), who showed that consumption of tall fescue by beef steers reduced liver and plasma Cu and decreased ceruloplasmin oxidase activity. In a 2-yr study conducted by Coffey et al. (1992), a decrease in serum Cu and ceruloplasmin was observed in steers that grazed *N. coenophialum*-infected tall fescue not supplemented with Cu, compared with steers supplemented with Cu

oxide needles. In our research, plasma Cu concentration of steers that grazed E+ tall fescue ranged from borderline normal ( $.7$  to  $1.1 \mu\text{g/g}$ ) to deficient ( $.2$  to  $.4 \mu\text{g/g}$ ) for cattle (Underwood, 1981). Decreased ceruloplasmin activity in steers that grazed E+ fescue in 1994 coincided with lower plasma Cu and reduced weight gains (Table 1) compared with that in steers on the E- fescue pastures.

This study provides evidence that steers that grazed E+ tall fescue had depressed immune function and lowered Cu status compared with steers that grazed E- tall fescue. Further investigation is needed to define this relationship and elucidate the interaction between Cu and the endophyte at the plant and animal levels.

## Implications

Steers that grazed endophyte-infected tall fescue during the stocker phase of production had lowered immunocompetence and lowered Cu status. Lowered immune function owing to the endophyte may lead to reduced stress tolerance and increased susceptibility to disease. The lowered Cu status of steers that grazed infected tall fescue was due perhaps to differences in forage Cu concentrations, reduced bioavailability of Cu, or reduced forage intake that led to reduced Cu intake. The expression of fescue toxicosis and lowered immune function in beef steers that graze endophyte-infected fescue may involve dietary Cu insufficiencies. Understanding these relationships can enhance animal health and improve utilization of infected tall fescue.

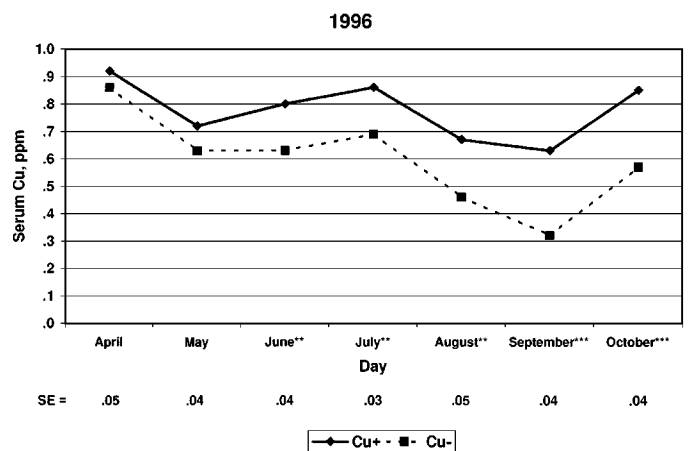


Figure 7. Serum Cu concentrations of steers that grazed endophyte-infected (E+) and endophyte-free (E-) tall fescue that were either supplemented or not with a Cu oxide bolus during 1996. Each point represents the mean of 24 steers per treatment. \*\* and \*\*\* indicate a difference between means at each time ( $P < .01$  and  $.001$ , respectively).

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