Induction of Fescue Foot Syndrome in Cattle by Fractionated Extracts of Toxic Fescue Hay

M. Williams, MS; S. R. Shaffer, MS; G. B. Garner, PhD; S. G. Yates, MS; H. L. Tookey, PhD;
L. D. Kintner, MS, DVM; S. L. Nelson, PhD, DVM; J. T. McGinity, MS, DVM

SUMMARY

Tall fescue (Festuca arundinacea Shreb) hay from a source known to cause “fescue foot” in grazing cattle was extracted with 80% ethanol. The ethanolic extract was further refined and fractionated into cation, anion, and neutral fractions by ion-exchange chromatography. The cation fraction was partitioned with alkaline-chloroform to give chloroform-extractable cation and residual cation fractions. All fractions plus the crude ethanolic extract were assayed for toxic activity by intraperitoneal injection into 12 calves (weighing 152.4 to 241.3 kg each) over a 14-day period. Clinical signs of fescue foot were observed on the 5th day in calves given the anion and crude ethanolic extracts. Lameness, swelling, and reddening of the rear coronary bands, discoloration of the tip of the tail, and other signs of fescue foot were seen. Microscopically, coronary bands and tail tips of affected calves had blood vessels with thick walls and small lumens. More severe clinical signs include lameness, loss of body weight, emaciation, arched back, rough coat, and gangrene of the tail tip, rear hooves, and ears. Sloughing of hooves and portions of the tail may occur.14 Microscopically, blood vessels in affected areas are congested, and some tissues contain perivascular hemorrhages.4 Blood vessels often have heavy, thick walls and small lumens. Perivascular edema is common. However, these changes have not always been present in field cases examined in Missouri.

Progress toward isolation and identification of the compound(s) causing tall fescue toxicosis has proceeded by the preparation of hay extracts and subsequent bioassay for toxic activity in cattle. The fescue foot syndrome was experimentally induced in cattle by intraruminal or intraperitoneal injection of ethanolic extracts of toxic tall fescue.24 Subsequent observations in our laboratory demonstrated that chemical fractionation of a toxic ethanolic extract into cation, chloroform-extractable cation, anion, and neutral fractions by ion-exchange chromatography was feasible. The present study was designed to assay these fractions for fescue foot toxin(s) by intraperitoneal injection into calves.

Materials and Methods

Extraction of Toxic Hay—Fall regrowth from a known toxic tall fescue (Ky-31) pasture was harvested, air-dried, and chopped into pieces 2 to 15 cm long. In a typical extraction, 27.2 kg of chopped hay was steeped in a 430-L stainless steel tank with 80% ethyl alcohol for 4 days. The hay residue was extracted a 2nd time with 208 L of solvent for 2 days and a 3rd time with 208 L of solvent for 1 day. The extracts were concentrated to a syrup at temperatures less than 40 C. From 176.9 kg of hay, 51.5 kg of concentrate was obtained. The concentrate was frozen before it was processed.

The following clarification procedure was used: The crude extract was centrifuged for 30 minutes at 23,300 x g (4 C) to remove a lipid layer (16.9% w/w). The aqueous layer was decanted from beneath the lipid layer and filtered through a 1.2-cm thick bed of sterile acid-washed filter aid on filter paper. The filtrates were subjected to a 2nd centrifugation for 1 hour (23,300 x g, 4 C) and filtered through sterile filter paper.

Preparation of Crude Ethanolic Extract—The volume equivalent of 43.6 kg of hay (9.46 L) of clarified extract was neutralized to pH 7 with sodium hydroxide and diluted to 14.4 L with sterile deionized water. The fraction

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was filtered through sterile acid-washed filter aid, placed in sterile 1-L intravenous (IV) infusion bottles at the daily dose level and frozen until used. This extract and all subsequent fractions were processed so that the maximal daily dose was contained in 400- and 600-ml volumes, respectively.

Preparation of Residual Cation and Chloroform-Extractable Cation Fractions—The volume equivalent of 65.3 kg of hay (14.2 L) was passed into a 10.2- by 121.9-cm cation-exchange resin bed (H-form) at an effluent flow rate of ca 25 ml/minute. After an exhaustive wash with deionized water, the column was eluted with 2 N ammonium hydroxide followed by a 50% ethanol elution to remove cations precipitated on the column. The ethanol and ammonia fractions were pooled and evaporated in vacuo at 50°C to ca 14 L. The concentrate was adjusted to pH 11 with 30% sodium hydroxide and extracted in 1-L portions 4 times with equal amounts of chloroform. Residual chloroform in the aqueous fraction was removed by evaporation to give the cation fraction. The combined chloroform extracts were evaporated to 2.5 L and mixed with 1 L of 0.1 N hydrochloric acid. Complete removal of chloroform produced a yellow oil, a precipitate, and an aqueous-hydrochloric acid fraction referred to as the chloroform-extractable cation fraction. The oil and precipitate were removed by centrifugation, but were not assayed. The residual cation and chloroform-extractable cation fractions were adjusted to pH 7 and diluted to 14.4 L with sterile deionized water. The chloroform-extractable cation fraction was passed through a 0.45-μm pore diameter filter, and the residual cation fraction was filtered through sterile acid-washed filter aid. The fractions were frozen in sterile IV bottles until used.

Preparation of Anion and Neutral Fractions—The neutral-anion fraction derived from the passage of the crude extract through the cation-exchange resin was passed in reverse flow through a 30.5- by 50.8-cm bed of anion-exchange resin (OH-form) at an effluent flow rate of ca 25 ml/minute. After an extensive wash with deionized water, the column was eluted with 6 N hydrochloric acid. Both the neutral and anion fractions were concentrated in vacuo to ca 10 L and adjusted to pH 7. The fractions were diluted to 14.4 L with sterile deionized water, filtered through sterile acid-washed filter aid, and frozen in sterile IV bottles until used.

Preparation of Saline Solution—Saline solution (0.89%) was purchased in 1-L IV bottles from a local veterinary supplier and was injected at the rate of 600 ml/calf/day.

Administration of Fractions—Twelve Hereford calves (steers and heifers) weighing between 152.4 and 241.3 kg each were acclimated to stanchions and handling. The calves were from cows which previously had signs of febrile lameness or loss of tail or both. The calves were kept in a heated barn and fed ad libitum alfalfa pellets (15% protein) during the experiment. Catheterization of the calves was achieved by shaving the right paralumbar fossa area, sterilizing the area, and inserting a 35- to 40-cm long, 4-mm OD piece of silicone rubber tubing through a trocar into the intraperitoneal cavity. The tubing was fitted with a syringe-receiving shank and anchored to the skin with 2 sutures around a gum rubber sleeve. The shank and exposed tubing were placed in a tobacco pouch sutured to the hide.

The calves were randomly allotted to 6 treatments, with 2 calves per treatment group. The starting dose level was to be half the maximal dose and increased every 2nd day until the maximal dose level was attained. The maximal dose level was to be 1.81 kg of hay equivalent (400-ml volume) of the crude ethanolic extract and 2.72 kg of hay equivalent of the fractions (600-ml volume). The 2 reasons for this increased dose were to intensify the challenge exposure to the calf or to compensate for possible loss of toxic activity during the fractionation procedure or both. However, in practice, some of the calves reacted severely to the injection, and the dose level was modified (Table 1).

Solutions for injection were thawed, and 50 mg of oxytetracycline per 100 ml of solution was added. The solutions were administered through a disposable IV set equipped with a drop-counting window and a flow control. The injection rate was ca 300 ml/hour. The catheters were rinsed with sterile saline solution before and after injections were done. When not in use, the catheter was capped and protected in the pouch.

Rectal temperatures were taken during the injection. When a rectal temperature was increased by 1.1°C, the calf was given 500 mg of oxytetracycline intramuscularly. During the last 7 days of the experiment, calves not eating well and showing distress were intraperitoneally given approximately 1 L of a 1% glucose-electrolyte solution plus 500 mg of oxytetracycline.

Results

Clinical signs of febrile foot were seen on the 5th day in those calves given the anion fraction and crude ethanolic extract (Tables 1 and 2). Swelling and reddening of the coronary bands were seen (Fig 1). The calves were lame. There was dark discoloration of the tail tips of the 2 calves given the anion fraction and the 2 calves given the crude ethanolic extract (Fig 2). Expressions of discomfort, trembling, and a reluctance to stand were common observations. Rectal temperatures were increased. Ruminal motility seemed to be absent in at least 2 calves, especially during the first days of the experiment when bloating occurred. Signs in the calves given the crude ethanolic extract

### TABLE 1—Record of Daily Hay Equivalents Injected Intraperitoneally into Calves Which Developed Clinical Signs of Fescue Foot

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5*</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay equivalents (kg) injected on experimental day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Day on which clinical signs of febrile foot were seen. ** Extract to which this calf was derived from 1972 grown hay; all other extracts were derived from 1970 grown hay. † Calf died on day 14.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calf No. (and sex)</th>
<th>Initial body weight (kg)</th>
<th>No. of days injected</th>
<th>Hay equivalent (kg) injected to time of appearance of clinical signs</th>
<th>No. of days from 1st injection to appearance of clinical signs</th>
<th>Gross</th>
<th>Microscopic</th>
<th>Pathologic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude ethanolic extract</td>
<td>282 (♂)</td>
<td>188.2</td>
<td>13</td>
<td>3.18</td>
<td>5</td>
<td>Lameness; dark discoloration of one or both rear coronary bands and of tail tip; peritonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>296 (♂)</td>
<td>158.8</td>
<td>10*</td>
<td>3.18**</td>
<td>5</td>
<td>Dark discoloration of one or both rear coronary bands and of tail tip; peritonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Residual cation</td>
<td>276 (♀)</td>
<td>169.2</td>
<td>14</td>
<td></td>
<td></td>
<td>Peritonitis and retroperitonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>296 (♂)</td>
<td>214.1</td>
<td>14</td>
<td>32.66</td>
<td>15</td>
<td>Lameness; dark discoloration of one or both rear coronary bands; retroperitonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Chloroform extractable cation</td>
<td>298 (♂)</td>
<td>187.8</td>
<td>14</td>
<td></td>
<td></td>
<td>Retroperitonitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>281 (♀)</td>
<td>171.0</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion</td>
<td>292 (♂)</td>
<td>190.1</td>
<td>10*</td>
<td>6.35</td>
<td>5</td>
<td>Lameness; dark discoloration of one or both rear coronary bands and of tail tip; peritonitis and retroperitonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
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<tr>
<td></td>
<td>246 (♂)</td>
<td>231.3</td>
<td>11*</td>
<td>5.44</td>
<td>5</td>
<td>Lameness; dark discoloration of one or both rear coronary bands and of tail tip; peritonitis</td>
<td>Hemorrhage of many hair follicles of tail tip; vessels congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td>299 (♀)</td>
<td>152.4</td>
<td>14</td>
<td></td>
<td></td>
<td>Peritonitis and retroperitonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
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<td>178.2</td>
<td>14</td>
<td></td>
<td></td>
<td>Peritonitis and retroperitonitis</td>
<td>Thickered walls and small lumens in many blood vessels of coronary band; perivascular edema often seen; coronary band vessels congested with sludged blood; hemorrhage of many hair follicles of tail tip; vessels of hair follicles congested with sludged blood; some perivascular hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Saline solution† (control)</td>
<td>251 (♂)</td>
<td>241.3</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>216 (♂)</td>
<td>208.7</td>
<td>14</td>
<td></td>
<td></td>
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</table>

* Calf died. ** This extract was derived from 1972 grown hay; all other extracts were derived from 1970 grown hay. † Dosage of 0.89% saline solution injected was 600 ml/calf/day.

Fig 1—Left rear hoof of calf (No. 282) affected with "fescue foot," showing a darkened, necrotic coronary band.

Fig 2—Tail of fescue foot-affected calf (No. 292), showing 5-cm long darkened discoloration of the tip. Switch hair has been clipped.
intensified until the 7th day, even though it was necessary to decrease the dose level on day 4 (Table 1) due to the calves' rapidly deteriorating condition (i.e., decreased feed consumption, respiratory distress, arched back, listlessness). With the decreased dose, a mild remission of the clinical signs occurred after the 8th day. Only 3.18 kg of hay equivalent was required to produce clinical signs in the calves given the crude ethanol extract, and 5.44 to 6.35 kg of hay equivalent, in the calves given the anion fraction (Table 2).

Signs of fescue foot were not observed in calves given either saline solution, chloroform-extractable cation, or neutral fractions (Table 2). Rectal temperatures and general condition of the calves generally remained normal except when complicated by surgical manipulation of the catheter and intermittent infections. Feed consumption for these calves was adequate after they became acclimated to the extract.

One calf (No. 296) given the residual cation fraction developed some clinical signs of fescue foot during the experiment. He developed a slight limp, both rear hooves appeared swollen between and below the dewclaws, and a lesion developed on the left rear coronary band after 15 days. However, the hay equivalents that had been given to cause signs were not commensurate with observations of the other calves. Microscopic sections of the blood vessels and coronary band were normal.

At necropsy, the calves given the anion fraction and the crude ethanolic extract had diffuse peritonitis. The major organs adhered to masses of fibrinous exudate. Scattered small foci of necrosis were seen in liver, kidney, and rumen. The abdominal cavity contained 3 to 12 L of serous exudate. Hearts were normal. The large joints of one calf given the anion fraction contained excess amounts of milky synovial fluid infected with bacteria of unknown genera. Microscopically, there was hemorrhage in many of the hair follicles of the tail tips and distention of capillaries and small vessels with blood. The vessels themselves were not necrotic and had a lumen. In 1 calf (No. 282) given the crude ethanolic extract, the epidermis of the tail tip was destroyed, and exudate from this defect formed a thick scab. The adjacent epithelium was thickened and infiltrated with neutrophils. Hair was readily removed (by rubbing with fingers) from the epidermis of both tail tips and coronary bands of the calves. Sections from inflamed coronary bands generally had heavy infiltration by neutrophils. In 3 calves, the blood vessels of the coronary band were conspicuous with heavy, thick walls and small lumens (Table 2; Fig 3). Peri-vascular edema was common. The digital arteries were microscopically normal. Coronary band sections from 1 calf (No. 246) given the anion fraction did not have significant lesions. Cause of death in 3 calves was listed as combinations of peritonitis, toxemia, and shock. The 4th calf (No. 282) was killed and necropsied (Table 1).

Calves given the residual cation and neutral fractions were killed and necropsied. All the calves had many abscesses containing pus in the peritoneal cavity near the right paralumbar fossa area. Cultures of the pus yielded Corynebacterium and Streptococcus. The kidneys of both calves given the residual cation fraction were encased in a mass of necrotic tissue. Cytoplasmic eosinophilic inclusions were in the epithelial cells of collecting tubules of the kidneys of 1 calf. Other major organs, glands, and lymph nodes were normal. Microscopic lesions were not observed in these tissues or coronary bands and digital arteries (Table 2).

Discussion

From the aqueous phase of an 80% ethanolic extract of toxic tall fescue hay, the causative agent(s) of the fescue foot syndrome was bound to an anion-exchange resin and eluted from the resin with 6 N hydrochloric acid. After elution, the hydrochloric acid-anion contain-
ing fraction was concentrated in vacuo at 60°C and neutralized to pH 7.0 for intraperitoneal injection. On the basis of hay equivalents, there did not seem to be greater than 50% loss of activity for the total process from aqueous crude extract to the anion fraction (Table 2). These observations indicate the causative agent(s) has at least one acidic moiety and is moderately heat and inorganic acid stable.

All fractions except the chloroform-extractable cation fraction, which was clear and yellow, were clear and dark. Upon injection, chromagens were deposited on the peritoneal lining as seen at necropsy by discoloration of the tissues. The chromagens, the salt content, and the continued presence of oxytetracycline undoubtedly contributed to the general peritonitis seen at necropsy.

Feed intake was markedly affected in calves given the anion and crude ethanolic extract during the injection period. In contrast, calves given the chloroform-extractable cation fraction were not so affected. There did not seem to be a correlation between feed intake and degree of peritonitis in the calves given the residual cation or the neutral fractions.

Peritonitis or retroperitonitis occurred in all calves given the anion and crude ethanolic extract during the injection period. In contrast, calves given the chloroform-extractable cation fraction were not so affected. There did not seem to be a correlation between feed intake and degree of peritonitis in the calves given the residual cation or the neutral fractions.

Peritonitis or retroperitonitis occurred in all calves given the anion and crude ethanolic extract, thus indicating the need for aseptic handling of the fractions. Sterilization of the chloroform-extractable cation fraction was achieved by filtration through a 0.45-μm pore diameter filter. All other fractions were filtered through sterile acid-washed filter aid before bottling and freezing, since they would not pass a 0.45-μm pore diameter filter. This technique was found to sterilize the fractions in preliminary experiments. Future fractionation of the toxic anion fraction should allow for filtration of all resulting fractions. However, should the toxic principle be proved heat stable, then autoclaving would be the method of choice.

With the intraperitoneal injection technique, relatively large amounts of extracts are needed. The animal must be surgically prepared, and there is concomitant risk of peritonitis. Therefore, chemical fractionation and concentration should facilitate aseptic handling of the toxin and the possible use of an infusion (intravenous) technique. A 5- to 7-day treatment would be sufficient to produce visible signs, provided adequate toxin is present. The short treatment period would reduce the possibility of synergistic or antagonistic effects between peritonitis and the toxic factor(s). However, the 2 calves given the neutral fractions and the 1 calf given the residual cation fraction had extensive peritonitis without any gross or microscopic evidence of fescue foot syndrome. With its shortcomings, the intraperitoneal injection technique is a usable bioassay for work in elucidating the toxic principle in tall fescue.

References