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# Chemical Quality, in Vitro Cellulose Digestion, and Yield of Tall Fescue Forage Affected by Mefluidide

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Tall fescue (*Festuca arundinacea* Schreb. "Ky 31") was treated with mefluidide at 0 and 0.28 kg/ha on April 1, April 15, April 29, or May 13 in 1976. Forage was sampled through Aug 9, and dry matter yields were obtained 2 weeks after application and on Sept 15. The April 29 and May 13 treatments had lowered cellulose content through July 8 and Aug 9, respectively. All treatments of mefluidide except the April 1 treatment increased crude protein content on Aug 9. In vitro cellulose digestion was increased ($P < 0.05$) and may be related to increased crude protein and decreased cellulose content. Dry matter yield was reduced ($P < 0.05$) only at 2 weeks after the April 1 and April 15 applications. Mefluidide treatments made between April 1 and May 13 enhanced forage quality. Data indicate that mefluidide affects tall fescue quality longer than previous considered.

Tall fescue (*Festuca arundinacea* Schreb.) is the predominant cool-season pasture species in the transition zone that separates the northern and southern regions in the eastern half of the United States. Kentucky 31 is the predominant cultivar. Tall fescue quality is often inadequate to produce maximum lean meat by ruminants. Forage quality decreases from the onset of reproductive growth until maturity (Blazer, 1964; Norman and Richardson, 1937). Total sugar and digestible energy decrease (Sullivan, 1969) and cellulose content increases (Phillips et al., 1954) as tall fescue matures. Burrus (1957) found that inhibition of the maturation process of tall fescue with frequent clippings (14-day intervals) produced a high-quality forage.

Mefluidide [*N*-[2,4-dimethyl-5-[[(trifluoromethyl)-sulfonyl]amino]phenyl]acetamide] is a plant growth regulator that inhibits seed head production of many cool-season grasses. Glenn et al. (1980) found that mefluidide applied on April 29 enhanced total water-soluble carbohydrate and crude protein concentration of tall fescue and decreased cellulose content through mid-May in 1975 and mid-June in 1976. The effect of mefluidide on quality later in the summer was not determined. Dry matter yield of tall fescue was decreased 21 days after treatment with 0.28 and 0.56 kg/ha mefluidide in 1975 and 0.56 kg/ha in 1976. However, there was no effect of mefluidide on dry matter yield by the second harvest.

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The objectives of this study were to examine cellulose content, crude protein content, and in vitro cellulose digestion of tall fescue through Aug 9 after treatment with mefluidide at several different dates. Dry matter yield of mefluidide-treated tall fescue was examined through Sept 15.

## MATERIALS AND METHODS

Kentucky 31 tall fescue was mowed and fertilized with 330 kg/ha 16–16–16 on March 23, 1976. Mefluidide was applied to the tall fescue at 0 or 0.28 kg/ha on April 1, April 15, April 29, or May 13. The tall fescue sward was young and vegetative on April 1 and in the late boot to early bloom stage on May 13. The plots were 2 by 7.6 m and were distributed in a randomized complete block design with four replications. The study was conducted at Lexington, KY, and with Maury silt loam (Typic Paleudalfs). Dry matter yields were obtained from a 0.9 by 6.7 m area through the middle of each plot 14 days after treatment and again on Sept 15. Immediately after harvest for yield determination the remaining portion of each plot was harvested. Grab samples were obtained from each plot on the day of but prior to treatment, May 13, July 11, July 8, and Aug 9. Precautions were taken to sample an area from each plot other than the area from which yields were obtained.

The samples were analyzed for cellulose and crude protein content and in vitro cellulose digestion was determined. The cellulose component of tall fescue was analyzed by the acetic–nitric acid (10:1) (v/v) method (Crampton and Maynard, 1938). Crude protein was calculated from total nitrogen determined by the Kjeldahl

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Table I. Donor Steer Diet for in Vitro Cellulose Digestion

| ingredient | % |
|---|---|
| ground alfalfa hay | 20.0 |
| cracked corn | 68.5 |
| soybean meal (44% crude protein) | 3.0 |
| liquid cane molasses | 7.0 |
| trace mineral salt[a] | 0.5 |
| dicalcium phosphate | 0.5 |
| vitamin premixture[b] | 0.5 |

[a] Composed of the following: zinc, 0.350%; manganese, 0.200%; iron, 0.200%; magnesium, 150%; copper, 0.030%; cobalt, 0.005%; iodine, 0.007%; salt 98.5%. [b] Composed of the following: vitamin A, 8818000 U.S.P. units; vitamin D, 1764000 I.C. units; vitamin E, 1100 INT units/kg of premixture.

method (Association of Official Analytical Chemists, 1975) and multiplied by 6.25.

For determination of in vitro cellulose digestion of tall fescue, equal quantities of grass from the four replications of each treatment were combined, and in vitro cellulose digestion was evaluated on 0.5-g samples of the composited forage. Cellulose content of the composited forage was determined before and after digestion. The in vitro system used for digestion of cellulose was a modification of the method of Baumgardt et al. (1962). Rumen fluid was obtained from a mature Angus steer fitted with a permanent rumen fistula and fed the maintenance level diet (Table I). Rumen fluid was collected 3 h after the evening feeding, strained through eight layers of cheesecloth, and placed in a thermos bottle preheated to approximately 39 °C. Anaerobic conditions were maintained in the laboratory by bubbling $CO_2$ through a buffer media while the rumen fluid was added (rumen fluid–buffer, 1:2 v/v). The buffer media had $MgSO_4$ substituted for $MgCl_2$ (McDougall, 1948). The pH of the rumen inoculum fluid was maintained in a range of 6.7–6.9, and glucose and urea were added at 0.05% w/v. The suspension was mixed in a $CO_2$ atmosphere while 30-mL portions were added to tubes containing a 0.5-g sample of forage. The incubation period was 24 h and $CO_2$ was continuously bubbled through each sample. After the incubation period 3 drops of saturated mercuric chloride solution was added to each sample to stop microbial activity. Each treatment was analyzed in triplicate and repeated with different rumen inoculum media.

## RESULTS AND DISCUSSION

Mefluidide applied on April 1 significantly reduced the cellulose content of tall fescue sampled on May 13 compared with the control (Table II). The April 15 application of mefluidide caused a reduction of cellulose content only on June 11. There was no effect on the samples obtained on May 13. This may have been due to mowing 2 weeks after application (April 29). Glenn et al. (1980) reported that an April 29 application of mefluidide continued to increase the quality of tall fescue after the forage had been mowed. However, the effect of mefluidide on quality may be reduced during the period immediately after mowing. Mefluidide applications made on April 29 and May 13 decreased the cellulose content through July 8 and Aug 9, respectively.

Glenn et al. (1980) reported that mefluidide application on April 29 reduced the cellulose content of tall fescue through May 21 in 1975 and June 24 in 1976. These data (Table II) also indicate that mefluidide decreased the cellulose content and that the effects last through July and August when mefluidide was applied on April 29 or May 13. Later mefluidide applications (April 29 and May 13) altered cellulose content longer than earlier applications (April 1 and April 15). This may be explained by the rapid growth rate of tall fescue in early April providing a greater rate of mefluidide metabolism.

Crude protein content of tall fescue was higher than the control on May 13, June 11, and July 8 when mefluidide was applied on April 1 (Table III). The April 15 application of mefluidide increased the crude protein content on June 11 and Aug 9. Neither cellulose nor crude protein content was changed with the April 15 application by May 13. Again, this observation may be confounded with

Table II. Percent Cellulose Content of Tall Fescue Forage following Treatment with 0 or 0.28 kg/ha Mefluidide

| application date | mefluidide, kg/ha | % cellulose for sampling date | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4/1 | 4/15 | 4/29 | 5/13 | 6/11 | 7/8 | 8/9 |
| 4/1 | 0 | 20.1a[a] | | | 21.7a | 24.9a | 24.1a | 24.4a |
| | 0.28 | 20.1a | | | 19.1b | 24.6a | 26.2a | 25.8a |
| 4/15 | 0 | | 18.5a | | 19.8a | 25.3a | 28.5a | 28.4a |
| | 0.28 | | 18.6a | | 18.9a | 21.7b | 26.0a | 25.2a |
| 4/29 | 0 | | | 18.3a | 22.4a | 22.7a | 26.5a | 25.0a |
| | 0.28 | | | 18.6a | 19.7b | 20.9b | 20.3b | 24.6a |
| 5/13 | 0 | | | | 20.9a | 25.3a | 26.7a | 25.1a |
| | 0.28 | | | | 22.1a | 21.7b | 21.1b | 22.7b |

[a] Means with the same letter within a column for each application date do not differ at $P = 0.05$ by the LSD test. Plots were harvested 2 weeks after the application data of mefluidide.

Table III. Percent Crude Protein Content of Tall Fescue Forage following Treatment with 0 or 0.28 kg/ha Mefluidide

| application date | mefluidide, kg/ha | % crude protein for sampling date | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4/1 | 4/15 | 4/29 | 5/13 | 6/11 | 7/8 | 8/9 |
| 4/1 | 0 | 26.3a[a] | | | 16.9b | 12.7b | 11.9b | 10.9a |
| | 0.28 | 27.3a | | | 21.4a | 15.9a | 12.8a | 11.6a |
| 4/15 | 0 | | 24.7a | | 16.1a | 13.9b | 13.1a | 11.1b |
| | 0.28 | | 23.8a | | 16.3a | 17.8a | 14.0a | 12.2a |
| 4/29 | 0 | | | 16.9a | 15.5a | 15.9a | 13.1a | 9.5a |
| | 0.28 | | | 16.9a | 15.6a | 16.3a | 15.5a | 11.0a |
| 5/13 | 0 | | | | 15.0a | 13.1a | 13.1b | 12.2b |
| | 0.28 | | | | 14.1a | 13.3a | 16.4a | 13.1a |

[a] Means with the same letter within a column for each application date do not differ at $P = 0.05$ by the LSD test. Plots were harvested 2 weeks after the application date of mefluidide.

Table IV. Percent in Vitro Cellulose Digestion of Tall Fescue Forage following Treatment with 0 or 0.28 kg/ha Mefluidide

| application date | mefluidide, kg/ha | % cellulose digestion for sampling date | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4/1 | 4/15 | 4/29 | 5/13 | 6/11 | 7/8 | 8/9 |
| 4/1 | 0 | 55.3a[a] | | | 46.8b | 53.1b | 38.5b | 46.2b |
| | 0.28 | 56.2a | | | 53.3a | 60.1a | 40.9a | 56.2a |
| 4/15 | 0 | | 58.2a | | 50.5a | 42.0b | 40.6a | 33.4b |
| | 0.28 | | 56.4a | | 49.1a | 48.6a | 39.5a | 45.5a |
| 4/29 | 0 | | | 45.3a | 47.7a | 53.7a | 42.6b | 41.0b |
| | 0.28 | | | 44.4a | 50.3a | 53.0a | 56.5a | 56.1a |
| 5/13 | 0 | | | | 42.3a | 40.0a | 38.3b | 37.6b |
| | 0.28 | | | | 41.0a | 44.0a | 48.1a | 42.5a |

[a] Means with the same letter within a column for each application date do not differ at $P = 0.05$ by the LSD test. Plots were harvested 2 weeks after the application date of mefluidide.

Table V. Dry Matter Yield (kg/ha) of Tall Fescue Forage following Treatment with 0 or 0.28 kg/ha Mefluidide

| application date | mefluidide, kg/ha | dry matter production, kg/ha, for harvest date | | | | |
|---|---|---|---|---|---|---|
| | | 4/15 | 4/29 | 5/13 | 5/27 | 9/15 |
| 4/1 | 0 | 578a[a] | | | | 2179a |
| | 0.28 | 163b | | | | 2227a |
| 4/15 | 0 | | 615a | | | 1915a |
| | 0.28 | | 427b | | | 1867a |
| 4/29 | 0 | | | 1425a | | 1983a |
| | 0.28 | | | 1265a | | 1751a |
| 5/13 | 0 | | | | 1619a | 1769a |
| | 0.28 | | | | 1715a | 1863a |

[a] Means with the same letter within a column for each application date do not differ at $P = 0.05$ by the LSD test.

mowing. Forage protein did remain above the control through Aug 9 after April 1 and April 15 applications even though most differences were not significant. Applications of mefluidide on April 29 and May 13 increased the crude protein content on July 8 and Aug 9.

Truelove et al. (1976) found that when cucumber leaf disks were preincubated with $2.9 \times 10^{-5}$ M mefluidide, incorporation of [$^{14}$C]leucine into protein was enhanced. Perhaps mefluidide stimulation of protein synthesis was responsible for the increased crude protein.

The April 1 application of mefluidide increased in vitro cellulose digestion on all sampling dates after application (Table IV). The April 1 application had no significant effect on crude protein or cellulose content sampled on Aug 9 (Tables II and III); therefore, the effect on in vitro cellulose digestion cannot be explained by decreased cellulose or increased protein contents. The April 15 application increased in vitro cellulose digestion and crude protein content on June 11 and Aug 9 (Table III), whereas cellulose content was decreased only on June 11 (Table II). In vitro cellulose digestion and crude protein content of tall fescue treated with mefluidide on April 29 and May 13 were increased on July 8 and Aug 9 (Table III). Cellulose content was decreased through this same period with May 13 applications of mefluidide, but April 29 applications did not affect cellulose content on Aug 9.

Forage quality of tall fescue deteriorates as the plant matures. Spears (1975) reported that in vitro digestibility of cellulose was greatest in tall fescue in a vegetative stage of growth and in vitro cellulose digestion decreased as the plant matured. Mefluidide is known to inhibit the maturation process of tall fescue (Chappell et al., 1977; Freeborg and Daniel, 1975; Gates, 1975; Hield and Henstreet, 1975), and this may be the mechanism by which mefluidide enhances tall fescue quality (Glenn et al., 1980). The relationship of in vitro cellulose digestion to crude protein ($r = 0.58$) and cellulose ($r = -0.53$) content indicates that the effect of mefluidide on these two quality parameters may partially explain the increased in vitro cellulose digestion of mefluidide-treated tall fescue. Effects of mefluidide on other parameters such as carbohydrate content may also be involved.

The April 1 and April 15 applications of mefluidide decreased tall fescue dry matter production 2 weeks after applications but had no effect on dry matter production on Sept 15 (Table V). The April 29 and May 13 applications of mefluidide did not reduce dry matter production at either harvest date. Nielson and Wakefield (1975) reported that turf grasses treated with mefluidide on May 1 produced significantly less top growth than the control for 7 weeks after application. This suggests differences in the effects of mefluidide on dry matter yield of turf grass (clipped frequently) and dry matter yields of tall fescue clipped less frequently to simulate pasture.

Dry matter production of tall fescue is outstanding when compared with other cool-season grasses in Kentucky. During May and June production is excessive for average stocking rates, and grazing animals are unable to consume amounts necessary to prevent reproductive development of tall fescue which is associated with deterioration of forage quality. Maintaining high yields during this period is not as important as preventing quality deterioration. Reductions in dry matter yields of tall fescue treated with mefluidide on April 1 or April 15 may be more than offset by enhanced forage quality.

Mefluidide increased the quality of tall fescue, while dry matter yield was reduced early in the season from the April 1 and April 15 applications. Improved quality from mefluidide treatments has been related to the ability of mefluidide to prevent reproductive development of tall fescue (Glenn et al., 1980). However, this only partially explains the quality response to mefluidide, since the effects on tall fescue quality remain long after the visual effects on the maturation process have disappeared. Feed digestibility is highly correlated to lean meat production of ruminant animals. The in vitro cellulose digestion response to mefluidide indicates the potential of mefluidide to improve tall fescue quality as a forage for ruminant animals.

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# Ammoniation Products of an Aflatoxin Model Coumarin

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Treatment of 5,7-dimethoxy-9-oxocyclopenteno[*c*]coumarin (**2**) with aqueous ammonia at 37 °C produces three major products identified as 3-(2-hydroxy-4,6-dimethoxyphenyl)-2-cyclopentenone (**3**), 3,5-dimethoxyphenol (**4**), and 3-(2-hydroxy-4,6-dimethoxyphenyl)-2-hydroxy-2-cyclopentenone (**5**). Compound **5**, which is not derived from **3**, further decomposes in aqueous ammonia to **4**. The keto group on the cyclopentene ring of **2** is required for ammonia-induced decomposition because 5,7-dimethoxycyclopenteno[*c*]coumarin (**1**) fails to react under conditions that degrade **2**.

Aflatoxin levels in highly contaminated corn can be effectively reduced by treatment with low concentrations of ammonia at atmospheric pressure (Brekke et al., 1977a, 1979). In this process, contaminated corn at 17.5% moisture content is treated with 1.5% gaseous ammonia at ambient temperatures ranging from 32 to 43 °C for 13 days. The corn is then dried at 40 °C to 10% moisture. The process reduces aflatoxin to below the FDA action level of 20 ng/g. Although biological data indicate that the process is feasible for detoxification (Brekke et al., 1977b; Norred, 1979; Southern and Clawson, 1980), the chemical fate of aflatoxin during the ammoniation process is not known.

In studies of the ammoniation of aflatoxin $B_1$ (Figure 1) at elevated temperature and pressure, the major products were identified as aflatoxin $D_1$, which results from lactone ring opening followed by decarboxylation, and a molecular weight 206 phenol that lacks the cyclopentenone ring of $D_1$ (Lee et al., 1974; Cucullu et al., 1976). Subsequent work using aflatoxin $B_1$ spiked peanut and cottonseed meals showed this process to be additionally complicated by the meals, because $D_1$ was found in only trace amounts (Lee and Cucullu, 1978). Use of radiolabeled aflatoxin $B_1$ allowed approximately 50% of the added toxin to be accounted for by several products, but the remainder appeared to be lost through volatilization (Lee et al., 1979). The present study was undertaken to further explore the sequence of reactions that occurs during ammoniation of aflatoxin as they relate to the corn decontamination process, which is carried out under considerably milder conditions than the peanut–cottonseed meal process. Model coumarins **1** and **2** (Figure 2), rather than aflatoxin, were used which allowed the reactions to be carried out on a scale that facilitated isolation and characterization of the products without the concomitant hazard in using carcinogenic aflatoxin. Also, structural requirements for reactivity as influenced by other functional groups could be easily studied by using model compounds. These compounds have been shown to be nonlethal and noncarcinogenic to rats at doses greatly exceeding the effective levels of aflatoxin $B_1$ (Wogan et al., 1971).

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## EXPERIMENTAL SECTION

**Analytical Procedures.** Infrared spectra (IR) were recorded in chloroform with a Beckman IR8 spectrophotometer. Ultraviolet spectra (UV) were recorded in ethanol with a Unicam SP 800 spectrophotometer. Mass spectra (MS) were obtained by electron impact at 70-eV ionizing electron energy and a source temperature of 200 °C with a Kratos MS 30 spectrometer. Samples were introduced by a direct insertion probe or a gas chromatograph (GC) inlet. For GC–MS, a 3-ft 3% OV-1 column was temperature programmed from 70 to 250 °C at 4 °C/min. The helium flow rate was 40 mL/min, and the GC was coupled to the MS by using a single-stage glass jet separator.

Proton nuclear magnetic resonance (NMR) spectra were obtained with a Varian HA-100 or Bruker WH-90 Fourier transform spectrometer. Carbon-13 NMR spectra were obtained with the Bruker instrument. Chemical shifts are reported as $\delta$ values in ppm downfield from internal tetramethylsilane. Column chromatographic separations were done on silica gel 60 (70–230 mesh; EM Laboratories). Thin-layer chromatography (TLC) was carried out on precoated 0.25-mm layers of silica gel 60, F-254 (EM Laboratories), with dichloromethane–acetone (4:1) as the developing solvent. Components were visualized under long- and short-wave UV light and with iodine vapor.

**Synthesis of Coumarins 1 and 2.** The procedures described by Asao et al. (1965) were employed for the synthesis of 5,7-dimethoxycyclopenteno[*c*]coumarin (**1**) and 5,7-dimethoxy-9-oxocyclopenteno[*c*]coumarin (**2**).