Alkaloid Affects in Vitro Dry Matter Digestibility of *Festuca* and *Bromus* Species

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Abstract

A field evaluation study was made on eight pasture grass species, 'Latar' orchardgrass (*Dactylis glomerata* L.), 'Fawn' tall fescue (*Festuca arundinacea* Schreb.), 'Manchar' bromegrass (*Bromus inermus* Leyss.), 'Regar' bromegrass (*B. biebersteinii* Roem and Schult.), 'Garrison' creeping foxtail (*Alopecurus arundinacea* Poir.), 'Meadow' foxtail (*A. pratensis* L.), 'Luna' pubescent wheatgrass [*Agropyron trichophorum* (Link) Richt.], and 'Greenar' intermediate wheatgrass [*A. intermedium* (Host) Beauv.]. Three harvests of each species were analyzed for in vitro dry matter digestibility (IVDMD) for 1975, 1976, and 1977. Fawn tall fescue and Manchar bromegrass had severely depressed IVDMD values in the second harvest of 1976 and both species and Regar bromegrass had low values for the third harvest of that year. The forages were analyzed for perloline alkaloid by a thin layer chrometography method and the depressed IVDMD values were found in the replicate samples containing perloline. The alkaloid could be toxic to livestock.

Dry matter digestibility is an important measure of forage quality which can be determined by in vitro and in vivo methods. The alkaloid perloline has been found to inhibit in vitro dry matter digestibility (IVDMD) in several studies of species of *Festuca* and *Lolium* (Bush et al. 1970, Gentry et al. 1969, Jeffreys 1964). In this research on irrigated forages, inhibited IVDMD and alkaloid concentrations were observed not only in *Festuca,* but also in *Bromus* species. On western rangelands, these grasses are generally grown on relatively small areas under irrigation and are frequently used as supplemental pasture for beef cattle (Nichols 1975).

Methods

The eight grass species studied were 'Latar' orchardgrass (*Dactylis glomerata* L.), 'Fawn' tall fescue (*Festuca arundinacea* Schreb.), 'Manchar' bromegrass (*Bromus inermus* Leyss.), 'Regar' bromegrass (*B. bierbersteinii* Roem and Schult.), 'Garrison' creeping foxtail (*Alopecurus arundinacea* Poir.), 'Meadow' foxtail (*A. pratensis* L.), 'Luna' pubescent wheatgrass [*Agropyron trichophorum* (Link) Richt.], and 'Greenar' intermediate wheatgrass [*A. intermedium* (Host) Beauv.]. These irrigated forages were established in the field at Cheyenne, Wyo., in the spring of 1974 as part of a large evaluation trial in 2.4 × 4.8 m plots. Two centimeters of water were applied to the grasses every 3 or 4 days. Each species was replicated twice. Ammonium nitrate was applied to the grasses each year near May 1 and July 1 at the rate of 76 kg N/ha. Harvests were made each year when the grass was at boot or heading stage on the first crop and when regrowth was 20 to 30 cm tall on succeeding crops. Forage from each plot area was clipped, weighed, and subsampled. The subsample of green forage was placed in a drying oven within 1 hour after harvest and dryed at 65° C to determine dry matter production. The forage IVDMD for each of three harvests per year during 1975 thru 1977 was determined by a modified Tilly and Terry (Tilly and Terry 1963) method. The alkaloid concentration in the 1976 harvests were determined by a thin layer chromatography (TLC) method as follows:

A 1-gram sample of dried and ground plant material was mixed with 0.3 g of sodium bicarbonate and 2 ml of water in a small beaker. The mixture was stirred with a glass rod for 2 minutes. Ten ml of a $CHCl_3$/MeOH (9:1) solution was added to the mixture and stirred with a magnetic stirrer for 30 minutes. The mixture was then filtered and the filtrate was evaporated *in vacuo* to a volume of 1 ml. The alkaloids were separated from other plant material by a silica gel TLC. Forty microliter aliquots were spotted and developed with the upper phase of a mixture of butanol/acetic acid/water (4:1:5). An iodoplatinic acid solution, used for visulization of spots, consisted of a mixture of 10 ml of a 5% platinum chloride, 5 ml of conc. HCl, and 240 ml of a 2% KI solution.

Results and Discussion

Festuca and *Bromus* species had significantly inhibited IVDMD in the second and third harvests of 1976 compared with other species in the study. While all grass species showed depressed IVDMD values for the first harvest of 1975, this was probably caused by the fiber content of the more mature forage compared with the re-growth of succeeding harvests of that year. During the study, severe inhibition of IVDMD did not occurr in Latar orchardgrass, Garrison creeping foxtail, Meadow foxtail, Luna pubescent wheatgrass, and Greenar intermediate wheatgrass. Therefore, IVDMD values were shown in Table 1 for a representative noninhibited species, Latar orchardgrass, and three inhibited species, Fawn tall fescue, Manchar brome, and Regar brome. The alkaloid analysis was done on the 1976 harvests of these four grasses. All of the 1976 samples with inhibited IVDMD showed perloline (Table 2) except for the second harvest of Manchar brome which contained only a trace of alkaloid that was not identifiable. This may have been due to a breakdown with time in the perloline alkaloid within the forage samples. Gentry et al. (1969) found much higher perloline concentrations in pasture forage than in 2-year old hay samples. The alkaloid analyses of this study were made 3 years after the IVDMD had been determined. Although Fawn tall fescue contained several unidentified alkaloids, inhibition of IVDMD in this species appeared to be only related to samples with identifiable perloline.

Perloline synthesis in plants has been shown to be associated with N fertilization and radiant energy (Noller and Rhykerd 1974). Since fertilization was made at the same time and rate each year, perloline buildup in tall fescue and bromegrass in 1976 was probably caused by differences in temperatures as compared with those in 1975 or 1977. The temperatures during the 1976 growing season were generally higher than those for 1975 or 1977 (Dept. of Commerce 1975-77).

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Table 1. In Vitro dry matter digestibility of four grass species at three harvests per year during 1975, 1976, and 1977 at Cheyenne, Wyo.[1].

Species	1975 Harvests			1976 Harvest			1977 Harvest		
	1	2	3	1	2	3	1	2	3
Latar orchardgrass	58.2a	62.5a	64.5a	67.5a	60.0a	66.5a	—	—	65.5b
Fawn tall fescue	51.2b	61.0a	65.2a	62.2a	41.5c	33.7b	58.0a	62.9a	66.4b
Manchar bromegrass	54.2ab	61.7a	62.5a	66.2a	48.2b	40.0b	63.1a	65.5a	75.9a
Regar bromegrass	59.5a	60.5a	68.0a	61.7a	63.0a	35.0b	65.3a	60.0a	73.5a

[1]Within a column values followed by the same letter are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 2. Alkaloids[1] in four grass species at three harvests in 1976.

Species	Rep.	1st Harvest			2nd Harvest			3rd Harvest		
		R_f	Relative amounts	Spot color	R_f	Relative amounts	Spot color	R_f	Relative amounts	Spot color
Latar orchardgrass	1	—[2]	—	—	—	—	—	—	—	—
Latar orchardgrass	2	—	—	—	—	—	—	—	—	—
Fawn tall fescue	1	0.12	++	Blue	0.10	++	Blue	0.10	++	Blue
		0.17	+++	Brown	0.15	+++	Brown	0.15	trace	Brown
		0.26	+	Blue	0.24	+	Blue	0.24	+++	Blue
Fawn tall fescue	2	0.11	++	Blue	0.09	++	Blue	0.11	++	Blue
		0.16	+++	Brown	0.15	++++	Brown	0.15	trace	Brown
		0.26	+	Blue	0.23	++	Blue	0.23	+++	Blue
Manchar bromegrass	1	—	—	—	trace not identifiable			0.15	+	Brown
Manchar bromegrass	2	—	—	—	trace not identifiable			0.15	trace	Brown
Regar bromegrass	1	—	—	—	—	—	—	0.15	+	Brown
Regar bromegrass	2	—	—	—	—	—	—	0.15	trace	Brown

[1]Known perloline samples showed an R_f value of 0.15 with a brown spot.
[2]—means none present.

The inhibition IVDMD associated with the presence of perloline in bromegrass species is unusual. These species can generally be depended upon for high quality forage. The observation reported here indicates that perloline syntheses in bromegrass, as well as fescues, can occur when summer temperatures are high. Livestock producers and agronomists need to be aware of this possibility.

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