# Barium in Forage Plants and in the Manure of Cattle Treated with Barium Boluses

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An atomic absorption spectroscopy procedure was developed for the determination of barium in the manure of barium-treated cattle. Although considerable background levels of barium were present, the difference between treated and untreated cattle was sufficient to allow for separation. Two weeks after treatment with a bolus containing barium, the tests for barium in the manure indicted that 70% of the cattle still retained an intact bolus. Background levels of barium averaged 17.5 ppm of dried manure, but plants available to the cattle contained from 4.4 to 25.2 ppm of barium.

With the development of the sustained-release bolus, containing an insecticide to control flies breeding in cattle manure (Miller et al., 1979), the question arose as to how well the bolus is retained in the bovine's intestinal system. The bolus is prepared with a high concentration of a heavy element, usually barium, in the form of barium sulfate ($BaSO_4$). The bolus, given orally, is generally assumed to be deposited in the reticulum because of its weight, and it should remain in that location while it slowly erodes and releases the insecticide. An analysis of the manure of treated cattle for barium should indicate whether the bolus was retained; however, barium is present in trace amounts in all plant material and could interfere in the separation of treated and untreated animals.

While the determination of barium in cattle manure has not been reported, emission spectrography has shown barium in barley and bush bean shoots (Chaudhry et al., 1977). Smith (1971) used neutron activation analysis to determine barium in wheat shoots. Results of these investigations, however, indicate that the amount of barium in forage-type plants (and presumably in cattle manure) would vary depending on the part of the plant analyzed and on the type of soil the plants were grown in. When 500 ppm of barium was added to the soil (Chaudhry et al., 1977), the barium was increased in the barley plants to over 2000 ppm (dry basis).

Our investigation was undertaken to determine whether by barium analysis of cattle manure we could determine which animals retained barium boluses and if the naturally occurring barium in the manure interfered with the determination. Also, we determined the source of the natural barium in the manure.

## EXPERIMENTAL SECTION

**Apparatus.** A Perkin-Elmer Model 403 atomic absorption spectrophotometer, equipped with a barium hollow cathode lamp (Westinghouse) and a nitrous oxide burner, was used to determine the barium. The instrument was operated in the "continuous mode", and the height of the atomization peak was measured. Other parameters on the spectrophotometer were a slit width of 0.3 nm and a spectral band of 4 Å. The wavelength counter was set at 277, with the range control at "visible" to give the correct 5536-Å wavelength. The recorder response control was set at 1 s, and the recorder set full-scale at 0.5 absorbance. Data were recorded on a Leeds-Northrup Speedomax-XL recorder set on the 1- or 2-mV scale. When the recorder was set full-scale at 0.5 absorbance and the recorder was set at 1 or 2 mV, the sensitivity was increased by factors of 10 or 20 over the usual 1.0 absorbance and the 10-mV recorder setting. While recorder noise increased, it was more than compensated for by the increased sensitivity to barium. It was critically important to adjust the nitrous oxide–acetylene flame, so that the rose-colored portion of the flame was 1.0 cm in height and the total flame height was about 45 cm, in order to obtain the maximum sensitivity for barium.

**Reagents.** The distilled water was purified by passage through a mixed-bed ion-exchange resin. Reagent-grade acids free of barium were used, and standard barium solutions were prepared from either reagent-grade barium chloride ($Ba\ Cl_2 \cdot 2H_2O$) or reagent-grade barium nitrate [$Ba(NO_3)_2$]. The weighed standards were diluted with the deionized water and sufficient reagent-grade potassium chloride and hydrochloric acid to give a final concentration of 2000 ppm of K solution with 1% HCl. The spectrophotometer was zeroed with the reagent blank to eliminate any possibility of the effect of trace amounts of barium in the reagents.

**Procedure.** All glassware and crucibles were originally cleaned in detergent and then rinsed with distilled water followed by a rinse in distilled deionized water. Between determinations, all crucibles were washed and then filled, with 3.7 N $HNO_3$, for 3–24 h before rinsing in distilled water and deionized distilled water. Determinations for barium were made on weighed samples of either wet cattle manure or dried forage. The moisture content of the manure was found to be a relatively constant 79–82%, but grass varied from 65 to 80% depending on season and moisture conditions; therefore, forage determinations were made by using oven-dried material. Three to five 1-g samples of manure or 200-mg samples of dried forage were weighed out for each test. Manure samples were dried for 1 h at 125 °C and then ashed at 500 °C for 7 h in a muffle furnace. The forage was similarly ashed without the drying period. After the ashed samples had cooled, 0.5 mL of 20% HCl was added slowly, and any residue in the bottom of the crucible was crushed with a glass rod. After soaking for about 30 min, the samples were further diluted with 9.5 mL of 2100 ppm of K solution. After every five test samples, a standard was aspirated as an internal check of the sensitivity of the spectrophotometer.

## RESULTS AND DISCUSSION

Initially, we attempted to wet ash the dried feces with concentrates of sulfuric acid and nitric acid, such as was used for the digestion of insects (Chamberlain, 1977), but digestion was incomplete and the soluble portion did not dilute satisfactorily. Even the use of larger amounts of acid

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Table I. Barium in Various Forage Plants and Cattle Manure

| sample | location | ppm of barium,[a] mean ± SD |
|---|---|---|
| Bermuda grass | Kerrville | 19.7 ± 0.60 |
| | Camp Stanley | 18.3 ± 0.83 |
| alfalfa (*M. sativa* L.) | New Mexico | 4.4 ± 0.38 |
| little bluestem (*An. scoparius frequens* F. T. Hubb) | Kerrville, no. 1 | 22.5 ± 1.97 |
| | Kerrville, no. 2 | 14.3 ± 0.79 |
| | Camp Stanley | 16.0 ± 0.59 |
| Texas winter grass (*S. leucotricha* Trin. + Rupr.) | Camp Stanley | 5.1 ± 0.60 |
| purple threeawn (*Ar. purpurea* Nutt.) | Camp Stanley | 25.2 ± 2.40 |
| cattle manure (alfalfa fed) | Kerrville | 13.7 ± 0.84 |
| cattle manure (grass fed) | Camp Stanley | 17.6 ± 1.38 |

[a] Based on dry weight.

Table II. Detection of Barium in Manure of Pasture-Held Cattle

| | | amount of barium, ppm of wet manure | |
|---|---|---|---|
| | animal no.[a] | pretreatment value, mean ± SD | 2 weeks posttreatment, mean ± SD |
| yearlings | 1 | 5.1 ± 0.18 | 6.8 ± 0.14 |
| | 2 | 2.8 ± 0.16 | 11.4 ± 0.19 |
| | 3 | 3.4 ± 0.09 | 14.3 ± 0.34 |
| | 4 | 4.8 ± 0.15 | 5.7 ± 0.12 |
| | 5 | 3.1 ± 0.12 | 24.1 ± 0.57 |
| 2 year olds | 6 | 3.7 ± 0.09 | 6.1 ± 0.12 |
| | 7 | 3.8 ± 0.03 | 2.2 ± 0.18 |
| | 8 | 2.2 ± 0.13 | 4.0 ± 0.15 |
| | 9 | 3.2 ± 0.14 | 6.9 ± 0.14 |
| | 10 | 3.2 ± 0.13 | 9.0 ± 0.19 |

[a] Mixed sex.

was unsatisfactory, possibly because the feces contained ca. 200 mg of dried material compared with the <5 mg of insect material.

A number of attempts were made to completely dissolve the residue. The increase of the ashing temperature, the use of strong NaOH or concentrated $H_2SO_4$, or the use of 20% HF has only minor effects on the determination of barium. The results of these tests suggested that a portion of the residue was some form of a fused silicate. When the ashing temperature was increased, the amount of detectable barium was decreased. When $Ba(NO_3)_2$ was added to filter paper and ashed at 500 °C, the recovery was 94–99%, but when Ba ($NO_3$) was ashed at 700 °C, the recovery was 45–70%. With manure, the recovery at 500 °C was 79% but at 700 °C only 22–35%. When HF was used to partially dissolve the residue, variable results were obtained.

Since it was necessary to show that the barium from the bolus could be detected in the manure of a steer, a test animal was given 200 mg of $BaSO_4$ by oral capsule on each of 10 consecutive days. The barium equaled 118 mg daily and was thought to approximate that released by a typical barium bolus. The average amount of barium in the wet manure 40–290 h after the start of the test averaged 11.7 ± 0.52 ppm for nine sampling times (five subsamples each) posttreatment. The five subsamples for each sampling time also enable an estimate of the reproductivity of the measurement technique. The coefficient of variation (variance as a percentage of the mean) for multiple observations on the same sample ranged from 1.7% to 5.3% for a mean of 3.7%. This level of variation was considered adequate for our purposes. Before treatment, the manure averaged 2.2 ± 0.14 ppm. Thus, the additional amount of barium contributed by the capsule was 9.5 ppm. Since the steer produced an average of 8058 g of manure daily, the added amount of barium provided by the capsule should have been 14.6 ppm, and with the natural barium the theoretical total should have been 16.8 ppm. Thus, by actual analysis of the manure 65% recovery (9.5/14.6) was shown, which is considerably below 79% recovery found when barium was added directly to manure.

The amount of natural barium in the wet cattle manure obtained from forage varied in most samples from 1.0 to 4.0 ppm. No detectable amounts of barium were found (less than 0.02 ppm) in the drinking water of the cattle. The variability in the amounts of barium in different forage species is shown in Table I. Alfalfa (*Medicago sativa* L.) had the lowest barium levels, and the manure of cattle fed on alfalfa had lower levels than grass-fed cattle. The highest amounts of barium were found in purple threeawn (*Aristida purpurea* Nutt.), a range grass native to the area. The wide range of barium in the wet feces is probably related to the availability, location, and choice of forage by the cattle.

Manure collected from three rumen-fistulated animals previously treated wth boluses was analyzed for barium. Two of the animals had been given 50-g barium boluses (75% $BaSO_4$) 2 months before collection of the manure; one had two boluses and the other one bolus. From the third animal treated 7 months previously, no bolus had been detected by physical examination for over 4 months. Analyses showed that wet manure from the animal with one bolus had 11.0 ppm of barium, from the animal with two boluses 11.3 ppm, and from the one with no apparent bolus 7.5 ppm of barium. These results were not in agreement with the physical erosion of the boluses because the single bolus was eroding at the rate of 0.10 g/day, and the two boluses were eroding at the rate of 0.25 g total/day. Thus, the barium in the manure of the animal with two boluses should have been more than twice that of the animal with a single bolus. The results from the animal with no apparent bolus suggest that some particles of earlier boluses remain in the reticulum since no other untreated animal fed on alfalfa had shown more than 5 ppm of barium in the wet manure. This factor may interfere with detection of the loss of a bolus. However, the results of this test clearly indicated that the presence of the barium boluses was detectable in the cattle 2 months after initial treatment.

The manure of 10 cattle held on range-type pasture, with the dominant grasses consisting of little bluestem (*Andropogon scoparius frequens* F. T. Hubb), purple threeawn (*Ar. purpurea* Nutt.), and some Texas winter grass (*Stipa leucotricha* Trin. and Rupr.), was examined for barium before treatment and 2 weeks after each animal had been given 50-g boluses containing 75% $BaSO_4$. Five of the animals were yearling cattle, and five were 2-year-old cattle. The two groups were a mixture of both sexes, but the males were steers.

The quantity of barium in the manure of these cattle before and after treatment is shown in Table II. The average amount of barium in the manure of the yearling cattle was 3.8 ± 0.47 ppm before treatment while that of the 2-year-old cattle was 3.2 ± 0.28 ppm, but after treatment the averages were 12.5 ± 3.30 and 5.6 ± 1.18 ppm, respectively. There was a significant (5% level) barium increase due to treatment of the yearling group but not of the 2 year olds. The larger size of the older animals

makes it more difficult to detect the 50-g bolus by barium analysis, presumably because the large animals have larger intestinal systems and eat more, thus diluting the barium. However, apparently some animals had not retained the bolus. In only one case was the barium content less after treatment than before treatment. This is an indication that the bolus was not retained. If the acceptance or rejection criteria is arbitrarily set at 1.5 times the pretreatment level, we could also conclude that the bolus was present in only seven of the ten aminals 2 weeks after treatment.

Although the vegetation on which animals are grazing will affect the background levels of barium detected in the feces, this method will be useful in future bolus retention studies provided that base-line data are collected to determine the background levels and statistical techniques can be developed as criteria to accept or reject a level of barium as indicative of the presence of the bolus.

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# Evaluation of Food Potential, Some Toxicological Aspects, and Preparation of a Protein Isolate from the Aerial Part of Amaranth (Pigweed)

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*Amaranthus* spp. (pigweed, water hemp, etc.) grows profusely in cropped fields in the midwest and is considered a troublesome weed. Vegetative parts of the plants are high in protein, calcium, potassium, iron, and ascorbic acid, indicating a high food potential, but also may contain appreciable amounts of nitrate and oxalate. Although the leaves and tender tops of these plants have been used for human consumption on three continents for centuries with no apparent problems, the potential for toxic effects cannot be ignored. Several fractions taken from fresh, cooked, and oven-dried plants were analyzed for nutrient content and also for levels of nitrate and oxalate. Results indicate that any nitrate and soluble oxalate are removed by extraction into the cooking water. The resulting food appears to be of good quality as determined by chemical analyses.

In recent years a number of articles have appeared in scientific as well as in the popular press extolling the virtues of amaranth as a potential crop for feeding a protein-hungry world (Cole, 1979; Marx, 1977; Olatunbosun, 1976; "Proceedings of the First Amaranth Seminar", 1977; "Proceedings of the Second Amaranth Conference", 1980; NAS, 1975). For centuries both vegetative and grain varieties of amaranth have been a dietary staple for humans in many tropical and subtropical countries spread over three continents. Because the leaf tissue wilts rapidly soon after harvest, amaranth greens are usually obtained from garden plots or from fence rows rather than from markets. Consequently, it is the poor, rural people in these countries, rather than the urban dwellers, who use these plants as potherbs.

Varieties of *Amaranthus* spp. known popularly as pigweed, redroot, water hemp., etc. grow profusely in the cultivated fields in Midwestern United States, especially in fields which are summer fallowed. While descriptions of these species may be found in lists of edible plants (Hall, 1973; McPherson and McPherson, 1977), farmers in this area consider them to be troublesome weeds.

For decades we have been cognizant that the vegetative parts of these plants may contain high levels of nitrate and that they have been implicated in numerous cases of livestock poisoning (Duckworth, 1975; Osweiler et al., 1969). In addition to high nitrate levels, usually there is a high level of oxalate which is linked also to livestock problems (Marshall et al., 1967) and problems in humans as well (Valyasevi and Dhanamitta, 1974; Hodgkinson, 1977). Therefore, *Amaranthus* spp. remains an enigma. The present study was undertaken with three main objectives: (1) to determine the levels of nitrate and oxalate present in varieties of amaranth found in the Midwest; (2) to make compositional analyses of the aerial parts of the common varieties of amaranth native to the Midwest; (3) to evaluate these plants as potential sources for a protein isolate which might have possible use for diet enrichment. A procedure for the preparation of such of a protein isolate from the total aerial parts of five common varieties of amaranth is given.

## MATERIALS AND METHODS

Dried samples of various fractions of mature *A. edulis* plants, a grain amaranth, grown at the University of Nebraska by using seed obtained from Nigeria, were made available to us. These samples were analyzed for the usual food constituents as well as for nitrate and oxalate content. All other studies were conducted on wild plants harvested at Lincoln, NE, or Waseca, MN, during the summer of 1980. These are *A. caudatus* (an ornamental variety), *A. retroflexus* (pigweed; redroot), *A. hybridus* (both red stem and green stem varieties), and *A. graecizans* (prostrate pigweed). The plants of upright habit were harvested when 1–2 m tall; stems were cut at 10 cm above the roots. The prostrate variety was harvested when the runners were at least 1 m long.

Total nitrogen was estimated according to the Kjeldahl procedure 46-12 approved by the American Association of Cereal Chemists in 1976 (AACC, 1969). Nitrate nitrogen

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