# EFFECT OF CALCIUM NUTRITION ON THE FORMATION OF CALCIUM OXALATE IN KIKUYU GRASS

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## ABSTRACT
The objective of this investigation was to study the formation of calcium oxalate crystals *in situ* in kikuyu grass (*Pennisetum clandestinum*) and to determine the effect of calcium uptake on the soluble and insoluble oxalate content of the grass. Oxalate crystals were more abundant in high-calcium leaves than in low-calcium leaves. Electron microscopy showed that the oxalate crystals are formed in association with globular electron-dense bodies in the vacuole of certain cells. The calcium content of kikuyu is increased by increasing the calcium level of the growth medium. However, the increased amount of calcium taken up by the plant is largely bound as insoluble calcium oxalate, which is poorly available to the grazing animal.

## KEYWORDS
Kikuyu, calcium, oxalate, animal nutrition, crystal idioblasts, electron microscopy

## INTRODUCTION
In terms of the requirements of high-producing dairy cattle, calcium concentrations and calcium to phosphorus ratios of kikuyu grass (*Pennisetum clandestinum*) are often well below critical limits (Miles *et al*., 1995). Furthermore, members of the genus *Pennisetum* have been shown to accumulate oxalates (McKenzie, 1985, Marais, 1990). Oxalic acid in plants often reacts with monovalent cations such as potassium or sodium to form soluble oxalate salts and sparingly soluble chelates in the presence of divalent cations such as calcium or magnesium. Of these chelates, calcium oxalate is the most stable and the least soluble. These insoluble crystals form in the vacuoles of specialized cells, called crystal idioblasts, which are often associated with the vascular system of the plant and tend to pass largely intact through the digestive tract of animals (Ward *et al*., 1979). Based on molar ratios and the assumption of preferential binding of calcium by oxalate, at least 95% of the calcium in kikuyu grass is bound as insoluble calcium oxalate and would be poorly available to ruminants (Marais, 1990).

Relatively large responses in forage calcium could be expected following fertilizer calcium applications to tropical pastures (Minson, 1990). Calcium deficiencies in animals on kikuyu could therefore be corrected by calcium fertilization provided the content of available calcium in the plant is increased.

The aim of this investigation was to study the formation of calcium oxalate crystals *in situ* and to determine the effect of calcium fertilization on soluble and insoluble oxalate concentrations in kikuyu.

## METHODS
Kikuyu tillers (200 mm long) were grown in a greenhouse and maintained continuously at 27$^0$C in aerated solution culture based on Medium II of Shive & Robbins (Hewitt, 1952). The calcium content of the medium was adjusted to either 0.40 mM or 2.85 mM and the two treatments arranged in a simple random design. After a growth period of six weeks the grass was sampled, divided into a leaf blade fraction and a leaf sheath plus stem fractions and analyzed for soluble and insoluble oxalate (Moir, 1953). The significance of the difference between the oxalate content of the high and low calcium treatments was assessed by analysis of variance.

The mid-portion of mature leaf blades of plants from both calcium treatments was sampled and prepared for transmission electron microscopy. Ultra thin sections were stained with uranyl acetate and lead citrate, and were examined and photographed with a Phillips 301 transmission electron microscope. Similar plant samples were also cryofractured in liquid nitrogen and prepared for scanning electron microscopy, using a Joel 6100 scanning electron microscope equipped with a Noran Voyager 2100 energy dispersive X-ray (EDX) microanalyser. X-ray elemental dot maps and line scans for calcium were made on the samples.

## RESULTS AND DISCUSSION
Transmission electron microscopy showed oxalate crystals in certain mesophyll cells associated with the vascular bundles in kikuyu leaves. Crystals were also occasionally observed in epidermal cells. Oxalate crystals were more abundant in leaves from the high-calcium treatment than in those receiving less calcium.

Developmental studies of crystal idioblasts have shown that calcium oxalate crystals are usually produced in membrane-like compartments within the vacuoles (Franceschi, 1984). Crystal formation in kikuyu leaf cells appears to occur in a similar manner. Electron-dense bodies, apparently of cytoplasmic origin, occur in the central vacuole of crystal cells and appear to give rise to the calcium oxalate crystals. Horner and Whitmoyer (1972) regarded the electron-dense bodies as compacted stores of membranes giving rise to crystal chambers which, apparently, determine the shape and size of the developing crystal.

Scanning electron microscopy of cryofractured leaf material showed that the electron-dense bodies are joined together in long strings, often by means of thin tube-like connections. These strings of bodies join centrally within the vacuole to form a mass of electron-dense material in which the calcium oxalate crystal develops. The position of the crystal within the central mass was verified by means of X-ray elemental dot maps and line scans for calcium.

The nature and function of the strings of bodies in crystal formation are uncertain. They do not appear to provide calcium to the central crystal chamber, since X-ray elemental scans on the strings were negative for calcium. They could, however, be involved in oxalic acid synthesis, or the transport of oxalic acid to the site of crystal formation.

Results presented in Table 1 show that by increasing the calcium level of the growth medium the calcium content of both kikuyu leaf and stem was increased. Although the total oxalate content of leaf and stem material remained constant, the plants grown in the medium with the high calcium level contained more insoluble calcium oxalate and less soluble oxalate in both leaf and stem than the low calcium treatment. These results suggest that the increased amount of calcium taken up from the high-calcium nutrient medium largely replaced the monovalent cations in soluble oxalate to form insoluble calcium oxalate, which would be poorly available to the grazing animal.

Although the high-calcium treatment reduced the molar ratio of insoluble oxalate to calcium in kikuyu, 84% of the calcium in the leaf and 66% of the calcium in the stem remained bound as insoluble calcium oxalate. It therefore appears unlikely that liming of kikuyu pastures would substantially improve the availability of calcium to ruminants on kikuyu. Direct supplementation of the animal with calcium should be more effective.

## REFERENCES

**Franceschi, V.R.** 1984. Developmental features of calcium oxalate crystal sand deposition in *Beta vulgaris* L.leaves. Protoplasma **120**:216-223.

**Horner, H.T. and Whitmoyer, R.E.** 1972. Raphide crystal cell development in leaves of *Psychotria punctata* (Rubiaceae). J. Cell Sci. **11**:339-355.

**Hewitt, E.J.** 1952. Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux, England. 86pp.

**Marais, J.P.** 1990. Effect of nitrogen on the oxalate and calcium content of Kikuyu grass (*Pennisetum clandestinum* Hochst). J. Grassl. Soc. South. Afr. **7**:106-110.

**McKenzie, R.A.** 1985. Poisoning of horses by oxalate in grasses. Pages 150-154 *in* Plant toxicology. Proceedings of the Australia - USA poisonous plant symposium, Brisbane, Australia, May 14-18, 1984.

**Miles, N., De Villiers, J.F. and Dugmore, T.J.** 1995. Macromineral composition of kikuyu herbage relative to the requirements of ruminants. J. S. Afr. Vet. Ass. **66**:206-212.

**Minson, D.J.** 1990. Forage in ruminant nutrition. Acad. Press, London. 208pp.

**Moir, K.W.** 1953. The determination of oxalic acid in plants. Queensland J. Agri. Sci. **10**:1-3.

**Ward, G., Harbers, L.H. and Blaha, J.** 1979. Calcium containing crystals in alfalfa: their fate in cattle. J. Dairy Sci. **62**:715-722.

**Table 1**
Effect of nutrient calcium level on the oxalate and calcium composition of kikuyu grass.

| | Leaf | | | Stem | | |
|---|---|---|---|---|---|---|
| Composition* (g.kg$^{-1}$) | Low Ca | High Ca | Sign. | Low Ca | High Ca | Sign. |
| Soluble oxalate | 17.3 | 13.6 | P<0.01 | 7.5 | 6.5 | NS |
| Insoluble oxalate | 7.1 | 10.8 | P<0.05 | 1.9 | 3.3 | P<0.01 |
| Total oxalate | 24.4 | 24.4 | NS | 9.4 | 9.8 | NS |
| Calcium | 2.47 | 5.10 | P<0.01 | 0.92 | 2.21 | P<0.01 |
| Ox:Ca** | 1.40 | 0.84 | P<0.01 | 0.94 | 0.66 | NS |

* Mean of 6 replicates
** Insoluble oxalate:calcium molar ratio