

## OXALATE ACCUMULATION IN FODDER CROPS AND IMPACT ON GRAZING ANIMALS-A REVIEW

MEENAKSHI GOYAL\*

Department of Plant Breeding and Genetics,  
Punjab Agricultural University, Ludhiana

\*(e-mail : meenakshigoyal@pau.edu)

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### SUMMARY

Oxalate content in some forage crops causes poisoning in ruminants under certain conditions. Many external and internal factors like plant species/variety, plant parts, seasonal variation, nitrogen fertilization and clipping interval etc. have a large effect on oxalate accumulation in forage plants and thus influence metabolism of grazing animals. In the present paper, an overall view of oxalate accumulation under the influence of these various factors in forage crops has been summarized. Different nitrogen sources vis-à-vis N doses alter the oxalate accumulation to different levels. Nitrate application resulted in higher contents of soluble and total oxalates than ammonium application. With increased harvesting interval, oxalate content showed a decreasing trend in some plants but reverse trend was reported in others. Ruminants tend to be more tolerant of oxalate than non-ruminants. A dose of 0.12 g oxalic acid/kg live weight/d resulted in mild degree of hypocalcaemia in sheep but prolonged grazing on some tropical grasses could result in severe hypocalcaemia in sheep and cattle. However, more research is required for a better understanding of the interactions between oxalate in different species and grazing animals.

**Key words :** Clipping interval, Growing environment, Fertilization, Forages, Oxalate

Oxalate is a dicarboxylic acid and can be found in a wide variety of plants. Oxalate is involved in various activities like coping metal toxicity, calcium regulation, quenching oxidative burst under pathogenic attack and involvement in programmed cell death. In spite of its variable functional role, high oxalate concentrations in plants are sufficient to induce poisoning in animals. Oxalate accumulates primarily as soluble oxalate, insoluble calcium oxalate or a combination of these two forms. Insoluble oxalate forms with calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ) and iron ( $\text{Fe}^{2+}$ ) ions and soluble oxalate usually forms with sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ) and ammonium ( $\text{NH}_4^+$ ) ions (Savage *et al* 2000). Soluble oxalate is one of a number of anti-nutrients in forage plants. It exerts its effects by binding calcium, magnesium and other trace minerals such as iron, making them unavailable for assimilation (Talapatra *et al.*, 1948; Watts, 1959; Gorb and Maksakow, 1962). This leads to disturbances in calcium and phosphorus metabolism and causes excessive mobilization of bone mineral. The demineralized bones become fibrotic, misshapen and thus causing lameness and 'bighead' in animals (McKenzie *et al.*, 1981).

In ruminant system, oxalate is metabolized in four possible ways. First, soluble oxalate may be

degraded by rumen bacteria (Allison *et al.*, 1977). Secondly, when  $\text{Ca}^{2+}$  is taken along with high soluble oxalate containing dietary feed,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  ions combine with oxalate ions in the rumen or intestine to form insoluble oxalate. These crystals are eliminated in the faeces and cannot be absorbed into the body. Thirdly, when  $\text{Ca}^{2+}$  is low in the diet, soluble oxalate remains soluble in the liquid portion of the intestine contents and is readily absorbed from the intestine into the bloodstream. If the oxalate ion concentration becomes very high in the blood being filtered by the kidney, it may combine with  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  to form insoluble oxalate crystals that may block urine flow and cause kidney failure (Lincoln and Black, 1980; Blaney *et al.*, 1982). Fourthly, insoluble oxalate from ingested plants may pass through the digestive tract without any harmful effect on the body metabolism (Ward *et al.*, 1979).

In general, oxalate poisoning is a complex issue. Induction of acute oxalate intoxication depends on several factors including the chemical form of oxalate, amount and quality of other feed consumed concurrently, age of animal, rate of consumption, total amount of oxalate consumed and adaptation to a diet containing oxalate (Burrows and Tyrl, 1989; Pickrell and Oehme, 2004 ; Radositits *et al.*, 2007). Dietary

oxalate, if consumed in large quantities, is well known to have potential toxicity (Panciera *et al.*, 1990; Sidhu *et al.*, 1996). Oxalate accumulation in plants and invariably toxicity in animals depends on many factors which are summarized below.

**Plant species/varieties**

Various tropical grasses contain soluble oxalates in sufficient concentration to induce calcium deficiency in grazing animals (Table 1). These include buffel grass (*Cenchrus ciliaris*), pangola grass (*Digitaria decumbens*), setaria (*Setaria sphacelata*), bathu (*Chenopodium album*) and kikuyugrass (*Pennisetum clandestinum*) and halogeton. Guinea grass (*Panicum maximum*), bajra (*Pennisetum glaucum*) and Napier Bajra Hybrid (*Pennisetum glaucum* x *Pennisetum purpureum*, NBH) contain oxalate content with in the safe limit, however, they may prove toxic if fed over for extended period of time (Rahman *et al.*, 2011). In another study on fodder crops, highest concentration of oxalate was found in bathu (3.48%- 5.98%) followed by NBH (2.58%- 5.62%). Guinea grass, bajra, and chari showed oxalate concentration ? 2%. In NBH, oxalate concentration was ?3% in 93% samples and >3% in 7% samples. Bathu was found to contain >3% oxalate in all the samples (Sidhu *et al.*, 2014; Table2). Total oxalate content in NBH varied from 34.5-39.7 g/kg DM and soluble oxalate from 15.6-21.2 g/kg DM, taking into the account of seasons and nitrogen inputs (Kaur *et al.*, 2016). Halogeton contained 17 to 30% soluble oxalate on dry plant weight basis (Cronin and Williams, 1965). In many developing countries, rice

TABLE 1  
Plant species and oxalate content

Plant species	Oxalate content (%)	Reference
Napier Bajra Hybrid	3.3-4.0	Kaur <i>et al.</i> , 2016
	2.58-5.62	Sidhu <i>et al.</i> , 2014
Guinea Grass	1.05-2.40	Garcia-Rivera and Morris (1955)
Pearl millet	0.63-1.98	Kaur <i>et al.</i> , 2012
Bathu	3.48-5.98	Sidhu <i>et al.</i> , 2014
Sorghum	0.92-1.70	Sidhu <i>et al.</i> , 2014
Setaria	2.61-4.98	Katoch, 2013
Napiergrass	3.24-4.78	Rahman <i>et al.</i> , 2006
Kikuyu grass	0.4-2.4	Marais (1997)
Baffel grass	1.3	Rahman <i>et al.</i> , 2013
	1.2-2.2	Playne (1976)

TABLE 2  
Oxalate concentration in forage crops collected from farmer's field

Plant Species	Oxalate (%)
Napier bajra hybrid (n=145)	2.58-5.62 (< 3% = 135; > 3% = 10)
Guinae grass (n=30)	1.05-2.40 (≤ 1% = 12; > 1% = 18)
Bajra (n=42)	0.63-1.98 (≤ 1% = 32; > 1% = 10)
Bathu (n=21)	3.48-5.98 (> 3% = 21)
Chari (n=50)	0.92-1.70 (≤ 1% = 38; > 1% = 12)

(Sidhu *et al.*, 2014)

straw is fed as a basal diet to ruminants. Rice leaves contained 3.0-6.0% oxalate depending on growth stage and culture conditions (Ji and Peng, 2005). The leaves of fodder beet contained up to 10% soluble oxalate (Libert and Franceschi, 1987). The soybean crop residue is a major source of dry roughage to livestock and being utilized as a fodder to greater extent in soybean cultivation area of Maharashtra, India. Feeding of soybean crop residues to ruminants resulted in incidents like oxalate poisoning. The crop residues were found to contain oxalate content varied from 1.1-1.7% (Ambore *et al.*, 2014).

Oxalate content may vary among varieties within the same species. Out of two varieties of NBH, oxalate level was higher in PBN-83 (2.62%) than PBN-233 (2.25%) (Sidhu *et al.*, 2014). Of two cultivars of setaria, Nandi contained low oxalate than Kazungula (Jones and Ford, 1972). When two varieties of napiergrass were compared at different cutting intervals, mean oxalate value was high in COP cultivar than K56351. In oxalate accumulating forage crops, attention should be made to select those cultivars that accumulate a low content of oxalate under the same growing conditions.

**Plant parts**

Leaves normally contain higher oxalate content than stem so cultivars with high proportion of leaves may also have a higher oxalate level than that of varieties with low proportion of leaves (Table 3). Compared to a non-dwarf variety, a dwarf variety of napiergrass exhibited higher oxalate content, though the difference was not significant (Rahman *et al.*, 2006). In a study on NBH, total oxalate content was found to be positively correlated to leaf/stem ratio in autumn season (Kaur *et al.*, 2016). In previous studies on kikuyugrass (Marais, 1990) and setaria (John and Ford, 1972), leaf tissue contained significantly more

TABLE 3  
Soluble oxalate content (% DM) in plant parts of some forage species

Species	Plant part	Soluble oxalate	References
Setaria	Leaf Blade	4.40	Jones and Ford (1972)
	Leaf sheath	2.90	
Kikuyugrass	Leaf	1.33	Marais (1990)
	Stem	0.39	
	Leaf	2.44	Marais <i>et al.</i> , (1997)
	Stem	0.98	
Napiergrass	Leaf	2.78	Rahman <i>et al.</i> , (2006)
	Stem	2.05	

oxalate than stem. However, no particular trend was observed in plant fractions in different seasons, in early summer oxalate content was higher in stem (3.54%) than leaf tissues (3.01%); whereas in the late summer leaf tissue indicated the highest values (Rahman *et al.*, 2006).

### Seasonal variation

Seasonal variation strongly affects the level of oxalate level in NBH. Among different harvest seasons (summer, monsoon, autumn, pre-winter), soluble oxalate content was highest in the summer (2.08%) i.e. the month of June and decreased afterwards in the subsequent harvest seasons in NBH (Kaur *et al.*, 2016). Higher values of oxalate during the month of June and July might be due to the peak in growth during summer and rainy seasons (Singh 2002). Similar trend of soluble oxalate at different harvest seasons was observed in setaria (Rahman *et al.*, 2014). In other pot study, oxalate content recorded the highest value (3.77%) in early summer samples and the lowest value (1.76%) in late autumn samples of napiergrass (Rahman *et al.*, 2006). Oxalate level of *Atriplex* species was 8.29 and 4.92% of dry weight in spring and fall seasons respectively (Abu-Zanat *et al.*, 2003). According to Sidhu *et al.* (2014), more oxalate content was observed in extreme winter and summer months than that of normal season in NBH.

### Nitrogen Fertilization

Total oxalate content varied non-significantly as N fertilization increased from 50-100 Kg/ha in NBH (Kaur *et al.*, 2016). Non-significant changes in oxalate content with N fertilization was also found in napiergrass (Rahman *et al.*, 2009b), kikuyugrass (William *et al.* 1991) and *Tetragonia tetragonoides*

(Ahmed and Johnson, 2000). In rhodegrass, guineagrass and sudangrass, oxalate content was not increased significantly with increased rate of N fertilization (Rahman *et al.*, 2008a). The results from this study suggest that these grasses do not use further addition of N fertilizer (Standard×2 or Standard×4) to form high content of oxalate salts. In addition, the levels of oxalate present with these grasses are quite low as far as toxicity to animals is concerned. However, these observations contradict the result of Jones and Ford (1972) where the oxalate content in setaria increased from 3.3% to 5.6% as N fertilizer (as urea) level increased from 0 to 200 kg/ha, respectively. In a recent study also oxalate content increased with N fertilization in bajra x Napier hybrid (*cv.* Phule jaywant) and was maximum at 125% of RDN (Sonane *et al.*, 2017). In another study on napiergrass, first cut was affected by N treatment but this effect was not consistent in subsequent cuttings (Rahman *et al.*, 2010a). In contrast with total oxalate, soluble oxalate content showed positive correlation with nitrogen fertilization doses in NBH (Kaur *et al.*, 2016; Table 2).

Among the different forms of N fertilizer like potassium nitrate, urea, ammonium nitrate, ammonium sulfate and manure, application of urea to forage did not result in excessive oxalate accumulation (Williams *et al.*, 1991; Rahman *et al.*, 2008b; Rahman *et al.*, 2009a). A study on NBH depicted higher oxalate accumulation with nitrate form of fertilization as compared to other two forms of fertilization (urea, ammonium sulphate) in each harvest (Kaur *et al.*, 2016). Similar findings were obtained in napiergrass grown in nutrient solution (Rahman *et al.*, 2009a). Nitrate application also resulted in higher oxalate accumulation in rice and some vegetable crops than did ammonium application (Ji and Peng, 2005; Zhang *et al.*, 2005; Xu *et al.*, 2006 Rahman and Kawamura, 2011). The mechanistic basis of oxalate accumulation in relation to nitrate/amide/ammonium treatment has long been a subject of interest but is little understood. It is reported that nitrate-N activates the oxalate accumulation in plants When N was supplied as nitrate to plants, a number of hydroxyl (OH<sup>-</sup>) ions were produced by catalysis with nitrate reductase and nitrite reductase. Increased level of OH<sup>-</sup> ions acted as a signal to stimulate organic acid biosynthesis (e.g, oxalic acid). The H<sup>+</sup> ion dissociated from the organic acid neutralized the excess level of OH<sup>-</sup> ion in order to maintain intracellular pH (Raven and Smith 1976, Allen *et al.* 1988) and remaining organic (COO<sup>-</sup>) ions may stimulate cation uptake (e.g, Na, Ca and Mg). A recent molecular study demonstrated that nitrate itself

acted as a direct signal to induce organic acid metabolism (Scheible *et al.*, 1997).

Ammonium application served as negative signal and inhibited oxalate accumulation (Ji 2004). The inhibition of oxalate accumulation by ammonium application was because of stress induced breakdown of the whole metabolism in plants (Britto *et al* 2001). Earlier studies also showed sole ammonium nutrition caused toxicity to many plant species (Rahman *et al.*, 2010b). Other possible reason of lower oxalate accumulation may be that plants grown with ammonium fertilization acidified the cytoplasm due to excretion of H<sup>+</sup> from plant roots which probably inhibit organic acid biosynthesis including oxalic acid (Schubert and Yan, 1997).

### Clipping interval

Clipping interval strongly influence oxalate content in different plants. In setaria more oxalate was present in young plant than in mature plant (Jones and Ford, 1972). In napiergrass, NBH and pearl millet crops, oxalate content declined as the harvest interval increased (Kaur *et al* 2012; Table 4). Rahman *et al.* (2009) reported that the oxalate concentrations in napiergrass declined with the age of the plant material. It may be due to more oxalate synthesis rate at early stages of plant growth in these species (Singh, 2002). The decline in oxalate content with maturity of the crop may be due to degradation of oxalate to carbonates or bicarbonates or due to dilution of oxalate. In contrast, some plant species such as rhubarb (*Rheum rhabarbarum*), halogeton and water hyacinth (*Eichhornia crassipes*), accumulated more oxalate content as plant matures. Halogeton became more toxic as growth proceeds, reaching a peak of toxicity at maturity (Torell *et al.*, 2005). Abu-Zanat *et al.* (2003)

TABLE 4  
Total oxalate content (g/100g DW) at different cutting interval of experimental crops

Crop/Variety	Height					
	50	100	130	150	200	Mean
Napiergrass/ COP	6.4	5.8	5.1	4.4	3.2	4.9
NBH/ PBN 233	4.1	3.1	2.8	2.1	1.9	2.8
Pearl Millet/ FBC 16	2.4	2.0	1.7	1.5	1.3	1.8

(Kaur *et al.*, 2012).

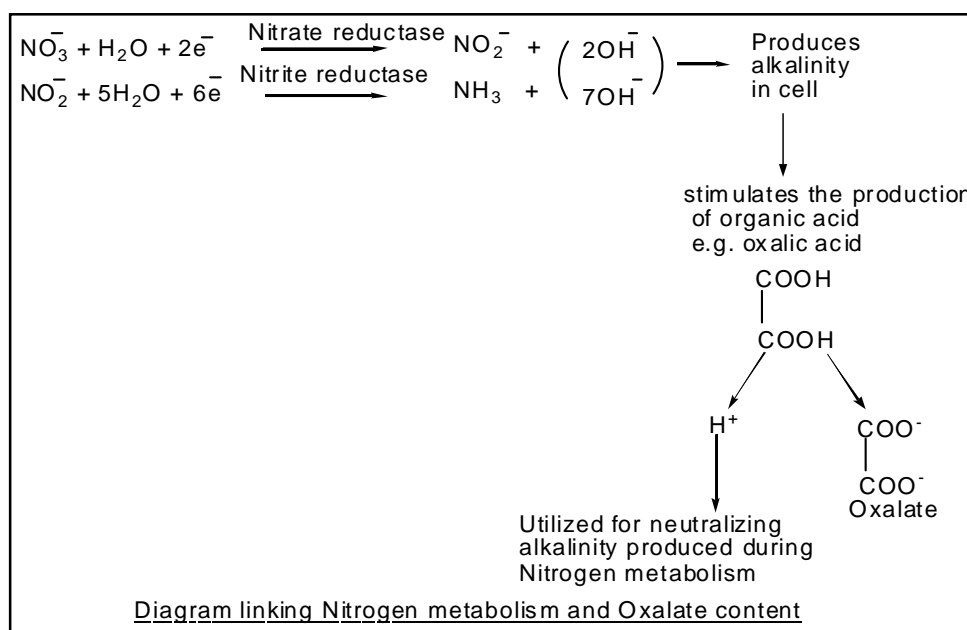
observed no significant difference in saltbush plant with clipping.

### Animal Type /Toxic level

Ruminants (e.g. goats) tend to be more tolerant of oxalate than non-ruminants (e.g. horses). Rumen bacteria degrade oxalate into formic acid and carbon dioxide rendering it non-toxic (Table 5). Prolonged grazing by cattle and sheep on some tropical grasses can result in severe hypocalcaemia (Seawright *et al.*, 1970). A mild degree of hypocalcaemia was observed in sheep fed at a dose of 0.12 g oxalic acid/kg live weight/d (Kyriazakis *et al.*, 1997). Panda and Sahu (2002) observed that the total oxalate intake at the level of 0.58% of the DM intake was harmless to bulls, but an increase to 1.19% created a negative balance of calcium. Instances of sheep poisoning have been documented owing to high oxalate contents in buffel grass (*Cenchrus ciliaris*) pastures (McKenzie *et al.*, 1988). Higher mortality in cattle and buffalo calves has been reported following feeding on napiergrass (*Pennisetum purpureum* cv. Pusa giant) that contained high levels of oxalic acid (3.01%) and low levels of calcium (Dhillon *et al.*, 1971; Sidhu *et*

TABLE 5  
Animal responses as affected by different levels of soluble oxalate (% DM)

Critical level	Incidence	Reference
4.0% or more	Toxic or even fatal to the cattle	Moir (1953)
2.0%	Negative calcium balance in cattle	Morris and Garcia-Rivera (1955)
6.9%	Acute toxicity in cattle	Seawright <i>et al.</i> , (1970)
3.01%	Toxicity in buffalo calves	Dhillon <i>et al.</i> (1971)
3.5%	Increased blood clotting time in calves	James (1978)
1.3-1.8%	Sub-clinical bone diseases in ponies	Cymbaluk <i>et al.</i> , (1986)
2.0% or more	Acute toxicity in ruminants	McKenzie <i>et al.</i> , (1988)
3.01%	Toxicity in cattle and buffalo calves	Sidhu <i>et al.</i> , (1996)
0.39-2.44%	Acute toxicity in ruminants	Marais (2001)
2.66-2.75%	Reduced serum calcium level in sheep	Wein Chang <i>et al.</i> , (2004)
3.0% or more	Reduced plasma calcium level in goat	Rahman <i>et al.</i> , (2011)



*al.*, 1996). An outbreak of acute oxalate intoxication in a flock of sheep was associated with consumption of *Seidlitzia rosmarinus* (Chenopodiaceae), with a mortality rate of about 19% (Aslani *et al.*, 2011). Soluble oxalate levels of 2.0% or more may lead to acute toxicosis in ruminants, while levels of 0.5% or more may induce nutritional hyperparathyroidism in horses (McKenzie *et al.*, 1988; Rahman *et al.*, 2012). Parathyroid hormone (PTH) is secreted in response to low blood  $\text{Ca}^{2+}$  levels, causing bone to release  $\text{Ca}^{2+}$  into the blood stream. Low levels of this hormone are secreted even when blood  $\text{Ca}^{2+}$  levels are high, but steep increase in the secretion of PTH occurred when  $\text{Ca}^{2+}$  levels fall below the normal range (Martin *et al.*, 1996). Sheep fed high oxalate-containing grasses showed lower blood calcium concentrations than sheep fed low oxalate-containing grasses (Rahman *et al.*, 2011). Symptoms of oxalate poisoning in cattle and buffalo calves are characterized by severe inappetence, straining, constipation, dryness of muzzle, dullness, increased blood clotting time, dyspnea with normal water intake and zero rumen motility (Sidhu *et al.*, 1996).

It is concluded from the study that oxalate accumulation in plants is a complicated process and depends upon a number of climatic and agronomic factors. External factors including seasonal variation, harvest interval, N fertilization, N forms interact invariably for elevated/declined level of oxalate in different forage crops. Forage species or cultivars having a lower tendency to accumulate oxalate should be selected for cultivation. Precautions should be made while feeding oxalate rich grasses which may result

in hypocalcaemia in ruminants. More biochemical studies on oxalate metabolism may further enlighten the processes to minimize oxalate level in forage crops

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