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Cyanogenic poisoning by spontaneous ingestion of star grass (*Cynodon nlemfuensis* var. *nlemfuensis* cv. 'Florico') in cattle[1]

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ABSTRACT.- Molossi F.A., Ogliari D., Morais R.M., Wicpolt N.S., Gheller E., Weber L. & Gava A. 2019. **Cyanogenic poisoning by spontaneous ingestion of star grass (*Cynodon nlemfuensis* var. *nlemfuensis* cv. 'Florico') in cattle.** *Pesquisa Veterinária Brasileira 39(1):20-24*. Laboratório de Patologia Animal, Departamento de Medicina Veterinária, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Lages, SC 88520-000, Brazil. E-mail: aldo.gava@udesc.br

This study reports the epidemiological data and the clinical-pathological condition of five outbreaks of cyanogenic poisoning in cattle spontaneously ingesting star grass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* cv. 'Florico'). In all outbreaks, the areas where the plant was previously fertilized with high concentrations of nitrogen and the properties adopted the silvipastoral system. The first clinical signs appeared between 10 and 15 minutes after the first introduction of cattle and were characterized by muscular tremors, dyspnea, moderate tympanism, staggering gait, forced breathing with open mouth, sternal recumbency followed by death after 15 to 30 minutes and/or recovery in a few hours after the signs started. In total, 43 cows have become ill and 18 died. Two necropsies were performed and no significant changes were found except for the presence of the plant near the esophageal sphincter region. No histological lesions were seen through microscopy. Green leaves of the star grass were collected from all properties where the outbreaks occurred and the test of the picro-sodium paper was performed, revealing red-brick coloration in 20 minutes after maceration of the leaves.

INDEX TERMS: Cyanogenic poisoning, star grass, *Cynodon nlemfuensis*, hydrocyanic acid, star grass cv. Florico, cattle, toxic plants, toxicoses.

RESUMO.- [**Intoxicação cianogênica pela ingestão espontânea de grama estrela (*Cynodon nlemfuensis* var. *nlemfuensis* cv. 'Florico') em bovinos.**] Descrevem-se os dados epidemiológicos e quadro clínico-patológico de cinco surtos de intoxicação cianogênica em bovinos que ingeriram espontaneamente grama estrela (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* cv. 'Florico'). Em todos os surtos, as áreas onde a planta se encontrava haviam sido previamente adubadas com altas concentrações de nitrogênio e as propriedades adotavam o sistema silvipastoril com *Eucaliptus* sp. Os primeiros sinais clínicos surgiram entre 10 e 15 minutos após a primeira introdução dos bovinos e caracterizou-se por tremores musculares, dispneia, timpanismo moderado, andar cambaleante, respiração forçada com a boca aberta, decúbito esternal seguido de morte após 15 a 30 minutos e/ou, recuperação em poucas horas após início dos sinais. No total, adoeceram 43 vacas e destas 18 morreram. Duas necropsias foram realizadas e não foram encontradas alterações significativas, exceto a presença da planta próxima a região do esfíncter esofágico. Através da microscopia não foram visualizadas lesões histológicas. Folhas verdes da grama estrela foram coletadas de todas as propriedades onde os surtos ocorreram e realizadas o teste do papel picro-sódico, o qual revelou coloração vermelho-tijolo em 20 minutos após maceração das folhas.

TERMOS DE INDEXAÇÃO: Intoxicação cianogênica, grama estrela, *Cynodon nlemfuensis*, bovinos, ácido cianídrico, grama estrela cv. Florico, plantas tóxicas, toxicoses.

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INTRODUCTION

Grasses of the genus *Cynodon*, belonging to the Poaceae family, came from Africa and were introduced in North America in the mid-eighteenth century (Harlan 1970). For more than 50 years, many breeding works have been carried out mainly at the universities of Georgia and Florida in the United States in order to better use the forage potential of this genus and to adapt it to the subtropical conditions of Southeastern United States. In Brazil, it is believed that this grass was introduced by private initiative to evaluate its behavior under Brazilian conditions; however, there are no records of how and where the genus *Cynodon* was introduced (Vilela & Alvim 1998). There are also many doubts about the recognition of the species used.

The genus *Cynodon* is divided into two groups, the Bermuda grass group (*Cynodon dactylon* (L) Pers.), in which several hybrids are available, such as Coastal, Alicia, Callie, Tifton 44, Tifton 68, Tifton 78, Tifton 85, Coastcroos and more recently the Florakirk. In the star grass group (*Cynodon nlemfuensis* Vanderyst, *Cynodon aethiopicus* Clayton and Harlan), the most used cultivars are McCaleb, Ona, Florico and Florona (Vilela & Alvim 1998). Florico (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* cv. 'Florico') and Florona cultivars (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* cv. 'Florona') are the most recent commercial star grass launches by the University of Florida in the USA (Mislevy et al. 1989a, 1989b). Florona cultivar was collected at the Experimental Station of the University of Florida in 1974 and submitted to evaluation trials from 1975 (Mislevy et al. 1989b). Florico cultivar, native to Kenya, was introduced in Puerto Rico in 1957 and later brought to Florida in 1972 (Mislevy et al. 1993a). Both cultivars were recorded in 1993 by Mislevy et al. (1993a, 1993b). Florico cultivar can be distinguished from other star grasses of the *nlemfuensis* variety by its hairiness and purplish-green color (Mislevy et al. 1989a). It presents high dry mass production and good response when it receives high levels of fertilization, but it has great potential for accumulation of cyanogenic glycosides, especially under high Nitrogen doses (N), especially during the early stages of plant development. In 16 years of testing in Ona, Florida, hydrocyanic acid (HCN) poisoning in cattle grazing in Florico grass (Mislevy et al. 1993a) was not observed. In Brazil, the presence of HCN in Florico grass was verified as a function of the age of grass cut, but the HCN dose did not exceed 109.01mg/kg (Castro 1998). In Santa Catarina, cattle breeding in a silvopastoral system recommended by Garcia et al. (2013) have been developed and star grass is one of the grasses used. The use of this system seems to favor the accumulation of hydrocyanic acid in the plant similar to what occurs with *Sorghum* spp. described by Vetter & Haraszti (1977).

In South Africa, other star grasses (*C. nlemfuensis* Vanderyst var. *robustus*, *C. aethiopicus* Clayton and Harlan) were also identified as cyanogenic (Kellerman et al. 1988). Most cyanogenic plants are harmless due to the low concentration of glycosides and low palatability. However, toxic grasses that are widely cultivated are highlighted due to their good acceptance by animals (Youssef & Maxie 2004).

In Brazil, several other cyanogenic plants of animal interest have been described, such as *Cnidoscolus phyllacanthus* (Oliveira et al. 2008), *Manihot glaziovii* (Tokarnia et al. 1994a) *M. glaziovii* Muell. Arg. (Amorim et al. 2005), *Manihot piauhyensis* (Canella et al. 1968), *Sorghum sudanense* (Juffo et al. 2012), *Sorghum halepense* (Nobrega Junior et al. 2006), *Passiflora foetida* (Carvalho et al. 2011), *Piptadenia macrocarpa* (Tokarnia et al. 1994b), *Piptadenia viridiflora* (Tokarnia et al. 1999), *Prunus sphaerocarpa* (Saad & Camargo 1967), *Prunus sellowii* (Gava et al. 1992) and Tifton 68 (Gava et al. 1997, Galindo et al. 2017).

The toxic principle of cyanogenic plants is the presence of cyanogenic glycosides, which through the action of intracellular plant enzymes and ruminal digestion are converted into hydrocyanic acid (HCN), one of the most known toxic compounds (Egekeze & Oehme 1980). HCN reversibly binds to the cytochrome c oxidase enzyme (CcOX) and disrupts oxygen transfer from red blood cells to somatic cells (Hibbs 1979, Way 1984, Leavesley et al. 2008). When ingested under the form of glycoside, the minimum lethal HCN dose for cattle and sheep is about 2mg/kg body weight (Radostits et al. 2002). Signs of histotoxic anoxia resulting from cyanogenic poisoning are observed within a few minutes after plant ingestion and death occurs within a few minutes to one hour after the onset of signs. Clinical signs are characterized by dyspnoea, anxiety, salivation, muscle tremors, incoordination, staggering gait, reddish mucous membranes, depression, tachycardia, seizure, decubitus and opisthotonus in the terminal phase (Kellerman et al. 1988, Radostits et al. 2002, Youssef & Maxie 2004). No significant macro and microscopic lesions are observed. The finding of leaves of the plant in the cardic region, not yet mixed with the ruminal content (Tokarnia et al. 2012), is fundamental for diagnosis.

The picrate test qualitatively evaluates the presence of HCN in plants, and is a fundamental tool for diagnosis. Slower reactions should not be disregarded, since there are cyanogenic glycosides of slower development. Treatment based on sodium nitrite and sodium thiosulphate is effective, but in most poisoning events, the timing for administration is short due to the rapid evolution of cases, but whenever possible, treatment should be performed as it is important in confirmation of diagnosis by cyanogenic plants (Tokarnia et al. 2012).

The aim of the present study was to evaluate the epidemiological, clinical and pathological aspects of five outbreaks of cyanogenic poisoning by spontaneous ingestion of star grass Florico cultivar in cattle.

MATERIALS AND METHODS

The collection of epidemiological data, the observation of clinical signs and the necropsy of two cows were carried out in visits to the five properties where the disease outbreak occurred. Viscera samples were collected and fixed in 10% formalin and processed for histological evaluation. Green star grass leaves were collected from all the properties where the outbreaks occurred and the picrate test described by Henrici (1926), quoted by Tokarnia et al. (2012) was performed. The test consisted of a strip of white paper immersed in solution composed of 5g sodium carbonate and 0.5g picric acid dissolved in 100ml distilled water. Leaves were macerated and placed in glass jars with lid, where the paper strip was fixed, which remained freely suspended above the plant material. Glass jars were held upright and the reaction was observed for 1 hour. The intensity of the reaction to the picrate test was classified taking into account the time of appearance of the red-brick color.

RESULTS

Five HCN poisoning outbreaks in cattle, four in Santa Catarina, in the municipalities of Água Doce (Properties 1 and 2), Santa Terezinha (Property 3) and Braço do Norte (Property 4) were diagnosed from 2015 to 2017. An outbreak occurred in Paraná, in the municipality of União da Vitória (Property 5). Epidemiological data from the five outbreaks are shown in Table 1. In four of the five properties, owners were informed that the cultivated grass was purchased from another property as being tifton specimens.

In all outbreaks, the areas where the plant was cultivated had been previously fertilized with high nitrogen concentrations and properties adopted the silvopastoral system with *Eucalyptus* sp. (Fig.1). The first clinical signs appeared between 10 and 15 minutes after the first introduction of the cattle in pasture exclusively constituted of star grass Florico cultivar and was characterized by muscle tremors, dyspnea, moderate tympanism, staggering gait, forced breathing with open mouth, sternal decubitus followed by death after 15 to 30 minutes and/or recovery within a few hours after the onset of signs. Two necropsies were performed and no significant changes were found except for the presence of the plant near the esophageal sphincter region. No histological lesions were observed in the various organs.

In all properties, star grass Florico cultivar samples collected to perform the picrate test showed a red-brick color change in 20 minutes after maceration of leaves.

Table 1. Data referring to outbreaks of spontaneous poisoning by Florico star grass

Outbreaks	County	Total cows in the picket/lot	Diseased	Deaths
1st	Água Doce/SC	11	11	3
2nd	União da Vitória/PR	10	10	4
3rd	Santa Terezinha /SC	16	16	8
4th	Braço do Norte/SC	30	3	2
5th	Água Doce/SC	27	3	1
Total		94	43	18

Fig.1. Star grass Florico cultivar, fresh and in silvopastoral system in the municipality of Santa Terezinha/SC.

DISCUSSION

The clinical-pathological condition observed in spontaneous poisoning by star grass Florico cultivar is in agreement with that described by other authors for cyanogenic plants (Saad & Camargo 1967, Canella et al. 1968, Kellerman et al. 1988, Gava et al. 1992, Tokarnia et al. 1994a, 1994b, 1999, Gava et al. 1997, Radostits et al. 2002, Youssef & Maxie 2004, Amorim et al. 2005, Nóbrega Junior et al. 2006, Riet-Correa & Méndez 2007, Oliveira et al. 2008, Carvalho et al. 2011, Juffo et al. 2012, Galindo et al. 2017).

In the present study, the majority of cows with clinical signs recovered spontaneously within a few hours. These animals probably stopped star grass consumption spontaneously due to the appearance of mild clinical signs, consuming doses lower than those consumed by animals that became seriously ill and died, or may be related to the management system adopted in properties. According to Radostits et al. (2002), hungry animals and/or those not used to ingesting cyanogenic plants are more prone to poisoning. Studies indicate that animals in contact with cyanogenic plants can tolerate increasing HCN doses due to the increased production of the rhodanase enzyme, which is important in the organism detoxification. In the present study, all outbreaks were the first contact of cattle with the plant and morbidity ranged from 10% to 100% and mortality from 3.7% to 50%, evidencing the susceptibility of animals to poisoning.

The use of the silvopastoral system seems to show that shadow associated with excess nitrogen on star grass Florico cultivar favors the synthesis of hydrocyanic acid. Vetter & Haraszti (1977) observed that the production of HCN in *Sorghum* spp. decreases gradually during its vegetative development, as the photosynthesis intensity increases, that is, the less photosynthesis the more HCN. Mislevy et al. (1993a) reported that the hydrocyanic acid potential of Florico star grass is high under heavy N fertilization, especially during the early stages of plant development.

The response to the picrate test occurred within 20 minutes, which differs from that obtained from green *Prunnus sellowii* leaves, a cyanogenic plant found in the Southeastern and Southern regions of Brazil, which occurred within 3 to 5 minutes (Gava et al. 1992). This is probably due to the fibrous character of Florico leaves, which makes it difficult to macerate them and consequently delay the reaction between the enzyme and the glycoside.

The differential diagnosis of cyanogenic poisoning should be made mainly in relation to other diseases or poisonings with super acute clinical evolution and without macroscopic and microscopic alterations, especially nitrate/nitrite poisoning. This poisoning is commonly observed in the southern region of Brazil in cattle that graze on grasses, mainly oat and ryegrass, well fertilized and under favorable climatic conditions (dry seasons followed by rains). The grazing time on these grasses is also an important data for differential diagnosis. While for poisoning with plant containing hydrocyanic acid, the grazing time is 10 to 20 minutes, nitrate/nitrite poisoning requires grazing time at least greater than one hour. Both conditions show clinical signs of respiratory difficulty and staggering gait; however, in nitrate/nitrite poisoning, there is a marked tachypnea, not observed in the same proportion in HCN poisoning. In addition, in HCN poisoning, mucous membranes are bright red and in nitrate/nitrite poisoning

brown (Hibbs 1979). In these cases, it is interesting to apply the picrate test to identify HCN and/or the diphenylamine test for nitrate in pastures. Another difference is that pastures with high nitrate/nitrite content maintain their toxicity after desiccation (Jönck et al. 2013) and grasses with HCN, such as Tifton 68, lose their toxic action after phenation (Galindo et al. 2017).

Among the toxic plants that cause rapid death in cattle in the southern region of Brazil, *Amorimia exotropica* stands out in the coastal region of Santa Catarina and Rio Grande do Sul. However, it is possible to distinguish them among epidemiological aspects (Gava et al. 1998, Pavarini et al. 2011). Urea poisoning is also included in the differential diagnosis, which also produces rapid death. Epidemiological data on the accidental access of cattle to urea and the alkalinity of the ruminal content should also be evaluated.

CONCLUSION

When cultivated in the shade, star grass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* cv. 'Florico') can accumulate cyanide acid and cause cyanogenic poisoning in cattle.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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