

Plant Poisoning Containing Hydrocyanic Acid in Cattle in Southern Brazil

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ABSTRACT

Background: Cyanogenic plants accumulate cyanogenic glycosides and release hydrocyanic acid (HCN). In Brazil, there have been reports of several plants that cause HCN poisoning in animals and lead to a fast death with few clinical signs and lesions on *post mortem* examination. Some cultivars of *Cynodon* spp. grasses cause HCN poisoning in cattle in Brazil. The objectives of this work were to report the occurrence of deaths by HCN poisoning in cattle as diagnosed by the veterinary pathology laboratory, describe the quantity of HCN in some cultivars of *Cynodon* spp., as well as, to describe one cultivar of genus *Cynodon* never reported as poisonous.

Materials, Methods & Results: The archives of the Veterinary Pathology Laboratory (LPV) at the Concórdia Campus of the Instituto Federal Catarinense (IFC) were reviewed, seeking cases with a diagnosis of hydrocyanic acid poisoning in cattle after *post mortem* examination. The amount of HCN present in some cultivars of the *Cynodon* genus was quantified due to the high frequency of poisoning cases. From the 1,235 *post mortem* examinations of cattle 28 (2.27%) were diagnosed with spontaneous hydrocyanic acid poisoning, 17 cases (60.7%) due to ingestion of *Prunus* sp. or *Manihot* sp., and 11 cases (39.3%) of *Cynodon dactylon* ingestion. Most animals were found dead, normally having presented no clinical signs. Macroscopic evaluation mainly showed a severe amount of unchewed and undigested leaves or grass mixed in the ruminal content presenting a bitter almond odor. It was possible to infer that, among cultivars of the *Cynodon* genus, Florakirk showed the highest levels of HCN compared ($P < 0.05$) with Star of Puerto Rico, Tifton 68, Tifton 44, and Coast-Cross. Furthermore, Tifton 85 and Jiggs showed undetected levels of HCN. Leaves showed the highest HCN levels when comparing different parts of the plant. Regarding conservation methods, hay showed undetectable levels of HCN.

Discussion: To the best of our knowledge, this work is the first description of HCN poisoning in cattle due to ingestion of *Cynodon dactylon* cultivar Florakirk. This condition is described with a fast-clinical course, with animals found dead with no premonitory clinical signs. Poisoned animals did not develop macroscopic or microscopic specific lesions. Poisoning can be suspected when animals die suddenly, with absence of lesions under necropsy, and large amounts of unchewed and undigested leaves or grass inside their forestomachs. The diagnosis can be established performing the Picrosodic paper test, either in the pasture, or in the ruminal content. Occasionally however, HCN can go undetected when this chemical compound volatilizes between death and necropsy after several hours. Of all the *Cynodon* cultivars evaluated, Florakirk was the most dangerous for animals. In contrast, Tifton 85 and Jiggs released no HCN. Leaves were the part of the plant presenting the highest concentration of HCN. This is a defense mechanism that the plant develops against the ingestion by animals. This condition can cause great economic losses to farmers with the loss of animals and the need for prevention by using cultivars without HCN or hay, as 2.27% (28) of deaths diagnosed by the Veterinary Pathology Laboratory in the west of Santa Catarina, Brazil, were due to HCN poisoning. Notably, Florakirk cultivar was identified as the most dangerous cultivar tested, with higher levels compared with Tifton 68. The Star of Puerto Rico cultivar showed similar levels of HCN as Tifton 68. Both cultivars are commonly cultivated in many farms in the south of Brazil.

Keywords: HCN, prussic, Florakirk, *Cynodon dactylon*, hay.

DOI: 10.22456/1679-9216.109475

Received: 25 November 2020

Accepted: 10 January 2021

Published: 22 February 2021

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INTRODUCTION

When cyanogenic glycosides, accumulated in cyanogenic plants, are submitted to enzymatic hydrolysis or when the damaged plant tissue (chewed or digested by bacteria) comes into contact with hydroxynitrile lyase, hydrocyanic acid, also known as prussic acid (HCN) is released [8].

HCN is rapidly absorbed when released in the forestomachs of ruminants, causing the inhibition of cellular respiration by binding with a trivalent iron in intracellular oxidative enzymatic systems. This leads to a decrease in energy production and cytotoxic hypoxia, progressing to cellular death [12,21].

In Brazil, there are reports of several plants that cause HCN poisoning in animals, all of them leading to fast death, with few clinical signs and lesions on *post mortem* examination [21]. One of the most common genera of pasture in Brazilian territory is *Cynodon* spp., which is described three species naturalized, that have good quality and capacity to be adapted. These characteristics have made these plants quite attractive to farmers [16,23] despite the potential for hydrocyanic acid poisoning.

In Brazil, HCN poisoning by spontaneous intake of *Cynodon* spp. grasses by cattle has been described [21]. Additionally, cases that occur due to the ingestion of Tifton 68 [9-10] and star grass [15] have been reported as well. Among other plants, *Prunus myrtifolia* (*sphaerocarpa*) and *Manihot* sp. were already known as potentially deadly [21]. Therefore, the objectives of this work were to report the occurrence of deaths by HCN poisoning in cattle as diagnosed by the Veterinary Pathology Laboratory, and describe the quantity of HCN in some cultivars of *Cynodon* spp., including cultivars never reported as potentially presenting HCN.

MATERIALS AND METHODS

Methodology

Data from the archives of the Veterinary Pathology Laboratory (LPV) at the Instituto Federal Catarinense (IFC), Campus Concórdia, were reviewed, evaluating cases with the diagnosis of hydrocyanic acid poisoning in cattle after *post mortem* examinations. Data such as images and the description of breed, sex, age, municipality, year, history, and macroscopic and microscopic findings were evaluated.

In the *post mortem* examination, organ samples were systematically gathered, fixed in 10% buffered

formalin¹, and routinely processed. Tissues were embedded in paraffin² wax, cut in 3-microns thick sections, and stained with hematoxylin and eosine². Samples of ruminal content and of the grasses and/or leaves of the plants were collected for the Picrosodic paper test.

The Picrosodic paper test was performed [21]. It consists of a strip of white paper soaked in Picrosodic solution, composed of 5 g sodium carbonate³ and 0.5 g picric acid⁴ dissolved in 100 mL distilled water. The leaves or grass were macerated and placed in lidded plastic bowls, where the paper strip was placed and remained freely suspended above the plant material. In some cases, the plant material was replaced by ruminal content or muscle. The plastic bowl was held upright and the reaction was observed for one hour. The intensity of the reaction to the Picrosodic paper test was considered positive when the paper acquired a red-brick color.

Quantification of hydrocyanic acid

The samples of the *Cynodon* spp. cultivars were obtained from the agrostological field of the IFC, originated from a germplasm bank. The evaluated cultivars were Coast-cross (*Cynodon dactylon*), Tifton 68 (*Cynodon nlemfuensis*), Tifton 85 (*Cynodon nlemfuensis*), Tifton 44 (*Cynodon nlemfuensis*), Florakirk (*Cynodon dactylon* cultivar Florakirk), Jiggs (*Cynodon dactylon*), and Star of Puerto Rico (*Cynodon nlemfuensis*). The samples from the agrostological field of the IFC were harvested considering the suitable age and size for cattle to graze. Three harvests were performed for each cultivar with an interval of seven days between harvests.

The samples were identified and selected according to the structures to be used: leaf, stem, *in natura* whole plant, and hay. Haymaking was carried out in an oven and dried until obtaining approximately 15% of dry matter. Quantification was performed for each cultivar sample [11]. Hydrocyanic acid extractions were conducted in a lidded plastic bowl where the samples were macerated in particles measuring 1 to 2 cm in length. A strip of white paper soaked in Picrosodic solution was placed over the bowls of all treatments, including a negative control (with no content, just the strip of white paper soaked on Picrosodic solution). The bowls were then incubated in an oven at 37 °C for 16 h.

After incubation, 1 cm² of each stripe was submerged in 5 mL of distilled water for 60 min, in

an oven at 37°C. Subsequently, the absorbance of the solution was determined using a spectrophotometer, at a wavelength of 510 nm. It was possible to calculate the amount of hydrocyanic acid in milligrams per kilogram (mg/kg), based on the result of the absorbance, according to the equation [11] ($\text{HCN mg/kg} = 396 * \text{absorbance} * 100 / Z$ [Z is the weight of the sample in milligrams]).

HCN was not measured in samples from areas diagnosed with outbreaks due to the significant difference in farm management, specially fertilizing regimes, type of soil, and grazing pressures. These factors would compromise results and comparisons. On the other hand, all samples measured from the agrostological field of the IFC were subjected to the same management, allowing the comparison of the results. Another reason is that outbreaks occurred in different years, and samples would be affected by different weather, also compromising the correlations.

Data analysis

The results of the quantification of HCN were tabulated and analyzed statistically using the Statistical Analysis System software⁵ as a completely randomized design. The HCN data were submitted to the Shapiro-Wilk and Levene normality tests. When data did not show a normal behavior, the NPAR1WAY procedure and the Kruskal Wallis test were used to analyze the part of the plant. The effect of the cultivar was evaluated using the GLM procedure, transforming the data (logarithmic transformation) since they were not homogenous. The means were compared by Tukey's test ($P < 0.05$).

RESULTS

Of the 1,235 bovine necropsies conducted in the Veterinary Pathology Laboratory (LPV), 28 (2.27%) were diagnosed as hydrocyanic acid poisoning. Different plant species were indicated as the source, 15 (53.6%) due to the ingestion of *Prunus* sp., 11 (39.3%) due to *Cynodon* sp., and 2 (7.1%) due to *Manihot* sp. Most deaths (24) occurred in outbreaks and 4 as isolated cases. Table 1 describes the stratification of municipalities, number of dead animals, year, number of animals per batch, species of cyanogenic plant, and mode of poisoning. All cases were diagnosed in the west of Santa Catarina.

The animals involved in the reported cases were dairy cattle (85.7%), female (100%), and adult (96.4%). In most cases, 57.1% (16/28), farmers found the animal

dead, always near the pasture or broken branches of *Prunus* sp. contaminating the pasture [Figure 1].

When the clinical course was extended, owners reported that the animals presented ataxia (11/28), paddling movement (5/28), acute dyspnea (4/28), drooling (4/28), and staggering when walking (1/28). The clinical course in one of the outbreaks extended for 3 h, from the first view of clinical signs and the last animal that evolved to death. Despite the clinical signs, most animals evolved to death in minutes.

Severe amount of unchewed and undigested leaves or grass mixed in the ruminal content (23/28) [Figure 2] was observed in the macroscopic examination. Other findings consisted of a bitter almond odor (23/28), petechiae in the mucosa of the trachea (4/28), large amounts of *Manihot esculenta* shell (2/28), and non-clotting of bright red blood (1/25) [Figure 3]. The macroscopic or microscopic examination showed no other lesions.

The Picrosodic paper test was performed in 85.7% (24/28) of the cases. The ruminal content and pasture were positive in 75% (18/24) of the cases. Only the pasture was positive in the others 6 cases (25%) [Figure 4]. Furthermore, the skeletal muscle was also positive in 4 cases (16.67%). Picrosodic paper test was not performed in 2 outbreaks, where 4 animals (16.67%) were subjected to *post mortem* examination, because a large amount of undigested *Prunus* sp. leaves was found mixed in the ruminal content and the extensive description of this tree shows cyanide glycosides.

Quantification of hydrocyanic acid

The hydrocyanic acid quantification allowed the conclusion that some cultivars of both species, *Cynodon dactylon* and *Cynodon nlemfuensis*, could release HCN. A comparison of the results of each analyzed cultivar (Figure 5) in the whole plant (stem and leaves) showed that Florakirk (119.7 mg/kg \pm 58.0) presented the highest levels of HCN ($P < 0.05$). Star of Puerto Rico (111.7 mg/kg \pm 56.4) and Tifton 68 (34.2 mg/kg \pm 30.1) showed intermediate levels of HCN, presenting higher levels ($P < 0.05$) than Tifton 44 (18.7 mg/kg \pm 24.6) and Coast-Cross (19.7 mg/kg \pm 21.8). Moreover, Tifton 85 and Jiggs released no HCN using the methodology applied in this study.

Statistically, the leaves (54.7 \pm 47.31) presented ($P < 0.05$) more HCN than the stem (32.1 \pm 48.82) and total *in natura* plant (39.1 \pm 55.94). When evaluating the hay made from the whole plant, no cultivar showed any detectable levels of HCN.

Table 1. Stratification of cities, number of deaths and of animals of the batch, species of cyanogenic plant and mode of poisoning.

City	Deaths	Total*	Plant	Poisoning
Concórdia	1	NI	<i>Prunus myrtifolia</i> (<i>sphaerocarpa</i>)	Contamination of pasture
Alto Bela Vista	1	NI	<i>Cynodon dactylon</i>	Pasture
Concórdia	3	NI	<i>Prunus myrtifolia</i> (<i>sphaerocarpa</i>)	Contamination of pasture
Concórdia	4	12	<i>Cynodon dactylon</i> cultivar Florakirk	Pasture
Irani	5	NI	<i>Prunus myrtifolia</i> (<i>sphaerocarpa</i>)	Contamination of pasture
Concórdia	3	14	<i>Prunus myrtifolia</i> (<i>sphaerocarpa</i>)	Contamination of pasture
Peritiba	1	NI	<i>Cynodon dactylon</i>	Pasture
Peritiba	1	NI	<i>Cynodon dactylon</i>	Pasture
Concórdia	4	10	<i>Cynodon dactylon</i> cultivar Florakirk	Pasture
Concórdia	2	30	<i>Manihot esculenta</i>	Feed base
Concórdia	3	17	<i>Prunus myrtifolia</i> (<i>sphaerocarpa</i>)	Contamination of pasture

*Total Batch; NI: not informed or not recorded in the archives.



Figure 1. Hydrocyanic acid poisoning in cattle. Environment. Pasture contamination by *Prunus* sp. broken tree branch.



Figure 2. Hydrocyanic acid poisoning in cattle. Rumen. Unchewed and undigested *Cynodon dactylon* cultivar Florakirk grass mixed in the ruminal content.



Figure 3. Hydrocyanic acid poisoning in cattle. Blood. Normal venous clotted blood (left) and non-clotting bright red venous blood (right).



Figure 4. Hydrocyanic acid poisoning in cattle. Picrosodic paper test. Control (left) and red brick color, positive for HCN (right).

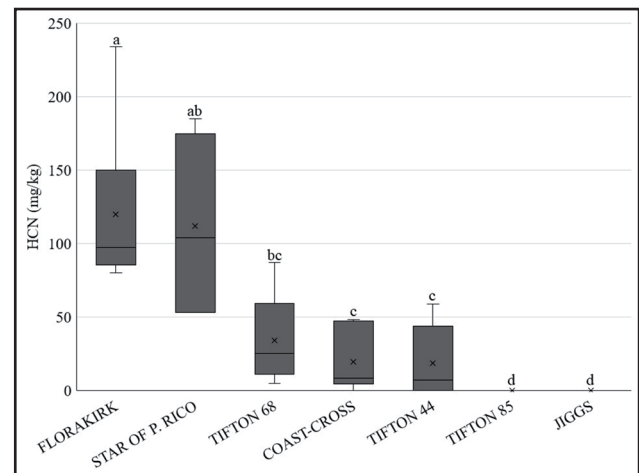


Figure 5. Amount of HCN present in each cultivar in the whole plant (stem and leaves). ^{a,b,c}Different letters demonstrate significant statistical difference ($P < 0.05$). ^{*}Mean value among the samples of the cultivar.

DISCUSSION

The diagnosis of hydrocyanic acid poisoning was done by associating history, clinical signs, macroscopic findings, and Picrosodic paper test results. The animals were predominantly adult, female, and dairy cattle. However, these cannot be inferred as predisposing factors. The Veterinary Pathology Laboratory is in a geographic area predominated by small dairy farms, therefore the laboratory routine activity is mainly represented by this category of animals [7].

The amount of plant capable of poisoning an adult bovine was described experimentally as 3.5 g/kg of *Prunus* sp. [10], 5 g/kg of *Manihot* sp. [6], and 10.3 g/kg of Tifton 68 [9]. These doses could trigger clinical signs and, in some cases, cause death. Considering that the cyanogenic plants have small poisonous doses, and the animals usually ingest large quantities of the plant, the clinical course is normally fast, explaining why farmers often found dead animals near the plants.

Considering that the Tifton 68 cultivar was described as toxic, triggering clinical signs with a dose superior to the aforementioned [9], an animal with a weight of 600 kg would have to ingest 6.18 kg of pasture. Therefore, considering our result that Tifton 68 presents 34.17 mg of HCN in 1 kg of plant, the animal must ingest 211.17 mg of HCN to be poisoned. The amount of HCN determined for each cultivar allowed the calculation of the amount of plant necessary to poison a 600 kg animal with each cultivar. The amount of Florakirk is 1.76 kg, Star of Puerto Rico is 1.89 kg, Tifton 68 is 6.18 kg, Coast-Cross is 10.74 kg, and Tifton 44 is 11.31 kg. The amount necessary for each cultivar indicates the high risk of Florakirk and Star of Puerto Rico cultivars.

The factors that influence the poisoning are toxicity and speed of ingestion [13]. Furthermore, when the lethal dose is not ingested, the animal metabolizes the HCN, which causes mild clinical signs of toxicity followed by recovery [9]. Thus, not all animals in the evolve to death in some outbreaks.

Clinical signs of the poisoning are hard to observe since the clinical course of poisoning takes minutes to few hours [5]. In one of the outbreaks diagnosed, the clinical course was of 3 h. However, in experimental poisoning with cyanogenic plants, the clinical signs began between 10 and 49 min after

ingestion and evolved to death in 30 min to 3 h, varying according to the dose [6,9].

The pasture base or contamination of the pasture by cyanogenic trees was the most frequent mode of poisoning. Many *Cynodon dactylon* are already described as having potential for hydrocyanic acid poisoning [21]. However, according to the best of our knowledge, this is the first report of 2 outbreaks of spontaneous HCN poisoning by the *Cynodon dactylon* cultivar Florakirk, which caused the death of 8 (28.6%) bovines in a total of 28 diagnosed with HCN poisoning, among the 1,235 (0.65%) bovines necropsied. The Star of Puerto Rico cultivar presented a higher average level of HCN when compared with Tifton 68. This last cultivar is well known to cause HCN poisoning among farmers, veterinarians, agronomists, and animal scientists. Moreover, Florakirk and Star of Puerto Rico cultivars were commonly found in farms in southern Brazil, making it necessary to communicate this high risk.

Other authors also observed the macroscopic findings of unchewed leaves mixed in ruminal content [9,13,15]. Furthermore, one case showed samples of *Manihot esculenta* shell. The shell is not a commonly toxic part of the plant but has been described to contain and release HCN [18].

The *post mortem* examination detected an evident bitter almond odor in the ruminal content in some cases, as well as petechial or ecchymotic and subepicardial hemorrhage in the tracheal mucosa, characteristics described in literature [17,19]. These hemorrhages are common and nonspecific lesions caused in the *peri mortem* period, and should not be considered as a characteristic lesion of any pathology. One case showed that the venous blood did not clot and was bright red, which occurs due to the presence of oxygen in the erythrocytes, marking the arterial and venous blood with these characteristics [4].

The Picrosodic paper test, indicating the presence of cyanogenic glycosides, is considered positive when the yellow color of the Picrosodic solution becomes brick-red, and can be performed in the chopped pasture or ruminal content [3]. The Picrosodic paper test was performed in 24 cases in this work, showing positive results for ruminal content and pasture (75%) or only the pasture (25%). This difference occurred because HCN is highly volatile and can be released from the ruminal content from the time of death to the *post mortem* examination.

Moreover, 4 of the cases tested positive in muscles, corroborating the findings in the literature, which reported the detection of HCN in muscle and liver samples as a suspicion of hydrocyanic acid poisoning [19].

Differential diagnosis of hydrocyanic acid poisoning in cattle can include other conditions with rapid death such as *Amorimia exotropa* (not described in west of Santa Catarina), *Nerium oleander* (not present in any of these farms), and nitrate-nitrite poisoning (dark brown blood not seen in any of these cases). Other diseases with hyper acute or acute behavior such as anthrax, electrocution, and ruminal blot by excessive ingestion of leguminous plants are suspected [13]. Characteristic lesions are usually found in the last three.

Determination of the concentration of HCN in different cultivars of the *Cynodon* genus revealed a HCN concentration of 53 to 185 mg/kg in Star of Puerto Rico (mean of 111.7 mg/kg), which is lower than the maximum level already described, 757.8 mg/kg [2]. On the other hand, Florakirk presented a mean of 119.7 mg/kg, with a variation of 80 to 234 mg/kg of HCN, a much higher value than the maximum already described, 68.4 mg/kg [22]. Data regarding the levels of HCN in the other cultivars were not found for comparison.

Statistically, Tifton 68 showed the highest levels of HCN ($34.2 \text{ mg/kg} \pm 30.06$) compared with Tifton 44 ($18.7 \text{ mg/kg} \pm 24.6$), Coast-Cross ($19.7 \text{ mg/kg} \pm 21.8$), Tifton 85 (0 mg/kg), and Jiggs (0 mg/kg). Tifton 44 and Coast-Cross released some HCN levels, requiring precaution when used as animal feed. Tifton 85 and Jiggs on the other hand, can be used without risk of hydrocyanic acid poisoning.

Factors such as precipitation, growth, climate, and nitrogen fertilization favor the concentration of HCN in the plant [20]. Moreover, the concentration of HCN declines with plant aging [22]. Excessive fertilizing can also cause an excessive amount of HCN in the plant. Since all cultivars were subjected to the same fertilization protocol, soil, weather, among other factors, comparison was possible among all cultivars tested.

Florakirk and Star of Puerto Rico Cultivars presented higher levels of HCN compared with other cultivars. Additionally, it is common for farmers in the state of Santa Catarina to subject the pasture to a much more excessive fertilization than the one practiced in the agrostological field of the IFC, especially with manure. Thus, one can presume that the cultivars received much more nitrogen in the farms compared to the IFC.

The results of different parts of the plants showed that the leaf presented a statistically higher concentration of HCN compared with the stem and *in natura* plant of the *Cynodon* genus. This corroborates studies conducted with *Sorghum vulgare* Pers. [14]. The higher concentration of HCN in the leaves can be associated with a mode of defense that the plant develops against ingestion by animals [3].

None of the cultivars showed detectable concentrations of HCN when the hay was evaluated, which is explained by the fact that the haymaking process is culminates in low availability of water, decreasing the concentration of cyanogenic glycosides thereby slowing or stopping the biochemical reactions [9]. However, some works have reported that even though the Picrosodic paper test on hay showed slow or no reaction, poisoning can still occur since the ruminal bacteria can hydrolyze the low concentration of the cyanogenic glycosides [1].

CONCLUSIONS

Hydrocyanic acid poisoning in cattle is an important cause of death, and can lead to great economic losses to producers. The present study is the first description of spontaneous HCN poisoning by *Cynodon dactylon* cultivar Florakirk. Of all *Cynodon* cultivars evaluated, Florakirk is the most dangerous for animals, followed by Star of Puerto Rico and Tifton 68 cultivars, both with similar values. Both cultivars are commonly cultivated in many farms in southern Brazil and the poisoning risk they poses is still ignored.

Tifton 85 and Jiggs cultivars released no HCN and are considered safe for use as pasture. The leaves are the part of the plant with the highest concentrations of HCN. When the hay of all the cultivars was analyzed, HCN was not found in any, and are thus considered safe for use as animal feed. However, it is still unclear if there is an environmental or management procedure of the pasture that can predispose higher concentration of cyanogenic glycosides and induce cattle poisoning.

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Funding. This work was supported by grants from the Instituto Federal Catarinense (IFC) and from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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