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Conditioned food aversion to control poisoning by *Ipomoea carnea* subsp. fistulosa in goats

Aversão alimentar condicionada para o controle da intoxicação por *Ipomoea carnea* subsp. fistulosa em caprinos

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ABSTRACT

Ipomoea carnea *is a toxic plant often ingested by livestock in Brazil. Three experiments were conducted to determine if conditioned food aversion was effective in reducing goats' consumption of* ***I. carnea****. In the first experiment, 10 mildly intoxicated goats that had been eating* ***I. carnea*** *were averted using LiCl (175 to 200mg kg^{-1} body weight). These intoxicated goats did not develop an aversion to* ***I. carnea****, demonstrating that the technique is not effective in goats that are already accustomed to consuming the plant. In the second experiment, 14 naïve goats were placed in a pasture with* ***I. carnea****, and averted after they ingested the plant. In this group the aversion persisted until the end of the experiment, 2 years and 8 months after the initial aversion. In another experiment, 20 goats were placed in a pasture with* ***I. carnea****, and after consuming the plant were averted with LiCl. The averted goats were transferred to Marajo Island and periodically observed over a 2 year period at 2-3 month intervals to determine if they were still averted. The averted goats did not ingest the plant while grazing in the pasture, whereas in 6 neighboring goat farms the prevalence of intoxication from* ***I. carnea*** *poisoning was estimated to be about 40%. These results demonstrated the efficacy of conditioned food aversion to avoid ingestion of* ***I. carnea*** *in formerly naïve goats that had only recently begun to ingest the plant.*

Key words: *poisonous plants, swainsonine, conditioned food aversion, lithium chloride.*

RESUMO

Para testar a técnica de aversão alimentar condicionada como método de controle para a intoxicação por ***I. carnea****, foram realizados 3 experimentos administrando cloreto de lítio (LiCl) na dose de 175-200mg kg^{-1} após a ingestão da planta por caprinos. No primeiro, foram induzidos à aversão 10 caprinos que tinham o hábito de ingerir a planta e com sinais clínicos da intoxicação. Apesar da realização de diversos tratamentos aversivos, após os animais ingerirem a planta, a aversão não foi eficiente, demonstrando que a técnica não é eficiente em caprinos que já estão habituados a ingerir a planta. No segundo experimento, 14 caprinos foram adaptados a ingerir a planta na pastagem e, após ingerirem a planta a campo, foram induzidos à aversão com LiCl. Neste grupo, a aversão persistiu até o fim do Experimento, 2 anos e 8 meses após a aversão. Em outro experimento, 20 caprinos foram adaptados a consumir* ***I. carnea*** *e, em seguida, induzidos à aversão com LiCl. Esses animais foram transferidos para uma propriedade na Ilha de Marajó, onde foram realizadas 9 visitas com intervalos de 2-3 meses para verificar a duração da aversão. Após 2 anos de observações, nenhum animal voltou a ingerir a planta na pastagem e não foram observados casos de intoxicação, enquanto que, em 6 propriedades vizinhas, a doença foi observada com uma prevalência de até 60%. Esses resultados demonstram a eficiência da aversão alimentar condicionada para evitar a ingestão de* ***I. carnea*** *em caprinos recém adaptados a ingerir a planta, nas regiões invadidas por esta planta e nas condições naturais da Ilha de Marajó.*

Palavras-chave: *plantas tóxicas, swainsonina, aversão alimentar condicionada, cloreto de lítio.*

INTRODUCTION

Ipomoea carnea subsp. fistulosa is a plant that contains the indolizidine alkaloid swainsonine, which causes a neurological disease characterized by lysosomal accumulation of oligosaccharides in ruminants, especially in goats. After the ingestion of the plant in large amounts, goats show chronic clinical signs indicating cerebellar and brain stem lesions including intention tremors, dysmetria, imbalance,

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and signs of cranial nerve derangements (ARMIÉN et al., 2007; OLIVEIRA et al., 2009). Some evidence suggests that animals that begin to feed on swainsonine-containing plants become habituated, and eat the plants compulsively regardless of palatability; further, through social facilitation, these animals induce grazing cohorts to consume the plants (ARMIÉN et al., 2007; OLIVEIRA et al., 2009). Currently, there is no treatment for poisoning cases and control methods are inefficient (OLIVEIRA et al., 2009). Conditioned food aversion can be used in ruminants to avoid eating poisonous plants (RALPHS AND OLSEN, 1990; PFISTER et al., 2002; ALMEIDA et al., 2009; PIMENTEL et al., 2012). In Brazil, lithium chloride (LiCl) has been used to induce aversion to consumption of ***Amorimia rigida*** (BARBOSA et al., 2008; PACIFICO DA SILVA & SOTO-BLANCO, 2010), ***Leucaena leucocephala*** (GORNIAK et al., 2008) ***Turbina cordata*** and ***I. carnea*** (PIMENTEL et al., 2012). This aversion may persist for up to three years, but can extinguish quickly if averted animals graze in the same area with non-averted animals that ingest the plant (RALPHS, 1997). The objectives of this study were to test conditioned food aversion as a method to reduce the consumption of ***I. carnea*** in goats, including those animals previously intoxicated as well as animals recently introduced to the plant. Additionally, one goal of the study was to determine the efficacy of this technique to reduce the incidence of ***I. carnea*** poisoning in a commercial goat farm under grazing conditions.

MATERIAL AND METHODS

Experiments 1 and 2 were performed on a farm located in the municipality of Castanhal, Pará state, located at S00°32'05.9'' W048° 39'60.4''; elevation 45m. An experimental 0.8ha paddock with vegetation composed of approximately 67% ***Panicum maximum*** cv. Tanzania, and 33% ***I. carnea*** was used for field tests of ***I. carnea*** ingestion. All goats used in this study were crossbred Spanish-type goats without definite ancestry and were raised on ranches in the general area of Castanhal. In Experiment 3 the goats were averted on a farm in Castanhal and then moved to a farm located on Marajó Island, municipality of Soure, state of Pará, located at S00°30'20.9'' W048°39'03.5'', elevation 18m.

Experiment 1. Ten crossbred goats, four adults and six juveniles, 6 months to one-year-old, were purchased in the municipality of Soure, Marajó Island, Pará, from a flock with numerous cases of poisoning. All these goats were showing mild clinical signs of poisoning from ingesting ***I. carnea***, such as mild intention tremors and weakness of the limbs, mainly when they were disturbed. Further, all animals were observed eating ***I. carnea*** in the paddocks, and all appeared to be habituated to eating the plant even with the availability of other desirable forage. The 10 animals were transferred to the experimental farm and adapted to the consumption of concentrate commercial food in individual stalls for 25 consecutive days. Simultaneously during this 25-day period, freshly harvested branches of ***I. carnea*** were offered to each goat by hanging the plant material from their stall wall for 4 hours each day. After this period the animals were introduced as a group into the paddock with ***I. carnea*** for 4 hours daily over a 15 day period, and an observer noted if each animal consumed ***I. carnea*** in the field.

For induction of aversion, 40 days after arrival in the farm, the animals were fasted overnight, in individual stalls. On day 1, 200g of leaves of freshly harvested ***I. carnea*** were offered in their stall for 10 minutes. Immediately thereafter the animals that ingested any amount of the plant received, by ruminal tube, a solution of LiCl at a dose of 175mg kg^{-1} bw diluted in 1 liter of water. After each treatment the animals were kept in stalls, separated from the others and at the end of the day, received concentrate feed in an amount equivalent to 1% of their body weight, and also freshly-cut grass ad libitum. Four hours later, the remaining feed was withdrawn and they were fasted overnight. All goats ate the offered plant on day 1, and after the LiCl treatment on day 1, did not eat any of the plant on days 2 or 3.

On day 4, all goats were released into the pasture for 4 hours each day for 40 days. Goats that were observed ingesting ***I. carnea*** in the field were returned to their stalls, again offered ***I. carnea*** in their stalls as before, and the aversion treatment was repeated with a dose of 200mg kg^{-1} of LiCl if they ate the plant. Animals that did not ingest the plant in the stalls, but ate it only in the pasture were handled in the pasture immediately after ingesting the plant, and treated with 200mg kg^{-1} of LiCl. During the 40 day pasture portion of the study, individual animals were given up to a maximum of 8 doses of LiCl.

Experiment 2. Twenty four crossbred goats were divided into 2 groups. Group 1 consisted of 14 goats, 10 adults and 4 juveniles (6-8 months-old). Group 2 consisted of 10 young goats (6-8 months-old). All animals were naïve to this plant. Goats from Group 1 were introduced into the experimental paddock, four hours a day for 30 days, and those that consumed at least 10 bites of ***I. carnea*** were given

an oral gavage in the pasture with LiCl at a dose of 200mg kg^{-1} bw. From the 31st day until the end of the experiment 2 years and 8 months later, the animals were introduced to the experimental paddock 2 days a week for 4 hours daily. Goats from Group 2 were also introduced in the experimental paddock and those that consumed more than 10 bites of ***I. carnea*** received 1 liter of water by ruminal tube. This group was observed in the same way as Group 1, but the groups did not graze simultaneously in the pasture.

Experiment 3. Twenty young crossbred goats, naïve to ***I. carnea***, were used. These animals were released in the experimental paddock for 15 days. After initiating consumption of ***I. carnea*** in the pasture, on day 16 the animals were given access to ***I. carnea*** in stalls, by offering branches of green plant (>200g of fresh plant) hanging on the walls of the stall for 1 hour per day for 5 consecutive days. On days 21 to 23, goats were exposed to the plant in their stalls, and those animals that ate any amount were dosed with LiCl at 200mg kg^{-1} bw as previously noted. After this 3-day aversion period, the plant was offered to all animals for 3 consecutive days, and no further dosing was done as all animals abstained. After inducing an aversion in the stalls, the goats were introduced into the experimental pasture for 4 hours daily for 45 consecutive days. During this 45-day period the animals were also challenged in the stalls by offering ***I. carnea*** as previously mentioned on grazing days 3, 5, 10, 15 and 30. After this 45-day grazing trial, the 20 averted goats were transferred to a farm on Marajó Island. Poisoning by ***I. carnea*** in goats had been previously diagnosed on this farm, and because of the severity of the poisoning all goats had been removed (OLIVEIRA et al., 2009). After the transfer, 9 visits over 32 months, with intervals of 2-3 months, were made to determine if the goats were still averted. On each visit the animals were observed grazing for 4 hours daily for 3 consecutive days in the paddocks. Further, the goats were placed into individual stalls, and freshly harvested branches of green ***I. carnea*** were offered for 3 days for 1 hour each day. All animals were examined during each visit including a specific clinical examination of the central nervous system. Additionally, a survey was made of the occurrence of outbreaks of poisoning by ***I. carnea*** in 6 neighboring farms that raised goats.

Swainsonine concentrations in ***I. carnea***. Aerial parts of ***I. carnea*** (10 samples) were randomly collected in the farms in Castanhal and Marajó Island to determine the levels of swainsonine by liquid chromatography and mass spectrometry using the methods described by GARDNER et al. (2001).

RESULTS

Experiment 1. After the first aversive treatment in stalls, all 10 goats began to eat the plant in the pasture on days 4 to 10 and were again averted. From the 12th to the 20th day, it was observed that most goats were still consuming ***I. carnea*** in the pasture, but did not consume the plant offered in the stalls. All goats except one ingested ***I. carnea*** and were averted in the pasture between days 21 to 35. In the end, after 40 days, 9 animals continued to ingest the plant despite being treated with LiCl from 2 to 8 times.

Experiment 2. Group 1 (Averted). On the 3rd day of grazing, 4 young animals spontaneously began to consume small amounts of ***I. carnea*** leaves even with good forage availability. The amount consumed by these animals increased gradually and in the days following, 5 adult animals also began to consume small amounts of plant. On the 13th day, the 9 animals were consuming large amounts of leaves, flowers and fruits of ***I. carnea*** and were treated with LiCl. These 9 treated animals did not consume the plant again during the entire 32 month observation period. The 5 adult goats that had never ingested the plant and were not treated also did not ingest ***I. carnea*** during the 32 month observation period.

Group 2 (Controls). By the 10th day of observation, 8 goats spontaneously consumed small amounts of ***I. carnea*** leaves. Over the next few days, the animals increased the quantity of leaves consumed, and by 13th day, all animals in this group were consuming the plant in varying amounts. After 23 days of observation all the animals were consuming large amounts of leaves, flowers and fruits, and soon after ingestion of the plant, were treated by ruminal tube with 1 liter of water. The animals continued to consume the plant in the pasture for 5 days and then these control animals were removed from further exposure to ***I. carnea*** before clinical signs developed.

Experiment 3. On the 5th day after being released in the paddock with ***I. carnea***, 3 goats started to ingest the plant. By day 10, 11 goats had ingested the plant and by the 15th day all animals had begun ingestion. All goats also ingested the plant when it was offered in the stalls for another 5 days. After 20 days of exposure to ***I. carnea*** in the pasture and in the stalls, the plant was offered for 3 days and all animals that ingested were averted. On the 1st day all goats ingested the plant and were treated with LiCl at the dose of 200mg kg^{-1} bw. On the 2nd day, 11 animals returned to eat small amounts of the plant and were treated again. Only 1 goat ingested the plant on the 3rd day and was treated again.

None of the goats consumed ***I. carnea*** during the 45 day grazing period in the experimental pasture, nor did they eat any of the plant in their stalls during the challenges. At that point, all goats were transferred in April, 2011 to the farm on Marajó Island. During the 9 observation periods spanning 32 months, none of the goats were observed to consume any ***I. carnea*** in the pasture. Four visits were conducted during the dry season (September and December 2011 and 2012), three in the rainy season (February and May, 2012 and March 2013) and 2 in the transition period between the dry and rainy season (July, 2011 and 2013). During the visits in the dry period, there was always an acute shortage of other available forage, yet ***I. carnea*** remained green and abundant throughout the paddocks. When the goats were observed grazing in areas heavily invaded by ***I. carnea***, the plant was not consumed. However, the goats consumed large amount of fruits and seeds of a tree known as "siriubeira" (***Avicennia germinans***), and leaves of another tree known as "turia" (***Drepanocarpus lunatus***). During visits in the rainy season and transition period there was always good forage availability, and ***I. carnea*** remained green and had flowers and fruits.

When ***I. carnea*** was offered in the stalls, all animals smelled the plant and some consumed small amounts or part of the stem, leaves, flowers and fruits, but no treatment with LiCl was given. No clinical signs of intoxication were observed during the clinical examinations of the animals.

During the experimental period, 9 goats kidded. Of the 18 kids born, 2 died shortly after birth. None of the kids began to ingest the plant and therefore were not averted. Nine goats died of different causes or were slaughtered by the landowner.

There were no clinical cases of poisoning by ***I. carnea*** noted during regular visits to 6 neighboring farms until the 7th visit (September 2012). However, during the 8th and 9th visits (December 2012 and March 2013, respectively) affected goats were observed on all farms. Clinical signs characteristic of ***I. carnea*** poisoning included hindlimb weakness, ataxia, broad based position, head tremors and falls. In one farm 9 out of 32 goats presented clinical signs. Most of these animals were slaughtered by their respective landowner before weight losses became severe.

Swainsonine concentrations in ***I. carnea***. Mean concentrations of swainsonine in ***I. carnea*** in the farm in Castanhal were 0.047±0.035%, ranging from not detected to 0.1% between different samples. On Marajó Island the average swainsonine concentration was 0.07±0.076%, ranging from not detected to 0.184%.

DISCUSSION

Experiment 1 demonstrated that goats that are already showing clinical signs of intoxication from ***I. carnea*** and are habituated to consuming the plant, do not respond to the aversive treatment. In contrast, in Experiment 2, goats that were naïve then exposed to the plant were successfully averted at the end of the initial exposure, and further, did not ingest the plant for at least 2 years and 8 months. This result suggests that livestock producers may be able to reduce losses substantially by culling goats that are habitually consuming the plant, and then introducing naïve animals that have recently been averted or that can be averted on site. The primary difference in the results of these two experiments is apparently the length of time that animals are exposed to the plant. It has been demonstrated that aversion is most effective when used with novel foods (RALPHS & PROVENZA, 1999). Horses and sheep previously intoxicated by the swainsonine-containing plants *Astragalus* and *Oxytropis* (locoweeds) may develop aversion to these plants, but in some animals, this aversion may not be as efficient as aversion induced in non-intoxicated animals (PFISTER et al., 2007).

During Experiment 1, after the aversive treatment in the stalls, goats continued to ingest ***I. carnea*** in the pastures, but rejected the plant when it was offered in the stalls. The context of the aversion in livestock is important (RALPHS & OLSEN, 1990; BURRITT & PROVENZA, 1997), as the pasture where the goats were challenged after the aversion was an unfamiliar environment to the animals, whereas the stalls were a familiar environment. Heifers averted to toxic ***Delphinium barbeyi*** with LiCl and placed into an unfamiliar environment began to ingest ***D. barbeyi*** when grazing, but the cattle did not eat the plant when the aversion was tested in the corral where they had been originally averted (RALPHS & OLSEN, 1990). further, BURRITT & PROVENZA) indicated that animals should be averted in the environment where they will graze to increase the persistence of the aversion.

In Experiment 2, it was observed that 5 adult animals did not begin to consume ***I. carnea***, demonstrating that not all animals kept in pastures invaded by the plant start to consume it spontaneously. However, all young animals of the two groups began rapidly to ingest the plant, even when there was other desirable forage available. In

general, young animals are more prone to try and eat unfamiliar foods (RALPHS & CHENEY, 1993). This fact should be taken into account in considering the use of conditioned aversions to reduce livestock losses to ***I. carnea***.

In Experiment 3, the introduction of goats previously averted to the plant effectively prevented consumption of ***I. carnea*** even under stressful grazing conditions. In previous visits to this same farm in earlier years, cases of intoxication were observed throughout the year, and in the dry season nearly 100% of the animals showed clinical signs (OLIVEIRA et al., 2009). The kids born on the farm to the experimental goats did not ingest the plant, suggesting that the aversion may be multi-generational (MIRZA & PROVENZA, 1990; SANGA et al., 2011), but this will require further verification. Further, our observations suggest that ***I. carnea*** is not a preferred forage species, and that goats avoid the plant except under certain conditions. Consumption of ***I. carnea*** appears to occur primarily during times of extreme scarcity of forage (i.e., drought or overgrazing), and younger goats may show a greater propensity to eat the plant than adults. In this experiment the maintenance of the aversion was facilitated apparently by the consumption of fruits of ***A. germinans*** and leaves of ***D. lunatus***, which were available even though other forage was in short supply. Forage availability is important to maintain aversion, and animals with a good supply of forage can maintain the aversion for up to three years (RALPHS, 1997).

When ***I. carnea*** was periodically offered to the goats in the stalls during visits to the farm on Marajo Island, some goats consumed small amounts of the plant, including flowers or fruits. Even so, the animals did not eat the plant while grazing, and the aversion was not extinguished. In other studies with livestock and toxic plants, sampling of the target plant has resulted in gradual extinction of the aversion (PFISTER, 2000). The context of offering the plant in the stalls where the aversion was first conditioned may have been important in maintaining the aversion. In addition, PFISTER (2000) reported that the aversion may be specific to different plant parts (leaves, flower, stem, petiole) or ripeness (green, dried or wilted).

The success of averting naïve goats to ***I. carnea*** in farms suggests that removal of goats that ingest the plant, followed by their substitution by averted goats may be an effective means to reduce goat losses. Another possibility may be the removal of goats that ingest the plant followed by the induction of aversion in each naïve replacement goat if it begins to eat ***I. carnea***. This technique was applied successfully to control poisoning in goats by ***T. cordata***, another swainsonine-containing plant (PIMENTEL et al., 2012). The use of other techniques such as removal of plants by pulling, plowing or using herbicides, or building fences to prevent animal access is economically infeasible on Marajo Island because most areas remain flooded for long periods each year, the production system is very extensive, and access may be difficult. Providing supplemental feed during the dry season may also be a way to prevent the onset of consumption of ***I. carnea***.

Despite the effectiveness of the aversion technique to control ***I. carnea*** poisoning, there are some factors that should be considered to maintain the aversion of the herd and prevent the initial ingestion by some goats. Young animals, especially those born after the start of an aversion program need to be observed for the consumption of the plant and, if necessary, averted. Other work has shown that the response by lambs to a toxin such as LiCl was more important than the influence of the mothers on what the lambs ate, because the lambs were conditioned by LiCl to avoid the plant whether or not their mothers ate the target food (PROVENZA et al., 1993). Averting young animals so that the aversion is maintained in the herd could be done every two months by offering the plant in the stalls or by observation of the animals during grazing. This technique was successfully used by PIMENTEL et al. (2012) to control ***T. cordata*** poisoning. Another important point is the availability of forage, particularly for livestock producers to avoid overgrazing and to provide, when possible, supplemental feed during the dry season. In an outbreak of poisoning ***I. carnea***, in the state of Paraiba, the aversion was effective in preventing consumption during the rainy season, even in animals that had ingested the plant for long periods, but the goats began again to ingest the plant during the dry season because of the severe shortage of forage (PIMENTEL et al., 2012).

In conclusion, conditioned food aversion can effectively prevent the ingestion of ***I. carnea*** in naïve or less experienced animals, whereas in animals that have been ingesting the plant for long periods this method of reducing losses is not effective. Intoxication by ***I. carnea*** in goats can be prevented by removing experienced and/or intoxicated animals that ingest the plant, followed by the introduction of relatively inexperienced and conditioned goats. However, it is necessary to maintain periodic surveillance to detect and condition aversions mainly in young goats that start to ingest the plant.

ETHICS COMMITTEE

The experiment was approved be the ethical committee on animal experimentation on the UFCG, process CEP 69-2013.

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