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Protection against *Amorimia septentrionalis* poisoning in goats by the continuous administration of sodium monofluoroacetate-degrading bacteria

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ABSTRACT

The intraruminal inoculation of sodium monofluoroacetate (MFA)-degrading bacteria has been proposed as a method to prevent poisoning by MFA-containing plants. In previous experiments, MFA-degrading bacteria were inoculated intraruminally before or concurrent with plant challenge, with both strategies conferring partial protection to poisoning. To evaluate the protection to *Amorimia septentrionalis* poisoning provided by the continuous inoculation of MFA-degrading bacteria isolated from plants and soils, 18 goats were divided into three experimental groups of six animals each: Group 1 goats received daily doses of a mixture of *Paenibacillus* sp. and *Cupriavidus* sp., and Group 2 goats received a mixture of *Ralstonia* sp. and *Burkholderia* sp., for 40 days, while Group 3 goats were not inoculated. Ten days after initiation of bacterial inoculation in Groups 1 and 2, all goats were challenged daily with 5 g/kg body weight of green leaves from *A. septentrionalis*. Four goats from Group 1 consumed the leaves throughout the 30-day consumption period and showed clinical signs such as transient tachycardia and engorgement of the jugular. The two remaining animals from Group 1 showed obvious signs of intoxication, and plant administration was suspended on days 17 and 19. The goats in Group 2 consumed the leaves throughout the 30-day study without showing signs of poisoning. The goats from Group 3 (control) manifested severe clinical signs of poisoning between the 3rd and 10th days following the start of the *A. septentrionalis* challenge. Under the conditions of this experiment continuous intraruminal administration of *Ralstonia* sp. and *Burkholderia* sp. provided complete protection to poisoning by *A. septentrionalis* in goats, while continuous intraruminal administration of *Paenibacillus* sp. and *Cupriavidus* sp. provided partial protection.

1. Introduction

Amorimia septentrionalis, commonly known as Tingui, contains sodium monofluoroactetate (MFA) and can cause acute heart failure associated with exercise in ruminants (Silva et al., 2015). *Amorimia* spp., together with *Palicourea* spp. and *Arrabidaea* spp., which also contain MFA, are responsible for causing approximately 50% of toxic plant deaths in Brazilian cattle, generating economic losses across the country (Pessoa et al., 2013).

The induction of resistance to poisoning by MFA-containing plants through treatment with MFA-degrading bacteria has been studied (Pessoa et al., 2013). Recent studies have shown increased resistance to poisoning by MFA-containing plants in goats inoculated with the MFA-degrading bacteria *Ancylobacter dichloromethanicus* and *Pigmentiphaga kullae*, which were isolated from the rumen content of goats (Pessoa et al., 2015). Furthermore, it was found that this resistance can be transmitted to susceptible animals by transferring the rumen content of those that are resistant (Silva et al., 2015).

The presence of MFA-degrading bacteria in soil and plants was shown by Twigg and King (1991), who isolated 24 MFA-degrading microorganisms in Australia. Camboim et al. (2012) isolated *Paenibacillus* sp. [whose 16S rRNA gene sequence is registered in the

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National Center for Biotechnology Information (NCBI) as ECPB01], *Burkholderia* sp. (ECPB02), *Cupriavidus* sp. (ECPB06), *Staphylococcus* sp. (ECPB04), *Ancylobacter* sp. (ECPB05), *Ralstonia* sp. (ECPB06) and *Stenotrophomonas* sp. (ECPB07) from Brazilian plants and soils, and demonstrated that each of them could degrade sodium fluoroacetate in culture. They suggested establishing populations of these bacteria in the rumen might prevent poisoning by MFA. This study aimed to evaluate the effectiveness of using MFA-degrading bacteria isolated from Brazilian plants and soils in the detoxification of MFA from *A. septentrionalis* in the rumen.

2. Material and methods

Nine female and nine male goats, ranging in age from 1 to 3 years weighing 20–40 kg, and raised in areas free of MFA-containing plants, were randomly divided into three groups of six animals each. Group 1 goats received a daily oral administration of a 60 ml solution containing *Paenibacillus* sp. isolated from soil samples and *Cupriavidus* sp. isolated from plants and soil (Camboim et al., 2012); this treatment lasted for a total of 40 days. The goats in Group 2 received a daily oral administration of a similar solution of *Ralstonia* sp. isolated from plants and soil and *Burkholderia* sp. isolated from soil (Camboim et al., 2012), also for the 40-day study period. Bacteria were not inoculated into Group 3 goats.

To obtain the bacterial solutions, bacteria were plated on blood agar with 5% defibrinated sheep blood and incubated in a bacteriological oven at 37 °C for 24–48 h. Each bacterium was diluted in 0.9% sterile saline solution until it reached level 1 cell density on the McFarland scale. The inoculum was prepared by mixing 50 mL of each bacterial solution; 60 mL of this mixture was inoculated intraruminally daily into each goat.

The *A. septentrionalis* provided to the animals was collected in the municipality of Teixeira (S7 ° 12.24 ′O37° 15.11′, elevation 749 m), which is located in the state of Paraiba, Brazil. It was harvested in the same location as plants used in previous experiments (Duarte et al., 2014; Pessoa et al., 2015; Silva et al., 2015). The average MFA concentration across 20 samples of *A. septentrionalis* leaves collected from that location was 0.002 ± 0.0009% (Lee et al., 2012).

Ten days following the start of daily intraruminal inoculation of the MFA-degrading bacteria solutions, all goats in all three groups started daily consumption of 5 g/kg doses of green leaves from *A. septentrionalis*. This dose was determined after previous daily administration of leaf at this rate to two goats resulted in clinical signs after 3 days. Two other goats that received daily doses at 3 g/kg of *A. septentrionalis* for 15 days did not show clinical signs. The leaves of *A. septentrionalis* were offered voluntarily to the goats, but for those that did not spontaneously consume the plant, it was administered by placing small amounts directly into their mouths. Feeding or administration of the *A. septentrionalis* continued for 30 days or until the animals demonstrated severe clinical signs of intoxication. Such signs included depression, reluctance to walk, muscle tremors, uncoordinated movement and sternal recumbence. This protocol was decided upon following a pilot experiment in which four goats, divided into two groups, received bacterial treatment for 10 days only and then began to consume the MFA-containing plant. In this pilot, the two goats that received a solution of *Paenibacillus* sp. and *Cupriavidus* sp. showed signs of intoxication 4–5 days after starting plant consumption, and the two goats that received a solution of *Ralstonia* sp. and *Burkholderia* sp., showed signs of intoxication 10 days after initial plant ingestion.

From the start of the experiment, the three groups of goats were observed daily, and measurements were taken of the heart and respiratory rates, ruminal movements and rectal temperatures. After the start of plant administration, the goats were forced to walk for 10 min, and those same parameters were measured before and after exercise.

Comparison of the mean dose of *A. septentrionalis* consumed between groups was performed using the non-parametric Mann–Whitney test, as the data did not show a normal distribution when using the Shapiro–Wilk test. The ingested dose of plant material and the time until the onset of clinical signs of intoxication were analyzed using Student's T test. Data analysis was performed with the aid of the R 3.0.3 program at the 5% significance level.

3. Results

Four goats in Group 1 ingested *A. septentrionalis* throughout the 30-day administration period, showing slight transient signs of intoxication (tachycardia and engorgement of the jugular) between the 15th and 20th days of plant consumption. The remaining two goats in Group 1 initially showed signs of intoxication on the 15th and 16th days of *A. septentrionalis* administration. These signs continued to worsen and on the 17th and 19th days, plant administration was stopped due to the risk of death. Goats from Group 2 consumed the plant for the full 30 days without showing signs of poisoning. The Group 3 control goats showed signs of severe clinical intoxication between the 3rd and 10th days of plant administration (Table 1), and feeding of it was stopped.

The total mean dose of plant ingested by goats in Group 1 (130 ± 31.14 g/kg) and Group 2 (150 ± 0.0 g/kg) was significantly different ($p = 0.031$ and $p = 0.035$, respectively) than the dose ingested by Group 3 (30.83 ± 15.94 g/kg). The doses consumed by Groups 1 and 2 were not significantly different ($p = 0.175$). Plant consumption was suspended in Group 3 after 6.16 ± 3.18 days because the goats showed severe signs of intoxication, and risked death. The period of plant consumption before clinical signs became apparent was significantly longer ($p = 0.000064$) in Group 1 goats than in Group 3 goats (Table 1). Group 1 goats also consumed (91.66 ± 18.07 g/kg) significantly more plant before the onset of clinical signs than did the Group 3 control animals (16.66 ± 6.05 g/kg) ($p = 0.000060$) (Table 2).

The clinical signs of intoxication observed in the two severely affected goats from Group 1 and all goats in Group 3 (control) were characterized initially by tachycardia and tachypnea, followed by engorgement of the jugular, positive venous pulse, and culminating with depression, reluctance to walk, muscle tremors, bellowing, uncoordinated movement and sternal recumbence. Most severely affected goats recovered over a period of 4.5 ± 0.57 days after cessation of plant ingestion, but two goats in Group 3 died 2 and 3 days after the onset of clinical signs. At necropsy, blood clots in the left ventricle of the heart and pulmonary edema were observed in these animals. Histologically, there was vacuolar-hydropic degeneration and necrosis of epithelial cells of the distal convoluted tubules in the kidneys.

Table 1
Time to onset of clinical signs of intoxication by *Amorimia septentrionalis* in goats inoculated with the MFA-degrading bacteria *Paenibacillus* sp. and *Cupriavidus* sp. (Group 1) and non-inoculated controls (Group 3)[a].

	Group 1						Group 3					
Goat number	1	2	3	4	5	6	1	2	3	4	5	6
Days to onset of clinical signs	20	15	24	20	15	16	3	5	4	2	2	4
$\overline{x}\overline{x}\pm\sigma$	18.33 ± 3.61[b]						3.33 ± 1.21					

[a] Goats in Group 2 did not become sick.
[b] Statistically significant difference observed with the control group ($P < 0.05$).