Applied Animal Behaviour Science 117 (2009) 197–200

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Conditioned aversion in sheep induced by *Baccharis coridifolia*

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ARTICLE INFO

Article history:
Accepted 9 December 2008

Keywords:
Baccharis coridifolia
Food aversion
Sheep

ABSTRACT

In Southern Brazil, Uruguay, Argentina, and Paraguay, the invasive weed *Baccharis coridifolia* often poisons naïve animals. Farmers prevent *B. coridifolia* poisoning using several unconventional methods to reduce ingestion: (1) burning plant material under an animals' nose, and having the animal inhale the resulting smoke; (2) rubbing the plant on the animals' muzzle and mouth; and (3) gradually introducing animals into *B. coridifolia*-infested pastures. To determine if *B. coridifolia* would condition an aversion, and to test the efficiency of these three aversive methods, 18 adult sheep were used to induce an aversion to corn, a novel food. In Group IBc, four sheep ingested 0.25 g/kg bw of fresh *B. coridifolia*. In Group OMBc, four sheep were treated by rubbing the plant in the mouth. In Group IHBc, four sheep inhaled smoke produced by burning *B. coridifolia*. In Group LiCl, two sheep were treated by oral gavage with 175 mg/kg lithium chloride (LiCl). In Group IA, two sheep received alfalfa by oral gavage. In Group IHLm, two sheep inhaled the smoke produced by burning ryegrass. On days 1–5, 10, 30, 60 and 90, 100 g of corn were offered to the animals. All sheep that ingested *B. coridifolia* or were treated with LiCl and one that inhaled smoke produced by burning *B. coridifolia* developed an aversion to corn for the whole experimental period. After 1 year, sheep from Groups IBc, OMBc, LiCl, and IA were transferred to a pasture with *B. coridifolia*, and observed for plant consumption. Sheep from group IBc that were treated with *B. coridifolia* the previous year, did not graze the plant. Sheep from the other groups ingested the plant occasionally, had anorexia, and two showed signs of digestive stress and died. Results demonstrate that *B. coridifolia* is as efficient as LiCl in conditioning an aversion to a previously unknown food.

1. Introduction

Conditioned aversion to a novel food is a powerful experimental tool to modify an animal's diet and has been used, for example, to prevent predation of livestock by coyotes and wolves, to prevent destruction of grains by rodents, and for treatment of alcoholism in humans (Ralphs and Provenza, 1999). Also, it has been investigated to prevent livestock from grazing poisonous plants such as *Delphinium barbeyi*, *Oxytropis sericea*, *Astragalus* spp., and *Pinus ponderosa* (Ralphs and Olsen, 1990; Pfister and Price, 1996; Ralphs, 1997; Pfister, 2000; Pfister et al., 2002, 2007). The mechanism responsible for the development of aversion is not well established, but it is suggested that animals learn which plants or food to eat and which to avoid through interactions between flavor (odor, taste and texture) and the post-ingestive consequences of nutrients and toxins (Provenza, 1996).

Baccharis coridifolia is a weed of the Asteracea family, known as mio-mio. It is one of the most important toxic plants in southern Brazil, Argentina, Paraguay and

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0168-1591/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.applanim.2008.12.006

Uruguay. Intoxication by mio-mio has been known since the Spanish colonizers introduced horses and cattle into South America. In 1653, the Jesuit priest Bernabé Cobo mentioned that in the provinces of Tucuman (Northern Argentina) and Paraguay horses died after the ingestion of a plant known by the indians as mio, and the only way to prevent the ingestion of the plant was by rubbing the plant in the mouth, lips and nose of the animals (Cobo, 1653). The spontaneous poisoning, causing severe digestive disturbances, occurs mainly in cattle, less frequently in sheep, and rarely in horses. In recent outbreaks reported in Brazil morbidity was 22% and 16.5% in cattle (Rissi et al., 2005) and sheep (Rozza et al., 2006), respectively, and the fatality rate was 100% in both species.

The poisoning occurs when animals raised in areas without the plant are transported to, and allowed to graze in, pastures infested by *B. coridifolia*. Intoxication risk increases considerably when recently transported animals are stressed, fatigued, hungry, or thirsty (Barros, 1998; Riet-Correa and Méndez, 2007). Animals raised in rangelands where mio-mio grows are rarely or never poisoned (Barros, 1998; Riet-Correa and Méndez, 2007). Various preventative measures have been recommended, but have not proven to be efficacious. Some livestock producers practice a form of aversive conditioning, in which animals are averted from eating the plant by burning plant material under an animals' nose, and having the animal inhale the resulting smoke to condition an aversive reaction (Barros, 1998; Riet-Correa and Méndez, 2007). Similarly, producers rub small quantities of fresh plant material on the animals' muzzle and mouth in order to condition a taste aversion and to avoid future consumption (Barros, 1998; Riet-Correa and Méndez, 2007). Another preventative measure is to introduce animals gradually into mio-mio-infested pastures. On the first day, grazing animals are rapidly moved through infested pastures; each succeeding day for 5–10 days the animals are gradually exposed for longer periods of time to mio-mio, until finally experienced animals will not consume the plant (Tokarnia et al., 2000).

B. coridifolia is a highly toxic plant. The lethal dose to cattle of the green plant during flowering varies between 0.25 and 0.50 g/kg body weight (Tokarnia and Döbereiner, 1975). It is 4–8 times more toxic in the flower stage than when sprouting. During the vegetative stage the lethal dose for cattle is 2 g/kg (Tokarnia and Döbereiner, 1975, 1976). Sheep are more resistant than cattle and need to ingest about double the quantity of plant material that cattle must ingest to be fatally intoxicated (Tokarnia and Döbereiner, 1976). All parts of the plant are toxic (Tokarnia and Döbereiner, 1975, 1976).

Conditioned aversion may be the mechanism by which animals avoid intoxication because animals raised in areas where the plant occurs do not ingest the plant. The toxins in *B. coridifolia* are macrocyclic trichothecenes, including roridin A and E, roridin A, D, and E, verrucarin A and J, and miotoxin A. These compounds are mycotoxins produced by soil fungi, primarily *Myrothecium roridum* and *M. verrucaria*, which are absorbed by the plant (Busam et al., 1985). It is unknown if some of these substances have an aversive effect or if the aversion induced by mio-mio is due to another substance.

The objective of this paper was to determine the efficacy of *B. coridifolia* to condition an aversion to a novel food in sheep. In addition, the study evaluated the effectiveness of various methods of administering *B. coridifolia* to animals to condition short- and long-term aversions.

2. Materials and methods

The capacity of *B. coridifolia* to induce aversion to a previously unknown food (corn) was tested by three different treatments: (1) by rubbing the plant in the mouth of the animals (oral manipulation, OM); (2) by inhalation (IH) of the smoke of the plant; and (3) by the ingestion of small amounts of *B. coridifolia* (IBc). For this, 18 adult Corriedale sheep, non pregnant ewes and males, weighting 32.5–39 kg, were divided at random in six groups. The experiment was performed in two steps: in the first sheep were treated with the different aversion methods and tested at various times until 90 days; in the second, 1 year or after the end of the aversion experiment, the animals were transported to a farm with pastures infested with *B. coridifolia* for a field test if the animals were averted to growing *B. coridifolia*.

In Group IBc, four sheep ingested 0.25 g/kg bw of fresh *B. coridifolia*. Four sheep from Group OMB were treated by rubbing the fresh plant in the mouth. In Group IHBc, four sheep inhaled smoke produced by burning fresh *B. coridifolia*. In Group LiCl, two sheep were given 175 mg/kg bw lithium chloride (LiCl) by oral gavage with a maximum volume of 0.5 L of tap water. In Group IA, two sheep received dried, ground alfalfa by oral gavage (0.25 g/kg). In Group IHLm, two sheep inhaled the smoke produced by burning dried *Lolium multiflorum*. To test the strength and persistence of the aversion, on days 1–5, 10, 30, 60 and 90, 100 g of corn was offered to the animals for 10 min. Animals were fasted for 15–16 h, then offered corn individually in their pens; refusals were weighed.

On the first day of the experiment each sheep, naïve to corn and maintained in individual pens, received 100 g of ground corn for 10 min. After this period the corn that was not ingested was weighed and each sheep received the treatment mentioned above (Dose 1). After the treatment the animals received commercial feed pellets at 1% of body weight (as fed basis). Water was offered *ad libitum* and the pellets were withdrawn at night.

On the second day of the experiment 100 g of corn was offered for 10 min to each experimental sheep. After this period the treatment was repeated (Dose 2) for any sheep that ingested any amount of corn. This procedure was repeated on day 3 (Dose 3).

On days 4, 5, 10, 30, 60 and 90 the animals were offered 100 g of corn for 10 min, and the amount consumed was recorded; no additional doses of any kind were given. After the final pen test on day 90, the sheep were maintained as a group on a native grass pasture at the Federal University of Pelotas. One year later, some treatment animals were used in a field study to determine the persistence of the aversion to mio-mio. Sheep from Groups IBc, OMBc, LiCl, and IA were transferred to a farm 180 km from the pen study. Animals were introduced to a 100 m^2 paddock containing

Table 1
Ingestion of novel corn (means ± S.E.) by sheep in various treatments[a] after initial exposure to corn (day 1) and on subsequent test days. Sheep were offered 100 g of corn for 10 m.

Treatment	Day 1	Day 2	Day 3	Day 5	Day 10	Day 30	Day 60	Day 90
Group IBc	16.1 ± 3^b	16.2 ± 16^a	3.2 ± 2^a	1.0 ± 0.6^a	1.5 ± 1^a	3.8 ± 4^a	2.5 ± 1^a	3.0 ± 1^a
Group IHBc	62.5 ± 23^b	50.4 ± 29^a	32.6 ± 19^a	54.8 ± 18^b	50.0 ± 29^b	75.0 ± 25^b	75.0 ± 25^b	75.0 ± 25^b
Group OMBc	31.6 ± 7^a	76.2 ± 10^b	93.1 ± 7^b	95.8 ± 4^b	91.5 ± 9^b	70.8 ± 17^a	87.7 ± 8^b	87.1 ± 8^b
Group LiCl	10.9 ± 4^a	0 ± 0^a	0 ± 0^a	0 ± 0^a	1 ± 1^a	0 ± 0^a	5.1 ± 5^a	3.3 ± 0.8^a
Group IA	22.8 ± 2^a	70.0 ± 30^b	100 ± 0^b	100 ± 0^b	51 ± 49^b	$5.5 \pm .5^a$	100 ± 0^b	57.8 ± 42^b
Group IHLm	100 ± 0^b	60.5 ± 40^b	36.9 ± 27^a	100 ± 0^b	65.4 ± 35^b	79.5 ± 21^b	100 ± 0^b	100 ± 0^b

Only preplanned comparisons were considered. For simplicity, the comparison shown here is Ingest *Baccharis* (IBc) vs. the other treatments; treatment means for IBc followed by a different superscript letter for other treatments indicates a significant ($P < 0.05$) difference on that test day for corn ingestion. LiCl can be considered a negative control, whereas Ingest Alfalfa (IA) can be considered a positive control.

[a] Treatments: IBc: oral dose of *B. coridifolia* at 50% of lethal dose; OMBc: oral manipulation with *B. coridifolia*; IHBc: inhalation of *B. coridifolia* smoke; LiCl (175 mg/kg): oral dose of LiCl; IA: oral dose of ground alfafa; ILm: inhalation of *L. multiflorum* smoke.

substantial mio-mio, with adequate forage availability. Mio-mio abundance was visually estimated at 20–25% of the botanical composition of the pasture. Animals were observed for plant consumption and presence of clinical signs. The animals grazed for 30 h in this paddock, under continuous observation during all daylight hours by observers blind to the treatment groups. All occurrences of mio-mio consumption were recorded. Two sheep that died were necropsied and studied histologically by current methods.

A mixed model repeated measures analysis was performed on the corn intake data using SAS (SAS Inst. Inc., Cary, NC; Version 9.1 for Windows). The repeated factor was days in the experiment. Sheep were a random factor and were nested within treatments. Even though sample sizes were small, the magnitude of the treatment effects were large, and the mixed model procedure was sufficiently robust to show treatment effects and day × treatment interactions ($P < 0.05$). Preplanned multiple comparisons were made after significant *F*-tests using the PDIFF procedure of SAS. No statistical evaluation of the pasture grazing study was done.

3. Results

All sheep that ingested *B. coridifolia* (IBc) or were treated with LiCl, and one sheep, from the Baccharis smoke inhalation treatment, developed aversions to corn for the whole experimental period. There was a treatment effect ($P < 0.05$) and a day × treatment interaction. Sheep in the IBc treatment did not differ from LiCl-treated animals in their intake of corn on any test day, indicating the strong and persistent aversion they developed. On the other hand, sheep in the other treatments often had higher corn intake than did the IBc animals, indicating relatively weak conditioning. Further, sheep in the IBc treatment differed on days 5–10, and days 60 and 90, from the alfalfa-treated control group, again demonstrating the strong aversion (Table 1). The IBc group also differed on most days from the other treatment groups such as those with smoke inhalation, showing that smoke inhalation was not a useful technique for most animals. Likewise, oral manipulation of *Baccharis* was not effective, and essentially had the same results as the alfalfa controls.

Sheep were not averted to corn with a single dose of *Baccharis*, rather it took several doses for animals to be conditioned (Table 1). In contrast, LiCl conditioned a strong aversion to corn after a single pairing.

When transferred 1 year later to a paddock with *B. coridifolia*, sheep from Groups IBc, OMBc, LiCl, and IA began grazing at approximately 10:00 a.m. Animals from Group IBc smelled *B. coridifolia*, but did not eat the plant. The other sheep eventually ingested the plant. During the morning of the following day sheep from Groups OMBc, LiCl, and IA stopped grazing. Sheep 5 from group OMBc and Sheep 14 from Group IA exhibited signs of abdominal pain, rubbing their abdomens on the ground or kicking their stomachs. These individuals did not graze again until 4:00 p.m. when they were removed from the paddock. Sheep 5 died sometime during the night and was found dead the next morning. Sheep 14 was depressed and mostly recumbent after being moved from the pasture. This animal died 38 h after being removed from the mio-mio pasture. Both sheep showed gross and histological lesions characteristic of the intoxication. Pathologically we observed congestion and hemorrhages of the rumen and abomasum, and ballooning degeneration and necrosis of the ruminal epithelium and congestion of the submucosa.

4. Discussion

The results of this experiment demonstrated that *B. coridifolia* is strongly aversive, conditioning aversions similar to that induced by LiCl. This strong aversion was induced when sheep were given oral doses equivalent to 50% of the lethal dose of mio-mio for two consecutive days. No aversion to corn was induced by the other techniques used by the farmers, i.e. oral manipulation with *B. coridifolia* or inhalation of *B. coridifolia* smoke, with one possible exception. From the groups not ingesting mio-mio or LiCl only, sheep 8 from the Baccharis inhalation group showed a reduction in corn intake, but this sheep also ingested only a small amount of corn on the first day of the experiment, suggesting that this might be an individual neophobic response to corn. Conditioned food aversion is probably due to negative gastrointestinal consequences (e.g., nausea) after the ingestion of a plant and the integration of sensory (flavor) and negative post-ingestive consequences (i.e., effects of nutrients or toxins on chemo-, osmo, or mechano-receptors in the gut and brain) (Wang and Provenza, 1996; Provenza, 1996). The strong aversion induced by mio-mio is apparently due to its toxic effects on the digestive system.

Intoxication by mio-mio is more common when recently transported animals are stressed, fatigued, hungry or thirsty or when there is low availability of forage in the areas infected by the plant. In these cases the reason for the occurrence of the intoxication is the ingestion of a large amount of the plant. Herbivores generally sample small quantities when they encountered a new plant (Freeland and Janzen, 1974). Thus, the ingestion of small amounts of the plant seems to be the mechanism of the naturally acquired aversion to mio-mio in animals born in areas with mio-mio, in animals introduced during good forage conditions in areas infested by mio-mio, or in areas with high availability of forage and/or with low amounts of the plant. A similar mechanism is suggested in goats that avoid ingesting *Coleogyne ramosissima* after the first exposure to current seasons growth, which contains high concentrations of tannins (Provenza et al., 1990).

The grazing portion of this study in a paddock infested with mio-mio suggests that strong aversions last for at least 1 year after treatment. Similarly, Ralphs (1997) found that aversions to toxic larkspur (*Delphinium barbeyi*) in cattle conditioned by high doses of LiCl lasted for at least 3 years. The failure to induce aversion by the traditional methods used by farmers (inhalation of mio-mio smoke or rubbing the plant in the mouth) suggests that these methods are not effective.

5. Conclusion

Mio-mio is strongly aversive and the aversion lasts for at least 1 year. This aversive effect is apparently responsible for the lack of *Baccharis* intoxication in livestock born in areas with mio-mio. Therefore, the aversion induced by non-toxic doses of the plant is an efficient way to prevent intoxication. In the future it will be necessary to repeat this experiment in cattle, as cattle are more susceptible to *B. coridifolia* intoxication and are more frequently affected (Barros, 1998).

Acknowledgement

We thank the CNPq for financial support (Grants No. 471588/2004-0 and No. 420012/2005-2).

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