

Video Article

How to Create Conditioned Taste Aversion for Grazing Ground Covers in Woody Crops with Small Ruminants

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Abstract

Conditioned taste aversion (CTA) is a learning behavior process where animals are trained to reject certain feed after gastrointestinal discomfort has been produced. Lithium chloride (LiCl) is the preferred agent used in livestock to induce CTA because it specifically stimulates the vomit center. In addition, LiCl is commercially available, and easy to prepare and administer using a drenching gun. Nevertheless, some factors have to be considered to obtain an effective long-lasting CTA, which allows small ruminants to graze during the cropping season. A key aspect is to use animals with no previous contact with the target plant (the plant chosen to be avoided; new feed). Due to their native neophobic feeding behavior, small ruminants can easily associate the negative feedback effects with the new feed, resulting in a strong and persistent CTA. The recommended doses are 200 and 225 mg LiCl/kg body weight (BW) for goats and sheep, respectively. To induce CTA, 100 g of the target plant should be individually offered for at least 30 min, and LiCl administered thereafter if the intake is greater than 10 g. Each time the animal eats the target plant without negative consequences, the CTA becomes weaker. Consequently, to minimize the risk of target plant consumption, it is essential to have sufficient palatable ground cover available. The presence of an alternative feed (of quality and quantity) prevents the accidental consumption of the target plant. A close monitoring of the flock is recommended to remove and re-dose any animal consuming more than 4 bites or 10 g of the target plant. At the beginning of each grazing season, check the CTA status of each animal before moving them to the crop.

Video Link

The video component of this article can be found at <http://www.jove.com/video/53887/>

Introduction

The use of ground cover between woody crop lines mitigates soil erosion and degradation and increases water, organic carbon and nitrogen retention¹⁻³. Additionally, ground cover maintains and increases biodiversity, supporting the balance between crop pests and their natural predators. Farmers tend to eliminate weeds by applying agrochemical products or using a reaper machine; thus reducing nutrient competition between crops and green cover. A cost effective way to control ground cover would be the use of small ruminant grazing. An additional benefit from animal grazing is the improvement in soil health and fertility. However, farmers are reluctant to implement this practice due to small ruminants damaging the crops by consuming young leaves and sprouts.

To prevent potential crop damage it is useful to induce conditioned taste aversion (CTA) in the sheep or goats within the flock or herd. The CTA is easily established for new feeds, due to the innate feed neophobia behavior^{4,5} of small ruminants, and because familiar feeds are positively associated with a "learned safety" status which is more difficult to change or manipulate⁶. Animals learn to reject a specific feed (conditioned stimulus) due to its negative post-ingestive effect (unconditioned stimulus). To induce CTA towards palatable and non-toxic plants, lithium chloride (LiCl; inductor agent) is orally administered after the animal consumes the target plant. While there are others inductor agents (e.g. apomorphine, ciclophosphamide, thiabendazole), LiCl showed the strongest and most persistent CTA due to its effect on the emetic system by stimulation of the chemoreceptor trigger zone area and gastrointestinal distress^{7,8} with mild signs of general discomfort. Lithium (Li) is absorbed from the upper gastrointestinal tract and distributed in the total body water space⁹. The animals can have a recovery period as short as two days^{7,10,11}.

The LiCl can be administered by mixing it with the food^{12,13}, in a gelatin capsule^{13,14} or in a solution administered orally by a drenching gun¹⁵⁻¹⁷. Although LiCl solution is caustic, no injury in mouth or esophagus was described. LiCl is used in the range of 100 to 400 mg LiCl/kg of body weight (BW), with better results (more persistent CTA) using higher doses^{16,18}. Nevertheless, considering the known dosage effects towards different species and breeds, the lethal effect in some cases start at 400 mg LiCl/kg BW. The recommended dosage for an effective long-term CTA begins at 200 mg/kg BW for goats and 225 mg/kg BW for sheep^{10,17,19}. Li used at these dosages is excreted within the first 4 days post-administration, mainly through urine (92 ± 4%), followed by feces (6.5 ± 1.3%) and milk (2.8 ± 0.4%)¹¹. The complete estimated withdrawal period for a single dose of LiCl in plasma is 9 and 11 days for sheep and goats, respectively. Due to the minimal Li excretion in milk, CTA cannot be naturally established in the suckling off-spring^{11,20}.

Long-term CTA persistence in sheep has been reported throughout an entire grazing season (3-4 months) when an alternative forage source was available^{14,21}, being re-established to an almost complete aversion with a single LiCl dose at the next grazing season (9 months later)¹⁴. Moreover, CTA persistences of 2 and 3 years have been reported in cows under pasture conditions, without the need for reinforcement doses, when the target feed is a toxic but palatable plant^{22,23}. The option of considering an alternative feed is critical for the animal to maintain the CTA against a non-toxic plant. Each time the animal consumes more than 10 g of the averted plant without resulting in gastrointestinal discomfort, the CTA would be compromised²⁴.

Protocol

The protocol described below for inducing CTA towards woody crops follows the animal care guidelines of the "Universitat Autònoma de Barcelona" (Bellaterra, Spain) and is approved by the Ethical Committee of Animal and Human Experimentation for sheep and goats (CEEAH, references 770 and 998 respectively).

1. Preparation of LiCl Dose

1. Make a 25% w/v (weight/volume) LiCl solution in distilled water. Alternatively, use clean tap water. The LiCl is extremely hygroscopic therefore it is necessary to take care when handling the powdered chemical.
 1. Weigh 250 g of LiCl and dissolve it in distilled water (LiCl concentrate solution). As the reaction is exothermic, wait until the solution has returned to room temperature before continuing. Pour the solution in a volumetric flask and add distilled water to bring it to 1,000 ml.
2. Calculate the volume of solution for each animal (individual dose).
 1. Weigh the animals to obtain their body weight (BW) and calculate the mL of LiCl needed for each animal following equation 1. The recommended dose for long-term persistence is 225 mg LiCl/kg BW in sheep¹⁰ and 200 mg LiCl/kg BW in goats¹⁷. However, other doses used for grazing are shown in **Table 1**.
 $(\text{kg BW} \times \text{LiCl dose in g/kg BW}) / \text{LiCl concentration in g/L} = \text{Volume of LiCl (L)} \text{ [Eq. 1]}$
 For example:
 Sheep = 60 kg BW; dose = 225 mg LiCl/kg BW; concentration of LiCl solution = 250 g/L
 $(60 \text{ kg} \times 0.225 \text{ g/kg}) / 250 \text{ g/L} = 0.054 \text{ L} = 54 \text{ mL}$
 Goat = 35 kg BW; dose = 200 mg LiCl/kg BW; concentration of LiCl solution = 250 g/L
 $(35 \text{ kg} \times 0.200 \text{ g/kg}) / 250 \text{ g/L} = 0.028 \text{ L} = 28 \text{ mL}$

LiCl dose	Mazorra <i>et al.</i> (2006) ¹⁹	Burritt <i>et al.</i> (2013) ³⁵
Low	100	125
Medium	150	150
High	225	175

Table 1: LiCl dosage range. Range of LiCl doses (mg/kg Body Weight, BW) used by different authors to induce feed aversion.

2. Animal Selection and Facilities

1. Choose adult, non-pregnant and dry animals, which have never previously eaten the target feed.
2. Do not induce the CTA to more than 20 animals at the same time, to better control their behavior. Initially, only induce CTA to 5 animals.
3. Allocate the animals in a place where the intake of the target feed can be recorded individually. Feed the animals once-a-day with a basal diet covering all their nutrient requirements and provide free access to water and to a mineral block. Assure an adequate salt intake to prevent LiCl toxic effects, since body cells can use Li instead of Na⁹.
4. If the facilities are new to the animals, give them 1 week of adaptation time to become familiar with the environment and the basal diet.

3. Aversion Induction

1. Remove the basal diet the evening before (day -1) and offer the target feed *ad libitum* to ensure that animals eat the target feed avidly on the following day¹².
2. On the following day (day 0), remove the orts in the morning and offer 200 g of target feed to each animal during 30 min. Weigh the orts thereafter.
3. If the animals consume more than 20-30 g, administer the corresponding calculated volume of LiCl (ml calculated in 1.2) with a drenching gun as soon as is practically possible, not waiting more than 1 hr after consumption. If animals consume less than 20-30 g, repeat the procedure the following day. Due to their innate neophobic feed behavior, animals could need more than 1 day to begin consuming the target feed^{10,17,25-27}.
4. After LiCl administration, wait 2 hr before offering the basal diet.
5. From day 1 to 3, do not offer the animals the target feed and check them periodically to detect any possible sign of severe illness, recording daily feed and water intake (by weight or by a check-list), respiratory rate and animal behavior. They could present dropped head and ears, inactivity, diarrhea, increased respiration rate and decreased water and diet intake during the days following the LiCl administration^{9-11,20,25}.

4. Aversion Validation

1. On days 4, 5, 6 and 7 after LiCl administration, repeat step 3.2.
2. If the animals consume <10 g (or <4 bites) of the target feed, consider the CTA established. The threshold between zero (no consumption) and 10 g could depend on the circumstance of the target plant (toxic or not).
3. If any animal consumes >10 g, administer a new LiCl dose and repeat step 4.1. If the animal persists in consuming the target plant after the second dose, eliminate it from the CTA group. Social facilitation (e.g. mother, siblings and close mates) can modify CTA behavior in livestock. Animals which have been averted to a target plant easily consume it again when they graze with non-averted animals²⁸.

5. Pasture Management

Note: The dose of LiCl used to induce CTA is completely excreted in a few days, mainly by urine.

1. Although Li is widely distributed on the earth's crust, in order to avoid any possible contamination in the organic crop, wait 9-11 days before moving the animals to the crop (clearance time established with Li pharmacokinetic)¹¹.
2. Ensure that the crop ground cover is abundant and palatable throughout the grazing period to prevent the animals from sampling the target feed. Graze treated animals separately from non-treated.
3. Allow the animals to graze a delimited plot of the entire surface to obtain a uniform control of the grass height. Delimit the plot by using portable electric or metallic fences or using a shepherd to graze the animals between specific crop lines.
4. Avoid overgrazing a plot, removing the animals before the ground cover becomes scarce (grass height of 5 cm)²⁹.

6. Re-establishing the CTA

1. Every year, before allowing the animals to return to graze the crop, repeat step 4.

Representative Results

The results described below were obtained by several studies in CTA toward woody crops in small ruminants conducted at the Universitat Autònoma de Barcelona and they provide evidence to establish the protocol proposed.

Doses of 175 and 200 mg LiCl/kg BW in goats and 200 and 225 mg LiCl/kg BW in sheep were successfully used to induce CTA against woody crops with a single administration, with the exception of two animals which did not completely swallow the administered LiCl dose^{10,17} (**Table 2**). Most CTA animals showed dropped heads, inactivity and decreased intake of the basal diet the day after LiCl administration^{10,11,17}; however, animals overcame these signs of gastrointestinal discomfort within 2 days^{10,11}. For all the doses used, the typical CTA behavior observed was that the animals refused to approach the feeding boxes, sniffed the plant material and rejected to eat or consumed <10 g of the target feed^{10,17}. In addition, differences between doses for each species were detected regarding the long-term persistence and the number of animals needing a reinforcing LiCl dose in the short-term. Animals which had received the lower dose (175 and 200 mg LiCl/kg BW for goats and sheep, respectively) showed shorter complete CTA persistence (intake of the target feed <10 g) and more animals needed to be reinforced to strengthen the CTA. Goats receiving the same LiCl dose (200 mg/kg BW) as sheep for inducing CTA against olive tree leaves, showed a longer complete CTA persistence¹⁷. On the other hand, CTA persistence differed by breed (Lacaune, Manchega and Ripollesa sheep breeds) using the 200 mg LiCl/kg BW but not when the 225 mg LiCl/kg BW dose was used¹⁰.

Although a single LiCl dose induced a complete CTA against a novel feed (*i.e.*, olive tree leaves or grapevine leaves and sprouts), it was not enough to induce CTA against a familiar feed (*i.e.*, concentrate and hay). Sheep which received a LiCl dose to induce CTA against concentrate only showed a slight decrease in the target feed intake compared to the control group on the following day (**Figure 1**). In addition, the repeated LiCl administration (up to 3 doses on consecutive days) during the learning period was not effective to induce CTA against the familiar feed³⁰.

The CTA induced against grapevine leaves with a 225 mg LiCl/kg BW single dose was complete (intake <10 g) through the first year (**Figure 2**). Nevertheless, during the following 2 years a re-enforcing dose was necessary to strengthen the CTA when ewes resumed consumption of the target feed. The CTA became weaker when the ground cover availability was scarce during the grazing season. A flock of 6 CTA ewes grazing (11 days; 24 hr/day) on a commercial vineyard plot (8.8 acres) with spontaneous ground cover reduced grass cover by 44 ± 4% (dry matter basis), leaving on the ground the more fibrous and less nutritive parts of the plants (**Table 3**)³¹.

A herd of 5 goats maintained an effective CTA (intake of the target feed markedly lower than the control group) against olive trees through 14 months after aversion was induced with a 200 mg LiCl/kg BW. A pasture trial of 30 min in a commercial olive grove (a plot of 156 m² with spontaneous grass cover and 5 olive trees) showed that CTA goats spent 3.1% of the pasture trial time in contact with the olive trees (behavior of sampling, smelling or contact could not be distinguished in the video recorded), whereas control goats spent 50.7%³².

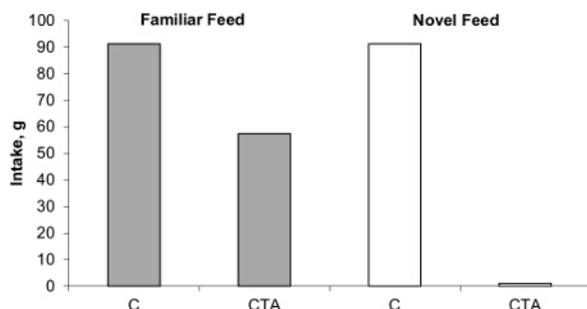


Figure 1: Familiar vs. Novel feed in CTA. Intake of the target feed after a single LiCl dose (225 mg/kg Body Weight, BW) to induce CTA against a familiar or a novel feed in the averted (CTA) and control (C) ewes. [Please click here to view a larger version of this figure.](#)

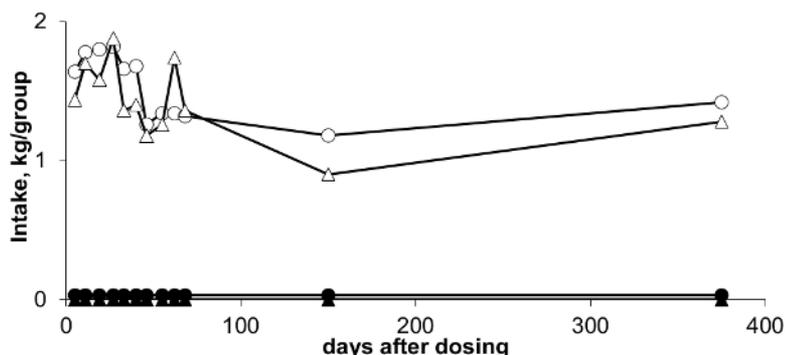


Figure 2: CTA persistence after LiCl administration. Group intake of grapevine leaves during the first year after inducing conditioned taste aversion (CTA). (○, Control Lacaune; ●, CTA Lacaune; △, Control Manchega; ▲, CTA Manchega). [Please click here to view a larger version of this figure.](#)

Species	N	LiCl dose	Target feed	Success ratio, % ¹
Goat	5	175 mg/kg BW	Olive tree	100 (5/5)
Goat	10	200 mg/kg BW	Olive tree	90 (9/10)
Sheep	20	200 mg/kg BW	Olive tree	95 (19/20)
Sheep	15	225 mg/kg BW	Olive tree	100 (15/15)
Sheep	44	225 mg/kg BW	Grapevine	100 (44/44)

¹Proportion of CTA induced animals with a single LiCl dose.

Table 2: CTA success ratio. Proportion of success for inducing conditioned taste aversion against woody crops with single doses of 175, 200 and 225 mg LiCl/kg Body Weight (BW) for goat and sheep.

Item, %	Before grazing	After grazing	P-value
Dry matter	28.6 ± 1.4	45.9 ± 5.8	0.066
Crude protein	11.4 ± 1.2	7.8 ± 0.5	0.071
Crude fiber	29.5 ± 0.9	35.1 ± 1.5	0.046
Neutral detergent fiber	46.2 ± 4.4	57.6 ± 3.0	0.077
Acid detergent fiber	28.0 ± 2.7	35.9 ± 2.5	0.041
Lignin acid detergent	4.2 ± 0.8	6.2 ± 1.1	0.075
Ash	8.9 ± 0.3	9.1 ± 0.5	0.788

Table 3: Ground cover chemical composition. Chemical composition (dry matter basis) of spontaneous ground cover of a commercial vineyard before and after grazing by averted ewes.

Discussion

The CTA is easily established in small ruminants if the target feed is a plant which the animal has never eaten before and does not contain an irreplaceable nutrient. Animals have a positive post-ingestive association with a non-toxic feed unless previous contact makes it difficult to change their perception of that particular feed^{7,33}. The condition taste aversion is produced because LiCl stimulates the emetic system producing malaise or gastrointestinal discomfort³⁴. It has been established that animals consuming LiCl increased rates of respiration, showed head and

ear droop, occasionally they would kick themselves in the stomach²⁵, and show decreased milk production²⁰ and food and water intake¹¹. However, these signs were overcome in a few days (2 to 4 days)^{11,20}.

The protocol can be slightly modified according to other CTA studies. Although we indicated that the LiCl should be administered immediately after consumption of the target feed, animals were able to associate intake with sickness within the first 4 hr post-administration²⁴. However, it is important to offer the target feed alone and temporarily separate (at least 1 hr after administration²⁴) from other feeds; such as the basal diet, to prevent cross-CTA. Although we can choose one of the doses indicated in Table 1, we have to consider that those studies did not evaluate long term CTA (<1 year), only the short-term (4 days)³⁵ and mid-term CTA (3 months)¹⁹. The use of adult animals is recommended due to young temperament in small ruminants which negatively affect the intensity and persistence of the CTA⁴.

Some key aspects have to be considered to obtain a successful CTA. Although the drenching gun is routinely used for deworming, the LiCl solution has to be applied with a higher volume than that of anthelmintic drugs (average 40 ml/animal). It is important to choose the correct size of the drenching gun, keep the gun in good condition (clean and lubricated) and carefully administer to the animal. To maintain the long-term aversion it is also important that the green cover is of high quality and palatable for the animals in order to avoid the erratic consumption of the target plant. It is stressed that the aversion becomes weaker every time the animal samples the averted food without suffering negative consequences^{25,36}. CTA might be difficult to establish in a flock of small ruminants if they live in an area where the predominant crop is the target plant (e.g., vineyards, orange trees). One of the reasons for this is that previous contact with the target plant might occur when animals graze or are fed pruning waste in shelter. The solution could be to bring animals from other regions or raise the replacement stock without contact with the target plant.

In the last few years, farmers have been encouraged to implement more sustainable farming techniques due to societal demands. The CTA methodology proposed would change the use of tilling tools or herbicides to a system with small grazing animals. An additional benefit with grazing small ruminants is an increase of pasture surface available since the cultivated lands were forbidden from grazing animals in the past. Mixing grazing animals and crop production enables farmers to obtain a higher value product while improving soil quality.

Disclosures

The authors have nothing to disclose.

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