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Sheep conditioned aversion: A sustainable alternative for vineyard floor management

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Abstract

The wine industry is facing new environmental challenges, particularly with respect to herbicide use. Integration of sheep into vineyards provides an opportunity to maximize landscape level production and reduce environmental impact of vineyard floor management. However, due to the high palatability of vine leaves integration of sheep during the key periods for grapevine production (growing season) is rare. The use of Lithium Chloride (LiCl) has proven to be useful for conditioning aversion influencing ruminants' diet. This paper analyses the use of LiCl as a mean to control the damage produced by ewes on vineyards while grazing. The sample consisted of 20 Milchschaf female sheep all subdue to a preconditioning period of consumption. Subsequently, the conditioning period included exposure to fresh vine leaves. Once the entire sample consumed this type of leaves, half of them were exposed to an aqueous solution of LiCl (200mg/kg). The effects of LiCl were monitored for a period of six days in a row after administrating the solution, then monthly for three months. It was proven that LiCl caused a significant decrease in the consumption of vine leaves. We suggest that the aversion conditioned by LiCl consists of a useful tool for reducing the damage provoked on vine leaves made by ewes while grazing, thus stimulating joint agricultural production.

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Introduction

The integration of crop and livestock systems has been recognized for its potential to reduce the environmental impacts associated with agriculture and improve farmer livelihoods (Niles *et al.*, 2017). Run under strict personal control, sheep and other species have been used to reduce the amount of non-useful vegetation in a certain type of crops. Particularly, grazing vineyards during the winter when the risk of plant structures damage is low and the supply of forage decreases is a practice commonly adopted in some viticulture areas such as New Zeeland (Dastgheib & Frampton, 2010).

In the field of animal production, vineyards and other fruit trees represent a great source of food, especially in areas where plantations are located on natural fertile soils (Coniberti *et al.*, 2018). Even most integrated crop and livestock systems research has focused primarily on cattle (Garrett *et al.*, 2017), integration of sheep into vineyards provides an opportunity to maximize landscape level production and potentially reduce environmental impact of grape growth by utilizing both systems synergistically (Niles *et al.*, 2017). The wine industry is facing new environmental challenges, particularly with respect to herbicide use. The use of under-trellis cover crops (UTCC) (100% of vineyard floor covered with grass), has been recently studied in vineyards from regions that often experience excessive water availability and/or fertile soils (Vanden Heuvel *et al.*, 2017). Those studies have demonstrated the potential use of UTCC, to limit grapevine water availability and so improve fruit composition in climates with precipitation above optimum for high quality wine production (Coniberti *et al.*, 2018). Full cover cropping has been demonstrated to be a suitable alternative to herbicide, and clearly adapted to a more environmentally friendly viticulture (Vanden Houvel *et al.*, 2017).

However, it's not always easy for winegrowers, to find a trade-off between cover crop ecosystem services and disservices (Coniberti *et al.*, 2018). The higher operation costs of interrow grass managements, compared to herbicide application, is one of the most important limitations for under trellis cover crop adoption.

Sheep integration into vineyards offers the potential to utilize the synergies of both systems not only to reduce external inputs and promote soil health, but also to increase farmer profit. However, due to the high palatability of vine leaves (Dastgheib & Frampton, 2010, Manuelian *et al.*, 2014) integration of sheep during vine dormancy is common, but integration during the key periods for grapevine production (growing season) is rare (Niles *et al.*, 2017).

Allowing sheep and fruit plantations to coexist during the entire production cycle would be highly beneficial. Besides the potential economic income generated by using vineyards for grazing, and the reduction of costs associated to management practices oriented to reduce competition and/or vineyard´s passability (herbicide applications and mowing), this natural approach of vineyard management may significantly reduce environmental impact of grapevine production (highly valuated by wine consumers). This combinate farm system may be especially relevant for small

The use of Lithium Chloride (LiCl) has proven to be useful for conditioning aversion influencing ruminants' diet in regards to avoiding toxic plants (Burritt *et al.*, 1989; Welzl *et al.*, 2001; Pacifico da Silva *et al.*, 2010; Pimentel *et al.*, 2012; Ruiz *et al.*, 2012), as well as other foods not indicated to be consumed (Welzl *et al.*, 2001; Ruiz *et al.*, 2010, 2018). Moreover, this tool can be used both individually as well as collectively (Ralphs *et al.*, 1999; Ruiz, 2015; Ruiz *et al.*, 2018).

Conditioning aversion produced by LiCl is widely used in cattle (Burrit *el al.*, 1989; Provenza, 1996; Riet-Correa *et al.*, 2001; Pfister *et al.*, 2002; Almeida *et al.*, 2009), showing similar results on goats, horses, and sheep (Ralphs *et al.,* 1990, 1992,1993, 1994; Pfister *et al.*, 1996, 2002; 2007; Duncan *et al.*, 2002; Gorniak *et al.*, 2008; Oliveira *et al.*, 2014), in regards to animal consumption training. It has proven to be very useful in avoiding the consumption of both toxic and highly palatable plants (Ralphs *et al.*, 1994; Dumont *et al.,* 1999; Manuelian *et al.*, 2010, 2014).

Aversion is developed by conditioning mechanisms by which the animals associate consuming certain foods with related consequences, all of this necessary to learn which foods might be healthy or toxic (Provenza *et al.*, 1992).

LiCl stimulates toxic plant´s action mechanisms generating vomiting and indigestion in which animals associate with the intake of the last food eaten (Ralphs *et al*., 1999; Pacifico da Silva *et al*., 2010; Manuelian *et al*., 2014). Certain reports suggest that intoxication by LiCl occurs at 500mg/kg. For this paper, a safe and useful dosage was used to generate the conditioning (200mg/kg PV). On this dosage, animals experience observable digestive symptoms (ex. drooling) recuperating after a few minutes (Ruiz *et al*., 2010, 2016).

Previous research has shown the usage of LiCl as an aversion to prevent the cutter from consuming olive tree leaves (Manuelian *et al*., 2010). These authors used LiCl in sheep and goats reporting effects in both species and monitoring their maintenance for up to four months. They also studied the effect of LiCl in several sheep breeds showing that race influences its lasting effect (Manuelian *et al*., 2014). Both of these experiences emerged in Europe while studies concerning the effects of LiCl in reducing the consumption of vine leaves in the American production context have not been produced yet.

The motivation of this study is based on providing novel information on the conditioned aversion with LiCl in sheep with the intention of preventing the consumption of vineyards while grazing. There are several publications that study this tool in different conditions and applied to different foods, but none in vineyards in South America. The aim of this paper is analyze the conditioned aversion as a tool to facilitate ewes grazing in vineyards. A specific protocol was designed using LiCl conditioning as an aversion mechanism, by which animals decreased their consumption reducing damaging effects on plantation sites facilitating the cohabitation of sheep in vineyards.

Material and methods

Animals and facilities

The experimental protocol applied was approved by la Comisión de Ética Animal de la Facultad de Veterinaria de la Universidad de la República (CEUA-Fvet-UdelaR). The experiment was conducted in INIA Las Brujas establishment located in Canelones (Uruguay) from November 2017 to February 2018. The animals were kept in cement floor establishments to prevent their access to vineyard plantations provided for the experiment as well as other foods that were found in that location. Twenty Milchschaf female sheep which weighted 44,6±7,8kg were used for the experiment. Half of the sample (n=10) were randomly selected as part of the group treated with an aqueous solution of LiCl while the rest (n=10) constituted the control group with the same handling but dosed with water.

Plants and Lithium Chloride (LiCl)

The vine branches (shoots) used for the experiment (*Vitis vinifera*) were provided by the establishment itself. These branches were hanged from wooden fences and then offered to ewes to eat. Bites were counted by time unit, branches' weight and the number of leaves before and after each trial. LiCl (Sigma® L4408, St. Louis, EE. UU.) was administrated intragastric diluted in water for 200mg/kg. This dosage of LiCl has proven not to generate adverse effects on animals (Burrit *et al*., 1989) recuperating after a few minutes (Ruiz *et al*., 2010, 2016).

Experimental Protocol

The protocol consisted of three phases.

Phase I: preconditioning (1 to 5 sessions). Animals were trained to eat palatable foods (soy-based ration) every morning (09.00 am) daily after fasting during night time staying in a cement floored setting. In individual premises, animals were offered ration for five minutes while registering grams consumed during that period. Once the sample ate ration for three consecutive days they were exposed to vine leaves freshly collected from that same location while counting bites, grams and amount of leaves consumed for five minutes.

Phase II: conditioning (6 to 13 sessions). At this stage, all animals have been consuming the plants for three consecutive days and therefore oral LiCl was administrated (200mg/kg) to half of the sample (n=10), while the other half was provided with water (n=10). After administration, the animals remained locked down for half an hour before being released into the field.

This lapse of time allowed them to experience the effects of LiCl associated with the last meal consumed. LiCl effect was monitored for six consecutive days while the animals were individually exposed to the plant evaluating its consumption (in case any animal consumed, a new dose of LiCl was administrated). Only once all the animals have shown not to consume the plant for three days consecutively this period finish.

Phase III: Monitoring. After confirming the effects of LiCl on the previous stage, animals were allowed to graze on vineyard free meadows within the establishment in which they were being held. For a period of three months (December, January, and February) consumption tests were monthly applied as a way to monitor the maintenance of the effects of LiCl on the consumption of vines overtime. Animals were allowed to wander through those meadows until 19:00 hours and afterward were placed together under a fasting regime during night time. The next morning (09.00 hours) they were released in fenced lines of vineyards while being recorded to be able to count the number of bites produced for five minutes. This phase concluded once there were no longer differences identified between both groups of animals.

Data analysis

Variance analysis was conducted to compare consumption sessions, and repeated measures data were applied for analyzing variance of consumption throughout days and in between groups (factors between groups: treatment; factors of repeated measures: consumption sessions). The most significant effects and interactions were analyzed by using Fisher´s *posthoc* test. Alfa values were situated at 0,05 and the results were expressed as mean±SEM.

Results

Effects of preconditioning over ration consumption

The consumption of ration during the five sessions of training were compared by repeated measures ANOVA showing that animals increased the amount of food consumed as days progressed ($F_{(4,72)}$=11,6, p<0,001).

The effects of LiCl in conditioning phase

By comparing consumption through ANOVA repeated measures significant effects were reported between the sixth and twelfth sessions. At the beginning of the conditioning phase, the animals did not consume as many leaves as they would as days went by. Animals consumed larger amounts of the plant measured in grams ($F_{(7,108)}$=58,1, p<0,001), bites ($F_{(6, 108)}$=78,9, p<0,001), and in the plant leaves ($F_{(6,108)}$=55,1, p<0,001).

The entire sample consumed leaves for three consecutive sessions, in session number twelve treated group were exposed to LiCl 200mg/kg. After LiCl was administrated, the treated group strongly reduced consumption while the control sustained its previous level. Every animal exposed to LiCl throughout different sessions needed new dosages (a unique dose of LiCl 200mg/kg was not enough to generate an aversion in any of the animals used for this experiment). Half of the animals needed two doses while the other half needed three doses of LiCl to stop the plants´ consumption.

Repeated measures ANOVA comparing groups after LiCl treatment through sessions show a significant effect of the treatment on grams consumed ($F_{(1,18)}$=174,7, p<0,001), bites ($F_{(1,18)}$=277,3, p<0,001) and plan leaves ($F_{(1,18)}$=284,4, p<0,001). This shows a clear effect of LiCl by which animals tend to reduce their consumption of the plant throughout sessions after treatment (Figure 1).

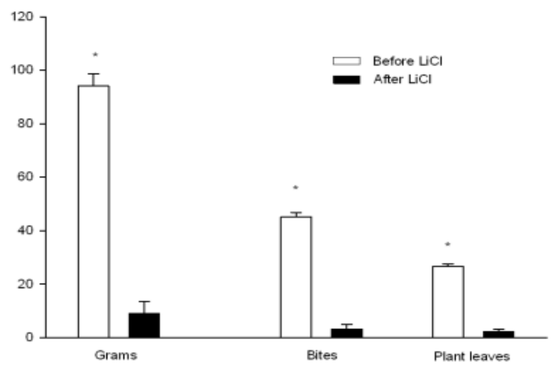

Fig. 1. Grams, bites and leaves of grapevine consumed before and after LiCl treatment. The data are expressed as the mean ± SEM.

Monitoring LiCl´s effect

All consumption sessions post-treatment with LiCl were compared included the monthly reports generated for three months, ANOVA´s repeated measures showed treatment effect ($F_{(1,9)}$=66,8, p<0,001), manifesting the effect of LiCl on the number of bites registered. Also, a Treatment per Session effect was identified ($F_{(8,72)}$=3,5, p<0,01), showing significant differences between sessions after the treatment and during the monitoring period (Figure 2). No significant differences were found in the last report (February) using Fisher´s *poshoc* test.

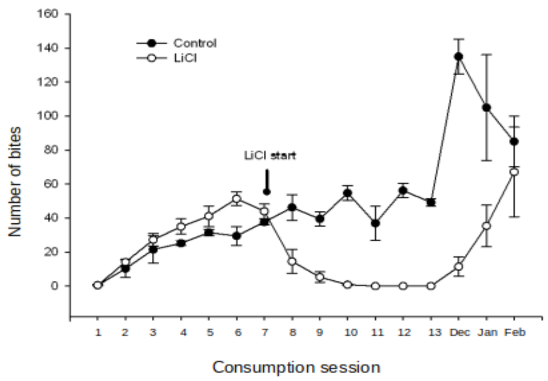

Fig. 2. Bites to grapevine trough the experiment. First six session were to train animals to eat the plant, in session seven start conditioning period which finished when all animals don't eat the plant. Then were monitored the effect for three month (December, January and February) until LiCl loose effect. The data are expressed as the mean ± SEM.

Discussion

According to it was previously reported (Meadows, 2008), in our study conditioned aversion by the use of LiCl has proven to be an effective tool for reducing the damage provoked by ewes in vineyards. Our results shows the significant effect of LiCl on sheeps vine leaves consumption, during training and more relevant in the vineyard.

At the beginning of the conditioning phase, the animals did not consume as many leaves as they would as days went by. Once the entire sample consumed these leaves for three consecutive consumption sessions, LiCl was orally administrated showing reduced consumption on behalf of the treated group while the control group sustained its previous level of consumption

Most specialized literature states that to achieve aversion to palatable commercialized ration on sheep, a dose of LiCl on 200mg/kg IG to be present (Mazorra *el al.*, 2006; Ruiz *et al.*, 2010, 2019, 2020; Manuelian *et al.*, 2016). Importantly enough, every animal exposed to LiCl throughout different sessions in our study needed new dosages. A unique dose of LiCl 200mg/kg was not enough to generate an aversion in any of the Milchschaf female sheep used for this experiment. Manuelian *et al.* (2014) identified that the persistence of the LiCl effect on 200mg/Kg depends on race but this difference can be overturned by augmenting the dosage to 225mg/kg. Manuelian *et al.,* 2013, working with Manchega and Lacaune breeds, reported that only needed one dose of 225mg/kg LiCl to generate aversion on sheep towards vine leaves. Similar results were also reported on goats when only a sole dose with 225mg/kg LiCl was needed to avoid the consumption of *Ipomoea carnea* (Pimentel *et al.*, 2012) Considering that the elevated cost of LiCl might be one of the main weakness of this tool in commercial settings, it is relevant to mention that in our study the approach was to apply various doses of LiCl 200mg/kg, instead increase the concentration. Even it was not evaluated, according to the results presented by Manuelian *et al.* (2014), it is possible that the average total LiCi required per animal to achieve the aversion in our study, could be reduced increasing the LiCl concentration.

In the same direction, sheep used in our experiment were not older that one year which can contribute to explaining the need to apply several dosages in some cases, taking into consideration the fact that eating skills learned in ruminants are influenced by age and younger ones are less neophobic (Ralphs *et al.*, 1992; Mirza *et al.*, 1992). These variables have also proven to influence the effect and longevity of the treatment with LiCl on cattle (Ralphs *et al.*, 1993), so it is also possible that LiCi needed to achieve aversion could be lower and/or effective for a longer period when using older animals.

In regard to our findings concerning grapevine leaves consumption over time there are two main elements to consider. First of all, the effect of LiCl could be confirmed for a period up to three months. Secondly, mention that neither during the conditioning phase or the monitoring phase at the vineyards started, grapes were not present. When analyzing the data concerning animal consumption in vineyards the evidence shows that they no longer consumed leaves (to which the aversion was generated) but instead were eating grapes whether ripe or not, ending in an indiscriminate consumption by the third month after treatment with LiCl had been applied. These results are similar to those found in Manuelian *et al.,* 2013 study, in which a different set of breeds was used and a larger dosage of LiCl was administrated prolonging LiCl´s effect over time. Moreover, in Manuelian *et al.*, 2010 LiCl at 200mg/kg was administrated to sheep and goats to prevent olive consumption evaluating its effects for a period of 144 days showing its presence until the end of the experiment. From other researches we conducted regarding aversion to LiCl on olive trees we were able to find the LiCl effect until day 164 (Ruiz *et al.*, 2020).

There are many possible strategies for integrating sheep into vineyards. The use of sheep in the vineyard during vine dormancy (Dastgheib and Frampton, 2010); seasonal integration using sheep on short time intervals to pluck leaves from vineyards to open up the grape canopy (Hawkes Bay Winegrowers Association 2010); Byproduct integration through feeding of grape pomace to sheep (Nistor *et al.,* 2014); and/or integration using sheep during longer periods during the growing season by training sheep with LiCl, masks or fencing (Niles *et al.,* 2017). Although all of them may be relevant for animal production, integrating the sheep into the vineyard during the growing season becomes particularly important for wine grapes production. It may provide an opportunity to reduce environmental impact of grapevine production, particularly with respect to herbicide use.

In this context, to continue investigating in regards to LiCl aversion becomes highly relevant since generating modeling diet protocols for production animals might bring a positive impact on production systems applied to commercial establishments. When referring to the applicability of this tool for joint production, economic factors must be considered. LiCl is expensive and therefore producers will need to conduct a cost-benefit assessment towards its expenses in pasture´s control; costs of applying a conditioning aversion system in their facilities and possible profit based on mixed production of sheep placed on commercial crop establishments.

Therefore, further investigations focused on the uses of LiCl aversion as well as relevant variables associated with this tool such as differentiated responses to LiCl according to race and age in Latinamerican context; how the effect depends on dosage volumes; its lasting effect and economic impact in our productive systems become key in the process of formalizing the use of LiCl for commercial purposes.

Conclusions

LiCl proves to have a significant effect on the consumption of vine tree leaves becoming a potentially useful tool to facilitate cohabitation between sheep and this specific type of crop. Applying this methodology onto commercial vineyards might contribute to generating a positive impact in producers´ income saving costs associated with underbrush control competing for soil resources with other plantations (herbicides, cost on machinery fuel, expert labor force salary and working hours, among others). Moreover, the nutritional value of vineyard line pastures that sheep consume might contribute to their weight gain; carry to term offspring and wool production depending on the type of animal located at the establishments.

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