Hayvansal Üretim 48(1): 21-25, 2007

Araştırma Makalesi

Conditioning Goats to Avoid Eating Young *Sarcopoterium Spinosum* Plants

Akın Pala[1*], Mehmet Mendeş[1], Yavuz Akbaş[2]

[1]Department of Animal Science, Canakkale Onsekiz Mart University, 17020 Çanakkale, Turkey

[2]Department of Animal Science, Ege University, Bornova, 35100 Izmir, Turkey

*e-posta: akin@comu.edu.tr; Tel.: +90 (286) 218 00 18 Ext.: 1349

Abstract

Major purpose of this study was to investigate whether German fawn goats can learn to refrain from eating *Sarcopoterium spinosum* plants. Conditioning goats to avoid eating their favorite plants such as young tree seedlings may be useful in preventing them from harming forests. Eight goats were divided in to two groups. Magnesium Hydroxide (MGH) treated plants were given to one group while hot red pepper treated plants were given to the other group. Animals were observed half an hour for each two hours during the day from 9 AM to 1 PM to record the number of reaches for the plants treated with MGH or hot red pepper (consume vs. not consume). Total of 224 records from eight goats were analyzed by using Generalized Estimating Equations (GEE). The analyses showed that the goats tended to consume more of the *Sarcopoterium Spinosum* plants (P=0.04) treated with MGH than those treated with hot pepper. The plants with no treatment (weed) were consumed in similar amounts by the two groups of goats (P=0.43). Differences between consumption of *Sarcopoterium Spinosum* plant and the non-treated plants were large within MGH and hot red pepper (P=0.001), indicating both of the substances were effective in having the goats refrain from consuming the plants. Goats can be conditioned to refrain from harming forests if the conditioning is done with an effective substance.

Keywords: Conditioned learning, taste aversion, goats, repeated measurements, GEE

Kecilerin *Sarcopoterium Spinosum* bitkilerini yemekten kaçınmaları için şartlandırılması

Özet

Bu çalışmanın amacı Alman Asil keçilerinin *Sarcopoterium spinosum* bitkisini tüketmekten kaçınmayı öğrenip öğrenemeyeceklerini araştırmaktır. Keçilerin taze ağaç fidanları gibi tercih edilen bitkilerin tüketilmesini engelleyecek şekilde şartlandırılması ormanlık alanlara zarar vermelerini engelleyebilir. Sekiz adet keçi iki gruba ayrılmıştır. Bir gruba acı biberle muamele edilmiş bitkiler verilirken diğer gruba Magnesium Hydroxide (MGH) ile muamele edilmiş bitkiler verilmiştir. Keçiler sabah 9 ve öğleden sonra 1 arası, her iki saatte bir yarım saat boyunca gözlenmiş, MGH veya acı kırmızı biberle muamele edilmiş bitkileri kaç defa yeyip yemedikleri kayıt edilmiştir. Analizlerin yapılmasında sekiz adet keçiye ait toplam 224 kayıt kullanılmıştır. MGH ve acı biberle muamele edilmiş bitkilerin tüketimlerinin zamana göre analiz edilmesinde Genelleştirilmiş Tahmin Eşitlikleri (Generalized Estimating Equations, GEE) yönteminden yararlanılmıştır. Yapılan analizler sonucunda keçilerin, MGH ile muamele edilmiş bitkileri, acı biberle muamele edilmiş bitkilere göre daha fazla tüketme eğiliminde oldukları gözlenmiştir (P=0.04). Diğer taraftan, herhangi bir muameleye tabii tutulmamış bitkilerin tüketilme sayıları bakımından, söz konusu gruplar arasında, istatistiksel olarak önemli olan bir farklılığa rastlanılmamıştır (P=0.43). Uygulama ne olursa olsun, *Sarcopoterium Spinosum* bitkisinin tüketimi ile herhangi bir uygulamanın olmadığı bitkilerin tüketimi arasındaki farklılığın istatistiksel olarak önemli olduğu görülmüştür (P=0.001). Bu bulgulardan hareketle, hem MGH, hem de acı biber ile muamele etmenin keçilerde etkili olduğu söylenebilir. Dolayısıyla, etkili maddeler kullanılmasıyla keçilerin genç bitkilere zarar vermelerinin önlenebileceği sonucuna varılabilir.

Anahtar kelimeler: Şartlanmış öğrenme, tat kaçınması, keçi, tekrarlanan ölçümler, GEE

Introduction

In nature, animals have different ways to fight predators. Some run, while some others secrete chemicals that give bitter taste or hallucinations to the predator. Fire bellied frog (Bombina bombina) for example, releases a substance called bombesin (Brown *et al*., 1977) which causes hallucinations in the predator and it no longer has a taste for the frog. Similarly, ingestion of Bufotenin in the skin of some common species of frogs can result in vivid hallucinations (Siegel and McDaniel, 1991) and several other animals, such as coyotes (Gustavson, 1974) and hawks (Brett, 1976) have been shown to learn aversions to certain preferred foods if consumption is followed by gastrointestinal malaise. In Africa, red hot pepper dust is used to keep

elephants away from the crop fields. Ruminants may avoid or prefer different plants depending on what happens after consumption (Burritt and Provenza, 1992; Duncan and Young, 2002; Kyriazakis *et al*., 1997). Barker *et al*. (1977) reported that Jersey cows administered lithium chloride solution after alfalfa pellets developed a strong aversion in a single trial and that aversion was maintained. However, they also reported that those administered lithium chloride after consuming sweet feed (golden grains flavored with molasses) either had aversion after four pairings of the food and the poison, or had no reduction in intake at all.

Duncan and Young (2002) reported that in free-ranging herbivores, any one feeding bout might include many different plant species (Fraser, 1997), and associating post-ingestive consequences with particular species appear unlikely. However, in the area of this study, goats prefer young leaves of the *Sarcopoterium spinosum* plant over all the other plant species.

Jimenez and Tapia (2004) defined conditioned taste aversion as a paradigm in which animals learn to avoid new tastes when they are associated with gastrointestinal malaise. In this study, a laxative substance, *Magnesium hydroxide* (MGH) or hot red pepper was used to condition the goats to refrain from eating young *Sarcopoterium spinosum* leaves. If the goats can be conditioned to avoid young *Sarcopoterium spinosum* leaves, which they prefer the most, they can be conditioned to avoid young seedlings. This may prevent their harmful effects to forest areas by consuming seedlings. Major purposes of this study were to investigate the differences between the effects of MGH and hot red pepper on aversion to *Sarcopoterium spinosum* and to determine the time it takes goats to be conditioned avoiding the plant.

Materials and Methods

Consumption of the plants was observed as a behavioral recording of the browsing and was recorded by the number of feedings on the plants or weed. When the animal switched from *Sarcopoterium spinosum* to the ordinary weed, a mark was made for *Sarcopoterium spinosum* and weed. If the animal switched back to *Sarcopoterium spinosum,* another mark was added for the *Sarcopoterium spinosum* consumption. In other words, goats consuming the plants were observed and marks were made on paper according to the kind of plant consumed. These marks ranged from zero to six, forming the values for the dependent variable.

Data used in this study consisted of 224 records and were collected from eight German Fawn goats maintained in the Yahya Cavus Research Center at Uvecik-Canakkale, Turkey. The city of Canakkale is located at latitude 40.1° N and longitude 26.4° E. Altitude is only 3m and the area is rather windy. Two different substances, hot red pepper and *Magnesium hydroxide*, were used to have the goats refrain from eating the *Sarcopoterium spinosum* plant, which is common in Canakkale area of Turkey. The study was done in the month of July, when the *Sarcopoterium spinosum* plant renews its leaves. Animals were tied in an area in two groups and were kept there for four days followed by a three day period in another close-by area. The tethers were 2 m long and the area that each goat can roam was 16 m^2. The *Sarcopoterium spinosum* plants were treated with either MGH or hot red pepper for the 1^{st} through 4^{th} days. After these four days of treatment, the goats were presented with clean plants with no application of MGH or hot red pepper by moving them to another area of *Sarcopoterium spinosum* and weeds, where they were kept during the 5^{th}, 6^{th} and 7^{th} days. Ordinary grass (called weed) was never treated with any of the substances.

The substances were applied by spraying them directly on the plants. Approximately 6-10 gr of the dry substances were diluted with 100 ml of water and the whole solution was applied to four plants, using a bottle of solution for each goat. There were total of 31 plants. Each goat had access to four plants while one of the goats had access to three plants only. The observations were recorded every two hours for half an hour during the day from 9 AM to 1 PM. Each goat was given access to water, 500 gr. commercially available concentrate feed and 1.2 kg dried vetch every day. Concentrate feed was given two times a day before milking. There were enough *Sarcopoterium spinosum* for all the animals for the whole period of the experiment.

Effects of the two substances were compared, and it is known that the animals prefer *Sarcopoterium spinosum* over ordinary grass available in the region. The "control group" was the eating behavior of the weed; if the goats were unaffected by any of the drugs, both group would consume the *Sarcopoterium spinosum* plant only, and consumption of the weeds would be meagerly small.

The GENMOD procedure of SAS (V8) for fitting Generalized Linear Models, a class of regression models for univariate responses with density from an exponential family (McCullagh and Nelder, 1989) was used for analyses (SAS Institute Inc. 1999). The data is

correlated in time and it was modeled via the REPEATED statement of SAS to use the Generalized Estimating Equations (GEE) method of Liang and Zeger (Liang and Zeger, 1986). The GEE method allows repeated measures analysis when the response variable is binary. ESTIMATE statement of SAS was used to test the differences between the substances and the days. Cumulative logit function was used for the GEE analysis because the distribution was multinomial. Lipsitz *et al*. (1994) reported how to extend GEEs to multinomial data. The statistical model used was:

$Y_{ikl} = \mu + A_i + B_k + C_l + AB_{ik} + e_{ikl}$

where Y_{ikl} = individual observation for *Sarcopoterium spinosum* or weed consumption (0, 1, … ,6 reaches for the plant).
μ = Overall mean,
A_i = effects due to the substance (Magnesium hydroxide or hot red pepper solution) in Model 1, or plant (*Sarcopoterium spinosum* or weed) within substance in Model 2,
B_k = effects due to days (1, 2, … ,7),
C_l = effects of the subject (all animals),
AB_{ik} = interaction effect between A and B,
e_{ikl} = random error term.

The errors were not assumed to be normally and independently distributed. Distribution was multinomial and dependent in time. The pepper estimates were used as reference and were set to zero due to the singular design matrix.

In addition, differences between *Sarcopoterium spinosum* consumption and the weed consumption (A_i) within different groups (MGH or pepper) were investigated to provide confirmation on the "control group". In other words, this analysis was added to test whether treatments had large effects in decreasing the *Sarcopoterium spinosum* consumption compared to the weed consumption. Because the goats prefer *Sarcopoterium spinosum* and not the weed, negative changes in consumption of *Sarcopoterium spinosum* indicates that the treatments by themselves were effective. This was done by including plant (*Sarcopoterium spinosum* or weed) within substance as a factor in the model instead of the fixed effect due to substance. From hereinafter, this will be referred to as Model 2.

Results

GEE parameter estimates of MGH for the number of reaches to the plants by the goats were not different for both for *Sarcopoterium spinosum* (P = 0.62) and weed (P = 0.28). The P values indicate if the estimates are significantly different than zero. A higher estimate suggests the animal reached more for the plant and lower numbers indicates aversion. The MGH estimate for goats consuming the *Sarcopoterium spinosum* was 0.41 times higher than the hot red pepper estimate of *Sarcopoterium spinosum* consumption. Though the estimates were not significantly different than zero, the contrast made between MGH and the hot red pepper on consumption of the *Sarcopoterium spinosum* plant resulted in a large difference (Chi-square= 4.25, P=0.04). The same contrast between the substances for weed was small (Chi-square= 0.63, P = 0.43). This shows that goats in the hot red pepper group avoided eating the *Sarcopoterium spinosum* plants more than the goats in the MGH group while there was no difference between MGH and hot pepper for consumption of the weed. Because there was no application of the substances on weed, this was expected and indicates that the treatment made a difference.

MGH estimate for goats consuming the weed was 0.48 times lower than the hot red pepper estimate of weed consumption. However, the estimate was not different than zero (P = 0.28) and the differences between the two substances did not differ (Chi-square=0.63, P=0.43). This was due to no application of any substance on the weed.

Evaluation of the days showed that GEE estimates for the *Sarcopoterium spinosum* plant were large in day one and day five (P = 0.001) as compared to other days (P>0.25). This main effect includes both MGH and pepper. Since the first day, treatment of the plants was started and the fifth day, goats were presented with clean plants with no application of MGH or hot red pepper. Effects of the other days were small (P > 0.25).

Effects of the treatment by day interaction were not significant for both plants and these GEE estimates are presented in Figure 1. MGH estimates are given in reference to the pepper estimates due to singular design matrix. For the *Sarcopoterium spinosum* plant, the applications differed only in the second day (MGH2 line; P = 0.03) and the effects were non-significant in the other days. Differences between the applications were large in the second day for weed consumption also (MGH1 line; P = 0.001). These effects were non-significant in the other days though they tended to be large in the fourth day (P = 0.08). The tendency in consumption of the plants in Figure 1 indicates that the condition effect might have occurred if the animals were

fed with treated plants longer than four days. There is a tendency because consumption of the treated plants decreases until day 5. This occurs especially for the MGH2 (*Sarcopoterium spinosum* consumption in the laxative group) line in day 5, which was the day the goats were presented with untreated plants. However, after the first day with the untreated plants, the animals tended to increase consumption of *Sarcopoterium spinosum*. The weed consumption seems to be increasing and decreasing at random, because only the *Sarcopoterium spinosum* plants were treated.

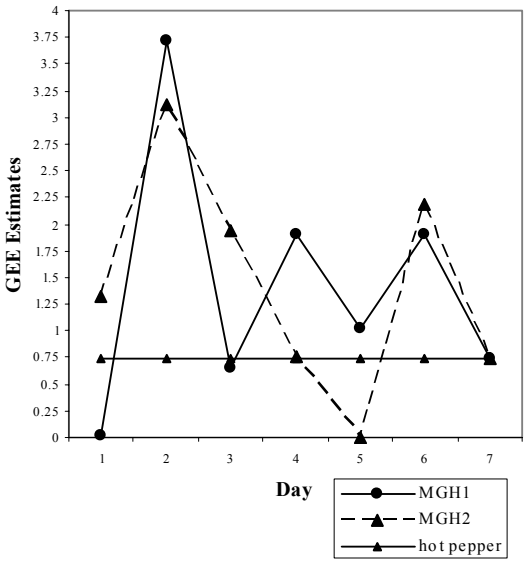

Figure 1. GEE Estimates for Magnesium Hydroxide and hot red pepper treated *Sarcopoterium spinosum,* and non-treated weed consumption (MGH1: Weed consumption in the magnesium hydroxide group, MGH2: *Sarcopoterium spinosum* consumption in the magnesium hydroxide group).

Differences for *Sarcopoterium spinosum* consumption between day one and day two were small (P = 0.53) while the differences between day one and day three, and day one and four were large (P = 0.001). Differences between days two and three were also large (P = 0.05). Significant differences among the days suggest that the animals' consumption of the *Sarcopoterium spinosum* plant changed when MGH and hot pepper were applied.

Model 2, which was used in measuring the control effect, resulted in large differences both in the MGH group and the pepper group (P = 0.001). Significant differences between the plants in MGH group indicates that MGH had large effects in decreasing *Sarcopoterium spinosum* consumption compared to the weed consumption, and those significant in the pepper group indicates that pepper had large effects in decreasing *Sarcopoterium spinosum* consumption compared to the weed consumption.

Discussion

Koh *et al*. (2003) reported that taste novelty strongly affects the speed and strength of taste aversion conditioning. The taste of the bitter substance used in this study may have been more novel to the goats. Duncan and Young (2002) reported that goats can learn to associate particular foods with particular post-ingestive effects and adjust their diets accordingly. However, this study indicates that direct effects (hot pepper) may be more efficient than post-ingestive consequences (MGH).

Results of this study suggest that animals may need to be conditioned longer than four days to permanently learn avoiding the plant in question. In contrast, Bills *et al*. (2003) worked with rats and reported that conditioning during short sessions produced strong conditioned taste aversions. Provenza *et al*. (2000) reported that lambs with 8 d exposure to *Astragalus bisulcatus* odor but not given LiCl (lithium chloride) consumed similar amounts of food, with and without the odor of *A. bisulcatus*, whereas lambs given LiCl showed a mild aversion to food with the odor during testing. The same authors reported also that lambs with 1 d exposure to the odor and given LiCl showed a strong but transient aversion to food with the odor. Short time conditioning may not be a permanent deterrent to herbivores because they continually sample foods and adjust intake. Barker *et al*. (1977) reported that Jersey cows administered lithium chloride solution after consuming sweet feed either had aversion after four pairings of the food and the poison, or had no reduction in intake at all. Conditioning may be harder if the food item is strongly favored by the organism. The differences between some of the days were highly significant. This warrants further research on the subject because the trend (Figure 1) and these statistics suggests that if the number of days for conditioning is increased to more than four, the aversion effect may grow. Follow-up studies may include longer periods of conditioning and investigate different doses.

Ruminants may avoid or prefer different plants depending on what happens after consumption (Burritt and Provenza, 1992; Kyriazakis *et al*., 1997). Parker

(2003) worked with rats and argued that taste aversion may require conditioned nausea while taste avoidance may be performed by fear only. The bitter taste in this study was more effective perhaps because it created more discomfort than the laxative substance. Any substance increasing the discomfort, or simply increasing time of the conditioning further may be more effective. Goats may refrain from consuming young tree seedlings if conditioned using an effective substance.

Conclusion

Bitter tasting substances may be more suitable for conditioning goats in avoiding normally preferred plants. Results of this study suggest that direct effects (hot pepper) may be more efficient than post-ingestive consequences (laxative). Goats conditioned to avoid normally preferred plants such as young tree seedlings may refrain from harming environmentally valuable forests.

References

Barker, L.M., Domjan, M., Best, M.R. 1977. Learning mechanisms in food selection. Baylor Univ. Press, Waco, Texas, USA.

Bills, C., Smith, S., Myers, N., Schachtman, T. R. 2003. Effects of context exposure during conditioning on conditioned taste aversions. Learning and Behavior 31: 369-377.

Brett, L.P., Hankins, W. G., Garcia, J. 1976. Prey-lithium aversions. III: Bueto hawks. Behav. Biology 17: 87-98.

Brown, M., Rivier, J., Vale, W. 1977. Bombesin: potent effects on thermoregulation in the rat. Science 196:998-1000.

Burritt, E.A., Provenza, F.D. 1992. Lambs form preferences for nonnutritive flavors paired with glucose. J. Anim. Sci. 70: 1133-1136.

Duncan, A.J., Young, S.A. 2002. Can goats learn about foods through conditioned food aversions and preferences when multiple food options are simultaneously available? J. Anim. Sci. 80: 2091-2098.

Fraser, M.D., Gordon, I.J. 1997. The diet of goats, red deer and South American camelids feeding on three contrasting Scottish upland vegetation communities. J. Appl. Eco. 34: 668-686.

Gustavson, C.R., Garcia, J., Hankins, W.G., and Rusiniak K.W. 1974. Coyote predation control by aversive conditioning. Science 184: 581-583.

Jimenez, B., Tapia, R. 2004. Biochemical modulation of NMDA receptors: role in conditioned taste aversion Neurochem. Res. 29: 161-8.

Koh, M.T., Wilkins, E.E., Bernstein, I.L., 2003. Novel tastes elevate c-fos expression in the central amygdale and insular cortex: Implication for taste aversion learning. Behav. Neuro Sci. 117: 1416-1422

Kyriazakis, I., Anderson, D.H., Duncan, A.J. 1997. Conditioned flavour aversions in sheep: the relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. Brit. J. Nutrit. 79:55–62.

Liang, K.Y., Zeger, S.L. 1986. Longitudinal data analysis using generalized linear models. Biometrika 73: 13-22.

Lipsitz, S.H., Kim, K., Zhao, L. 1994. Analysis of repeated categorical data using generalized estimating equations. Statistics in Medicine 13: 1149 -1163.

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models, New York: Chapman and Hall.

Parker, L.A. 2003. Taste avoidance and taste aversion: Evidence for two different processes. Learning and Behavior 31: 165-172.

Provenza, F.D., Kimball, B.A., Villalba, J.J. 2000. Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. Oikos 88: 424.

SAS Institute Inc., 1999. SAS OnlineDoc®, Version 8, Cary, NC: SAS Institute Inc..

Siegel, D.M., McDaniel, S.H. 1991. The frog prince: tale and toxicology. Am. J.Orthopsyc. 61: 558-62