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Manipulating Sheep Browsing Levels on Coyote Willow *(Salix exigua)* with Supplements[1]

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Summary

Macronutrients and additives have been used to suppress or promote livestock intake of upland tannin-containing browse species, but to our knowledge this technique has not been applied to sheep that feed on tannin-rich species in riparian areas. The objective of this study was to determine the effect of four supplement regimes on coyote willow (*Salix exigua*) intake by sheep during the dormant and growing seasons. Twelve Western White Face lambs (48 kg ± 4.5 kg) were placed in individual pens and assigned to one of four treatments which consisted of a basal diet of sudangrass and supplements predicted to either suppress (whole corn or quebracho tannin) or promote (cottonseed meal or polyethylene glycol, PEG) willow intake. Each of the four supplements was tested with dormant and growing willow in a Latin rectangle design with three periods and six lambs per group. Basal diet (sudangrass) intake was not affected by either promoter nor suppressor treatments in either season. Cottonseed meal effectively promoted intake of willow compared to the control and PEG treatments ($P< 0.05$) in the dormant season. No difference was detected between the control, quebracho-tannin, and whole-corn treatments, although the latter tended to depress dormant-willow intake of lambs. None of the treatments altered intake of coyote willow in the growing-season trials. Protein and possibly corn-based supplements may be effective tools to manipulate sheep browsing levels of *Salix exigua* but need to be tested in a field setting before management strategies with supplementation can be applied.

Key Words: Targeted Grazing, Tannins, Cottonseed Meal, Whole Corn, Polyethylene Glycol, Quebracho Tannin

Introduction

Willows (*Salix spp.*) are generally considered an important component of riparian areas because they influence biological diversity, water quality/quantity, and aquatic/terrestrial food webs and habitats of these ecosystems (Kauffman et al. 1983a, b; Mitsch and Gosselink 1986; Elmore and Bescheta 1987; Case and Kauffman 1997; Clary and Kruse 2004). Consequently, protection of willow populations from excessive browsing by domestic livestock is a common riparian management objective on rangelands of western North America. Nonetheless, in irrigated valleys or in areas where production of meadow grass hay is important, willow thicket encroachment from watercourse margins onto stream floodplains and terraces is considered undesirable. In such situations, hay farmers seek to control the spread of this species because it hinders the hay harvesting process and competes aggressively for water and other resources with the herbaceous understory (Gulickx et al. 2007). Thus, depending on site-specific management objectives, ranchers could manage livestock grazing to either promote or contain the growth of willow populations. This study investigated the feasibility of manipulating sheep preference for coyote willow by feeding supplements that were predicted to either suppress or stimulate intake levels of this woody plant.

Feed-supplementation strategies have been used repeatedly to modify livestock preference for woody species that contain secondary compounds (Silanikove et al. 1994, 1996; Gilboa et al. 2000; Provenza et al. 2000; Titus et al. 2000, 2001; Landau et al. 2002; Villalba et al. 2002a, b, c; Provenza et al. 2003; Villalba and Provenza 2005; Dziba et al. 2007). For example, concentrate supplements rich in starch, such as corn, can decrease intake and digestibility (Hanna et al. 1989; Pordomingo et al. 1991) and reduce preference for low-digestible forage, while a small quantity of condensed tannins added to the diet as supplement can induce animals to eat less of foods containing tannins (such as willows) when overall dietary protein is low (Villalba et al. 2002a, c). On the other hand, additives, such as polyethylene glycol (PEG) or supplemental protein, can induce goats and lambs to increase intake of tannin-rich species (Petersen and Hill 1991; Silanikove et al. 1996; Gilboa et al. 2000; Landau et al. 2002; Villalba et al. 2002a, c). To our knowledge, no prior studies have examined the effects of feed supplementation on intake levels of sheep that have access to willow, a plant genus, which usually contains high levels of tannins (Palo 1984; McWilliam et al. 2005).

The objective of this study was to determine the feasibility of: a) promoting sheep browsing on willow by offering supplements high in protein or containing a compound that binds tannins (i.e., PEG); and b) suppressing sheep browsing on willow by offering supplements high in energy or containing an external source of tannins. Specifically, we hypothesized that willow intake by sheep would be stimulated with cottonseed meal or PEG supplements and suppressed with corn or quebracho tannin.

Materials and Methods

Experimental Design

Our experiment was conducted in February and July 2006 at the New Mexico State University Campus Farm in Las Cruces, N.M. The Institutional Animal Care and Use Committee at New Mexico State University approved all procedures used in this experiment. Twelve Western White Face ewe lambs (48.1 kg ± 4.5 kg BW) were housed in individual pens (2 cm x 5m) with access to shelter and fresh water and were randomly divided into two groups. One group received supplements expected to suppress willow intake (SUP), while the other group received supplements predicted to enhance willow intake (PRO). A 3 x 6 Latin Rectangle experimental design was used to allow each animal within a group to consume both supplements and the control diet (n = 2 animals per treatment group per period). The three SUP treatments consisted of either: basal diet + whole corn; basal diet + quebracho tannin; or basal diet alone (control). A small amount of soybean meal was added to the ration of animals in the control (basal diet alone) and tannin (basal diet + quebracho tannin) treatments to ensure that all SUP diets were isonitrogenous (Table 1). The three PRO treatments consisted of either: basal diet + cottonseed meal; basal diet + PEG; or and basal diet alone (control).

Table 1. Percent composition of experimental diets predicted to suppress (SUP) or promote (PRO) willow browsing (*Salix exigua*) by sheep.

	SUP diets			PRO diets		
	Control	Energy	Tannin	Control	Protein	PEG[a]
	DM basis					
Sudangrass hay	97.49	64.83	91.03	83.56	76.66	76.04
Ground corn	0.00	0.00	0.00	15.39	0.00	20.07
Whole corn	0.00	34.11	0.00	0.00	0.00	0.00
Cottonseed cake	0.00	0.00	0.00	0.00	22.30	0.00
Soybean meal	1.47	0.00	2.43	0.00	0.00	0.00
PEG	0.00	0.00	0.00	0.00	0.00	2.84
Quebracho tannin (80%)	0.00	0.00	5.50	0.00	0.00	0.00
Mineral vitamin premix[b]	1.04	1.06	1.04	1.05	1.04	1.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
DM%	92.00	91.00	93.00	91.00	92.00	92.00
CP%[c]	6.87	6.87	6.87	6.61	15.00	6.61
ME (Mcal/kg)[c]	1.73	2.22	1.73	2.00	2.00	2.00

[a] Polyethylene Glycol, Molecular weight 6000 g/mol
[b] Mineral oil 1.5%; limestone 33.74%; dicalcium phosphate 33.23%; salt 12.70%; ammonium sulfate 4.06%; EDDI 13.51%; selenium 0.54%; vitamin A 0.26%; vitamin E 0.46%.
[c] Estimated from NRC (1985)

A reduced amount of ground corn was added to the ration of animals in the control (basal diet alone) and PEG (basal diet + PEG) treatments to ensure that all PRO treatments were isoenergetic (Table 1). Treatment order was randomly determined for each animal in both the SUP and PRO groups. The study was conducted during the dormant season (February 2006) and repeated during the growing season (July 2006). Twelve different lambs from the same flock were used in each test.

Treatments

Ground sudangrass (*Sorghum vulgare var. sudanense*) hay was offered as the basal diet during the February 2006 trials to simulate the quality of dormant-season, herbaceous-rangeland forage. The sudangrass offered during the growing-season trials was current season growth harvested from a local farm and expected to have a higher nutritional value (up to 17-percent CP; NRC 1985) than that used for the dormant-season trials. In both trials, sudangrass hay was offered at 1.7-percent BW.

The SUP diets were isonitrogenous (Table 1) and contained either 5-percent quebracho tannins or corn at 0.4 percent BW mixed with ground sudangrass hay (control animals received hay only). The PRO diets were isoenergetic (Table 1) and consisted of sudangrass hay plus 20 g of polyethylene glycol 6000 g/mol (Sigma Aldrich, St. Louis, Mo.) or cottonseed meal (15-percent CP).

The SUP and PRO supplements were mixed with the basal sudangrass diet (Table1), which was offered in the evening (7:00 p.m.) and removed two hours before feeding willow (8:00 a.m.) the following morning. Amount of feed rejected was recorded daily to the nearest gram. Each period included a 5-day, diet-adaptation period followed by a 7-day trial period. Adaptation time was assumed to serve as a washout period to avoid carry over effects.

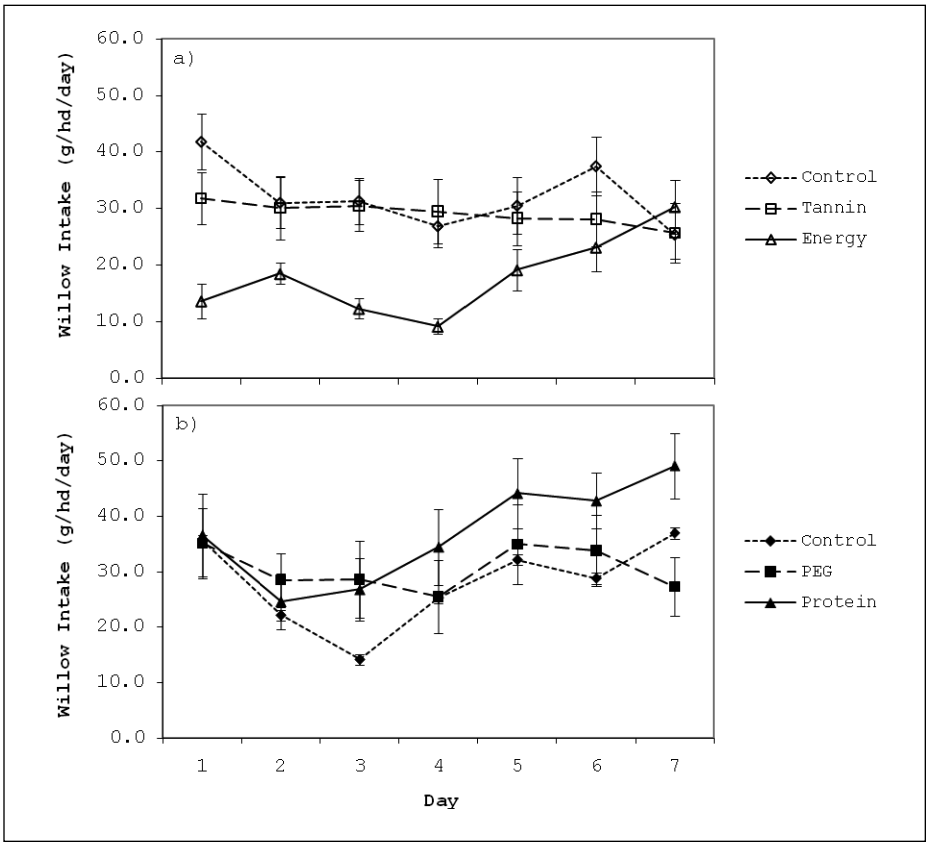
Willow Intake

Coyote willow (*Salix exigua*) branches were harvested from a designated site in the Rio Grande Valley about 8.5 km north of our experiment site and stored in a walk-in refrigerator at 4°C. Stems were clipped between 55 cm to 80 cm in length and collected from the grazeable height range of sheep (<1.5m) every 10m along the 100m-long, designated-harvest site.

Approximately 1200 grams of willow were removed from the refrigerator one hour prior to feeding tests each day. Each animal was offered 100 grams of willow per day in each of the three 7-day periods. Willow biomass offered was assumed to be sufficient to allow *ad libitum* intake during the 15-minute feeding trial period. Willow stems were stapled to a 1.5 m wooden stand at a 45-degree angle 50 cm to 95 cm from the ground. Stands were secured in each pen for a 15-minute feeding bout and then removed. The weight of the willow, plus the stand, was recorded before and after each individual feeding bout to the nearest gram. The difference in weight was calculated as willow consumed by each animal. A subset of willow stems (30 g) placed outside the pens was used to monitor water loss from willow stems and adjust intake estimates.

Twelve willow samples (55 cm to 80 cm, 20 g to 35 g) were collected during the dormancy (stems only) and growing season (stems and leaves), ground, and analyzed for condensed tannins using the vanillin-HCl method of Burns (1971) as modified by Price et al. (1978) at the end of each trial. Leaf-to-stem weight ratio of the growing-season willow samples was determined using 30 samples from 55 cm to 80 cm in length collected at the same site. Leaves were removed from stems and samples and dried at 50°C for 72 hours. Percent contribution to total sample weights of leaves and stems was calculated.

Figure 1a. Mean willow intake of lambs receiving diets that were predicted to suppress (a) or promote (b) willow intake (SUP and PRO treatments) across days during the dormant season. SUP diets consisted of sudangrass alone (Control), sudangrass + quebracho tannins (Tannins), or sudangrass + whole corn (Energy). Tannin and Control diets contained small amounts of soybean meal to ensure that all SUP diets were isonitrogenous. PRO diets consisted of sudangrass alone (Control), sudangrass + polyethylene glycol (PEG), or sudangrass + cotton seed meal (Protein). PEG and Control diets contained small amounts of ground corn to ensure all PRO diets were isoenergetic. Error bars indicate SEM.



Statistical Analyses

Willow- and basal-diet intake were analyzed separately for dormant and growing-season trials and for PRO and SUP groups. The effects of treatments during each season and within each group were analyzed in a 3 by 6 Latin Rectangle. The two blocking factors were time period (3 levels), and lamb (6 levels). A repeated measures analysis of days (7 levels) was conducted for each animal-time period combination. An autoregressive covariance AR(1) structure was assumed (Littell et al. 2006). The model allowed for heterogeneous variance for each lamb. The analyses were performed using Proc Mixed in SAS version 8.2 (SAS Institute Inc., 1999, Cary, N.C.). Statistical significance was considered at the $P = 0.05$ level.

Results and Discussion

During dormancy, willow offered consisted of bare twigs without leaves and contained 7.5 percent ± 1.2 SD condensed tannin. The sudangrass offered contained 51-percent TDN, 1.80 Mcal ME, and 6.3-percent CP (Table 1).

Intake of dormant willow by lambs was not suppressed when supplemented with whole corn or quebracho tannin ($F = 1.20$; $P = 0.35$). A day effect was observed ($P = 0.03$, Figure 1a) along with a day by treatment interaction ($P < 0.01$, Figure 1a) that reflected initial suppression of willow intake by diets containing corn, which tended to decrease over time within periods. No difference among treatments was observed on basal-diet consumption ($P = 0.21$) (Table 2). On average, lambs consumed 87-percent ± 5 SD of their offered diet (1.7-percent BW) (Table 2).

Our results differ from other studies that reported whole corn decreased forage intake, digestibility, and grazing time because animals met energy requirements sooner (Hanna et al. 1989; Pordomingo et al. 1991). Villalba et al. (2002c) reported a decrease in consumption of tannin-rich forage with corn supplementation, although their high-energy treatment contained 2.75 Mcal/kg of ME, which was somewhat higher than the 2.22 Mcal/kg of ME in high energy diets in this study. Suppressor diets containing higher ME content may lead to stronger willow intake depression than what was observed in this study.

Supplements predicted to promote willow intake affected the levels of dormant willow consumed by lambs ($P = 0.05$, Table 2). Cottonseed meal increased dormant-willow intake over the control treatment ($F = 4.59$, $P = 0.03$, Table 2). A day effect was observed ($P = 0.01$, Figure 1b), which reflected the tendency of lambs receiving cottonseed meal to consume increasing amounts of willow as trial days progressed. Animals receiving PEG exhibited intermediate levels of willow intake, which was not significantly higher than the control treatment (Table 2). No difference was observed in basal-diet consumption among treatments ($P = 0.62$, Table 2). On average, lambs consumed 93-percent ± 4 SD of their offered diet (1.7-percent BW, Table 2).

These results agree with previous research, demonstrating that lambs are better able to cope with foods containing high concentration of tannins if they are fed supplements that help offset nutrient losses incurred during metabolic detoxification processes (Freeland and Janzen 1974; Silanikove et al. 1997; Dziba et al. 2007). In addition to offsetting detoxification costs, supplemental protein may stimulate rumen-microbial activity and consequently increase digestibility, thereby reducing the negative feedback associated with ingestion of low-quality feeds containing tannins (Villalba et al. 2002c; Provenza et al. 2003).

Our results differ, however, from previous studies that observed a significant increase in the intake of tannin-rich foods when receiving PEG (Silanikove et al. 1994, 1996; Gilboa et al. 2000; Provenza et al. 2000; Titus et al. 2001; Villalba et al. 2002a, c). Our results also disagree with an earlier study conducted by Villalba et al. (2002c) who

Table 2. Mean daily intake (%) of willow and basal sudangrass ration for lambs receiving diets predicted to either suppress (SUP) or promote (PRO) consumption of dormant and growing coyote willow.

	Dormant Season Experiment				Growing Season Experiment			
	Willow[c]		Forage		Willow		Forage	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control[a]	32.0	21.3	92.2	14.8	74.9	31.1	99.7	1.2
Tannin	29.2	22.9	83.0	21.6	82.0	24.5	99.4	2.2
Energy	18.0	15.9	87.5	17.7	78.7	29.6	100.0	0.0
Control[b]	27.9[a]	26.5	89.5	23.9	74.8	30.4	100.0	0.0
PEG	30.6[ab]	28.6	91.1	19.1	74.8	29.1	100.0	0.0
Protein	37.0[b]	28.9	97.6	7.6	72.4	30.5	100.0	0.0

[a] SUP diets: control, quebracho tannin, whole corn
[b] PRO diets: control, Polyethylene glycol (PEG 6000 g/mol), cottonseed meal
[c] Column means followed by the same letter or without letters were not significantly different, $P < 0.05$ (Least Significant Difference Test).

determined that PEG was more effective than macronutrients for promoting intake of foods containing tannins. This apparent inconsistency may be due to the fact that tannin content in coyote willow during the dormant season was about half (7.5 percent) of that in the tannin-rich (15 percent) diets used by Villaba et al (2002 a, c). Alternatively (although unlikely), ground corn used in the PEG and control rations, to ensure that all PRO diets were isoenergetic, could have altered rumen pH (Murphy et. al 1994) thus inducing a slight decrease in willow intake, which may have neutralized the predicted effects of PEG. Our results suggest that nitrogen-rich supplements hold the most promise as promoters of dormant-willow intake.

Willow leaves and stems during the growing season trials contained an average of 15.2- percent ± 1.91 SD and 4.0-percent ± 1.34 SD condensed tannins, respectively, and accounted for 55 percent and 45 percent of average-sample DM weights. Thus, the average concentration of condensed tannins in the growing-season willow (stems and leaves) was 10.16 percent.

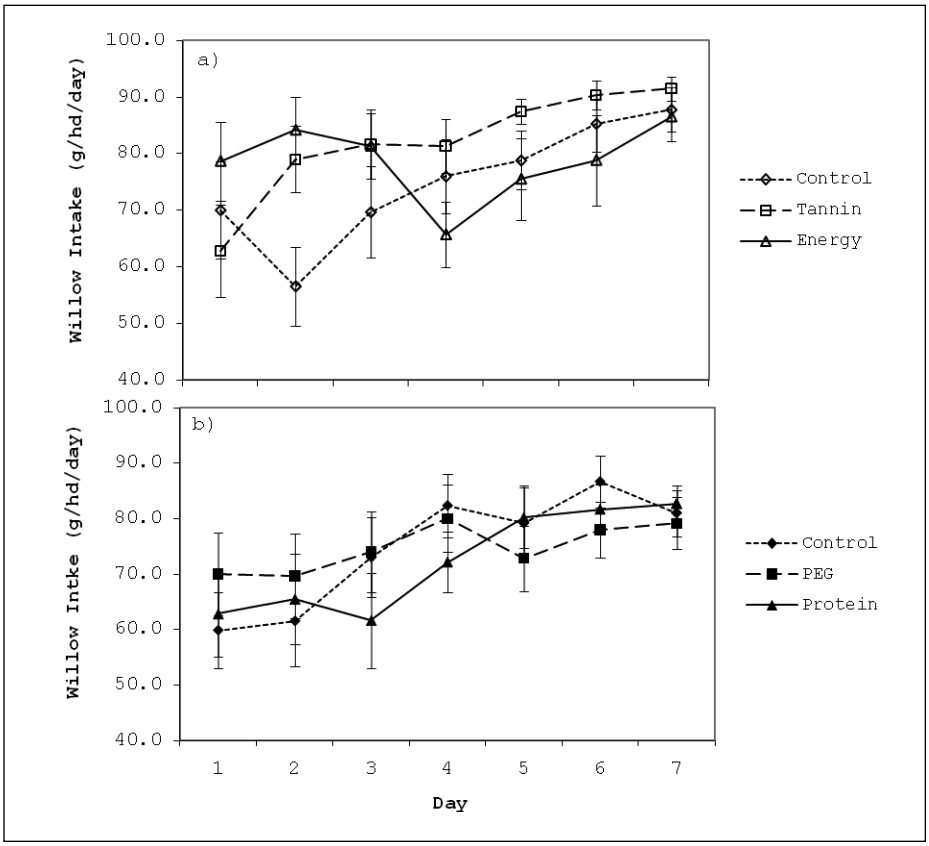
Willow intake did not differ among SUP treatments ($F = 0.23$, $P = 0.8$, Table 2) in the growing-season trials. A day effect was observed ($P = 0.04$, Figure 2a), which apparently reflected a transient, willow-intake suppression on day 4 for lambs fed the corn supplement. No difference among treatments was observed in basal-diet consumption ($P = 0.18$, Table 2).

Not surprisingly, lambs consumed almost 2.5 times more willow in summer than in winter (Table 2), which possibly reflected differences in nutrient concentration and palatability of green leafy material vs. dry stems. Treatments predicted to suppress willow intake (SUP) were not effective in altering sheep preference for willow in summer, although a transient depression in intake of lambs receiving corn appeared to occur on day 4. Difficulties in delivering quebracho tannins in the basal-diet mixture may have influenced our results. Further research is needed to test the effects of added tannins in supplements that conceal their astringency and preclude animal sorting.

Willow intake did not differ among PRO treatments ($F = 0.69$, $P = 0.53$, Table 2) during the growing season. A day effect was observed ($P < 0.01$, Figure 2b), which apparently reflected a decrease in willow intake in lambs receiving PEG supplement on days 5 to 7. Basal-diet consumption did not differ among treatments and was almost 100 percent each day (Table 2).

Increased nitrogen content of green willow and sudangrass may have improved willow palatability and possibly offset the negative effects of increased tannin concentrations, overshadowing any effects of PRO supplements in the growing-season study. Coyote willow contains up to 16.5-percent CP in the growing season (D.Cram, unpublished data) and recently harvested sudangrass hay can contain up to 17-percent CP (NRC 1985). The high levels of willow intake during the summer trials (animals avidly consumed most of the branches offered) strongly suggest that the use of supplements to boost willow intake during the growing season may be unnecessary.

Figure 1b. Mean willow intake of lambs receiving diets that were predicted to suppress (a) or promote (b) willow intake (SUP and PRO treatments) across days during the growing season. SUP diets consisted of sudangrass alone (Control), sudangrass + quebracho tannins (Tannins), or sudangrass + whole corn (Energy). Tannin and Control diets contained small amounts of soybean meal to ensure that all SUP diets were isonitrogenous. PRO diets consisted of sudangrass alone (Control), sudangrass + polyethylene glycol (PEG), or sudangrass + cotton seed meal (Protein). PEG and Control diets contained small amounts of ground corn to ensure all PRO diets were isoenergetic. Error bars indicate SEM.



Conclusions

Protein supplementation with cottonseed meal was effective at increasing intake of dormant-coyote willow by sheep when the quality of the alternative forage was low. More research is needed to identify opportunities to suppress sheep consumption of dormant-coyote willow with whole corn. Feasibility of manipulating coyote-willow preference of sheep using supplements during the growing season appears much less likely. Sheep in this study consumed almost all willow branches offered during the growing season, regardless of the supplement fed.

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