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Communication

Effects of Packing Density and Inoculation with Lactic Acid-Producing Bacteria to Evaluate the Potential for North American Elderberry (*Sambucus canadensis* L.) Fodder as Silage

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Abstract: Commercial elderberry production requires complete pruning in late fall to maintain productive canes. For integrated farms (livestock and crops), this biomass has potential as ensiled fodder for ruminant livestock. The objectives of this study were to determine the forage nutritive value of late-season (November) pruned elderberry (*Sambucus canadensis* L. "Rogersville") fodder when ensiled. A 2 × 2 factorial laboratory silo experiment was conducted testing two packing densities with or without inoculation with lactic acid-producing bacteria silage inoculant to determine effects on silage nutritive values and fermentation parameters. Pre-ensiled elderberry fodder, composited from plants over 2000 m^2, averaged 5.6% crude protein, 62.5% acid detergent fiber (ADF), 72.5% neutral detergent fiber, 11.4% non-fiber carbohydrates, 53% total digestive nutrients, and 52% relative feed value (RFV). The two packing densities were 160.2 kg dry matter/m^3 and 240.3 kg dry matter/m^3. Packing density did not affect any nutrient characteristics of the ensiled fodder. Acid detergent fiber was greater ($p = 0.01$) in un-inoculated silage, resulting in lower ($p < 0.01$) RFV for un-inoculated silage. Only lactic acid concentration was affected by packing density with greater concentrations ($p = 0.04$) in high-density silos. Inoculant affected several fermentation parameters with greater concentrations of ($p < 0.01$) propanediol, ($p = 0.01$) propanol, and ($p < 0.01$) acetic acid, while un-inoculated silages had greater concentrations of ($p = 0.03$) ammonia-nitrogen, ($p < 0.01$) lactic acid, ($p = 0.02$) succinic acid, and ($p < 0.01$) ethanol. Overall, late-season elderberry fodder was successfully ensiled, but nutritive value was low. Packing density did not affect nutritive value but did increase lactic acid concentration. Inoculation improved the RFV by reducing ADF, and though acetic acid production was greater in inoculated silage, total acid concentration was not affected.

Keywords: agroforestry; fodder; silage; silvopasture; elderberry

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1. Introduction

Elderberry (genus *Sambucus*) refers to 10 species of shrubs and small trees from the family *Adoxaceae* found natively in temperate and sub-tropical regions around the world. Fruit from elder trees have a long history of medicinal and nutritional uses in many cultures [1], and commercial production in the United States is growing in popularity due to the health benefits of secondary plant compounds [2]. Additionally, elderberry is a robust woody perennial that can easily be incorporated into a wide variety of agroforestry systems. This creates an opportunity to investigate the viability of utilizing biomass as a source of fodder for livestock as part of a multi-functional polyculture system with animals.

Commercial elderberry production typically requires aggressive pruning in late fall to maintain productive canes the following year. The pruned material is waste and is discarded or composted. Instead of pruning, integrated farms could graze the standing residue, however, the effect of grazing on subsequent berry production is unknown. Additionally, many elderberry species have potentially toxic concentrations of cyanogenic glycosides, which may also present challenges with fresh fodder grazing [3]. Thus, a potential solution may be to ensile the late-season biomass, both to utilize the material and avoid the risk of any cyanide toxicity. There is little information available about the nutritional value of elderberry biomass as fresh fodder for livestock, much less the suitability of the residue for ensiling [4,5].

Digestible components of the fodder and water-soluble carbohydrates are the main substrates that drive the fermentation process and production of volatile fatty acids during ensiling [6]. Increased volatile fatty acid (VFA) concentration results in lower pH and, therefore, greater silage stability [7]. Lactic acid-producing bacteria (LAB) are among the fermentative microbial groups that are active early on in ensiling [8]. Since low pH results in greater silage stability and LAB produce lactic acid, inoculation of forages or fodder with LAB has been utilized. At levels equal to or exceeding the indigenous population (e.g., 10^6 colony forming units (cfu)/g fresh crop), LAB can increase the speed of pH decrease and reduce the final silage pH [9]. Given the unknown ability of late-season biomass to readily ensile, we hypothesized that inoculation of LAB would increase the stability of this material indicated by lowering silage pH.

The packing density of the silage is also an important factor for reducing dry matter losses in silages. Greater packing density reduces the porosity of silage, excluding oxygen and increasing the rate at which oxygen moves through the silage during filling and storage and controls the rates of plant and microbial respiration that result in DM loss [10]. For precision harvested forages ensiled and stored in bunkers, 243 kg DM/m^3 has been recommended [10], while the recommendation for baled silages is 162 kg of DM/m^3 [11]. It is unknown how elderberry residue will be ensiled by producers on the farm, so it was appropriate to evaluate different packing densities for the effectiveness of fermentation.

The objectives of this study were to (1) describe the nutritional characteristics of North American elderberry (*Sambucus canadensis* L.) residue harvested in a commercially relevant stage of production (late-season), and (2) determine if LAB inoculation and increased packing density improve fermentation and increase fermentation products of late-season elderberry biomass. Our hypothesis was that increased packing density and inoculation would increase total acid production in ensiled elderberry fodder.

2. Materials and Methods

2.1. Harvest and Ensiling

The study was a completely randomized design with a 2 × 2 factorial treatment structure. Fodder material was harvested, and laboratory silos were packed on 23 November 2020 at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri (USA). Plant material was utilized from an existing randomized trial plot (approximately 2000 m^2 area) of North American elderberry. Elderberry plots were managed according to commercial standards throughout the year, with mulching and ground-pruning in February 2020 and spring application of herbicide and fertilizer. Fruit was harvested in early September. Canes with attached remaining biomass from "Rogersville" cultivar were pruned at ground level. The average ambient temperature over the course of the day was 3.3 °C (±1.1 °C). Composited material was passed through a commercial mulch chipper attachment (Wallenstein BX42), achieving approximately 2–4 cm chop size. Additionally, 5–50 g sub-samples were collected and frozen for chemical analysis.

Treatments for this study consisted of either inoculated or un-inoculated elderberry material packed at either a high or low packing density. Each treatment combination was replicated and 4 replicates. Targeted packing densities were 160.2 kg/m^3 and 240.3 kg/m^3. This produced an inoculated high-density treatment and inoculated low-density treatment, and un-inoculated high- and low-density treatments.

Sixteen laboratory polyvinyl chloride (PVC) silos were constructed (10.2 cm diameter × 29.2 cm height) with rubber end caps and metal brackets to secure the caps, which allowed for 2.4 L of volume for each laboratory silo. One week prior to the start of the study, samples of elderberry canes were chopped and dried at 60 °C in a forced air oven for 48 h to determine dry matter to predict and estimate packing density treatments. Using the predictor DM content, the amount of fresh chopped material needed to achieve the treatment packing densities of 160.2 and 240.3 kg/m^3 was calculated. Chopped material was passed through a mesh screen with large (5.1 × 7.6 cm) squares to screen any prohibitively large pieces of material. This was then weighed out and inoculated with sample silage inoculant mixed at a rate of 0.5 g/L to provide inoculant at a rate of 4.4 mL/kg of material. Elderberry fodder was inoculated with SiloSolve FC (CHR Hansen; *Lactiplantibacillus buchneri* LB1819, *Lactococcus lactis* O224; 150,000 cfu/g forage). Un-inoculated silage was sprayed with water at the same rate to provide equivalent levels of moisture between treatments. Laboratory silos were packed and sealed and set in a dry indoor location with a temperature maintained at 10 °C (range 7.2 to 12.8 °C) for fermentation. After 60 days of ensiling, the silos were open, contents removed and frozen for analysis.

2.2. Nutritive Analyses

Approximately 150 g of pre- and post-ensiling material was dried at 60 °C in a forced air oven for 48 h to determine DM content. Approximately 150 g of dried material was express shipped to Dairy One Forage Laboratory (Ithaca, NY, USA) for nutritional analysis (crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fiber carbohydrates (NFC), total digestible nutrients (TDN), net energy for lactation (NEL), net energy for maintenance (NEM), net energy for gain (NEG), relative feed value (RFV)) and minerals using the following methods. Crude protein was determined via combustion using a CN928 Carbon/Nitrogen Determinator (Leco Corporation, St. Joseph, MI, USA) [12] Official Method 990.03). Neutral detergent fiber and ADF extractions were made using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) and ANKOM filter bag technique [13,14]. Non-fiber carbohydrates were calculated as 100-(CP + NDF + ether extract + ash). Total digestible nutrients were calculated by Dairy One using summative energy equations [15]. Net energy for lactation, NEG, and NEM were also calculated by Dairy One [15]. Relative feed value was calculated by multiplying digestible dry matter by dry matter intake and then dividing by 1.29 [16]. For mineral analysis, samples were digested using CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control (CEM, Matthews, NC) then analyzed by Thermo iCAP Pro XP ICP (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Approximately 150 g of post-ensiled sample were shipped fresh to Rock River Laboratory (Watertown, WI, USA) for analysis of fermentation parameters conducted by the following methods. For pH, forage samples were mixed with deionized water and read with a combination pH electrode. Volatile fatty acids (VFA) were extracted from the sample in a 1:10 ratio of sample and deionized water, centrifuged, and the supernatant was combined with calcium hydroxide and copper sulfate, centrifuged again, and the supernatant analyzed by high-performance liquid chromatography (HPLC) equipped with a reverse-phase ion exclusion column and a refractive index detector (Waters Corporation, Milford, MA). For ammonia-nitrogen (NH_3-N), the same supernatant produced as in the VFA procedure, and was analyzed with Skalar San++ Segmented Flow SA 5000 Analyzer (Skalar, Breda, The Netherlands) (based on modified Berthelot reaction [17]).

2.3. Statistical Analyses

Silage chemical composition and fermentation parameters were analyzed using SAS 9.4 PROC GLIMMIX (SAS Inst. Inc., Cary, NC, USA) to test the effects of packing density, inoculation, and density × inoculation in ANOVA for a 2 × 2 factorial arrangement. The LSMEANS option was used to generate individual treatment means. Significance was declared at $p \leq 0.05$, and tendencies were declared at $p \leq 0.10$.

3. Results

3.1. Pre-Ensiled Nutrient Composition

Nutritive values for pre-ensiled elderberry fodder and nutritive value for tall fescue (Schedonorus arundinaceus (Schreb.) Dumort) from National Academies of Sciences, Engineering, and Medicine Nutrient Requirements of Beef Cattle [18] are provided in Table 1. Compared to tall fescue, elderberry fodder has a low CP (5.6). The level of NDF in elderberry fodder is comparable to tall fescue, but ADF levels are 55.1% greater than tall fescue. Elderberry fodder was lesser in all minerals compared to tall fescue, with the exception of Cu.

Table 1. Nutritive value for pre-ensiled elderberry fodder and NASEM [1] tall fescue values for comparison.

	CP [2]	**ADF**	**NDF**	**NFC**	**TDN**
			%		
Fresh elderberry fodder	5.6	62.5	72.5	11.4	53
Tall fescue	9.2	40.3	65	n/a [3]	n/a

	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn	Mo
			%					ppm		
Fresh elderberry fodder	0.3	0.1	0.1	0.6	0.04	48	18	5	50	0.5
Tall fescue	0.5	0.2	0.2	1.7	0.21	n/a	21	5	90	1

[1] NASEM, National Academies of Sciences, Engineering, and Medicine Nutrient Re-quirements of Beef Cattle.
[2] CP, crude protein; ADF, acid detergent fiber; NFC, non-fiber carbohydrates; TDN, total digestible nutrients.
[3] n/a, not available.

3.2. Post-Ensiled Nutrient Composition

Post-ensiled nutrient composition of elderberry fodder packed at two densities and with or without inoculant is shown in Table 2. There were no interactions between packing density and inoculation. No variables were affected by density, though CP had a tendency ($p = 0.06$) to be greater in high-density laboratory silos compared to low-density. Inoculation affected ADF, with greater ($p = 0.01$) ADF concentrations in un-inoculated fodder silage.

3.3. Fermentation Parameters

Fermentation parameters of elderberry fodder packed at two densities and with or without inoculant are in Table 3. There were no interactions between packing density and inoculation. For the density treatment, trends were observed for NH_3-N ($p = 0.07$) with greater values in the high-density treatment, and lactic acid was greater ($p = 0.04$) in the high-density treatment. Moisture did not differ among treatments and averaged 55.2%. No differences were observed among treatments for pH, and pH averaged 4.6. Parameters that were greater ($p < 0.05$) in inoculated silage were acetic acid, 1, 2 propanediol, and propanol, while NH_3-N, lactic acid, succinic acid, and ethanol were greater ($p < 0.05$) in un-inoculated silage.

Table 2. Nutritional values for elderberry silage by main effect and interaction of density (High and Low) and inoculation (Yes or No) treatments.

Treatment	**CP [1]**	**ADF**	**NDF**	**NFC**	**TDN**	**NEL**	**NEM**	**NEG**	**RFV**
Density	%	%	%	%	%	McaL/kg	McaL/kg	McaL/kg	%
High	5.9	67.5	77.5	6.2	51.8	0.67	0.9	0.36	43.8
Low	5.6	67.7	77.5	6.4	51.8	0.67	0.9	0.36	43.5
SEM [2]	0.09	0.25	0.47	0.46	0.16	0.018	0.006	0.006	0.35
p-Value	0.06	0.56	0.99	0.68	1	0.92	1	1	0.62
Inoculant									
Yes	5.8	67.1 [b3]	77.1	6.7	51.8	0.69	0.9	0.36	44.4 [a]
No	5.7	68.1 [a]	77.8	5.9	51.6	0.66	0.89	0.36	42.9 [b]
SEM	0.09	0.25	0.47	0.46	0.16	0.018	0.006	0.006	0.35
p-Value	0.85	0.01	0.27	0.27	0.3	0.27	0.2	0.58	<0.01
Density × Inoculant									
High × Yes	6	66.7	77.1	6.4	51.8	0.69	0.9	0.36	44.8
High × No	5.8	68.3	77.8	5.9	51.8	0.66	0.9	0.36	42.8
Low × Yes	5.6	67.4	77	6.9	52	0.69	0.91	0.36	44
Low × No	5.7	67.9	77.9	5.9	51.5	0.66	0.89	0.36	43
SEM	0.13	0.35	0.66	0.65	0.23	0.026	0.008	0.009	0.49
p-Value	0.28	0.15	0.87	0.71	0.3	0.92	0.51	1	0.33

[1] CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; TDN, total digestible nutrients; NEL, net energy for lactation; NEM, net energy for maintenance; NEG, net energy for gain; RFV, relative feed value. [2] SEM, Standard error of the mean. [3] Lowercase letters, means without a common letter differ ($p < 0.05$).

Table 3. Fermentation values for elderberry silage by main effect and interaction of density and inoculation treatments.

	Moisture	NH_3-N	pH	Lactic Acid	Acetic Acid	Propionic Acid	Succinic Acid	Formic Acid	Ethanol	Propanediol	Propanol	Butanediol	Total Acids	Total Alcohol
Density	%	% of N		%	%	%	%	%	%	%	%	%	%	%
High	55.5	0.27	4.6	0.83 [a]	1.24	0.33	0.06	0.05	0.99	0.49	0.08	0.45	2.51	2.02
Low	55.0	0.23	4.6	0.62 [b]	1.43	0.25	0.07	0.03	1.07	0.48	0.05	0.44	2.39	2.04
SEM [1]	0.22	0.014	0.10	0.065	0.132	0.039	0.008	0.035	0.091	0.076	0.011	0.015	0.165	0.166
p-Value	0.10	0.07	0.64	0.04	0.33	0.18	0.67	0.75	0.56	0.95	0.16	0.65	0.62	0.90
Inoculant														
Yes	55.3	0.22 [b3]	4.6	0.50 [b]	1.78 [a]	0.29	0.05 [b]	0.01	0.71 [b]	0.77 [a]	0.09 [a]	0.44	2.62	2.02
No	55.2	0.27 [a]	4.7	0.96 [a]	0.89 [b]	0.29	0.08 [a]	0.07	1.35 [a]	0.20 [b]	0.04 [b]	0.45	2.28	2.04
SEM	0.22	0.014	0.10	0.065	0.132	0.039	0.008	0.035	0.091	0.076	0.011	0.015	0.165	0.166
p-Value	0.56	0.03	0.50	0.01	0.01	0.98	0.02	0.24	0.01	0.01	0.01	0.74	0.17	0.92
Density × Inoculant														
High × Yes	55.7	0.26	4.6	0.54	1.68	0.29	0.05	0.02	1.36	0.82	0.11	0.45	2.58	2.02
High × No	55.3	0.28	4.7	1.13	0.79	0.36	0.08	0.08	1.35	0.16	0.05	0.46	2.44	2.01
Low × Yes	55.0	0.19	4.6	0.46	1.87	0.29	0.06	0.00	0.78	0.73	0.07	0.44	2.66	2.02
Low × No	55.0	0.27	4.6	0.79	0.98	0.21	0.08	0.06	1.36	0.23	0.04	0.44	2.12	2.07
SEM	0.31	0.019	0.14	0.092	0.186	0.055	0.011	0.050	0.128	0.107	0.016	0.022	0.233	0.235
p-Value	0.54	0.16	0.76	0.18	0.99	0.20	0.67	0.98	0.64	0.46	0.55	0.82	0.40	0.90

[1] SEM, standard error of the mean. [2] Butyric acid and butenol were not detected in the samples. [3] Lower case letters, means without a common letter differ ($p < 0.05$).

4. Discussion

4.1. Pre-Ensiled Nutrient Composition

The nutritive value of North American elderberry fodder was compared to tall fescue, the common forage species fed in the mid-South USA, a region in which elderberry is adapted. Values for CP in elderberry fodder were lesser than tall fescue and, if fed alone, would not meet the nutrient requirements of growing ruminants (12% CP) or pregnant cows (7–8% CP) [18].

Elderberry fodder's nutritive value was lower than other fodders in the literature. Smith et al. [19] ensiled willow (*Salix viminalis* L.) and observed pre-ensiled levels of 16.7% CP, 57.3 NDF%, and 41.0% ADF. Baertsche et al. [20] ensiled several intensively managed hardwood species, including ailanthus (*Ailanthus altissima* Mill.), aspen (*Populus tremula* L.), black alder (*Alnus glutinosa* (L.) Gaertn.), black locust (*Robinia pseudoacacia* L.), birch (*Betula platyphylla* Suk.), elm (*Ulmus Americana* L.), green ash (*Fraxinus pennsylvanica* Marshall), honeylocust (*Gleditsia triacanthos* L. (Fabaceae)), poplar (*Populus* spp.) and (*Salix viminalis* L.). For those species, ADF ranged from 23.39 to 9.34%. [20]. Crude protein ranged from 9.86% for willows to 23.87% for nitrogen-fixing black locust [20]. However, hardwood species utilized by Baertsche et al. [20] were managed with short rotation, trees were harvested twice per year (mid-June and regrowth in early August), trees were harvested at 30 to 60 cm of either initial growth or regrowth, 5 to 10 cm from the ground. In the case of elderberry, only one biomass harvest occurs per year in late fall. Baertsche et al. [20] also observed high ADF and lignin values in species with a low leaf-to-stem ratio since leaves contain greater amounts of nutrients compared to stems. Elderberry utilized in this study was harvested in a dry fall when much of the leaf biomass had senesced, and therefore, most of the biomass was the stem.

In addition, mineral content was considerably lesser in elderberry fodder used in this study compared to tall fescue. Few studies have evaluated the mineral content of fodder species. Baertsche et al. [20] observed mineral content ranging from 0.6–0.14 Ca, 0.08–0.19 P, 0.42–0.76 Mg, 1.98–4.12 K, 0.03–0.08 Na% to 151–275.2 Fe, 32.5–105.4 Zn, 6.4–12.9 Cu, and 74.7–187 Mn ppm, indicating large amounts of variation for the 10 tree species. Other than species, several other factors can influence mineral uptake and composition, including soil fertility, pH, and type.

4.2. Post-Ensiled Nutrient Composition

Inoculation reduced ADF levels in ensiled elderberry fodder as a result of greater fermentation of the cellulose fraction, as NDF was not different among treatments. A meta-analysis of homofermentative and heterofermentative lactic acid bacteria inoculation of corn silage observed reductions in ADF, but no change in NDF [21].

4.3. Fermentation Parameters

Packing silage densely is important for the exclusion of oxygen, at greater densities, porosity is reduced, and oxygen cannot penetrate through the silage, this exclusion prevents the growth of detrimental aerobic microorganisms that can spoil silage, and nutrients are preserved [22]. Low oxygen environments are optimal for lactic acid-producing bacteria which resulted in greater lactic acid concentrations in high-density silos compared to low-density silos. Currently, no packing density recommendations exist for elderberry fodder, it could be practical for producers to bale and wrap the fodder or use piles/bunkers options, the study results show some advantage of greater lactic acid concentrations with greater packing densities.

Moisture averaged 55.2%, which is within the range of recommended silage moisture for forages [23]. The average pH was 4.6, which is similar to typical values for legumes and grass silages [24]. Baertsche et al. [20] observed a pH range of 4.66–6.45 for 10 ensiled tree species.

Several fermentation parameters differed with the inclusion of inoculation. A meta-analysis of 130 peer-reviewed papers examined the effects of inoculation (for temperate grasses and legumes, and tropical grasses) with LAB revealed increased lactic acid and reduced pH, acetate, and NH_3-N [25]. Greater values for 1,2 propanediol are also expected with LAB inoculation. However, the inoculant in this study also included *Lactiplantibacillus buchneri*, which has been shown to convert lactic acid to acetate and 1, 2 propanediol [26] and convert 1, 2 propanediol to 1, propanol [27].

Inoculated silage had greater acetic acid concentrations, while un-inoculated silage had greater concentrations of lactic acid. The inoculant used in this study contained both *Lactiplantibacillus buchneri* and *Lactococcus lactis.* The substrate for *Lactococcus lactis* is sugars and the product is lactic acid, while *Lactiplantibacillus buchneri* utilizes lactic acid and sugars to create acetic acid [28]. Several inoculants utilize both strains as *Lactococcus lactis* quickly ferments sugars to lactic acid that rapidly reduces pH, preventing growth of undesirable microorganisms, thereby, reducing DM loss, though with low acetic acid concentrations, these forages lack stability [28]. Therefore, *Lactiplantibacillus buchneri* may be used with *Lactococcus lactis* as *Lactiplantibacillus buchneri* is active after the initial fermentation period is over and can convert lactic acid and remaining sugars to acetic acid [28]. There is no data available to qualify the sugar content of the elderberry fodder in this study, however, it is probable that sugar content was low, and while *Lactococcus lactis* may have produced lactic acid, that lactic acid and any other available sugars were converted to acetic acid by *Lactiplantibacillus buchneri*. Though total acids did not differ between inoculated and un-inoculated, there might be some advantages to silage stability by inoculating elderberry silage.

5. Conclusions

Despite the lateness of the season and minimal amount of leaf biomass, the elderberry canes did successfully ensile to produce a moderately-low quality feed resource. The nutrient and fermentation profile suggest that elderberry fodder could be incorporated into a maintenance diet in ruminants, potentially providing a winter feed resource. This study raises additional questions that should be explored to fully understand the potential of elderberry silage, including aerobic stability, silage palatability, and cyanogenic glycoside content before and after ensiling. Furthermore, additional research should evaluate the optimal time to cut and ensile the fodder to maximize nutritive value without compromising subsequent elderberry harvest yields.

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