Research Note

MODELS OF LABORATORY MINI-SILOS TO STUDY THE FERMENTATION OF WILTED ALFALFA (MEDICAGO SATIVA) USING A HOMOLACTIC BACTERIAL INOCULANT[1,2]

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To study and understand silage fermentation, there is a need for research using laboratory mini-silos that permit the control of variables and the assessment of different experimental treatments through replication. Research tools, such as mini-silos, are cost effective alternatives that require a small sample size and reduced labor (Solórzano et al., 2016b). Most laboratory silos require manual packing. As an alternative, Cherney et al. (2004) reported the use of vacuum-sealed plastic bags to ensile whole-plant corn. However, it may be possible to use vacuum-sealed glass jars as experimental mini-silos to study the fermentation characteristics of forages. Vacuum packing may allow higher throughput during mini-silo packing and may improve the consistency of packing density. The objective of this study was to evaluate the nutrient content, fermentation and aerobic stability (AS) characteristics of alfalfa (*Medicago sativa*) silage when ensiled in three different types of laboratory mini-silos for 91 d with or without homolactic bacterial inoculation (HBI).

Alfalfa (*Medicago sativa*) was grown and harvested at a commercial crop farm in Jefferson County, Wisconsin. The whole plant forage was swathed and allowed to wilt to a moisture content of approximately 50%. Wilted alfalfa was chopped to a theoretical length of cut (TLC) of 20 mm and transported to Fitchburg, Wisconsin, for further processing. Particle size distribution was determined using the Penn State particle size separator (Heinrichs and Kononoff, 1996). The proportions of material that remained on the surface of sieves with pore sizes 19.04, 7.85 and 1.27 mm were 47.2, 23.6 and 21.2%, respectively, while 8% reached the bottom pan. One-half of the vegetative material was inoculated using a water soluble HBI at a rate of 1.1 g/t of wilted matter with a product supplying >$9.1x10^{10}$ CFU/g containing *Lactobacillus plantarum, Enterococcus faecium*, *Pediococcus acidilactici, Pediococcus pentosacesus,* and *Lactococcus lactis* (MikropHerm WF*,* Madison, WI)[6]. The other half of the vegetative material received the same amount

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of water only. The liquids were applied using a hand sprayer while the forage was mixed manually. Four samples of non-inoculated wilted herbage were collected prior to ensiling and stored at -18° C until analyzed. To evaluate the fermentation process, the nutritional content of the resulting silages and repeatability of results, three types of mini-silos (Figure 1) were used: A) 3 L capacity PVC mini-silo fitted with a one-way mechanism to vent gas (Figure 2); B) 1 L capacity PVC mini-silo fitted with a one-way mechanism to vent gas; and C) 1 L glass mini-silo vacuum sealed using a Food Saver FM2001 (Sunbeam Corp., Boca Raton, FL 33431 USA) with a wide mouth jar adaptor. Four mini-silos of each type were assigned to each of two microbiological additives: No HBI (NON) and HBI (HBI). The combination of the type of mini-silo and microbiological additive resulted in six experimental treatments to be evaluated: 1) 3 L PVC mini-silo with no HBI (3L-NON); 2) 3 L PVC mini-silo with HBI (3L-HBI); 3) 1 L PVC with no HBI (1L-NON); 4) 1 L PVC with HBI (1L-HBI); 5) 1 L glass mini-silo with no HBI (1LGJ-NON), and 6) 1 L glass mini-silo with HBI (1LGJ-HBI). The 3 L PVC mini-silos were filled with about 1,200 g of the wilted alfalfa containing approximately 13.2% water soluble carbohydrates (WSC) (DM basis). The 1 L PVC or glass mini-silos were filled with about 300 g of the same wilted forage. The PVC mini-silos utilized in this study used a one-way mechanism to vent gas, thus differing from those reported by Solórzano et al. (2016b).

Alfalfa was fermented for 91 d at a temperature of 20 to 23 °C. Upon opening the mini-silos, silages were weighed; temperature was measured using a 12 cm Taylor thermometer (model 5989) placed in the middle of each mini-silo for 60 s, sampled and subsequently analyzed for nutrient content and fermentation products. Pre-ensiled forage and silage samples were analyzed by wet chemistry for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), amylase treated neutral detergent fiber adjusted for ash content (aNDFom), ash, undigested neutral detergent fiber adjusted for ash content (uNDFom), WSC, non-fibrous carbohydrates (NFC) and in vitro determination of 30 h neutral detergent fiber digestion (NDFD30) (Dairyland Laboratories, Inc. Arcadia, WI). Additionally, fermentation characteristics (pH, lactic acid, acetic acid, propionic acid, butyric acid, iso-butyric acid, total volatile fatty acids (VFA), ethanol, methanol, propanol, propanediol, butanol and ammonia nitrogen (NH_3-N) were analyzed by wet chemistry at the commercial laboratory cited. Lactic acid as a proportion of the total acids and the lactic:acetic ratio were calculated. The silage DM recovered at silo opening divided by the DM mass ensiled, multiplied by 100 was used to calculate the percentage of DM recovery (% DMR). Data pertaining to nutrient content of pre-ensiled wilted forage and silages resulting from the six treatments were analyzed using the GLM procedure of SAS (SAS Institute, 2004) in a completely randomized design (CRD) with six treatments and four replicates per treatment. Silage temperature at opening the mini-silos and DMR were analyzed as a CRD with six treatments replicated four times. Mean separation was conducted using Tukey's Test.

Silage aerobic stability was determined by monitoring temperature at 6 h intervals during 174 h (Honig, 1986). Approximately 100 g of each silage were loosely placed in styrofoam containers and exposed to air in thermally insulated chambers. A 12 cm Taylor thermometer (model 5989) was placed in the middle of each sample. Aerobic stability was defined as the time after mini-silo opening for silage temperature to reach 3º C above ambient. Data were analyzed using the GLM of SAS (SAS Institute, 2004) as a split plot design using mini-silo as the repetitive measure replicated four times with a factorial arrangement of treatments: six treatments x 30 time points when temperature was recorded. Mean separation was conducted using Tukey's Test.

Ensiling did not change ($P>0.05$) the DM content, which varied from 50.41% for the wilted forage to an average of 48.71% for the alfalfa silage (Table 1). Ensiling alfalfa increased ($P<0.05$) ADF from 35.15% DM for the wilted forage to an average of 37.31% DM

TABLE 1.—*Nutritional characteristics of pre-ensiled (wilted) alfalfa and 91-d silage resulting from using three types of mini-silos, without (NON) and with (HBI) homolactic bacteria inoculant.*[1]

		3 L PVC		1 L PVC		1 L GJ			
Item[2], %	Pre-Ensiled	NON	HBI	NON	HBI	NON	HBI	SE	P<
DM	50.41	48.67	48.96	48.75	48.40	48.68	48.80	0.61	0.35
CP	17.85	19.05	18.53	18.02	18.68	17.92	18.32	0.31	0.10
NDICP	2.86	3.05	2.97	2.88	2.99	2.87	2.93	0.05	0.10
ADF	35.15c	36.33bc	36.71b	37.39ab	36.87b	38.51a	37.09ab	0.33	0.01
aNDF	41.10	41.36	41.15	42.23	42.26	42.48	41.08	0.46	0.14
aNDFom	38.63	38.69	38.69	39.24	38.74	40.09	39.98	0.57	0.32
uNDFom	19.95	19.89	20.34	20.53	20.44	21.06	21.03	0.33	0.12
NDFD30	48.35	48.60	47.44	47.69	47.23	47.43	47.39	0.66	0.69
WSC	13.18a	4.01b	4.06b	3.73b	3.94b	4.04b	3.83b	0.14	0.01
Ash	10.29b	11.19ab	10.44ab	11.26a	10.48ab	10.97ab	10.36ab	0.20	0.01
NFC	33.28	31.32	32.51	31.57	32.29	31.08	31.48	0.53	0.09

[1]Within a row, means with different letters differ P<0.05.

[2]Dry matter (DM), crude protein (CP), neutral detergent insoluble CP (NDICP), acid detergent fiber (ADF), amylase treated neutral detergent fiber (aNDF), amylase treated neutral detergent fiber adjusted for ash content (aNDFom), undigested neutral detergent fiber adjusted for ash content (uNDFom), in vitro determination of 30 h neutral detergent fiber digestion (NDFD30), water soluble carbohydrates (WSC), ash, and non-fibrous carbohydrates (NFC).

TABLE 2.—*Fermentation characteristics of 91-d alfalfa silage resulting from using three types of mini-silos, without (NON) and with (HBI) homolactic bacteria inoculant.*[1]

	3 L PVC		1 L PVC		1 L GJ			
	NON	HBI	NON	HBI	NON	HBI	SE	P<
pH	4.53ab	4.40b	4.70a	4.40b	4.68a	4.40b	0.04	0.01
Lactic acid, %	5.14ab	5.86a	4.36b	4.51ab	4.47b	5.63ab	0.30	0.01
Acetic acid, %	1.87ab	1.56bc	1.68a	0.96c	1.97ab	1.24c	0.09	0.01
Total acids, %	7.01ab	7.41a	6.04bc	5.47c	6.44abc	6.87ab	0.26	0.01
Lactic:Total acids, %	72.95bc	78.59ab	72.25c	82.44a	69.42bc	81.96a	1.71	0.01
Lactic:Acetic	2.88bc	3.88ab	2.61bc	4.70a	2.27c	4.54a	0.31	0.01
Ammonia, % CP	5.74ab	5.05bc	6.40a	4.50bc	6.18a	4.25c	0.31	0.01
Ethanol, %	0.34ab	0.37a	0.28c	0.22c	0.34ab	0.29a	0.01	0.01

[1]Propionic acid, butyric acid, iso-butyric acid, ethanol, methanol, propanol, propanediol and butanol were analyzed; however, they were not detected at levels above 0.01% of the DM.

[2]Within a row, means with different letters differ P<0.05

TABLE 3.—*Dry matter recovery, temperature at opening and density 91-d alfalfa silage resulting from using three types of mini-silos, without (NON) and with (HBI) homolactic bacteria inoculant.*[1]

	3 L PVC		1 L PVC		1 L GJ			
	NON	HBI	NON	HBI	NON	HBI	SE	P<
DMR[2], %	96.4	97.0	96.6	96.5	95.7	95.1	1.01	0.80
Temperature at opening of mini-silo, °C	20.4ab	19.6b	20.3a	18.45ab	20.0a	18.45b	0.37	0.03
Density, kg DM/m^3	194.2a	194.2a	154.3c	154.3c	158.5b	158.5b	0.0	0.01
Volume of mini-silo, ml	3,088.9	3,088.9	972.0	972.0	946.25	946.25		
Dry matter ensiled, g	600	600	150	150	150	150		

[1]Within a row, means with different letters differ P<0.05
[2]Dry matter recovery

FIGURE 1. Laboratory mini-silos evaluated, from left to right: 1 L PVC, 1 L glass, 3 L PVC.

for the silages. An exception was the ADF content of the silage from the 3L-NON treatment, which did not differ ($P>0.05$) from that of the wilted forage. The highest ADF content was for the silage ensiled in the 1LGJ-NON (38.51% DM) which was significantly higher ($P<0.05$) compared with the wilted forage (35.15% DM) or the silage from the 3 L

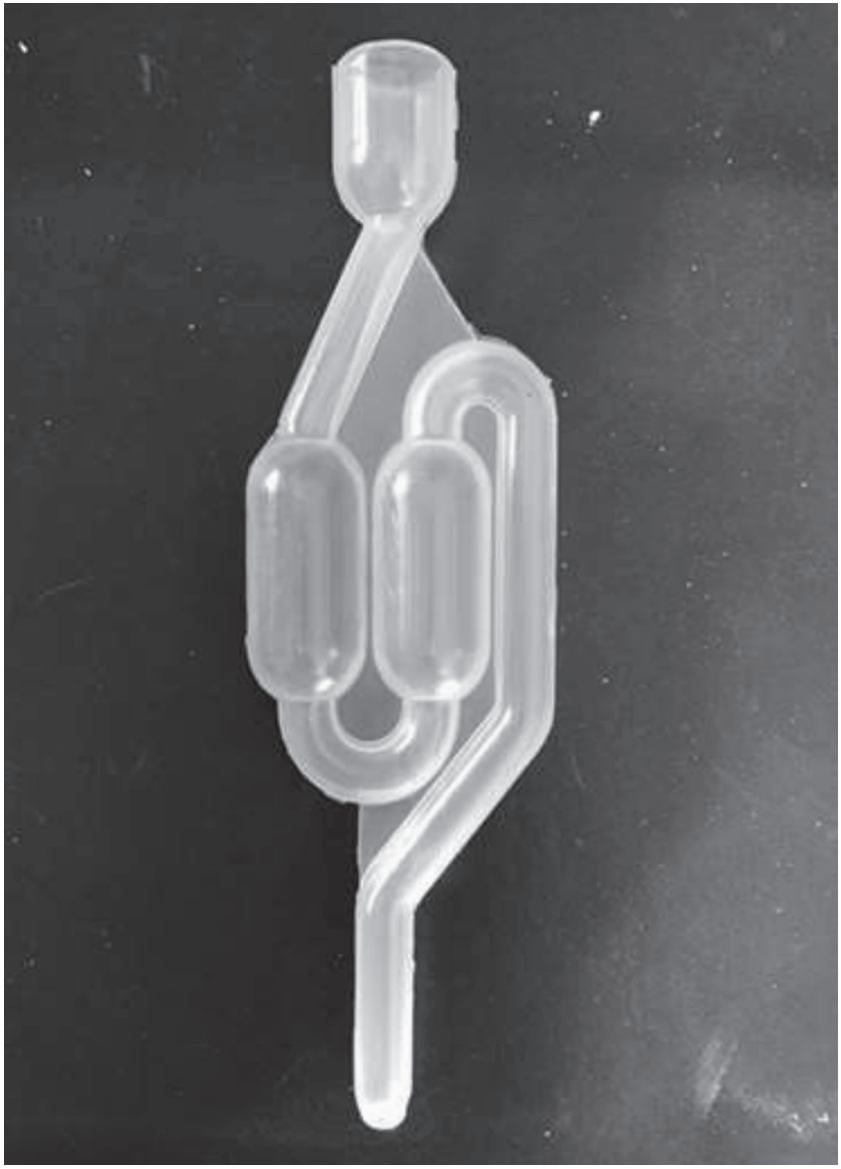

FIGURE 2. S-shaped airlock water valve used as a one-way mechanism to vent gas in the 1 L and 3 L PVC mini-silos.

PVC regardless of inoculation (36.33 and 36.71% DM) or the 1 L PVC with HBI (36.87% DM). These differences in ADF content suggest that fermentation needs to be studied in conjunction with the nutritional content of the resulting silages in order to better understand the effects of ensiling on the nutritional characteristics of the resulting silages.

TABLE 4.—*Average temperature across 30 time points of 91-d alfalfa silage resulting from using three types of mini-silos, without (NON) and with (HBI) homolactic bacteria inoculant, and then exposed aerobically during 174 h.*[1]

	3 L PVC		1 L PVC		1 L GJ			
	NON	HBI	NON	HBI	NON	HBI	SE	P<
Temperature, °C	18.4b	17.2bc	18.2a	18.4a	20.2b	20.2b	0.38	0.01

[1]Within a row, means with different letters differ P<0.05

Fermentation could have been influenced by HBI or the type of mini-silo, as there were no statistical differences between the non-inoculated 1 L mini-silos. Ensiling decreased (P<0.05) WSC from 13.18% to an average of 3.94% DM and tended to decrease (P<0.10) NFC from 33.28% to an average of 31.71% DM compared with the wilted forage. There were no other treatment differences in nutrient content due to inoculation. Fermentation profiles were affected by the addition of the microbial additive, and the effects appear to be related to the size of the mini-silo and not related to the type (PVC vs. glass). Inoculation decreased (P<0.05) silage pH in the 1 L mini-silos, whether the vessel was PVC or glass, but in the 3L mini-silos numerical differences were detected only (Table 2). Lactic acid was numerically higher (5.33 vs. 4.66% DM) for the inoculated silages but did not differ within the same type of mini-silo. Acetic acid (1.84 vs. 1.25% DM), pH (4.64 vs. 4.40) and ammonia (6.11 vs. 4.60% of CP) were lower (P<0.05) for the inoculated silages in the 1 L mini-silos. The ratio of lactic: acetic (2.44 vs. 4.62) and the proportion of lactic acid to total acids (70.84 vs. 82.20) were increased (P<0.05) by inoculation in the 1L mini-silos. In the 3L mini-silo, the contents of acetic acid and ammonia were numerically lower for the inoculated silage, while the contents of lactic acid, total acids, lactic as a proportion of total acids, the ratio of lactic: acetic and ethanol were numerically higher, but statistical differences were not detected. Independent of the size of the mini-silo, 1 L or 3 L, the fermentation characteristics of the resulting alfalfa silage were similar to those reported in the literature (Hassanat et al., 2013; Coblentz et al., 2016; Ke et al., 2017). Ward (2019) reported, based on approximately 1,500 legume silage samples analyzed at a commercial laboratory, that the optimal DM range for legume silage fermentation is 32% to 40%. Either below or above this DM range is not conducive to proper silage fermentation. Also, microbial inoculants may not perform as expected when DM content is outside this range. In the present study, the high DM content of the wilted alfalfa did not impair fermentation or the effects of microbial inoculation on fermentation. The DMR was not affected by treatment and averaged 96.2% across all treatments. Fermentation results from this study suggest that 1L mini-silos replicated four-times are suitable for the detection of treatment differences in alfalfa silage, which differs from the conclusion by Solórzano et al. (2016b) with triticale silage. For 3L mini-silos, however, greater replication may be needed to determine treatment differences. These could be explained by the packing densities achieved in the 1L (156 kg DM/m^3) vs. 3L (194 kg DM/m^3) mini-silos (Table 3). The bulkiness (NDF content) of each type of forage influences packing density. Solórzano et al. (2016b) achieved packing densities with triticale of approximately 34.7 kg DM/m^3 whereas in the present study we achieved densities >154 kg DM/m^3 using the same type of mini-silo. We agree with the conclusion reached by Solórzano et al. (2016a) that treatment differences in fermentation characteristics due to inoculation have a greater opportunity to be expressed in stressed silages, or in this case, lower density packed mini-silos compared with mini-silos packed with a high density that are more conductive to proper and efficient fermentation. By contrast, the mini-silo

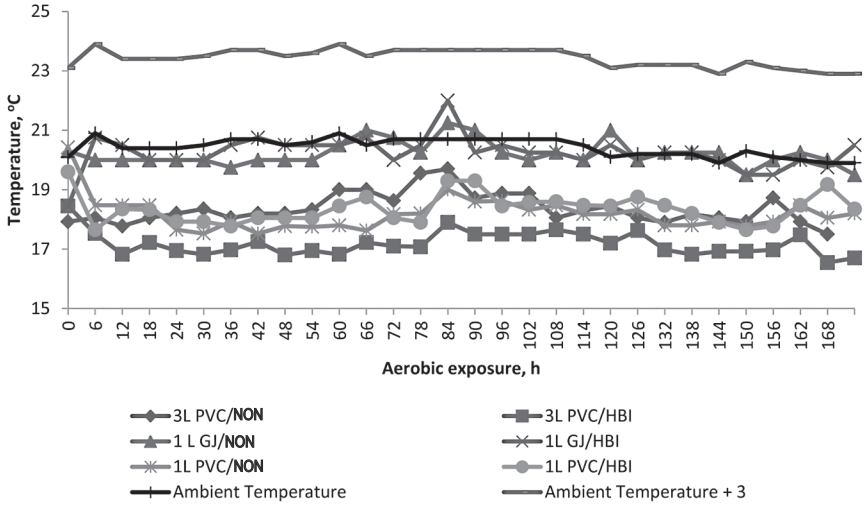

FIGURE 3. Temperatures of 91-d alfalfa silage resulting from using three types of mini-silos, without (NON) and with (HBI) homolactic bacteria inoculant, and exposed aerobically during 174 h upon opening of the mini-silo

temperatures at opening were lower ($P<0.05$) only for the inoculated 3L PVC mini-silos (Table 4). All silages were stable during 174 h of aerobic exposure (Figure 3) possibly due to strong homolactic fermentation, as evidenced by the resulting low pH, high content of lactic acid, low content of ammonia and despite having a high DM content.

Inoculation exerted a positive influence on the fermentation process of alfalfa, wilted to 50% DM, which was detected in the 1 L, but not in the 3L mini-silos. Treatments did not influence the aerobic stability of silage. Treatments in mini-silos as small as 1 L replicated four times are suitable for the study of the fermentation process of forages, such as alfalfa, whether they are hand packed or vacuum sealed, as they allow the detection of treatment differences in fermentation characteristics that are statistically significant.

LITERATURE CITED

Cherney, D.J.R., J.H. Cherney and W.J. Cox, 2004. Fermentation characteristics of corn forage ensiled in mini-silos. *J. Dairy Sci.* 87: 4238-4246.

Coblentz, W.K., K.P. Coffey and E.A. Chow, 2016. Storage characteristics, nutritive value, and fermentation characteristics of alfalfa packaged in large-round bales and wrapped in stretch film after extended time delays. *J. Dairy Sci.* 99: 3497-3511.

Hassanat, F., R. Gervais, C. Julien, D.I. Massé, A. Lettat, P.Y. Chouinard, H.V. Petit and C. Benchaar, 2013. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, N-balance, and milk production. *J. Dairy Sci*. 96: 4553-4567.

Heinrichs, J. and P. Kononoff, 1996. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. The Pennsylvania State University, College of Agriculure Science, Cooperative ExtensionTechnical Bulletin DAS 02-42. Honig, H., 1986. Evaluation of aerobic stability. Proc. of the Eurobac Conference, Uppsala, Sweden pp. 76-82.

Ke, W.C., W.R. Ding, D.M. Xu, L.M. Ding, P. Zhang, F.D. Li and X.S. Guo, 2017. Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. *J. Dairy Sci.* 100: 8958-8966

SAS Institute, 2004. SAS/STAT 9.1. User's Guide: SAS Institute, Inc. Cary, NC 27513-2414, USA.

Solórzano, L.C., L.L. Solórzano and A. Rodríguez-Carías, 2016a. Characterization of triticale silage inoculated with homolactic bacteria and exposed to aerobic stress during storage. *J. Agric. Univ. P.R.* 100 (2): 155-170. https://doi.org/10.46429/jaupr.v100i2.12728

Solórzano, L.C., L.L. Solórzano and A. Rodríguez-Carías, 2016b. Triticale silage treated or not with homolactic acid bacteria ensiled in laboratory mini-silos. *J. Agric. Univ. P.R.* 100 (2): 183-201. https://doi.org/10.46429/jaupr.v100i2.12730

Ward, R., 2019 (17-June). Forage dry matter drives fermentation. Retrieved from: https://hoards.com/article-25760-forage-dry-matter-drives-fermentation.html