INOCULANT EFFECTS ON BACTERIAL COMMUNITY, VOC PRODUCTION, AEROBIC STABILITY, AND FIBER DEGRADABILITY OF ALFALFA-GRASS SILAGE

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INTRODUCTION

- Alfalfa-grass silage can exhibit reduced aerobic stability when ensiled at high DM content.
- Inoculants combining facultative and obligate heterofermentative LAB offer a dual benefit by improving fermentation and enhancing aerobic stability.
- Inclusion of exogenous fibrolytic enzymes to bacterial inoculants can aid in the release of sugars that fuel fermentation, while improving fiber accessibility to microorganisms.
- Volatile organic compounds (VOCs) are valuable indicators of fermentation quality and spoilage processes, as their production is closely linked to microbial activity.

OBJECTIVES

To evaluate the effects of an inoculant on the fermentation, bacterial community, aerobic stability, NDF degradability, and VOC profile of alfalfa-grass silage, compared to untreated and chemically treated silages.

MATERIALS AND METHODS

Alfalfa-grass
35.9% DM

CTRL
water

INO
MAGNIVA Platinum 3: *L. buchneri* 40788 at 75,000 cfu/g of FM, *L. hilgardii* I-4785 at 75,000 cfu/g of FM, and *P. pentosaceus* 12455 at 100,000 cfu/g of FM, xylanase, and beta-glucanase

CHEM
sodium benzoate, sodium propionate, and sodium nitrite at 3 L/t of FM

Ensiled in 7-L silos at 190 kg/m^3 of DM for 0, 2, 5, 10, 20, 40, 80, or 160 d

- Parameters evaluated (5 replicates): pH, aerobic stability, LAB counts by PCR, plate yeast counts at 20 d, bacterial composition (16S rRNA), organic acids (HPLC), VOCs (GC/MS), and *in situ* NDF degradability (CTRL and INO at 0 and 160 d).
- Aerobic stability: time (h) until silage temperature rises 2°C above ambient.
- Data were analyzed by ensiling duration using Kruskal-Wallis and Dunn's post hoc.
- Microbial and VOC differential abundance was assessed using DESeq2 in R.

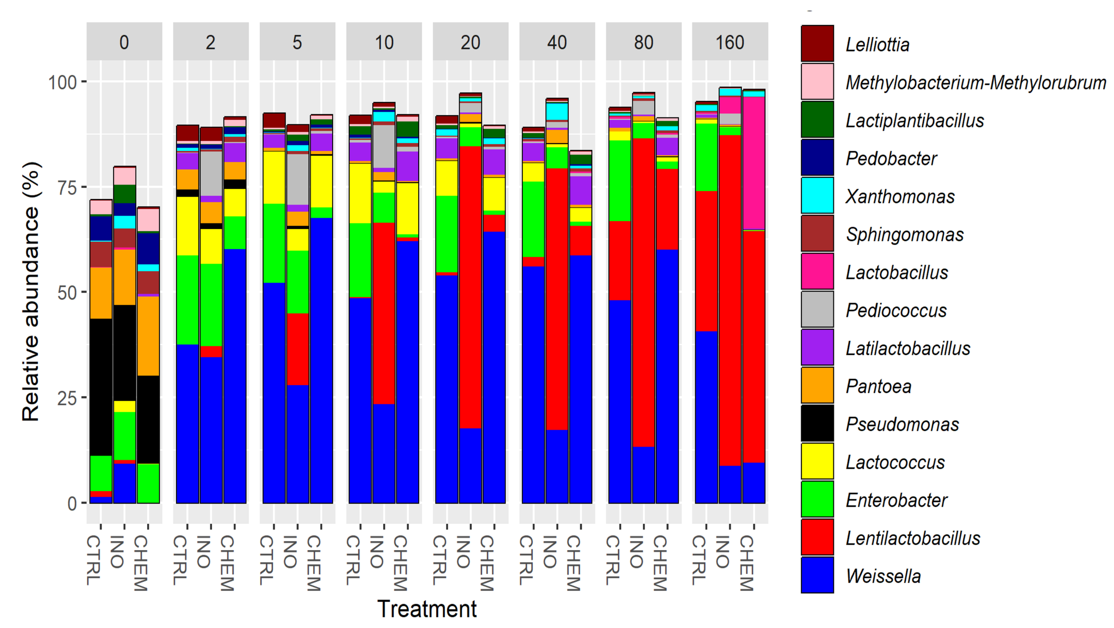

Figure 1. Relative abundance of the 15 most abundant bacterial genera after 0, 2, 5, 10, 20, 40, 80, and 160 days in untreated silage (CTRL) and silages treated with a bacterial inoculant (INO) or a chemical additive (CHEM).

Days of ensiling	Treatment	aNDF (g/kg of DM)	NDFD (% of NDF) 24 h	30 h	48 h	240 h
0	CTRL	421.8	44.2	56.6	65.9	76.7
	INO	432.5	47.3	59.1	66.2	74.5
SEM		4.5	1.5	1.2	0.9	1.03
P-value		0.347	0.754	0.347	0.917	0.25
160	CTRL	452.7	42.4	52.6	68.0	75.9
	INO	438.8	42.9	53.3	73.4	80.4
SEM		3.1	0.9	1.1	1.2	1.1
P-value		0.028	0.602	0.754	0.028	0.016

Table 1. The aNDF concentration and the *in situ* degradability of NDF (NDFD) in 24, 30, 48, and 240 h after 0 and 160 days of ensiling in untreated silage (CTRL) and silages treated with a bacterial inoculant (INO).

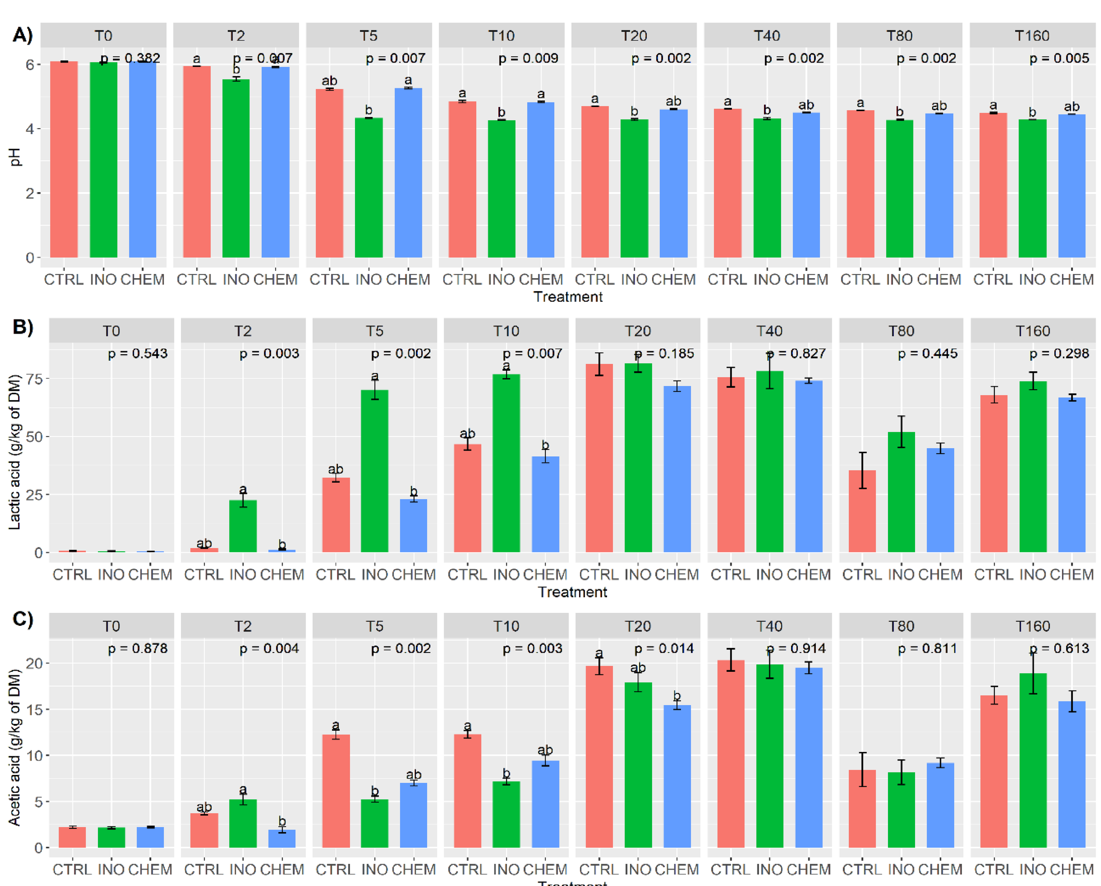

Figure 2. The A) pH levels and concentrations of B) lactic acid and C) acetic acid after 0, 2, 5, 10, 20, 40, 80, and 160 days of ensiling in untreated silage (CTRL) and silages treated with a bacterial inoculant (INO) or a chemical additive (CHEM).

Figure 3. The levels of A) propionic acid B) 1,2-propanediol C) ethanol after 0, 2, 5, 10, 20, 40, 80, and 160 days of ensiling in untreated silage (CTRL) and silages treated with a bacterial inoculant (INO) or a chemical additive (CHEM).

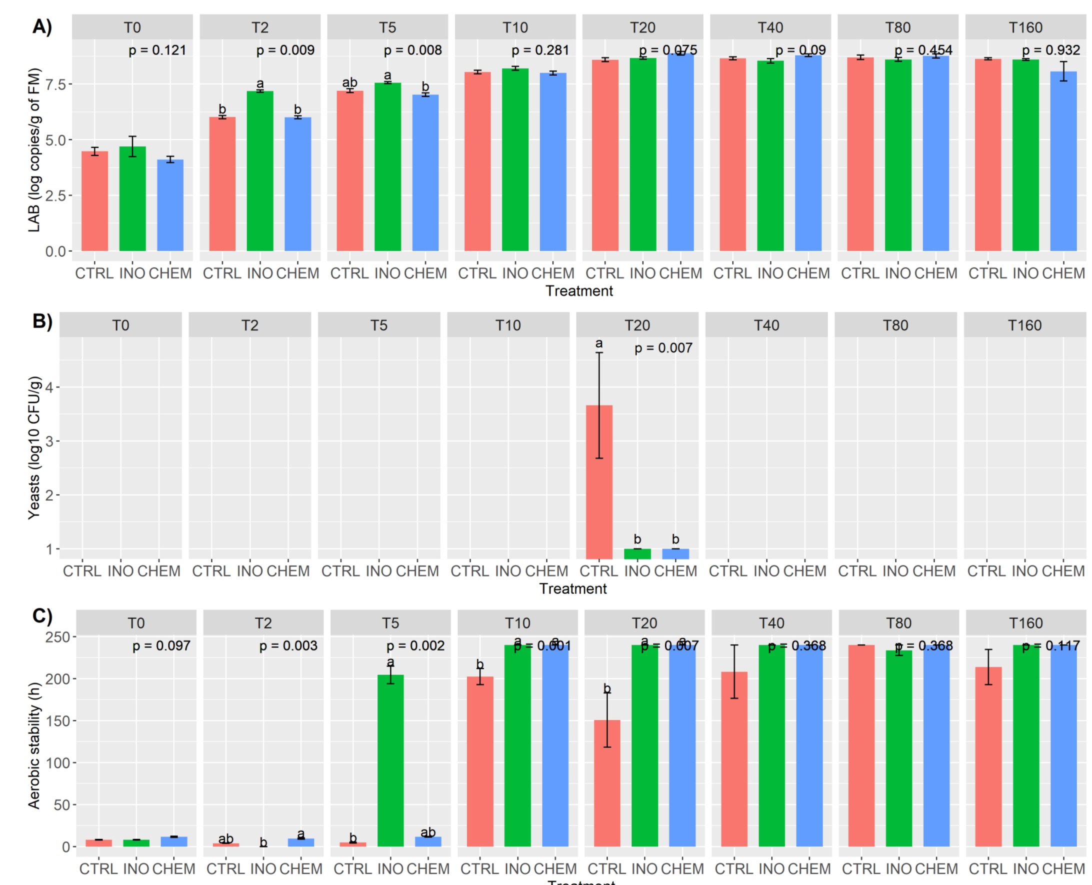

Figure 4. A) LAB counts, B) yeast counts, and C) aerobic stability after 0, 2, 5, 10, 20, 40, 80, and 160 days of ensiling in untreated silage (CTRL) and silages treated with a bacterial inoculant (INO) or a chemical additive (CHEM).

RESULTS

- Bacterial community shifts: INO had a greater relative abundance of *Lentilactobacillus* and *Pediococcus* than CTRL and CHEM, and a lower abundance of *Enterobacter* than CTRL but not CHEM at several timepoints (Figure 1).
- Enhanced fiber degradability: After 160 d, INO had higher NDF degradability than CTRL at both 48 h and 240 h (Table 1).
- Faster pH decline: INO enhanced lactic acid production during the first 10 d, leading to lower pH values than CTRL and CHEM during early fermentation (Figure 2).
- Enhanced heterofermentation: INO increased the production of 1,2-propanediol (Figure 3) and acetic acid measured by GC/MS during the later stages of ensiling (Figure 5).
- Suppressed yeast growth: yeasts were below the detection limit of 1.00 log cfu/g of FM in both INO and CHEM at 20 d (Figure 4)
- Improved aerobic stability after short-term ensiling: At 5 d, INO (204 h) was significantly more stable than CTRL (5 h) and numerically more stable than CHEM (12 h). At 10 and 20 d, both INO and CHEM were more stable than CTRL (Figure 4)
- Reduced undesirable VOCs: INO had lower levels of VOCs linked to undesirable fermentations, such as acetone, 2-butanone, and esters compared to CTRL (Figure 5).

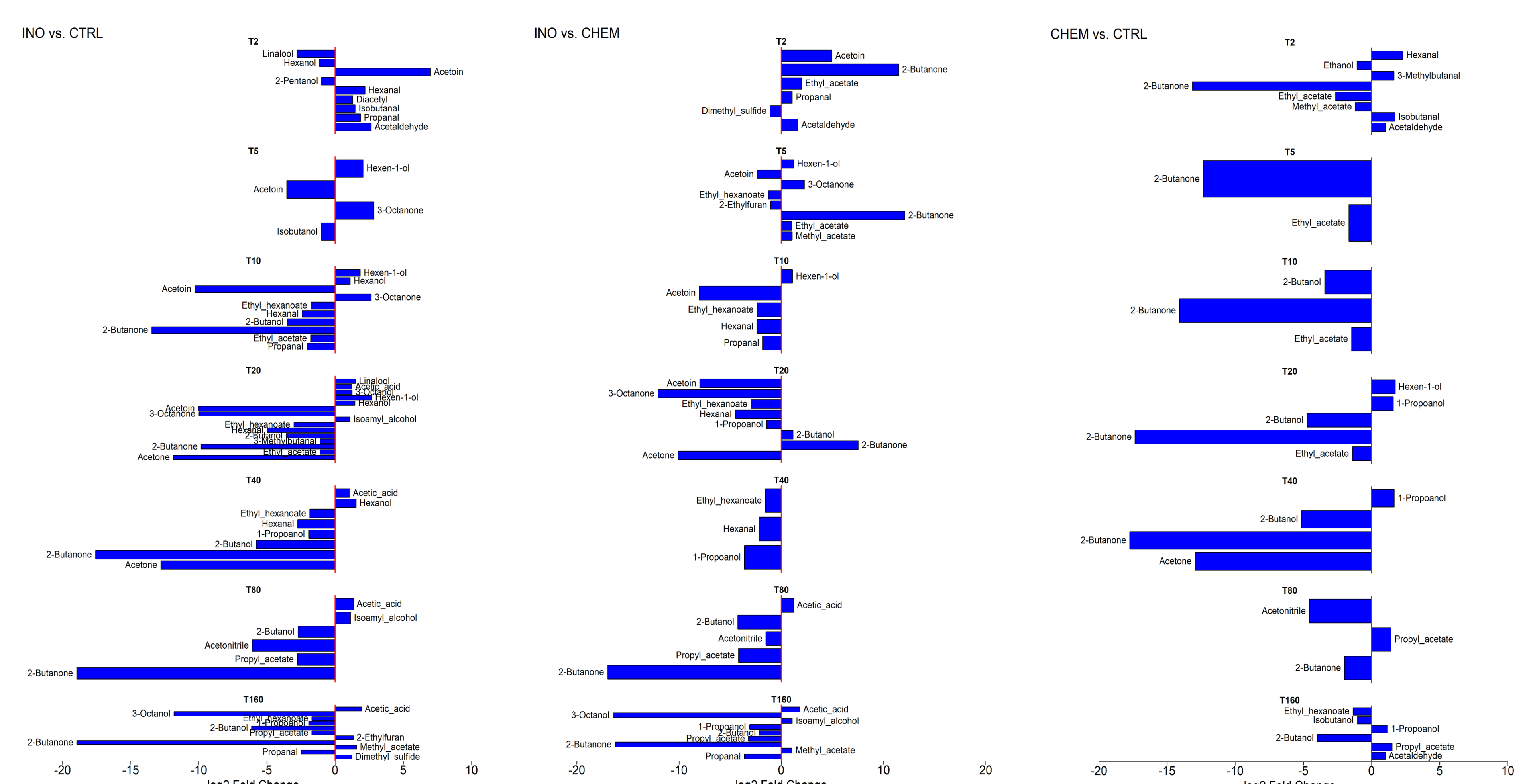

Figure 5. Differential levels of VOCs after 2, 5, 10, 20, 40, 80, and 160 days of ensiling in silage treated with a bacterial inoculant (INO) vs. untreated silage (CTRL), in INO vs. silage treated with a chemical additive (CHEM), and in CHEM vs. CTRL. Only VOCs with a log2 fold change > 1 or < -1 are displayed.

CONCLUSIONS

- The inoculant effectively dominated the bacterial community, accelerating the pH reduction.
- Inoculation suppressed yeasts and *Enterobacter*, enhancing aerobic stability and reducing the levels of VOCs associated with undesirable fermentations.
- Inoculation improved NDF degradability.