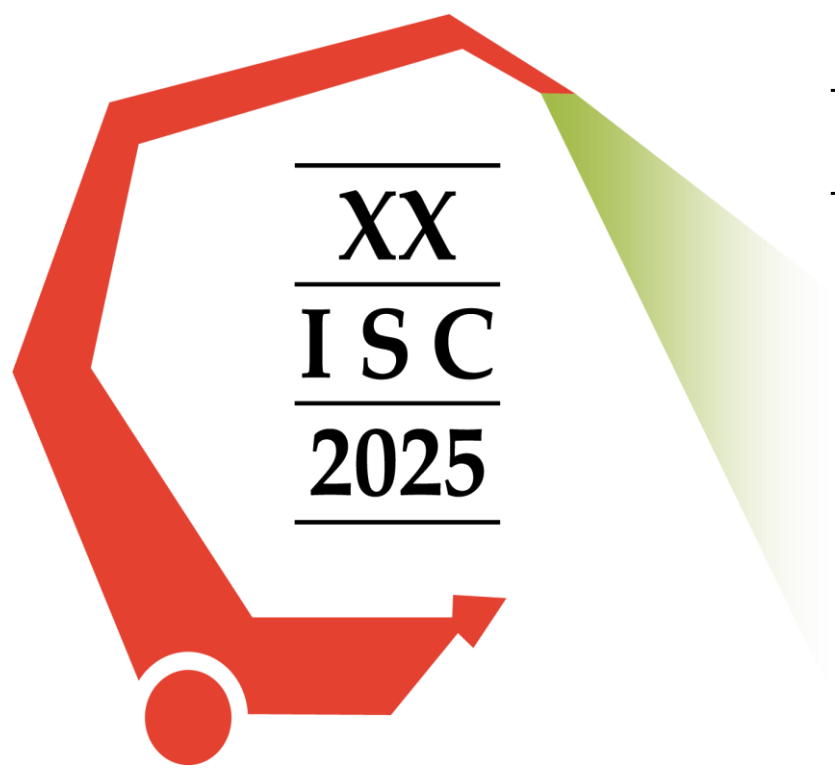




# BACTERIAL DYNAMICS IN MIXTURES BASED ON FORAGE CACTUS AND SORGHUM SILAGE, WITH OR WITHOUT FECAL CONTAMINATION DURING AEROBIC EXPOSURE

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## Introduction

### Forage Cactus Overview

- » *Nopalea cochenillifera Salm Dyck* is vital in arid regions.
- » High in non-fibrous carbohydrates (NFC); low in dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP).
- » Can cause digestive issues (e.g., soft stools) when fed in large proportions or as the sole feed.

### Microbial Contamination

- » Digestive disturbances may stem from pathogenic microorganisms introduced during feed preparation.
- » Post-harvest handling is a critical contamination point.

### Sorghum Silage as a Complement

- » *Sorghum bicolor L. Moench* silage helps suppress microbial growth.
- » Ensiling preserves nutrients and supports feed availability during pasture shortages.
- » Characterized by homolactic fermentation with stable pH (3.8–4.2).

Thus, we hypothesize that the pH of the ensiled mass inhibits the growth of potentially pathogenic microorganisms in diets containing high proportions of forage cactus, thereby improving its hygienic quality.

(Pereira et al., 2021).  
(Sato et al., 2016; Paulino et al., 2021).  
(Dos Santos et al., 2023; Lucena et al., 2023).

## Objectives

- » Accordingly, the present study aims to evaluate the growth of potentially pathogenic microorganisms in mixtures of forage sorghum silage and fresh forage cactus, with or without fecal contamination, depending on the period of aerobic exposure.

## Materials and Methods

### Experimental Setup

- » Location: Forage Laboratory, CCA/UFPB, Campus II – Areia, Paraíba, Brazil
- » Design: Randomized  $2 \times 2 \times 2$  factorial:
  - » Forage cactus proportions: 80% and 20% (fresh matter basis)
  - » With or without fecal contamination
  - » Aerobic exposure: 0 and 24 hours
- » Replications: 4 per treatment group

### Fermentation Profile – Sorghum Silage

- » pH: 3.9
- » Lactic acid: 72.80 g/kg DM
- » Acetic acid: 35.68 g/kg DM
- » Propionic acid: 14.10 g/kg DM
- » Butyric acid: 0.017 g/kg DM
- » Ammoniacal nitrogen: 16.29 g N-NH<sub>3</sub>/kg total nitrogen

### Genomic DNA Extraction & Processing

- » Sample prep: 25 g sample + 225 mL sterile saline (0.85%), homogenized, filtered, centrifuged
- » DNA extraction: Powersoil Pro DNA Kit
- » Quality check: Agarose gel electrophoresis (1%) and fluorometry (Qubit)
- » PCR amplification: V3–V4 regions of 16S rRNA gene
- » Library prep: Nextera XT Index Kit Set A + AMPureXP magnetic bead purification

### Sequencing & Bioinformatics

- » Platform: Illumina MiSeq, 500-cycle V2 kit
- » Processing: Qiime2
  - » Sequence import (forward & reverse)
  - » Quality filtering: Dada2 (Q > 20)

### Statistical Analysis

- » Test used: PERMANOVA
- » Diversity evaluated:
  - » Alpha diversity
  - » Beta diversity

## Results

### ASV Detection:

- » 461 ASVs in the 80% forage cactus samples
- » 1267 ASVs in the 20% forage cactus samples
- » Increased alpha diversity in contaminated 20% mixture at 0 hours

### Richness & Evenness:

- » Higher in 80% mixtures
- » Lower in 20% mixtures after 24h aerobic exposure
- » No significant impact from contamination

### Dominant Phyla:

- » *Bacillota*, *Pseudomonadota*, and *Bacteroidota* in 80% mixtures
- » *Bacillota* dominant at 0h in 20% samples; *Pseudomonadota* dominant at 24h

### Family-Level Composition:

- » 80% cactus at 0h: *Lactobacillaceae*, *Acetobacteraceae*
- » 80% cactus at 24h: Increase in *Clostridiaceae*
- » 20% contaminated at 0h: *Lactobacillaceae*, *Lachnospiraceae*
- » 20% at 24h: *Acetobacteraceae* dominance

### Main Genera Identified:

- » 80% cactus at 0h: *Weissella*, *Acetobacter*, *Gluconobacter\_A*, *Lactiplantibacillus*
- » 20% cactus at 24h: *Acetobacter*, *Gluconobacter*
- » Acetic acid bacteria linked to ethanol presence under aerobic conditions

## Conclusions

- » The mixture with 80% forage cactus showed lower microbial diversity compared to the mixture with 20% forage cactus. However, the higher proportion of forage cactus was associated with higher-risk microbial groups, such as *Clostridiaceae*. On the other hand, the mixture with 20% forage cactus exhibited a greater presence of acetic acid bacteria. Therefore, based on the hygienic quality of the mixtures, the use of the mixture with 20% forage cactus is recommended.

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