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EFFECT OF A SILAGE INOCULANT CONTAINING *L. DIOLIVORANS* ON CO_2 EMISSIONS OF CORN SILAGE DURING ANAEROBIC AND AEROBIC STORAGE

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Background & Objective

- Silage contributes to climate-relevant emissions (CO_2, nitrous gases, volatile organic compounds (VOCs)) and dry matter losses during both anaerobic and aerobic storage phases, impacting environmental and economic sustainability
- Silage inoculants based on heterofermentative lactic acid bacteria (LAB) can reduce greenhouse gases (GHG) and VOC emissions, especially under aerobic conditions during reheating, enhancing aerobic stability and livestock productivity

Aim: to investigate the effect of a LAB inoculant on fermentation quality, aerobic stability and GHG emissions in corn silage

Conclusion

The study demonstrates the effect of an LAB mixture containing L. diolivorans on aerobic stability and CO_2 emission losses during the reheating process after a short storage period. Thus, appropriate LAB mixtures have the potential to be part of emission reduction strategies in livestock production.

Results

- For the mini silos (MS), lactic acid and acetic acid were increased, and pH was significantly reduced in INO (LAB-treated silage) compared to CON (untreated silage) at both opening days (OD); Aerobic stability was improved at OD14 (CON: 1.3 d; INO: 8.0 d), while both CON and INO remained stable at OD90 (Table 1)
- CO_2 emissions during the anaerobic storage (OD14 and 90) were 2.9g and 4.5g for CON and 3.5g and 6.0g for INO (+20 % vs. CON) in the MS

Table 1: Silage parameters of the corn silages after opening day 14 (OD14) and 90 (OD 90) for control (a) and treated LAB mixture (b) ensiled in mini silos (MS)

Parameter	14OD		90OD	
	a	b	a	b
DM_c (%)	44.4	43.6	43.3	42.5
LA (% DM)	1.74^a	2.58^b	2.13^a	2.45^b
AA (% DM)	0.59^a	1.67^b	0.97^a	3.13^b
pH	4.38^a	3.97^b	4.18^a	3.97^b
WL (% DM)	1.77	2.33	2.83	3.67
AS (days)	1.30^a	8.00^b	>14	>14

(*DM_c = DM corrected, LA=Lactic acid; AA= Acetic acid; AS= Aerobic stability (max. length: 14 days); WL= Weight losses; ^{a,b} symbolize significant differences (*P<0.05)*)

- For the mini silos with integrated injection lance (MSIL), CON started to heat up after about one day, the INO remained stable during the 14 d of aerobic exposure (Figure 1, left); CO_2 emissions demonstrate similar pattern; CO_2 concentration of CON (146,000ppm) were significantly higher than to INO (7,600ppm), (Figure 1, right). At OD90, aerobic stability and CO_2 emissions were very low (CON: 7,500 and INO: 7,200ppm) because there was no reheating in both treatments
- Calculating the mass of emitted CO_2 based on the sum of air exhausted from the MSIL and the mean CO_2 concentration during 14 days of reheating, 192g CO_2 was emitted from 0.75kg FM corn silage ensiled in each MSIL. In contrast, only 9.7g CO_2 was emitted from one MSIL of the LAB treatment

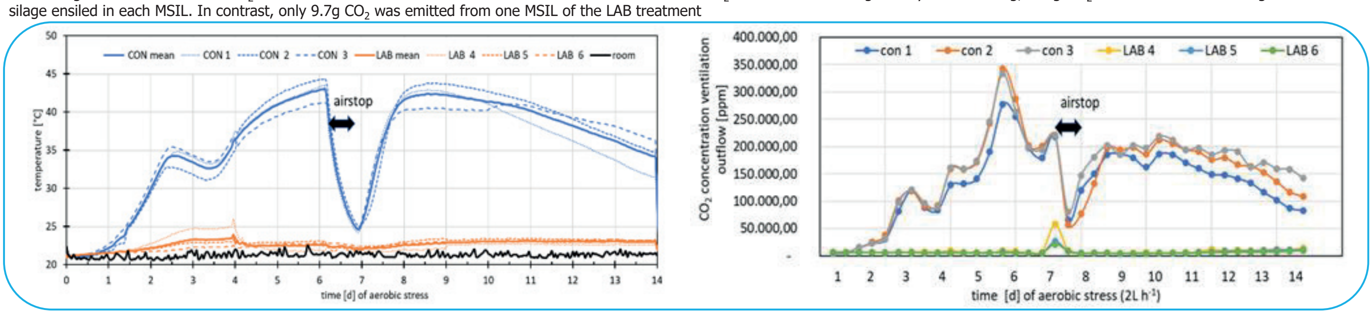

Figure 1: Silage temperature (left) and emitted CO_2 concentrations in the exhaust air (right) during aerobic stability test at opening day (OD) 14 for control (CON 1-3) and treated samples (LAB4-6) ensiled in the mini silos with injection (MSIL). Note: The air injection failed at day 7 for 14h, so the temperature and CO_2 concentrations dropped immediately

Methods

- Corn silage (41% DM) was ensiled in 1.5 L mini silos (MS) and in 1.5 L mini silos with integrated injection lance (MSIL) at 20°C for 14 and 90 days at 20°C in three replicates
- Treatments consisted of an untreated control (CON) and treated (INO) with a mixture of homo- and heterofermentative LAB (Bonsilage Speed M: *L. diolivorans, L. buchneri and L. rhamnosus*; application rate of 250.000 CFU/ g FM)
- Aerobic stress was simulated after 7 days of storage for 24h; and for MS aerobic stability was assessed using Honig's (1990) method; and for MSIL, aerobic stability test was simulated by injection of 2 L air per hour (Figure 2 a, b) and air exhaustion twice daily, which leads to very intensive air stress
- CO_2 emissions were monitored for MSIL during anaerobic storage and aerobic stability test
- Statistical evaluation: the data were examined by SAS, including Kruskal-Wallis test for significant differences (P<0.05) between CON and INO

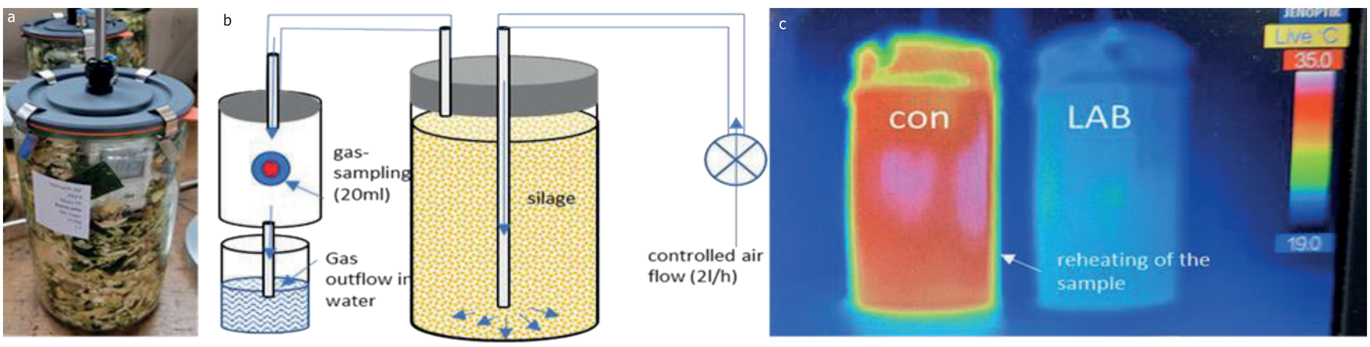

Figure 2: Experimental setup for aerobic stability and gas sampling using controlled airflow through the silage (a, b); thermogram of two mini silos (control vs. treatment) after 7 days of aerobic test (c)

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Influence of *L. diolivorans* inoculant on the carbon footprint of corn silage in a biogas plant

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Introduction

- Silage inoculant could improve fermentation and reduce spoilage.
- Research on the carbon footprint (CF) effects of additives is still limited.
- This study investigates the impact of a inoculant based on homo- and heterofermentative lactic acid bacteria (LAB) containing *L. diolivorans, L. buchneri*, and *L. rhamnosus* on the CF of corn silage in a biogas system.

Methodology

Material: Corn (36.2%DM) inoculated with an application rate of 250.000 CFU/gFM on a dairy farm in north-western Germany.

Stored into two silos:

- control ("untreated silage")
- test ("treated silage")

Characterization: Lactic and acetic acid concentration, number of yeasts and moulds, aerobic stability (ASTA), and biogas production

LCA

Software: Open LCA

Databases: ecoinvent, and bioenergiedat

Emissions: IPCC (2019)

Impact assessment method: IPCC 2021.

Impact category: Global Warming Potential (GWP 100 non-fossil CO2 uptake and emission).

Ensiling: From corn cultivation to delivery at the biogas plant.

Biogas production: includes corn cultivation, ensiling, and generation of heat and electricity from the biogas plant (90% corn silage and 10% manure).

Results

The test silo showed a 62% reduction in weight loss, an extended ASTA and a reduced number of yeasts and moulds compared to the control

Table 1: Characterization of the test and control silos.

Parameter	Unit	Control	Test
Weight losses	% FM	19.75	7.59
DM	g / kg	331.77 ± 26.9 ^{A}	348.01 ± 22.09 ^{A}
Lactic acid	% DM	5.79 ± 1.97 ^{A}	4.31 ± 1.01 ^{B}
Acetic acid	% DM	1.40 ± 0.33 ^{A}	3.58 ± 0.48 ^{B}
Yeasts	CFU/ g FM	27200 ± 18074 ^{A}	180 ± 130 ^{B}
Moulds	CFU/ g FM	192060 ± 305436 ^{A}	100 ± 0 ^{A}
ASTA	h	72.40 ± 16.99 ^{A}	223.20 ± 16.35 ^{B}
Biogas	Nl/kgTS	664.91 ± 27.97	680.21 ± 30.38

The emissions from the test during the ensiling process and biogas production are approximately 14.5% and 18.2% lower compared to the control, primarily due to reduced emissions associated with weight losses.

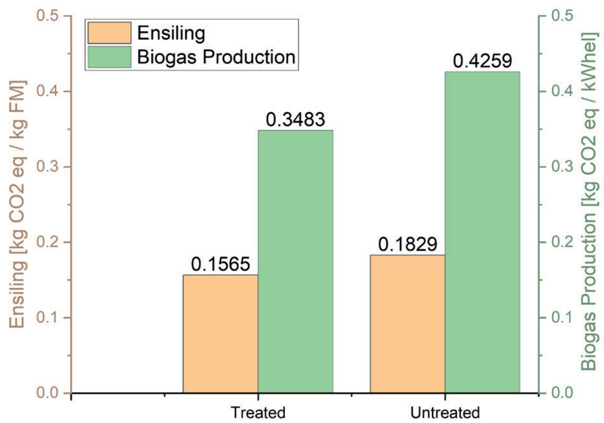

Figure 1: Greenhouse gas emissions for ensiling of corn silage and biogas production fed with corn silage (treated vs. untreated).

Conclusion

The LAB mixture improved silage quality, reduced weight loss, and increased methane yields, helping to lower the carbon footprint of biogas production from corn silage through better preservation and process efficiency.

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EFFECT OF DIFFERENT SILAGE INOCULANTS ON DIGESTIBILITY PARAMETERS AND METABOLIZABLE ENERGY *IN VIVO* – A META-ANALYSIS

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BACKGROUND & OBJECTIVE

- Livestock production significantly contributes to global GHG emissions, with cattle as the main source via enteric fermentation
- A promising strategy for mitigation emissions is to improve the forage quality and digestibility of silages by using lactic acid bacteria (LAB) based inoculants

Aim: to assess the effect of different LAB-inoculants on digestibility and the derived energy in a meta- analysis compiled from *in vivo* digestibility studies in sheep

RESULTS

- All three LAB inoculants showed significant higher estimated marginal means of the ME (INO1: 10.59 vs. 10.37; INO2: 10.20 vs. 9.77; INO3: 11.57 vs. 11.18), compared to untreated controls, see Figure 1
- Results align with previous studies showing improved fermentation, digestibility, and ME intake from LAB-treated silages
- The positive effects of inoculants on silage digestibility are reinforced by the revised GfE system (2023), which reflects the new ME prediction of highly digestible silages more realistically; these results support their potential role in strategies to reduce emissions through improved feed conversion

MATERIAL & METHODS

- This meta-analysis includes 14 *in vivo* digestibility studies performed between 1996-2002 in Germany using three LAB inoculant mixtures (INO) vs. untreated control (CON)
    - INO1: Mixture of homofermentative LAB (*L. rhamnosus* DSM 7133 and *E. lactis* DSM 7134, application rate: 100,000 CFU/g FM)
    - INO2: Mixture of homo- and heterofermentative LAB (*L. rhamnosus* DSM 7133, *L. plantarum* DSM 12836, *P. pentosaceus* DSM 12834, *L. buchneri* DSM 12856 and *L. brevis* DSM 12835, application rate: 100,000 CFU/g FM)
    - INO3: Mixture of homo- and heterofermentative LAB (*L. buchneri* DSM 12856, *L. plantarum* DSM 12837 and *P. pentosaceus* DSM 12834, application rate: 250,000 CFU/g FM)
- Silages differ in type (alfalfa, grass, maize), number of cut, DM and maturity (defined as crude fiber (CF)), see Table 1 and were ensiled for 90 days; digestibility and metabolized energy (ME) were determined via standardized guidelines (GfE 1991/2001)
- Statistical analysis: linear mixed models including the random effect 'study' and the fixed effect 'silage type' were created to evaluate the effect of the silage inoculant (INO1/INO2/INO3) on the ME in contrast to the CON; the significance level was 5 %

Table 1. Overview of the used silages regarding dry matter (DM), crude fiber (CF) range and silage inoculant (INO1/INO2/INO3)

	n studies	DM range (%)	CF range (% DM)	Silage inoculant
Grass 1st cut	6	22-50	21.7-27.4	INO1; INO2
Grass 2nd cut	1	51	27.7	INO2
Alfalfa 1st cut	2	35-56	26.6-27.2	INO1
Alfalfa 2nd cut	1	40	28.0	INO2
Maize	4	25-40	17.7-25.6	INO1; INO2; INO3

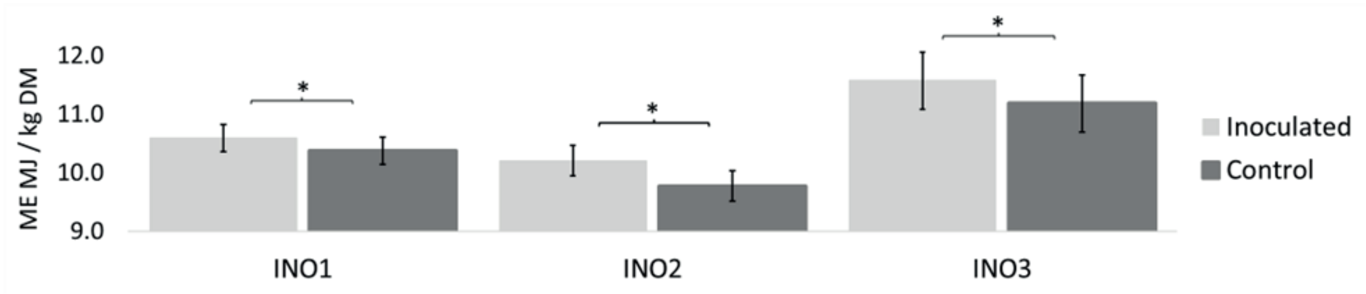

Figure 1. Estimated marginal means and standard error of the metabolizable energy (ME) of the three silage inoculants (INO1, used in alfalfa (A), grass (G), maize (M); INO2, used in A,G,M; INO3, used in M) and their controls (P<0.05).

CONCLUSION

This meta-analysis of 14 studies confirms that all three tested silage LAB-inoculants improved digestibility and metabolizable energy across forage types, supporting more efficient and sustainable milk production.

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