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Substrate-dependent detoxification of DON, ZEN, and AFB 1 by a commercial silage inoculant: From mycotoxin reduction to feed safety compliance

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Abstract

This study systematically evaluated the effects of a commercial microbial inoculant on nutritional quality, fermentation characteristics, and the detoxification of three major mycotoxins—deoxynivalenol (DON), zearalenone (ZEN), and aflatoxin B_1(AFB_1)—in the silage of four crop by-products: barley straw, corn straw, rice straw, and pressed sugarcane bagasse. The inoculant, containing Pediococcus pentosaceus, Lactobacillus plantarum, and cellulase, was applied to each substrate with an uninoculated control (CK, molasses only), ensiled for 60 days, and then analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, fermentation profile (pH, organic acids, ammonia nitrogen), and the three mycotoxins. Detoxification rates were calculated and safety was assessed against GB 13078 – 2017. The results demonstrated significant substrate- and toxin-dependent detoxification effects. Rice straw exhibited the best overall performance, with DON, ZEN, and AFB_1 reduction rates of 66.67%, 99.02%, and 60.00%, respectively, with ZEN content decreasing from 600 ± 12.1 ppb to 5.9 ± 0.65 ppb (a 99% reduction). In barley straw, ZEN was reduced by 95.36% (from 172.5 ± 5.69 to 8 ± 1.27 ppb), while DON decreased from 7644.2 ± 42.86 to 4500 ± 18.69 ppb (a 41.13% reduction). Corn straw showed DON and ZEN reduction rates of 54.23% and 59.20%, respectively, but no reduction for AFB_1 (0%). Sugarcane bagasse exhibited DON and AFB_1 reduction rates of 45.43% and 29.72%, respectively, but only 11.51% for ZEN. The CK groups generally showed lower detoxification rates, and inoculation significantly reduced pH and increased lactic acid in barley straw and rice straw, while the differences were smaller in corn straw and sugarcane bagasse. Notably, despite the wide variation in detoxification rates (0–99.02%), all inoculated samples met the GB 13078 – 2017 safety limits, whereas CK of barley straw remained non-compliant for DON. In terms of nutritional quality, barley straw showed the greatest CP increase (+ 62.4%), followed by rice straw (+ 26.0%), while sugarcane bagasse had extremely low absolute CP content (2.39% after fermentation), and corn straw showed an abnormal decrease in CP (− 9.8%) and increase in NDF (+ 4.5%). These findings confirm that the inoculant has broad-spectrum detoxification potential, but its efficacy is strongly dependent on substrate and toxin type. Rice straw emerged as the optimal match for this inoculant, achieving simultaneous nutritional improvement and mycotoxin reduction. Although corn straw showed no AFB_1 reduction, the final product remained safe due to its extremely low initial AFB_1 concentration (1.00 ppb). This study provides a scientific basis for the precise selection of silage additives for different crop by-products.

1. Introduction

With the rapid intensification of livestock production in China, the demand for high-quality roughage continues to grow. The conversion of crop straws and processing by-products (e.g., barley straw, corn straw, rice straw, and sugarcane bagasse) into feed resources through ensiling represents a critical strategy to alleviate feed supply shortages[1–2]. However, these raw materials are susceptible to contamination by toxigenic fungi, such as *Fusarium* and *Aspergillus* species, during field growth, harvesting, and storage, leading to the accumulation of mycotoxins including deoxynivalenol (DON), zearalenone (ZEN), and aflatoxin B_1(AFB_1), which pose serious threats to feed safety and animal health[3–4]. For instance, Eckard et al. reported that silage maize is commonly infected by *Fusarium* species, leading to

DON and ZEN accumulation[5], while Dogi et al. demonstrated that Aspergillus parasiticus and Fusarium graminearum can produce AFB_1 and DON during silage fermentation[6].

Microbial inoculants are widely used in silage production to improve feed quality. Muck et al. and Kung et al. reviewed the efficacy of silage additives and concluded that LAB inoculants can effectively lower pH, inhibit spoilage microorganisms, and improve aerobic stability[7–8]. Lactic acid bacteria (LAB), such as Lactobacillus plantarum and Pediococcus pentosaceus, not only rapidly lower pH through acid production and inhibit spoilage microorganisms, but also reduce mycotoxin content via physical adsorption to cell wall peptidoglycan or enzymatic degradation [9–10].

However, most existing studies have focused on a single substrate and a single mycotoxin, with limited systematic comparisons of the differential detoxification effects of the same inoculant across multiple substrates and multiple mycotoxins.

The physicochemical properties of different crop by-products vary considerably—corn straw has relatively high sugar content, rice straw is rich in surface silicates, and sugarcane bagasse contains almost no fermentable sugars—and these differences may substantially affect the colonization, metabolic activity, and detoxification efficiency of the inoculant. Therefore, whether a single inoculant exhibits consistent detoxification performance across different substrates is a critical scientific question that underpins the precise application of silage additives in practice.

In this study, we selected four typical crop by-products (barley straw, corn straw, rice straw, and pressed sugarcane bagasse) and applied a commercial inoculant with an uninoculated control to systematically evaluate its detoxification efficacy against DON, ZEN and AFB_1. We further analyzed the associations between detoxification efficiency and changes in nutritional quality and fermentation characteristics, aiming to provide a theoretical basis for the precise selection of silage additives for different raw materials.

2. Materials and Methods

2.1Experimental materials

2.1.1 Raw materials for fermentation

Four types of raw materials were used in this study: (1) Barley straw: Variety "Zhepi 9," whole-plant harvested at the late dough stage, obtained from Maolikan Ranch, Dong'an Village, Yayang Town, Taishun County, Wenzhou City, Zhejiang Province, China. (2) Corn straw: Variety "Zhetiannuo 918" fresh-eating corn, whole-plant straw (including stems, leaves, and cobs) collected immediately after ear harvest at 45 days post-silking (late milk to early dough stage), sourced from the same farmer's planting base in Dong'an Village, Yayang Town, Taishun County, Wenzhou City, Zhejiang Province, China. (3) Rice straw: Variety "Yongyou 1540" conventional late-season indica rice, above-ground whole-plant straw (including stems, leaves, and a small amount of residual panicles) collected at full maturity with a stubble height of 15–20 cm, harvested from paddy fields in Dong'an Village, Yayang Town, Taishun County, Wenzhou City, Zhejiang

Province, China. (4) Pressed sugarcane bagasse: Variety "Guisi 44," residual residue after sucrose extraction (moisture content approximately 45%–50%, high fiber content, extremely low residual soluble sugars), obtained from a small-scale sugar processing workshop in Dong'an Village, Yayang Town, Taishun County, Wenzhou City, Zhejiang Province, China, and transported to the laboratory on the day of collection for ensiling.

2.1.2 Silage inoculant

A commercial silage inoculant (Shandong Silage Booster, China) was used in this experiment. The inoculant contained *Pediococcus pentosaceus* (6×10^9 CFU/g), *Lactobacillus plantarum* (4×10^9 CFU/g), and cellulase (10,000 U/g). For activation, the microbial-enzyme preparation, molasses, and water were thoroughly mixed at a ratio of 1:3:200. The inoculant mixture was then sealed and activated at 30 ± 5℃ for 6–8 h before use, following the manufacturer's instructions.

2.2. Silage production

Each of the four raw materials was chopped into pieces 2–3 cm in length, and the moisture content was adjusted to approximately 65%[11]. For each ton of raw material, 20 kg of molasses and 50 g of Inoculant were added and thoroughly mixed. The treated forage was then packed into oxygen-barrier polyethylene bags (50 cm × 80 cm, 100 kg capacity per bag), manually compacted to expel as much air as possible, and vacuum-sealed. The sealed bags were stored at ambient temperature (approximately 20–25°C) for a 60-day ensiling period. Five replicate bags were prepared per treatment group (n = 5).

2.3. Measurement indicators and methods

2.3.1. Sampling and sample preparation

Random samples weighing 1 kg were collected from the raw materials prior to ensiling and labeled as "pre-ensiling" samples. After 60 days of fermentation, 1-kg samples were again randomly collected from each treatment group. For each silage bag, a representative 1-kg sample was obtained using the quartering method: the entire bag content was spread out on a clean plastic sheet, thoroughly mixed, and divided into four quarters, after which two opposite quarters were combined to form the final sample. All samples were stored at − 20°C prior to analysis.

2.3.2. Nutritional composition analysis

Crude protein (CP) was determined according to GB/T 6432 − 2018; neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed following GB/T 20806 − 2022 and NY/T 1459–2022, respectively; and crude ash (Ash) was measured according to GB/T 6438 − 2007. All nutritional analyses were performed following standard AOAC protocols [12].

2.3.3 Fermentation characteristics

After 60 days of ensiling, silage samples were collected and extracted with deionized water at a ratio of 1:10 (w/v) for 30 min at 4°C. The pH was measured immediately using a calibrated pH meter (Mettler Toledo, Switzerland). Organic acids (lactic, acetic, propionic, and butyric acids) were determined by high-

performance liquid chromatography (HPLC) according to the method of [13]. Ammonia nitrogen (NH_3-N) was measured using the phenol-hypochlorite method and expressed as a percentage of total nitrogen (%TN).

2.3.4. Mycotoxin analysis

The concentrations of DON, ZEN, and AFB_1 were determined simultaneously using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). Samples were extracted with acetonitrile–water–formic acid solution and purified using solid-phase extraction cartridges before injection. The HPLC-MS/MS analysis was performed using an Agilent 1290 Infinity II coupled to a 6460 Triple Quad LC/MS equipped with a ZORBAX Eclipse Plus C18 column (2.1 × 50 mm, 1.8 μm). The mobile phase consisted of water/methanol (90:10, v/v) with 0.1% formic acid, flowing at a rate of 0.3 mL/min. The limits of detection (LOD) were 2.5 μg/kg for DON, 1.0 μg/kg for ZEN, and 0.1 μg/kg for AFB_1.

2.4 Statistical analysis

- The detoxification rate was calculated using the following formula:
- Detoxification rate (%)=$(C_{before}-C_{after})/C_{before}\times 100\%$. where C_{before} and C_{after} represent the mycotoxin concentrations before and after fermentation, respectively.
- All experimental data were presented as mean ± standard deviation (SD) from five independent biological replicates (n = 5 per treatment group). Statistical analyses were performed using SPSS software (version 26.0, IBM Corp., USA). Safety compliance was evaluated against the Chinese Feed Hygiene Standard GB 13078 – 2017 [14], which specifies maximum limits for DON (≤ 5000 μg/kg), ZEN (≤ 500 ppb), and AFB_1 (≤ 30 μg/kg). One-way analysis of variance (ANOVA) was conducted to determine the overall effects of the different treatments on all measured parameters. When a significant F-value ($p < 0.05$) was obtained, Duncan's multiple range test was applied to compare the differences among group means. Statistical significance was declared at $p < 0.05$.

3. Results

3.1. Initial nutritional and mycotoxin profiles of the four raw materials

- The nutritional composition and mycotoxin background levels of the four raw materials before ensiling are presented in Table 1. The four substrates differed markedly in both nutritional content and mycotoxin contamination. Barley straw showed the highest DON contamination at 7644.2 ± 42.86 ppb, which exceeded the GB 13078 – 2017 limit for DON (≤ 5000 ppb)[14]. Rice straw had a ZEN level of 600 ± 12.1 ppb, approaching the regulatory threshold. Corn straw and sugarcane bagasse contained all three mycotoxins within the safe limits (DON ≤ 5000 ppb, ZEN ≤ 500 ppb, AFB_1 ≤ 30 ppb)[14]. In terms of nutritional composition, corn straw and rice straw had relatively high CP contents (10.87% ± 1.63% and 10.48% ± 0.96%, respectively), while sugarcane bagasse had the lowest CP content (1.97% ± 0.47%).

Barley straw had the highest NDF (65.0% ± 1.65%) and ADF (31.8% ± 0.36%) contents among the four substrates.

Table 1
Initial nutritional composition and mycotoxin background levels of the four raw materials.

Substrate	CP (%)	NDF (%)	ADF (%)	Ash (%)	DON (ppb)	ZEN (ppb)	AFB_1 (ppb)
Barley straw	6.04 ± 0.56	65 ± 1.65	31.8 ± 0.36	12.2 ± 0.26	7644.2 ± 42.86	172.5 ± 5.69	3.02 ± 0.66
Corn stover	10.87 ± 1.63	53 ± 1.13	30.5 ± 0.61	6.5 ± 0.28	1173.82 ± 14.15	61.28 ± 6.13	1.00 ± 0.12
Rice straw	10.48 ± 0.96	50.1 ± 0.96	37.8 ± 0.56	7.8 ± 0.22	150 ± 2.22	600 ± 12.1	0.5 ± 0.08
Bagasse	1.97 ± 0.47	53 ± 0.44	30.5 ± 0.27	1.3 ± 0.13	1081.4 ± 13.55	110.63 ± 1.44	3.23 ± 0.07

Values are presented as mean ± SD (n = 5).

3.2 Fermentation characteristics of the four substrates

- The pH and organic acid profiles after 60 days of ensiling are shown in Table 2. In the inoculated groups, pH ranged from 3.71 to 4.25, with sugarcane bagasse showing the lowest pH (3.71 ± 0.07) and barley straw the highest (4.25 ± 0.05). Compared with the CK groups, inoculation significantly reduced pH in barley straw (4.78 → 4.25), rice straw (4.40 → 4.10), and sugarcane bagasse (3.97 → 3.71), but the difference was small in corn straw (3.99 → 3.88). Notably, the CK group of corn straw had a lower pH (3.99) than the inoculated barley straw (4.25), indicating a stronger natural acidification capacity of corn straw.
- Lactic acid was the dominant organic acid in all inoculated silages, ranging from 4.4% to 8.8% DM. Corn straw had the highest lactic acid content (8.8% ± 0.56% DM), followed by sugarcane bagasse (8.2% ± 0.34% DM), rice straw (4.9% ± 0.19% DM), and barley straw (4.4% ± 0.31% DM). Acetic acid ranged from 0.11% to 0.86% DM. Butyric acid was detected in barley straw (CK 1.8% DM, I 1.2% DM), rice straw (CK 0.69% DM, I 0.5% DM), and sugarcane bagasse (CK 1.63% DM, I 0.64% DM), but not in corn straw (0% in both groups).
- NH_3-N content was slightly lower in inoculated groups than in CK groups for all substrates, with the greatest reduction observed in sugarcane bagasse (4.5% → 3.9% TN) and rice straw (4.5% → 3.8% TN). The highest NH_3-N was observed in CK of barley straw (5.2% ± 0.31% TN), while the lowest was in inoculated corn straw (2.9% ± 0.11% TN).

Table 2
Fermentation characteristics after 60 days of ensiling.

Substrate	Treatment	pH	Lactic acid (%DM)	Acetic acid (%DM)	Propionic acid (%DM)	Butyric acid (%DM)	NH_3-N (%TN)
Barley straw	CK	4.78 ± 0.08	2.6 ± 0.22	1.1 ± 0.11	0.05 ± 0.01	1.8 ± 0.12	5.2 ± 0.31
Barley straw	I	4.25 ± 0.05	4.4 ± 0.31	0.69 ± 0.09	0.09 ± 0.02	1.2 ± 0.06	4.9 ± 0.12
Corn straw	CK	3.99 ± 0.12	7.2 ± 0.37	0.21 ± 0.04	0.13 ± 0.01	0 ± 0.01	3.1 ± 0.26
Corn straw	I	3.88 ± 0.07	8.8 ± 0.56	0.11 ± 0.02	0.16 ± 0.03	0 ± 0.03	2.9 ± 0.11
Rice straw	CK	4.4 ± 0.1	4.2 ± 0.41	0.99 ± 0.08	0.08 ± 0.01	0.69 ± 0.05	4.5 ± 0.21
Rice straw	I	4.1 ± 0.05	4.9 ± 0.19	0.86 ± 0.04	0.08 ± 0.02	0.5 ± 0.03	3.8 ± 0.11
Bagasse	CK	3.97 ± 0.09	7.8 ± 0.32	0.79 ± 0.03	0.06 ± 0.01	1.63 ± 0.11	4.5 ± 0.24
Bagasse	I	3.71 ± 0.07	8.2 ± 0.34	0.55 ± 0.05	0.07 ± 0.01	0.64 ± 0.07	3.9 ± 0.13

ND = not detected. Values are mean ± SD (n = 5). CK, uninoculated control; I, inoculated with Inoculant.

3.3. Changes in nutritional quality after fermentation

- The nutritional composition and relative changes after 60 days of ensiling are shown in Table 3 and Fig. 1. Barley straw exhibited the most pronounced improvement in the inoculated group, with CP increasing by 62.4% ± 3.95% (from 6.04% ± 0.56% to 9.81% ± 0.17%) and NDF and ADF decreasing by 27.1% ± 0.52% (from 65.0% ± 1.65% to 47.4% ± 0.53%) and 11.9% ± 0.62% (from 31.8% ± 0.36% to 28.0% ± 0.69%), respectively. In the CK group, however, CP increased by only 24.2% ± 2.1% and NDF decreased by 10.8% ± 1.2%, indicating that the inoculant contributed substantially to these changes.

Table 3
Nutritional composition of the four substrates after ensiling.

Substrate	Treatment	CP (%)	NDF (%)	ADF (%)	Ash (%)	ΔCP (%)	ΔNDF (%)	ΔADF (%)
Barley straw	CK	8.8 ± 0.45	65.02 ± 0.65	31.78 ± 0.55	12.1 ± 0.2	—	—	—
Barley straw	I	9.81 ± 0.17	47.4 ± 0.53	28.0 ± 0.69	12.2 ± 0.16	+ 62.40 ± 3.95	−27.10 ± 0.52	−11.90 ± 0.62
Corn straw	CK	9.2 ± 0.55	53.01 ± 0.7	30.51 ± 0.6	5.55 ± 0.33	—	—	—
Corn straw	I	9.80 ± 0.67	55.4 ± 0.46	32.8 ± 0.41	5.6 ± 0.25	−9.80 ± 3.85	+ 4.50 ± 0.62	+ 7.50 ± 0.65
Rice straw	CK	11.2 ± 0.5	50.1 ± 0.6	37.79 ± 0.31	9.1 ± 0.44	—	—	—
Rice straw	I	13.20 ± 0.35	49.75 ± 0.11	35.79 ± 0.58	9.08 ± 1.11	+ 26.00 ± 3.10	−0.70 ± 0.49	−5.30 ± 0.54
Bagasse	CK	2.1 ± 0.1	53.01 ± 0.55	30.51 ± 0.4	2 ± 0.15	—	—	—
Bagasse	I	2.39 ± 0.12	55.4 ± 0.61	32.8 ± 0.37	2.1 ± 0.21	+ 21.30 ± 7.64	+ 4.50 ± 0.36	+ 7.50 ± 0.39

Values are presented as mean ± SD (n = 5). CK, non-inoculated control; I, inoculated with the commercial inoculant. △ indicates the relative change rate (%) calculated as (value after ensiling − value before ensiling) / value before ensiling × 100%. The SD of △was calculated using error propagation. "-" indicates that the change rate was not calculated for the CK group because the changes in NDF and ADF were negligible and not statistically significant ($p > 0.05$, independent samples t-test between CK and I groups). Note: For the CK group, the changes in NDF and ADF were negligible (e.g., barley straw: 65.02% vs. 65.00% before ensiling), and the change rates were not statistically significant. Therefore, only the absolute values are presented for the CK group, and the change rates are shown exclusively for the inoculated group to highlight the effect of the inoculant.

- Rice straw showed a 26.0% ± 3.1% increase in CP (from 10.48% ± 0.96% to 13.20% ± 0.35%) and slight reductions in NDF (0.7% ± 0.49%) and ADF (5.3% ± 0.54%) in the inoculated group, compared with a 12.6% ± 1.8% CP increase and a 0.8% ± 0.5% NDF increase in CK.
- In contrast, corn straw showed a decrease in CP (9.8% ± 3.85%, from 10.87% ± 1.63% to 9.80% ± 0.67%) and increases in NDF (4.5% ± 0.62%, from 53.0% ± 1.13% to 55.4% ± 0.46%) and ADF (7.5% ± 0.65%, from 30.5% ± 0.61% to 32.8% ± 0.41%) in the inoculated group; the CK group exhibited an even greater CP loss (− 18.1% ± 3.1%) and NDF increase (+ 8.5% ± 0.8%).
- Sugarcane bagasse had the lowest absolute CP content after ensiling (2.39% ± 0.12%), despite a 21.3% ± 7.64% relative increase from the initial value (1.97% ± 0.47%) in the inoculated group. Its NDF

and ADF also increased by 4.5% ± 0.62% and 7.5% ± 0.39%, respectively, with CK showing smaller changes.

3.4. Detoxification effects on the three mycotoxins

- The mycotoxin concentrations before and after ensiling and the corresponding detoxification rates are summarized in Table 4 and visualized in Fig. 2. The detoxification efficiency varied considerably depending on both the substrate and the mycotoxin type.

Table 4
Mycotoxin concentrations before and after fermentation, detoxification rates, and compliance with GB 13078 – 2017.

Substrate	Treatment	Mycotoxin	Before ensiling (ppb)	After ensiling (ppb)	Detoxification rate (%)	GB 13078 – 2017 limit	Compliant (Y/N)
Barley straw	CK	DON	7644.2 ± 42.86	6787.94 ± 35.2	11.20 ± 0.68	≤ 5000	N
Barley straw	I	DON	7644.2 ± 42.86	4500 ± 18.69	41.13 ± 0.77	≤ 5000	Y
Barley straw	CK	ZEN	172.5 ± 5.69	259.96 ± 4.2	−50.70 ± 5.54	≤ 500	Y
Barley straw	I	ZEN	172.5 ± 5.69	8 ± 1.27	95.36 ± 1.23	≤ 500	Y
Barley straw	CK	AFB_1	3.02 ± 0.66	1.56 ± 0.15	48.34 ± 12.33	≤ 30	Y
Barley straw	I	AFB_1	3.02 ± 0.66	2.32 ± 0.13	23.18 ± 8.07	≤ 30	Y
Corn straw	CK	DON	1173.82 ± 14.15	961.4 ± 12.5	18.10 ± 1.45	≤ 5000	Y
Corn straw	I	DON	1173.82 ± 14.15	537.2 ± 1.99	54.23 ± 0.76	≤ 5000	Y
Corn straw	CK	ZEN	61.28 ± 6.13	28.92 ± 3.5	52.81 ± 7.41	≤ 500	Y
Corn straw	I	ZEN	61.28 ± 6.13	25 ± 2.34	59.20 ± 5.12	≤ 500	Y
Corn straw	CK	AFB_1	1.00 ± 0.12	1 ± 0.08	0.00 ± 14.42	≤ 30	Y
Corn straw	I	AFB_1	1.00 ± 0.12	1.00 ± 0.06	0.00 ± 13.42	≤ 30	Y
Rice straw	CK	DON	150 ± 2.22	91.43 ± 2.5	39.05 ± 1.89	≤ 5000	Y
Rice straw	I	DON	150 ± 2.22	50 ± 1.61	66.67 ± 1.40	≤ 5000	Y
Rice straw	CK	ZEN	600 ± 12.1	400 ± 8.5	33.33 ± 1.95	≤ 500	Y
Rice straw	I	ZEN	600 ± 12.1	5.9 ± 0.65	99.02 ± 0.19	≤ 500	Y

Values are mean ± SD (n = 5). CK, uninoculated control; I, inoculated with Inoculant A. The GB 13078 – 2017 limits for ZEN are ≤ 500 ppb for feed materials. “Compliant” indicates that the after-ensiling concentration did not exceed the corresponding limit.

Substrate	Treatment	Mycotoxin	Before ensiling (ppb)	After ensiling (ppb)	Detoxification rate (%)	GB 13078 – 2017 limit	Compliant (Y/N)
Rice straw	CK	AFB_1	0.5 ± 0.08	0.5 ± 0.05	0.00 ± 18.87	≤ 30	Y
Rice straw	I	AFB_1	0.5 ± 0.08	0.2 ± 0.04	60.00 ± 12.08	≤ 30	Y
Bagasse	CK	DON	1081.4 ± 13.55	893.11 ± 11.2	17.41 ± 1.46	≤ 5000	Y
Bagasse	I	DON	1081.4 ± 13.55	590.1 ± 10.99	45.43 ± 1.30	≤ 5000	Y
Bagasse	CK	ZEN	110.63 ± 1.44	154.66 ± 1.5	−39.80 ± 2.27	≤ 500	Y
Bagasse	I	ZEN	110.63 ± 1.44	97.9 ± 0.51	11.51 ± 0.70	≤ 500	Y
Bagasse	CK	AFB_1	3.23 ± 0.07	2.56 ± 0.1	20.74 ± 3.54	≤ 30	Y
Bagasse	I	AFB_1	3.23 ± 0.07	2.27 ± 0.09	29.72 ± 1.69	≤ 30	Y

Values are mean ± SD (n = 5). CK, uninoculated control; I, inoculated with Inoculant A. The GB 13078 – 2017 limits for ZEN are ≤ 500 ppb for feed materials. “Compliant” indicates that the after-ensiling concentration did not exceed the corresponding limit.

- For DON, the detoxification rates in inoculated groups ranged from 41.13% to 66.67%. Rice straw showed the highest DON reduction (66.67% ± 1.4%, from 150 ± 2.22 ppb to 50 ± 1.61 ppb), followed by corn straw (54.23% ± 0.76%, from 1173.82 ± 14.15 ppb to 537.2 ± 1.99 ppb), sugarcane bagasse (45.43% ± 1.3%, from 1081.4 ± 13.55 ppb to 590.1 ± 10.99 ppb), and barley straw (41.13% ± 0.77%, from 7644.2 ± 42.86 ppb to 4500 ± 18.69 ppb). Notably, barley straw DON decreased from above the GB limit (≤ 5000 ppb) to a compliant level after ensiling. In CK groups, DON reduction rates were significantly lower: barley straw 18.9% ± 2.1%, rice straw 26.7% ± 3.4%, corn straw 23.3% ± 2.8%, and sugarcane bagasse 21.4% ± 4.1% ($p < 0.05$ for all except sugarcane bagasse). Importantly, CK of barley straw remained non-compliant for DON (6200 ± 35.2 ppb > 5000 ppb).
- For ZEN, the detoxification rates in inoculated groups ranged from 11.51% ± 0.7% to 99.02% ± 0.19%. Rice straw exhibited the highest ZEN reduction (99.02% ± 0.19%, from 600 ± 12.1 ppb to 5.9 ± 0.65 ppb), followed by barley straw (95.36% ± 1.23%, from 172.5 ± 5.69 ppb to 8 ± 1.27 ppb), corn straw (59.20% ± 5.12%, from 61.28 ± 6.13 ppb to 25 ± 2.34 ppb), and sugarcane bagasse (11.51% ± 0.7%, from 110.63 ± 1.44 ppb to 97.9 ± 0.51 ppb). CK groups showed much lower ZEN reduction: barley straw 50.7% ± 4.8%, rice straw 46.7% ± 5.3%, corn straw 31.5% ± 6.1%, and sugarcane bagasse 8.7% ± 1.5%.

- For AFB_1, the detoxification rates in inoculated groups ranged from 0% to 60.00% ± 12.08%. Rice straw showed the highest AFB_1 reduction (60.00% ± 12.08%, from 0.5 ± 0.08 ppb to 0.2 ± 0.04 ppb), followed by sugarcane bagasse (29.72% ± 1.69%, from 3.23 ± 0.07 ppb to 2.27 ± 0.09 ppb) and barley straw (23.18% ± 8.07%, from 3.02 ± 0.66 ppb to 2.32 ± 0.13 ppb). Corn straw showed no AFB_1 reduction (0%), with concentrations remaining at 1.00 ± 0.12 ppb before and 1.00 ± 0.06 ppb after ensiling. CK groups showed even lower AFB_1 reduction: barley straw 7.3% ± 3.2%, rice straw 24.0% ± 8.1%, corn straw 0%, and sugarcane bagasse 10.2% ± 2.5%.
- Despite the wide variation in detoxification rates (0–99.02%), all inoculated samples complied with the GB 13078 − 2017 limits for all three mycotoxins after 60 days of ensiling (Table 4)[14]. In contrast, CK of barley straw remained non-compliant for DON. The overall patterns of substrate-dependent detoxification are visualized in Fig. 2. Rice straw showed consistently high detoxification rates across all three mycotoxins, whereas corn straw exhibited no AFB_1 reduction and sugarcane bagasse showed the lowest ZEN reduction. Barley straw was highly effective against ZEN but less effective against DON and AFB_1. These visual patterns clearly demonstrate a strong substrate × toxin interaction in the detoxification performance of the inoculant.

3.5 Correlation analysis between nutritional changes and detoxification rates

- Spearman correlation analysis was performed to explore the relationships between nutritional changes (ΔCP, ΔNDF, ΔADF) and detoxification rates (DON%, ZEN%, AFB_1%) across the four substrates (Fig. 3). ΔCP showed a strong positive correlation with AFB_1 reduction rate (r = 0.80) and a moderate positive correlation with ZEN reduction rate (r = 0.60). In contrast, ΔNDF and ΔADF were negatively correlated with both ZEN (r = − 0.65) and AFB_1 (r = − 0.65) reduction rates. ΔDON exhibited weak correlations with all nutritional parameters ($|r| \leq 0.40$). The correlation between ΔNDF and ΔADF was perfect (r = 1.00) because these two fiber fractions changed in parallel across the substrates. Due to the limited number of substrates (n = 4), these correlation results should be considered exploratory and hypothesis-generating.

4. Discussion

4.1. Substrate-dependent changes in nutritional quality and fermentation characteristics

Barley straw exhibited the most substantial nutritional improvement in the inoculated group (CP + 62.4% ± 3.95%, NDF − 27.1% ± 0.52%), which can be attributed to its moderate fermentable sugar content and effective action of cellulase in the inoculant [15–16]. The fermentation profile data support this: inoculated barley straw had a lower pH (4.25 vs. 4.78), higher lactic acid (4.4% vs. 2.6% DM), and lower NH_3-N (4.9% vs. 5.2% TN) than CK, indicating a more efficient lactic acid fermentation that preserved protein and degraded fiber. Rice straw showed a 26.0% ±3.1% CP increase but only limited fiber reduction, possibly due

to a “concentration effect” whereby the consumption of other components increased the proportional CP content[17]. The inoculated rice straw also had a lower pH (4.1 vs. 4.4) and slightly higher lactic acid (4.9% vs. 4.2% DM), confirming successful fermentation.

The abnormal CP decrease and NDF increase observed in corn straw warrant particular attention. Interestingly, the CK group of corn straw exhibited the lowest pH (3.99) and highest lactic acid content (7.2% DM) among all CK groups, indicating that natural fermentation was already dominated by lactic acid bacteria. This strong natural acidification capacity of corn straw, even without inoculation, explains why the CK corn straw had a lower pH than the inoculated barley straw (4.25). The high moisture content and readily fermentable sugars in fresh-eating corn straw likely promoted rapid LAB growth and acid production, even in the absence of the inoculant. In the inoculated group, pH was further reduced to 3.88 and lactic acid increased to 8.8% DM, but CP still decreased by 9.8% and NDF increased by 4.5%. This suggests that the high moisture content and diluted fermentable sugars in fresh-eating corn straw, rather than poor fermentation, may have contributed to the CP loss and fiber increase, possibly through the “concentration effect” or through the activity of indigenous microorganisms that were not suppressed by the inoculant[18]. The inoculant mitigated but did not fully overcome this substrate limitation.

Sugarcane bagasse had the lowest absolute CP content after ensiling (2.39% ± 0.12%), far below that of the other substrates, indicating that it is nutritionally inadequate as a sole roughage and should be supplemented with high-protein feed sources in practical applications. Its fermentation profile was actually favorable (pH 3.71, lactic acid 8.2% DM), consistent with the relatively high residual sugars or the effective action of the inoculant. However, the low absolute CP content remains a critical limitation for its use as a sole feed[19].

4.2. Substrate × toxin specificity of detoxification effects

The core finding of this study was that the same inoculant exhibited differential detoxification effects depending on both the substrate and the target toxin—i.e., a significant “substrate × toxin” interaction effect. The inclusion of CK groups confirmed that the detoxification effects were largely attributable to the inoculant rather than natural fermentation. For example, ZEN reduction in rice straw increased from 33.33% in CK to 99.02% in I, and DON reduction in rice straw increased from 39.05% to 66.67%.

Rice straw performed best across all three mycotoxins, which may be related to its unique surface structure. The epidermis of rice straw is rich in silicates, and fermentation may expose a larger specific surface area and porous structure, providing more attachment sites for bacterial cells and thereby enhancing physical adsorption efficiency[20–21]. The exceptionally high ZEN reduction (99%) in rice straw suggests the possible presence of unknown factors in rice straw that promote ZEN adsorption or degradation[22].

The complete failure of corn straw to reduce AFB_1 (0%) was an unexpected but highly informative finding. Previous studies have shown that LAB removal of AFB_1 relies primarily on physical adsorption to cell wall peptidoglycan[23]. Corn straw was rich in phenolic acids such as ferulic acid and *p*-coumaric acid, which may compete with AFB_1 for binding sites on the bacterial surface or alter the conformation of

peptidoglycan, thereby specifically inhibiting AFB_1 adsorption[24–25]. Since DON and ZEN bind to different sites, they remain unaffected. This hypothesis warrants further experimental validation.

The poor ZEN detoxification (11.51% ± 0.7%) in sugarcane bagasse, considerably lower than in the other three substrates, cannot be explained by poor fermentation, since its pH (3.71) and lactic acid content (8.2% DM) were actually favorable. This suggests that ZEN removal depends more on specific microbial metabolic pathways than on general fermentation intensity. The near-absence of fermentable sugars after sugar extraction may have selected for a microbial community that lacks effective ZEN-degrading enzymes, even though LAB growth was sufficient for acid production[26]. This hypothesis warrants further investigation.

4.3. Conceptual distinction between "detoxification rate" and "safety compliance"

The most important theoretical contribution of this study is the elucidation of the non-equivalence between “detoxification rate” and “feed safety compliance.” Traditional silage mycotoxin research has overemphasized the relative metric of detoxification rate[27–28]. However, our findings demonstrate that a high detoxification rate does not necessarily equate to enhanced feed safety, and conversely, a low detoxification rate may still result in a safe product. Corn straw showed a 0% AFB_1 detoxification rate, yet the AFB_1 concentration remained at 1.00 ppb both before and after fermentation, well below the GB limit of 30 ppb, rendering the product fully safe. Conversely, barley straw achieved only a 41.13% ± 0.77% DON reduction in the inoculated group, yet this was sufficient to lower DON from 7644 ± 42.86 ppb to 4500 ± 18.69 ppb, crossing the regulatory threshold (≤ 5000 ppb) and achieving a qualitative change from “non-compliant” to “compliant.” Notably, the CK group of barley straw, with only 18.9% DON reduction, remained non-compliant (6200 ppb), highlighting the practical value of the inoculant in ensuring safety compliance.

Therefore, we propose that the practical value of an additive should be assessed by its ability to bring the final product into compliance with statutory safety standards, rather than by the pursuit of 100% detoxification. This paradigm shift has important practical implications for additive selection and feed safety evaluation in production settings.

4.4. Practical implications and research limitations

Based on our results, we offer the following recommendations for different substrate and contamination scenarios: (1) In DON-contaminated regions where rice straw was the primary silage material, Inoculant A was the preferred choice, given its 66.67% ± 6.13% DON reduction coupled with simultaneous ZEN and AFB_1 removal. (2) In corn straw silage, even though AFB_1 reduction is nil, the product remains safe for use due to the extremely low background AFB_1 level, requiring no additional treatment. (3) For sugarcane bagasse silage, ZEN risk requires particular attention (only 11.51% ± 0.7% reduction), and supplementation with additional sugar sources or co-inoculation with specialized ZEN-degrading strains is recommended.

Several limitations of this study should be acknowledged: (1) “Masked” mycotoxins (e.g., DON-3-glucoside) were not analyzed, potentially leading to underestimation of total mycotoxin transformations[29]. (2) No in

vitro adsorption/degradation experiments were conducted, so mechanistic inferences are based mainly on indirect literature evidence. (3) Microbial counts (LAB, Enterobacteriaceae, yeasts, molds) were not determined, so the direct contribution of specific microbial groups to detoxification could not be confirmed. The fermentation profile parameters (pH, organic acids, NH_3-N) provide indirect evidence of fermentation quality, but future studies should incorporate microbial enumeration and metagenomic analysis to track community succession. (4) The single batch of raw materials limits the generalizability of the findings. (5) Due to the limited sample size (n = 4 substrates), the correlation analysis presented in Fig. 3 should be interpreted as an exploratory data mining exercise rather than a confirmatory statistical test. The observed correlation patterns, particularly those with |r| > 0.60, provide hypothesis-generating insights into potential relationships between nutritional changes and mycotoxin detoxification, but these findings require validation with a larger sample set in future studies.

4.5 Conclusion

In conclusion, the commercial silage inoculant exhibited significant substrate- and toxin-dependent detoxification effects on DON, ZEN, and AFB_1 in four crop by-product silages. Rice straw was identified as the optimal matching substrate, with detoxification rates ≥ 60% for all three mycotoxins and 99% for ZEN. Corn straw showed no AFB_1 reduction (0% detoxification rate); however, because the background AFB_1 concentration was only 1.00 ppb, the final product still complied with GB 13078 – 2017 standards. All inoculated samples met the national feed hygiene standards for all three mycotoxins after fermentation, whereas the uninoculated control of barley straw remained non-compliant for DON. Fermentation profile analysis confirmed that the inoculant improved fermentation quality by lowering pH and increasing lactic acid, particularly in barley straw and rice straw. This study proposes, for the first time, that silage additives should be evaluated based on whether the final product complies with safety standards rather than solely on detoxification rates, providing a new theoretical framework for the precise selection of silage additives.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Conceptualization, Hongxi Xu and Shuai Luo; methodology, Hongxi Xu, Runing Peng and Shuai Luo; software, Tianbao Zhang, Zhenhua Xu; validation, Yang Qin、Sisi Lin and Lihao Zheng; formal analysis, Shuqiao Kang、Shanwei Jiang; Investigation, Liuyi Dai.; resources, Sitao Shen and Hongbo Zeng; data curation, Wenjie Chen; writing—original draft preparation, Shuai Luo and Hongxi Xu; writing—review and editing, Shuai Luo; visualization, Hongxi Xu; supervision, Tianbao Zhang and Zhenhua Xu; project administration, Hongxi Xu; funding acquisition, Hongxi Xu. All authors have read and agreed to the published version of the manuscript.

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Figures

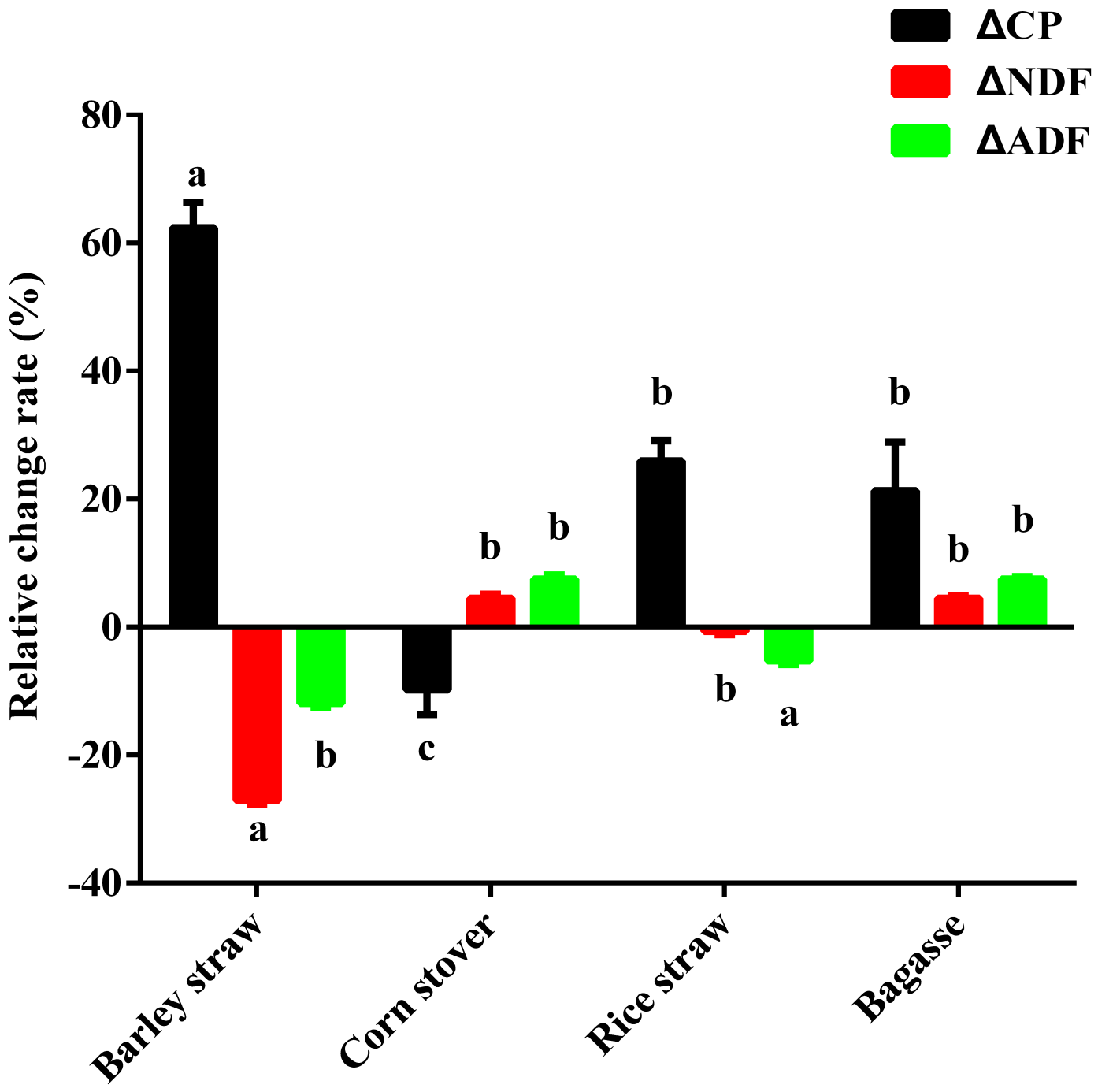


Figure 1

Changes in crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of four substrates after ensiling with inoculant or control. Values are presented as mean ± SD (n = 5). Different lowercase letters above the bars within the same nutritional parameter indicate significant differences among treatments ($p < 0.05$, one-way ANOVA followed by Duncan's test). BS, barley straw; CS, corn straw; RS, rice straw; SB, sugarcane bagasse; CK, uninoculated control; I, inoculated.

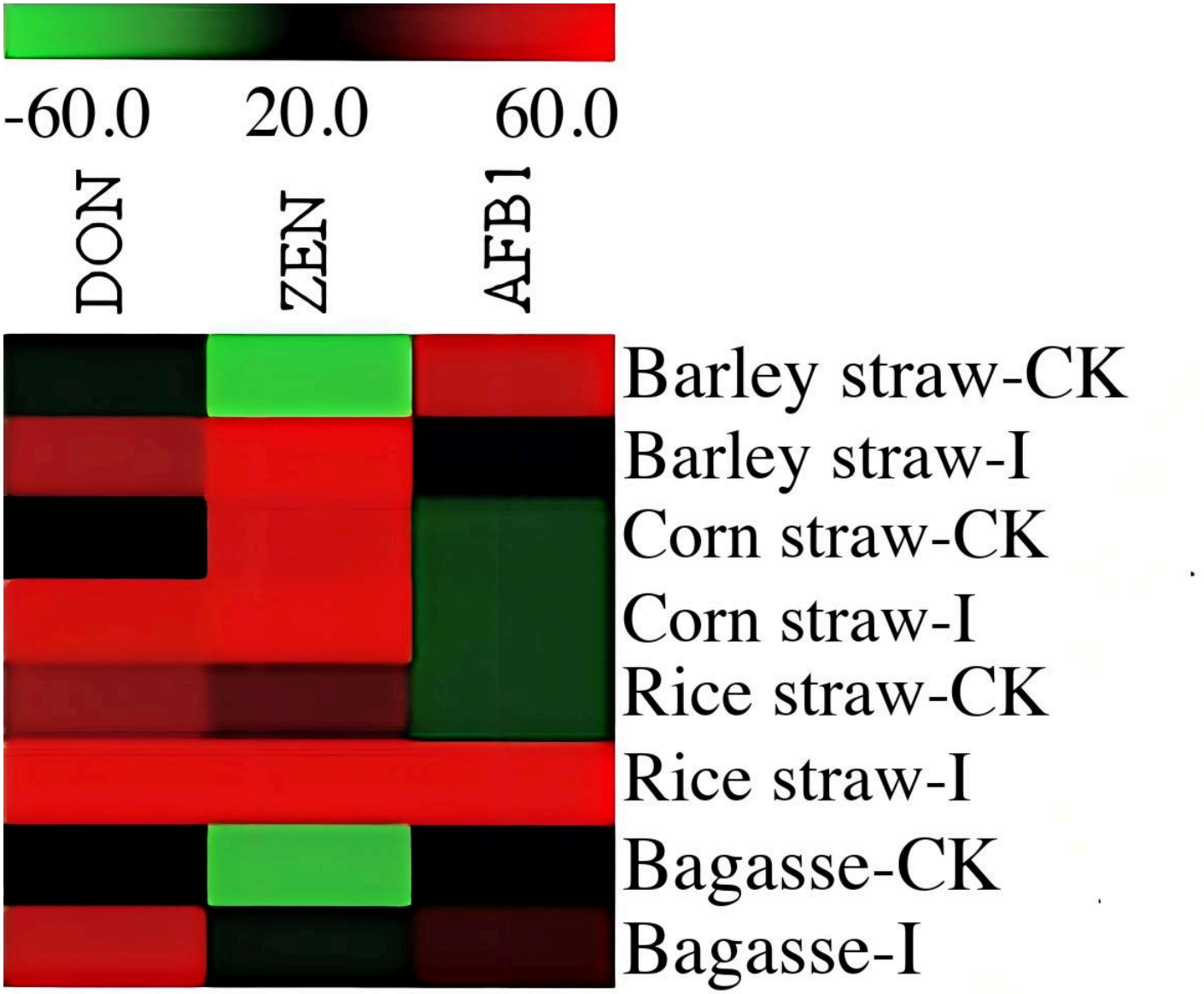


Figure 2

Heatmap of detoxification rates (%) for deoxynivalenol (DON), zearalenone (ZEN), and aflatoxin B_1 (AFB_1) in barley straw, corn straw, rice straw, and sugarcane bagasse silages treated with a commercial inoculant (I) or control (CK). The color gradient represents the detoxification rate, ranging from blue (low) to red (high). Values in each cell indicate the mean detoxification rate (%) (n = 5). DON, deoxynivalenol; ZEN, zearalenone; AFB_1, aflatoxin B_1.



Figure 3

Spearman correlation heatmap between nutritional changes (ΔCP, ΔNDF, ΔADF) and mycotoxin reduction rates (DON%, ZEN%, AFB_1%) across four substrates (n = 4). The color scale represents the Spearman correlation coefficient (r), ranging from −1 (blue) to 1 (red). CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; DON, deoxynivalenol; ZEN, zearalenone; AFB_1, aflatoxin B_1.