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Research Article

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Effects of Different Additives on the Fermentation Profile and Aerobic Stability of Total Mixed Ration Silage Containing Wet Distillers Bran Plus Solubles: An Experimental Study

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ABSTRACT
Wet Distillers bran plus solubles is a high-moisture agro-industrial by-product widely used in ruminant diets; however, its rapid spoilage limits storage and transport. This experimental and applied study evaluated the effects of different biological and chemical additives on the fermentation profile, microbial populations, dry matter losses, and aerobic stability of total mixed ration silage containing wet Distillers bran plus solubles. A total mixed ration was treated with no additive (control), a heterofermentative lactic acid bacteria inoculant, sodium benzoate, or a blend of organic acids and ensiled for 45 days. Fermentation characteristics, microbial counts, dry matter losses, and aerobic stability were evaluated after the ensiling period. All treatments resulted in adequate fermentation, with final pH values within the expected range. Chemical additives reduced dry matter losses, whereas the biological additive increased lactic acid bacteria counts without improving aerobic stability. This study provides original experimental evidence under controlled conditions, focusing exclusively on the applied evaluation of additive effects in total mixed ration silage containing wet Distillers bran plus solubles.

List of Abbreviations

DM	:	Dry Matter
LAB	:	Lactic Acid Bacteria
SB	:	Sodium Benzoate
TMR	:	Total Mixed Ration
WDBS	:	Wet Distillers Bran Plus Solubles

Keywords: Aerobic Stability, Fermentation Characteristics, Microbial Populations, Silage Preservatives, Wet Distillers Bran Plus Solubles

Introduction

Wet Distillers bran plus solubles is a high-moisture by-product incorporated into ruminant diets. When included in total mixed ration silage, preservation efficiency depends on fermentation dynamics and microbial activity, and inadequate fermentation may result in nutrient losses and reduced silage stability [1,2].

Silage additives may influence fermentation products, microbial populations, and dry matter losses in high-moisture substrates. Biological additives, such as heterofermentative lactic acid bacteria, and chemical additives, including preservatives and organic acids, may exert different effects during ensiling [3,4].

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However, comparative experimental information regarding the effects of these additives on wet Distillers bran plus solubles–based total mixed ration silage remains limited.

Therefore, this study experimentally evaluated the effects of different biological and chemical additives on fermentation characteristics, microbial populations, dry matter losses, and aerobic stability of total mixed ration silage containing wet Distillers bran plus solubles.

Materials and Methods

Experimental design and treatments

The experiment followed a completely randomized design with four treatments and four replicates per treatment. Treatments consisted of:

- Control without additives
- Heterofermentative lactic acid bacteria inoculant
- Sodium benzoate and
- A blend of organic acids, following procedures commonly adopted in silage additive evaluation studies [5,5].

Total Mixed Ration Formulation and Ensiling

The total mixed ration was formulated to include wet Distillers bran plus solubles as the primary ingredient, combined with dry components to achieve adequate dry matter content for ensiling. The treated material was packed into laboratory-scale silos, compacted, sealed, and stored at ambient temperature for 45 days, as described by Kleinschmit et al [4].

Chemical and Microbiological Analyses

Dry matter and fermentation characteristics, including pH and organic acid concentrations, were determined following standard analytical procedures [5]. Microbial populations of lactic acid bacteria, yeasts, and molds were quantified using selective culture media [7].

Dry Matter Losses and Aerobic Stability

Dry matter losses were calculated based on differences between initial and final silo weights. Aerobic stability was evaluated by monitoring silage temperature during exposure to air, with stability defined as the time required for silage temperature to exceed ambient temperature by 2 °C [3].

Statistical Analysis

Data were analyzed using analysis of variance with treatment as a fixed effect. Mean comparisons were performed when significant effects were detected ($p < 0.05$).

Results

Chemical Composition of the Total Mixed Ration before Ensiling

The chemical composition of the total mixed ration prior to ensiling is presented in Table 1. Dry matter content reflected the high inclusion of Wet Distillers bran plus solubles, resulting in a moisture level typical of high-moisture total mixed rations. Crude protein, neutral detergent fiber, ether extract, and ash concentrations were similar across treatments, as additives were applied only at the time of ensiling. These results confirm that all treatments originated from the same basal ration and that differences observed after ensiling were attributable to additive effects rather than initial compositional variation.

Table 1: Chemical composition of raw ingredients in TMR

Items	DM, %	CP, DM%	NDF, DM%	Ash, DM%
Tropical grass silage	24.79±0.04	4.62±0.29	77.00±0.11	7.18±0.20
Cottonseed hulls	85.42±0.23	4.79±0.39	76.30±0.13	2.92±0.10
Soybean hulls	85.41±0.00	9.48±0.38	76.70±0.20	3.97±0.00
Ground corn	84.94±0.00	8.84±0.19	10.40±0.08	1.33±0.05
WDBS	35.96±0.07	25.39±0.20	35.71±0.21	11.70±0.00

DM, Dry matter; CP, Crude protein; NDF, Neutral detergent fiber; WDBS, Wet distillers bran plus solubles

Fermentation Profile after 45 days of Ensiling

Fermentation characteristics of total mixed ration silage after 45 days of ensiling are summarized in Table 2. Final pH values were within the range considered adequate for total mixed ration silage and did not differ among treatments, indicating successful acidification across all silages. Lactic acid concentrations were comparable among treatments, whereas chemical additives influenced the production of secondary fermentation products. The organic acid treatment increased propionic acid concentration relative to the control, while sodium benzoate resulted in intermediate values. The heterofermentative lactic acid bacteria treatment showed greater butyric acid concentration compared with chemical additive treatments. Ammonia nitrogen concentrations remained low and were not affected by additive type, indicating limited proteolysis during ensiling.

Table 2: Designed raw ingredients DM rate in the TMR

Items	Designed dry matter inclusion (%)
Tropical grass silage	7
Cottonseed hulls	9
Soybean hulls	12
Ground corn	19
WDBS	50
Premix mineral-vitamins	3
Total	100

WDBS, Wet Distillers bran plus solubles

Microbial Populations after Ensiling

Microbial populations after ensiling are shown in Table 3. Silages treated with the heterofermentative lactic acid bacteria inoculant exhibited higher lactic acid bacteria counts compared with the control and chemical additive treatments. Yeast and mold populations were low across all treatments, with no significant differences detected among them. These results indicate effective establishment of the biological inoculant without corresponding increases in spoilage-associated microorganisms. The consistently low yeast and mold count across treatments suggest a low risk of aerobic deterioration at silo opening.

Table 3: The chemical composition of TMR before ensiling

Items	Treatments1			
	CON	LB	SB	BOA
DM, %	47.74±0.05	47.63±0.003	47.25±0.05	46.50±0.08
CP, DM%	18.00±0.13	17.61±0.05	18.70±0.36	18.61±0.52
NDF, DM%	34.81±0.06	35.71±0.04	35.60±0.10	36.70±0.08
Ash, DM%	9.87±0.06	11.08±0.10	10.08±0.18	10.08±0.01

CON, Control; LB, Lentilactobacillus buchneri; SB, Sodium benzoate; BOA, Blend of organic acids

Dry Matter Losses during Ensiling

Dry matter losses during the ensiling period differed among treatments (Table 4). Silages treated with chemical additives exhibited lower dry matter losses compared with the control, whereas the biological additive resulted in intermediate losses. These differences indicate that additive type influenced fermentative efficiency and the extent of substrate loss during storage.

Table 4: Chemical composition of 45 days ensiled TMR treated with heterolactic bacteria or chemical additives

Items	Treatments[1]				SEM[2]	P-value
	CON	LB	SB	BOA		
DM, %	46.5^{a}	46.6^{a}	45.8^{b}	46.0^{ab}	0.150	0.008
CP, DM%	17.9	18.5	18.6	18.5	0.249	0.189
NDF, DM%	35.2^{a}	35.6^{a}	32.9^{b}	34.9^{a}	0.473	0.006
Ash, DM%	9.85	10.1	10.6	10.4	0.250	0.203

DM, Dry matter; CP, Crude protein; NDF, Neutral detergent fiber.
[1]CON, Control; LB, Lentilactobacillus buchneri; SB, Sodium benzoate; BOA, Blend of organic acids
[2]SEM, Standard error of the mean
^{ab}For same row means with different superscripts differ ($p<0.05$), used Tukey's test

Aerobic Stability during Air Exposure

Aerobic stability results are presented in Figure 1. During air exposure, silage temperatures remained close to ambient temperature across all treatments. No differences in aerobic stability were observed among treatments, indicating that additive application did not influence resistance to aerobic deterioration under the evaluated conditions.

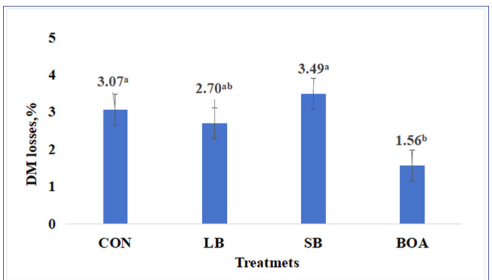

Figure 1: Dry matter losses of 45 days ensiled TMR treated with heterolactic bacteria or chemical additives. CON, Control; LB, Lentilactobacillus buchneri; SB, Sodium benzoate; BOA, Blend of organic acids. Lowercase letters above the bars indicate statistical difference between treatments, statistically using the Tukey test at 5% probability

Discussion

Adequate fermentation across all treatments indicates that the basal composition of the total mixed ration containing wet Distillers bran plus solubles provided suitable conditions for ensiling. Similar final pH values among treatments suggest that acidification was sufficient to stabilize the silage regardless of additive application, which is consistent with reports showing that total mixed ration silages with adequate fermentable substrates may reach stable pH values without strong additive effects [1,2].

The absence of differences in lactic acid concentration among treatments indicates that the primary fermentation pathway was not substantially altered by additive inclusion. This response suggests that endogenous lactic acid bacteria populations and substrate availability were sufficient to drive lactic fermentation, limiting the potential for additives to further increase lactic acid production, as previously observed in well-formulated total mixed ration silages [2].

Chemical additives influenced secondary fermentation products, particularly propionic acid concentration, which was increased in silages treated with organic acids. This effect reflects the direct antimicrobial action of organic acids and their capacity to suppress undesirable microorganisms associated with nutrient losses during ensiling (Danner et al., 2003). Sodium benzoate resulted in intermediate responses, which is consistent with its mode of action as a preservative rather than as a compound that actively modifies fermentation pathways [6].

The greater butyric acid concentration observed in silages treated with the heterofermentative lactic acid bacteria inoculant suggests increased heterofermentative activity. Although butyric acid is often associated with clostridial fermentation, the concentrations observed in the present study remained within acceptable limits for total mixed ration silage. This indicates that the observed increase likely reflects altered fermentation pathways rather than clostridial spoilage, particularly given the low ammonia nitrogen concentrations observed across treatments [1,7].

The increased lactic acid bacteria count in silages treated with the biological inoculant confirm effective microbial establishment, as expected when inoculants are applied at adequate rates [4]. However, this increase did not translate into improved aerobic stability. The lack of additive effects on aerobic stability may be explained by the consistently low yeast and mold populations observed across all treatments. When spoilage-associated microorganisms are already suppressed, the potential for additives to further enhance aerobic stability is inherently limited [4].

Dry matter losses differed among treatments, with chemical additives reducing losses compared with the control. This finding indicates greater preservation efficiency and reduced substrate degradation during ensiling. Lower dry matter losses likely reflect inhibition of undesirable fermentative pathways that generate gaseous losses, supporting previous observations that chemical preservatives can be effective in minimizing nutrient losses in high-moisture silages [6,3].

In contrast, the biological additive resulted in intermediate dry matter losses, suggesting that increased microbial activity did not necessarily translate into improved preservation efficiency. This highlights that enhanced microbial growth alone is not sufficient to minimize nutrient losses and underscores the importance of additive mode of action when targeting preservation efficiency in total mixed ration silage systems [5].

Overall, the present results demonstrate that additive effects in total mixed ration silage containing wet Distillers bran plus solubles are additive-specific and context-dependent. Under controlled experimental conditions where baseline fermentation is already adequate, chemical additives provide greater benefits in reducing dry matter losses, whereas biological additives primarily influence microbial populations without improving aerobic stability.

Conclusion

Under controlled experimental conditions, total mixed ration silage containing wet Distillers bran plus solubles was successfully preserved across all treatments. The use of chemical additives consistently reduced dry matter losses, indicating greater preservation efficiency and improved control of fermentative pathways associated with nutrient degradation. In contrast, the heterofermentative lactic acid bacteria inoculant increased lactic acid bacteria populations but did not result in improved aerobic stability or reduced dry matter losses.

These findings demonstrate that additive effects in this silage system are additive-specific and strongly dependent on baseline fermentation conditions. When adequate fermentation is achieved by the basal ration, chemical preservatives provide greater benefits for minimizing nutrient losses, whereas biological additives primarily modify microbial populations without additional gains in aerobic stability. This study provides robust experimental evidence supporting the targeted use of chemical additives to enhance preservation efficiency in high-moisture total mixed ration silage containing wet Distillers bran plus solubles.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical statements

Not applicable.

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