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Effects of Different Dry Matter Levels and Additives on the Fermentation Quality and *In Vitro* Digestibility of Legume-Cereal Forage Crop Mixture Silage

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ABSTRACT

This study aimed to evaluate the effects of different dry matter (DM) levels and additive applications on the fermentation characteristics, chemical composition, microbiological profile, aerobic stability, and *in vitro* digestibility of silages prepared from a mixture of legume and cereal forage crops. The forage material, consisting of a legume-cereal mixture, was divided into two portions after harvest. One half was directly ensiled with the addition of various additives, while the other half was artificially wilted at 30 °C for 3 hours prior to additive application and ensiling. The additives tested in this study included: (1) Control (no additive), (2) Homofermentative lactic acid bacteria (3) Heterofermentative lactic acid bacteria (4) Sodium diacetate and (5) Resin. The treated silages were vacuum-sealed in plastic bags (seven replicates per group) and incubated for 60 days under laboratory conditions. After fermentation, a 7-day aerobic stability test was performed. Chemical, microbiological, and *in vitro* evaluations were carried out on both fresh and fermented silage samples.The results demonstrated that additive applications significantly improved silage quality by increasing DM, pH, and water-soluble carbohydrate (WSC) contents, while reducing NH_3-N/TN ratio and dry matter lost (DML) ($P<0.05$). Similar positive effects were observed with wilting. Additives did not significantly influence the organic acid profile (lactic, acetic, propionic, and butyric acids) ($P>0.05$). In terms of chemical composition, additives increased crude protein (CP) and neutral detergent fiber (NDF) contents while decreasing crude fiber (CF), acid detergent fiber (ADF), and hemicellulose (HCEL) ($P<0.05$). Microbiological analysis showed that additives reduced yeast populations and enhanced lactic acid bacteria (LAB) counts ($P<0.05$). Aerobic stability was also improved, particularly through reduced pH increases during air exposure. However, neither additives nor wilting had significant effects on in vitro gas production, metabolic energy (ME), or organic matter digestibility (OMD) ($P>0.05$). In conclusion, additives and the drying process had a positive effect on silage quality. In particular, resin and heterofermentative LAB additives stood out in terms of increasing microbial stability, inhibiting yeast growth, and delaying aerobic spoilage.

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Introduction

Qualitative roughage production in animal husbandry is one of the cornerstones of sustainable productivity and animal welfare. One of the most common practices to ensure that roughage is available in consistent quality throughout the year is silage production. Silage is a type of feed obtained by fermenting forage crops under anaerobic conditions and storing them, characterized by high moisture content and high digestibility. However, the nutrient content and stability of silage are significantly influenced by the characteristics of the initial material and the processes involved in the silage production process (McDonald et al., 1991; Kung et al., 2018).

Forage crops utilized in silage production exhibit different fermentation characteristics due to species and intraspecific variations. Legume and cereal mixtures have emerged as notable alternatives in ruminant nutrition in recent years due to their balanced protein and energy content. However, the high buffering capacity and moisture content of these mixtures can challenge achieving the desired level of fermentation (Weinberg and Muck, 1996). Therefore, in silage production, it is necessary to both regulate the dry matter content of the feed material and optimise the fermentation process using microbial and chemical additives.

The wilting process, which is applied to increase the dry matter level, reduces the moisture content of the silage material, limiting the growth of undesirable fermentative bacteria and creating a more favorable environment for lactic acid bacteria (LAB) (Wilkinson and Davies, 2013). However, the use of additives to direct the silage microbiology is also of great importance in preventing aerobic instability and nutrient losses. Homofermentative LAB strains reduce the growth of proteolytic microorganisms by causing a rapid pH decline, while heterofermentative LAB strains increase aerobic stability by suppressing yeast and mold growth through acetic acid production (Filya, 2003; Koc et al., 2017; Besharati et al., 2021). Among chemical additives, sodium diacetate (SDA), the salt form of acetic acid, has the potential to enhance silage stability due to its broad-spectrum antimicrobial effect (Kung et al., 2003; Akça et al., 2019; Esen et al., 2024).

In recent years, plant-based compounds containing phytochemicals have been gaining increasing attention in silage technology as environmentally friendly and natural additives. In this context, natural plant products such as resin, with their rich phenolic component structure, both limit microbial growth and contribute to the preservation of nutrients through their antioxidant effects (Keeling and Bohlmann, 2006; Franco and Rinne, 2023a¸ Franco and Rinne, 2023b). However, studies on the use of resin as a silage additive are limited, and further research is needed in this area.

In this context, this study examined the effects of different additives (homofermentative LAB, heterofermentative LAB, sodium diacetate, and resin) on the fermentation characteristics, chemical and microbiological composition, aerobic stability, and *in vitro* digestibility parameters of silage obtained from a legume-cereal forage crop mixture.

Materials and Methods

Plant Material and Preparation of Silages

The legume and cereal forage crop mixture that makes up the trial material was harvested on May 28, 2024. According to the botanical composition evaluated after harvesting, the forage mixture consists of 28% legumes and 72% cereals. The legume group consists of 11% Hungarian vetch and 17% forage pea, while the cereal group comprises 23% triticale, 22% oat, and 27% wheat.

Following harvesting, 100 kg of material was brought to the laboratory, half of which was immediately ensiled with the addition of additives. The other half was artificially wilted for 3 hours at an air temperature of 30 °C and ensiled with the addition of additives. The composition and microbial count of the feed materials were evaluated prior to ensiling. Samples were taken from both dry matter contents before the treatment was applied. A CAS CVP 260 PD brand vacuum machine was used to vacuum-pack the samples.

Silage Additive

- Control: No additive
- Homofermentative LAB (^{Ho}LAB): (1188 Pioneer®, USA). Contains *Lactobacillus plantarum* and has been added to silage at a rate of $3x10^6$ cfu/g in accordance with the manufacturer's recommendation.
- Heterofermentative LAB (^{het}LAB): (NCIMB 40788–CNCM I-4323; Lalsil AS, Lallemand Inc., Canada). Contains *Lactobacillus buchneri* and was added to silage at a rate of $3x10^8$ cfu/g according to the manufacturer's recommendation.
- Sodium diacetate (SDA): Contains acetate and acetic acid (E262) (Alfasol) 5 g/kg
- Resin: GCC Protek's colophony resin (gum rosin) 0.78 g/kg

A total of 70 packages, with 7 replicates per group, were left to ferment under laboratory conditions (26±2 °C) for 60 days. An aerobic stability test was applied to the silage opened on the 60th day for 7 days.

Chemical and Microbiological Analyses

Chemical and microbiological analyses were performed on the initial materials and feed samples obtained on the 60th day of silage. pH measurements were performed according to the method described by Chen et al. (1994). Dry matter (DM), crude protein (CP), crude ash (CA), and crude fiber (CF) analyses were determined according to the methods reported by Akyıldız (1984). Lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) analyses were conducted according to the Supelco (1998) method. Water-soluble carbohydrate (WSC) levels were analyzed according to the protocols proposed by Dubois et al. (1956), while ammonia nitrogen (NH_3-N) levels were analyzed according to the methods proposed by Anonymous (1986). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents, as well as hemicellulose (HCEL) value, were calculated based on the Goering and Van Soest (1983) methodology, using the difference between NDF and ADF as the basis. Microbiological analyses were conducted in accordance with the standard analysis procedures reported by Seale et al. (1990) for LAB, yeast, and mold counts.

In Vitro Gas Production

The Gas Production (Hohenheim) Technique developed by Menke and Steingass (1988) was used to evaluate the *in vitro* digestibility characteristics of silage samples. In this method, approximately 0.200 ± 0.010 g of each feed sample was taken and placed at the bottom of special 100 mL glass syringes. To ensure the accuracy of the experiments, blank groups and standard feed samples were also included in the gas production calculations. All feed samples were tested in triplicate. To initiated fermentation, 10 mL of rumen fluid and 20 mL of buffer solution mixture were added to each syringe, which were then incubated in a water bath at 39 °C. Gas production was measured at 3, 6, 12, 24, and 48 hours of incubation. The amount of gas produced was collected from the syringes using a separate syringe and analyzed using a methane analyzer (Sensors Europe Analysentechnik GmbH, Erkath, Germany) to determine methane (CH_4) production.

Based on the obtained data, the metabolic energy (ME) level of the feeds were calculated using the formulas reported by Blümmel and Ørskov (1993), while the organic matter digestibility (OMD) was calculated using the method proposed by Menke et al. (1979).

$$ME = 2.2 + 0.136 \times GP + 0.0057 \times CP + 0.00029 \times EE (1)$$

$$OMD = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times CA (2)$$

Statistical Analyses

In the statistical evaluation of the data, one-way analysis of variance was used to determine the differences between groups, and Duncan's multiple comparison test was used to compare group effects. Differences were considered significant at P≤0.05. Statistical analyses were performed using software (SPSS v18).

Results

The analysis results related to the fermentation parameters of silage prepared from legume and cereal forage crops are presented in Table 1. Upon analysis of Table 1, it was determined that the additives had no significant effect on the DM content of the silages (P>0.05). However, both wilting and the use of additives significantly affected the pH, NH_3-N/TN ratio, WSC, and DML values (P<0.05). In general, additives significantly reduced the NH_3-N/TN ratio, while increasing WSC content and DML. Wilting application also resulted in higher pH, WSC, and DML values compared to unwilted groups (P<0.05). Regarding organic acids, no significant changes were observed in AA, PA, and BA concentrations (P>0.05); however, the LA content was significantly influenced by both additive treatments and wilting (P<0.05).

The results regarding the chemical composition of legume–cereal silages are presented in Table 2. Statistical analysis revealed that both wilting and additive applications, as well as their interaction, significantly affected all chemical parameters (CP, CF, CA, NDF, ADF, ADL, and HCEL) (P<0.05). In general, the use of additives resulted in significant alterations in nutrient composition, leading to increases in CP and NDF, while some treatments showed reductions in CF, CA, ADF, and HCEL compared to the control (P<0.05). Wilting application also caused significant increases in CP, CF, CA, NDF, ADF, ADL, and HCEL values compared with unwilted silages (P<0.05).

The results related to the microbiological characteristics of silages are presented in Table 3. Statistical analysis indicated that wilting had no significant effect on yeast populations (P>0.05), whereas the use of additives significantly reduced yeast counts compared with the control (P<0.05). On the other hand, wilting application significantly influenced LAB populations, resulting in higher counts in wilted silages compared to unwilted ones (P<0.05). In addition, the effect of additives on LAB counts was found to be statistically significant (P<0.05), and certain additives contributed to the increase of LAB populations.

The results of the aerobic stability test for silages are presented in Table 4. The results of the aerobic stability test indicated that the use of additives significantly decreased the pH values of silages during the 7-day aerobic exposure period (P<0.001). In contrast, wilting significantly increased the aerobic pH values compared to unwilted silages (P<0.001). Moreover, a significant interaction between wilting status and additives was observed (P<0.001), suggesting that the effectiveness of additives in controlling pH varied depending on the wilting status. However, the effects of additives and wilting on yeast and mold counts were not statistically significant (P > 0.05).

The *in vitro* GP, CH_4, ME, and OMD values of silages opened on day 60 are presented in Table 5. The additive supplementation had a significant effect on the GP_3, GP_6, and GP_{24} values (P<0.05), whereas GP_{12} was mainly affected by the interaction between wilting and additives (P< 0.05).

Table 1. Results of analysis of fermentation parameters of silage opened on the 60th day of fermentation

WS	Additive	DM%	pH	NH_3-N/TN g/kg DM	WSC g/kg DM	LA g/kg DM	AA g/kg DM	PA g/kg DM	BA g/kg DM	DML %
Unwilted	FM	26.93	6.38	-	15.45	-	-	-	-	-
	Control	26.29	3.98a	33.87b	14.57de	31.47	5.99	0.06	0.43ab	3.55a
	^{Homo}LAB	25.29	3.87d	37.83ab	11.47e	27.71	5.07	0.02	0.78a	3.10abcd
	^{Het}LAB	25.89	3.89cd	39.21ab	15.10de	32.32	5.64	0.03	0.76a	2.90bcd
	SDA	25.74	3.91bc	40.00a	12.59e	28.58	6.49	0.01	0.21ab	3.20abc
	Resin	25.30	3.90bcd	38.29ab	18.31de	26.98	5.22	0.03	0.23ab	3.30abc
Wilted	FM	31.85	6.52	-	18.00	-	-	-	-	-
	Control	24.75	3.99a	36.64ab	13.49de	28.43	4.54	0.03	0.39ab	3.35ab
	^{Homo}LAB	25.75	3.93b	35.72ab	21.30cd	27.43	5.34	0.01	0.04b	2.80cd
	^{Het}LAB	25.65	3.93b	37.07ab	30.20b	32.87	5.93	0.01	0.39ab	2.60d
	SDA	25.14	3.92bc	39.00ab	40.83a	29.56	6.41	0.01	0.09ab	3.30abc
	Resin	24.90	3.92bc	37.28ab	29.03bc	26.91	5.24	0.02	0.67ab	2.90bcd
SEM		0.199	0.008	0.466	2.131	0.557	0.179	0.004	0.065	0.068
P										
WS		0.345	0.000	0.334	0.000	0.628	0.569	0.069	0.063	0.005
Additive		0.924	0.000	0.029	0.000	0.006	0.147	0.099	0.067	0.000
WS*Additive		0.752	0.009	0.218	0.000	0.508	0.461	0.520	0.009	0.179

WS: Wilting status, FM: Fresh material, ^{Homo}LAB: Homofermentative lactic acid bacteria, ^{Het}LAB: Heterofermentative lactic acid bacteria, SDA: Sodium diacetate, DM: Dry matter, NH_3-N: Ammonia nitrogen, TN: Total nitrogen, WSC: Water-soluble carbohydrate, LA: Lactic acid, AA: Acetic acid, PA: Propionic acid, BA: Butyric acid, DML: Dry matter loss. SEM: Standard error of mean, $^{a-e}$ Differences between groups with different letters in the same column are statistically significant.

Table 2. Results of chemical analysis of silage opened on the 60th day of fermentation (DM%)

WS	Additive	CP	CF	CA	NDF	ADF	ADL	HCEL
Unwilted	FM	17.09	38.78	8.25	73.25	33.01	5.77	40.24
	Control	17.13c	38.83g	8.85cd	73.35f	33.06c	5.76h	40.23gh
	HomoLAB	16.17de	38.24h	10.51ab	73.48f	32.77d	5.46ı	40.70f
	HetLAB	16.14de	38.89fg	10.49ab	72.29g	32.15g	6.74e	40.14h
	SDA	15.62e	38.89fg	9.69bc	74.72b	33.52b	5.37j	41.19e
	Resin	16.06de	41.59a	6.79f	75.86a	33.51b	8.07c	42.34b
Wilted	FM	16.17	38.24	8.45	73.48	32.78	5.46	40.70
	Control	17.18bc	41.31b	8.05de	74.56c	31.71h	9.58a	42.83a
	HomoLAB	17.41b	39.81d	5.55g	73.96e	33.71a	6.10g	40.25g
	HetLAB	18.08a	39.04ef	10.54a	74.21d	32.39e	6.65f	41.82d
	SDA	16.14de	40.58c	10.72a	74.10de	32.16g	8.42b	41.94c
	Resin	16.64cd	39.14e	7.88e	74.06e	32.28f	6.86d	41.73d
SEM		0.169	0.250	0.394	0.204	0.151	0.302	0.208
P								
WS		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Additive		0.000	0.000	0.000	0.000	0.000	0.000	0.000
WS*Additive		0.000	0.000	0.000	0.000	0.000	0.000	0.000

WS: Wilting status, FM: Fresh material, HomoLAB: Homofermentative lactic acid bacteria, HetLAB: Heterofermentative lactic acid bacteria, SDA: Sodium diacetate, DM: Dry matter, CP: Crude protein, CF: Crude Fiber, CA: Crude Ash, ADF: Acid-detergent fiber, NDF: Neutral detergent fiber, ADL: Acid-detergent lignin: HCEL: Hemicellulose, SEM: Standard error of mean, a–j Differences between groups with different letters in the same column are statistically significant.

Table 3. Results of microbiological analysis of silage opened on the 60th day of ensiling (cfu/g DM)

WS	Additive	LAB	Yeast
Unwilted	FM	4.47	7.48
	Control	3.66b	4.02ab
	HomoLAB	4.33ab	3.30b
	HetLAB	4.25ab	3.40b
	SDA	3.87b	3.50b
	Resin	4.13ab	3.20b
Wilted	FM	5.08	7.22
	Control	4.07ab	4.55a
	HomoLAB	4.01ab	3.20b
	HetLAB	4.84a	3.70ab
	SDA	4.46ab	3.50b
	Resin	4.23ab	3.10b
SEM		0.081	0.106
P			
WS		0.026	0.307
Additive		0.029	0.001
WS*Additive		0.088	0.393

WS: Wilting status, FM: Fresh material, HomoLAB: Homofermentative lactic acid bacteria, HetLAB: Heterofermentative lactic acid bacteria, SDA: Sodium diacetate, LAB: Lactic acid bacteria, SEM: Standard error of mean, a–b Differences between groups with different letters in the same column are statistically significant.

Table 4. Aerobic stability test results for silages

WS	Additive	pH	Yeast cfu/g DM	Mould cfu/g DM
Unwilted	Control	4.49cd	4.65ab	2.00
	HomoLAB	4.46d	4.55ab	0.00
	HetLAB	4.35de	4.40ab	0.00
	SDA	4.31de	4.30b	0.00
	Resin	4.96a	4.93a	0.00
Wilted	Control	4.66bc	4.10b	1.95
	HomoLAB	4.30de	4.30b	0.00
	HetLAB	4.23e	4.22b	1.75
	SDA	4.18e	4.20b	2.00
	Resin	4.69b	4.20b	1.85
SEM		0.054	0.069	0.314
P				
WS		0.001	0.009	0.103
Additive		0.000	0.488	0.438
WS* Additive		0.001	0.400	0.693

WS: Wilting status, DM: Dry matter, HomoLAB: Homofermentative lactic acid bacteria, HetLAB: Heterofermentative lactic acid bacteria, SDA: Sodium diacetate, SEM: Standard error of mean;a–e Differences between groups with different letters in the same column are statistically significant.

Table 5. *In vitro* gas production rates (ml/200 mg DM), ME, and OMD results of silages opened on the 60th day of fermentation

WS	Additive	GP_3	GP_6	GP_{12}	GP_{24}	CH_4	ME	OMD
Unwilted	Control	11.50abc	18.00ab	21.50a	32.50b	4.94ab	6.74b	51.64b
	^{Homo}LAB	12.00abc	15.50ab	24.00a	33.00b	4.68bc	6.85b	52.17b
	^{Het}LAB	7.00c	12.50b	18.00ab	32.00b	4.07cd	6.71b	51.27b
	SDA	8.00bc	13.50b	14.00b	25.00c	3.52d	5.75c	44.76c
	Resin	16.00a	21.50a	24.00a	39.00a	5.73a	7.67a	57.22a
Wilted	Control	9.50bc	17.00ab	20.50ab	31.50b	4.84bc	6.60b	50.69b
	^{Homo}LAB	8.00bc	15.50ab	19.00ab	31.00b	4.43bc	6.60b	50.63b
	^{Het}LAB	8.00bc	14.50ab	18.00ab	29.00bc	4.04cd	6.34bc	49.48bc
	SDA	12.00abc	19.50ab	22.00a	31.00b	4.79bc	6.58b	50.40b
	Resin	13.00ab	17.50ab	20.00ab	31.00b	4.57bc	6.59b	50.44b
SEM		0.659	0.682	0.794	0.786	0.135	0.107	0.688
P								
WS		0.200	0.518	0.747	0.021	0.576	0.037	0.077
Additive		0.000	0.018	0.158	0.000	0.000	0.000	0.001
WS× Additive		0.009	0.051	0.046	0.000	0.000	0.001	0.001

WS:Wilting status, ^{Homo}LAB: Homofermantative lactic acid bacteria bacteria, ^{Het}LAB: Heterofermantative lactic acid bacteria bacteria, SDA: Sodium diacetate, GP: Gaz production, CH_4: Methane (ml), ME: Metabolic energy (MJ/Kg, DM), OMD: Organic matter digestibility (%); $^{a-c}$ Differences between groups with different letters in the same column are statistically significant, SEM: Standard error of mean

During early incubation times (3 and 6 hours), the Resin group showed the highest gas production values (16.00 and 21.50 ml/200 mg DM, respectively), while silage treated with SDA showed the lowest values (8.00 and 13.50 ml/200 mg DM, respectively). After 24 hours of incubation, gas production was the highest in the Resin group (39.00 ml/200 mg DM), while the SDA group showed the lowest value (25.00 ml/200 mg DM).

Methane production was also significantly affected by the additives ($P<0.001$). The lowest value was detected in silage treated with SDA (3.52 ml), while silage treated with resin displayed the highest methane production (5.73 ml). A strong interaction effect ($P<0.001$) indicated that methane reduction was influenced by both the wilting process and the additive applied.ME and OMD values were significantly improved by additives ($P<0.01$).

Resin additive resulted in the highest ME (7.67 MJ/kg DM) and OMD (57.22%) values, while SDA showed the lowest ME (5.75 MJ/kg DM) and OMD (44.76%) values. In contrast, the control, ^{Homo}LAB, and ^{Het}LAB groups presented moderately high values that were not statistically different from from each other. Although wilting alone had a limited effect, it significantly affected GP_{24} and ME values ($P<0.05$). Furthermore, significant interactions between wilting and additives were observed for ME and OMD ($P<0.01$), indicating that the effectiveness of additives varied depending on the wilting process of silage.

Discussion

In this study, the effects of dry matter levels and different additives on fermentation parameters, chemical composition, microbiological composition, aerobic stability, and *in vitro* digestibility in silage prepared from legume and cereal mixed forage crops were comprehensively evaluated. Considering the many factors that influence silage quality, the data obtained revealed that these factors interact in a complex manner.

At the beginning of the study, significant differences were observed in the contents of fresh and wilted silage materials. In particular, while the DM ratio was 26.93% in the fresh group, this ratio increased to 31.85% after a 3-hour wilting process. This finding underlines the importance of the wilting process in enhancing the suitability of silage material for ensiling. The optimal DM level is generally reported to be between 30–35%, and this range is considered critical for a good fermentation process (Kung et al., 2018; Muck et al., 2018). Additionally, the WSC level increased in the wilted material. This increase may positively influence fermentation by providing a more accessible energy source for microorganisms (Weinberg and Muck, 1996).

The fact that the epiphytic LAB count was higher in wilted material than in fresh material indicates that the wilting process may be decisive in increasing the activity of the natural LAB population. However, no difference was observed in yeast and mold growth, which is a positive indicate that the silage material was hygienic and of high quality at the initial stage.

The pH values of silages were examined, and it was found that the additive and wilting process were effective. The pH value was below the critical limit of 4.2 in all groups, indicating that fermentation was successful. It was observed that the pH decreased further with homo- and heterofermentative LAB additives. It is known that homofermentative LAB (e.g., *lactobacillus plantarum*) rapidly lower pH through high levels of lactic acid production, thereby preventing aerobic spoilage (Filya, 2003). Heterofermentative species, on the other hand, support aerobic stability through acetic acid production but may cause a slower pH decrease (Driehuis et al., 1999).

Ammonia nitrogen (NH_3-N/TN) levels remained low, particularly in the treated and additive-treated groups, indicating that proteolytic activity was inhibited and protein preservation was more effective (Kleinschmit et al., 2005). The resin additive did not increase NH_3-N levels, indicating that the resin did not promote protein degradation. The antimicrobial structure of the resin (Keeling and Bohlmann, 2006) suggests that this additive may limit proteolytic degradation by suppressing the growth of saprophytic bacteria.

Lactic acid levels changed depending on both the type of additive and the wilting process. The highest LA production was obtained in the wilted and SDA added group, indicating that SDA did not inhibit fermentation and may have supported the suitable microbial flora. On the other hand, higher AA production was observed in the ^{het}LAB-added groups, supporting the notion that these additives contribute to aerobic stability (Akça et al., 2019; Esen et.al. 2024). Propionic and butyric acid values were low in all groups, indicated that undesirable clostridial fermentation did not occur.

Additives have caused significant differences in the chemical composition of silage. In particular, CF, NDF and ADF levels have been affected by the addition of additives. The preservation of fiber fractions is important in terms of the animal's rumination time, salivation and rumen function (Van Soest, 1994). The ^{het}LAB additive contributes to the preservation of high values in fiber components, while the SDA additive significantly increases these values. This can be interpreted as SDA limiting microbial activity and thereby restricting cellular degradation. Additionally, the absence of significant changes in ADL levels with the resin additive indicates that lignin degradation is independently of additives.

There was no significant difference between the groups in terms of crude protein content, but the wilting process reduced protein loss with some additions. This shows that the wilting process better preserves protein structure by limiting the activity of proteolytic enzymes (Kung et al., 2018).

Microbiological analyses have revealed the significant effects of additives, particularly in terms of LAB and yeast growth. ^{het}LAB and resin additives have effectively limited yeast growth. Various studies have highlighted that the terpenoid compounds contained in the resin inhibit the growth of yeast and mold by causing damage to their cell walls (Kairenius et al., 2022; Franco and Rinne, 2023a,b). In terms of LAB counts, the heterofermentative additive is prominent; this additive has further increased the LAB population, particularly in the wilting material, thereby enhancing fermentation efficiency.

In a 7-day test on the aerobic stability of silage, the effects of additives in limiting aerobic deterioration were clearly observed. The resin and ^{het}LAB additives-maintained pH stability and reduced the yeast population. The yeast count, indicated as a measure of aerobic stability, remained below 10^5 cfu/g, indicating that spoilage was prevented (Wilkinson and Davies, 2013). In this context, it can be concluded that the resin additive is a potential natural and effective preservative alternative to existing additives.

The *in vitro* gas production, ME and OMD results of silages were examined, and it was observed that the interactions between additives and wilting were significant. Silage prepared with resin additives reached the highest GP and ME values. This can be explained by the resin's potential to preserve digestible components. Additionally, a direct relationship between GP and CH_4 production was observed, indicating active ruminal fermentation (Getachew et al., 2004). However, the increase in methane production should be evaluated from an environmental perspective; it is recommended that resin additives be integrated into environmentally friendly feed strategies with appropriate dosages and combinations (Jayanegara et al., 2012).

Conclusion

The results of the research have shown that additives and the wilting process positively affect silage quality. Resin and ^{Het}LAB have been particularly effective in increasing microbial stability, inhibiting yeast growth, and delaying aerobic spoilage. Resin improved digestibility parameters and energy values, but also increased methane production. This highlights the need for further *in vivo* studies to clarify the long-term effects on animal performance and environmental sustainability.

Declarations

Ethical Consideration

The study does not require ethics committee approval**.**

Author Contribution Statement

The authors declare that they have contributed equally to this manuscript.

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Conflict of Interest

The author declared that there is no conflict of interest.

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