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Effects of Native *Lactobacillus brevis* (MF098783) Strain on the Fermentation Profile, Aerobic Stability, and Digestibility of Wheat Straw Silage

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ABSTRACT

This study aimed to evaluate the effects of the heterofermentative *Lactobacillus brevis* (MF098783) strain on the fermentation characteristics, aerobic stability, chemical and microbiological composition, and in vitro digestibility of wheat straw silage. Wheat straw was ensiled with three different concentrations of L. brevis (10^6, 10^8, and 10^9 cfu/g), forming the treatment groups (WSLB6, WSLB8, WSLB9), and these were compared with a control group (WS). A total of 64 silage samples were fermented under anaerobic conditions for 90 days. After fermentation, physical (pH, temperature, color), chemical (DM, CP, EE, CF, ADF, NDF, etc.), microbiological (LAB, yeast, mold), energy, and in vitro digestibility parameters (IVOMD, GP, ME, NEL) were analyzed. Statistical analyses were performed using ANOVA and Duncan's multiple range test with SAS software. The L. brevis inoculation significantly reduced the pH values of the silages ($P<0.001$), indicating improved fermentation quality. The WSLB6 and WSLB8 groups showed increased crude protein and ether extract contents, and significantly lower ADF, ADFom, and crude fiber values ($P<0.05–0.001$) compared to the control. These groups also demonstrated higher metabolizable energy (ME), net energy (NEL, NEM), and in vitro digestibility. Microbiological analyses revealed reduced yeast counts and no mold growth in the inoculated groups, whereas the control had higher yeast levels. Post-aerobic stability assessments confirmed better preservation and microbial control in WSLB6 and WSLB8, reflected by lower pH and yeast counts. In conclusion, *Lactobacillus brevis* inoculation, particularly at 10^6 and 10^8 cfu/g concentrations, significantly enhanced the fermentation quality, nutritional value, and digestibility of wheat straw silage. These findings suggest that L. brevis holds strong biotechnological potential as a silage additive for enhancing feed quality and supporting sustainable livestock production.

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Introduction

Silage is an alternative roughage with high nutritional value, produced by fermenting various feed crops under anaerobic conditions. During this process, carbohydrates are converted into organic acids such as lactic acid, contributing to the preservation of silage. Silage, primarily made from crops like corn, wheat, and sorghum, is widely preferred due to its low labor requirements, long-term storage capability, and ease of preparation (Demir and Elmalı, 2016; Kızılşimşek et al., 2016; Özdemir and Okumuş, 2021). Wheat serves not only as a staple food for humans but also as an important feed source for livestock. Its high protein, carbohydrate, and fiber content, along with its ease of transport, wide adaptability, and lower water requirements, make it widely used in animal husbandry (Atak, 2017).

Lactic acid bacteria (LAB) have been reported to significantly impact silage fermentation quality, aerobic stability, and animal performance (Muck 2010; Shah et al., 2017, 2018; Kim et al., 2018). Bacterial inoculants, commonly used in silage production, typically include microorganisms from the genera *Lactobacillus*, *Pediococcus*, and *Enterococcus* naturally present in the silage environment. These inoculants facilitate the fermentation process based on the chemical and physical properties of the ensiled material, thereby improving the microbiological quality, stability, and nutritional value of the silage (McDonald et al., 1991).

Studies aimed at improving silage digestibility have shown that species such as *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and

Pediococcus acidilactici have a significant impact on silage fermentation. The use of these bacterial species as additives not only improves silage quality but also offers a sustainable alternative to address feed scarcity (Roberto et al., 2016).

This study aimed to evaluated the effects of the heterofermentative lactic acid bacterium *Lactobacillus brevis* on the fermentation process, aerobic stability, microbial development and *in vitro* digestibility of wheat straw silage.

Materials and Methods

Wheat straw for silage was obtained from the research fields of the Field Crops Department at Kırşehir Ahi Evran University (Latitude: 39.1286"N, Longitude: 34.1078"E). The wheat was harvested at the dough stage, chopped into lengths of 2.5–3.5 cm. After chopping, 1000 g of plant material was placed in 2 kg plastic bags, and heterofermentative *Lactobacillus brevis* MF098783, isolated from pickles, was sprayed at concentrations of 1×10^6, 1×10^8, and 1×10^9 cfu/g. After inoculation, the air in the bags was vacuumed using a vacuum device (Packtech PT-VKM-CPRO). A total of 64 silage samples were prepared with 8 replicates per group and fermented for 90 days in a dark laboratory environment at 18.5 ± 2 °C. The experimental groups were as follows (WS), wheat straw silage + *Lactobacillus brevis* 10^6 (WSLB6), wheat straw silage + *Lactobacillus brevis* 10^8 (WSLB8), and wheat straw silage + *Lactobacillus brevis* 10^9 (WSLB9). Four groups of three parallel samples each were taken from the silages whose fermentation process was completed; (1) chemical [dry matter (DM), crude ash (CA), ether extract (EE), crude protein (CP), crude fiber (CF), ADF (acid detergent fiber), NDF (neutral detergent fiber), water soluble carbohydrate (WSC)], (2) calculated parameters and energy values [DCP: Digestible crude protein, TDN: Total digestible nutrient, DE: Digestible energy, ME: Metabolic energy, NE_L: Net energy-lactation, NE_M: Net energy-maintenance, NE_G: Net energy-gain], (3) physical (temperature, color, pH), (4) microbiological parameters (lactic acid bacteria, yeast and mold counts), (5) *in vitro* digestibility and (6) statistical analyzes . DM, CP, EE, CA analysis according to AOAC (2006) standard procedures; while CF, ADF and NDF were analyzed according to Van Soest et al. (1991) (ANKOM 200 Fiber Analyzer); pH values were determined according to Chen et al. (1994); and WSC contents were measured as explained by AOAC (1990). After the silage samples were opened, *L**, *a**, and *b** color values were measured from three different parts of the silage with the Konica-Minolta CR-410 colorimeter. Based on the *a** and *b** values, the Chroma (C*, saturation index) and hue angle (h°) values were calculated according to the method described by Filik, (2020). In this study, lactic acid bacteria, yeast, and mold counts contained in the silages were determined by the method reported by Seale et al. (1990). On the fifth day after opening, the CO_2 value and pH values were measured as described by Ashbell et al. (1991). The metabolizable energy and protein values of silages with total carbohydrate (TC), hemicellulose, nitrogen free extract (NFE) and nitrogen free carbohydrate (NFC) were calculated as reported by Filik (2020). The relative feed value (RFV) and relative forage quality (RFQ) were calculated according to the reported by Kılıç and Abdiwali (2016) and Filik (2020). *In vitro* organic matter digestibility (IVOMD) and energy values of the silage samples were determined using the ANKOMRF Gas Production System (Ankom Technologies, Macedon, NY, USA) as described by Filik (2020). Statistical analyses were performed using the software package SAS (2001), variance analyse were conducted using the General Linear Model (PROC GLM) procedure according to the randomized plot experimental design. Linear relationships between groups were tested using orthogonal polynomial contrasts in the same software, and differences between groups were assessed using Duncan's Multiple Comparison Test (Genç and Soysal, 2018).

Results and Discussion

Physical analyses included temperature, pH, color parameters (*L**, *a**, *b**, ΔE*, h, C*), and water-soluble carbohydrate (WSC) contents. According to the results presented in Table 1, pH values were 6.56 for the control group (WS), and 4.73, 4.90, and 4.86 for WSLB6, WSLB8, and WSLB9, respectively. The *Lactobacillus brevis* inoculants significantly reduced pH levels ($P<0.001$), indicating that bacterial inoculation effectively lowered silage pH, as intended. The addition of lactic acid bacteria accelerates lactic acid fermentation in silage, leading to an increase in acidity (approximately pH 4), which is a critical factor in silage preservation by inhibiting the growth of undesirable microorganisms (Bozkurt Kiraz, and Kutlu, 2016; Muck, 2010). Similarly, Ertekin and Kızılşimşek (2020) reported a significant reduction in pH silages containing *Lactobacillus brevis* (pH 5.99). Desta et al. (2020) found that pH values in inoculated corn silages dropped to the 4.0–4.5 range, while the control group's pH remained above 5.0. The water-soluble carbohydrate (WSC) values were 28.13 °Brix for the control group, 27.28 for WSLB6, 28.10 for WSLB8, and 27.10 for WSLB9, with no statistically significant differences ($P>0.05$). Yang et al. (2006) noted that WSC is an important substrate for fermentation, but inoculants generally don't alter WSC levels. No statistically significant differences were observed in color parameters (*L**, *a**, *b**, ΔE*, h, C*) among the groups ($P>0.05$), indicating that *Lactobacillus brevis* application did not affect the visual properties of the silage. İnce and Vurarak (2019) reported that inoculants didn't affect color parameters in alfalfa silages, supporting these findings.

Chemical analyses included dry matter (DM), organic matter (OM), crude ash (CA), crude protein (CP), crude fat (CF), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), and ash-free ADF/NDF (ADFom, NDFom) contents (Table 2). Dry matter contents were 938.95 g/kg for the control group, 938.10 for WSLB6, 937.70 for WSLB 8, and 940.00 for WSLB 9, with no statistically significant differences. This suggests that *Lactobacillus brevis* application prevented dry matter loss but did not significantly increase content. Organic matter (OM) contents were 93.35% for the control, 92.86% for WSLB6, 92.80% for WSLB8, and 92.84% for WSLB9, with differences considered statistically significant at the threshold level CA contents were significantly higher in experimental groups compared to the control (6.65%) ($P<0.05$). This suggests that *Lactobacillus brevis* application may increase mineral content.

Table 1. Physical analysis of wheat straw silage

Parameters	WS	WSLB6	WSLB8	WSLB9	P
°C	22.78 ± 0.25	22.88 ± 0.09	23.05 ± 0.21	23.15 ± 0.21	0.5615
pH	6.56^{a} ± 0.00	4.73^{c} ± 0.02	4.90 ^{b} ±0.02	4.86^{b} ± 0.08	<0.001
WSC Brix°	28.13 ± 0.45	27.28 ± 0.26	28.10 ± 1.17	27.10 ± 1.22	0.7746
L *	47.61 ± 2.93	49.93 ± 6.66	46.84 ± 1.46	51.34 ± 1.16	0.8245
a *	3.71 ± 0.40	2.77 ± 0.43	3.29 ± 0.17	3.95 ± 1.23	0.6460
b *	17.40 ±0.87	16.90 ± 2.02	15.99 ± 0.59	18.55 ± 0.94	0.5401
ΔE *	50.84 ± 3.00	52.80 ± 6.93	49.60 ±1.52	54.78 ± 1.71	0.8029
h	77.95 ± 1.30	80.39 ± 1.45	78.38 ± 0.40	78.15 ± 3.59	0.8228
C *	17.81 ± 0.87	17.14 ± 2.01	16.32 ± 0.60	19.08 ± 0.99	0.4739

°C: Celsius; WSC: Water-soluble carbohydrate value; L: Lightness (light-dark color); a*: Redness; b*: Yellowness; ΔE*: Total color difference; h: Hue angle; C*: Chromaticity or saturation. Values with different superscripts (a, b, c) in the same column indicate statistically significant differences (P<0.01) *

Table 2. Nutrient analysis of wheat straw silage

Parameters	WS	WSLB6	WSLB8	WSLB9	P
DM (g/kg)	938.95^{b} ± 0.15	938.10^{b} ± 0.80	937.70^{b} ± 0.30	940.00^{a} ± 0.10	0.0677
OM (%)	93.35^{a} ± 0.19	92.86^{b} ± 0.01	92.80^{b} ± 0.07	92.84^{b} ± 0.00	0.0499
CA (%)	6.65^{b} ± 0.19	7.15^{a} ± 0.01	7.20^{a} ± 0.07	7.16^{a} ± 0.00	0.0499
CP (%)	10.84^{b} ± 0.06	11.17^{a} ± 0.13	11.36^{a} ± 0.04	10.77^{b} ± 0.00	0.0130
EE (%)	4.60^{c} ± 0.00	4.81^{b} ± 0.06	5.00^{a} ± 0.00	4.71^{b} ± 0.03	0.0030
CF (%)	28.72^{a} ± 0.14	27.42^{b} ± 0.12	26.90^{b} ± 0.09	28.31^{a} ± 0.17	0.0024
ADF (%)	37.09^{a} ± 0.02	34.17^{c} ± 0.17	34.29^{cb} ± 0.09	34.94^{b} ± 0.29	0.0009
ADFom (%)	30.44^{a} ± 0.21	27.03^{b} ± 0.17	27.09^{b} ± 0.02	27.78^{b} ± 0.29	0.0007
NDF (%)	64.21^{a} ± 0.55	63.67^{a} ± 0.01	63.24^{a} ± 0.06	38.28^{b} ± 0.07	0.1739
NDFom (%)	57.56^{a} ± 0.36	56.52^{c} ± 0.13	56.04^{c} ± 0.13	57.01^{b} ± 0.01	0.0193

DM: Dry Matter (g/kg); OM: Organic Matter (%); CA: Crude Ash (%); CP: Crude Protein (%); EE: Ether Extract (%); CF: Crude Fiber (%); ADF: Acid Detergent Fiber (%); NDF: Neutral Detergent Fiber (%); ADFom: Ash-free ADF; NDFom: Ash-free NDF. Values with different superscripts (a, b, c) in the same column indicate statistically significant differences (P<0.01).

Wang et al. (2009) noted that during lactic acid fermentation, the conversion of organic matter (e.g., sugars) to lactic acid may slightly reduce OM content, while the relative concentration of minerals in dry matter increases, elevating ash content. Crude protein (CP) contents were 10.84, 11.17, 11.36, and 10.77 for the control, WSLB6, WSLB8, and WSLB9, respectively, with WSLB6 and WSLB8 showing higher values than the control and WSLB9. Abdullah, (2023) reported increased CP content in wheat straw silages treated with *L. plantarum* (11.67 for WSLP9), but this increase was not observed in WSLP9 in this study, suggesting that optimal inoculant concentration may be required for protein preservation. Miron et al. (2001) indicated that silage inoculants promote microbial protein synthesis, preserving CP concentration. Ether Extract (EE) contents were highest in WSLB8 (5.00%) and lowest in the control (4.60%), with statistically significant differences. Soycan Önenç et al. (2022) reported that ethanol extract increases EE content in silages, supporting the effect of *Lactobacillus brevis* on lipid metabolism. Crude fiber (CF) contents were lower in WSLB6 (27.42%) and WSLB8 (26.90%) compared to the control (28.72%) and WSLB9 (28.31%), indicating that *Lactobacillus brevis* effectively degrades fiber fractions. The control (WS) and WSLB9 groups had the highest crude fiber (CF) content, while WSLB6 and WSLB8 showed lower values, suggesting that LAB additives aid in cellulose breakdown, thereby improving digestibility. Nishino et al. (2003) demonstrated that certain *Lactobacillus* strains positively affect silage fermentation quality by producing enzymes that break down cellulose and hemicellulose, thereby reducing fiber components and enhancing feed digestibility. ADF and ADFom values were lower in WSLB6 and WSLB8 compared to the WS and WSLB9 (P<0.001), indicating partial degradation of cellulosic structures by *Lactobacillus brevis*. Xu et al. (2024) reported that LAB inoculants reduce ADF content, improving digestibility. NDF and NDFom values showed no significant differences among groups (P>0.05), indicating limited effects of *Lactobacillus brevis* on NDF. Zhou et al. (2024) noted that lactic acid bacteria application generally does not alter NDF content but still improves overall silage quality. Borreani et al. (2018) reported that lower ADF and NDF contents positively affect feed intake and nutrient digestion, thereby enhancing animal performance.

Energy values are critical for evaluating the feeding potential of silages in livestock (Table 3). Total carbohydrate (TC) values were lower in WSLB6 (76.89%) and WSLB8 (76.45%) compared to the control (77.91%) and WSLB9 (77.37%), with statistically significant differences. Metabolizable energy (ME), net energy lactation (NE_L), and net energy maintenance (NE_M) values were higher in WSLB6 and WSLB8 (2.18-1.36) compared to the control and WSLB9 (2.16-1.34) (P<0.05). This indicates that WSLB8 is a suitable feed source for ruminants due to its high energy content. Harper et al. (2017) noted that silages with high DE and ME provide advantages for milk and meat production. Net energy gain (NE_G) values were highest in WSLB8 (0.75) and lower in the control and WSLB9 (0.73), though the difference was statistically marginal. Nitrogen-free extract (NFE) and non-fiber carbohydrate (NFC) values showed no significant differences among the groups (P>0.05). Reed et al. (2021) emphasized that high-energy silages are critical for meeting energy requirements during lactation.

Digestible dry matter (DDM), total digestible nutrients (TDN), relative feed value (RFV), and relative forage quality (RFQ) were used to evaluate the feeding quality of the silages (Table 4). DDM values were higher in WSLB6 (62.29%) and WSLB8 (62.19%) compared to WSLB9 (61.69%) and the control (60.01%). TDN, RFV, and RFQ values were also higher in WSLB8 (60.34, 91.49, 93.09%, respectively) compared to other groups, with statistically significant differences ($P<0.05$). This highlights WSLB8's superior digestibility and nutritional value. Brown and Garcia (2020) noted that silages with high DDM and TDN contents increase milk yield. Mertens and Grant (2020) emphasized that TDN is directly related to silage digestibility. Dry matter intake (DMI) values showed no significant differences among the groups.

Microbiological analyses included dry matter, lactic acid bacteria (LAB), yeast, mold, and pH values (Table 5). Dry matter contents were highest in WSLB9 (50.24%) and lowest in the control (47.60%), with statistically significant differences ($P<0.001$). This indicates that LAB addition improved fermentation quality, reducing dry matter losses. Xu et al. (2024) reported that LAB inoculants reduce dry matter loss, increasing efficiency. Lactic acid bacteria counts were $1.50*10^5$ cfu/g in the control and $1.00*10^5$ cfu/g in WSLB8. Yeast was observed in the control and WSLB6 groups, with no mold detected in any group.

Post-aerobic stability microbiological results (Table 6) showed that the control group had a high pH_2 value (6.57), while WSLB6, WSLB8, and WSLB9 had lower pH values ($P<0.001$).

This indicates that lactic acid bacteria inoculants increased silage acidity, thereby improving aerobic stability. McDonald et al. (1991) and Weinberg and Muck (1996) reported that low pH levels positively affect aerobic stability by suppressing harmful microorganisms. No significant differences were found in CO_2 production among the groups. The control group had the highest yeast count, consistent with its higher pH. Microbiological analyses after aerobic stability showed that the added microbial strain effectively prevented spoilage. Pitt and Leibensperger (1987) noted that lowering pH and maintaining an anaerobic environment significantly limit mold growth, particularly in inoculated silages.

Table 3. Energy values of wheat straw silages

Parameters	WS	WSLB6	WSLB8	WSLB9	P
NFE (%)	49.20 ± 0.12	49.47 ± 0.04	49.46 ± 0.11	49.06 ± 0.19	0.1962
NFC (%)	13.71 ± 0.80	13.22 ± 0.07	13.20 ± 0.04	13.20 ± 0.04	0.7760
TC (%)	77.90^{a} ± 0.25	76.89^{cb} ±0.07	76.45^{c} ± 0.02	77.37^{b} ± 0.02	0.0055
DE (Mcal /kg)	2.66^{b} ± 0.00	2.65^{b} ±0.01	2.66^{a} ± 0.00	2.63^{b} ± 0.00	0.0298
ME (Mcal /kg)	2.16^{b} ± 0.01	2.18^{a} ± 0.01	2.18^{a} ± 0.00	2.16^{b} ± 0.00	0.0200
NE_L (Mcal /kg)	1.34^{b} ± 0.00	1.36^{a} ± 0.01	1.36^{a} ±0.00	1.34^{b} ± 0.00	0.0097
NE_M (Mcal /kg)	1.30^{b} ±0.00	1.32^{a} ± 0.01	1.32^{a} ± 0.00	1.30^{b} ±0.00	0.0097
NE_G (Mcal /kg)	0.73^{b} ± 0.01	0.74^{b} ±0.00	0.75^{a} ± 0.01	0.73^{b} ± 0.01	0.0635

NFE: Nitrogen-free extract (g/kg); NFC: Non-fiber carbohydrate (g/kg); TC: Total carbohydrates (g/kg); DE: Digestible energy (Mcal/kg); ME: Metabolizable energy (Mcal/kg); NE_L: Net energy lactation (Mcal/kg); NE_M: Net energy maintenance (Mcal/kg); NE_G: Net energy gain (Mcal/kg). Values with different superscripts (a, b, c) in the same column indicate statistically significant differences ($P<0.01$).

Table 4. Relative feed value and feed quality of wheat straw silages

Parameters	WS	WSLB6	WSLB8	WSLB9	P
DDM (%)	60.01^{c} ± 0.02	62.29^{a} ± 0.03	62.19^{b} ± 0.07	61.69^{b} ± 0.22	0.0009
DMI (%)	1.87^{a} ± 0.02	1.88^{a} ± 0.00	1.90^{a} ± 0.00	1.87^{a} ± 0.00	0.2564
TDN (%)	59.68^{b} ± 0.07	60.11^{a} ± 0.14	60.34^{a} ± 0.05	59.63^{b} ± 0.02	0.0093
RFV	86.95^{c} ± 0.54	91.00^{b} ±0.19	91.49^{a} ± 0.00	89.42^{b} ± 0.35	0.0049
RFQ	90.68^{b} ± 0.67	92.10^{a} ± 0.21	93.09^{a} ± 0.02	90.65^{b} ± 0.04	0.0201

DDM: Digestible Dry Matter (%); DMI: Dry Matter Intake (% of live weight); TDN: Total Digestible Nutrients (%); RFV: Relative Feed Value; RFQ: Relative Forage Quality. Values with different superscripts (a, b, c) in the same column indicate statistically significant differences ($P<0.01$).

Table 5. Microbiological analyses and dry matter of wheat straw silage

Parameters	WS	WSLB6	WSLB8	WSLB9	P
ADM	47.60^{c} ± 0.16	49.26^{b} ± 0.07	49.05^{b} ± 0.02	50.24^{a} ± 0.37	<0.0001
LAB (10^5 cfu/g)	1.50 ±0.50	N/A	1.00 ± 0.00	N/A	0.4226
Yeast (10^5 cfu/g)	1.00 ± 0.00	1.00 ± 0.00	N/A	N/A	N/A
Mold (10^5 cfu/g)	N/A	N/A	N/A	N/A	N/A

ADM: Air-Dry Matter (g/kg); Values with different superscripts (a, b, c) in the same column indicate statistically significant differences ($P<0.01$).

Table 6. pH_2, CO_2 and microorganism results of wheat straw silages after aerobic stability

Parameters	WS	WSLB6	WSLB8	WSLB9	P
pH_2	6.57^{a} ± 0.00	4.86^{b} ± 0.02	4.86^{b} ± 0.02	4.86^{b} ± 0.03	<.000
CO_2	4.28 ± 0.26	3.96 ± 0.31	3.40 ± 0.13	3.84 ± 0.18	0.1965
Yeast (10^5 cfu/g)	2.67 ± 0.67	1.00 ± 0.00	N/A	N/A	0.3377

pH_2: Aerobic Stability post-pH CO_2, Aerobic Stability Post-CO_2, Yeast 10^5 cfu/g: Post-aerobic stability yeast.

Table 7. Total gas amount of wheat straw silage measured in 24 hours with the In vitro Gas Production

Parameters	WS	WSLB6	WSLB8	WSLB9	P
IVOMD (%)	34.23 ± 0.05	37.67 ± 0.48	36.65 ± 0.20	34.49 ± 2.21	0.1711
MEgp (MJ/kg ^{DM})	5.15 ± 0.02	5.69 ± 0.19	5.53 ± 0.08	5.18 ± 0.87	0.1741
NE_L (MJ/kg ^{DM})	2.62 ± 0.00	3.00 ± 0.06	2.89 ± 0.02	2.64 ± 0.25	0.1804
GP (ml/200 mg ^{DM})	21.00 ± 0.05	25.03 ± 0.57	23.80 ± 0.23	21.28 ± 2.62	0.1754

IVOMD: In vitro Organic Matter Digestibility; ME: Metabolizable Energy; NE_L: Net Energy Lactation; GP: Gas Production.

In vitro gas production technique results (Table 7) evaluated *in vitro* organic matter digestibility (IVOMD), metabolizable energy (MEgp), net energy lactation (NE_L), and gas production (GP) of wheat straw silages. The WSLB6 group had the highest IVOMD (37.67%), while the control and WSLB9 groups had similar IVOMD values, and WSLB8 had a lower value. For MEgp, WSLB6 had the highest value (5.69 MJ/kg ^{DM}), while the control and WSLB9 had lower values. For NE_L, WSLB6 had the highest value (3.00 MJ/kg ^{DM}). For GP, WSLB6 had the highest GP (25.03 ml/200 mg ^{DM}). Muthia et al. (2021) reported a gas production of 19.1 mL/g for wheat straw silage after 24 hours of incubation, which is comparable to the in vitro results in Table 7.

Conclusion

This study evaluated the effects of *Lactobacillus brevis* (WSLB6, WSLB8, and WSLB9) inoculants on the physical, chemical, microbiological, and digestibility properties of wheat straw silage. The findings demonstrate that *Lactobacillus brevis* strains significantly improved silage quality in multiple aspects. Notably, WSLB6 and WSLB8 groups exhibited significantly lower pH levels ($P<0.001$), enhancing silage preservation and preventing the growth of undesirable microorganisms. The lack of significant differences in color parameters and water-soluble carbohydrate values indicates that the inoculants did not affect the visual properties or carbohydrate stability of the silage. Chemical analyses showed higher crude protein and crude fat contents in WSLB6 and WSLB8 compared to the control, indicating a positive contribution of the inoculants to protein and lipid metabolism. Lower ADF, ADFom, and crude fiber values in these groups ($P<0.001$) suggest that microbial activity effectively degraded fiber fractions, thereby increasing feed digestibility. The limited change in NDF values indicates that *Lactobacillus brevis* primarily affects cellulosic components. In terms of energy content, WSLB6 and WSLB8 exhibited higher DE, ME, NE_L, and NE_M values ($P<0.05$), confirming their suitability for ruminant nutrition. In vitro gas production analyses highlighted WSLB6 as the most effective treatment, with the highest IVOMD, MEgp, NE_L, and GP values. In conclusion, silages inoculated with *Lactobacillus brevis*, particularly WSLB6 and WSLB8, showed significant improvements in fermentation quality, nutrient content, energy value, and digestibility. These findings suggest that *Lactobacillus brevis* is a promising biotechnological additive for enhancing feed quality and animal performance in livestock production.

Declarations

Author Contribution Statement

Authors declare that they have contributed equally to the article.

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Conflict of Interest

The authors declare no conflict of interest.

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References

Abdullah, S. A. A. (2023). The effects of the use of lactobacillus plantarum in wheat produced silage on silage quality and fermentation (Master's thesis, Kirşehir Ahi Evran University (Türkiye).

Atak, M. (2017). Wheat and Turkish wheat village varieties. Mustafa Kemal University Journal of Agricultural Faculty, 22(2), 71–88.

AOAC (Association of Official Analytical Chemists), (1990). Official methods of analysis of the Association of Official Analytical Chemists. Arlington, VA: AOAC

AOAC (Association of Official Analytical Chemists), (2006). Official Methods of Analysis. 18th 437 Washington, DC. AOAC. 2263

Ashbell, G., Weinberg, Z. G., Azrieli, A., Hen, Y., & Horev, B. (1991). A simple system to study the aerobic determination of silages. Canadian Agricultural Engineering, 34, 171-175. https://library.csbe-scgab.ca/docs/journal/33/33_2_391_raw.pdf

Borreani, G., Tabacco, E., Schmidt, R. J., & Kung Jr, L. (2018). Silage review: Factors affecting dry matter intake and animal performance. *Journal of Dairy Science, 101*(5), 3950-3964.

Brown, L. A., & Garcia, J. M. (2020). Feed quality and weight gain in beef cattle: A longitudinal field study. Journal of Agricultural Science, 55(3), 321–335.

Bozkurt Kiraz, A., & Kutlu, H. R. (2016). Bakteriyel İnokulant Kullanımının Silajlarda Fermantasyon Özellikleri Üzerine Etkileri. Harran Tarım Ve Gıda Bilimleri Dergisi, 20(3), 230-238. https://doi.org/10.29050/harranziraat.259118

Chen, G., Weinberg, Z. G., & Ashbell, G. (1994). the effect of lactic acid bacteria inoculants on the fermentation of corn and wheat silage. Journal of Industrial Microbiology, 13(3), 132–136.

Demir, P.A, & Elmalı, D.A (2016). Silage Usage and Economic Necessity in Cattle Farms in Hatay Province. Animal Health Production and Hygiene, 5(1), 432-437.

Desta, ST, Urge, M., & Tadesse, Y. (2020). Effect of different inoculants on fermentation quality and aerobic stability of maize silage. *African Journal of Agricultural Research, 16* (2), 173-182.

Ertekin, İ., & Kızılşimşek, M. (2020). Effects of lactic acid bacteria inoculation in pre-harvesting period on fermentation and feed quality Properties of alfalfa silage. Asian-Australasian Journal of Animals sciences, 33(2), 245–253. https://doi.org/10.5713/ajas.18.0801

Filik, G. (2020). Biodegradability of quinoa stalks: The potential of quinoa stalks as a forage source or as biomass for energy production. Fuel, 266, 117064.

Genç S, Soysal Mİ. 2018. Parametric and Nonparametric Post Hoc Tests. Black Sea Journal of Engineering and Science, 1 (1), 18-27. Retrieved from https://dergipark.org.tr/tr/pub/bsengineering/issue/38497/448288

Harper, M. T., Oh, J., Giallongo, F., Roth, G. W., & Hristov, A. N. (2017). Inclusion of wheat and Triticale silage in the diet of lactation dairy cows. Journal of Dairy Science, 100(8), 6151-6163.

İnce, A., & Vurarak, Y. (2019). An approach to color change and quality relation in roughages. Journal of Agricultural Journal of Agricultural Sciences, 25(1), 21–28. https://doi.org/10.15832/ankutbd.538982.

Kızılşimşek, M., Mokhtari, N.E.P, Erol, A., Öztürk, Ç., & Gürkan, L. (2016). Effects of isolates known to have high lactic acid production ability on in vitro gas production values and forage quality traits of corn silage. Journal of Field Crops Central Research Institute, 25(SPECIAL ISSUE-2), 285-288.

Kılıç U, Abdiwali MA. 2016. Alternatif Kaba Yem Kaynağı Olarak Şarapçılık Endüstrisi Üzüm Atıklarının İn Vitro Gerçek Sindirilebilirlikleri ve Nispi Yem Değerlerinin Belirlenmesi. Kafkas Universitesi Veteriner Fakültesi Dergisi 2016; 22(6). https://doi.org/10.9775/kvfd.2016.15617

Kim, DH, Amanullah, SM, Lee, HJ, Joo, YH, Han, OK, Adesogan, AT, & Kim, SC (2018). Effects of hybrid and bacterial inoculation on fermentation quality and fatty acid profile of barley silage. Animal Science Journal, 89(1), 140-148. https://doi.org/10.1111/asj.12923

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). The biochemistry of silage (2nd ed.). Chalcombe Publications.

Mertens, D. R., & Grant, R. J. (2020). Digestibility and intake. Forages: the science of grassland agriculture, 2, 609-631.

Miron, J., Adin, G., & Nikbachat, M. (2001). Ensiling forage sorghum at different maturity stages and with addition of lactic acid bacteria. *Animal Feed Science and Technology, 91* (1-2), 99-108.

Muck, R. E. (2010). Silage microbiology and its control through additives. Revista Brasileira de Zootecnia, 39, 183-191. https://doi.org/10.1590/S1516-35982010001300021

Muthia, D., Laconi, E.B., Ridla, M., Jayanegara , A., Ridwan , R., Fidriyanto, R., & Ramdani, H. (2021, June). Effect of combining autoclave and ammoniation on nutrition value and in vitro digestibility of rice straw In IOP Conference Series: Earth and Environmental Science (Vol. 788, No. 1, p. 012052). IOP Publishing.

Nishino, N., Hattori, R., & Sakazaki, T. (2003). Effects of a new lactic acid bacteria strain on the fermentation quality of silage. *Animal Feed Science and Technology, 108* (1-4), 193-202.

Özdemir, M., & Okumuş, O. (2021). Some Silage Studies Made in Turkey in The Last Five Years. Erciyes Journal of Agricultural and Animal Sciences, 4(2), 30-39.

Pitt, R. E., & Leibensperger, R. Y. (1987). The effectiveness of silage inoculants: a systems approach. Agricultural systems, 25(1), 27-49. https://doi.org/10.1016/0308-521X(87)90097-7

Reed, H., Mueller, B., Groves, C. L., & Smith, D. L. (2021). Impact of foliar fungicides on disease and silage quality of brown Midrib corn hybrids in Wisconsin. Plant Health Progress, 22(4), 503-508.

Roberto, F., Diana, C., Julio, P., Rueda, D., Johana, Z., Diego, R., ... & Selvanayagam , M. (2016). Identification and characterization of Lactobacillus bacterial General most prevalent used to improve silage digestibility is important forage species for livestock sector. Journal of Applied Pharmaceutical Science, 6(1), 035-041.

SAS, (2001). Statistical Analysis System. User's Guide: Statistics, Version 8.2. SAS Institute, Cary, NC, USA.

Seale, D. R., Pahlow, G., Spoelstra, S. F., Lindgren, S., Dellaglio, F., & Lowe, J. F. (1990). Methods for the microbiological analysis of silage. In Proceeding of the Eurobac Conference; Sveriges Lantbruksuniv: Uppsala, Sweden, 1990; pp. 147–164.

Shah, A.A., Xianjun, Y., Zhihao, D., Siran, W., & Tao, S. (2017). Effects of lactic acid bacteria on inhibition characteristics, chemical composition and aerobic stability of King grass. The Journal of Animal & Plant Sciences, 27(3): 2017, Page: 747-755. thejaps.org.pk/docs/v-27-03/08.pdf

Shah, A.A., Xianjun, Y., Zhihao, D., Junfeng, L., & Shao, T. (2018). Isolation and molecular Identification of lactic acid bacteria from King grass and their application to improve the fermentation quality of sweet sorghum. World Journal of Microbiology and Biotechnolog, 34, 1–13.

Soycan Önenç, S., Yücel, B., & Turan Uçman, A. (2022). Effects of adding ethanol extract of propolis on the fermentation quality, aerobic stability, fatty acid profile, and in vitro digestibility of alfalfa silage. Fermentation, 8(8), 412. https://doi.org/10.3390/fermentation8080412.

Van Soest P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Wang, J., Wang, S., Wu, D., Zhang, Y., & Wei, B. (2009). Effects of bacterial inoculants on fermentation quality and aerobic stability of whole crop wheat silage. *Animal Feed Science and Technology, 151* (3-4), 221-229.

Weinberg, Z.G. & Muck, R.E. (1996). New trends and opportunities in the development and use of inoculants for silage, FEMS Microbiology Reviews, Volume 19, Issue 1, October 1996, Pages 53–68, https://doi.org/10.1111/j.1574-6976.1996.tb00253.x

Xu, J., Ma, J., Sa, R., Sui, H., Wang, X., Li, Q., ... & Niu, H. (2024). Effects of lactic acid bacteria inoculants on the nutrient composition, fermentation quality, and microbial diversity of whole-plant soybean-corn mixed silage. Frontiers in Microbiology, 15, 1347293.

Yang, H. Y., Wang, X. F., Liu, J. B., Gao, L. J., Ishii, M., Igarashi, Y., & Cui, Z. J. (2006). Effects of water-soluble carbohydrate content on silage fermentation of wheat straw. Journal of bioscience and bioengineering, 101(3), 232-237.

Zhou, Y., Feng, Q., Li, Y., Qi, Y., Yang, F., & Zhou, J. (2024). Adding rumen microorganisms to improve fermentation quality, enzymatic efficiency, and microbial communities of hybrid Pennisetum silage. Bioresource Technology, 410, 131272. https://doi.org/10.1016/j.biortech.2024.131272