Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effect of Adding Lactic Acid Bacteria to Maize Silage on Nutritive Guality, Fermentation Properties and in Vitro Digestibility

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ARTICLE INFO
Research Article
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Received: 30 March 2023 / Revised: 05 June 2023 / Accepted: 09 June 2023 / Online: 24 Oct 2023

Cite this article
AYDIN S S, DENEK N (2023). Effect of Adding Lactic Acid Bacteria to Maize Silage on Nutritive Guality, Fermentation Properties and in Vitro Digestibility. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(4): 1050-1058. DOI: 10.15832/ankutbd.1273724

ABSTRACT
This study aimed to determine the effects of adding lactic acid bacteria to maize silage on the nutritional quality, fermentation properties, and its *in vitro* organic matter digestion (IVOMD). Pre-fermented juices (PFJ) prepared from different water-soluble carbohydrate (WSC) sources at the rate of 5% and commercial homofermentative and heterofermentative lactic acid bacteria (LAB) were added to silages. Groups were designed as (I) control, (II) Glucose-PFJ, (III) Fructose-PFJ, (IV) Sucrose-PFJ, (V) Molasses-PFJ, (VI) Homofermentative LAB (HoLAB) and (VII) Heterofermentative LAB (HetLAB). Lactic acid bacteria (LAB) count, lactic acid (LA), acetic acid (AA), LA/AA ratio, pH and yeast values of the natural fermented lactic acid bacteria liquids prepared by adding 5% of different easily soluble carbohydrate sources to meadow grass showed significant variations. The differences among the groups in the crude ash (CA), acid detergent fiber (ADF), IVOMD and methane (CH_4) values of the silage groups prepared by adding PFJ were also found to be statistically significant. The differences in the fermentation characteristics of the silages (pH, ammonia-nitrogen (NH_3-N), LA, AA, LA/AA, CO_2, and total yeast mold after aerobic stability) were statistically significant too. When all parameters were examined, it was concluded that the addition of PFJ, which is prepared by adding 5% fructose to the meadow grass plant, to the maize silage has positive effects on IVOMD, ME, CH_4, LA and yeast-mold and can be used instead of commercial inoculants.

Keywords: Epiphytic microorganisms, Inoculant, *In vitro* digestibility, Methane, Mikrobiota

1. Introduction

The maize plant is the predominant grain crop for ensiling worldwide, compared to other forage crops due to its fermentation efficiency in animal nutrition. Since the maize plant has a proportionally higher dry matter content, a low buffer capacity (resistance to acidification) and sufficient water-soluble carbohydrate (WSC) content necessary for lactic acid (LA) fermentation, it can be easily ensiled. The use of bacterial inoculants in ensiling provides a rapid pH decrease by producing lactic acid, preventing the growth of undesirable epiphytic microorganisms, reducing proteolysis and increasing dry matter gain (Muck 2013).

Lactic acid bacteria (LAB) are divided into two main groups according to the types of saccharolytic fermentation (Axelsson 1998). Members of the mandatory homofermentative (HoLAB) or facultative heterofermentative LAB group include *Lactobacillus acidophilus, L. delbrueckii, L. helveticus, L. farciminis, L. lactis and L. bovis*. Only in some special cases (when there is insufficient sucrose in the environment), microorganisms in this group, called facultative heterofermentative LAB (HetLAB), acquire heterofermentative properties. The most important members of this group are *L. plantarum. L. alimentarius, L. casei, L. curvatus, L. sakei, L. paralimentarius and L. pentosus* are also found in this group. Members of the mandatory HetLAB group include *L. brevis, L. buchneri, L. fermentum, L. reuteri, L. fructivorans, L. sanfranciscensis* and *Leuconostoc mesenteroides*. The effects of bacterial inoculants used as silage additives on silage fermentation vary according to the characteristics of the LAB contained in the inoculant. Results are inconsistent regarding silage fermentation of Homofermentative LAB use in maize silage (Kleinschmit & Kung 2006). Although the use of HetLAB increases aerobic stability by preventing the growth of yeasts in the silage, fermentation losses were observed due to CO_2 formed during fermentation (Blajman et al. 2020). Another aspect to consider is the number of bacteria in the product and per gram of silage. A comparison of the products available on the market for silage additives reveals a range of 10^5 to 10^6 cfu/g of silage (DLG 2011; Aragón 2012). Natural fermented lactic acid bacteria liquid (PFJ) is more effective in LA production especially in silages with a high moisture content, since it contains many epiphytic LAB species that act synergistically, can be prepared in a practical and economical way and used as an alternative to commercial LAB inoculants. Its use has become widespread in recent years due to its benefits (Sun et al. 2021). This study was conducted to determine the effects of the addition of LAB to maize silage on the silage quality, its fermentation properties and it's *in vitro* organic matter digestion.

2. Material and Methods

2.1. Study design and silage preparation

In this study, the maize plant was used as the raw silage material. The buffering capacity of the fresh maize plant used in the present study was determined according to the method reported by Playne & McDonald (1966). The total number of LABs in the fresh silage material and in the experimental groups was measured in four replications per group according to the tempo automatic bacterial count test method reported by Güney & Ertürk (2020). The addition-free maize plant constituted the control group in the study, while the experimental groups consisted of maize silages to which fructose PFJ (F-PFJ), molasses PFJ (M-PFJ), sucrose PFJ (S-PFJ), glucose PFJ (G-PFJ), commercial HoLAB, and HetLAB inoculants were added. The PFJs used in this study were prepared according to the method reported by Masuko et al. (2002). After the PFJs were added to the meadow grass plant with pure water at a ratio of 1:1 and passed through the blender, 5% of different easily soluble carbohydrate sources (glucose, fructose, molasses, and sucrose) were added to the resulting liquid and left to incubate at 30°C for five days. The microbiota content of the PFJs were determined according to the Shotgun method (Sun et al. 2021). The PFJs, HoLAB and HetLAB inoculants were applied to the silages at 1 mL/kg. Inoculants were used by inoculating 1 mL of silage with 10cc distilled water to ensure homogeneity. The commercial HoLAB inoculant that was used as an additive contained *Lactobacillus plantarum* DSM 18112, *Lactobacillus plantarum* DSM 18113, *Lactobacillus plantarum* DSM 18114, *Lactobacillus plantarum* ATCC 55943, *Enterococcus faecium* ATCC 55593 and *Enterococcus faecium* ATCC 53519 water. HetLAB inoculant used as additive contained the *Lactobacillus buncheri* ATCC PTA-2494 strain.

Control and experimental groups were compressed into 1.5-liter glass jars in 4 replicates. The silages were fermented in a dark environment for 60 days before opening. During this time, the silages were stored at room temperature.

2.2. Fermentation profile analysis

After the silages were opened, a 3-5 cm section was discarded from the top of the jar. After the silages were poured into a container, approximately 25 g of silage sample was mixed homogeneously with 100 mL of distilled water with the help of a blender. The pH value of the shredded silage liquid was rapidly recorded with pH meter measuring device. In addition to the silage liquid, 0.1 mL of 1M HCl was added to the tubes prepared for ammonia nitrogen analysis. For the analysis of LA and volatile fatty acids (VFA), 0.25 mL of 25% metaphosphoric acid was added to the prepared tubes. The tubes prepared for ammonia nitrogen, LA and VFA assessment were stored in the deep freezer until analyses. NH_3-N/TN determination of the silage samples were performed according to the method reported by Broderick & Kang (1980). VFA such as propionic acid (PA), acetic acid (AA) and butyric acid (BA) and LA were determined as reported by Suzuki & Lund (1980). For this reason, high performance liquid chromatography (HPLC) analyser (Shimadzu LC-20 AD HPLC pump, Icsep Coregel (87H3 colon), Shimadzu SIL-20 ADHT Autosampler, Shimadzu cto-20ac Colum oven, Shimadzu SPD M20A Detector (DAD), Türkiye) was used. The silages obtained in the study were subjected to aerobic stability test in order to determine the CO_2 production values. For this purpose, silages were exposed to oxygen for 5 days according to the method developed by Ashbell et al. (1991).

While the raw nutrient contents of the silages (such as dry matter, crude ash, crude protein) were determined according to the method reported by AOAC (2005), the ADF and NDF analyzes of the silages were performed as reported by Van Soest et al. (1991). Before the raw nutrient analysis, the silages were dried at room temperature and ground in a laboratory mill to pass through a 1 mm sieve and made ready for analysis.

The gas production values of the silages and alfalfa herbage were determined through the method described by Menke & Steingass (1988) using four glass syringes as replicates. The rumen fluid used in the analysis was collected with the help of a rumen pump from 2 rams who were provided with a training diet (60% forage, 40% concentrate) for 2 weeks. The in vitro organic matter digestibility (IVOMD) (g/kg OM) and metabolizable energy (ME) (MJ/kg DM) of silages were calculated using equations reported by Menke et al. (1979) as:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times Gp + 0.057 \times CP + 0.0029 \times CP2,$$
$$IVOMD\ (\%) = 14.88 + 0.889 \times Gp + 0.45 \times CP + 0.0651 \times XA,$$

Where; CP is CP in g/100 g DM, crude ash in g/100 g DM and gas production is the net gas production (mL) from 200 mg DM after 24 h of incubation. After recording 24-h gas production values, gas inside the syringe was measured by three-way syringe system and total gas was injected into computer-assisted infrared methane gas meter (Sensor Europe GmbH, Erkrath, Germany) and then methane content was determined as a percentage of 24 h the total amount of gas formed (Goel et al. 2008). Yeast and mold contents of silages were determined using the method reported by Filya et al. (2000).

2.3. Statistical analysis

One Way Analysis of Variance (One Way Anova) was used to determine whether the data obtained from the groups were significantly different. Duncan's multiple comparison tests were used to control the significance of the differences among the

groups, and for this purpose, the SPSS (1991) software program was used.

3. Results and Discussion

The amount of LAB, LA, AA, LA/AA ratio, pH, total yeast and mold values of the PFJs prepared by adding different easily soluble carbohydrate sources (glucose, fructose, molasses and sucrose) to the meadow grass plant are presented in Table 1. Microbiota graphics of the LAB liquids prepared by adding different easily soluble carbohydrate sources (fructose, sucrose and molasses) to the meadow grass plant are shown in Figure 1.

When the microbiota contents of the PFLs prepared by adding 5% easily soluble carbohydrate sources (fructose, molasses, sucrose and glucose) to the meadow grass plant were examined, it was observed that they were of different types and ratios. As indicated in Figure 1, in S-PFJ, *Lactiplantibacillus plantarum* 38%, *Lactiplantibacillus pentosus* 19%, *Lactiplantibacillus paraplantarum* 2%, *Lactiplantibacillus plagiomi* 2%, *Lactiplantibacillus argentoratensis* 2%, *Lactiplantibacillus argentoratensibacillus* 2% *fermocicillus* 7%, *Levilactobacillus brevis* 2%, *Levilactobacillus parabrevis* and *Levilactobacillus hammesii* species were identified.

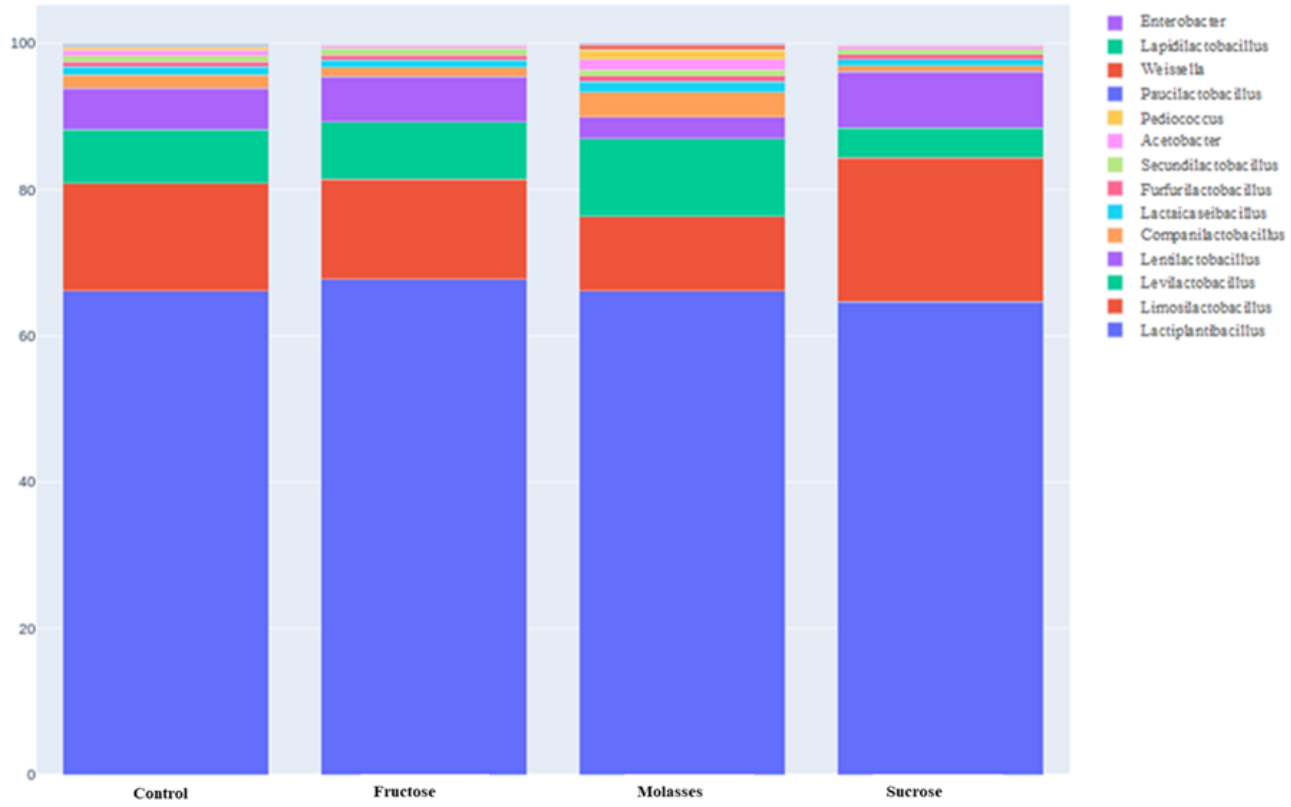

Figure 1- Microbiota graphs of LAB liquids prepared by adding 5% easily soluble carbohydrate sources (fructose, sucrose and molasses) to the meadow grass plant

As shown Figure 1, *Lactiplantibacillus plantarum* 41%, *Lactiplantibacillus pentosus* 19%, *Lactiplantibacillus paraplantarum* 2%, *Lactiplantibacillus plagomomi 2%*, *Lactiplantibacillus argentoratensis* 2%, *Lictiplantibacillus argentoratensibacillus* 2% *fermoscinercillus* 5%, *Levilactobacillus brevis* 2% and *Levilactobacillus zymae* 5% species were identified in the microbiota content of F-PFJ.

When the microbiota content of M-PFJ were examined in Figure 1, *Lactiplantibacillus plantarum* 42%, *Lactiplantibacillus pentosus* 18%, *Lactiplantibacillus paraplantarum* 3%, *Lactiplantibacillus argentoratensis* 2%, *Limosilactobacillus levbuchilactiobacillus* 8%, *brevis* 4%, *Levilactobacillus zymae* 5%, *Companilactobacillus kimchii* 5% *Lactobacillus pontis, Lactobacillus frumenti, Levilactobacillus parabrevis, Levilactobacillus hammesii, Levilactobacillus namurensis* and *Levilactobacillus senmaizukei* species were identified.

Similarly, Parvin & Nishino (2010) detected *Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus brevis* and *Lactococcus lactis* species in Rhodes grass in their study. In a study by Fabiszewska et al. (2019) *Lactiplantibacillus plantarum, Lactobacillus acidophilus, E. faecium, P. Acidilactici, P. pentosaceus Lentilactobacillus buchneri, Limosilactobacillus reuteri, Lacticaseibacillus casei Levilactobacillus zymae, Apilactobacillus kunkeei, Levilactobacillus*

acidifarinae, *Levilactobacillus namurensis*, *Levilactobacillus brevis*, *Levilactobacillus spicheri*, *Fructilactobacillus fructivorans*, *Fructilactobacillus fructivorans* and *Levilactobacillus hammesii* were identified as key species used to effectively and vigorously improve silage quality. Similar species were detected in the PFJs of the present study.

Table 1- Fermentation values of PFJs prepared by adding 5% different easily soluble carbohydrate sources to the meadow grass plant

Groups	*LAB*	*LA*	*AA*	*LA/AA*	*pH*	*Yeast*	*Mold*
G-PFJ	12.52^{a}	754.77^{a}	75.05^{b}	10.05^{a}	3.48^{b}	7.03^{c}	<10
F-PFJ	12.33^{b}	351.16^{d}	45.88^{d}	7.80^{b}	3.29^{c}	7.60^{b}	<10
S-PFJ	12.01^{d}	411.01^{c}	71.79^{c}	5.80^{d}	3.25^{d}	7.82^{a}	<10
M-PFJ	12.18^{c}	700.00^{b}	108.95^{a}	6.40^{c}	3.72^{a}	5.82^{d}	<10
SEM	0.058	52.946	6.75915	0.493	0.055	0.235	…
P-Value	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**…**

$^{a-d:}$ Values with different letters in the same column were found to be different (P<0.05); **LAB:** Lactic acid bacteria log10 cfu/mL, **LA:** Lactic acid g/kg DM, **AA:** Acetic acid g/kg DM, **Yeast:** log10 cfu/mL, **Mold:** log10 cfu/mL.

When the fermentation values such as LAB number, LA, AA, LA/AA ratio, pH and yeast of the PFJs prepared by adding 5% different easily soluble carbohydrate sources to the meadow grass plant were examined, the differences among the groups were statistically significant. In this study, when the LAB values in the natural LAB fluids were examined, the lowest LAB value was obtained from the S-PFJ, and the highest value was obtained from the G-PFJ. The total LAB values in the PFJ obtained in this study were found to be higher than the values obtained in the study of Bureenok et al. (2005a), and similar to the values reported in the study of Aydın & Denek (2022). It is known that LAB species degrade different carbohydrates at different levels. The higher the molecular weight of the carbohydrate type, the lower the level of fermentation, that is, degradation. More complex carbohydrates such as sucrose and polysaccharides are more difficult to break down than monosaccharides, and microbial and plant enzymes play an important role in this breakdown process (Kılıç 1986). Detection of the lowest LAB count in the sucrose PFJ group supports this hypothesis. Bureenok et al. (2005b) found the highest LAB value in the glucose supplemented group and the lowest LAB value in the S-PFJ group, which is consistent with the current study. When the LA values of the natural LAB fluids were examined, the highest LA value was found in the G-PFJ group, and the lowest LA value was found in the F-PFJ group. A possible reason for the difference could be attributed to the fact that LAB can decompose glucose into LA more efficiently than other carbohydrate sources (Müller & Lier 1994). The fact that the highest amount of LA was in the G-PFJ group supports this statement. While the highest AA value in the natural LAB fluids was detected in the M-PFJ group, the lowest AA value was observed in the F-PFJ group. This result can be explained by the fact that molasses contains not only sucrose but also nitrogenous compounds that can be used by microorganisms (Otero et al. 1993). Our results are in accordance with these reported by Bureenok et al. (2005a). When Table 1 is examined, the LA/AA ratio in the PFJ groups was within the range of 5.80-10.05. It is reported that homolactic fermentation occurs when the LA/AA ratio is greater than 3.0 and heterolactic fermentation occurs when the LA/AA ratio is less than 3.0 (Zhang et al. 2010). In this study, the LA/AA ratio being greater than 3 in all groups reveals that homolactic is more intense than heterolactic activity. When the pH values of the PFJs were examined, the lowest value (3.25) was obtained in the S-PFJ, and the highest value (3.72) was obtained in the M-PFJ group. Differences in pH values in PFJs may be due to the type and amount of LAB used, the plant species, the easily soluble carbohydrate source and amount, and the fermented incubation time (Can 2010). According to Denek et al. (2011), the pH values (3.45-3.76) in PFJs prepared by adding glucose were comparable with that of the present study. When Table 1 was examined, the highest yeast value in the PFJs was obtained from the S-PFJ, and the lowest yeast value was obtained from the M-PFJ. In addition, molds were not found in any PFJ group. The determination of the lowest yeast and the highest acetic acid values in the M-PFJ group can be explained by the fact that the amount of acetic acid formed as a result of LAB fermentation has an inhibitory effect on the growth and activity of yeasts (Ali et al. 2020). Yeast and mold values in PFJ prepared by adding 2% glucose to alfalfa plant are similar to the values in this study (Tao et al., 2017). The analyses of the maize plant used as silage raw material in this study are shown in Table 2.

Table 2- Analysis values of the maize plant used as silage material in the study

Silage Material	***BC***	***DM***	***CA***	***CP***	***ADF***	***NDF***	***IVOM***	***ME***	***CH_4***	***LAB***	***Yeast***	***Mold***
Maize	210	28.15	6.70	7.95	26.25	56.14	58.72	8.61	9.94	$7.2*10^{6}$	$2*10^{6}$	$4.2*10^{5}$

BC: Buffer capacity; **DM:** Dry matter, %; **CA:** Crude ash, DM%; **CP:** Crude protein, DM%; **ADF:** Acid detergent insoluble fiber, DM%; **NDF:** Neutral detergent insoluble fiber, DM%; **IVOMD:** *In vitro* organic matter digestion, %; **ME:** Metabolizable energy MJ/kg DM; **CH_4:** *In Vitro* methane gas, %

The nutrient content and IVOMD, ME and *in vitro* CH_4 values of the silages prepared by adding LAB liquid to the maize plants are presented in Table 3.

Table 3- Nutrient content and IVOMD, ME and CH_4 values of the silages prepared by adding LAB liquid to the maize plants

Groups	*DM*	*CA*	*CP*	*ADF*	*NDF*	*IVOMD*	*ME*	*CH_4*
Control	27.84	6.09^{b}	7.25	25.28^{a}	54.56	56.21^{d}	8.59	10.28^{a}
F-PFJ	27.20	6.28^{b}	7.25	24.33^{a}	46.85	66.86^{a}	9.81	7.33^{c}
M-PFJ	27.44	6.13^{b}	7.37	24.88^{a}	46.77	64.27^{abc}	9.73	9.16^{ab}
S-PFJ	28.07	6.23^{b}	7.37	25.26^{a}	49.15	61.22^{bc}	9.09	9.01^{ab}
G-PFJ	27.51	6.80^{a}	7.44	23.78^{ab}	42.84	63.88^{abc}	9.26	8.65^{bc}
HoLAB	27.15	6.40^{ab}	7.28	20.79^{bc}	51.06	65.60^{ab}	9.13	8.07^{bc}
HetLAB	26.51	6.51^{ab}	7.77	19.28^{c}	52.74	60.15^{cd}	9.12	8.23^{bc}
SEM	0.152	0.063	0.045	0.550	1.141	0.809	0.122	0.222
P	0.125	0.021	0.100	0.002	0.077	0.001	0.105	0.004

$^{a-c:}$ Values with different letters in the same column were found to be different (P<0.05); **DM:** Dry matter, %; **CA:** Crude ash, DM%; **CP:** Crude protein, DM%; **ADF:** Acid detergent insoluble fiber, DM%; **NDF:** Neutral detergent insoluble fiber, DM%; **IVOMD:** *In Vitro* organic matter digestion, %; **ME:** Metabolizable energy MJ/kg DM; **CH_4:** *In Vitro* methane gas, %.

When Table 3 was examined, the differences among the groups in the CA, ADF, IVOMD and CH_4 values of the silages were statistically significant (P<0.05), while the differences in DM, CP, NDF and ME values were not significant (P>0.05). When the CA values of the silages were examined, an increase was observed in G-PFJ group due to the addition of additives compared to the controls. ADF values of the silages were found to be statistically significant in all groups. ADF values of HoLAB and HetLAB groups showed a decrease compared to the other groups. The hypothesis that HetLAB strains produce ferulate esterase and that ferulate esterase can reduce the cell wall coverage (Ding et al. 2019) was in agreement with the present study. Jalč et al. (2009a) reported that inoculants reduced ADF and NDF levels in their silage study using three microbial inoculants (*Lactobacillus plantarum* CCM 4000, *L. fermentum* LF2 and *Enterococcus faecium* CCM 4231). When the IVOMD values of the silages were examined, increases were observed in all experimental groups compared to the control group, but the highest IVOMD value was observed in the group with the addition of F-PFJ. It is considered that the main fermentation product in the silages was LA and therefore IVOMD values were increased. While Sucu (2009) reported that LAB inoculants increased the OM digestibility of maize silage, Altınçekiç (2006) reported that LAB inoculants significantly reduced the *in vitro* OM digestibility of maize silage. However, depending on the type of microorganism, strain, and substrate used, *in vitro* responses to the effects of silage inoculants have varied considerably (Ellis et al. 2016). When the CH_4 values of the silages were examined, the lowest value was observed in the F-PFJ group, and the highest value was observed in the control group. Research reveals that LAB has varying impacts on CH_4 production. According to Cao et al. (2011), the application of the *L.* plantarum Chikuso-1 inoculant along with vegetable residual silage resulted in a 46.6% decrease in methane generation when compared to the control group. Significant drops in CH_4 were also observed, according to Jalč et al. (2009b), after inoculating grass silage with *E. faecium* (CCM 4231), *L. fermentum*, or *L. plantarum* (CCM 4000). Nevertheless, Jalč et al. (2009c) discovered no significant increases in CH_4 for the same LAB when used as an inoculant with maize. Huyen et al. (2020) found that the ryegrass silage prepared by adding *Lactiplantibacillus plantarum* (LMG P-20353), *Lactiplantibacillus plantarum* (CECT 4528), *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. lactis* (DSM 33083) strains reduced the CH_4 value. These results are consistent with that reported by Doyle et al. (2019). It has been hypothesized that LAB can reduce CH_4 production in ruminants by altering rumen fermentation, directly inhibiting rumen methanogens, inhibiting specific rumen bacteria that produce H_2 or methyl-containing compounds that are substrates for methanogenesis, and producing bacteriocins that inhibit methanogens or by affecting other rumen microorganisms that produce substrates required for methanogenesis (Doyle et al. 2019).

Within the scope of this study, the fermentation characteristics of the silages prepared by adding PFJ and commercial HoLAB and Het LAB inoculant to the maize plants and the correlation results of the analyses are provided in Tables 4 and 5.

Table 4- The effect of the silages prepared by adding LAB liquid to the maize plants on fermentation properties

Groups	*NH_3-N*	*CO_2*	*PH*	*LA*	*AA*	*LA/AA*	*PA*	*BA*	*Yeast-Mold*
Control	8.57^{a}	2.34^{ab}	3.61^{b}	49.96^{d}	10.38^{d}	4.80^{b}	-	-	9.66^{b}
F-PFJ	8.52^{ab}	2.28^{ab}	3.63^{b}	58.84^{a}	14.83^{b}	3.96^{f}	-	-	3.85^{f}
M-PFJ	7.51^{c}	1.66^{ab}	3.61^{b}	56.90^{b}	12.90^{c}	4.60^{c}	-	-	6.13^{d}
S-PFJ	7.67^{bc}	1.28^{b}	3.62^{b}	26.85^{f}	5.90^{f}	4.41^{e}	-	-	9.10^{c}
G-PFJ	7.92^{abc}	1.05^{b}	3.73^{a}	39.44^{e}	8.76^{e}	4.50^{d}	-	-	8.95^{c}
HoLAB	6.57^{d}	4.26^{a}	3.63^{b}	54.87^{c}	4.78^{g}	11.47^{a}	-	-	10.24^{a}
HetLAB	7.95^{abc}	1.30^{b}	3.81^{a}	24.37^{g}	18.35^{a}	1.32^{g}	-	-	5.70^{e}
SEM	0.151	0.290	0.017	2.560	0.867	0.550	-	-	0.433
P	**0.001**	**0.028**	**0.000**	**0.000**	**0.000**	**0.000**	**-**	**-**	**0.000**

^{a,b,c,d,e,f,g}: Values with different letters in the same column were found to be different (P<0.05); **NH_3-N/TN:** Ammonia nitrogen; **CO_2:** Carbon dioxide, g/kg DM; **LA:** Lactic acid, g/kg DM; **AA**: Acetic acid, g/kg DM; **PA:** Propionic acid, g/kg DM; **BA:** Butyric acid, g/kg DM, **Yeast-Mold:** log10 cfu/g

Table 5- Correlation relationship between the fermentation properties and yeast and mold values of the silages prepared by adding LAB liquid to the maize silages

Silage Parameters		*pH*	*NH_3-N*	*LA*	*AA*	*CO_2*	*Yeast-Mold*	*IVOMD*	*ME*	*CH_4*
pH	**PC**	1	0.173	-0.528**	0.415*	-0.197	-0.151	-0.148	-0.075	-0.149
	P		0.379	0.004	0.028	0.314	0.444	0.454	0.705	0.448
NH_3-N	**PC**		1	-0.036	0.446*	-0.192	-0.368	-0.435*	-0.147	0.216
	P			0.856	0.017	0.327	0.054	0.021	0.456	0.270
LA	**PC**			1	-0.086	0.404*	-0.136	0.371	0.246	-0.071
	P				0.665	0.033	0.491	0.052	0.206	0.721
AA	**PC**				1	-0.278	-0.839**	-0.068	0.202	-0.177
	P					0.153	0.000	0.729	0.303	0.368
CO_2	**PC**					1	0.219	0.127	-0.042	-0.061
	P						0.263	0.518	0.831	0.759
Yeast-Mold	**PC**						1	-0.299	-0.462*	0.420*
	P							0.122	0,013	0.026
IVOMD	**PC**							1	0.818**	-0.475*
	P								0.000	0.011
ME	**PC**								1	-,181
	P									,356
CH_4	**PC**									1

PC: Pearson correlation; ***:** The correlation is significant at the 0.05 level; ****:** The correlation is significant at the 0.01 level; **NH_3-N/TN:** Ammonia nitrogen; **CO_2:** Carbon dioxide, g/kg DM; **LA:** Lactic acid, g/kg DM; **AA:** Acetic acid, g/kg DM; **IVOMD:** *In Vitro* organic matter digestion, %; **ME:** Metabolizable energy, MJ/kg DM; **CH_4:** *In Vitro* methane gas, %, **Yeast-Mold:** log10 cfu/g

When the fermentation properties (pH, NH_3-N, LA, AA, LA/AA, CO_2, and total yeast-mold after aerobic stability) of the silages prepared by adding LAB liquid to the maize plants were examined, the differences among the groups were statistically significant (P<0.05).

The pH values of the silages were within the range of 3.61-3.81. The reason for the highest pH value (3.81) in the silage group with heterofermentative additives was that in the HetLAB silage plant structure, secondary products are produced such as ethyl alcohol, acetic acid, diacetyl and carbon dioxide, as well as lactic acid, which is the main product from WSCs. When the correlation of analyses of the silages was examined in Table 5, the positive correlation (R:0.415) between silage pH and AA supported this finding. In addition, the reason for the low pH value in all silage groups is related to the low buffering capacity of the maize plant and its sufficient WSC content for fermentation. Moreover, it is thought that the bacterial inoculants used ensure silage fermentation with rapid pH decrease by using WSCs in the plant.

When the NH_3-N values of the silages were examined, a decrease was observed in M-PFJ, S-PFJ and HoLAB groups compared to the control group. The lowest NH_3-N value was identified in the group with the addition of the HoLAB additive. As a result of the LA production rate and rapid pH decrease in the silo environment depending on the decrease in the efficiency of proteolytic enzymes, proteolysis decreases and thus the degradation of proteins also decreases (Reich & Kung 2010). Carpintero et al. (1979) reported that the silage NH_3-N/TN% value should be lower than 11% to be classified in the good quality silage class. McDonald et al. (1991) reported that lower pH values inhibited protein degradation in silages. Therefore, in the experiment, all maize silages had low ammonia-N contents.

When the LA and AA values of the silages were examined, the highest LA value (58.84) was found in the group in which F-PFJ was added to the maize silage, and the lowest LA value (24.37) was found in the group with the HetLAB addition to the maize silage. The highest AA value (18.35) of the silages was identified in the group with the HetLAB inoculant addition. The silage with the lowest AA value (4.26) was found in the HoLAB group. Bernardi et al. (2019) reported that the addition of the HoLAB inoculant increases LA and decreases AA and that the addition of the HetLAB inoculant increases AA by reducing LA. These results support the findings of the present study. In Table 4, the LA/AA ratio of the silages was within the range of 1.32-11.47. The highest homofermatative activity was found in the group with the HoLAB addition. The highest heterolactic activity was observed in the group with the HetLAB inoculant addition. Studies that added inoculants to maize silage support the results of the current study (Bernardi et al. 2019; Xu et al. 2021). In the present study, on day 5 of aerobic stability, the CO_2 formation amounts of the silages varied between 1.05 and 4.26 g/kg DM, and the highest value was detected in the group with the HoLAB inoculant supplementation while the lowest value was detected in the group with G-PFJ added to the maize silage and in the group with the HetLAB inoculant addition. Ali et al. (2020) reported that the amount of acetic acid produced by HetLAB fermentation in silages has an inhibitory effect against microorganisms that cause silage deterioration, inhibits the growth and activity of yeast and molds, and reduces CO_2 production, that is, improves aerobic stability values. When the correlation table is examined, the negative correlation of AA between CO_2 and yeast-mold supports this statement. In the present study, the highest CO_2 and total yeast and mold amounts after aerobic stability and the lowest AA value were identified in the group with the added HoLAB inoculant. Adesogan & Arriola (2020) reported that HoLAB inoculants reduce the amount of AA that inhibits yeast production but increase the amount of LA and decrease aerobic stability. The report that increased levels of LA are used as a substrate for yeast growth and that LA is decomposed by yeasts to CO_2 and water is in line with the present study (Adesogan & Arriola 2020). In most of the studies examining the effect of inoculant use on the aerobic stability of silage, aerobic stability was negatively affected by the addition of HoLAB (Filya 2002; Filya & Sucu 2010; Filya et al. 2004).

4. Conclusions

This study aimed to determine the effects of the addition of LAB to maize silage on the silage quality, its fermentation properties and its *in vitro* organic matter digestion.

When all parameters were examined, it was concluded that the addition of F-PFJ, which was prepared by adding 5% fructose from easily soluble carbohydrate sources to the meadow grass plant, to maize silage has the most positive effects on silage fermentation and *in vitro* organic matter digestion.

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