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Effects of Use of Lactic Acid Bacteria Isolated from Whole Crop Corn as Inoculant on Corn Silage Fermentation and Aerobic Stability

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This study was conducted to determine the effects of lactic acid bacteria (LAB) that were isolated from whole crop corn on silage quality and aerobic stability when added to corn silages. Experimental groups were designed as follow: C (control), the group with no added inoculant; LD, the group with added *Lactobacillus delbrueckii*; LP, the group with added *Lactobacillus plantarum* and LB, the group with added *Lactobacillus buchneri*, a commercial inoculant. On days 5, 10, 15, 30 and 60 of fermentation, three jars from each group were opened and samples were taken to perform microbiological and chemical analyses.

In the study, control group, lactic acid (LA) and acetic acid (AA) levels (g/kg^{-1} Dry matter) reached the highest values on day 30, whereas in the groups with added inoculants, levels of these organic acids rapidly increased as from 5th day, butyric acid (BA) level was high in the first few days of fermentation and dramatically decreased towards the end of the experiment (P<0.001). During aerobic stability test performed at the end of the experiment, no difference was observed between the groups in terms of pH, LA, total bacteria (PCA) levels and yeast, while mold was not detected in any of the groups.

As a result; the use of LB, LD and LP as silage inoculants positively affected the fermentation, and they rapidly increased the lactic acid level of silage.

Key Words: *Silage, lactic acid bacteria, inoculant, aerobic stability*

Mısır Hasılından İzole Edilen Laktik Asit Bakterilerinin İnokulant Olarak Kullanımının Mısır Silajı Fermantasyonu ve Aerobik Stabilite Üzerine Etkileri

Bu araştırma, mısır silajlarına katılan farklı laktik asit bakterilerinin (LAB) silaj kalitesi ve aerobik stabilite üzerine etkilerini belirlemek üzere yapılmıştır. Silaj materyali olarak mısır hasılı kullanılmış ve hiçbir katkı yapılmayan grup Kontrol, *Lactobacillus delbrueckii* katılan grup (LD), *Lactobacillus plantarum* katılan grup (LP) ve ticari inokulant olarak *Lactobacillus buchneri* katılan grup (LB) deneme gruplarını oluşturmuştur. Fermantasyonun 5, 10, 15, 30 ve 60. günlerinde her gruptan üç kavanoz açılmış ve örnekler alınarak mikrobiyolojik ve kimyasal analizler yapılmıştır.

Araştırmada, kontrol grubunda laktik asit (LA), asetik asit (AA) düzeyleri (g/kg^{-1} KM) 30. günde en yüksek değere ulaşırken, inokulant katılan gruplarda bu organik asitler 5. günden itibaren hızla yükselmiş, bütirik asit (BA) düzeyi fermantasyonun ilk günlerinde yüksek bulunurken, deneme sonuna doğru önemli ölçüde azalmıştır (P<0.001). Deneme sonunda yapılan aerobik stabilite testinde pH, LA, toplam bakteri (PCA) düzeyleri ile maya bakımından gruplar arasında farklılık görülmemiş (P>0.05) ve grupların hiç birinde küf tespit edilememiştir.

Sonuç olarak; silajlara inokulant katılmasının fermantasyonu olumlu yönde etkilediğini, ortamdaki laktik asit düzeyini hızla artırdığını söylemek mümkündür.

Anahtar Kelimeler: *Silaj, laktik asit bakterileri, inokulant, aerobik stabilite*

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Introduction

Although Turkey has a significant potential for livestock, its shortage in forage production is remarkably high due to both unfavorable climatic conditions and mismanagement of grasslands and pastures. This has an impact on feeding ruminants sufficient and balanced rations and thus efficiency and profitability of ruminants. In the case of insufficient forage production, silage, one of cultivated forage crops, becomes more important. Good silage required by farmers are energy and protein-rich feed which are fondly consumed by animals and contribute to animal health and product quality. The feed material suitable for this purpose should be harvested at the right time and ensilaged correctly. In addition, various silage additives for improving silage quality are of great interest to researchers and manufacturers (1). For good ensilage, lactic acid bacteria (LAB) should dominate fermentation medium (2). In the case of farm practices, silage contacts with oxygen during feed-out, which is unavoidable. Especially during warmer seasons, oxygen activates mainly yeasts and molds as well as other aerobic

microorganisms in the environment which cause spoilage of silages. Such microorganisms consume sugar and fermentation products in the medium and cause silages to warm up. In recent years, microbial additives have been widely used to improve aerobic stability of silage and prevent yeast and mold growth. These additives work with lactic acid bacteria (LAB) naturally existing in the material ensiled, and ensure that a very fast and efficient fermentation process takes place inside a silo (3). In a study by Sucu and Filya (4), the authors added two different inoculants in corn silage with low dry matter and found that lactobacilli increased and yeasts and molds decreased in the silage at the end of ensilage period. Filya et al. (5) demonstrated that inoculants significantly improved fermentation characteristics of corn silage and increased aerobic stability.

The aim of this study was to determine the effects of *L. delbrueckii* (LD), *L. plantarum* (LP) which were isolated from corn in milk-dough stage, and of commercial inoculant *L. buchneri* (LB) on silage fermentation, aerobic stability, microbial population and silage quality.

Materials and Methods

Leaf and tassel samples of corn (Zea mais) in milk-dough stage were collected from a total of 16 fields in villages of the province of Elazig and brought to the laboratory. LAB were isolated using appropriate methods (6). Selected LAB colonies were then transferred to API 50 CHL (Lactobacillus identification medium, CHL broth, Bio-Mérieux) and MRS (Man–Rogosa–Sharpe) Broth. LD and LP were identified and made available for use as inoculants in corn silage (7).

Whole crop corn used in the study was harvested at dough stage and chopped by a conventional silage machine to a theoretical cut length of 1.5-2.0 cm. LAB prepared as inoculants were transferred into saline (deionized water) and their concentrations were adjusted so that bacterial load would be $1.0x10^{-6}$ colony forming units (cfu)/g. Then, they were uniformly sprayed over 20 kg of silage material on a clean tarpaulin, before the silage material and LAB were thoroughly mixed, filled in 1.8 liter anaerobic jars. Then, the jars were tightly sealed and stored at ambient temperature (at an average temperature of 22 °C and relative humidity of 34). Silage material was prepared in a total of 60 jars, including 15 for each of different groups. In the experiment, experimental groups were designated as follow: 1) group control (C) (no additives); 2) the group with added *L. delbrueckii (*group LD); 3) the group with added *L. plantarum* (group LP); 4) the group with added commercial inoculant *L. buchneri* (group LB). On day 5, 10, 15, 30 and 60 of the experiment, three jars from each group were opened and sampled for microbiological and chemical analyses.

Raw nutrients were determined by methods reported in AOAC (8), and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) levels by ANKOM 220 fiber analyzer (9).

For pH measurements of silages, 25 g of sample was taken from each jar and mixed with 100 mL of distilled water, homogenized in stomacher and its pH was measured with a pH meter (Hanna HI 221). The pH of the filtrate was measured, then it was transferred to a 50 mL centrifuge tube and centrifuged for 10 min at 6000 rpm, then transferred to a vial. Lactic acid (LA), acetic acid (AA) and butyric acid (BA) concentrations of silages were determined by HPLC and calculated as g/kg Dry matter (DM). The HPLC system (Shimadzu, Kyoto, Japan) included pumps (LC-10 ADVP), a PDA detector (SPD M10 VP), a column oven (CTO-10 ASvp), an auto sampler (SIL-10 ADVP) and inertsil ODS-3 column (15×4.6 mm, 5 mm). HPLC column was adjusted to 30°C and 1 mL/min so that 5 mM H_2SO_4 (pH= 4.0) and methanol (90:10, v/v) ratio were achieved.

Aerobic stability tests of silages were conducted using the method developed by Ashbell et al. (10). According to this method, silages in the jars opened on day 60 of the experiment were placed in aluminum trays and allowed to stand at the air at room temperature (at an average temperature of 22 °C and relative humidity of 34) for 5 days. At the end of day 5, visual mold, pH, LA, yeast and mold (10^{-1} cfu) counting were performed. Also, silage materials remaining in the trays at the end of day 5 were placed in 1.5 liter PET bottles, and CO_2 production was determined according to the method developed by Ashbell et al. (10).

Statistical analyses were performed using SPSS 15.0 package program and the main effects of different LAB and fermentation times on examined parameters were established using One-Way Anova test. Duncan multiple comparison test was applied to determine the differences between the groups. All microbiological data were converted into $\log_{10}$ cfu/g.

Results

DM and pH values of the experimental groups are shown in Table 1. As seen in the table, DM levels of whole crop corn varied in the range of 30.55 and 30.90%, and this value dropped to an average level of 28% on day 60. According to Table 1, pH of silage fresh material was 5.40, the highest pH value 4.58, was obtained for group C on day 5 of fermentation, whereas pH values lower than 4.00 were obtained for the other 3 groups ($P<0.001$). Crude protein (CP), crude fat (CF), crude ash (CA) and organic matter (OM) values of the experimental groups are given in Tables 2 and 3. CP and OM levels of group C and group LD were found to be statistically similar at the beginning and end of the experiment ($P>0.05$), whereas CP and OM levels of groups LP and LB decreased toward the end of the experiment ($P<0.01$). During the experiment, CF level remained unchanged in the groups, whereas CA levels of groups LP and LB increased towards the end of the experiment ($P<0.01$). In view of Table 4, in which NDF and ADF values of the groups are given, the highest NDF value 51.67% was obtained for group LP on day 5

of the fermentation, and the difference between the groups was found to be significant (P<0.05). During the experiment, there was no change in ADF, NDF and hemicellulose values of the control group, and an increase was observed in ADL on day 60 (P<0.001). Table 5 shows that LA level of fresh silage material was in the range of 6.72 to 7.37 g/kg DM, and increased to 8.59, 76.15, 74.91 and 64.72 g/kg DM for groups C, LD, LP and LB on day 5, respectively. The highest LA level in the groups with added inoculants was determined on day 30 and the values obtained decreased on day 60 (P<0.001). On day 5 of the experiment, no difference was observed in terms of AA between the groups, and the highest AA value was determined in group LD on day 10, in three groups with added inoculants on day 15, and in group LB on day 30 and 60 (P<0.001). High BA values were obtained in the experimental groups on day 5 and 10 according to the periods, and very low values were determined on day 15, 30 and 60 (P<0.01). Data of aerobic stability test conducted on day 60 of the research are given in Table 6. Accordingly, among the study groups, the lowest pH value of 4.02 was observed in group LB, while the highest pH value of 4.84 was observed in group LP. No difference was observed between the experimental groups in terms of LA, total bacteria and yeast, while mold was not detected in any of the groups.

Table 1. Dry matter (%) and pH profiles of experimental groups

	Fresh Material	5th day	10th day	15th day	30th day	60th day	P
Dry Matter							
C	30.69±0.32	28.67±0.35	28.01±0.60	26.15±1.66	26.92±0.50	27.73±0.09	0.30
LD	30.56±0.61	32.27±0.93	28.28±0.50	28.50±0.18	28.20±0.12	28.28±0.72	0.426
LP	30.55 ± 0.37^{A}	27.49 ± 0.05^{B}	27.04 ± 0.14^{B}	27.17 ± 0.19^{B}	27.31 ± 0.13^{B}	27.67 ± 0.11^{B}	0.000
LB	30.90 ± 0.40^{A}	29.12 ± 0.17^{B}	27.62 ± 0.13^{C}	27.87 ± 0.19^{C}	28.02 ± 0.36^{C}	27.85 ± 0.40^{C}	0.000
P	0.936	0.416	0.225	0.438	0.68	0.261	
pH							
C	5.40 ± 0.04^{A}	$4.58\pm0.01^{a,B}$	3.97 ± 0.02^{C}	$3.90\pm0.01^{c,D}$	4.02 ± 0.01^{C}	4.03 ± 0.01^{C}	0.000
LD	5.40 ± 0.06^{A}	$3.97\pm0.00^{b,B}$	3.95 ± 0.01^{B}	$3.94\pm0.00^{b,B}$	4.01 ± 0.01^{B}	3.99 ± 0.01^{B}	0.000
LP	5.40 ± 0.04^{A}	$3.98\pm0.00^{b,B}$	3.98 ± 0.01^{B}	$3.96\pm0.01^{ab,B}$	4.01 ± 0.00^{B}	4.01 ± 0.00^{B}	0.000
LB	5.40 ± 0.06^{A}	$3.99\pm0.00^{b,B}$	3.98 ± 0.01^{B}	$3.97\pm0.01^{a,B}$	4.02 ± 0.00^{B}	4.03 ± 0.00^{B}	0.000
P	1.000	0.000	0.561	0.001	0.330	0.003	

a, b, c: Values in the same column with different letters are significantly different (P<0.001).
A, B, C, D: Values in the same row with different letters are significantly different (P<0.001).
C: Control, LD: *L. delbrueckii* , LP: *L. plantarum*, LB: *L. buchneri* group.

Table 2. Crude protein and crude fat profiles of experimental groups (%)

	Fresh Material	5th day	10th day	15th day	30th day	60th day	P
Crude Protein							
C	5.85±0.27	5.63±0.21	5.93±0.08	5.33±0.05	5.63±0.41	5.34±0.44	0.740
LD	6.04±0.46	5.19±0.11	6.53±1.59	5.22±0.74	5.28±0.13	5.54±0.15	0.750
LP	6.31 ± 0.30^{A}	5.73 ± 0.36^{AB}	5.13 ± 0.26^{B}	5.48 ± 0.23^{B}	4.99 ± 0.09^{B}	5.31 ± 0.20^{B}	0.034
LB	6.12 ± 0.10^{A}	5.79 ± 0.08^{AB}	5.10 ± 0.08^{C}	5.34 ± 0.35^{BC}	4.79 ± 0.12^{C}	4.99 ± 0.31^{C}	0.005
P	0.775	0.271	0.580	0.977	0.122	0.637	
Crude Fat							
C	2.74±0.27	3.98±1.16	3.57±0.06	3.36±0.76	2.79±0.17	2.12±0.01	0.314
LD	2.56±0.12	2.22±0.41	3.57±0.99	2.70±0.07	2.94±0.34	2.18±0.24	0.347
LP	4.59±1.07	3.97±0.19	2.47±1.21	2.59±0.79	3.00±0.05	2.13±0.05	0.280
LB	3.85±0.29	3.62±0.19	3.59±0.93	3.24±0.51	2.99±0.12	2.04±0.01	0.144
P	0.109	0.232	0.777	0.754	0.875	0.895	

A, B, C: Values in the same row with different letters are significantly different (P<0.001).
C: Control, LD: *L. delbrueckii*, LP: *L. plantarum*, LB: *L. buchneri* group.

Table 3. Crude ash and organic matter profiles of experimental groups (%)

	Fresh Material	5th day	10th day	15th day	30th day	60th day	P
			Crude Ash				
C	7.09±0.06	7.10±0.22	6.83±0.39	7.33±0.07	7.27±0.17	7.75±0.04	0.103
LD	7.07±0.33	6.97±0.15	7.37±0.19	7.13±0.23	7.33±0.17	7.32±0.28	0.771
LP	$7.15±0.30^{B}$	$7.06±0.15^{B}$	$6.86±0.13^{B}$	$7.36±0.08^{B}$	$7.32±0.10^{B}$	$7.85±0.08^{A}$	0.009
LB	$7.19±0.29^{AB}$	$7.09±0.17^{AB}$	$6.85±0.10^{B}$	$6.68±0.16^{B}$	$7.07±0.01^{AB}$	$7.58±0.13^{A}$	0.036
P	0.988	0.929	0.348	0.044	0.513	0.185	
			Organic Matter				
C	90.85±0.54	87.62±0.74	88.50±0.67	89.11±0.29	87.77±0.64	87.48±0.29	0.455
LD	90.26±0.45	89.10±0.27	88.72±0.12	89.51±0.86	87.74±1.29	88.51±0.56	0.263
LP	$90.38±0.30^{A}$	$88.74±0.09^{A}$	$87.98±1.25^{B}$	$89.89±0.01^{A}$	$87.28±0.52^{B}$	$87.89±0.12^{B}$	0.009
LB	$89.99±0.18^{A}$	$88.98±0.04^{AB}$	$90.20±0.11^{A}$	$89.84±0.19^{A}$	$87.89±1.18^{BC}$	$87.29±0.12^{C}$	0.006
P	0.512	0.031	0.453	0.628	0.971	0.115	

A, B, C: Values in the same row with different letters are significantly different (P<0.001).
C: Control, LD: *L. delbrueckii*, LP: *L. plantarum*, LB: *L. buchneri* group.

Table 4. NDF, ADF, ADL and hemicellulose profiles of experimental groups (%)

	Fresh Material	5th day	10th day	15th day	30th day	60th day	P
			NDF				
C	51.10±0.68	$50.59±0.21^{ab}$	49.74±0.54	51.20±0.76	50.53±0.07	52.07±1.62	0.497
LD	49.36±1.28	$49.11±0.39^{b}$	50.31±0.61	49.53±1.07	49.53±0.55	50.27±1.58	0.937
LP	$48.71±0.80^{B}$	$51.67±0.24^{a\,A}$	$50.48±0.97^{AB}$	$51.74±0.63^{A}$	$50.33±0.14^{AB}$	$52.20±0.57^{A}$	0.020
LB	48.49±1.27	$49.54±0.88^{b}$	48.53±0.74	50.18±1.20	49.61±0.23	52.14±0.59	0.112
P	0.340	0.028	0.305	0.399	0.114	0.630	
			ADF				
C	30.92±0.63	$32.90±0.17^{ab}$	32.70±0.87	$32.50±0.17^{b}$	33.30±0.06	32.88±0.52	0.065
LD	$29.69±1.03^{B}$	$32.00±0.58^{\,bA}$	$33.73±0.47^{A}$	$32.70±0.52^{b,A}$	$33.22±0.47^{A}$	$32.71±0.62^{A}$	0.011
LP	$29.74±0.58^{D}$	$33.80±0.23^{a\,BC}$	$32.90±0.29^{C}$	$35.19±0.41^{a,A}$	$34.57±0.41^{AB}$	$32.85±0.24^{C}$	0.000
LB	$28.82±0.48^{C}$	$32.50±0.17^{b,AB}$	$32.60±0.35^{AB}$	$33.00±0.69^{b,AB}$	$34.30±0.52^{A}$	$31.75±0.98^{B}$	0.001
P	0.294	0.028	0.479	0.015	0.104	0.583	
			ADL				
C	$6.13±0.12^{b,B}$	$6.20±0.12^{b,B}$	$6.40±0.35^{b,B}$	$6.70±0.06^{b,B}$	$6.60±0.00^{b,B}$	$9.10±0.17^{a,A}$	0.000
LD	$6.30±0.06^{b,CD}$	$6.37±0.09^{b,CD}$	$7.30±0.05^{a,B}$	$6.11±0.29^{b,D}$	$6.99±0.11^{a,BC}$	$9.20±0.46^{a\,,A}$	0.000
LP	$7.00±0.21^{a,B}$	$7.14±0.18^{a,B}$	$6.89±0.06^{ab,B}$	$7.95±0.29^{a,A}$	$7.17±0.03^{a,B}$	$8.03±0.12^{b\,,A}$	0.001
LB	$6.33±0.03^{b,B}$	$6.41±0.01^{b,B}$	$7.20±0.23^{a,A}$	$7.41±0.12^{a,A}$	$7.30±0.17^{a,A}$	$7.70±0.32^{b,A}$	0.001
P	0.005	0.002	0.063	0.002	0.007	0.017	
			Hemicellulose				
C	20.18±0.44	17.69±0.27	17.04±0.59	18.70±0.93	17.23±0.09	19.19±1.41	0.079
LD	19.66±1.97	17.11±0.56	16.57±0.92	16.83±0.67	16.31±0.78	17.56±2.02	0.526
LP	$18.97±0.28^{A}$	$17.87±0.43^{AB}$	$17.58±1.00^{AB}$	$16.54±0.47^{BC}$	$15.77±0.32^{C}$	$19.35±0.81^{A}$	0.010
LB	$19.67±1.21^{AB}$	$17.04±0.72^{BC}$	$15.93±0.44^{C}$	$17.18±0.51^{BC}$	$15.31±0.29^{C}$	$20.93±1.29^{A}$	0.005
P	0.909	0.616	0.519	0.185	0.071	0.603	

a, b, c: Values in the same column with different letters are significantly different (P<0.001).
A, B, C: Values in the same row with different letters are significantly different (P<0.001).
C: Control, LD: *L. delbrueckii*, LP: *L. plantarum*, LB: *L. buchneri* group.

Table 5. LA, AA and BA profiles of experimental groups (g/kg DM)

	Fresh Material	5th day	10th day	15th day	30th day	60th day	P
Lactic acid							
C	7.37±0.18^{D}	8.59±2.49^{b,C}	10.55±1.88^{c,C}	11.87±1.01^{b,B}	63.59±1.84^{c,A}	46.44±0.98^{c,C}	0.000
LD	6.72±0.43^{D}	76.15±5.25^{a,B}	68.78±2.21^{b,C}	92.81±1.72^{a,A}	103.27±1.04^{a,A}	71.44±0.45^{a,C}	0.000
LP	7.37±0.18^{D}	74.91±4.14^{a,C}	66.52±1.06^{b,B}	93.42±1.20^{a,AB}	98.03±0.87^{ab,A}	68.25±0.32^{ab,C}	0.000
LB	6.82±0.21^{D}	64.72±4.61^{a,C}	83.71±2.14^{a,B}	90.21±1.26^{a,AB}	94.07±2.59^{b,A}	65.15±2.58^{b,C}	0.000
P	0.253	0.000	0.000	0.000	0.000	0.000	
Acetic acid							
C	6.41±0.41^{C}	8.06±0.75^{B}	6.18±0.18^{c,C}	6.85±0.54^{b,BC}	13.51±0.27^{b,A}	8.00±0.55^{bc,B}	0.000
LD	7.39±0.51^{C}	10.18±0.62^{B}	12.47±0.51^{a,A}	12.23±0.71^{a,A}	13.59±0.16^{b,A}	8.81±0.25^{b,BC}	0.000
LP	6.26±0.26^{C}	7.96±0.99^{B}	10.60±0.18^{b,A}	11.03±0.35^{a,A}	11.50±0.13^{c,A}	7.02±0.08^{c,BC}	0.000
LB	7.39±0.50^{D}	8.43±1.27^{CD}	9.85±0.49^{b,C}	11.90±0.16^{a,B}	14.58±0.16^{a,A}	12.37±0.69^{a,B}	0.000
P	0.190	0.368	0.000	0.000	0.000	0.000	
Butyric acid							
C	0.17±0.02	0.27±0.14^{c}	0.58±0.33^{c}	0.54±0.40	0.58±0.15	0.26±0.04	0.642
LD	0.17±0.08^{B}	3.57±0.05^{b,A}	4.06±0.24^{a,A}	0.70±0.36^{B}	0.57±0.16^{B}	0.25±0.06^{B}	0.000
LP	0.17±0.02^{B}	4.41±0.27^{a,A}	4.24±0.07^{a,A}	0.72±0.31^{B}	0.42±0.04^{B}	0.40±0.09^{B}	0.000
LB	0.17±0.88^{C}	4.14±0.18^{ab,A}	2.14±0.06^{b,B}	0.44±0.03^{C}	0.44±0.03^{C}	0.31±0.07^{C}	0.000
P	1.000	0.000	0.000	0.902	0.415	0.416	

a, b, c: Values in the same column with different letters are significantly different (P < 0.001).
A, B, C: Values in the same row with different letters are statistically significantly (P < 0.001).
C: Control, LD: *L. delbrueckii*, LP: *L. plantarum*, LB: *L. buchneri* group.
LA: Lactic acid, AA: Acetic acid, BA: Butyric acid.

Table 6. Aerobic stability of experimental groups

	DM (%)	pH	LA (g/kg DM)	PCA (10^1cfu g^{-1})	Yeast (10^1cfu g^{-1})	CO_2 (g/kg DM)
C	48.08±0.09	4.23±0.12	17.61±0.18	18.35±0.23	7.93±0.23	0.011±0.005
LD	47.93±0.10	4.28±0.08	16.02±0.60	16.78±0.65	8.54±0.06	0.013±0.004
LP	47.89±0.13	4.84±0.34	17.03±0.47	17.60±0.35	8.19±0.32	0.008±0.003
LB	47.67±0.12	4.02±0.01	16.88±0.29	17.92±0.32	8.36±0.03	0.010±0.002
P	0.148	0.067	0.113	0.107	0.229	0.630

LA: Lactic acid, PCA: Total bacteria, LA, PCA and Yeast 10^1cfu g^{-1} levels.
Cfu: Colony-forming units. CO_2: g/kg DM
C: Control, LD: *L. delbrueckii*, LP: *L. plantarum*, LB: *L. buchneri* group.

Discussion

In this study, in which quality characteristics of corn silages with added LA bacteria were investigated, mean DM levels of silages were determined as about 31% at the beginning of the experiment, whereas DM levels declined in all groups on day 60 and came down to levels of 28% on average. No statistical difference was determined in these values between the groups by weeks. When intragroup evaluations were made, a statistically significant decrease in DM level was observed toward the end of fermentation in groups with added LP and LB (P<0.001). Tabacco et al. (11) prepared silages by adding LB and LP to corn and sorghum silages and reported that there was no statistically significant difference in DM level of silages at the end of a fermentation period of 90 days. Previous studies reported that addition of LAB to silages reduced DM losses in anaerobic phase (1).

pH value is one of the most important criteria in determining silage quality. In view of Table 1, a mean pH value of 5.4 was obtained for fresh forages, while on day 5 of the experiment, pH values of 4.58, 3.97, 3.98 and 3.99 were obtained for group C, LD, LP and LB, respectively. According to the findings of this research, addition of inoculant to corn silage can be said to cause a more rapid drop in the pH-value. In silages prepared by addition of LB and LP to whole crop corn, Tabacco et al. (11) found a pH value of 3.57 for control group and values of 3.74 and 3.57 for other groups, respectively, on day 90, and demonstrated that pH value of the group with added LB was higher than pH values of the other two groups (P<0.05). Filya (2) conducted a study by adding LB and LP to corn silage and found pH values of 3.72, 4.13, 3.75 and 3.88 for control, LB, LP and LB+LP groups, respectively on day 90 of the research, where the value obtained for LB was higher than the other groups and statistically significant (P<0.05). Other studies also reported a rapid decrease in silage pH after the addition of LAB to silages (1).

Throughout the study, it was determined that CP and OM levels decreased as from day 10 of the fermentation in groups LP and LB with added inoculants and crude

ash value increased in the same groups on day 60 (P<0.001) (Table 2 and 3). These values suggest that LP and LB broke down CP and OM. In some studies, different inoculants were added to corn silages and the inoculants were reported to have no significant effect on CP and CA (5, 12). Acosta Aragon et al. (1) reported that addition of inoculants to silages increased CP level, while it had no effect on CF and CA levels.

Data related to the levels of NDF, ADF, ADL and hemicellulose, which are cell wall elements of forage, are presented in Table 4. On day 5 of the research, the highest NDF value was observed for group LP and the difference was found to be statistically significant (P<0.05), while such difference was insignificant in the following weeks. The highest ADF value was obtained for group LP on day 5 and 15 of the research, and the difference was found to be statistically significant (P<0.05). With regard to their silages prepared by adding different inoculants, Tabacco et al. (11) concluded that inoculants had no effect on NDF, ADF and ADL values of the silages. Acosta Aragon et al. (1) reported that addition of inoculants to silages had no effect on NDF (P>0.05), and decreased the ADF content (P<0.05), whereas Polat et al. (12) suggested that addition of LAB + enzyme to corn silages did not change ADF content, however, significantly reduced NDF (P<0.01), ADL and cellulose content (P < 0.05).

Addition of different LAB to silages rapidly decreased pH level, whereas rapidly increased LA concentration. In view of data in Table 5, high LA concentrations were determined for the groups with added inoculant on day 5, and the highest concentration was obtained on day 30 (P < 0.001). However, LA concentration of group C with no added inoculant was low during the first several weeks, reached the highest level on day 30, however, it was less than the levels of other groups (P<0.001). AA values obtained in this study are given in Table 5. In view of Table 5, the highest AA value was observed for group LD on day 10 and the difference was found to be statistically significant (P<0.001). AA level in the groups with added LAB on day 10 and day 15 was determined to be higher than that in group C (P < 0.001). In their study conducted using corn and sorghum silages, Tabacco et al. (11) used LP and LB as inoculants, and at the end of a conservation period of 90 days, they found similar levels of LA in control group with no added LAB and the groups with added LP, and a lower level of LA in LB group. In the same investigation, AA level was found to be higher in LB group. Previous studies determined that addition of LAB to silages increased LA and organic acid levels of silages (2) accordingly metabolizable energy level of forage was improved (1).

In view of BA levels in the study groups (Table 5), the highest BA value was obtained in the groups with added LAB on day 5 and 10, and these values steadily declined and dropped down to very low values on day 60.

Corn silages are rich in starch and sugar so when they are exposed to air, they become prone to aerobic decomposition due to the effect of oxygen. Decomposition is more common, especially in hot climates. Because yeast and mold growth would be faster between 20 and 30 °C (13, 14). In the aerobic stability test performed on day 60 of this research, the lowest pH value of 4.02 was observed in group LB, while the highest pH value of 4.84 in group LP (P>0.05). No statistically significant difference was found between the groups in terms of LA, total bacteria and yeast, and no mold growth was observed. These values suggested that the silages were of good quality. Addition of bacterial inoculants to silages was reported to have improved aerobic stability (1, 2, 15). In a study by Hu et al. (16) conducted by addition of LB 40788 to corn silage, the groups with added inoculants were found to have higher rates of acetic acid compared to the control group, and very low levels of yeast has been reported, and it was concluded that the inoculants improved aerobic stability. Addition of homofermentative LAB to silages was reported to improve aerobic stability (17), however, there are also research findings which showed that domination of a medium by homolactic acid bacteria adversely affects aerobic stability (18). In a study conducted by mixing homo- and heterofermentative LAB, Acosta Aragon et al. (1) determined that internal temperature of silage began to increase in the control group with no added inoculant at 66 hrs after silages were exposed to air, that it increased by +2 ºC with respect to the temperature of the environment at 84 hrs, and such increase was more than +14 ^{0}C at 186 hrs. In the same study, the authors stated that the temperature increase in silages at 156 hrs was +2 ^{0}C in the groups with added inoculant, and this value increased to +6 ^{0}C at 234 hrs, and they concluded that inoculants protected silages for 72 hrs longer, as compared to the control group.

In conclusion, in this study, which investigated the effects of addition as inoculants of LAB to silages on silage quality, addition of LAB to silages caused a rapid decrease in pH and a rapid increase in LA level. It was seen that in LD group, for which LD were selected from fresh whole crop corn and added to silages, pH value was lower, the group's DM, CP, OM and CA levels during fermentation period did not decrease, its LA level was higher, as compared to other groups. These results suggest that LD bacteria should be considered as inoculants. Inoculants were found to have no effect on aerobic stability. However, this result may change if the experiment is conducted in warmer environment, rather than at room temperature, or if the silages are allowed to stand longer than 5 days. This should be taken into account in further studies.

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