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Effect of Supplementing Corn Silage with Commercial (Bonsilage-Mais) and Pre-Fermented Lactic Acid Bacteria Inoculant on Silage Quality

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Abstract: This study was aimed to determine the effects of supplementing corn silage with commercial lactic acid bacteria (LAB) inoculant and pre-fermented LAB juice (PFJ) on silage quality. Silage groups other than the control group were designed by adding commercial LAB inoculant (Bonsilage-Mais, 2.5 × 10^5cfu/g of fresh material), and 0.1% (3× 0^5cfu/g of fresh material), 0.3% (9×10^5cfu/g of fresh material), and 0.5% (1.5×10^6cfu/g of fresh material) PFJ. Each silage treatment group was prepared in four replicates. When compared to the control group, the addition of 0.3%, 0.5% PFJ and commercial LAB inoculant decreased the dry matter content of the silages, while the commercial LAB inoculant increased the crude protein content (P<0.05). When compared to the control silage, commercial LAB inoculant and PFJ additives (0.1, 0.3, and 0.5%) increased the ammonia nitrogen, lactic acid (LA), and acetic acid (AA) values of the silages (P<0.05). Butyric acid (BA) was not detected in the silages. As a result, it was concluded that 0.3% or 0.5% PFJ additives can be used as silage additives instead of commercial LAB inoculant.
Keywords: Commercial lactic acid bacteria inoculant, corn silage, pre-fermented juice

Mısır Silajına Ticari (Bonsilage-Mais) ve Doğal Laktik Asit Bakteri İnokulantı Katkısının Silaj Kalitesi Üzerine Etkisi

Öz: Bu çalışma mısır silajına ticari laktik asit bakteri (LAB) inokulantı ve doğal fermente edilmiş laktik asit bakteri sıvısı (Pre-FermentedJuice-PFJ) katkılarının silaj kalitesi üzerine etkilerini belirlemek amacıyla yapılmıştır. Silaj grupları, katkısız grubun yanı sıra ticari LAB inokulantı (Bonsilage-Mais, 2.5x10^5cfu/g taze materyal), %0.1 (3x10^5cfu/g taze materyal), %0.3 (9x10^5 cfu/g taze materyal) ve %0.5 (1.5x10^6cfu/g taze materyal) PFJ ilave edilerek oluşturulmuştur. Her bir silaj muamele grubu dörder tekerrür olacak şekilde hazırlanmıştır. Kontrol grubu ile kıyaslandığında; ticari LAB inokulantı ile %0.3 ve %0.5 PFJ katkılarının silajların kuru madde içeriklerini azalttıkları, ticari LAB inokulantı katkısının ham protein içeriğini arttırdığı (P<0.05) tespit edilmiştir. Kontrol silajı ile kıyaslandığında; ticari LAB inokulantı ile PFJ katkılarının (%0.1, 0.3 ve 0.5) silaj amonyak azotu (NH_3-N/TN), laktik asit (LA) ve asetik asit (AA) değerlerini arttırdıkları görülmüştür (P<0.05). Bu çalışma kapsamında hazırlanan silajların hiçbirinde bütirik asit (BA) tespit edilmemiştir. Sonuç olarak, silaj fermantasyonunu güçlendirmesi adına mısır bitkisinden hazırlanacak silajlara laktik asit ve asetik asit değerlerini arttırmalarından dolayı ticari LAB inokulantı yerine %0.3 veya %0.5 PFJ katkılarının kullanılabileceği kanaatine varılmıştır.
Anahtar kelimeler: Mısır silajı, pre-fermented juice, ticari laktik asit bakteri inokulantı

Introduction

Chemical additives and bacterial inoculants are commonly used to improve silage fermentation (Tao et al., 2020). Bacterial silage inoculants are commercial products that generally contain *Lactobacillus plantarum*, *Streptococcus*, *Enterococcus faecium*, and various *Pediococcus* species, either solely or in various mixtures (Filya, 2000). Commercial silage inoculants improve silage fermentation by reducing the pH, acetic acid (AA), butyric acid (BA), ammonia nitrogen, and ethanol levels of silages, while increasing the ratio of its lactic acid (LA) and LA:AA content (Duni'Ere et al., 2013). LA bacteria (LAB) are an important group of microorganisms for silage fermentation. However, the expensive price of LAB inoculants limits their use (Rabaioli Rama et al., 2020). Therefore, pre-fermented juice (PFJ), which is fermented LA liquid, has begun to be used as an alternative to commercial silage inoculants in recent years, since it is inexpensive and easily prepared when compared to commercial LAB inoculants (Ohshima et al., 1997a; Wang et al., 2009). There have been studies showing that even when commercial LAB inoculants are ineffective, PFJ reduces the pH, ammonia nitrogen, and BA levels of the silage (Nishino and Uchida, 1999, Wang et al., 2009).

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The aim of this study was to determine the effect of supplementing corn silage with commercial LAB inoculant (Bonsilage-Mais, 2.5×10^5cfu/g of fresh material) and 0.1% (3×10^5cfu/g of fresh material), 0.3% (9×10^5cfu/g of fresh material) and 0.5% (1.5×10^6cfu/g of fresh material) PFJ on silage quality. Also; this study was conducted to investigate which doses of PFJ can be used instead of commercial LAB inoculant (Bonsilage-Mais) in corn silages.

Material and Method

The silage material was obtained from the Harran University Livestock Research Unit by harvested at dough stage. Silage samples were chopped (sized 1-2 cm) size and added, on the basis of fresh weight, commercial LAB inoculants (Bonsilage-Mais, 2.5×10^5cfu/g of fresh material), and 0.1% (3×10^5cfu/g of fresh material), 0.3% (9×10^5cfu/g of fresh material) and 0.5% (1.5×10^6cfu/g of fresh material) PFJ and ensiled four replicate in 1.5 liter-glass jars.

The mixture was prepared in a way to ensure that 1g/ton of commercial LAB inoculant that contained *L. plantarum*, *P. Pentosaceus*, and *L. buchneri* was applied to the corn forage by diluting it with water (2.5×10^5cfu/g of fresh material). The application of commercial LAB inoculant rate was in accordance with the level of LAB in the inoculant as determined by the manufacturer. The PFJ was prepared according to the method given by Masuko et al. (2002). The number of commercial LAB inoculants and PFJ LABs used as a silage additive were determined according to the method of Masuko et al. (1992). In the control group, as with all of the silages that contained additives, distilled water was added at the same rate as that in the additive in the group, where the maximum amount of additive was added, to ensure dry matter (DM) stability. All the silage groups were opened at the end of the 60-day fermentation period, and the pH value of the silages was immediately measured (Polan et al., 1998). Fleig Points of the silages were calculated according to Kilic (1984).

Fleig points = 220+(2×DM,%-15)-(40×pH).

Where Fleig points denote values between 85 and 100, very good quality; 60 and 80, good quality; 55 and 60, moderate quality; 25 and 40, satisfying quality; <20 worthless.

Ammonia nitrogen (NH_3-N/TN) analysis was determined using the Kjeldahl method by Broderick and Kang (1980). The volatile fatty acid and LA contents of the silages were determined using a high-performance liquid chromatography device using the method of Suzuki and Lund (1980). The nutrient contents of the silage material and silage samples [DM, ash, and crude protein (CP) contents] were analyzed according to the method of the Association of Official Analytical Chemists, (2005) and their acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed based on the method of Van Soest et al. (1991).

The data was analyzed by using the general linear model procedure of SPSS (1991). Differences among the meanswere determined by the Duncan multiple comparison test at a significance level of P<0.05. For this purpose, the SPSS software package (1991) was used.

Results

The DM, ash, CP, ADF, and NDF values of silage material were found to be 30.68%, 5.78%, 8.32%, 33.11%, and 61.05%, respectively. While the count of commercial LAB inoculant (Bonsilage-Mais) was found 2×10^8cfu/mL, the count of LAB of PFJ was found 3×10^8cfu/mL.

The effects of commercial LAB inoculant and PFJ treatments on the chemical composition of corn silage are shown in Table 1.

Table 1. The effects of PFJ and commercial LAB inoculant treatments on the chemical composition of corn silage

Group	DM	CA	CP	NDF	ADF
Control	30.81±0.19 ^{a}	6.81±0.17	8.13±0.01^{bc}	56.10±0.36	32.05±0.34^{a}
Commercial LAB inoculant	29.55±0.19^{c}	6.72±0.10	8.24±0.08^{ab}	56.83±0.24	31.53±0.64^{a}
0.1% PFJ	30.60±0.09^{a}	6.79±0.20	8.34±0.01^{a}	55.03±0.26	30.93±0.34^{ab}
0.3% PFJ	30.08±0.09^{b}	6.69±0.09	8.11±0.04^{bc}	54.30±0.46	29.66±0.50^{b}
0.5% PFJ	29.40±0.08^{c}	6.53±0.09	8.07±0.08^{c}	55.07±0.42	31.31±0.24^{a}
SEM	0.139	0.059	0.031	0.248	0.252
P value	0.000	0,660	0.018	0.001	0.016

^{a,b,c}: Values with different letters were found different in each column; **Commercial LAB inoculant:** Commercial lactic acid bacteria inoculant; **PFJ:** Fermented natural lactic acid bacteria juice (Pre- Fermented Juice); **FM:** Fresh material; **DM:** Dry matter, %; **CA:** Crude ash, % DM; **CP:** Crude protein, % DM; **ADF:** Acid detergent fiber, % DM; **NDF:** Neutral detergent fiber, % DM; **SEM:** Standard error of means.

The highest DM values were determined from control (30.81%) and 0.1% PFJ (30.60%) groups, while the lowest values were from commercial LAB inoculants (29.55%) and 0.5% PFJ (29.40%) groups (P<0.05). The highest CP values were determined from the 0.1% PFJ (8.34%) group, while the lowest value was from 0.5% PFJ (8.07%) group (P<0.05). The NDF values of the silages statistical difference was not observed (P>0.05), while the lowest was obtained ADF value 0.3% PFJ (29.66% DM) group (P<0.05).

The effects of commercial LAB inoculant, PFJ treatments on pH, organic acid, and ammonia nitrogen content of silages are given in Table 2.

Table 2. The effects of PFJ and commercial LAB inoculant treatments on the fermentation characteristics of corn silage

Group	pH	Fleig Point	NH_3-N	LA	AA	PA	BA
Control	3.78±0.01^{ab}	115.32±0.48^{a}	3.00±0.05^{d}	67.80±0.51^{c}	12.43±0.41^{b}	0.38±0.01^{a}	**ND**
Commercial LAB inoculant	3.74±0.01^{b}	114.69±0.64^{a}	3.90±0.17^{b}	72.64±1.05^{b}	19.21±0.63^{a}	0.35±0.00^{b}	**ND**
0.1% PFJ	3.81±0.02^{a}	113.90±0.56^{a}	4.84±0.10^{a}	84.10±0.69^{a}	18.55±0.57^{a}	0.38±0.00^{a}	**ND**
0.3% PFJ	3.74±0.02^{b}	115.36±0.80^{a}	4.10±0.16^{b}	86.10±0.51^{a}	18.94±0.62^{a}	0.32±0.01^{c}	**ND**
0.5% PFJ	3.80±0.02^{a}	111.80±0.68^{b}	3.49±0.12^{c}	85.00±0.68^{a}	18.27±0.40^{a}	0.34±0.01^{b}	**ND**
SEM	0. 009	0. 396	0. 150	1.735	0.621	0.006	**ND**
P value	0.020	0.008	<0.001	<0.001	<0.001	<0.001	**ND**

a,b,c,d; ** Values with different letters were found different in each column; **Commercial LAB inoculant: Commercial lactic acid bacteria inoculant; **PFJ:** Fermented natural lactic acid bacteria juice (Pre- Fermented Juice); **FM:** Fresh material; **NH_3-N/TN:** Ammonia nitrogen rate in total nitrogen (TN) content % NH_3-N/TN; **LA:** Lactic acid, g/ kg DM;**AA:** Acetic acid, g/kg DM; **PA:** Propionic acid, g/kg DM; **BA:** Butyric acid, g/kg DM; **ND:** Not determined; **SEM:** Standard error of means.

The pH values of the silages prepared in the study were found to be in the range of 3.74 to 3.81, and the lowest pH values were determined from commercial LAB inoculants (3.74) and 0.3% PFJ (3.74) additives. The lowest NH_3-N/TN value was found in the control silages (3.00% NH_3-N/TN), the highest value was obtained from the 0.1% PFJ (4.84% NH_3-N/TN) group (P<0.05). The highest LA values were determined from 0.3%, 0.5%, and 0.1% PFJ addition groups (86.10, 85.00, and 84.10 g/kg DM) respectively. The lowest LA value was found in the control group (67.80 g/kg DM) (P<0.05). The AA value of control silage (12.43 g/kg DM) was lower than commercial LAB inoculant, 0.1% PFJ, 0.3% PFJ, and 0.5% PFJ additives. The propionic acid (PA) value control and %0.1 PFJ silage groups were higher than commercial LAB inoculant, 0.3% and 0.5% PFJ additive groups (P<0.05). The BA was not detected in any of the silages.

Discussion and Conclusion

In this study, the DM values of the silages were decreased with the commercial LAB inoculants and PFJ additives. Similarly, Nishino et al. (2003) reported that supplementing corn silage with commercial *L. buchneri* reduced the DM values. In the other hand Weinberg et al.(1988) and Denek et al.(2011) reported that supplementing commercial LAB inoculant and PFJ additives to legume silages increased the DM values. But in this study all DM values of silages generally acceptable for good silages. In this study, the addition of LAB inoculant and 0.1% PFJ additives increased the CP values, while the addition of 0.3% and 0.5% PFJ decreased them. Arslan et al. (2020) reported that the addition of 7.5 and 15 mL/kg PFJ to the grass silages decreased the CP values of the silages compared with the control silages. In silage studies conducted with trefoil plants, PFJ additives added at different concentrations were reported to have no effect on the CP contents of the silages (Wang et al; 2009; Denek et al., 2012).

In this study, NDF values were found to be similar by the addition of commercial LAB inoculants and PFJ, but a decrease was observed in the ADF values. Ranjit and Kung (2000) reported that silages that were prepared with the addition of two different commercial LAB inoculants and added to corn plants harvested during the milk stage did not affect the CP, NH_3-N/TN, or ADF values, while one of the inoculants (*L. plantarum*) reduced the silage NDF content when compared to the control silage. It can be said that the reason for the difference in the values on NDF and ADF is due to the plant species used in the experiments, their harvest periods, mixing ratios and ecological conditions.

In this study, compared with the control silage, commercial LAB inoculant and PFJ additives had no effect on the pH values (3.74-3.81). Aksu et al. (2004) reported that the addition of commercial LAB inoculant increased the pH values of corn silages. In this study the pH values of silages found between 3.7-4.2, and these values are the embracedof good quality silages (Kung and Shaver, 2001). Fleig points very well determined for all groups. Because Fleig points is related to dry matter and pH values of silages, silages with high dry matter and low pH values have higher Fleig points.

In this study, the NH_3-N/TN values of the silages with addition of commercial LAB and PFJ were increased compared with control silage. Filya et al. (2006) reported that the addition of commercial LAB inoculants to the corn plants don't affect the NH_3-N/TN values of the corn silages. According to the former studies, PFJ additives were reported to reduce the NH_3-N/TN values of the silages (Shao et al., 2007; Denek et al., 2011; Güney et al., 2018). In this study, the increased ammonia nitrogen values of the silages may have been caused by the destruction of the proteins in the silo by *Clostridial* bacteria (Slotner and Bertilison, 2006). However, Carpintero et al. (1979) reported that the NH_3-N/TN value of the silage obtained in their study (3.00%-4.84% NH_3-N/TN) was 11% lower than the NH_3-N/TN value accepted as that of high-quality silage, and that the resultants of in our study silages were in the category of good quality silages. Also, Kleinschmid et al. (2005) reported that the use of LAB inoculant in corn silages had no effect on the DM contents and fermentation properties of the silages. This result can be explained by the high use of content and adequate LAB content for fermentation of corn material (Meeske and Basson, 1998; Ranjit and Kung, 2000).

In this study, compared with the control silage, the addition of commercial LAB inoculant and PFJ increased the LA values of the silages. The increasein LA was found to be more prominent and high with 0.3% PFJ additive when compared with the commercial LAB inoculant. This result can be explained that commercial LAB inoculants contain fewer varieties, while PFJ contains both homofermentative and heterofermentative bacteria species (Ohshima et al., 1997a, 1997b). Nkosi et al. (2009) reported that when prepared by adding commercial LAB inoculant (Bonsilage-Mais) to corn silages, the LA, AA, and PA concentrations were increased, while there was a decrease in the BA and the amount of carbon dioxide values. On the other hand, Palic et al. (2011) reported that the same commercial LAB inoculant created no statistical difference in the DM values of the silages and there was an increase in the LA and AA values. Driehuis et al. (1999) and Nishino et al. (2004) reported that *L. buchneri* increased the AA value of silage. It has been reported that AA is a fungicidal agent such as propionic acids (Kayembe et al. 2013) and that AA level in DM with a rate of 1.5%-3.0% inhibits the growth of yeast and mold fungi. There is a positive correlation among silage aerobic stabilization and silage organic acid level and acetic acid obviously acts as an inhibitor for of the growth of spoilage organisms (Kayambe et al. 2013).

According to the results of this study, it was concluded that PFJ applications, which are more economical at the levels of 0.3% or 0.5%, can be used as silage inoculants instead of the expensive commercial LAB inoculants in corn silages. In addition, it was concluded that *in vivo* studies should be done in order to use PFJ application instead of commercial LAB inoculant in practice.

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