Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1303220

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 4: 402-410 / July 2023

PERFORMANCE OF NEW LACTIC ACID BACTERIA STRAINS AS INOCULANTS ON THE MICROORGANISM COMPOSITION DURING FERMENTATION OF ALFALFA SILAGE CONTAINING DIFFERENT DRY MATTER CONTENT

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Abstract: Alfalfa is the most planted perennial legume in the world due to its high nutritive value, protein content, productivity, and digestibility in addition to high vitamin and mineral content. It is also one of the hardest plants to ensile owing to its low reducible sugar and dry matter (DM) contents and high buffering capacity. In this study, the effects of inoculation with *Lactobacillus bifermentans* which is homofermentative and *Lactobacillus brevis* which is heterofermentative on the silage fermentation of different DM containing alfalfa forage. Alfalfa forages were unwilted, or wilted for 9 or 24 hours in order to achieve low (L), moderate (M) and high (H) DM contents. As a result of the research, it was determined that wilting improved the fermentation properties, decreased the pH value, and increased dry matter recovery. Microbial inoculation decreased the pH value, increased the dry matter recovery, and decreased the number of undesirable enterobacteria in silage. As a result of the research, it was determined that the wilting and inoculation should be applied for successful fermentation of alfalfa silage. It was also determined that inoculation with *L. bifermentans* gave the highest crude protein (CP) content while *L. brevis* gave the highest dry matter recovery (DMR).

Keywords: Alfalfa, *L. bifermantans*, *L. brevis*, Lucerna, Inoculant, Wilting

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Received: May 26, 2023
Accepted: June 14, 2023
Published: July 01, 2023

Cite as: Akbay F, Günaydın T, Arıkan S, Kızılşimşek M. 2023. Performance of new lactic acid bacteria strains as inoculants on the microorganism composition during fermentation of alfalfa silage containing different dry matter content. BSJ Agri, 6(4): 402-410.

1. Introduction

The main limited factors for livestock production in Türkiye are related to shortage of feed production and inadequate quality of feed source. Sufficient quality of feed may be produced from pastures and field production of forages in crop rotation system, however, both of them are insufficient for feeding livestock properly in Türkiye. The most vital and crucial problem to be urgently solved is about quality feed production amount (Canbolat, 2012). Green forage production and hay making are the methods for production quality forage for animal nutrition, but it is obvious that they are far from meeting the deficit in feed need by themselves, especially a large term of the year. Ensiling and preserving green forage without a significant loses from quality for all the year round may be one of the best way for solving the problem.

Alfalfa is known as the queen of fodder plants due to its high adaptation ability (NLO, 2010), high yield due to its multi-cutting, high palatability for livestock and high protein content compared to many other forage crops as well as high essential nutrients such as vitamins and minerals necessary for the ruminant nutrition (Tharanathan and Mahadevamma 2003; Arndt et al., 2015). Even though alfalfa is extensively used for silage processing, however, it is known as hard to ensile successfully plant (Liu et al., 2016) due to its low content of water-soluble carbohydrate, high buffering capacity (Dewhurst et al., 2003; Yang et al., 2004; Li and Wan, 2005; Muck et al., 2007) and in many cases insufficient lactic acid bacteria (LAB) on its epiphytic flora. Additionally, frequent rains in some regions especially during the first and the last cuttings periods prevent field drying of fresh forage, reduce its nutritional value and increase the risk of the reproduction of unwanted microbes. Therefore, need of ensiling process arises after a short wilting treatment or without wilting, rather than completely drying alfalfa particularly mowed at the beginning or at the end of the growing season of the plant. Primarily, dry matter (DM) content significantly influences silage fermentation profile (Xiccato et al., 1994). Low dry matter (DM) content (25%) generally causes high pH and significant DM loss compared to higher DM content (McDonald et al., 1991) and moreover may elicit protease activity and clostridial growth in silage, limiting its use as animal feed (Wan et al., 2021). Wilting may help to preserve the silage properly reducing the number of undesirable microorganisms

(Tao et al., 2021) which compete for fermentable carbohydrates against lactic acid bacteria. On the other hand, excessive wilting reduces the nutritional value of silage (Muck, 1987). Current study was carried out to determine the effects of wilting and different LAB inoculation, selected among 695 isolates obtained from the results of the TUBITAK project, on the fermentation characteristics of alfalfa silage.

2. Materials and Methods

Bilensoy-84 alfalfa cultivar grown under irrigated conditions in farmer's field was used as crop material. Fourth cut alfalfa plants at 50% flowering stage were mowed in the early morning hours and the weeds in the forage were removed. Two different lactic acid bacteria isolated from Türkiye's grassland flora within the scope of a project supported by the Turkish Scientific and Technical Research Organization (TUBITAK) were used as inoculant microorganisms. *Lactobacillus bifermentans* isolate with code number LS-65-2-1 was homofermentative while *Lactobacillus brevis* with code number LS-55-2-2 was heterofermentative in terms of sugar metabolism.

The wilting process was applied to the alfalfa forage on the purpose of defining the effects of different dry matter content on the fermentation profile and behavior of bacterial strains used in the study. After harvesting, the alfalfa plants were wilted for 9 and 24 hours in the shade at outdoor after chopped into approximately 2-3 cm theoretical length. The targeted DM contents were approximately 30% and 35% for moderate (MDM) and high (HDM) DM content, respectively, compared to untreated alfalfa containing low DM (LDM) content around 25%. Accordingly, three different DM content as control (LDM), moderate (MDM) and high (HDM) DM containing silage were evaluated in the study.

Previously regenerated two LAB strains (*L. bifermentans* and *L. brevis*) in MRS broth media were inoculated to different DM containing alfalfa forage after chopping and wilting, at a rate of 10^7 cfu/g fresh forage. Bacteria containing medium were diluted in 20 ml of distilled water and sprayed on the tops of the chopped plant and was homogeneously mixed all over the forage by hand wearing sterile gloves. Only 20 ml of distilled water non-inclusive LAB strain was added for control group. Approximately 400 g of green material was compressed into plastic silage bags, approximately 99.9% of the air in the silage packages was removed with a vacuum machine and then sealed automatically. Totally 27 vacuumed silage packages were prepared, for three DM content (LDM, MDM and HDM), three bacterial inoculations (Control, *L. bifermentans* and *L. brevis* LAB) and three replications. In order to be able to compare silage properties of the forages, forage samples were taken both before (T_0) and 60 days after (T_{60}) ensiling. For microbiological and chemical analysis as well as DM and pH measurements, 20 g samples were taken from the fresh material from T_0 and T_{60} forages, added 180 ml of Ringer solution and mixed with a blender at high speed for one minute. The blended samples (silage extracts) were filtered through Whatman 54 filter paper and a pH measurement was made immediately. Microorganism counts were made from the same filtered samples by making ten-fold dilution series. Serially diluted filtered extracts were analyzed for LAB, enterobacteria, yeasts and molds. LAB numbers were determined by pour plating in de Man, Rogosa, Sharpe agar with double overlaying for anaerobic conditions and plates were incubated at 32 °C for 48 to 72 h. Enterobacteria counts were enumerated by pour plating in violet-red bile glucose agar with a single overlay and plates were incubated at 36 °C for 18 h. Acidified malt extract agar after autoclaving by adding 85% lactic acid was used for yeasts and molds determination and plates were incubated aerobically at 32 °C for 48 to 72 h. The DM content of fresh forages and silage samples were determined by drying in a 70 °C forced-air oven for 48 h. Dried samples of fresh forage and silages were ground to prepare the samples for chemical analyzes with a laboratory mill using a 1-mm sieve. The nitrogen content of the samples was determined by the Kjeldahl method and multiplied by the coefficient of 6.25 to calculate the crude protein content (AOAC, 1990).

The data were analyzed according to the factorial design (wilting and different LAB inoculation) in randomized parcels using the JMP statistical program and the differences between the applications were determined by using LSD test.

3. Results and Discussion

As described in Table 1, DM content, wilting applications, LAB inoculations and their interactions were found to be significant in terms of pH value of forage before ensiling (T_0). The pH value of the uninoculated (Control) alfalfa forage was 6.48 which is the highest compared to *L. bifermentans* (LS-65-2-1) and *L. brevis* (LS-55-2-2) strains inoculations whose pH values were 6.44 and 6.40, respectively. Wilting procedure caused a significant increase of alfalfa forage before ensiling, probably yeast and mold growth on chopped forage during wilting (Wilkinson and Davides, 2013).

There was a significant interaction between wilting and LAB inoculation (Figure 1a). The pH values of T_0 samples were increased by wilting but these raises changed dependently on the LAB used. For example, pH changes in *L. bifermentans* form LDM to MDM were higher than that of other applications. Similarly, pH changes of Control from MDM to HDM were bigger than that of *L. bifermentans* and *L. brevis*. Eventually, it can be manipulated that wilting caused increases in pH values however raises were changed depending on the LAB applications. In other words, LAB strains behaved differently in different DM content resulting a significant interaction.

Table 1. pH values of different DM containing and LAB inoculated alfalfa silages

	pH (T_0)				pH (T_{60})			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	6.28^{de}	6.31^{de}	6.86^{a}	6.48^{A}	5.01	4.68	4.59	4.76A
L.bifermentans	6.25^{e}	6.32^{d}	6.77^{b}	6.44^{A}	4.54	4.66	4.55	4.58B
L. brevis	6.26^{de}	6.28^{de}	6.67^{c}	6.40^{B}	4.62	4.58	4.52	4.57B
Mean	6.27^{C}	6.30^{B}	6.77^{A}	6.44	4.73	4.64	4.55	4.64
LSD	W:0.04** LAB:0.04*WXLAB:0.06**				W: ns LAB:0.17** WXLAB: ns			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

Figure 1. a) pH value of WX LAB interaction b) Enterobacteria count (T_0) of WX LAB interaction.

This finding was in accord with some authors. As an example, Agarussi et al. (2019) determined that wilting would modify the pH during the fermentative process, and the combination of a microbial inoculant and wilting would not change the pH value of its silages.

Resulting silage pH (T_{60}) is one of the most important parameters for evaluating fermentation quality. Silage pH value for leguminous forage is considered sufficient when it falls between 4.5 and 4.9. (Davies et al., 2005). However, reaching this value is quite hard for legumes silages, especially in LDM content. LAB inoculation caused a significant decrease in pH value of silages (Hanagasaki, 2020) regardless to wilting procedure for increasing DM content. In other words, it can be clearly said that bacterial inoculations as well as wilting procedure are a critical factor in lowering the pH value to a satisfactory extend of the alfalfa silage. There were not significant differences between inoculated LAB strains in terms of pH value of T_{60} silages but some researchers found different results from inoculation studies of alfalfa silages. For example, Kızılşimşek et al. (2020) reported that *L. brevis* culture was more effective in lowering the pH level in wilted silages. Muck and Kung (1997) noted that lower pH was obtained in silages treated with homofermentative bacteria in legume plants compared to control. According to the results obtained from the study, it can be said that wilting procedure is a very effective method of reducing the pH of alfalfa silage. It has been revealed that silage obtained from wilted and increased DM content of alfalfa crop positively affects silage quality (Gül et al., 2015). The DM content and bacteria inoculant had significant (P<0.01) effects on crude protein (CP) content of both T_0 and T_{60} alfalfa forage and silage samples (Table 2).

Table 2. Crude protein values of different DM containing and LAB inoculated alfalfa silages

	CP (T_0)				CP (T_{60})			
	DM Content				DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	20.46	20.47	20.73	20.55^{b}	18.55	19.04	19.92	19.17b
L.bifermentans	22.19	20.84	22.76	21.89^{a}	19.34	20.37	21.28	20.33a
L. brevis	21.35	20.56	22.93	21.62^{ab}	18.44	17.88	19.83	18.72b
Mean	21.33^{ab}	20.59^{b}	22.14^{a}	21.35	18.77^{b}	19.10^{b}	20.35^{a}	19.41
LSD	W:1.08** LAB: 1.08** WXLAB: ns				W:1.03** LAB: 1.03** WXLAB: ns			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

Table 3. Dry matter ratio and dry matter recovery values of different DM containing and LAB inoculated alfalfa silages

	DM (T_0)				DM (T_{60})			
	DM Content				DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	24.73	30.73	35.41	30.29	22.6	26.9	33.5	27.67
L.bifermentans	25.51	31.36	35.75	30.88	22.6	28.2	34.3	29.35
L. brevis	24.71	30.33	36.14	30.40	23.6	29.5	32.5	28.53
Mean	24.99^{c}	30.81^{b}	35.77^{a}	30.52	23.91^{c}	28.19^{b}	33.44^{a}	28.52
LSD	W:0.65** LAB: NS WXLAB: ns				W:2.60** LAB:ns WXLAB:ns			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

The CP content of T_0 samples ranged from 20.59 to 22.14% for wilting procedures and from 20.55 to 21.89% for inoculation applications while that of T_{60} samples changed between 18.77-20.35% and 18.72-20.33%, respectively.

It can be clearly said that *L. bifermentans* inoculation had the highest CP content of resulted silages (T_{60}) compared to both *L. brevis* inoculation and control application. This increase can be explained by the fact that LAB inoculated silages have less protein degradation compared to control silages, which are affected by many factors such as pH level, buffering capacity, and VFA content (Ertekin and Kızılşimşek, 2020). Similarly, Silva et al. (2016) found that CP content increased with the application of inoculation LAB. The CP contents increased significantly in HDM treatment compared to the LDM. Similarly, Kızılşimşek et al. (2020) reported that alfalfa plants that wilted for 3 hours achieved higher CP content than those that were not wilted. Blajman et al. (2022) found that CP content was higher in alfalfa silages wilted for 21 hours. Lee et al. (2021) pointed out that the CP content of rye plant wilted for 12 hours was higher than that of non-wilted rye. In contrast, Muck (1987) reported that excessive wilting causes the nutritional value of silage to decrease. Similarly, Zhang et al. (2020), Wan et al. (2021), and Liu et al. (2021) indicated that CP contents decreased by wilting. This situation is related to the wilting conditions such as duration of wilting or environment temperature etc. and can also be explained by cultivar properties.

The DM contents of fresh material (T_0) and resulting silages (T_{60}) did not significantly change by LAB inoculants, but DM contents increased significantly by wilting (P<0.01) as it is supposed to be (Table 3). When the DM values of silages opened on the 60th day were examined, it was determined that there were small dry matter losses. The losses associated with fermentation in the silo seem like mostly due to CO_2 production. The amount of DM lost during fermentation depends on both the dominant microbial species and WSC content of the crop material (Oliveria et al., 2017).

Both inoculations and different DM content did not significantly affect the DMR values, statistically (Table 4). However, it can be said that the increase in the dry matter content of alfalfa caused an increase in DMR compared to the low DM content. In other words, it was determined that plants with high dry matter content had more dry matter recovery which is preferred in terms of economic perspective (Uslu et al., 2017). Moreover, it can be said that dry matter recovery is low in uninoculated (control) silages compared to LAB inoculation applications, and especially the least dry matter loss occurs in *L. brevis* (LS-55-2-2) strain inoculation.

Jatkauskas and Vrotniakiene (2016) explained that dry matter recovery increased significantly in alfalfa silages which are treated by different bacterial strains as inoculants. Similarly, Kızılşimşek et al. (2020) and Günaydın et al. (2023) reported that bacterial inoculation is effective in reducing dry matter expected losses.

Although there was no significant difference according to bacterial applications, lactic acid bacteria numbers among the bacterial inoculants in T_0 varied between 3.94 and 3.96 (log_{10} cfu/g silage). (Table 5). On the 60th day after ensiling, it was determined that there was no

statistical difference in the number of the LAB in terms of the bacterial inoculations, and the number of lactic acid bacteria varied between 5.42 and 5.49 (log_{10} cfu/g silage). Although no statistical difference was observed, the highest LAB count at the fresh material (T_0) and resulting silages (T_{60}) was obtained from the control application.

Table 4. Dry matter recovery values of different DM containing and LAB inoculated alfalfa silages

	DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean
Control	91.29	87.43	94.65	91.12
L.bifermentans	90.45	89.90	95.95	92.13
L. brevis	95.50	97.15	89.98	94.21
Mean	92.41	91.53	93.53	92.49
LSD		W: ns LAB: ns WXLAB: ns		

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

Table 5. Numbers of lactic acid bacteria in different DM containing and LAB inoculated alfalfa silages

	LAB (T_0)				LAB (T_{60})			
	DM Content				DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	4.09	3.50	5.10	4.22	5.06	5.50	5.90	5.49
L.bifermentans	3.27	3.21	5.37	3.94	5.09	5.20	5.99	5.42
L. brevis	3.39	3.30	5.19	3.96	5.20	5.02	6.06	5.43
Mean	3.58^B	3.34^B	5.22^A	4.04	5.09^B	5.31^B	5.98^A	8.17
LSD	W:0.30** LAB: ns WXLAB: ns				W: 0.27** LAB: ns WXLAB: ns			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

Ertekin and Kızılşimşek (2020) have explained that the highest lactic acid bacteria count in the alfalfa plant, in which five different bacterial inoculants were applied together with the control, were obtained from the control applications in fresh alfalfa (T_0) and ensiled during six hours (T_6) and ensiled during 75 days (T_{75}). However, the researchers reported that *L. brevis* isolate gave the highest LAB number in the openings 12 and 24 hours after ensiling, followed by *L. bifermentans* (LS-65-2-1) isolate. It can be clearly stated that there is no parallelism between the number of LAB and pH values. In other words, the high lactic acid bacteria count in control does not mean that the lowest pH value would be obtained from the control. Therefore, it has become understood that the ability of lactic acid bacteria to function effectively in the silo is related to the microorganism composition in the silo and the lactic acid production potential of LABs train individuals. Accordingly, Kızılşimşek et al. (2020) stated that bacterial inoculation and wilting increased lactic acid production resulting in decline in silage pH level. Before ensiling, the number of LAB in LDM and MDM alfalfa was 3.58 and 3.34 (log10 cfu/g silage), respectively, which was substantially lower than that in HDM, which was 5.22 (log10 cfu/g silage), indicating that the greater the DM content, the higher LAB counts in forage.

Correlatively, Tyrolová and Výborná (2011) stressed that the LAB population increased with the wilting applications. Similar results were obtained from resulting silage (T_{60}) which are about 100 times much more than T_0 samples. The LAB count was 5.09 and 5.31 (log_{10} cfu/g silage) for LDM and MDM, respectively, and 5.98 (log_{10} cfu/g silage) in HDM silages in T_{60}. Hence, Rangrab et al. (2000) concluded that the LAB population increased from 5.28 to 6.88 log cfu/g with increasing DM of alfalfa silages. Kızılşimşek et al. (2016), reported that many different microflora groups are competing with each other in silage and that LAB groups need to be dominant in the raw material before ensilage in order to obtain high-quality silage.

When inoculation is taken into consider, the number of enterobacteria among the bacterial inoculants in fresh material (T_0) varied between 6.16 and 6.46 (log_{10} cfu/g silage), and there was no statistical difference between applications (Table 6). Generally, enterobacteria numbers are high in the first stages of ensiling, but their numbers are tended to decrease in the silo as the fermentation continues (Bolsen et al., 1996). Similarly, the study determined that the presence of enterobacteria at the beginning of ensiling decreased from 6.29 to 3.85 by means of fermentation.

Table 6. Numbers of enterobacteria in different DM containing and LAB inoculated alfalfa silages

	Enterobacteria (T_0)				Enterobacteria (T_{60})			
	DM Content				DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	5.16^{e}	5.12^{e}	8.20^{ab}	6.16	4.18	4.19	4.28	4.22^{A}
L.bifermentans	5.27^{de}	5.28^{de}	8.25^{a}	6.26	3.42	3.49	3.54	3.48^{C}
L. brevis	5.74^{cd}	5.95^{c}	7.71^{b}	6.46	3.53	4.10	3.91	3.85^{B}
Mean	5.39^{B}	5.45^{B}	8.05^{A}	6.29	3.71	3.93	3.91	3.85
LSD	W:0.29** LAB: ns WXLAB:0.52**				W: ns LAB:0.30** WXLAB:ns			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

Table 7. Numbers of yeast in different DM containing and LAB inoculated alfalfa silages

	Yeast (T_0)				Yeast (T_{60})			
	DM Content				DM Content			
Bacteria Inoculant	LDM	MDM	HDM	Mean	LDM	MDM	HDM	Mean
Control	6.93^{d}	7.09^{cd}	7.38^{bc}	7.13	6.11^{bc}	5.93^{bc}	5.15^{e}	5.73
L.bifermentans	6.99^{cd}	6.08^{e}	7.58^{b}	6.88	6.16^{b}	5.80^{bcd}	5.41^{de}	5.79
L. brevis	6.32^{e}	7.16^{bcd}	8.07^{a}	7.18	6.89^{a}	5.64^{cd}	5.10^{e}	5.87
Mean	6.74^{B}	6.78^{B}	7.67^{A}	7.06	6.39^{A}	5.79^{B}	5.22^{C}	5.80
LSD	W:0.26** LAB: ns WXLAB:0.45**				W:0.26** LAB:ns WXLAB:4.57**			

DM= dry matter, W= wilting, LAB= lactic acid bacteria inoculation, W X LAB= wilting X LAB inoculation interaction, LDM= control with low DM content, M= moderate DM content, H= high DM content, **=P<0.01, *=P<0.05 statistically significant, ns= non-significant, LSD= least significant difference.

The enterobacteria count in untreated silages in T_{60} was 4.22 ($\log_{10}$ cfu/g silage), while this value significantly decreased to 3.85 in *L. brevis* (LS-55-2-2) and 3.48 in *L. bifermentans* inoculations, showing that *L. bifermentans* (LS-65-2-1) inoculation was more effective than *L. brevis* (LS-55-2-2). Since enterobacteria can compete with lactic acid bacteria for water-soluble sugars (WSC), this is an undesirable microorganism to form in silage (Muck, 2010; Coblentz and Muck, 2012; Gomes et al., 2021). It was determined that the enterobacteria count was insignificantly higher in MDM and HDM than LDM content silage. It can be speculated that the content of WSC in the ensiled material increases proportionally by wilting, which may cause an increase in the number of enterobacteria. These findings were similar to Keklik (2020), who concluded that the number of enterobacteria is higher in alfalfa silage with higher DM content.

DM content and interaction of DM content and bacteria inoculant had significant (P<0.01) effects on concentrations of yeast (T_0; T_{60}) (Table 7).

The yeast count of LAB and wilting interaction in fresh material are shown in Figure 2a. In general perspective, yeast counts were increased in HDM content. However, L.bifermentans application induced a decrease in yeast number in MDM causing an interaction between applications. In Figure 2b, LAB inoculants have shown different effects in the number of yeast count depending on the DM content. ^{Homo}LAB tends to increase the level of lactic acid, whereas ^{heto}LAB increase the level of acetic acid and butric acid. Acetic acid is known to improve aerobic stability by inhibiting the formation of yeast and molds (Muck et al., 2018). At the beginning of the fermentation, a very high (7.06 $\log_{10}$ cfu/g silage as mean) yeast number was counted. It was determined that there was no statistical difference among bacteria applications resulting silage (T_{60}); however, the presence of yeast decreased compared to initial fresh materials.

Kızılşimşek et al. (2016), reported that the number of yeasts decreased at the end of ensiling and the presence of yeast in silages may change depending on the ensiling conditions. In addition, yeast count increased as DM content increases before ensiling (T_0), but this changed in mature silages (T_{60}), and the yeast count decreased as DM content increases. This situation may have resulted from the higher number of LAB growth in the higher DM content. Agarussi et al. (2019) found that the decrease in LAB in silos opened on the seventh day of ensiling caused the dominance of enterobacteria, yeasts, and clostridia.

5. Conclusion

As a result of the study, the combination of lactic acid bacteria (LAB) inoculation and increasing DM content by wilting improved the fermentation profile of alfalfa silages compared to have high moisture content forage. The DM contents of fresh material (T_0) and resulting silages (T_{60}) did not significantly change by LAB inoculants, but DM contents increased significantly by wilting. Additions, the amount of LAB and the CP content and DM recovery in the silages increased by wilting. *L. bifermentans* (LS-65-2-1) was the best strain among the applications, with the highest CP content, the lowest enterobacteria, and the lowest yeast counts while *L.*

brevis (LS-55-2-2) isolate was better in terms of dry matter recovery. Satisfying low pH values were achieved by inoculation of both strains.

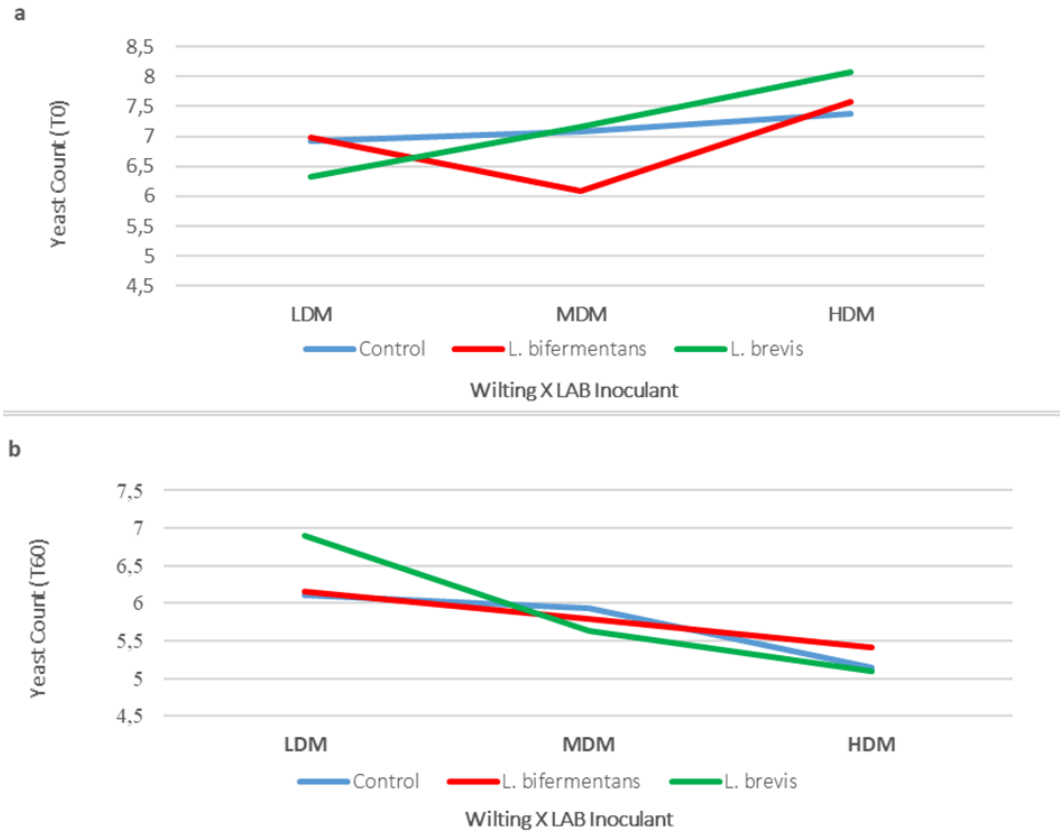

Figure 2. a) yeast count (T_0) of WX LAB interaction, b) yeast count (T_{60}) of WX LAB interaction.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	F.A.	T.G.	S.A.	M.K.
C	30	20	10	40
D	50			50
S	40	10	10	40
DCP	50	25	25	
DAI	60			40
L	70			30
W	60			40
CR	50	10	10	30
SR	50	10	10	30
PM	40	20		40
FA				100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

The microbial materials used in this study were isolated within the scope of the 110O694 project supported by TUBITAK.

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