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Effects of Inoculation of *Lactobacillus plantarum* at Different Doses on Triticale (*Triticosecale wittmack*) Silage on Quality, Fermentation and Aerobic Stability Properties and Feed Value

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ABSTRACT

This study was conducted to determine the effects of different doses of *Lactobacillus plantarum* (LP) inoculation into triticale silage on fermentation, quality, feed value, and aerobic stability. This study used three doses of LP bacteria strains (MF098786 strain) isolated from homemade pickles as inoculants. As LP dose, 1×10^6, 1×10^8 and 1×10^9 cfu/mL levels were used. The LP inoculation was applied by spraying onto by using a sterile injector at 1 mL per 1 kg material. The prepared silages were incubated for 60 d. The treatment groups in the study consisted of triticale control (TC), 1×10^6 (LP^6T), 1×10^8 (LP^8T) and 1×10^9 cfu/kg DM (LP^9T) LP inoculated triticale. The LP inoculation of triticale silage improved silage fermentation, chemical and microbiological properties, silage quality, and feed value, and aerobic stability of the product, regardless of dose application. This application did not change the silage's organic matter, ash, and hemicellulose contents but decreased the crude fiber, neutral detergent fiber, and acid detergent fiber contents. While there was no significant change in color parameters in all silages, a decrease in the ultimate pH value, and improvement in Flieg score and RFV were detected. The LP inoculation into triticale silage increased the number of lactic acid bacteria and decreased the number of yeast in the silages. This application improved the total digestible nutrient and energy values of LP^9T silage compared with other silages. When LP doses were evaluated within themselves, it was determined that all doses gave almost similar results in terms of the parameters studied. However, when the data obtained from the research are evaluated as a whole, LP inoculation at the level of 1×10^9 cfu/mL can be recommended to triticale silage, because of the positive effects of silage on total digestible nutrient, digestible energy, metabolizable energy, and net energy contents.

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Introduction

Silage-making is a popular and feasible method for the long-term preservation of green fodder under anaerobic conditions. This method is the simplest and cheapest way to keep green fodder fresh (Soundharrajan et al., 2021). This is possible with a good fermentation. When good fermentation does not occur, undesirable microorganisms can grow in silages, such as clostridia, enterobacteria, yeast and mold (Ávila & Carvalho, 2020; Kim et al., 2021). These microorganisms not only reduce the nutritional quality of silage, but also threaten animal health. This can situation inevitably cause serious health problems in people who consume the products of these animals (Kim et al., 2021). To minimize the risks in silage production and to obtain quality silage, knowing the properties of the silage material and understanding the conditions of the ensiling process are prerequisites (Oladosu et al., 2016; Wilkinson & Rinne, 2018).

Triticale (*Triticosecale wittmack*) is a cool-climate cereal plant genetically obtained by the hybridization of wheat and rye. It is widely grown in Türkiye and in many other countries. In addition to grain yield, it has become an interesting forage plant because of the high quality forage obtained from the unit area (Sucu & Çifci, 2016). Apart from the grain production, triticale also has several uses in grazing, hay production, and silage. The nutritional content of the roughage is also quite good. Although it varies according to growth periods, it contains 10-15% crude protein on average (Kılınç & Gökkuş, 2022). When evaluated as silage, it has been reported that the triticale plant has a similar feed value to wheat, rye, and barley, and the crude protein content of its silage is between 8.30% and 11.50% (González-Alcántara et al., 2020). Harper et al. (2017) reported that triticale silage can partially replace corn silage in the total mixed rations of dairy cows.

The triticale plant is a good source for silage production because of its early heading and high protein content. However, effective strategies are needed to improve silage quality, increase feed value, and preserve for a long time (Sucu & Çifci, 2016; Soundharrajan et al., 2021). As a strategy, many researchers suggest taking advantage of inoculants that can use water-soluble carbohydrates in the environment and increase the level of lactic acid in the silage environment for silage production (Soundharrajan et al., 2021). Lactic acid bacteria are the most important inoculant that serves this purpose. These convert the easily soluble carbohydrates in the silage into lactic acid. This lactic acid contributes to the improvement of fermentation quality by rapidly lowering the pH of the silage (Turan & Önenç, 2018; Soundharrajan et al., 2021; Jung et al., 2022; Ma et al., 2022).

The number of studies on improving the feed value of triticale silages using lactic acid bacterium is quite limited. Moreover, what kind of effects *Lactobacillus plantarum* (LP), which is a lactic acid bacteria, will have on promising plants such as triticale and at what dose will be used has not yet been revealed. Therefore, the aim of this study was conducted to determine the effects of different doses of *L. plantarum* inoculation into triticale silage on silage quality, feed value, and aerobic stability.

Materials and Methods

The plant material of the experiment, triticale (*Triticosecale wittmack*), was grown in an experimental area of the Field Crops Department of Kırşehir Ahi Evran University (1090-m asl., 39º08'N, and 34º06'E). The *Lactobacillus plantarum* bacteria strain (strain MF098786) used as an inoculant in the study was isolated, identified, and stocked from homemade pickles as described by Erdem et al. (2021). These stock cultures were stored at -80 °C in MRS (Merck *Lactobacillus* Agar acc. to de Man, Rogosa) containing 35% (v/v) glycerol in the laboratory. Analyses were performed at Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology.

In the study, as LP dose, 1×10^6, 1×10^8, and 1×10^9 cfu/mL levels were used. The treatment groups in the study consisted of triticale control (TC), 1×10^6 (LP^6T), 1×10^8 (LP^8T), and 1×10^9 cfu/kg DM (LP^9T) *L. plantarum*-inoculated triticale. Each treatment group was prepared as eight replicates, and three of them were used in the analysis depending on chance. When triticale reached 25-30% DM content in the heading stages (June 15), it was harvested by hand and withered for 8-10 h under laboratory conditions to reach 35-40% DM content. The plants brought to the laboratory were chopped with a butcher knife to a length of 2–3 cm. After the chopping process was completed, a sample was taken from the fresh material for analysis. The chemical composition and calculated energy value of the fresh material before ensiling are presented in Table 1.

The obtained materials were filled in PVC bags with a size of 250×200 mm, a capacity of 1 kg, and an oxygen permeability of 1.13 cc/m^2/day, each containing 500 g of material. The LP inoculation was applied by spraying onto with a sterile injector at 1 mL per 1 kg material. To prepare the stock cultures for use, they were thawed at 4°C, cultivated on MRS agar, and incubated at 37°C for 3 days. An equal amount of sterile distilled water was sprayed on the uninoculated control group. After the filling and inoculation process was completed, the sample bags were closed with a vacuum sealer and kept at ambient temperature 22-25°C for 60 d.

Table 1. The chemical composition and energy values of fresh triticale before ensiling

Items	Fresh triticale
Dry matter, %	100.00
Organic matter, %	94.40
Ash, %	6.01
Crude Protein, %	11.04
Ether extract, %	4.31
Crude fiber, %	27.50
Acid detergent fibers, %	35.16
Neutral detergent fibers, %	63.36
Hemicellulose[1], %	28.20
Non-fiber carbohydrates[1], %	15.27
Total digestible nutrients[1], %	59.97
Digestible energy[1], MJ/kg DM	11.063
Metabolizable energy$_1$, MJ/kg DM	9.072
Net energy-lactation[1], MJ/kg DM	5.645
Net energy-maintenance[1], MJ/kg DM	5.475
Net energy-gain[1], MJ/kg DM	3.076
Relative feed value[1]	90.31

[1)] It was determined by calculation.

After 60 days of fermentation, the silages were opened. To evaluate the opened silages, physical, chemical, and microbial analyze and aerobic stability tests were performed. Dry matter (DM, method no. 934.01), ash (method no. 942.05), and total protein content using the Kjeldahl procedure (method no. 988.05) of silage samples were determined according to AOAC (2000). The ether extract (EE; Am 5-04) content was analyzed using the ANKOM XT15 Extraction System AOCS (2005). The crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were analyzed using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA) according to the method of Van Soest et al. (1991). Organic matter (OM) was calculated by subtracting total ash value from the DM. The hemicellulose (Hcel) content was calculated as the difference between NDF and ADF. The content of non-fiber carbohydrates (NFC) was calculated as specified by NRC (2001).

$$\mathrm{NFC\%} = \mathrm{DM} - (\mathrm{Ash} + \mathrm{CP} + \mathrm{EE} + \mathrm{NDF})$$

The pH values of the silages were determined using a pH meter (Eutech Instruments pH 700). The total soluble solid (TSS) content was estimated by pressing a few drops of silage juice on the surface of a digital sucrose refractometer (HI 96801, Hanna Instruments Deutschland GmbH, Vöhringen, Germany) with a sensitivity of 0.2 Brix, at room temperature. Measurements were recorded as %brix (Singh et al., 2020).

The *L**, *a**, and *b** color parameter properties were measured from three different parts of the silage using a Konica-Minolta CR-410 color meter. These data were recorded on the following scales: (*L**) brightness (100: white, 0: black), (*a**) from red to green (-*a**: green, +*a**: red), and (*b**) from yellow to blue (-*b**: blue, +*b**: yellow).

Chroma (*C**, saturation index) and hue angle (h^o) values were calculated using the following formulas (AMSA, 2012).

$$(C)ab = [(a)^2 + (b)^2)]^{1/2}$$

$$(h)ab = \text{arctangent}\,(b/a)$$

Flieg score (FS) was calculated using the formula reported by Kılıç (2006).

$$FS = 220 + (2 \times DM\% - 15) - 40 \times pH$$

Total digestible nutrient (TDN), dry matter (DDM), and dry matter intake (DMI; % of BW) values were calculated using the following formulas (NRC, 2001). The relative feed value (RFV) is according to the formulas given by Jeranyama and Garcia (2004).

$$TDN\ (\%) = 50.41 + 1.04 \times CP - 0.07 \times CF$$

$$DDM\ (\%) = 88.9 - [0.779 \times ADF\%]$$

$$DMI\ (\%\ \text{of Body Weight}) = 120/[NDF\%]$$

$$RFV = [DDM \times DMI]/1.29$$

Digestible energy (DE), metabolizable energy (ME), net energy lactation (Oliveira et al., 2021), net energy maintenance (NE_m), and net energy gain (NE_g) values were calculated by using the formulas given in NRC (2001).

$$DE, MJ/kg\ DM = [TDN\% \times 0.04409] \times 4.184$$

$$ME, MJ/kg\ DM = [0.82 \times DE] \times 4.184$$

$$NEl\ (MJ/kg\ DM) = [0.0245 \times TDN(\%) - 0.12] \times 4.184$$

$$NEm\ (MJ/kg\ DM) = [1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12] \times 4.184$$

$$NEg\ (MJ/kg\ DM) = [1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65] \times 4.184$$

Lactic acid bacteria, yeast, and mold numbers were determined using the plate count method as described by Seale et al. (1990). A stock solution was prepared by mixing 10 g of the silage sample in sterile 90 mL of 0.85% NaOH solution. After preparing dilutions in a logarithmic series with this stock material, planting was performed in sterile Petri dishes. As the incubation medium, MRS agar (Merck *Lactobacillus* Agar acc. to de Man, Rogosa, and Sharpe for microbiology) was used for *Lactobacillus* and malt extract agar (MEA, Merck, Darmstadt, Germany) was used for mold and yeast. Sterile MRS and MEA agar were poured into approximately 15 mL sterile Petri dishes after cooling at 45°C. After the incubation of Petri dishes at 30°C for 3 days, the number of colonies of *Lactobacillus* spp., yeast, and mold that developed was determined. The determined *Lactobacillus* spp, yeast, and mold numbers were converted to logarithmic coliform units (log10 cfu/g).

The pH, development in the number of microorganisms, and carbon dioxide production were considered criteria in the determination of aerobic stability, on the fifth day after the silage samples were opened (Ashbell et al., 1991). For carbon dioxide measurement, 1.5-liter soft drink bottle (polyethylene terephthalate) and a 600 mL glass beaker were used. To ensure air circulation, two holes with a diameter of 1 cm, one at the top and one at the bottom of the plastic bottle, were drilled. A 200 g wet silage sample was placed on the pet bottle, which constitutes the upper part of the apparatus. The silage was loosely placed in this part. A 100 mL 20% KOH was placed in the glass beaker to form the lower part. The pet bottle was placed in the beaker with the lid down and not touching the KOH solution. Gas exchange in the system was provided only through a hole in the upper part. The apparatus was maintained in this manner under laboratory conditions for five days. The produced carbon dioxide was absorbed into KOH and then titrated with 1-N HCl to determine its amount (Ashbell et al., 1991).

Statistical analysis of all data was performed by one-way analysis of variance (ANOVA) using the SPSS package program (ver. 25.0, SPSS Inc., USA). The means were compared using Duncan's test at $P < 0.05$ level.

Results and Discussion

The chemical composition of the experimental silages is shown in Table 2. The LP application to triticale silage decreased the OM content of silages numerically in LP^6T and LP^8T silages compared with the control, and increased it in LP^9T silage (P>0.05). However, there was no statistically significant difference between the OM data. The highest OM content was obtained in the LP^9T silage (936.56) and the lowest in the LP^8T silage (932.93). The highest ash value was obtained in the LP^8T silage (67.07) and the lowest ash value was obtained in the LP^9T silage (63.45). LP application to triticale silage decreased the HP content in LP^6T and LP^8T silages numerically compared with the control, but this decrease was insignificant. This indicates that controlled fermentation occurs with less proteolysis in LP-inoculated silages than in uninoculated silages. The CP content of LP^9T silage (109.88) was higher than that of other silages (P<0.01). The LP treatment decreased the EE value in triticale silages (P<0.01). The lowest EE value was obtained in the LP^8T silage (40.15). The LP application to triticale silage caused significant changes in the structural carbohydrate content of the silages. The CF values of the silages showed statistically similar values in the control (TC), LP^6T, and LP^8T silages, whereas the LP^9T silage had a lower CF value than the other groups (P<0.01). The LP application significantly decreased the ADF content of triticale silages compared with control silage. This result is compatible with that of Sucu and Çifci (2016), who reported that the fiber fraction in triticale silage decreased with LAB+enzyme application. Similarly, Ozduven et al. (2010) reported that enzyme and bacteria + enzyme mixture inoculations reduced the cell wall content of silages by 30-35%. There was no difference in ADF content between the LP inoculated silages. The LP inoculation decreased the NDF content of the silages compared with the control, but the decrease in the LP^9T silage was insignificant. It did not create a statistically

significant difference in the Hcel content of silages. While LP application increased the NFC content of LP[6]T and LP[8]T silages compared with the control, it did not affect it in the LP[9]T silage. Turan and Önenç (2018) reported that the lactic acid produced in the silage rapidly decreases the pH, because of which the proteolytic activity may be limited and the ADF and NDF contents may decrease. O'Kiely (2011) reported the CP, ADF, and NDF content of triticale silage as 92-95, 345-363 and 556-567 g kg^{-1} DM, respectively. Harper et al. (2017) reported the DM, NDF, ADF, and CP contents of triticale silages as 30.7, 51.1, 32.9, 17.3%, respectively. Huuskonen et al. (2020) reported the OM, CP, and NDF values of triticale silage as 949, 98 and 496 g kg^{-1} DM, respectively. González-Alcántara et al. (2020) reported the OM, HP, NDF, and ADF contents of triticale silage as 878.95, 90.60, 666.77, and 358.73 g kg^{-1} DM, respectively. The differences between the nutrient content of triticale silage obtained in this study and those reported in the literature may be due to differences in the maturity period and harvest time of the plant and the applied processes.

It is generally used as a visual scoring system in the evaluation of silages in terms of color. However, in recent studies, the CIELAB Color System has been used to evaluate the color of silages (İnce and Vurarak, 2019; Çayıroğlu et al., 2020). This method provides a more objective and numeric evaluation opportunity than the visual scoring system. Although the color of the quality silage varies according to the plant variety, it is desired to be in different tones from light olive green to brownish olive. Black and very dark colors are not normal.

The color properties of the silages at time opening are presented in Table 3. The *L**, *a**, and *b** values were determined to be between 42.81 and 47.63, 2.04 and 3.38, 15.47 and 17.68, respectively. The highest *L** value of 47.63 was determined in the LP[8]T silage. It is thought that the high brightness value could be an effective factor in animal preferences (Sahar et al., 2022). The *C** and *h*° values were calculated between 15.62 and 18.02, and 79.40, and 82.68, respectively. The LP treatment had no effect on the color parameters of triticale silage.

The TSS, DM, and pH_{0d} contents of silages at the time of opening and quality class values according to Flieg scores are given in Table 4. The TSS and DM contents of the triticale silages were between 26.38 and 28.90 Bx%, 45.07 and 46.79%, respectively. The pH_{0d} values and Flieg scores were between 4.59 and 5.60, 74.67 and 111.66, respectively. The TSS is the most important energy source for maintaining the fermentation quality of silage. Lactic acid bacteria in silage can convert water-soluble fractions of total soluble solids into organic acids, thereby lowering the pH and preserving the feed (Jung et al., 2022). Although the TSS values of the triticale silages tended to decrease compared with those of the control, they were statistically insignificant. Ma et al. (2022) reported similar results. The LP inoculation of triticale silages significantly reduced the DM content of the silages, but the dose had no effect on the reduction. The LP inoculation significantly reduced the pH_{0d} values of the silages at the time of opening.

Table 2. The chemical composition of the experimental silages, g/kg DM

	OM	ash	CP	EE	CF	ADF	NDF	Hcel	NFC
TC	935.60	64.40	107.54b	44.90a	256.92a	313.47a	554.20a	240.73	228.97b
LP[6]T	933.75	66.25	107.49b	42.73b	253.16a	299.68b	523.34b	223.67	260.20a
LP[8]T	932.93	67.07	106.31b	40.15c	253.56a	296.85b	522.63b	225.78	263.85a
LP[9]T	936.56	63.45	109.88a	42.82b	248.00b	301.12b	547.79a	246.67	236.08b
SEM	0.640	0.640	0.512	0.655	1.285	2.457	5.603	4.162	5.817
P Value	0.131	0.131	0.013	0.006	0.025	0.003	0.013	0.081	0.004

OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; ADF, acid detergent fiber; NDF, neutral detergent fiber; Hcel, hemicellulose; NFC, non-fiber carbohydrates; [a,b,c] Means with different superscripts in the same row differ significantly (P<0.05).

Table 3. Color properties of silages at time opening

	*L**	*a**	*b**	*C**	h^o
TC	44.60	2.16	15.47	15.62	82.09
LP[6]T	42.81	2.42	15.85	16.05	81.21
LP[8]T	47.63	3.38	17.68	18.02	79.40
LP[9]T	44.11	2.04	15.86	16.00	82.68
SEM	1.029	0.230	0.427	0.453	0.578
P Value	0.434	0.147	0.272	0.242	0.208

*L**, lightness; *a**, redness; *b**, yellowness; *C**, chroma or saturation; h^o, hue angle.

Table 4. Total soluble solid, dry matter, and pH_{0d} contents, and quality classification according to Flieg score

	TSS, Bx%	DM, %	pH_{0d}[1]	Flieg score[2]	Silage quality
TC	28.90	46.79a	5.60a	74.67c	Good
LP[6]T	27.85	45.28b	4.84b	102.06b	Excellent
LP[8]T	26.38	45.13b	4.59c	111.66a	Excellent
LP[9]T	26.73	45.07b	4.60c	111.04a	Excellent
SEM	0.409	0.267	0.106	3.911	-
P Value	0.101	0.049	0.001	0.001	-

TSS, The total soluble solid; DM, dry matter.; [1)] pH_{0d}, the pH value determined as soon as the silages are opened.; [2] >80, excellent; 61-80, good; 41-61, medium; 21-40, weak; 0-20, poor; [a,b,c.] Means with different superscripts in the same row differ significantly (P<0.05).

Table 5. The number of lactic acid bacteria, yeast, and mold at the time of opening of the silages

	Lactic acid bacteria, log10 cfu/g	Yeast, log10 cfu/g	Mold, log10 cfu/g
TC	5.763b	5.36a	Not detected
LP^{6}T	6.227a	4.02b	Not detected
LP^{8}T	6.037a	4.30b	Not detected
LP^{9}T	6.168a	3.72b	Not detected
SEM	0.060	0.220	Not detected
P Value	0.004	0.004	Not detected

[a,b] Means with different superscripts in the same row differ significantly (P<0.05).

Table 6. Digestible dry matter, dry matter intake, and relative feed value of silages

	DDM, %	DMI, % of body weight	RFV[1]	Silage RFV quality
TC	64.48b	2.165b	108.25b	III. quality
LP^{6}T	65.56a	2.295a	116.53a	III. quality
LP^{8}T	65.78a	2.295a	117.08a	III. quality
LP^{9}T	65.44a	2.190b	111.13b	III. quality
SEM	0.191	0.023	1.432	
P Value	0.003	0.008	0.004	

[a,b] Means with different superscripts in the same row differ significantly (P<0.05). DDM, digestible dry matter; DMI, dry matter intake; RFV, relative feed value; [1)] According to the roughage classification method, the RFV value "V" (< 75) indicates poor quality to be rejected; (75-86), IV. Quality; (87-102), III. Quality; (103-124), II. Quality; (125-151) "prime" quality; and (>151) refers to the best quality.

Table 7. Total digestible nutrients and energy contents of silages

	TDN, %	DE, MJ/kg DM	ME, MJ/kg DM	NEl, MJ/kg DM	NEm, MJ/kg DM	NEg, MJ/kg DM
TC	59.80b	11.030b	9.045ab	5.625b	5.450b	3.050b
LP^{6}T	59.82b	11.035b	9.045ab	5.625b	5.455b	3.050b
LP^{8}T	59.69b	11.015b	9.025b	5.615b	5.435b	3.040b
LP^{9}T	60.10a	11.090a	9.090a	5.660a	5.490a	3.090a
SEM	0.059	0.011	0.010	0.007	0.008	0.008
P Value	0.006	0.004	0.071	0.007	0.013	0.026

[a,b] Means with different superscripts in the same row differ significantly (P<0.05); TDN, total digestible nutrient; DE, digestible energy; ME, metabolizable energy; NE_l, net energy-lactation; NE_m, net energy-maintenance; NE_g, net energy-gain.

The most significant pH_{0d} decrease was determined in the LP^{8}T and LP^{9}T groups, but no difference was found between these two groups. Jung et al. (2022) determined the pH_{0d} value between 6.10 and 6.20 in uninoculated triticale silages and between 4.10 and 4.20 in silages inoculated with lactic acid bacteria. Ma et al. (2022) determined the pH_{0d} values at the end of fermentation to be 3.98 and 4.64 in triticale silages harvested at different periods. Soundharrajan et al. (2021) reported pH_{0d} values between 4.18 and 4.71 in triticale silages inoculated with *L. rhamnosus* and *L. paracasei*. Researchers reported the pH_{0d} values of silages without inoculant between 5.85 and 6.14. The difference between pH_{0d} values determined in this study and the literature reports may be due to the difference in the vegetation period of the silage material and the difference in the inoculant used.

The numbers of lactic acid bacteria, yeast, and mold at the time of opening of the experimental silages are given in Table 5. The LP inoculation to triticale silage increased the lactic acid bacteria count of the silages, but decreased the yeast count. The dose of LP had no effect on lactic acid bacteria or yeast counts. No mold was grown in any experimental silage at the time of opening. Mold growth was inhibited at low pH levels due to LP inoculation, but yeast growth could not be completely prevented. Turan and Önenç (2018) stated that the presence of yeast determined in the silages may be due to the presence of yeast in the fresh material since there is no possibility of air entering the silage medium during fermentation. These results show that LP inoculation increases the LAB number and decreases the pH rapidly, thus preventing the growth of unwanted microorganisms in the silage. There are many studies available to support this idea. Turan and Önenç (2018), Soundharrajan et al. (2021), Jung et al. (2022), and Ma et al. (2022) reported that lactic acid bacteria convert easily soluble carbohydrates in silage to lactic acid, and lactic acid contributes to the improvement of fermentation quality by rapidly lowering the pH of the silage.

Digestible dry matter, dry matter intake, and relative feed value of the triticale experimental silages are given Table 6. The LP Inoculation increased the DDM values of the silages compared with the control. Similarly, the addition of LP increased the DMI and RFV values of the silages compared with the control. However, the increase in these values was significant in LP^{6}T and LP^{8}T levels, but insignificant for LP^{9}T levels. All silages were in the third quality class in terms of RFV quality.

The total digestible nutrients (TDN) and energy values of silages are given in Table 7. The LP inoculation increased the TDN value of the LP^{9}T silage compared with control and other groups, but the TC, LP^{6}T, and LP^{8}T silages had similar values. Similarly, the LP inoculation increased the DE, MEl, NEm, and NEg values of the silages compared with the control and other groups, whereas the TC, LP^{6}T and LP^{8}T silages did not differ in terms of the energy value. When the trial silages were examined in terms of ME value, it was not determined that LP inoculation did not create a significant difference among in the silage groups. Huuskonen et al. (2020) reported the ME value of triticale silage as 9.6 MJ kg^{-1} DM.

Table 8. The pH, carbon dioxide, yeast, and mold values in silages after the aerobic stability test

	pH_{0d}[1]	pH_{5d}[1]	CO_2, g/kg DM	Yeast, log10 cfu/g	Mold, log10 cfu/g
TC	5.60a	5.79a	5.35a	7.51a	Not detected
LP^6T	4.84b	4.98b	3.46b	5.90c	Not detected
LP^8T	4.59c	4.93b	3.08b	7.11a	Not detected
LP^9T	4.60c	4.82b	2.77b	6.50b	Not detected
SEM	0.106	0.105	0.403	0.167	-
P Value	0.001	0.001	0.025	0.001	-

[1] pH_{0d}, the pH value determined as soon as the silages are opened; pH_{5d}, the fifth-day pH value; [a,b,c] Means with different superscripts in the same row differ significantly ($P<0.05$).

Aerobic stability of silages is defined as the number of hours that the temperature of a silage exposed to air, remains 1°C to 2°C below ambient temperature (Addah, 2022). It is evaluated using the results of measurements made with thermal cameras, as well as the chemical and microbiological changes that occur in silage (Carvalho et al., 2021). In this study, the method proposed by Ashbell et al. (1991) was used as a determinant in the evaluation of aerobic stability. In this method, aerobic stability was evaluated by examining the pH, carbon dioxide (CO_2) production, and yeast and mold growth on the 5th day after the silages were opened. The pH, CO_2, yeast, and mold values of the silages determined after five days of aerobic stability are given in Table 8. The LP inoculation reduced the pH of the silages on the fifth day (pH_{5d}) compared with the control. However, the LP dose had no effect on this decrease. As a result of the five-day test to determine the aerobic stability of silages, the CO_2 production values were found to be lower in the treatment groups than in the control. In other words, the LP inoculation decreased the fifth day CO_2 production value of silages. This decrease continued as the LP dose increased, but there was no statistical difference between the treatment groups. The LP inoculation decreased the fifth day yeast counts of the silages compared with the control group. This reduction was more pronounced in LP^6T and LP^9T silages. There was no difference between the TC and LP^8T silages in terms of the fifth day yeast counts. No mold was detected in silages after 5 days of aerobic stability testing.

Conclusion

The LP inoculation of triticale silage improved silage fermentation, chemical, and microbiological properties, silage quality, and aerobic stability of the product, regardless of the dose application. In fact, the number and efficiency of LAB in silage increased with LP inoculation. The lactic acid produced by LAB contributed to the improvement of silage fermentation and quality by rapidly lowering the silage pH. In addition, this application contributed to the degradation of plant cell wall elements (CF, ADF, NDF, and Hcel). This improved the DE, ME, and NE value of the silages. Similarly, LP treatment increased the DDM, DMI, and RFV value of the silage, and aerobic stability was maintained for five days after the silage was opened. When the LP doses were evaluated within themselves, it was determined that all doses gave almost similar results in terms of the parameters studied. However, when the data obtained from the research are evaluated as a whole, LP inoculation at the level of $1*10^9$ cfu/mL can be recommended to triticale silage, especially because of the positive effects of silage on TDN, DE, ME, and NE contents.

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