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Effects of Different Doses of *Lactobacillus brevis* Addition to Oat (A*vena sativa* L.) Silage on Physical and Chemical Properties, Aerobic Stability, in Vitro Organic Matter Digestibility and Energy Value of Silage

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A B S T R A C T

This study aimed to determine the effects of the addition of different doses of *Lactobacillus brevis* to oat silage on the physical and chemical properties, aerobic stability, in vitro organic matter digestibility, and energy value of the silage. In the study, the treatment groups were oat control (OC), 1x10^6 (Lb6), 1x10^8 (Lb8) and 1x10^9 cfu/kg dry matter (Lb9) *L. brevis* inoculated oats. Inoculation of *L. brevis* into oat silage increased the number of lactic acid bacteria and decreased the pH of the silage at the time of opening. This inoculation decreased the organic matter, acid detergent fiber and neutral detergent fiber contents of the silages at opening compared to those of OC, while increasing the dry matter and crude protein contents. The *L. brevis* inoculation into oat silage decreased the pH values and yeast counts on the fifth day after opening the silages compared to OC, without affecting the amount of CO_2 production and mold count. The inoculation did not affect the in vitro organic matter digestibility and metabolizable energy value of silage; but increased the net energy lactation value compared to OC. When the *L. brevis* doses are evaluated independently, it can be said that 1*108 and 1*109 cfu/kg DM doses contributed more effectively to oat silage, so any of these doses can be preferred.

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Introduction

Oat (*Avena sativa* L.) is one of the most important feed and food crops worldwide, characterized by its tolerance to saline-alkali environments, drought, cold and stress (Tahir et al., 2023). Since it is highly palatable and is a good feed raw material, it is used both as hay and silage to feed ruminant animals (Jia et al., 2021). Its preservation as roughage causes a decrease in its feed value. Therefore, it is necessary to develop both silage production methods and technique as an alternative method to preserve the feed value of oats. Ensiling of oat plants can provide better quality and reliable feed production than keeping them as roughage (Kuter et al., 2023). However, ensiling oat plants is very difficult compared to maize, due to its low water-soluble carbohydrate and high moisture content. In addition, low compaction density, which causes air to leak in, also reduces silage quality. All these prevent the development of an effective fermentation in oat silage, which leads to a decrease in silage quality (Jia et al., 2021; Kuter et al., 2023). However, quality silages can also be obtained from oat plants provided that appropriate silage making techniques are applied, and this can facilitate the availability of quality roughage for ruminant animals throughout the year (Kuter et al., 2023). However, effective strategies are needed to improve the silage quality of oat plants, to increase the feed value of the product and prolong its preservation (Cheng et al., 2022). Indeed, the prevalence of undesirable microorganisms such as aerobic bacteria, yeasts and molds in silages, generally during ensiling or after exposure to air after opening, is a matter of concern all over the world. To eliminate this worrying situation and to optimize the fermentation process, some inoculants, including lactic acid bacteria, are added to the silage. The main purpose of adding these inoculants to silages is to restructure the bacterial community composition so that lactic acid bacteria become dominant (Cheng et al., 2022). Lactic acid bacteria provide a rapid acidification by converting the water-soluble carbohydrates in silage into organic acids, especially lactic acid, thus preventing the development of microorganisms that cause spoilage (Cheng et al., 2022; Tahir et al., 2023). In recent years, lactic acid bacteria strains with different characteristics have been used to improve fermentation quality and increase feed value in silages (Oliveira et al., 2017). One of them is *Lactobacillus brevis*, a heterofermentative lactic acid bacterium. This bacterium has been shown to improve product quality by manipulating silage fermentation, prevents yeast and mold growth, and thus increases both the feed value and aerobic stability of silage (Xu et al., 2017).

Although various studies have been carried out on oat silage, no research on the effects of *L. brevis* addition has been found. Therefore, this study aimed to determine the effects of adding *L. brevis* to oat silage, with various concentrations, on nutrient composition, physical and chemical properties, aerobic stability, in vitro organic matter digestibility and energy value of silage.

Materials and Methods

Silage oats, which constituted the plant material of the study, were grown in the experimental fields of Kırşehir Ahi Evran University, Department of Field Crops (1090 m a.s.l., 39º08'N and 34º06'E). The plant was harvested by hand when the grains reached the dough stage (about 35-40% dry matter content) and chopped with a laboratory-type chopper into pieces approximately 5.0-6.0 cm long. After chopping, fresh material was sampled and analyzed for nutrients. The nutritional composition of the fresh oat plant before ensiling is presented in Table 1. The *L. brevis* used as inoculant was isolated, identified and stocked from homemade pickles for another study as described by Erdem et al. (2021). The stock cultures were stored in MRS (Merck Lactobacillus Agar acc. to de Man, Rogosa) containing 35% (v/v) glycerol at -80°C. Analyses were performed in the laboratories of our university.

In the study, *L. brevis* was used at levels of $1x(10)^6$, $1x(10)^8$, and $1x(10)^9$ cfu/mL. The treatment groups in the study consisted of oat control (OC), *L. brevis*-inoculated oats at concentrations of $1x(10)^6$ (Lb6), $1x(10)^8$ (Lb8), and $1x(10)^9$ cfu/kg dry matter (Lb9). The chopped oat plants were packed into polyethylene bags (250×200 mm, 1 kg capacity, 1.13 cc/m^2/day oxygen permeability) in two layers, each containing 500 g mass. The filled bags were inoculated according to the treatment groups and vacuum sealed. Prepared silage samples were left for fermentation in the laboratory at 22-25°C ambient temperature for 60 days. At the end of the sixty-day fermentation period, the opened silages were subjected to chemical, physical, microbial, in vitro digestibility, and aerobic stability tests. Dry matter (DM), ash and crude protein (CP) content of silages were analyzed according to the methods of AOAC (2000); ether extract (EE) was analyzed according to the methods of AOCS (2005) using ANKOM XT15 ES. Crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of silages were determined using ANKOM 200 Fiber Analyzer according to the method reported by Van Soest et al. (1991). Organic matter (OM) was calculated by subtracting the total ash value from dry matter (DM). Nitrogen-free extract (NFE) was calculated by difference. Hemicellulose content was calculated as the difference between NDF and ADF. Starch analysis was determined by a polarimetric method (ISO, 1997).

The pH values of the silages were determined using a pH meter. Total soluble solids (TSS) content was determined by placing a few drops of silage water on the surface of a digital sucrose refractometer (HI 96801, Hanna Instruments) at room temperature with a sensitivity of 0.2 °Brix. Measurements were recorded as %brix (Singh & Choudhary, 2021). Flieg score (FS) was calculated by the following formula (Kılıç, 2006).

$$\mathrm{FS} = 220 + (2 * \%\mathrm{DM} - 15) - 40 * \mathrm{pH} \quad (1)$$

The relative feed value (RFV) of silages was determined according to the formulas given by Rohweder et al. (1978). The digestible dry matter (DDM) content and dry matter intake (DMI; % of body weight) values required in the calculation of RFV were calculated using the following formulas.

$$\mathrm{DDM}\ (\%) = 88.9 - [0.779 * \mathrm{ADF}\%] \quad (2)$$

$$\mathrm{DMI}\ (\%\ \text{of body weight}) = 120/[\mathrm{NDF}\%] \quad (3)$$

$$\mathrm{RFV} = [\mathrm{DDM} * \mathrm{DMI}]/1.29 \quad (4)$$

After the silage samples were opened, L^*, a^*, and b^* color values were measured from four different parts of the silage with a Konica-Minolta CR-410 colorimeter. These data were recorded on the following scales: (L^*) brightness (0: black, 100: white), (a^*) red to green ($+a^*$: red, $-a^*$: green), and (b^*) yellow to blue ($+b^*$: yellow, $-b^*$: blue). Using a^* and b^* values, the Chroma (C^*, saturation index) value was calculated using the following formulas (King et al., 2023).

$$\mathrm{Croma}\ (C)_{ab} = [(a)^2 + (b)^2)]^{1/2} \quad (5)$$

Lactic acid bacteria, yeast and mold counts of silages were determined by the plate counting method as described by Seale et al. (1990), and colony counts were expressed logarithmically (log10 cfu/g).

The pH, microorganism counts, and CO_2 production of silages on the fifth day after opening were evaluated as indicators of aerobic stability (Ashbell et al., 1991). The pH value and microorganism count on the fifth day after opening the silages were determined as described previously. The CO_2 content of silages was determined as described by (Ashbell et al., 1991). For this purpose, a 1.5-liter polyethylene terephthalate water bottle and a 600 mL glass beaker were used. To ensure air circulation in the system, two holes with a diameter of one cm were drilled in the top and bottom of the plastic bottle. Two hundred grams of silage sample was placed loosely in the upper part of the apparatus, namely, the plastic bottle. In the glass beaker forming the lower part, 100 mL of 20% KOH was added. The plastic bottle was then placed in the beaker with the cap down and not touching the KOH solution. Gas exchange in the system was provided only through the hole at the top. The device was kept in laboratory conditions for five days. The CO_2 produced during aerobic exposure and absorbed by KOH was titrated with 1-N HCl, and the details were calculated as described by Ashbell et al. (1991).

$$\mathrm{CO_2}\ (\mathrm{g/kg\,DM}) = 0{,}044 * \mathrm{T} * \mathrm{V}/(\mathrm{A} * \mathrm{FM} * \mathrm{DM}) (6)$$

Where, T: Volume in HCl used in the titration (mL), V: Total amount of 20% KOH (mL), A: Amount of KOH in solution (mL), FM: Mass of fresh material (kg), DM: Dry matter content (%).

Table 1. Nutritional composition of fresh oat plant before ensiling

Items	Fresh oat
Dry matter (g/kg fresh plant)	332.0
Organic matter (g/kg DM)	931.6
Crude Protein (g/kg DM)	111.7
Ether extract (g/kg DM)	56.0
Ash (g/kg DM)	68.4
Nitrogen-free extracts	560.2
Acid detergent fibers (g/kg DM)	328.6
Neutral detergent fibers (g/kg DM)	582.8
Hemicellulose (g/kg DM)	248.8
Starch (g/kg DM)	14.31

In the study, ANKOM^{RF} Gas Generation System (Ankom Technologies, Macedon, NY, USA) consisting of 250 mL glass bottles equipped with a connected module was used to determine in vitro organic matter digestibility (IVOMD), metabolizable energy (ME_{GP}), and net energy lactation (NE_L) content of silages (Menke & Steingass, 1988). When the ambient pressure inside each unit rose above 1.5 kPa, the module's valve automatically released the gas collected above the glass bottles. The internal pressure of the glass bottles was measured at 5-minute intervals. For use in the system, a buffer solution was prepared according to Menke & Steingass (1988) and mixed with rumen fluid at a ratio of 4:1 (80 mL artificial saliva solution + 20 mL rumen fluid) to form buffered rumen fluid. Artificial saliva solution was prepared as indicated in the method. Rumen fluid content was obtained from 3 heads of male Simmental calves, approximately 2 years old, and 450-500 kg live weight. These calves were slaughtered in a private integrated meat plant and fed with a ration prepared with 65% concentrate and 35% alfalfa grass. The rumen contents were brought to the laboratory in a thermos filled with carbon dioxide at 39 °C within 15 minutes. After ensuring the temperature and pH controls (6.23-6.28) in the laboratory, the substance was filtered through four layers of cheesecloth and used fresh. For the test, 1 g of sample was weighed into a bottle preheated in a water bath at 39 °C. 100 mL of buffered rumen fluid was added and incubated in a water bath at 39 °C for 24 hours. Each sample was analyzed in three replicates during the in vitro gas production phase. The measured gas pressure was converted into moles of gas produced using the ideal gas law equation (Equation (6)). The gas produced was converted to milliliters using Avogadro's law equation (Equation (7)).

$$n = (pV)/(RT) \quad (7)$$

$$GP, mL = n \times 22.4 \times 1000 \quad (8)$$

Where, n: Gas produced in moles (mol), p: Pressure in kilopascals (kPa), V: Void volume in the glass bottle in liters (L), T: Temperature in Kelvin (K), R: Gas constant (8.314472 L-kPa/K/mol).

The IVOMD, ME_{gp} and NE_l values of the silages were calculated by using the amounts of gases produced, as specified by Menke & Steingass (1988).

$$IVOMD\ (\%) = 14,88 + 0,8893 * GP + 0.448 * CP + 0,651 * ash \quad (9)$$

$$ME_{gp}, MJ/kg\ DM = (2,20 + 0,136 * GP + 0,057 * CP + 0,0029 * CP^2) \quad (10)$$

$$NE_l\ (MJ/kg\ DM) = (0,54 + 0,0959 * GP + 0.038 * HP + 0.001733 * CP^2) \quad (11)$$

Where, GP: 24-hour net gas production (mL/200mg DM), CP: Crude protein (%),

Nutrient content, all physical properties, feed value data, aerobic stability indicators, IVOMD, MEgp and NEl values of the silages used in the experiment were statistically analyzed using SAS (SAS, 2001). Differences between means were determined by the Duncan multiple comparison test.

Results and Discussion

The post-opening nutritional composition of the silages is given in Table 2. The addition of *L. brevis* to oat silage caused significant changes in the nutrient content of silage. While OM, ADF, and NDF contents of silages decreased compared to OC, crude protein, starch, and crude fat contents increased. There was no difference in hemicellulose content compared to OC. Among *L. brevis* inoculated treatment groups, the lowest OM content was obtained in the Lb6 treatment group (920.4 g/kg DM); an increase in OM content was determined as the dose of *L. brevis* increased ($P<0.05$). The *L. brevis* application increased the crude protein content of silages compared to OC. As a matter of fact, while the protein content of OC was 102.2 g/kg DM, it was between 111.1 g/kg DM and 115.6 g/kg DM in the treatment groups. There was no difference between Lb6 and Lb8 in terms of protein content ($P>0.05$). Post-opening starch content of oat silages was higher in Lb8 and Lb9, compared to OC and Lb6. The ADF and NDF contents of silages ranged from 319.0 to 335.0 and 528.2 to 556.4 g/kg DM, respectively. The *L. brevis* inoculation significantly decreased the ADF content of silages compared to OC ($P<0.01$). When NDF content was analyzed, the Lb9 group silage was lower than the other silages, while OC, Lb6, and Lb8 gave similar results. Ilavenil et al. (2015) reported that inoculation decreased the ADF, NDF, and ash contents of oat silage compared to OC in their study to determine the effects of *L. plantarum* inoculation on the feed value of oat silage.

Table 2. Post-opening nutritional composition of silages, g/kg DM

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
Organic matter	925.0^{a}	920.4^{b}	921.4^{b}	923.4^{ab}	0.723	0.044
Ash	75.05^{b}	79.56^{a}	78.58^{a}	76.56^{ab}	0.723	0.044
Crude protein	102.2^{c}	111.1^{b}	111.5^{b}	115.6^{a}	1.865	0.001
Ether extract	52.19^{c}	54.20^{c}	60.07^{b}	62.98^{a}	1.662	0.001
Starch	60.81^{b}	61.87^{b}	73.87^{a}	76.45^{a}	2.811	0.025
NFE	495.2^{a}	491.6^{a}	487.9^{ab}	481.3^{b}	2.092	0.029
ADF	335.0^{a}	328.2^{b}	319.0^{c}	322.3^{c}	2.350	0.003
NDF	551.9^{ab}	556.4^{a}	542.6^{b}	528.2^{c}	4.252	0.011
Hemicellulose	216.9^{ab}	228.3^{a}	223.6^{a}	205.9^{b}	3.421	0.033

NFE, nitrogen-free extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; ^{a,b,c} Means with different superscripts in the same row differ significantly (P<0.05).

Table 3. The post-opening pH, total soluble solids, relative feed value, flieg score and quality class values based on flieg score of silages

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
Dry matter, %	39.86^{d}	41.58^{c}	41.81^{b}	42.17^{a}	0.230	0.001
pH_0	5.45^{a}	4.95^{c}	4.98^{c}	5.32^{b}	0.058	0.001
TSS, °Brix	19.58	18.83	18.95	18.75	0.154	0.219
RFV[1]	105.9^{b}	105.9^{b}	109.8^{a}	112.4^{a}	1.072	0.006
[1]RFV quality class	2nd quality	2nd quality	2nd quality	2nd quality	-	-
Flieg score	66.62^{c}	90.27^{a}	89.61^{a}	76.44^{b}	2.615	0.001
[2]FS quality class	Good	Excellent	Excellent	Good	-	-

pH_0, pH values of silages at opening time; TSS, Total soluble solids; RFV, relative feed value; FS, flieg score; ^{a,b,c} Means with different superscripts in the same row differ significantly (P<0.05); [1] The RFV values below 75 refer to the fifth quality, 75-86 to the fourth quality, 87-102 to the 3rd quality, 103-124 to the 2nd quality, and 125-150 to the 1st quality.; [2] >80, excellent; 61-80, good; 41-61, satisfactory; 21-40, moderate; 0-20, bad.

In contrast to our study, the researchers reported a decrease in the crude protein content of silages compared to OC. In agreement with this study, Blajman et al. (2023) reported that CP content improved with the addition of microbial inoculants to vetch-oat silages. However, contrary to our study and other literature reports, based on the results of their meta-analysis, Oliveira et al. (2017) reported that inoculation of lactic acid bacteria into silages did not affect the CP content of silages. Rafiuddin et al. (2021) reported that the addition of an inoculant composed of lactic acid bacteria and enzyme reduced the ADF and NDF content of oat silage at the time of opening. Similarly, Jalč et al. (2009) reported that the addition of three different microbial inoculants (*Lactobacillus plantarum, L. fermentum,* and *Enterococcus faecium*) in corn silage decreased the ADF and NDF content of silage. In contrast, Oliveira et al. (2017) reported that inoculation of silages with lactic acid bacteria reduced the lignin concentration of silages but did not affect the NDF concentration. The differences between the results obtained in this study and those reported in the literature may be due to the type of silage plant, harvest time, type and application rate of inoculant used in silage and other silage management practices. Oliveira et al. (2017) also supports this view, stating that the response to inoculants in silages can be affected by many factors, including plant species, type, and application rate of the inoculant and other silage management practices.

The post-opening pH, total soluble solids, relative feed value, flieg score, and quality class values based on flieg score of silages are given in Table 3. The *L. brevis* inoculation of oat silage increased the DM content of the silages post-opening compared to OC (P<0.01). This finding supports the widespread view that the addition of lactic acid bacteria to silages increases the DM concentration. Indeed, based on the results of their meta-analysis, Oliveira et al. (2017) reported that inoculating lactic acid bacteria into silages increased the DM concentration of silages. Similar results were reported by Tobia et al. (2008) and Rafiuddin et al. (2021). Tobia et al. (2008) attributed the increase in DM concentration in silages to the loss of effectiveness of undesirable microorganisms such as *Clostridium*, yeast, and mold in the silage environment. In our study, the higher protein content of the silages after opening compared to OC supports this view. Similarly, Rafiuddin et al. (2021) reported that the addition of an inoculant consisting of lactic acid bacteria and enzymes combination increased the DM content of oat silage at the time of opening. As expected from other lactic acid bacteria, the *L. brevis* inoculation also decreased the pH_0 value of silages compared to OC (P<0.01). In studies conducted with different silage materials (Blajman et al., 2023; Chai et al., 2022; Miao et al., 2019; Rafiuddin et al., 2021), it was reported that the inoculation of different lactic acid bacteria decreased the pH value of silages. Among the treatment groups, the lowest pH value occurred in Lb6 silage (4.95), followed by Lb8 (4.98) and Lb9 (5.32) silage. The pH values of Lb6 and Lb8 silages were statistically similar. The TSS or Brix refers to the percentage by mass of total soluble solids of a pure aqueous sucrose solution. The higher Brix values may indicate the availability of total soluble sugars (sucrose, glucose and fructose) needed for lactic acid bacteria to rapidly dominate and initiate fermentation during the ensiling process (Singh & Choudhary, 2021). In our study, the TSS value was found to be between 18.75 to and 19.58 °Brix, and the *L. brevis* inoculation did not affect the TSS value of oat silage (P>0.05). The addition of *L. brevis* to oat silage increased the RFV content of the silages compared to OC in Lb8 and Lb9 silages but not in Lb6 silage. The RFV values of the silages were determined between 105.9 and 112.4. In terms of RFV values, all silages were in the second quality class. The *L. brevis* inoculation of oat silage increased the post-opening flieg scores of the silages compared to OC. The highest value in terms of flieg score was obtained in Lb6 silage (90.27) and was statistically similar to Lb8 silage (89.61). These two silages were in the "excellent" quality class, while Lb9 and OC silages were in the "good" quality class. These data were lower than the values (112-117) reported by Rafiuddin et al. (2021) who added a combination of lactic acid bacteria and enzymes to oat silage. These differences may be due to the inoculant used, the harvest time of the silage plants, the maturity period, and differences in vegetation.

The *L**, *a**, *b**, and *C** parameter values reflecting the color characteristics of silages are given in Table 4. The *L. brevis* inoculation of oat silage did not cause any change in the color parameters of the silage. The *L** value represents brightness feeds with high *L** value are more preferred by animals (Sahar et al., 2022). In this study, the *L** values were determined between 37.72 and 45.00, and there was no difference among silages (P>0.05). The *a** values were determined between 3.93 and 4.55, *b** values were determined 13.02 and 15.71, and C^*_{ab} values were determined 13.60 and 16.36. Consistent with this study, Çayıroğlu (2024) reported *L**, *a**, and *b** values in triticale silages inoculated with *Lactobacillus plantarum* as 42.81 and 47.63, 2.04 and 3.38, 15.47 and 17.68, respectively. İnce & Vurarak (2019) determined the *L** value to be 43.56 and the *a** value to be 4.1 in vetch-triticale mixture silages and reported that these values decreased to 28.8 and 2.2 in spoiled silages, respectively. The researchers also stated that spoiled silages lost their bright appearance.

The post-opening lactic acid bacteria, yeast and mold counts of the silages are given in Table 5. The inoculation of *L. brevis* into oat silage increased the post-opening lactic acid bacteria count of the silages compared to OC (P<0.01), but did not affect the yeast and mold count (P>0.05). The highest lactic acid bacteria content was obtained in Lb6 silage (6.62 cfu/g). There was no difference between the other silage groups Lb8 and Lb9. These results are in agreement with Blajman et al. (2023), and Chai et al. (2022) who reported that inoculation of lactic acid bacteria into silage increases the lactic acid bacterial community of silage. Indeed, Blajman et al. (2023) reported that a lactic acid bacteria mixture increased the total bacterial load in vetch-oat silages. Chai et al. (2022) reported that inoculation of *Lentilactobacillus buchneri* and *Lactiplantibacillus plantarum* into oat silage restructured the bacterial composition and improved the quality by reducing the pH, yeast, and mold content. They emphasized that plant species, climate, geographical location and fertilizers used may be effective in structuring this.

In this study, changes in pH value, CO_2 production, yeast and mold growth on the 5th day after opening of silages were accepted as indicators of aerobic spoilage. Higher CO_2 production and pH values indicate increased growth of spoilage microorganisms. This indicates that the shelf life of silage is short (Ashbell et al., 1991). The pH, CO_2, yeast, and mold counts of the silages after the aerobic stability test of five days are given in Table 6. The *L. brevis* treatment of oat silage decreased the pH value and yeast count on the fifth day after opening compared to the OC group (P<0.01).

The lowest pH_{5d} value was obtained in the Lb8 silage (4.96) and the lowest yeast count was obtained in the Lb6 silage (4.38). Although the *L. brevis* application to oat silage numerically decreased the CO_2 production value and the number of molds in the silage, these decreases were found to be statistically insignificant (P>0.05). These findings are in agreement with Aydın & Denek (2023) who reported that the addition of *Lactobacillus bundler* to maize silage reduced yeast and mold growth after aerobic stability test but did not affect the amount of CO_2 production.

Table 4. Parameter values reflecting the color properties of silages

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
*L**	42.68	43.37	45.00	37.72	1.480	0.365
*a**	4.47	4.33	4.55	3.93	0.305	0.922
*b**	14.54	14.71	15.71	13.02	0.595	0.513
C^*_{ab}	15.23	15.35	16.36	13.60	0.638	0.556

*L**, lightness; *a**, redness; *b**, yellowness; *C**, Chroma or saturation index.

Table 5. Post-opening lactic acid bacteria, yeast and mold quantities of silages

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
LAB community, cfu/g	6.39^c	6.62^a	6.47^b	6.49^b	0.027	0.001
Yeast, cfu/g	5.98	5.85	5.83	5.89	0.045	0.716
Mold, cfu/g	1.77	3.53	0.00	0.00	0.692	0.219

LAB, lactic acid bacteria; ^{a,b,c} Means with different superscripts in the same row differ significantly (P<0.05).

Table 6. The pH, carbon dioxide production, yeast, and mold growth in silages after the aerobic stability test

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
pH_{5d}	5.52^a	5.10^b	4.96^c	5.17^b	0.054	0.001
CO_2, g/kg DM	4.15	2.77	3.58	3.21	0.266	0.352
Yeast, cfu/g	6.44^a	4.38^c	4.78^{bc}	5.06^b	0.240	0.001
Mold, cfu/g	3.53	0.00	1.77	1.77	0.753	0.487

The pH_{5d}, the fifth-day pH value; ^{a,b,c} Means with different superscripts in the same row differ significantly (P<0.05).

Table 7. In vitro organic matter digestibility and energy values of silages

Variables	OC	Lb6	Lb8	Lb9	SEM	P Value
IVOMD, %	68.38	70.17	69.45	66.05	1.226	0.720
ME_{Gp}, MJ/kg DM	12.55	12.77	12.67	12.96	0.064	0.183
NE_L, MJ/kg DM	7.14[c]	7.59[b]	7.53[b]	7.88[a]	0.086	0.003

IVOMD, in vitro digestible organic matter; ME_{GP}, metabolizable energy calculated from gas production; NE_L, net energy lactation calculated from gas production; [a,b,c] Means with different superscripts in the same row differ significantly ($P<0.05$).

In contrast, Miao et al. (2019) reported that inoculation of *L. buchneri* into corn silage decreased the CO_2 production of silages but increased mold growth compared to OC. Blajman et al. (2023) reported that a lactic acid bacteria mixture improved aerobic stability and slowed down the growth rate of yeast and mold in vetch-oat silages. In this study, the significant decrease in the fifth day pH value and yeast count of the silages compared to OC can be considered as gains in terms of aerobic stability. Additionally, although not statically significant, the numerical decrease in CO_2 production value and mold growth also contribute to these gains.

In vitro organic matter digestibility, metabolizable energy and net energy lactation contents of silages are given in Table 7. Inoculation of different doses of *L. brevis* in oat silage did not affect the IVOMS and ME_{GP} values of the silage compared to OC. However, it increased the NE_L value. The IVOMS values of the silages were found between 66.05 and 70.17%. The highest digestibility value was found in Lb6 silage. The ME_{GP} and NE_L values calculated from gas production were between 12.55 and 12.96, and 7.14 and 7.88 MJ/kg DM, respectively. The highest ME_{GP} and NE_L values were determined in Lb9 silage. These results are consistent with Aydın & Denek (2023) who reported that the addition of *Lactobacillus buncher* to corn silage did not affect the IVOMS and ME_{GP} value. Rafiuddin et al. (2021) reported that increasing the inoculant level in oat silage increased the IVDMD of silage. The differences between the studies in terms of IVOMD, ME_{GP} and NE_L values of silages may be due to the differences in the type of plant, harvest time, maturity and inoculants used in the studies.

Conclusion

The *L. brevis* inoculation of oat silage increased the dry matter, crude protein and crude fat contents in the silage by contributing to the breakdown of plant cell wall components, especially ADF and NDF. This application increased the lactic acid bacteria density in silages, which decreased silage pH, and improved silage quality and feed value. In addition, aerobic stability results were promising for improving the shelf life of silages. In conclusion, inoculation of *L. brevis* into oat silage improved the chemical and microbiological properties, and aerobic stability of silage without causing any change in the physical properties. Upon evaluation, it can be said that the doses of *L. brevis* at 1×10^8 and 1×10^9 cfu/kg DM doses showed more effective results in oat silage in terms of the studied parameters; therefore, any of these doses can be preferred.

Declarations

Ethical Approval Certificate

Ethics committee permission is not required for the experimental procedures of this study.

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Conflict of Interest

The authors declare no conflict of interest.

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