Is It Possible to Improve the Fermentation and Nutritional Quality of Wheat Straw Silage by Replacing Commercial Inoculant with Kefir?

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ABSTRACT

The current study aimed to determine fermentation quality, aerobic stability, and enzyme soluble organic matter (ELOS) of wheat straw silage by replacing homofermentative (HM) and homofermentative+heterofermentative (HM+HT) lactic acid bacteria (LAB) inoculants with kefir as silage additives. For this purpose, commercially available Biotal Plus II (HM LAB), Biotal Buchneri 500 (HM+HT LAB), and MYStarter KF (KF) were used as silage additives. Four kg of wheat straw, about 400 g/kg, and 6.0 log cfu of inoculants or kefir were used in each treatment group and replicate. Including the control group (CON), a total of 12 laboratory-type silos (3 replicates and 4 groups) were opened after 45 days. The dry matter (DM), crude ash (CA), acid detergent lignin (ADL), and water-soluble carbohydrate contents of silages were not affected by the addition of HM LAB, HM+HT LAB, and KF (P>0.05). The KF group had the lowest pH value (4.32), NH3-N content (71.97 g/kg TN), and higher lactic acid content (43.11 g/kg DM). The crude protein (CP) ratio was decreased in HM LAB (5.95%) and HM+HT LAB (5.63%) groups and increased in the KF group (4.54%, P<0.001). An improvement (by lowering 17.02%) of NDF was only observed in the KF group (P<0.001). The ELOS and ME in HM LAB, HM+HT LAB, and KF groups were increased (P<0.001). The lowest carbon dioxide (3.42 g/kg DM) and yeast (5.50 log10 cfu/g) were observed in the KF and CON group, respectively. According to research findings, kefir could be an alternative silage additive to commercially available inoculants and could improve wheat straw silage’s nutritional quality instead of them.

Ticari İnokulantların Yerine Kefir Kullanarak Buğday Samanı Silajının Fermentasyon ve Besin Kalitesini İyileştirmek Mümkün müdür?

ÖZET

Bu çalışma homofermentatif (HM) ve homofermentatif + heterofermentatif (HM+HT) laktik asit bakterileri (LAB) yerine kefir kullanmanın buğday samanı silajlarının fermentasyon kalitesi, aerobic stabilitesi ve enzimde çözünen organik madde (EÇOM) miktarına olan etkisini araştırmayı amaçlamaktadır. Bu amaçla ticari olarak kullanılan Biotal Plus II (HM LAB), Biotal Buchneri 500 (HM+HT LAB) ve MYStarter KF (KF) silaj katkı maddesi olarak kullanılmıştır. Her muamele grubunda yaklaşık 400 g/kg kuru maddeye sahip 4 kg buğday samanı ve 6.0 log kob oranında silaj katki maddesi yada kefir kullanılmıştır. Kontrol grubu (KON) dahil toplama 12 adet laboratuvar tipi silo (3’er tekerrür ve 4 grup) 45 gün sonra açılmıştır. Silajların kuru madde (KM), ham kül (HK), asit deterjan lignin (ADL) ve suda çözünebilir karbondiikrat içeriği HM LAB, HM+HT LAB ve KF ilavesi sonrası değişmemiştir (P>0.05). KF grubu en düşük pH (4.32) ve NH3-N (71.97 g/kg TN) ve en yüksek laktik asit (43.11 g/kg KM) içeriğine sahiptir. Ham protein (HP) oranı HM LAB (%5.95) ve HM+HT LAB (%5.63) gruplarında azalırken KF

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INTRODUCTION

Wheat straw (WS), the second-largest agricultural residue, still contains some beneficial nutrients for ruminants and is mostly used as cost-effective animal feed in developing countries to cover the roughage deficiency. However, WS is classified as low-quality forages due to its high fiber fraction, low digestibility, and voluntary intake (Wiedmeier et al., 2002; Shahryari et al., 2018). WS's nutritional value which is also vary depending on the variety, growing conditions, and maturity stages, is far from meeting ruminants' dietary needs. On the other hand, its competitive prices make it a step forward to other commodities. However, intact WS is not an ideal feed source for ruminants (Chekani-Azar and Chekani-Azar, 2010). Thus, several methods are used to improve WS quality, such as physical (grinding, stream processing), chemical (alkaline treatments or other chemicals, such as sulfur dioxide, urea, or chlorine), and biological methods (fungal treatment, inoculants, or enzymes) either solitary or in combination (Eser, 2016; Gado et al., 2017; Ordaz, 2017; Ayaşan et al., 2020). It has not been enough progressed to improve WS quality by using physical or chemical methods. Therefore, the application of biological methods has become more common in this field.

Ensiling, a complex biochemical process, is based on the fermentation of water-soluble carbohydrates (WSC) by lactic acid bacteria (LAB) under anaerobic conditions (Kung et al., 2003). During the ensiling period, forage type, WSC content, lignification degree, and interaction between inoculants (LAB and/or enzymes) and ensiled forage affect fiber degradation. It was stated that the availability of volatile fatty acids (VFA) decreases the pH of ensiled forage and increased the availability of sugars fermented by the LAB population through ensiling process results in the nutritive quality of silage (Zwielehner et al., 2014). Moreover, fibrolytic enzymes and inoculants benefit animal performance, resulting in dry matter intake, improved organic matter digestibility, and microbial protein synthesis (Zwielehner et al., 2014).

Schnirer and Jonsson (2011) draw our attention to the ingredients of an excellent starter culture for well-preserved silage, and they suggested using a combination form of yeast and lactic acid bacteria. A number of authors have considered the effects of yeast to control mould growth by depleting oxygen, especially the initial phase of the ensiling, and inhibiting mould growth by decreasing pH through the secretion of organic acids also support this suggestion (Gamba et al., 2016; Droby and Wisniewski, 2018; Gonda et al., 2019). Recent research findings have also shown that the addition of kefir, which has heterolactic properties, into silage reduces nutrient losses and positively affects aerobic stability (Gonda et al., 2019; Koç et al., 2020). Given this aspect, kefir can be used as a silage additive due to its unique aspects, cheap and its heterofermentative properties, as an alternative to commercial inoculants. This study aimed to investigate the improving possibilities of WS silage by replacing inoculants with kefir and comparing fermentation and nutritional quality.

MATERIALS and METHODS

The current study was conducted at the Animal Feed and Nutrition Laboratory of Tekirdag Namik Kemal University in 2017. WS (Triticum aestivum L.) straw, which dry matter (DM) contents were 931.3 g/kg, was obtained from the experimental area of the Field Crops Department and transferred into the laboratory for silage preparation and further analysis. To prepare laboratory-scale silages, WS was chopped 2–3 cm long, water was added to yield approximately 400 g/kg DM content, and allowed WS at least 1 h to absorb added water (Nakashima et al., 1993). Then, approximately 4 kg of WS spread in a thin layer on a clean nylon cover with a 4 m² surface area. Commercially available Biotal Plus II (HMLAB: Lallemand Inc., USA; contains Pediococcus pentosaceus 12455, Propionibacterium freudenreichii R2435 strains and β-glucanase, xylanase and glucotomanase enzymes), Biotal Buchneri 500 (HM+HTLAB: Lallemand Inc., USA; contains Pediococcus pentosaceus 12455, Lactobacillus buchneri NCIMB 40788 strains and β-glucanase, xylanase and glucotomanase enzymes) inoculants, and MYStarter KF kefir (KF: contains Lactococcus
lactis subsp. lactis biovar diacetylactis, Lactobacillus brevis, Leuconostoc mesenteroides subsp. mesenteroides ve Saccharomyces cerevisiae strains) were applied at 6.0 log cfu/g theoretically, in each treatment group, and 3 replicates. A 0.074 g HM-LAB, 0.039 g HM-HT-LAB, and 1.5 g kefir were weighed and dissolved in 20 ml tap water. Homogenized inoculants and kefir were then applied by hand sprayer, mixed silage well with wearing sterile gloves in each replicate, and then vacuumed and sealed by a vacuum sealer (CAS CVP-260PD).

The vacuumed packs, stored at an ambient temperature of 25-30 °C, were opened at the end of 45 days ensiling, pH, DM, WSC, and lactic acid content of silages was determined immediately (Anonymous, 1986; Chen et al., 1994; Koç and Çaşkuntuna, 2003).

The proximate analysis of WS silages was performed according to Weende’s analysis by using AOAC (1990) methods. Briefly, DM of WS silages was determined by drying samples at 102 °C overnight, and crude ash (CA) content was determined by igniting the silage samples in a muffle furnace at 550 °C for 3 h. The nitrogen (N) content of WS silages was measured by the Kjeldahl method and multiplied by 6.25 to get the crude protein (CP) ratio. The Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of WS silages was determined according to Van Soest et al. (1991). Enumeration of LAB, yeast, and mould of silages were determined using MRS (de Man, Rogosa and Sharpe) and potato dextrose agar, according to Seale et al. (1990). The aerobic stability of WS silages was determined according to Ashbell et al. (1991). The two-stage enzymatic digestion method described by Tilley and Terry (1963) was used to evaluate enzyme soluble organic matter (ELOS) of WS silages. The cellulose, obtained from Trichoderma viride (Onozuka R-10, Merck, Darmstadt, Germany), and pepsin (0.7 FIP-U/g, Merck, Darmstadt, Germany) enzymes were used in enzymatic digestion studies. Approximately 300 mg of a sample taken into crucibles and added 30 ml pepsin (2 g of pepsin dissolved in 1 L of 0.1 N HCl) to pre-treated for 24 h at 40°C in the first stage. Then 30 ml cellulose buffer solution (3.3 g cellulose dissolved in 1 L of acetate buffer solution: Solution A: 5.9 ml Acetic acid in 1 L distilled water; Solution B: 13.6 g Sodium acetate + 1 L distilled water; w/w: 400/600) was added to crucibles and incubated. At the end of the incubation, samples were filtered, dried at 105 °C at least 3 h, and burned at 550 °C (Özkın, 2016). The ELOS was calculated between the weight differences of dried and burned samples after incubation. The following equations estimated the ELOS and ME of WS silages (Cömert Acar et al., 2018):

\[
\text{ELOS, g/kg} = \text{DW-BW}/\Sigma \times 1000 \quad (1)
\]

\[
\text{EUROS, g/kg} = 1000 \times \text{ELOS} \quad (2)
\]

\[
\text{ME (MJ/kg DM)} = 14.27 - (0.0120 \times \text{EUROS}) + (0.0023 \times \text{CP}) - (0.0147 \times \text{CA}) \quad (3)
\]

Where DW: dry weight of the sample (105 °C); BW: burn weight of sample (550°C); SW: sample weight; EULOS: enzyme insoluble organic matter; CP: crude protein; CA: crude ash.

The effect of treatments on fermentation quality and nutritive value of WS silages were analyzed using the GLM procedure of Minitab (2014) statistical package programs, and least-squares means were compared using Tukey’s multiple comparison tests. The following statistical model was used:

\[
Y_{ij} = \mu + a_i + e_{ij} \quad (4)
\]

Where \(Y_{ij}\) = observed value; \(\mu\) = overall mean; \(a_i\) = effect of inoculants or kefir; \(e_{ij}\) = effect of the experimental error.

**RESULTS**

The chemical and microbiological composition of pres-ensiling material and fermentation quality and chemical composition of WS silages is given in Table 1 and 2.

Table 1 Chemical and microbiological composition of pre-ensiling material

| Parameters (Parametreler) | SM (BM) |
|---------------------------|---------|
| DM, g/kg (KM, g/kg)       | 387.3   |
| pH (pH)                   | 7.60    |
| CP, g/kg DM (HP, g/kg KM) | 58.7    |
| CA, g/kg DM (Ham kül, g/kg KM) | 62.9 |
| NDF, g/kg DM (NDF, g/kg KM) | 624.8  |
| ADF, g/kg DM (ADF, g/kg KM) | 432.8  |
| ADL, g/kg DM (ADL, g/kg KM) | 42.6   |
| Hsol, g/kg DM (Hemiselilıoz, g/kg KM) | 192 |
| Cell, g/kg DM (Seli̇lıoz, g/kg KM) | 390.2 |
| WSC, g/kg DM (SCK, g/kg KM) | 18     |
| ELOS, g/kg DM (ECOM, g/kg KM) | 312.9  |
| ME, MJ/kg (ME, MJ/kg KM) | 5.24    |
| Lactobacilli, log10 cfu/g | 5.74    |
| Yeast, log10 cfu/g (Maya, log10 kob/g) | 5.69 |
| Mould, log10 cfu/g (Küf, log10 kob/g) | 0 |

SM: Starting Material, DM: Dry Matter, CP: Crude Protein, CA: Crude Ash, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, Hsol: Hemicellulose, Cell: Cellulose, WSC: Water Soluble Carbohydrate, ELOS: Enzym Soluble Organic Matter, ME: Metabolizable Energy

The DM, CA, ADL, and WSC contents of silages were not affected by the addition of HM-LAB, HM-HT-LAB, and KF (P>0.05). Compared to control silages (Table 2), the KF group had the lowest pH value: an increased pH value was observed in HM-LAB while (P<0.001) HM-HT-LAB did not affect pH (P>0.05). The CP ratio of silages was decreased in HM-LAB (5.95%) and


**Table 3 Microbiological composition of WS silages**

| Parameters (Parametreler) | Treatments (Muameleler) | P |
|---------------------------|-------------------------|---|
|                           | CON | KON | HM-LAB | HM-HTLAB | HM-HTLAB | KF | P |
| DM, g/kg (KM, g/kg)       | 382.2±12.3 | 384.0±2.8 | 385.4±2.2 | 381.5±0.8 | NS |
| pH (pH)                   | 4.50±0.02 | 4.60±0.02 | 4.52±0.00 | 4.32±0.02 | *** |
| CP, g/kg DM (HP, g/kg KM) | 63.9±1.3 | 60.1±0.14 | 60.3±1.4 | 66.8±0.8 | *** |
| CA, g/kg DM (Kül, g/kg KM) | 67.4±0.6 | 66.9±0.2 | 68.1±0.6 | 67.9±0.8 | NS |
| NDF, g/kg DM (NDF, g/kg KM) | 676.2±19.1 | 719.8±36.3 | 738.3±4.8 | 561.1±39.3 | *** |
| ADF, g/kg DM (ADF, g/kg KM) | 416.0±11.4 | 381.1±14.3 | 380.9±16.3 | 359.2±19.7 | ** |
| ADL, g/kg DM (ADL, g/kg KM) | 49.1±6.1 | 41.4±4.7 | 45.7±5.2 | 41.7±3.7 | NS |
| H$_{es}$, g/kg DM (Hemi, g/kg KM) | 260.2±18.3 | 337.8±5.01 | 357.5±21.1 | 201.9±22.0 | *** |
| Cell, g/kg DM (Sol, g/kg KM) | 366.9±6.4 | 339.8±19.0 | 335.1±11.1 | 317.5±23.0 | * |
| WSC, g/kg DM (SC, g/kg KM) | 5.91±1.21 | 8.12±1.55 | 7.33±1.20 | 5.94±1.59 | NS |
| NH$_{3}$N, g/kg TN (NH$_{3}$-N, g/kg TN) | 125.06±7.5 | 95.21±7.02 | 113.84±0.06 | 71.97±2.19 | *** |
| LA, g/kg DM (LA, g/kg KM) | 20.86±0.26 | 40.87±2.29 | 38.40±2.34 | 43.11±2.93 | *** |
| ELOS, g/kg DM (EČOM, g/kg KM) | 328.4±12.9 | 365.7±7.8 | 361.3±5.9 | 347.6±0.5 | *** |
| ME, MJ/kg DM (ME, MJ/kg KM) | 5.37±0.14 | 5.82±0.09 | 5.75±0.08 | 5.60±0.02 | NS |

The microbiological composition and aerobic stability parameters of silages were given in Tables 3 and 4. In all treatment groups, mould was not detected (P>0.05). Compared to the control group, HMLAB, HM-HTLAB, and KF increased the Lactobacilli and decreased the yeast number of WS silages (P<0.001). The highest Lactobacilli and lowest yeast number was detected in the KF and HM-HTLAB group, respectively (Table 3). Compared to the CON group, the pH value decreased only in the KF group after 5 days of aerobic stability test (P<0.001). The lowest CO$_{2}$ (3.42 g/kg DM) and yeast (5.50 log10 cfu/g) were observed in the KF and CON group, respectively (Table 4).

**DISCUSSION**

The current study set out to investigate the improving possibilities of WS silage by replacing inoculants with kefir and comparing fermentation and nutritional quality. Several reports have shown that an adequate substrate for LAB, DM, and WSC is required to produce stable silages (Li et al., 2016; Tao et al., 2017; Zhang et al., 2018). While the pH and WSC content of starting material was decreased after 45 days of the ensiling period, the DM content was not affected. Also, silage additives affected WS silages’ pH in all treated groups (P<0.001). The main objectives of adding enzymes into silages inoculants are to increase WSC supply.
Table 4 Aerobic stability parameters of WS silages

| Parameters (Parameteretreler) | Treatments (Muameleler) | CON | KON | HMLAB | HMHTLAB | KF | P | P |
|-----------------------------|--------------------------|-----|-----|-------|---------|----|---|---|
| pH (pH)                     |                          | 4.59±0.01<sup>a</sup> | 4.69±0.01<sup>b</sup> | 4.75±0.01<sup>a</sup> | 4.44±0.01<sup>c</sup> | ***|
| CO<sub>2</sub>, g/kg DM (CO<sub>2</sub>, g/kg KM) |                          | 5.14±0.15<sup>a</sup> | 4.62±0.07<sup>b</sup> | 5.08±0.23<sup>a</sup> | 3.42±0.07<sup>c</sup> | ***|
| Yeast, log10 cfu/g (Maya, log10 kób/g) |                          | 5.50±0.01<sup>c</sup> | 5.68±0.01<sup>c</sup> | 5.90±0.01<sup>b</sup> | 6.68±0.00<sup>c</sup> | ***|
| Mould, log10 cfu/g (Küf, log10 kób/g) |                          | 0    | 0    | 0     | 0       | NS |

<sup>a,b,c</sup> Values within a row with different superscripts differ significantly at <i>P</i><0.05
NS: Not significant: ***: <i>P</i><0.001

and promote better fermentation by LAB and partial degradation of fiber during the ensiling period, especially when the WSC of pre-ensiled material was below the recommended value (Ordaz, 2017; Yuan et al., 2017). It was stated that the sugar content of silage was increased as a result of partial fermentation of fiber (hemicellulose and cellulose) by enzymatic activity during the ensiling period (Kung et al., 2003). The high sugar content of silages allows fermenting of them by the LAB population of silages and yielding lower pH results. The decrease in pH has also been reported by Filya and Sucu (2007) and Aktürk and Gümüş (2020).

The breakdown of proteins by plant enzymes was continued during the ensiling period: the decrease in pH increases due to extending the proteolytic activity during the active fermentation stage. The NH<sub>3</sub>-N content, an indicator of protein breakdown, was significantly affected by HM LAB, HM-HT LAB, and KF (<i>P</i><0.001). Due to the low NH<sub>3</sub>-N amount in the KF group, the CP ratio is higher than the CON and inoculated groups (Todorov et al., 1997; Demirel et al., 2003). Also, it was stated that the CP ratio of silages could be increased in the reduction of NDF (Babaeinasab et al., 2015). Moreover, many researchers reported that the positive effect of addition LAB into silage increased the CP ratio (Nkosi and Meeske, 2010; Nkosi et al., 2011; Babaeinasab et al., 2015). These results are consistent with those research findings.

Results of cell wall components are summarized in Table 2. Numerically, but not significantly, the ADL content of treated groups was decreased (<i>P</i><0.05). It was stated that LAB could degrade NDF and ADF content of forages due to increasing hydrolyzing capacity (Rajabi et al., 2017). Several researchers reported that LAB inoculation with or without enzyme could degrade cellulose into sugars and promote the LAB population, resulting in cell wall losses (Djordjevic et al., 2016; Liu et al., 2016). Moreover, a decrease in cell wall components generally results in higher OMD and ME content of silages. Thus, it was expected that a decrease in the ADF and NDF ratio of silages. Parallel to this expectation, ADF content decreased by LAB and enzyme activity. On the other hand, NDF only was reduced in the KF group. An increased in HM LAB and HM-HT LAB may be related to clustering the simple sugar after hydrolysis in the silo. The obtained results are partially consistent with those results.

Data obtained in the aerobic exposure are presented in Table 4. The highest CO<sub>2</sub> level was found in HM + HT LAB group with 5.08 ± 0.23 g/kg DM (<i>P</i><0.001). As previously stated, the population of LAB, the composition of inoculants, concentration of organic acid, and WSC of ensiling material affect the aerobic stability of silages (Tao et al., 2017). Besides, several researchers have been reported that second-generation inoculants improve the aerobic stability of silages (Nishino et al., 2004; Reich and Kung, 2010). Second-generation inoculants, such as <i>L. buchneri</i> and <i>Propionibacteria</i>, are known as antimycotic agents and inhibit acid-tolerant yeasts in the silo by converting lactic acid into acetic acid and WSC into propionic acids (Weseh, 2013). It has also been indicated that silage’s aerobic stability reduced when yeast was added into the silage with or without LAB (Weinberg et al., 1999). In the current study, the amount of yeast in the KF group is mainly related to <i>S. cerevisiae</i>, found in the kefir’s natural flora, and improved aerobic stability of WS silages by decreasing pH.

In this study, a biological method was emphasized to improve plant-derived lignocellulosic material’s nutritional value, rich in lignin and cellulose. The effect of the inoculation of HM LAB, HM-HT LAB, and kefir on fermentation and WS silages’ nutritional quality was investigated. While a decrease in the ADF and ADL composition of silages was observed in all treated groups, the decrease in NDF was observed only in the KF group. In all treated groups, the ELOS and ME of silages were improved. Overall, this study strengthens the idea that the addition of kefir in WS silages increased their aerobic stability due to its significant effect on the pH and CO<sub>2</sub> level. Also, the findings of this research provide insights for kefir could be an alternative silage additive to commercially available inoculants and could improve WS’s nutritional quality instead of them. However, further studies should also be examined by carrying...
out in sacco degradability and in vivo digestibility experiments to better understand the implications of kefir on the nutritional quality of silage.

Researchers Contribution Rate Declaration Summary
The authors declare that they have contributed equally to the article.

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REFERENCES
Aktürk B, Gümüş H 2020. Effects of Lactic Acid Bacteria Inoculant on Quality, Fermentation Profile and Nutritive Value of Alfalfa Silage at Different Ensiling Period. Ankara Üniversitesi Vet Fak Derg 67(3): 281-287.

Anonymous 1986. The Analysis of Agricultural Material. Reference Book, London, UK, 427-428 pp.

AOAC 1990. Official Methods of Analysis. 15th Edition. Association of Official Analytical Chemist, Washington DC.

Ashbell G, Weinberg ZG, Azrieli A, Hen Y, Horev B 1991. A Simple System to Study the Aerobic Deterioration of Silages. Can Agric Eng 33: 391-393.

Ayaşan T, Cabi E, Esen S, Kader Esen V, Eseceli H 2020. Effect of Arbuscular Mycorrhizal Inoculation on the Quality and In Vitro Gas Production of Einkorn Wheat Straw. S Afr J Anim Sci, 50(3): 415-420.

Babaeinasab Y, Rouzehan Y, Fazaehi H, Rezaei J 2015. Chemical Composition, Silage Fermentation Characteristics, and In Vitro Ruminal Fermentation Parameters of Potato-Wheat Straw Silage Treated with Molasses and Lactic Acid Bacteria and Corn Silage. J Anim Sci 93(9): 4377-4386.

Chekani-Azar V, Chekani-Azar S 2010. Utilization of Wheat Straw in Sheep: Using an Applicable Method of Chemical Treatment. J Agrobio 27(2): 93-102.

Chen J, Stokes MR, Wallace CR 1994. Effects of Enzyme-Inoculant Systems on Preservation and Nutritive Value of Hay, Crop and Corn Silages. J Dairy Sci 77: 501-512.

Çömert Acar M, Özçelam H, Şayan Y, Soycan Önenç S 2018. The Accuracy of Pepsin-Cellulase Technique for Estimating the In Vivo Metabolizable Energy Values of Maize Silage and Dry Forages. J Anim Prod 59 (2):49-53.

Demirel M, Cengiz F, Erdoğan S, Çelik S 2003. A Study on Silage Quality and Rumen Degradability of Mixed Silages Containing Different Levels of Sudan grass and Hungarian Vetch. Turk J Vet Anim Sci 27(4): 853-859.

Djordjevic S, Mandić V, Stanojevic D 2016. The Effect of Bacterial Inoculant on Chemical Composition and Fermentation of Alfalfa Silage. Biotech Anim Hus 32(4): 413-423.

Droby S, Wisniewski M 2018. The fruit microbiome: A new frontier for postharvest biocontrol and postharvest biology. Postharvest Biol Technol 140: 107-112.

Eser S 2016. İnokulant ve Enzim İlaşının Farklı Samanların Besleme Değeri Üzerine Etkileri. Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü Zootekni Ana Bilim Dalı, Yüksek Lisans Tezi, 31 sy.

Fliya I, Sucu E 2007. Bazı Biyolojik ve Kimyasal Katki Maddelerinin Msur, Sorgum ve Buğday Silajlarının Fermantasyon, Mikrobiyal Flora ve Aerobik Stabilite Üzerine Etkileri. 4. Ulusal Hayvan Besleme Kongresi 25-28 Haziran 2017, Bursa, 116-120 sy.

Gado HM, Elghandour MMY, Cipriano M, Odongo NE, Salem AZM 2017. Rumen Degradation and Nutritive Utilization of Wheat Straw, Corn Stalks and Sugarcane Bagasse ensiled with Multienzymes. J Appl Anim Res 45(1): 485-489.

Gamba RR, Caro CA, Martinez OL, Moretti, AF, Giannuzzi L, De Antoni GL, Peláez AL 2016. Antifungal effect of kefir fermented milk and shelf life improvement of corn arepas. Int J Food Microbiol 235: 859-92.

Gonda M, Garmendia G, Rufo C, León Peláez Á, Wisniewski M, Droby S, Vero S 2019. Biocontrol of Aspergillus flavus in Ensiled Sorghum by Water Kefir Microorganisms. Microorganisms 7(8): 253.

Koç F, Coşkuntuna L 2003. The Comparison of the Two Different Methods on the Determination of Organic Acids in Silage Fodders. J Anim Prod 44(2): 37-47.

Koç F, Karapınar B, Okuyucu B, Koruyucu Erdem D 2020. Kefir İlaşının Yonca Silajlarının Fermantasyon Özellikleri ve Aerobik Stabilitesi Üzerine Etkileri. KSÜ Tarım ve Doğa Derg 23 (2): 535-542.

Kung JL, Stokes MR, Lin C 2003. Silage Additives. (Silage Science and Technology. American Society of Agronomy Inc., Crop Science Society of America Inc., Soil Science Society of America Inc., Publishers, Madison, Wisconsin, USA: Ed. Buxton DR, Muck RE and Harrison JH) 305-360.

Li P, Ji S, Hou C, Tang H, Wang Q, Shen Y 2016. Effects of Chemical Additives on the Fermentation Quality and N Distribution of Alfalfa Silage in South of China. Anin Sci J 87(12):1472-1479.

Liu Q, Li X, Seare TD, Zhang J, Shao T 2016. Effects of Lactobacillus plantarum and Fibrolytic Enzyme on the Fermentation Quality and In Vitro Deterioration of Silages. J Anim Prod 42(2): 37-47.

Djordjevic S, Mandić V, Stanojevic D 2016. The Effect of Bacterial Inoculant on Chemical Composition and Fermentation of Alfalfa Silage. Biotech Anim Hus 32(4): 413-423.
Digestibility of Total Mixed Rations Silage Including Rape Straw. J Integr Agric 15(9): 2087-2096.

Minitab 2014. Minitab I: Statistical Software for Windows, Release 17. Minitab Inc. USA.

Nakashima Y, Örskov ER, Adebawole EA, Ambo K 1993. Enzymatic Manipulation of Straw Quality: Experience on Straw Upgrading. Proceeding of the International Conference on Increasing Livestock Production through Utilization of Local Resources 18-22 October 1993, Beijing-China, 139-152 pp.

Nishino N, Wada H, Yoshida M, Shiota H 2004. Microbial Counts, Fermentation Products, and Aerobic Stability of Whole Crop Corn and a Total Mixed Ration Ensiled with and without Inoculation of Lactobacillus casei or Lactobacillus buchneri. J Dairy Sci 87(8): 2563-2570.

Nkosi BD, Kanengoni AT, Thomas R 2011. Effects of Ensiling Total Mixed Potato Hash Ration with or without Bacterial Inoculation on Silage Fermentation and Nutritive Value for Growing Pigs. J Anim Vet Adv 10(13): 1667-1672.

Ordaz S 2017. Fibrolytic Enzymes and Silage Inoculants to Improve the Nutritive Value of Silage. University of Vermont, The Faculty of Graduate College, USA, MSc Thesis, 108 pp.

Özkan M 2016. Hücre Duvarını ve Nişastayı Parçalayan Enzimlerin Fig-Yulaf Karışımı Silajların Fermentasyon, Aerobik Stabilite ve In Vitro Organik Madde Sınırlıbilişliği Üzerine Etkileri. Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü Zootekni Ana Bilim Dalı Yüksek Lisans Tezi, 18-19 sy.

Rajabi R, Tahmasbi R, Dayani O, Khezri A 2017. Chemical Composition of Alfalfa Silage with Waste Date and its Feeding Effect on Ruminal Fermentation and Microbial Protein Synthesis in Sheep. J Anim Physiol Anim Nutr 101(3): 466-474.

Reich LJ, Kung JL 2010. Effects of Combining Lactobacillus buchneri 40788 with Various Lactic Acid Bacteria on the Fermentation and Aerobic Stability of Corn Silage. Anim Feed Sci Technol 159(3-4): 105-109.

Schnürer J, Jonsson A 2011. Pichia anomala J121: a 30-year overnight near success biopreservation story. AvL 99: 5-12.

Seale DR, Pahlow G, Spoelstra SF, Lindgren S, Dellarlfo F, Lowe JF 1990. Methods for the Microbiological Analysis of Silage. Proceeding of the Eurobac Conference 12-16 August 1986, Uppsala-Sweden, 147 pp.

Shahryari Z, Fazaelipoor MH, Setoodeh P, Nair RB, Taherzadeh MJ 2018. Utilization of Wheat Straw for Fungal Phytase Production. Int J Recyc Org Waste Agric 7: 345-355.

Tao L, Zhou H, Zhang N, Si B, Tu Y, Ma T, Diao Q 2017. Effects of Different Source Additives and Wilt Conditions on the pH Value, Aerobic Stability, and Carbohydrate and Protein Fractions of Alfalfa Silage. Anim Sci J 88(1): 99-106.

Tilley JMA, Berry RA 1963. A Two-Stage Technique for the In Vitro Digestion of Forage Crops. J Brit Grasal Soc 18(2): 104-111.

Todorov NA, Pavlov DH, Djouvinov DS 1997. Effect of Hybrid, Maturity and Grain Content on Rumen Degradability of Maize Silage. Türkiye 1. Silaj Kongresi, 16-19 September 1997, Bursa, 127-134 pp.

Van Soest PJ, Robertson JB, Lewis BA 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J Dairy Sci 1991 74: 3583-3597.

Weinberg ZG, Szakacs G, Ashbell G, Hen Y 1999. The Effect of Lactobacillus buchneri and L. plantarum, Applied on Ensiling, on the Ensiling Fermentation and Aerobic Stability of Wheat and Sorghum Silages. J Indus Microbiol Biotechnol 23(3): 218-222.

Weshe A 2013. Effects of Silage Inoculants on Silage Fermentation, Aerobic Stability and Animal Performance. University of Alberta Department of Agricultural, Food and Nutritional Science, Canada, PhD Thesis, 33-38 pp.

Wiedmeier RD, Provenzat FD, Burritt EA 2004. Effect of Formic Acid and Potassium Diformate for Fungal Phytase Production. J Anim Feed Sci 7: 345-359.

Yu Y, Wen A, Dong Z, Desta ST, Shao T 2017. Effects of Formic Acid and Potassium Diformate on the Fermentation Quality, Chemical Composition and Aerobic Stability of Alfalfa Silage. Grass and Forage Sci 72(4): 833-839.

Zhang Q, Yu Z, Wang X, Tian J 2018. Effects of Inoculants and Environmental Temperature on Fermentation Quality and Bacterial Diversity of Alfalfa Silage. Anim Sci J 89(8): 1085-1092.

Zwielehner JC, Schoendorfer K, Schatzmayr G 2014. A Meta-analysis of Lactobacillus kefiri DSM 19544 and Lactobacillus brevis DSM 23231 as Silage Inoculant in Whole Plant Maize Silage. Proceeding of the International Scientific Conference on Probiotics and Prebiotics 24-26 June 2014, Budapest-Hungary, 173 p.