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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), NH_3-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.

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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 kullanımının buğday samanı silajlarının fermentasyon kalitesi, aerobic stabilitesi ve enzimde çözünen organic madde (EÇOM) miktarına olan etkisini araştırmayı amaçlamaktadır. Bu amaçla ticari olan 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 katkı maddesi yada kefir kullanılmıştır. Kontrol grubu (KON) dahil toplamda 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 karbondidrat içeriği ^{HM}LAB, $^{HM+HT}$LAB ve KF ilavesi sonrası değişmemiştir ($P>0.05$). KF grubu en düşük pH (4.32) ve NH_3-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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(%4.54) grubunda artmıştır (P<0.001). Nötral deterjan lif (NDF) içeriğinde iyileşme sadece KF (%17.02) grubunda gözlemlenmiştir (P<0.001). EÇOM ve ME değerleri HMLAB, HM+HTLAB ve KF gruplarında artmıştır (P<0.001). En düşük karbondioksit (3.42 g/kg KM) ve maya (5.50 log10 kob/g) değerleri sırasıyla KF ve KON gruplarında gözlemlenmiştir. Araştırma sonuçlarına göre, kefir ticari inokulantlar yerine silaj katkısı olarak alternatif olabilir ve ticari inokulantlar yerine kullanıldığında buğday samanı silajının besin değerini arttırabilir.

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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).

Schnürer 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 Namık 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 m^2 surface area. Commercially available Biotal Plus II (**HMLAB**; Lallemand Inc., USA; contains *Pediococcus pentosaceus* 12455, *Propionibacterium freudenreichii* R2435 strains and *β*-glucanase, xylanase and gluctomanganase enzymes), Biotal Buchneri 500 (**HM+HTLAB**; Lallemand Inc., USA; contains *Pediococcus pentosaceus* 12455, *Lactobacillus buchneri* NCIMB 40788 strains and *β*-glucanase, xylanase and gluctomanganase 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 Çoşkuntuna, 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 (Özkan, 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):

ELOS, g/kg= (DW-BW)/SW×1000 (1)

EULOS, g/kg= 1000- ELOS (2)

ME (MJ/kg DM) = 14.27-(0.0120×EULOS)+(0.0023×CP)-(0.0147×CA) (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; μ = overall mean; a_i= effect of inoculants or kefir; e_{ij}= effect of the experimental error.

RESULTS

The chemical and microbiological composition of pre-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

Çizelge 1.Başlangıç materyalinin kimyasal ve mikrobiyolojik kompozisyonu

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
H_{cell}, g/kg DM *(Hemiselüloz, g/kg KM)*	192
Cell, g/kg DM *(Selüloz, g/kg KM)*	390.2
WSC, g/kg DM *(SÇK, g/kg KM)*	18
ELOS, g/kg DM *(EÇOM, g/kg KM)*	312.9
ME, MJ/kg DM *(ME, MJ/kg KM)*	5.24
Lactobacilli , log10 cfu/g *(Lactobacilli, log10 kob/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, H_{cell}: Hemicellulose, Cell: Cellulose, WSC: Water Soluble Carbohydrate, ELOS: Enzyme 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

$^{HM+HT}$LAB (5.63%) groups, while the CP ratio was increased by 4.54% in the KF group (P<0.001). The ADF contents of silages treated with inoculants and kefir were decreased (P<0.01). An improvement (by lowering 17.02%) of NDF was only observed in the KF group (P<0.001). The lowest hemicellulose (P<0.001) and cellulose (P<0.05) content was recorded in the KF group. The NH_3-N and LA contents of treated groups were significantly different from the CON group (P<0.001). The KF group had lower NH_3-N content (71.97g/kg TN) and higher LA content (43.11 g/kg DM) than the control and other inoculated groups. Both inoculants and kefir significantly affected ELOS and ME of WS silages (P<0.001). The ELOS and ME in ^{HM}LAB, $^{HM+HT}$LAB, and KF groups were increased 11.35, 8.38; 10.02, 7.08; 5.84, 4.28%, respectively.

Table 2 Fermentation quality and chemical composition of WS silages
Çizelge 2 Buğday samanı silajlarının fermantasyon kalitesi ve kimyasal bileşimi

	Treatments *(Muameleler)*				
Parameters (*Parametreler)*	CON *KON*	^{HM}LAB *^{HM}LAB*	$^{HM+HT}$LAB *$^{HM+HT}$LAB*	KF *KF*	P *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^{b}	4.60±0.02^{a}	4.52±0.00^{b}	4.32±0.02^{c}	***
CP, g/kg DM *(HP, g/kg KM)*	63.9±1.3^{b}	60.1±0.1^{d}	60.3±1.4^{c}	66.8±0.8^{a}	***
CA, g/kg DM *(Kül, g/kg KM)*	67.4±0.6	66.9±0.2	68.1±0.6	6.79±0.8	NS
NDF, g/kg DM *(NDF, g/kg KM)*	676.2±19.1^{b}	718.9±36.6^{ab}	738.3±4.8^{a}	561.1±39.3^{c}	***
ADF, g/kg DM *(ADF, g/kg KM)*	416.0±11.4^{a}	381.1±14.3^{b}	380.9±16.3^{b}	359.2±19.7^{b}	**
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_{cell}, g/kg DM *(Hemiselüloz, g/kg KM)*	260.2±18.3^{b}	337.8±5.01^{a}	357.5±21.1^{a}	201.9±22.0^{c}	***
Cell, g/kg DM *(Selüloz, g/kg KM)*	366.9±6.4^{a}	339.8±19.0^{ab}	335.1±11.1^{ab}	317.5±23.0^{c}	*
WSC, g/kg DM *(SÇK, 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±7.50^{a}	95.21±7.02^{c}	113.84±0.06^{b}	71.97±2.19^{d}	***
LA, g/kg DM *(LA, g/kg KM)*	20.86±0.26^{c}	40.87±2.29^{b}	38.40±2.34^{ab}	43.11±2.93^{a}	***
ELOS, g/kg DM *(EÇOM, g/kg KM)*	328.4±12.9^{c}	365.7±7.8^{a}	361.3±5.9^{ab}	347.6±0.5^{b}	***
ME, MJ/kg DM *(ME, MJ/kg KM)*	5.37±0.14^{c}	5.82±0.09^{a}	5.75±0.08^{ab}	5.60±0.02^{b}	***

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05
DM: Dry Matter, CP: Crude Protein, CA: Crude Ash, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, H_{cell}: Hemicellulose, Cell: Cellulose, WSC: Water Soluble Carbohydrate, NH_3-N: Ammonia Nitrogen, TN: Total Nitrogen, LA: Lactic Acid, ELOS: Enzyme Soluble Organic Matter, ME: Metobalizable Energy, NS: Not significant; *: P<0.5; **: P<0.01; ***: P<0.001

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 $^{HM+HT}$LAB 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 content 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 3 Microbiological composition of WS silages
Çizelge 3 Buğday samanı silajlarının mikrobiyolojik kompozisyonu

	Treatments *(Muameleler)*				
Parameters (*Parametreler)*	CON *KON*	^{HM}LAB *^{HM}LAB*	$^{HM+HT}$LAB *$^{HM+HT}$LAB*	KF *KF*	P *P*
Lactobacilli, log10 cfu/g *(Lactobacilli, log10 kob/g)*	4.94±0.01^{d}	5.82±0.01^{b}	5.74±0.01^{c}	5.92±0.01^{a}	***
Yeast, log10 cfu/g *(Maya, log10 kob/g)*	5.60±0.02^{a}	5.33±0.02^{b}	5.07±0.01^{d}	5.15±0.01^{c}	***
Mould, log10 cfu/g *(Küf, log10 kob/g)*	0	0	0	0	NS

^{a,b,c} Means in the same row with different superscripts differ significantly at P<0.05
NS: Not significant; ***: P<0.001

Table 4 Aerobic stability parameters of WS silages
Çizelge 4 Buğday samanı silajlarının aerobik stabilite parametreleri

	Treatments *(Muameleler)*				
Parameters (*Parametreler)*	CON *KON*	^{HM}LAB *^{HM}LAB*	$^{HM+HT}$LAB *$^{HM+HT}$LAB*	KF *KF*	P *P*
pH *(pH)*	4.59±0.01^{c}	4.69±0.01^{b}	4.75±0.01^{a}	4.44±0.01^{d}	***
CO_2, g/kg DM *(CO_2, g/kg KM)*	5.14±0.15^{a}	4.62±0.07^{b}	5.08±0.23^{a}	3.42±0.07^{c}	***
Yeast, log10 cfu/g *(Maya, log10 kob/g)*	5.50±0.01^{d}	5.68±0.01^{c}	5.90±0.01^{b}	6.68±0.00^{a}	***
Mould, log10 cfu/g *(Küf, log10 kob/g)*	0	0	0	0	NS

^{a,b,c} Values within a row with different superscripts differ significantly at $P<0.05$
NS: Not significant; ***: $P<0.001$

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_3-N content, an indicator of protein breakdown, was significantly affected by ^{HM}LAB, $^{HM+HT}$LAB, and KF ($P<0.001$). Due to the low NH_3-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 ($P>0.05$). 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 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_2 level was found in $^{HM+HT}$LAB group with 5.08 ± 0.23 g/kg DM ($P<0.001$). 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 *L.buchneri* and *Propionibacteria,* 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 *S. cerevisiae*, 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_2 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.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

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