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Research Article / Araştırma Makalesi

Effect of Single or Combined Homo- and Heterofermentative Silage Additives on the Quality, Nutritive Value, and In Vitro Digestibility of Ensiled Wheat Harvested at Early Dough Stage of Maturity

Tekli veya Kombine Homo ve Heterofermentatif Silaj Katkı Maddelerinin Erken Hamur Olgunluk Aşamasında Hasat Edilen Silaj Buğdayının Kalitesi, Besin Değeri ve İn Vitro Sindirilebilirliği Üzerine Etkisi

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Abstract: The present study was conducted to evaluate the effect of single or combined homo- and heterofermentative silage additives on silage quality, nutritional composition, feed value, and *in vitro* digestibility of ensiled wheat harvested at early dough stage of maturity. The study was carried out as a completely randomized design with a 2 × 2 factorial arrangement of two levels of homofermentative silage inoculant (0 or 0.8 mg/kg) and two levels of heterofermentative silage inoculant (0 or 500 mg/kg) consisting of 4 groups with 4 replicates in each group. Control group received no silage additive. Remaining groups received either HMF, HTF, or a combination of both silage additives (HMF + HTF). pH and Flieg point of silage prepared with HMF and HTF alone or in combination were respectively lower and greater compared to control group. Nutritional composition, feed value, and *in vitro* true dry matter and organic matter digestibilities were unaffected among the treatments. In conclusion, the study shows that the application of single or combined HMF and HTF inoculants yields well-preserved wheat silage whereas the nutritional composition and *in vitro* digestibility may remain unaffected.

Keywords: Digestibilty, Inoculant, Nutritive value, Silage quality, Wheat..

Öz: Bu çalışma, erken hamur olum döneminde hasat edilen buğdayın tekli ya da kombine homofermentatif (HMF) veya heterofermentatif (HTF) mikrobiyal inokulantlar ile silolanmasının silaj kalitesi, yem değeri ve in vitro sindirilebilirlik üzerine etkilerini araştırmak amacıyla yapılmıştır. Denemede iki faklı düzeyde homofermentatif (0 veya 0,8 mg/kg) ve/veya heterofermentatif (0 ve 500 mg/kg) silaj inokulantı kullanılarak 2 × 2 deneme deseni uygulanmıştır. Her deneme grubu dört tekrar grubundan oluşturulmuştur. Kontrol grubuna herhangi bir silaj katkısı uygulanmazken; diğer gruplara HMF, HTF ya da bu ikisinin kombinasyonu (HMF + HTF) uygulanmıştır. Silaj inokulantı uygulanan grupların kontrol grubuna kıyasla daha düşük pH ve daha yüksek Flieg puanına sahip olduğu belirlenmiştir. Besin madde bileşimi, yem değeri ve in vitro kuru madde ve organik madde sindirilebilirliği açısından gruplar arasında fark oluşmamıştır. Sonuç olarak tekli ya da kombine HMF ve HTF inokulantların buğday silajının daha iyi korunabilmesini sağladığı ancak besin madde bileşimi ve in vitro sindirilebilirliğini etkilemediği belirlenmiştir.

Anahtar Kelimeler: Buğday, İnokulant, Beslenme değeri, Silaj kalitesi, Sindirilebilirlik.

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Introduction

Feeding practices of farmers in Turkey giving preference to cereals and straws instead of forages creates the shortage of forages and roughages (Arslan & Erdurmuş, 2012). It has resulted in an increase in the cultivation area of cereal crops compared to the forage crops (TÜİK, 2022). Wheat continues to be a major crop in Turkey especially for cereals and silage due to double cropping system in which wheat sown in winter is harvested in early or late spring to clear the fields

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for summer crops (Başkavak et al., 2008). Depending on the stage of maturity, wheat provides considerable dry matter (DM) with reasonable nutritive value for animal production (Filya, 2003a). Being a less expensive crop for ensiling, wheat has been considered an alternative for traditional silage crops i.e., grass and corn. However, the ensiling of wheat is an arduous task since it contains comparatively less water-soluble carbohydrates and starch. Additionally, the whole crop ensiling often ends in poor fermentation and aerobic stability resulting in higher butyrate concentration in the silage (Kaiser et al., 2003).

There are different strategies to improve the ensiling and to reduce the losses occurring at storage and feedout phases. Ensiling process of wheat can be improved using various silage additives of chemical or biological origin. Biological additives are easy-to-use and are not corrosive, therefore, the application of biological or bacterial additives is useful compared to their chemical counterparts. Studies have reported that the bacterial silage inoculants help in the fermentation, silage preservation, protection against pathogenic bacterial, and improvement of aerobic stability of silage that would other undergo spoilage thereby causing a loss in the nutritive value of the silage (Başkavak et al., 2008; Sucu and Filya, 2016). In general, bacterial silage additives are based on lactic acid bacteria (LAB) that may be homo- or heterofermentative. In practice, homofermentative (HMF) and heterofermentative (HTF) LAB silage additives are used to improve the fermentation and aerobic stability (Filya, 2003b), respectively. Although there are studies describing the use of HMF or HTF silage additives on the microbial composition and nutrient digestibility of ensiled bread wheat, there is still room for further study to evaluate the silage quality, nutritive value, and nutrient digestibility of wheat silage. In addition, a limited number of studies are available describing the wheat silage preserved using HMF and HTF silage additives. Therefore, the present study investigated the effect of single or combined application of HMF and HTF silage additives on the quality, nutritional composition, and nutrient digestibility of ensile durum wheat.

Materials and Methods

Location of the study

The study was conducted at the agricultural land of the Center for Agriculture, Livestock and Food Research, Burdur Mehmet Akif Ersoy University, Turkey in the western Mediterranean region of Turkey located 1280 m above the sea level.

Study design and experimental groups

The study was carried out as a completely randomized design with a 2 × 2 factorial arrangement of two levels of homofermentative silage inoculant (0 or 0.8 mg/kg) and two levels of heterofermentative silage inoculant (0 or 500 mg/kg) consisting of 4 groups with 4 replicates in each group. Control group received no silage additive. Remaining groups received either HMF, HTF, or a combination of both silage additives (HMF + HTF). The HMF silage additive consisted of *Lactobacillus plantarum* and *Enterococcus faecium* (Pioneer® brand 1188; Corteva Agriscience, Inc., IN, US) whereas HTF silage additive was comprised of *Lactobacillus buchneri* (Pioneer® brand 11A44; Corteva Agriscience, Inc., IN, US).

Wheat was sown by broadcasting in the mid of November 2021 (230 kg seed/hectare). Diammonium phosphate was used to fertilize the land (100 kg/hectare). Wheat was cultivated under dryland condition without irrigation. Wheat was harvested at the end of June 2022 at early dough stage of maturity. Fresh weight per m^2 and average plant height were measured by harvesting the forage using a quadrant at three different sites in the field. Additionally, three samples were collected to assess the nutritional composition of the forage.

Homofermentative silage additive consisted of 4 strains of *Lactobacillus plantarum* (2.5 × 10^{10} cfu/g *Lactobacillus plantarum* LP286 DSM 4784 ATCC 53187, 2.5 × 10^{10} cfu/g *Lactobacillus plantarum*

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LP318 DSM 4785, 2.5 × 10^{10} cfu/g *Lactobacillus plantarum* LP319 DSM 4786, and 2.5 × 10^{10} cfu/g *Lactobacillus plantarum* LP346 DSM 4787 ATCC 55943) and 2 strains of *Enterococcus faecium* (1.25 × 10^{10} cfu/g *Enterococcus faecium* SF301 DSM 4789 ATCC 55593 and 1.25 × 10^{10} cfu/g *Enterococcus faecium* SF202 DSM 4788 ATCC 53519) (Pioneer® brand 1188; Corteva Agriscience, Inc., IN, US).

Heterofermentative silage additive was comprised of 1.0 × 10^{11} cfu/g *Lactobacillus buchneri* LN4637 ATCC PTA-2494 (Pioneer® brand 11A44; Corteva Agriscience, Inc., IN, US).

Silage preparation, ensiling, opening, and physical quality assessment of ensiled wheat

Harvested wheat forage was cut to a particle size of 1.5-2.5 cm and vacuum packed in plastic bags (250 g in each bag) for ensiling after respective applications of silage additives. Control group was ensiled without the application of any additive whereas, prior to vacuum packing, silage additives were applied to the respective groups in accordance with the manufacturer's recommendations. Wheat forage was allowed to ensile for 120 days. The ensiled wheat forages were opened, and physical characteristics of each silage was assessed in terms of color (three-point scoring; 0 to 2), structure (four-point scoring; 0 to 4), and odor (15-point scoring; 0 to 14) following the DLG scoring method developed by German Agricultural Society (Deutsche Landwirtschafts Gesellschaft). A panel of three experts was employed to assess the physical quality of ensile wheat forage. The scores were summed up and categorized as follows according to the average score of the panel: bad (0 to 4 points), moderate (5 to 9 points), good (10 to 15 points), and excellent (16 to 20 points).

Fermentation characteristics and acidity of ensiled wheat forage

Following the assessment of physical quality, fermentation characteristics of ensiled wheat forages were evaluated in terms of Flieg point, pH, and ammonia nitrogen (NH_3-N). pH and dry matter (DM) of ensiled wheat forages were measured to calculate the Flieg point of each silage according to the method described by Dong et al. (2017) using equation below:

$$\text{Flieg point} = 220 + (2 \times \text{DM\%} - 15) - (40 \times \text{pH})$$

To measure the pH, 100 g of ensiled wheat forage was blended in 100 mL distilled water for 5 minutes, filtered through 4 layers of cheese cloth, and glass electrode of pH meter (Apera Instruments, LLC., Columbus, OH, US) was immersed into the filtrate to measure the pH of wheat silages.

The NH_3-N was measured according to the method previously described by Meeske et al. (2002). Briefly, 50 g silage was homogenized in 250 ml 0.1 N sulphuric acid followed by filtration of the homogenate through a four-layer cheesecloth. Finally, the filtrate was subjected to distillation and titration according to Kjeldahl method described by AOAC (2000).

Nutritive value of fresh and ensiled wheat forage

The DM of freshly harvested and ensile wheat forages were dried in hot air oven at 105ºC for 8 hours. Crude protein (CP), ether extract (EE), and crude ash were analyzed using AOAC (2000) methods. Crude fiber (CF), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom), and acid detergent lignin (ADL) were analyzed using automatic fiber analyzer (ANKOM A2000 Fiber Analyzer, ANKOM Technology, NY, US). Other fractions were calculated according to the equations reported by Horrocks and Vallentine (1999) as follows:

Non-structural carbohydrates (%, DM basis) = 100 – (aNDFom + CP + Ash + EE)

Hemicellulose (%, DM basis) = aNDFom% – ADFom%

Digestible DM (%, DM basis) = 88.9 – (0.779 × ADFom%)

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DM intake (%, DM basis) = 120 ÷ aNDFom%

Relative feed value (%, DM basis) = DDM% × DMI% × 0.775

Net energy for lactation = [1.044 – (0.0119 × ADFom%)] × 2.205

Total digestible nutrients = (–1.291 × ADFom%) + 101.35

Total carbohydrates (%, DM basis) = DM% – (CP% + Ash% + EE%)

Cellulose (%, DM basis) = ADFom% – ADL%

In vitro rumen digestibility of fresh and ensiled wheat forage

Fresh and ensiled wheat forages were subjected to incubation in ANKOM Daisy^{II} incubator to investigate the *in vitro* true DM and OM, digestibilities. For this purpose, the samples in duplicates were placed in bottles of Daisy^{II} incubator containing ruminal fluid as inoculum from a slaughtered cow. The samples, packed in ANKOM F57 filter bags, were incubated for 24 and 48 hours. All the procedures were conducted under anaerobic conditions using carbon dioxide gas to ensure the anaerobic environment at each stage. *In vitro* true digestibilities were calculated for DM and OM.

Statistical analysis

The data were tested for normality followed by logarithmic or square root transformation of non-normalized traits. The data were subjected to two-way analysis of variance applying the general linear model procedures using a statistical software package SPSS (IBM Corp., Armonk, NY, US) according to the following model:

$$Y_{ijk} = \mu + s_i + l_j + e_{ijk}$$

Where:

Y_{ijk} = phenotypic value of the trait for the k^{th} group of silages belonging to i^{th} HMF and j^{th} HTF silage additives;

μ = mean value of the trait for a given population;

s_i = effect of i^{th} HMF additive (i = 1, 2);

l_j = effect of l^{th} HTF additive (j = 1, 2);

e_{jkl} = effect of experimental error.

Confidence interval was assumed at 95% (P < 0.05) for significant different among the means. Tukey's test was applied as post-hoc test to separate the significantly different means in case of significant interactions. Results were presented as mean ± pooled standard error of the mean.

Results

All the silages were of excellent quality (Table 1). No difference was noted in the quality traits of the silages. There was a significant interaction between HMF and HTF for silage pH (P < 0.001) and Flieg point (P < 0.001) of ensiled wheat. Application of HTF reduced the pH of wheat silage compared to control group that further declined with the inclusion of HMF + HTF silage additives. An opposite trend was seen for Flieg point of wheat silages. Besides these, nutritional composition (Table 2), feed value, and *in vitro* digestibilities (Table 3) remained unaffected across the groups.

Discussion

Silage quality is dependent on the rapid pH decline, temperature, and other factors related to the packing and plant material intended for ensiling process. In the present study, pH was lower in wheat ensiled with single or combined HMF and HTF silage additives compared to control group. Similarly, Flieg point was greater in wheat silage prepared with HMF and HTF silage additives applied alone or in combination. These findings are consistent with those of Filya (2003b) who reported a decrease in pH of ensiled wheat with HMF and HMF + HTF silage additives. Likewise, Zhang et al. (2009) reported a decrease in the pH of alfalfa silage prepared with single or combined HMF and HTF silage inoculants.

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Table 1. Physical characteristics of wheat ensiled with single or combined homo- and heterofermentative silage additives.

Item	pH	NH_3-N[§]	Odor	Structure	Color	DLG Score	Flieg point
Homofermentative inoculant							
Not added	4.42	0.137	12.83	4.00	1.92	18.75	113.10
Added	3.99	0.161	12.83	4.00	1.92	18.75	132.06
P-value	<0.001	0.298	0.999	0.999	0.999	0.999	<0.001
Heterofermentative inoculant							
Not added	4.32	0.125	12.67	4.00	1.92	18.58	117.20
Added	4.09	0.172	13.00	4.00	1.92	18.92	126.96
P-value	<0.001	0.239	0.282	0.999	0.999	0.397	<0.001
SEM	0.02	0.07	0.20	0.00	0.08	0.26	0.94
Interaction means							
Control	4.64[a]	0.112	13.00	4.00	2.00	19.00	104.74[c]
HMF[1]	3.99[c]	0.139	12.33	4.00	1.83	18.17	132.06[a]
HTF[2]	4.20[b]	0.162	12.67	4.00	1.83	18.50	121.46[b]
HMF + HTF	3.98[c]	0.183	13.33	4.00	2.00	19.33	132.46[a]
SEM	0.04	0.03	0.29	0.00	0.12	0.37	1.34
HMF × HTF	<0.001	0.158	0.050	0.999	0.195	0.056	<0.001

[§] Relative of total nitrogen
[1] HMF = homofermentative
[2] HTF = heterofermentative

However, there was no effect on NH_3-N content of silages in the present study as opposed to Filya (2003b) who reported that the addition of HTF alone or in combination with HMF reduces the NH_3-N of ensiled wheat. Zhang et al. (2009) reported that the addition of HMF and HTF silage additives alone or in combination had no effect on the NH_3-N of alfalfa at d 2, 5, 9, 15, and 30, however, it significantly decreased at d 90 in silage prepared with a combination of HMF and HTF (HMF + HTF). Similarly, HMF or HTF silage inoculants reduced the pH and NH_3-N of potato hash silage (Nkosi et al., 2010). It seems that the increase in Flieg point of ensiled wheat in the HMF, HTF, and HMF + HTF groups was contributed by the pH of the silages since the DM was not different among the groups. The quality of all the silages was categorized as 'excellent' according to the DLG scoring method based on odor, structure, and color of the silage. This might be attributed to a rapid decrease in the pH of wheat silages under the action of HMF and HTF silage additives that helped in the preservation of silages via effective fermentation by producing acetic acid.

In our study, application of silage additives alone or in combination had no effect on the nutritional composition, feed value, and *in vitro* DM and OM digestibility of wheat silages. There are a limited number of studies describing the effect of HMF and HTF silage additives alone or in combination on wheat silage. Most studies have focused on the microbiological quality, fermentation characteristics, and of wheat silages while there is no study describing the nutritional composition and feed value of wheat silages. Consistent with our findings, Filya (2003b) reported that the *in situ* nutrient digestibility of wheat silage prepared with single or combined HMF and HTF silage additives remain unaffected. Similarly, Zhang et al. (2009) reported that the application of HMF and HTF silage inoculants alone or in combination had no effect on *in situ* DM, NDF, and ADF digestibility of alfalfa silages.

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Table 2. Nutrient composition Physical characteristics of wheat ensiled with single or combined homo- and heterofermentative silage additives (%, dry matter basis).

Item	Nutrients[1]											
	DM	CF	EE	CP	Ash	Total CHO	ADFom	ADL	aNDFom	NFC	HEC	CEL
Homofermentative inoculant												
Not added	42.45	23.83	4.26	9.77	7.84	20.58	26.79	4.31	47.05	31.08	20.26	22.48
Added	43.33	23.54	3.84	9.40	7.33	22.76	27.86	4.93	47.35	32.09	19.49	22.92
P-value	0.121	0.702	0.326	0.721	0.182	0.167	0.151	0.156	0.471	0.257	0.281	0.367
Heterofermentative inoculant												
Not added	42.50	23.62	4.24	9.22	7.56	21.48	27.02	4.56	46.59	32.39	19.58	22.46
Added	42.78	23.75	3.85	9.94	7.62	21.37	27.63	4.68	47.79	30.82	20.16	22.95
P-value	0.331	0.831	0.570	0.025	0.686	0.758	0.392	0.765	0.259	0.413	0.639	0.323
SEM	0.25	0.33	0.30	0.25	0.20	0.37	0.48	0.28	0.56	0.48	0.32	0.33
Interaction means												
Control	42.67	23.64	4.64	9.05	7.95	21.03	25.90	4.18	46.38	31.97	20.48	21.72
HMF[2]	43.33	23.60	3.85	9.39	7.16	22.93	28.14	4.94	46.81	32.79	18.67	23.20
HTF[3]	42.23	24.02	3.88	10.48	7.74	20.13	27.68	4.44	47.71	30.20	20.03	23.24
HMF + HTF	43.33	23.48	3.82	9.41	7.50	22.60	27.58	4.93	47.88	31.38	20.30	22.65
SEM	0.63	0.42	0.43	0.35	0.34	0.49	0.67	0.40	0.51	0.64	0.34	0.46
HMF × HTF	0.670	0.892	0.799	0.239	0.392	0.585	0.212	0.744	0.393	0.314	0.418	0.055

[1] DM = dry matter, CF = crude fiber, EE = ether extract, CP = crude protein, Total CHO = total carbohydrates, ADFom = ash-free acid detergent fiber, ADL = acid detergent lignin, aNDFom = ash-free neutral detergent fiber after amylase treatment, NFC = non-fibrous carbohydrates, HEC = hemicellulose, CEL = cellulose
[2] HMF = homofermentative
[3] HTF = heterofermentative

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Table 3. Feed value and *in vitro* digestibility of wheat ensiled with single or combined homo- and heterofermentative silage additives.

Item	Feed Value [1]			IVTDMD[2]			IVTOMD[3]		
	DDM	DMI	RFV	NE_L	TDN	24 h	48 h	24 h	48 h
Homofermentative additive									
Not added	68.03	2.55	134.44	1.60	66.77	60.88	63.91	61.23	65.64
Added	67.20	2.53	131.76	1.57	65.39	57.73	63.14	60.68	63.59
P-value	0.151	0.367	0.605	0.151	0.151	0.137	0.870	0.239	0.192
Heterofermentative additive									
Not added	67.85	2.58	135.66	1.59	66.47	60.22	63.16	61.10	65.83
Added	67.38	2.51	131.07	1.58	65.69	58.39	63.89	60.81	64.89
P-value	0.392	0.397	0.502	0.392	0.392	0.405	0.794	0.341	0.218
SEM	0.37	0.18	1.61	0.01	0.61	0.93	0.42	0.29	0.49
Interaction means									
Control	68.73	2.58	137.43	1.62	67.92	61.25	63.24	61.38	65.40
HMF[4]	66.98	2.56	132.88	1.56	65.03	59.18	63.08	60.82	63.26
HTF[5]	67.34	2.52	131.52	1.58	65.62	60.52	64.59	61.07	65.87
HMF + HTF	67.42	2.51	131.15	1.58	65.75	58.28	63.19	60.54	63.92
SEM	0.52	0.23	4.28	0.02	0.87	1.13	0.57	0.45	0.71
HMF × HTF	0.120	0.318	0.483	0.120	0.120	0.541	0.892	0.477	0.623

[1] DDM = digestible dry matter (%, DM basis), DMI = dry matter intake (% body weight), RFV = relative feed value, NE_L = net energy for lactation (Mcal/kg), TDN = total digestible nutrients (%, DM basis)
[2] IVTDMD = *in vitro* true dry matter digestibility
[3] IVTOMD = *in vitro* true organic matter digestibility
[4] HMF = homofermentative
[5] HTF = heterofermentative

Unlike our findings, the application of HMF or HTF silage additives reduced the DM, aNDFom, and ADF while increasing the CP of potato hash silage. However, the DM and OM digestibilities were not affected by the application of inoculants (Nkosi et al., 2010). Similar findings were reported by Zhang et al. (2021) in response to single or combined HMF and HTF silage additives.

Conclusions

Under the conditions of the present study, it is concluded that the application of homo- and heterofermentative silage inoculants alone or in combination yields well-preserved wheat silage harvested at early dough stage of maturity. Nutritional composition, feed values, and *in vitro* dry matter and organic matter digestibilities may remain unaffected. Further studies involving the *in situ* and *in vivo* nutrient digestibilities may present the true picture on the effect of wheat silage prepared with homo- and heterofermentative silage inoculants alone or in combination.

Conflict of Interest

No commercial funding was acquired for this study that may be construed as a potential conflict of interest.

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