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The Effects of Hazelnut Husk Supplementation on Silage Quality, Deterioration, and *In Vitro* Digestion Parameters in Second Crop Maize

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ABSTRACT

This research investigates the effects of hazelnut husk on the low dry matter (DM) maize silage quality, microbial profile, deterioration, fiber components, and digestion parameters. Second crop maize was harvested at the milk stage of maturity (26.61% DM) and ensiled in laboratory silos with or without ground hazelnut husk. A total of 18 jars of silage were made utilizing two treatments (control silage and 15% hazelnut-contained silage), three different opening dates, and three replicates. All silage analyses were completed in all of the opening periods. Hazelnut husk increased the ($p<0.05$) silage DM, pH, ash, protein, and cellulose fractions content while only decreasing ($p<0.05$) hemicellulose. The lactic acid, propionic acid, acetic acid, and butyric acid in silages were reduced ($p<0.05$) by the hazelnut husk. The addition of hazelnut husk to the silages increased ($p<0.05$) the population of lactobacilli but had no influence ($p>0.05$) on the yeast-mold population. Hazelnut husk increased ($p<0.05$) aerobic stability in maize silages. Hazelnut husks reduced *in vitro* gas production value, digestible organic matter, metabolic energy, and net energy lactation values, but increased protozoa in the rumen ($p<0.05$). The hazelnut husk demonstrated a potential hygroscopic property in low DM maize silage by increasing silage DM and improving fermentation efficiency, as well as air stability during feedout.

Keywords: Hazelnut husk, Maize silage, Low dry matter, Fermentation, Air stability, Nutritive value

1. Introduction

The hazelnut tree (Corylus avellana, Betulaceae) is a valuable tree in the Mediterranean basin, particularly Turkey, which produces 60% of the world's hazelnuts. Turkey is the leading producer, producing approximately 776,000 t hazelnuts in shell in 2019, followed by Italy, Azerbaijan, and the United States, which produced approximately 98,530; 53,739; and 39,920 t, respectively. France and Spain, for example, have much smaller production capacities of 11,660 t and 12,370 t. The European Union's total hazelnut production capacity is 160,683 t. Germany, Italy, and France are major European importers. Total hazelnut production surpassed 1 million tons, demonstrating the global popularity of hazelnuts (FAOSTAT 2020). The husk, or green, leafy covering of hazelnuts, covers up to one-third of the hazelnut's exterior surface (Dok 2014). After harvest, the green leafy coverings of hazelnuts are separated from the shell in the first step of processing (Solmaz 2017) and are typically discarded (Piccinelli et al. 2016). The majority of this waste is ligno-cellulose shells, which are obtained after cracking the kernel and are used as a heat source, mulch, and furfural production in the industrial production of pigment. Commercially viable compounds found in hazelnut side products could be used to create valuable coproducts. Hazelnut shells contain phenolic compounds that have natural antioxidant properties (Stévigny et al. 2007). Candellone et al. (2019) suggested using hazelnut skin for its natural antioxidant properties observed that including hazelnut skin in the diet of rabbits improved their oxidative status and immune function. Cetinkaya and Kuleyin (2016) investigated the digestibility of different hazelnut internal skins in order to recommend hazelnut fruit skin as a roughage replacement in ruminant nutrition. Cetinkaya and Kuleyin (2016) proposed that the internal fruit skin of various hazelnut species could be used as a substitute roughage for ruminant nutrition due to

its crude and digestible nutritive qualities. Caccamo et al. (2019) investigated the impact of hazelnut skin in dairy ewes' diets on the biological and sensory qualities of ovine cheeses, finding that the by-product had a significant impact on the cheeses' lipid content, fatty acids (FAs), and gustatory profiles. Another study found that substituting hazelnut skin for dried beet pulp had no effect on feed intake or milk output in lactating ewes, but did improve the atherogenic index and health-promoting unsaturated FAs in ewe milk while lowering the milk protein percentage and viable cells (Campione et al. 2020). Furthermore, Renna et al. (2020) found that hazelnut skin contains a high concentration of antioxidants and that feeding this byproduct to dairy cows can significantly increase the vitamin E content of the milk. According to Renna et al. (2020), despite being a minor percentage of dry matter (DM) intake, using hazelnut skin in the cow's diet improves the sustainability of the acquired milk in terms of the food-feed challenge and lowers the daily cost of the diet. Maize (*Zea mays*) silage is a common feed source in many animal agricultural practices. It meets a significant portion of the energy and fiber daily requirements of dairy cows when ensiled at optimal DM (28-35%) concentrations (Bell et al. 2007). However, in some situations, such as during the prolonged rainy season (Haigh 1997; Bell et al. 2007) or when maize is sown as a second crop in midsummer, achieving an optimal DM content before harvest can be difficult (Khorvash et al. 2006; Ranjbari et al. 2007). In all these situations, effluent generation has been considered a nutrient loss, so finding alternative solutions for raw sewage reduction or elimination should be a top priority. Since the maize plant is not optimal for wilting, one technique is to combine additives with ensiled low-DM maize (Muck et al. 2018). Various cereal straws, wheat bran, soy bran flakes, dried meadow grass, dried alfalfa grass, legume stalks, soybean husks, cereals, dried sugar beet pulp, bentonite, zeolite, whey powder, dried molasses, newspapers or waste papers are the additives for this purpose (McDonald et al. 1991; Jones & Jones 1996; Khorvash et al. 2006; Barmaki et al. 2018).

So far, most of the trials (Cetinkaya & Kuleyin 2016; Caccamo et al. 2019; Campione et al. 2020; Renna et al. 2020) have investigated the nutritional value of hazelnut inner skin as a diet ingredient. To our knowledge, there has yet to be a study on the nutritional benefits of the hazelnut's outer skin when utilized as a silage additive. For this reason, this study investigates how incorporating hazelnut husk into second crop maize forage affected silage fermentation, microbiological structure, aerobic stability, cell wall components, and *in vitro* digestion parameters.

2. Material and Methods

In the study, the hazelnut husk was obtained from Düzce Province's Gölyaka District, Kuyudüzü District. The husks were crushed and passed through a 2 mm sieve before being used in the experiment. A maize variety called DKC-7211 (Monsanto Gıda ve Tarım Tic. Ltd. Sti.) with a growth period of 120 days was used as silage material in 2018. A maize plant was cultivated in the area between latitudes 40°13' 35.1" north and longitudes 28°51' 48.8" east. In 2018, the total precipitation was 65.5 mm, the average temperature was 24 °C, and the relative humidity was 64%. According to the soil analysis results, the soil was heavy and medium textured, had a pH of 7.9, and was not saline. The soil had a low organic matter content but was free of lime, was rich in available phosphorus and potassium, and had a sufficient quantity of nitrogen. To determine plant height, ten plants from a specific row were cut. Five out of ten sampled plants were separated into stem, leaf, and ear fractions to determine their percentages in a whole-plant weight. Forage samples were collected from a 5.7 m^2 center area of each plot to assess forage yields. 500 g of fresh plant material was collected and dried for 48 hours at 75 °C to calculate hay yields. As a result of these measurements, the plant height was 2.78 m, the forage yield was 5990 kg/da, the DM yield was 1660 kg/da, and the ear/stalk ratio was 29.2%.

Maize was harvested when it reached the milk stage of maturity and chopped through a 2 cm screen with a standard forage harvester. The freshly chopped raw maize plant was then immediately delivered to the laboratory. It is homogeneously combined with ground hazelnut husk at a rate of 15% and ensiled into 1.5-liter special laboratory type jars (Weck, Wehr, Oflingen, Germany). For each application, two treatments (control and 15% husk supplemented), three opening dates (8, 21, and 60 days), and a total of 18 jars of silage were prepared as three parallels. For the control and hazelnut husk supplemented groups, each jar was filled with approximately 1.3 and 1.1 kg (fresh weight) of chopped forage, respectively. The packing densities were 230 and 248 kg/DM/m^3, respectively. On the eighth, twenty-first, and sixty-first days after ensiling, three jars from each treatment (control silages and silages with 15% husk supplemented) were opened, and pH, DM, and microbiological tests were performed on all silages on the same day. The 40 g wet silage sample was mixed with 360 mL of distilled water, shaken for 3 minutes in a stomacker, then filtered using Whatman paper. It was then centrifuged at 12000 rpm for 15 minutes before being transferred to sterile eppendorf tubes. The samples were kept at -20 °C in a deep freezer. A pH meter (Sartorius PB-20, Goettingen, Germany) was used to measure the silage pH directly from the silage juice. Each replicate's subsamples were composited, and the DM was measured by oven-drying at 65 °C for 48 hours. The dried samples were then milled to pass through a 1-mm screen in a laboratory mill (Elmeksan, E.M.S. 101-TIP, Turkey). Analytical DM was prepared for chemical analysis by oven-drying previously dried silage samples at 100 °C for 4 hours. All samples were analyzed for ash

(method 942.05, AOAC 2002), and ether extract (method 942.05, AOAC 2002). The sodium sulfite addition method without amylase was used to analyze neutral detergent fiber (NDF) and acid detergent fiber (ADF), and the results were given with the remaining ash (Van Soest et al. 1991). The difference between NDF and ADF was used to calculate hemicellulose (HCEL). The DM disappearance (DMD) in silages in the form of gases and effluents was quantified by weight difference, with respect to Cai and Ohmomo (1995). Gas chromatography was used to assess the organic acid and ethanol content of the fresh forage and silages (Agilent Technologies 6890N Network GC System, 7683 B Series Injector). In the analysis, a capillary column (Stabilwax®-DA; Crossbond "Carbowax"-PEG for acidic chemicals, 30 m, 0.25 mm ID, 0.25 m df, maximum program temperature of 260 °C) suited for the specified gas chromatography was utilized. The lactic acid content of the fresh forage and silages were determined using the spectrophotometric method described earlier by Barker and Summerson (1941). Fresh forage and silage microbiological analyses were performed in quadruplicate (per treatment for each replicate) and the results were presented on a fresh and wet silage basis. Lactobacilli colonies were cultivated on Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, U.K.), and yeast and mold were cultivated on malt extract agar (Difco, Detroit, MI) that had been acidified with lactic acid to pH 4.0. Plates were incubated at 30 °C for 3 days. The $\log_{10}$ transformation was applied to all microbiological data. An aerobic deterioration test (5 days of air exposure) was performed at the end of the ensiling period, according to Ashbell et al. (1991). As previously described, silage samples were examined for pH, yeast, and mold.

An *in vitro* ruminal fermentation study was conducted to evaluate the rumen fermentation properties of hazelnut husk as a maize silage supplement. Rumen fluid was taken from ruminally cannulated 600 kg Holstein non-lactating dairy cows (n=2) before morning feeding. The animals were cared for and handled in accordance with the Turkish Directorate of Provincial Agriculture and Forestry's welfare and ethics standards for experimental animals. The donor cows were fed a 50/50 combination of maize silage and concentrate to ensure that the rumen fluid had a balanced cellulolytic and amylolytic activity. Rumen fluid was taken before morning feeding and immediately transported to the laboratory after being placed in a warm Thermos flask (39 °C). Rumen fluid was squeezed through cheesecloth and placed in a 39 °C Erlenmeyer flask. For *in vitro* rumen fermentation, the Menke and Steingass (1988) method was used to create a buffer combination (comprising micro-and macro-elements, a reducing agent, and a resazurin reduction indicator). In a warmed bottle (39 °C), particle-free rumen fluid and buffer were combined and continuously gassed with CO_2. As incubation vessels, glass syringes (Fortuna®, Häberle Labortechnik, Germany) were used. Each syringe contained 30 mL of rumen fluid-buffer medium. Each syringe had about 200 mg of dry feed sample. The samples were incubated in triplicate. As blanks, triplicates of bottles with no substrate were employed. The total volume of gas produced was measured at 3, 6, 12, 24, 48, and 96 hours. The quantities of generated gas were computed in the Neway computer program using the model $y= a+b (1-e^{-ct})$ provided by Orskov and McDonald (1979).

In the model;

a = gas amount of easily soluble fractions, mL

b = gas production amount of insoluble fractions, mL

c = gas production rate of insoluble fractions (b) ($hour^{-1}$)

a+b = potential gas production, mL

t = incubation time, hours (h)

y = amount of gas produced during t

The organic matter digestibility and metabolic energy (ME) contents of silages were calculated according to the formulas reported by Menke & Steingass (1988) for forages.

OMD (%)= 15.38+0.8453xGP+0.0595xHP+0.0675xCA (1.1)

ME (MJ/kg DM)= $2.20+0.1357xGP+0.0057xCP+0.0002859xEE^2$ (1.2)

GP: net gas production (mL) after 24 hour incubation period

CP: Crude protein value (%) in DM

EE: Ether extract value (%) in DM

CA: Crude ash value (%) in DM

The following formula was used to calculate the net energy lactation (NE_L) content of silages (Menke et al. 1979).

NE_L (MJ/kg DM)= 0,101xGP+0,051xCP+0,112xEE (1.3)

GP: net gas production (mL) after 24 hour incubation period

CP: Crude protein value (%) in DM

EE: Ether extract value (%) in DM

The protozoa count was performed after 96 hours of incubation. To count protozoa, one milliliter of rumen fluid was combined with 49 milliliters of rumen protozoa counting solution (2.02% formalin and 15.15% glycerol). Using the Boyne et al. (1957) approach, diluted ruminal fluid samples were counted using a Fuchs Rosenthal counting chamber.

The data from silage quality were analyzed using a completely randomized design with three replications and the SAS GLM procedure for analysis of variance (Statistical Analysis System, version 6.0). Tukey's test was used to test for differences between means, when $p<0.05$, differences between treatments were regarded as significant, and when $0.05<p<0.10$, considered to be significant.

3. Results

The chemical composition of fresh maize and hazelnut husk are shown in Table 1. Fresh maize chemical structure is distinguished by a low DM (26.6), crude protein (6.7%), fat (2.0%) and low fibrous fractions (43.7, 26.7 and 2.7% for NDF, ADF, and ADL, respectively). Hazelnut husk's chemical structure is distinguished by a low protein content (6.3%) and crude fat (0.81 percent), as well as a high pH (7.9), DM (77%) and fibrous fractions (67.6, 53.4, and 26.7% for NDF, ADF, and ADL, respectively).

Table 1- The chemical composition of fresh maize and hazelnut husk

Item	*Maize*	*Hazelnut husk*
pH	5.95	7.90
DM,%	26.61	76.98
OM, % DM	94.59	93.67
CP, % DM	6.74	6.27
EE, % DM	2.01	0.81
CCEL, % DM	21.80	31.48
CA, % DM	5.41	5.81
NDF, % DM	43.76	67.55
ADF, % DM	26.68	53.36
ADL, % DM	2.71	26.65
HCEL, % DM	17.08	14.19

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CCEL: Crude cellulose, CA: Crude ash, NDF: Neutral detergent fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent lignin, HCEL: Hemicellulose

The results obtained from the chemical composition of silages are given in Table 2. The DM content of silages varied between 25.35% and 33.05% in control and hazelnut husk treated silages, respectively. Hazelnut husk had a high absorbency potency, which increased ($p<0.05$) the DM content of low DM maize silage (25.5%) by adding 15% hazelnut husk (32.5%). While opening days had no effect on the DM content of the control silages ($p>0.05$), there was an increasing trend in the DM content of the hazelnut husk-treated silages ($p=0.07$). The highest OM content was found in control silages ($p<0.05$). The crude ash content of hazelnut-treated silages was found to be higher than that of control silages ($p<0.05$). The highest ($p<0.05$) crude protein content was found in hazelnut husk treated silages on the 21st day of ensiling. On day 60, the crude protein content of all silages was comparable ($p>0.05$). The ether extract content of the silages did not differ between treatments ($p>0.05$). Regardless of treatment, the NDF content of maize silages decreased as the ensiling dates progressed ($p<0.05$). The control silages had the lowest ($p<0.05$) levels of CCEL, NDF, ADF, and ADL. The HCEL content of the hazelnut husk-treated silages was lower ($p<0.05$) than that of the control silages.

Table 2- The effect of the hazelnut husk additive on the chemical composition (% DM) of maize silages

Parameters	*Treatment*	*Ensiling days*			*SEM*	*P value*		
		8	21	60		Day	Trt	Day*Trt
DM, %	Control	25.77c	25.80c	25.35c	0.328	0.07	*	*
	Hazelnut husk	31.12b	32.50ab	33.05a				
DMD	Control	2.67c	2.43d	3.47a	0.046	*	*	*
	Hazelnut husk	2.82b	1.86e	2.43d				
OM	Control	94.41a	94.46a	94.48a	0.093	NS	*	NS
	Hazelnut husk	93.93b	94.12ab	93.94b				
CA	Control	5.59b	5.54b	5.52b	0.093	NS	*	NS
	Hazelnut husk	6.05a	5.87ab	6.07a				
CP	Control	6.28c	6.99b	7.19b	0.112	*	*	*
	Hazelnut husk	6.86b	7.62a	7.11b				
EE	Control	3.56	3.90	4.10	0.344	NS	NS	NS
	Hazelnut husk	2.98	3.92	3.76				
CCEL	Control	24.95d	24.00e	22.29f	0.189	*	*	*
	Hazelnut husk	31.51a	30.51b	28.55c				
NDF	Control	47.01b	45.53b	39.77c	0.794	*	*	*
	Hazelnut husk	53.93a	49.12b	48.17b				
ADF	Control	28.24c	28.25c	26.28c	0.469	*	*	*
	Hazelnut husk	40.70a	36.52b	36.12b				
ADL	Control	2.79e	3.09d	2.02f	0.04	*	*	*
	Hazelnut husk	10.25a	9.25b	8.15c				
HCEL	Control	18.77a	17.28a	13.49b	0.697	*	*	*
	Hazelnut husk	13.23b	12.60b	12.05b				

SE: Standard error, DM: Dry matter, DMD: Dry matter disappearance, OM: Organic matter, CA: Crude ash, CP: Crude protein, EE: Ether extract, CCEL: Crude cellulose, NDF: Neutral detergent fiber; ADF: Acid detergent fiber, ADL: Acid detergent lignin, HCEL: Hemicellulose, NS: Not significant, *p<0.05. The differences between the averages indicated with different letters on the same row are significant (p<0.05)

The results obtained from the fermentative properties and microbial structure of silages are given in Table 3. Hazelnut husk-treated silages had the highest (p<0.05) pH and the lowest (p<0.05) levels of lactic acid, acetic acid, propionic acid, and butyric acid. On the 60th day of ensiling, the control silages had the lowest (p<0.05) silage pH. The final pH of the silages ranged from 3.64 to 3.78, indicating that they had all been well preserved. There was no difference in the ethanol content between treatments (p>0.05). After 60 days of ensiling, hazelnut husk application increased LAB counts (p<0.05), but had no effect on mold counts (p>0.05). Although no yeast growth was detected in any of the silages, the mold counts increased in control silages as the ensiling period progressed (p<0.05).

Table 3- The effect of the hazelnut husk additive on the pH, organic acids (g/kg DM), ethanol (g/kg DM) concentrations and microbial structure (cfu/g DM) of maize silages

Parameters	*Treatment*	*Ensiling days*			*SE*	*P value*		
		8	21	60		Day	Trt	Day*Trt
pH	Control	3.84a	3.79b	3.64c	0.020	*	*	*
	Hazelnut husk	4.00a	3.85b	3.78b				
Ethanol	Control	0.09	0.10	0.12	0.002	NS	NS	NS
	Hazelnut husk	0.08	0.10	0.10				
LA	Control	43.21d	50.85bc	56.33a	0.563	*	*	*
	Hazelnut husk	40.33e	48.26c	52.72b				
AA	Control	26.39a	17.73c	13.44de	0.382	*	*	*
	Hazelnut husk	23.08b	14.92d	11.63e				
PA	Control	2.04a	1.03d	1.23e	0.029	*	*	*
	Hazelnut husk	1.82b	0.94d	0.96d				
BA	Control	0.24ab	0.25ab	0.26a	0.006	*	*	*
	Hazelnut husk	0.18c	0.18c	0.22b				
LAB	Control	9.14a	8.34b	7.00c	0.104	*	*	*
	Hazelnut husk	9.07a	8.99a	7.42c				
Yeast	Control	0	0	0	0	NS	NS	NS
	Hazelnut husk	0	0	0				
Mold	Control	3.37	4.77	4.97	0.355	NS	NS	NS
	Hazelnut husk	4.51	4.25	4.15				

DM: Dry matter, LA: Lactic acid, AA: Acetic acid, PA: Propionic acid, BA: Butyric acid, SE: Standard Error, NS: Not significant, *p<0.05. cfu: colony-forming unit, LAB: Lactic acid bacteria. The differences between the averages indicated with different letters on the same row are significant (p<0.05)

Changes in pH, CO_2 production, yeast and mold number, and visual assessment of mold were evaluated as maize silage deterioration markers (Table 4). When the deterioration characteristics of maize silages were compared, hazelnut husk treated silages had the lowest ($p<0.05$) CO_2 production and visible molding, while there was no treatment effect ($p>0.05$) on mold and yeast count.

Table 4- Effect of hazelnut husk additive on the deterioration of maize silages

Parameters	*Control*	*Hazelnut Husk*	*SE*	*P value*
pH	3.64b	3.79a	0.022	*
CO_2 (g/kg DM)	3.42a	1.79b	0.651	*
LAB (cfu/g DM)	7.26b	8.51a	0.225	*
Mold+Yeast (cfu/g DM)	7.18	7.59	0.115	0.07
Visible molding**	3	2		

SE: Standard error, CO_2: Carbon dioxide, DM: Dry matter, LAB: Lactic acid bacteria, cfu: Colony-forming unit, *p<0.05.
**It is a visual assessment of the molding stage of silages with numbers ranging from 1 to 5. 1: no mold, 2: very little mold in spots, 3: speckled mold on the surface, 4: locally moldy areas, the surface partly covered with mold, and 5: a silage whose surface is totally covered with mold, has a strong odor, and the particles are stuck together. These assessments were completed by two people and then averaged. The differences between the averages indicated with different letters on the same row are significant (p<0.05)

Table 5 shows the ruminal fermentation pattern markers as pH value and *in vitro* gas production at various hours, as well as OMD, ME, and NEL values and protozoa counts in rumen fluid at 96 hours. Total gas production gradually increased in all treatment groups from 3 to 96 hours ($p<0.05$). Starting at 3 hours and continuing until 96 hours, the addition of hazelnut husk decreased ($p<0.05$) total gas production. Adding hazelnut husks reduced the amount of easily fermentable DM ("a" value) slightly ($p>0.05$, Table 5). The "b" value, which was 45.23 mL in the control silage, was reduced to 31.89 mL with the addition of husk, while the a+b value was reduced from 50.28 mL to 35.92 mL with the addition of hazelnut husk ($p<0.05$, Table 5). The addition of hazelnut husk to maize silage had no effect ($p>0.05$, Table 5) on the gas production rate constant ("c"). When compared to control silages, the addition of hazelnut husk to maize silage decreased the *in vitro* OMD, ME, and NEL while increasing the protozoa count ($p<0.05$).

Table 5- Effect of hazelnut husk additive on the *in vitro* gas production (mL) and parameters, rumen pH, organic matter digestion (OMD, %), metabolic energy (ME, MJ/kg DM) and net energy lactation levels (NEL, MJ/kg DM), protozoa populations (cfu/g) of maize silages

Parameters	*Control*	*Hazelnut husk*	*SE*	*P value*
Incubation time (hour)				
3	10.67a	7.64b	0.399	*
6	16.67a	11.17b	0.660	*
12	26.17a	15.95b	0.623	*
24	35.83a	24.29b	0.712	*
48	45.17a	31.23b	1.136	*
72	48.83a	33.79b	1.552	*
96	50.42a	35.54b	1.934	*
Gas production parameters				
a	5.04	4.03	0.631	NS
b	45.23a	31.89b	1.489	*
a+b	50.28a	35.92b	0.099	*
c	0.18	0.05	1.972	NS
pH	6.61	6.64	0.044	NS
OMD (%)	53.67a	44.24b	0.706	*
ME (MJ/kg DM)	7,95a	6.31b	0.111	*
NE_L (MJ/kg DM)	4.45a	3.24b	0,080	*
Protozoa (cfu/g)	5,42b	5.59a	0.030	*

SE: Standard error, cfu: Colony-forming unit, NS: Not significant, *$p<0.05$. a: the volume of gas formed at the beginning (mL), b: the volume of gas formed over time (mL), a+b, total gas output (mL), c: Constant gas output rate (mL/hour), The differences between the averages indicated with different letters on the same row are significant ($p<0.05$)

4. Discussion

Hazelnut husk is rich in complex carbohydrates, has a high DM content (76.98%, Table 1), and is largely composed of cellulose (Jones & Jones 1996). As a result, it may be an effective supplement to improve the DM content of the silage material. This research examined how hazelnut husk affected silage fermentation, microbial structure, aerobic stability, cell wall components, and the *in vitro* digestibility of second crop maize forage harvested at low DM (26.61%).

The chemical content of maize silage studied in this investigation was comparable to that reported by others working with low DM maize silage (Özdüven et al. 2009; Barmaki et al. 2018, Altınçekiç & Filya 2018). According to Xu et al. (2012), total carbohydrate was the most abundant component in all hazelnut shells, accounting for 93.4% to 96.7%, with crude fiber accounting for more than 85%. Protein was the second most abundant component in the shells (2.1-4.0%), followed by ash (0.8-2.0%) and oil (0.3-0.7%). Renna et al. (2020) investigated the use of hazelnut skin as a dietary source for dairy cows. They observed that hazelnut skin has high ether extract (224 g/kg DM), NDF (530 g/kg DM), and ADF (492 g/kg DM) concentrations, but low CP (62 g/kg DM) and lignin concentrations (24 g/kg DM). According to Ozcan & Kılıc (2018), hazelnut husk pellets contained 9.32% DM crude protein and 0.84% DM ether extract. In one experiment, various hazelnut hull varieties were evaluated as a source of ruminant feed (Cetinkaya &

Kuleyin 2016). In that same study, the chemical composition did not differ between varieties with the exception of crude fat, which ranged from 17% to 21%, and DM, CP, NDF, ADF, and ADL, which were 91.1, 5.9, 30.3, 48.6, and 25.4%, respectively. In our study, we discovered that the crude fat content of hazelnut outer skin is lower (0.81% of DM, Table 1) than the findings of Renna et al. (2020) and Cetinkaya & Kuleyin (2016), but similar to the findings of Xu et al. (2012) and Ozcan & Kılıc (2018). Crude protein, on the other hand, was comparable to Renna et al. (2020), but lower than Ozcan and Kılıc's (2018) findings and higher than Xu et al. (2012) and Cetinkaya & Kuleyin's (2016) findings.

The addition of dry ingredients to moist silage resulted in an increase in DM content due to the dilution effect (Khorvash et al. 2006). Higher DM content in silages may be important not only for silage quality, but also for maintaining high feed consumption in dairy cows, as moist silage has been shown to reduce feed intake (Lahr et al. 1983). Silage fermentation tends to generate volatiles, which causes the apparent DM of the silage to decline, particularly in comparison to fresh material (Porter & Murray 2001). Higher DM preservation as a result of hazelnut husk application could be attributed to the additive's increased fermentation efficiency (Woolford 1983). This also supports the finding that hazelnut husk-treated silages had the lowest ($p<0.05$) DMD, particularly on the 21st day of ensiling (Table 2). All silages had a higher CP content (7.1%) than fresh forage (6.74%), indicating that the ensiling process protects forage by allowing for controlled fermentation with less proteolysis in silages (McDonald et al. 1991). Cell wall decomposition in silages is thought to be caused by the acidic content in the silage, and hydrolysis of hemicellulose (Kung et al. 2003) can increase the availability of energy for fermentation in the silo (McDonald et al. 2001).

In the current study, using small-scale experimental silos in a laboratory setting, low pH values indicated good fermentation for maize silages with DM content as low as 26% DM, even without additive. Lactic acid bacteria use water-soluble carbohydrates (WSC) to yield LA during the ensiling process. The acidity of the silage finally rises, and the pH falls as a result (McDonald et al. 1991). Silages harvested at an early stage of maturity, on the other hand, can be difficult to achieve low pH and good fermentation on a commercial scale without the use of an additive due to their low WSC and DM content (McDonald et al. 1991, Khorvash et al. 2006). Paydaş et al. (2019) obtained similar results by adding 15% pistachio outer shell to maize silage, which reduced the ammonia nitrogen, butyric acid, propionic acid, and lactic acid concentrations.

Control silages were found to be less stable after air exposure when compared to hazelnut husk treated silages (Table 4), most likely due to the high lactic acid content and mold count (Table 3), as well as the high moisture content of control silages (Table 2). When the forage is ensiled below 30% DM, an additive may be required to prevent the growth of clostridia. If the DM content is low, effluent will be produced. The effluent from silage production can become highly harmful as it is a perfect medium for microorganisms, but it also represents a loss of energy and nutrients (Woolford 1983). When the silages were exposed to air, the number of molds increased in comparison to the number of mold + yeast in the silages that were not exposed to air (Table 3). Sugar and lactic acid consumption by molds raise the pH and accelerate the process of aerobic instability, resulting in high DM losses during the feed out phase (McDonald et al. 1991).

Since we found no previous research on the use of hazelnut husks as a silage additive and how it affects the nutritive value of silage, we used straws, hay, and the hulls of some plants with similar nutritive values to hazelnut husk to compare the study findings. Notable change in gas volume were observed between treatments, as seen in Table 5. The reduction in gas production observed in hazelnut husk treated silages in the current study was similar to that observed in Babaeinasab et al. (2015), who used a potato and wheat straw mixture in maize silage, and Denek et al. (2017), who used pistachio byproduct in maize silage and revealed that additives that increased silage DM content decreased *in vitro* gas production value. Niderkorn et al. (2020) found that including hazelnut pericarps (Corylus avellana L.), which are high in procyanidin type condensed tannins, in a basal diet reduced total gas production. Although the tannin content of hazelnut husk and its effect on rumen microbiota were not examined in this study, Xu et al. (2012) investigated the tannin content of hazelnut shell from different cultivars and harvest years and discovered that condensed tannins ranged from 3.7 to 8.3 (% leucocyanidin equivalent), and total tannin content ranged from 3.6 to 7.9 (mg TAE g^{-1}). Because condensed tannin has been suggested to have biological functions on rumen fermentation, a decrease in gas production from diets supplemented with condensed tannin extract was predicted due to its impact on rumen microbes (Bueno et al. 2020). The current study found that adding hazelnut husks reduced the "a" value slightly ($p>0.05$, Table 5) which was expected due to high fibrous fractions of hazelnut husk (Table 1). The "b" value, which indicates the gas volume produced by the silage's slow fermenting fraction, and the "a+b" values, which represent total gas production and describe the gas volume that varies with time, were both dramatically reduced in hazelnut husk-supplemented silages ($p<0.05$, Table 5). By adding bay leaf and molasses, Atalay (2009) obtained comparable results with alfalfa silage. The tannin in the bay leaf, according to the author, has a negative effect on rumen microbes and causes the production of indigestible compounds with tannin

during the ensiling and fermentation processes. The "c" constant (0.049 mL/hour, Table 5) values of the silages obtained in this investigation were also determined by Babaeinasab et al. (2015), Sariçiçek and Kiliç (2009), and Salem et al. (2013). However, the results of Babaeinasab et al. (2015) potato pulp and straw added to maize silage and Sariçiçek and Kiliç (2009) straw added to maize silage were found to be higher than the results of this study. In the current study, the addition of hazelnut husk reduced the organic matter digestion of silages by 17.6% when compared to the control silage ($p<0.05$). This is due to higher NDF, ADF, and ADL contents in hazelnut husk supplemented silages ($p<0.05$, Table 2) than in control silages, while crude protein content was lower ($p<0.05$, Table 2), resulting in a decrease in *in vitro* gas production value and organic matter digestion. According to Rezaei et al. (2014), while supplying more crude protein to rumen bacteria enhances gas production, the provision of extra cell wall components reduces gas production. Furthermore, gas production is directly related to the amount of carbohydrates available to rumen bacteria (Blummel & Orskov 1993). Similar to our results, chestnut tannin reduced the organic matter digestibility of alfalfa silage by 5.1% (Tabacco et al. 2006). The current study found that the reduction in ME and OMD content in hazelnut husk treated silages was similar to the Paydaş et al. (2019) study, which revealed that the *in vitro* OMD and ME of maize silage prepared with 15% pistachio outer shell (52.63% and 7.96 MJ/kg DM, respectively) were lower than the control silages (54.07% and 8.17 MJ/kg DM, respectively). In another study, adding lucerna hay to low DM maize silage reduced apparent nutrient digestibilities (Barmaki et al. 2018). Similar results were also obtained in other studies by Atalay (2009), Babaeinasab et al. (2015), and Denek et al. (2017). While some absorbent additives reduce silage effluent production, they may also reduce the nutritious content of the silage (Khorvash et al. 2006). The addition of different absorbent additions, such as milled barley (5-15%) and powdered whey (5-15%), to low DM maize silage, however, increased its nutritional value (Khorvash et al. 2006). In the current experiment, the presence of rumen protozoa in control silages was determined to be 5.42 cfu/g (Table 5). This value was discovered to be compatible with Benchaar et al. (2007) findings. However, it was found to be greater than the reports of Chahaardoli et al. (2018), but Zhang et al. (2016) were found to be lower than the report. Protozoa account for more than half of the rumen microbes in terms of biomass. They have a numerical value of 10^5 to 10^7 per milliliter of rumen fluid (Williams & Coleman 1997). Protozoa consume starch in the rumen and inhibit rumen bacteria from using it excessively, i.e., converting it to volatile FAs. Furthermore, certain protozoa consume cellulolytic bacteria (Galindo et al. 2016). Protozoa are known to coexist with 9-25% of ruminal methanogens (McAllister & Newbold 2008). Higher protozoa levels in hazelnut supplemented silages were associated with lower silage ME, NEL, and OMD levels ($p<0.05$, Table 5). These findings could support the theory that an increase in the number of protozoa in the rumen can reduce cellulolytic bacteria, affect rumen pH stabilization, increase free ammonia levels and methanogenesis, and reduce the digestive efficiency of various diets, particularly those high in fiber content (Makkar 2005). However, definite conclusions are difficult to draw because various factors influence silage digestibility, energy content, and the number of microbial community. The state of various microorganisms in the rumen, as well as how ammonia and volatile FAs are regulated in the rumen, should be evaluated.

5. Conclusions

According to the results of the current study, when hazelnut husk was added at a rate of 15% to the second-crop maize plant, which was harvested at 26.61% DM, the DM was increased to 33%, which is the ideal level for silage fermentation. Hazelnut husk demonstrated a potential hygroscopic property for maize silage that increased silage DM. Simultaneously, the addition of hazelnut husk reduced silage DM losses, propionic and butyric acid, and mold-yeast activity, while also improving aerobic stability. Hazelnut husk enhanced the cell wall components of maize silage due to its fibrous structure. The hazelnut husk addition increased silage fermentation characteristics and provided the desired attributes in quality silage. As a result of its chemical features, hazelnut husk may represent a new silage addition capable of raising the low DM of maize used as a second crop to the optimal DM ratio. However, the addition of hazelnut husk reduced the ME, NEL, and OMD levels of maize silage, which are indicators of silage nutritional value. Future research in large-scale silos under field conditions will be beneficial in determining how hazelnut husk affects silage quality. Furthermore, animal feeding studies would be desirable in demonstrating the benefits of the hazelnut husk additive on silage quality.

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