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Influence of harvesting periods and additives on the quality of silage from common reed (*Phragmites australis*) and cattail (*Typha* spp.)

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ABSTRACT. The present study determined how additives and harvesting periods affect the quality of silage from common reed (*Phragmites australis*) and cattail (*Typha* spp.). Both species were harvested separately and together before and after flowering (June and August). Shredded plants (1–3 cm) were ensiled in four groups: one control and three experimental groups (addition of 5% molasses, 5% crushed barley, or 0.5% formic acid). For each harvest period and treatment, 11 jars were filled and incubated for 3 months. The silages were examined for their physical and chemical characteristics, volatile fatty acid and mycotoxin contents, gas production, and organic matter digestibility. The interaction between harvest period, species, and treatment significantly affected ($P \le 0.007$) crude protein content, Flieg score, NH_3-N/Total N, and *in vitro* gas production in silages. Specifically, the August harvest combined with molasses treatment significantly reduced acid detergent fibre content in silages ($P \le 0.019$). Cattail silage had significantly lower contents of crude fibre ($P < 0.001$), acid detergent fibre ($P \le 0.006$), and neutral detergent fibre ($P < 0.001$) compared to common reed silage. The addition of 5% molasses to common reed and cattail silages significantly increased lactic acid levels ($P < 0.001$). The effect of harvest period on gas and methane production proved to be insignificant regardless of the treatment-species interaction. In conclusion, treating *Typha* harvested in June with 5% molasses can be recommended as the most optimal wetland plant silage. Furthermore, the addition of 5% molasses positively affected the nutrient content and Flieg score for silages from both species harvested in August.

Introduction

Global warming has caused irrigation problems for forage crops, forcing the use of locally adapted forage plants such as common reed (*Phragmites australis*) and cattail (*Typha* spp.) in Turkey. Common reed contains 17% crude protein (CP) and 50% total digestible nutrients and can be harvested up to three times a year in Japan, yielding 1 kg/m^2 dry matter (Asano et al., 2014). These aquatic and semi-aquatic plants do not exert negative effects on animal nutrition, although their chemical composition varies depending on harvest time, region, and climate. Musa and Gaba (2022) have reported that cattail is a valuable roughage for ruminants during feed shortages, but increased lignification during the dry season reduces CP and metabolizable energy (ME) content, making silage a practical solution to preserve nutrients. In addition, John et al. (2022) reported that replacing sorghum straw with *Typha domingensis* silage in beef cattle diets exerted no adverse effects.

Asano et al. (2018) found that the dry matter content of *Phragmites* spp. harvested in Japan was 19.8% in May, 23.6% in June, and 25.1% in July, with May silage having a significantly lower pH (5.71). The genera *Typha* and *Phragmites* show substantial roughage potential, with their dry matter primarily consisting of structural carbohydrates. As these plants mature, their dry matter content increases and soluble carbohydrate levels decrease, which improves fermentation in the ensiling material (Silva et al., 2017). Lactic acid bacteria rapidly convert sugars into organic acids, thereby lowering the pH and preserving the forage (Wakano et al., 2019). However, highly lignified plants like cattail contain low levels of soluble carbohydrates and may require additives to obtain effective silage quality. Crushed barley and molasses are both sources of soluble carbohydrates, but they affect fermentation processes differently. It was reported that the addition of 5% barley to tomato grass silage significantly increased lactic acid levels compared to the supplementation of 5% molasses (Tekin and Kara, 2020).

Limited information is available regarding additives for ensiling aquatic plants. A recent study by de Evan et al. (2023) found that the addition of urea, molasses, and formic acid to *Typha latifolia* silage increased volatile fatty acid and NH_3-N concentrations, leading to unfavourable fermentation. Conversely, Doležal (2015) observed that formic acid, when used as a silage additive, resulted in reduced dry matter loss in maize and sugar beet pulp silage. In addition, Yuan et al. (2017) noted that formic acid significantly decreased the pH value, levels of butyric acid, ethanol, and NH_3-N, as well as the abundance of undesirable microorganisms in alfalfa silage. These findings suggested that formic acid improved silage quality and stability by creating a more favourable fermentation environment.

The objective of this study was to explore the potential of common reed (*Phragmites australis*) and cattail (*Typha* spp.) as alternative feed sources in Türkiye's arid lake region, where these plants are abundant. Biomass suitable for forage was acquired in Gölhisar, Türkiye, and the effects of different harvest dates (June and August) and silage additives (molasses, crushed barley, or formic acid) on silage quality were evaluated. Mycotoxin analysis was also conducted to confirm the quality and safety of the silages.

Material and methods

Plant materials, chemical analysis, and biomass determination

Common reed (*Phragmites australis*) and cattail (*Typha* spp.) were collected from Lake Gölhisar, Burdur, Türkiye, with *Typha angustifolia* and *Typha domingensis* being the predominant species. Biomass measurements were conducted using the quadrate technique (1×1 m) (Wetzel and Likens, 2001) in June and August in consecutive years. The harvested plants were weighed in the field to determine fresh weight (g/m^2), then dried for two weeks at room temperature, followed by one night drying at 65 °C to determine dry weight (g/m^2). During these harvest periods, nutrient analyses of various plant parts (upper and lower parts of stem, leaves and flowers, if available) were carried out according to AOAC International (2000), with crude fibre analysis using the method described by Crampton and Maynard (1938).

Treatments and silage procedure

The silages were prepared by harvesting common reed and cattail before and after the flowering stage. The plants were cut into 2–3 cm pieces, transferred into 1-l jars, and divided into four groups: a control group and three groups treated with additives (0.5% formic acid, 5% crushed barley, or 5% molasses). For each treatment and harvesting period (June and August), 1-l jars were tightly filled with 750–850 g of the sample. A total of 264 jars were used in the experiment, with harvesting, transporting, shredding, and filling completed in approximately 8 h. The jars were weighed before and after filling, and subsequently sealed and left to ferment in the laboratory for 3 months.

Physical and chemical analysis of silages

Physical attributes like colour, texture, and odour were assessed using the DLG silage evaluation key. The pH was measured using an Hi917hN pH meter (Hanna Instruments Inc., Woodschet RI, USA) after mixing 25 g of sample with 100 ml of distilled water for 20 min. The Flieg score was calculated using the following formula (Dong et al., 2017):

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

Metabolizable energy levels were calculated using three formulas (Güngör et al., 2008):

$$3309.5 - 35.64 \times CF\% \text{ (kcal / kg DM)};$$

$$239 \times (14.70 - 0.150 \times ADF\%) \text{ (kcal / kg DM)};$$

$$3464.7 - 58.10 \times ADF\% + 27.99 \times CF\% \text{ (MJ / kg DM)};$$

where: DM – dry matter, CF – crude fibre, ADF – acid detergent fibre.

The NH_3-N/Total N ratio was determined using a VAP001217 Kjeldahl distillation device (C. Gerhardt GmbH & Co., Königswinter, Germany), with CP and ammonia analysed by the Kjeldahl method (Filya et al., 2006). Nutrient analyses were performed according to AOAC International (2000), and crude fibre (CF), acid detergent fibre (ADF), and neutral detergent fibre (NDF) analyses were performed following the methods of Crampton and Maynard (1938) and Goering and Van Soest (1970), respectively.

Analysis of lactic acid and volatile fatty acids

Each sample (50 g) was homogenised in 450 ml of distilled water, filtered through gauze, and a 5 ml aliquot was acidified with 12 N H_2SO_4. The sample was then centrifuged at 26 000 × g, 4 °C, for 30 min to prepare it for HPLC injection using an Agilent 7697A HT12440002 Headspace sampler (Agilent, Santa Clara, CA, USA) (Tjardes et al., 2000). HPLC validation involved injecting standards of lactic, acetic, propionic, and butyric acids, both individually and in combination, using a 1 ml/min ODC-4 column (250 mm × 4.6 mm, 5 µm) with a mobile phase adjusted to pH 3 using orthophosphoric acid (Aktaş et al., 2005).

In vitro gas production technique

The research was approved by the Kayseri Erciyes University Experimental Ethics Committee (Approval No: 2014-9-14/134).

Rumen fluid was collected from 3 cows fed a diet comprising 60% roughage and 40% concentrate, filtered through gauze, and maintained at 39 °C under CO_2. Plant samples were incubated with rumen fluid and buffer in glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschie, Germany) (Menke and Steingass, 1988). Each syringe contained three 200 mg dry samples and 30 ml of rumen fluid-buffer mixture, heated in a water bath at 39 °C. Gas production was measured using three blank syringes (rumen fluid + buffer mixture, no sample).

Determination of total gas and methane production

After 24 h of incubation, the total gas volume was measured using a syringe scale. A methane analyser (Sensors Europe GmbH, Germany) was connected to the syringe outlet, and the accumulated gas was introduced by pressing the piston. The analyser displayed methane concentration as a percentage of the total gas volume, which was used to calculate methane production (Kara, 2015; Kara et al., 2015). The formula used to determine methane production was as follows:

$$\text{production of methane (ml/0.2 g DM)} = \text{total gas production (ml/0.2 g DM)} \times \text{methane volume (\%)}.$$

Determination of metabolizable energy and organic matter digestibility

The metabolizable energy (ME) and organic matter digestibility (OMD) contents in the silages were calculated using the following equations (Menke and Steingass, 1988):

$$ME \text{ (MJ / kg DM)} = 2.20 + 0.136 \times GP + 0.057 \times CP;$$

$$OMD \text{ (g / kg DM)} = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times CA;$$

where: DM – dry matter; GP – 24 h total gas production (ml/200 mg), CP – crude protein (g/kg DM), CA – crude ash (g/kg DM).

Mycotoxinanalysis

Analyses of total aflatoxin (AF), deoxynivalenol (DON) and zeralenone (ZEN) levels in silage samples were performed using appropriate commercial kits (Helica Biosystems, Inc., California, USA, Product Codes: 981AFL01LM, 941DON01M, 951ZEA01HS) based on ELISA methodology (Biotek Instruments, Inc., Vermont, USA, Model No: ELX - 800).

Statistical analysis

Statistical analysis was performed using Minitab 16.1 (Minitab Inc., USA). Descriptive statistics were computed, and a model (Yijklm = µ + ai + bj + cijklm) was employed to evaluate factors affecting silage quality, where µ is the overall mean, ai is the species effect, bj is the treatment effect, and cijklm is the harvesting period effect. A general linear model and analysis of variance (GLM, ANOVA) were applied, with significant factors further examined using the Tukey test.

Results and discussion

The biomass measurements (g/m^2) of *Typha* spp. and *Phragmites australis* at different periods are shown in Table 1. The biomass of *Phragmites australis* was more than 2 times higher compared to the total dry biomass of *Typha* spp. Both plants showed an approximate 5% increase in DM during the same period for two consecutive years. This could be due to the high adaptability of *Phragmites australis*, an invasive species, to harsh conditions worsened by climate change, as well as its rapid spread (Fouad et al., 2023).

While the effect of period on CP, EE, CA and CF was negligible, the interaction between period and treatments was significant ($P \leq 0.001$), probably due to the impact of the growth period on nutritional value. The combined effect of period, species and treatment was significant ($P \leq 0.005$) for the CP content. Period and treatment significantly affected the ADF and NDF ($P \leq 0.019$) values, while period and species significantly altered only the ADF content ($P \leq 0.025$). In contrast, the interaction between species and treatment did not significantly influence the analysed parameters, highlighting the dominant role of the growth period on ADF.

Table 1. Biomass values of *Phragmites australis* and *Typha spp*. at different periods, g/m^2

	Typha spp.			*Phragmites australis*		
	Fresh weight	Dry weight	DM, %	Fresh weight	Dry weight	DM, %
1st year June	ND	1005.33	ND	ND	2280.00	ND
1st year August	7363.11	1832.00	24.88	7317.77	3286.22	44.90
2nd year June	10495.56	2340.00	22,29	12727.11	4908.00	38.56
2nd year August	7874.22	2332.00	29.61	11976.89	5981.33	49.94
Average	8577.63	2051.73	23.91	10673.93	4556.67	42.68

DM – dry matter; ND – not detected

Table 2 presents *Typha* spp. and *Phragmites australis* nutrient analyses for upper and lower parts of stem, and leaves for June and August. Both species flowered in August. Fresh cattail in August had better nutrient content (CP: 7.27, CF: 36.11%) in terms of animal nutrition compared to June. This could be due to the contribution of the flower to the CP of the whole plant. The CP and CF contents in *Phragmites australis* shoots were 14.93 and 19.27%, respectively (Wang et al., 2022). However, these values excluded root and stem parts. The soluble carbohydrate content for both plants ranged from 0.11 to 2.89% during the periods examined.

Table 3 presnts the effects of silage treatments on DM, crude ash (CA), CP, ether extract (EE), CF, ADF, and NDF contents according to plant species and harvest period on a DM basis. Period, species, and treatment interaction significantly affected DM (65°C) ($P = 0.001$), likely due to differences in plant development stage and leaf proportions. The importance of the species and treatment interaction at 105°C DM may be attributed to the properties of the additives used. The significance of the period and species interaction in terms of CA may also be linked to the developmental stage of the plants ($P < 0.001$). Species and treatment had a significant effect on the CP, EE, and CF values ($P \leq 0.039$). Additionally, the interaction between period and species was significant for all nutritional values ($P \leq 0.001$).

Table 2. Nutrient contents of *Phragmites australis* and *Typha* spp. stem during the June and August harvest periods, DM basis, %

	DM	CA	CP	EE	CF	WSC
June period						
Phragmites australis						
upper part of stem	95.46	7.94	6.03	1.83	44.92	34.74
lower part of stem	94.73	6.40	3.40	2.00	43.48	39.45
leaves	93.75	11.81	19.89	2.21	27.69	32.15
whole	93.04	7.63	10.8	1.99	40.76	32.38
Typha spp.						
upper part of stem	93.60	6.27	ND	3.05	41.19	ND
lower part of stem	92.88	10.56	ND	1.53	44.19	ND
leaves	92.88	10.41	ND	3.25	40.47	ND
whole	93.99	6.34	6.62	2.61	40.14	38.28
August period						
Phragmites australis						
upper part of stem	94.92	10.95	2.76	4.05	44.40	32.76
lower part of stem	94.61	5.72	2.08	2.00	45.87	38.94
flower	94.46	7.83	10.55	5.33	30.08	40.67
leaves	93.66	16.04	13.15	4.09	29.26	31.12
whole	94.17	12.02	6.28	3.15	37.45	35.27
Typha spp.						
upper part of stem	92.96	7.09	9.86	4.86	35.01	36.14
lower part of stem	92.95	9.11	5.06	3.77	37.53	37.48
flower	93.92	5.79	10.46	11.49	27.61	38.57
leaves	92.67	7.59	ND	5.51	27.25	ND
whole	92.45	5.38	7.27	5.30	36.11	38.39

DM – dry matter; CA – crude ash; CP – crude protein; EE – ether extract; CF – crude fibre; WSC – water soluble carbohydrates; ND – not detected

Table 3. Effect of silage treatments on chemical composition, acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents by plant species and harvest period, dry matter, %

	DM (65 °C)	DM (105 °C)	CA	CP	EE	CF	ADF	NDF
Period								
June	26.24 ± 0.31^{b}	94.32 ± 0.10	9.65 ± 0.17	8.94 ± 0.28^{a}	3.69 ± 0.16	41.94 ± 0.40	51.12 ± 0.63^{a}	78.36 ± 0.72^{b}
August	36.72 ± 0.31^{a}	94.55 ± 0.10	9.62 ± 0.17	7.73 ± 0.28^{b}	3.90 ± 0.16	41.15 ± 0.40	48.47 ± 0.63^{b}	80.66 ± 0.72^{a}
P-value	<0.001	0.132	0.902	0.003	0.384	0.172	0.004	0.028
Species								
Phragmites australis	41.89 ± 0.38^{a}	94.80 ± 0.12^{a}	9.59 ± 0.21^{b}	7.65 ± 0.34^{b}	3.24 ± 0.20^{b}	47.28 ± 0.49^{a}	51.88 ± 0.78^{a}	82.84 ± 0.88^{a}
Typha spp.	23.02 ± 0.38^{c}	93.79 ± 0.12^{b}	8.52 ± 0.21^{c}	9.01 ± 0.34^{a}	4.62 ± 0.20^{a}	35.12 ± 0.49^{c}	48.42 ± 0.78^{b}	75.44 ± 0.88^{b}
Mix	29.53 ± 0.37^{b}	94.72 ± 0.12^{a}	10.79 ± 0.21^{a}	8.34 ± 0.34^{ab}	3.52 ± 0.20^{b}	42.24 ± 0.49^{b}	49.09 ± 0.78^{b}	80.25 ± 0.88^{a}
P-value	<0.001	<0.001	<0.001	0.025	<0.001	<0.001	0.006	<0.001
Treatment								
Control	29.29 ± 0.44^{b}	94.20 ± 0.14^{b}	9.23 ± 0.24	6.77 ± 0.39^{b}	3.30 ± 0.23^{b}	43.91 ± 0.56^{a}	54.45 ± 0.90^{a}	82.90 ± 1.02^{a}
5% molasses	32.38 ± 0.44^{a}	94.20 ± 0.14^{b}	9.97 ± 0.24	9.20 ± 0.39^{a}	4.36 ± 0.23^{a}	39.90 ± 0.56^{c}	47.06 ± 0.90^{b}	75.18 ± 1.02^{c}
5% barley crush	32.69 ± 0.43^{a}	94.77 ± 0.14^{a}	9.57 ± 0.24	8.61 ± 0.39^{a}	3.89 ± 0.23^{ab}	40.32 ± 0.56^{bc}	48.94 ± 0.90^{b}	78.16 ± 1.02^{bc}
0.5% formic acid	32.56 ± 0.43^{a}	94.56 ± 0.14^{ab}	9.76 ± 0.24	8.76 ± 0.39^{a}	3.61 ± 0.23^{ab}	42.05 ± 0.56^{ab}	48.73 ± 0.90^{b}	81.80 ± 1.02^{ab}
P-value	<0.001	0.018	0.179	<0.001	0.019	<0.001	<0.001	<0.001
P-value period*species	0.001***	0.283	0.001***	0.001***	0.001***	0.001***	0.025*	0.116
period*treatment	0.001***	0.471	0.307	0.001***	0.039*	0.001***	0.019*	0.001***
species*treatment	0.001***	0.012*	0.781	0.001***	0.278	0.077	0.514	0.337
period*species*treatment	0.001***	0.345	0.632	0.005**	0.694	0.374	0.338	0.180

DM – dry matter, CA – crude ash, CP – crude protein, EE – ether extract, CF – crude fibre. Data are presented as mean values ± SEM. SEM – standard error of the mean. ^{abc} – means within a column with different superscripts are significantly different at $P < 0.05$; ; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

The observed increase in % DM (65°C) as the harvest period progressed was similar to the findings in a study on maize (Yıldız et al., 2011). Research on wetland plants is limited, and interactions between period, treatment, and species have not been extensively explored. Papathanasiou et al. (2021) reported that *Phragmites australis* from Lake Mikri Prespa, Greece had DM and NDF contents of 67.08 and 46.3%, respectively, with CP contents of 8.38% in early August and 5.06% in late October. These findings on the effects of the harvest period on CP content are comparable to our results. Saeed et al. (2019) reported that treating *Phragmites* (2–3 cm) with 1.5% formic acid increased silage DM by 1.15% and CP by 2.11%, while reducing EE by 0.63%. Similarly, in this study, treatment with 0.5% formic acid significantly increased DM and CP contents ($P < 0.01$), while EE content remained unaffected. Recently, a study concerning wetland plant feed value has been published; however, it compared nutritional parameters of green and silage. Rufai and Trinidad (2019) found that *Typha* in both its green and silage forms had higher CA content and lower CF and NDF contents compared to sorghum straw, with the acid detergent fibre (ADF) content of *Typha* silage comparable to that of sorghum straw. Additionally, June silages with a CP proportion of 8.94% are consistent with previous results concerning maize harvested at the dough stage (Yıldız et al., 2011). Beyzi et al. (2023) reported ADF and NDF contents of untreated *Phragmites* silage at 43.20 and 65.45%, respectively. The findings of this study are consistent with those of Beyzi et al. (2023) regarding CA, but slightly higher in terms of ADF and NDF contents, potentially due to variations in the harvest period.

The effects of silage applications on the Flieg score, physical properties of silages, silage pH and selected silage acids by plant species and harvest period are shown in Table 4. While the interaction between period, species and treatment had a significant effect on the Flieg score ($P \leq 0.007$), the interaction between species and treatment did not show a significant effect. However, the interaction between species and treatment was significant for physical properties ($P \leq 0.007$), indicating the importance of the harvest period for different species. Additionally, the interaction between period and species was significant ($P \leq 0.001$) regarding the pH value. The interaction between period and treatment also significantly affected lactic acid ($P \leq 0.001$) and butyric acid levels ($P \leq 0.002$). Specifically, the 5% molasses treatment in June significantly increased lactic acid concentration in silage in all plant species compared to the other treatment groups and the control ($P < 0.001$).

Table 4. Effect of silage treatments on the Flieg score, physical properties of silages, pH and selected silage acids by plant species and harvest period, dry matter, %

	Flieg score	Physical properties	pH	Butyric acid	Propionic acid	Acetic acid	Lactic acid
Period							
June	68.76 ± 2.50[b]	17.96 ± 0.21[a]	4.71 ± 0.04	0.85 ± 0.11[a]	0.02 ± 0.01[b]	1.06 ± 0.14[a]	0.96 ± 0.21
August	86.95 ± 2.50[a]	17.31 ± 0.21[b]	4.81 ± 0.04	0.49 ± 0.11[b]	0.08 ± 0.01[a]	0.45 ± 0.14[b]	1.05 ± 0.21
P-value	<0.001	0.030	0.122	0.025	0.006	0.004	0.764
Species							
Phragmites australis	105.27 ± 3.09[a]	17.77 ± 0.26	4.63 ± 0.05[b]	0.53 ± 0.13	0.04 ± 0.01	0.32 ± 0.17[b]	1.36 ± 0.26
Typha spp.	61.43 ± 3.06[b]	17.60 ± 0.25	4.74 ± 0.05[b]	0.92 ± 0.13	0.07 ± 0.01	0.91 ± 0.17[ab]	0.95 ± 0.26
Mix	66.86 ± 3.04[b]	17.55 ± 0.25	4.93 ± 0.05[a]	0.56 ± 0.13	0.03 ± 0.01	1.02 ± 0.17[a]	0.70 ± 0.26
P-value	<0.001	0.805	0.001	0.090	0.184	0.014	0.203
Treatment							
Control	65.16 ± 3.57[c]	17.40 ± 0.30	5.02 ± 0.06[a]	0.65 ± 0.15[ab]	0.09 ± 0.02	1.25 ± 0.20	0.66 ± 0.30[b]
5% molasses	96.58 ± 3.57[a]	17.66 ± 0.30	4.33 ± 0.06[c]	0.39 ± 0.15[b]	0.04 ± 0.02	0.53 ± 0.20	2.55 ± 0.30[a]
5% barley crush	70.25 ± 3.51[bc]	17.44 ± 0.29	4.33 ± 0.06[c]	1.02 ± 0.15	0.03 ± 0.02	0.60 ± 0.20	0.55 ± 0.30[b]
0.5% formic acid	79.43 ± 3.51[b]	18.06 ± 0.29	4.71 ± 0.06[b]	0.61 ± 0.15[ab]	0.04 ± 0.02	0.63 ± 0.20	0.26 ± 0.30[b]
P-value	<0.001	0.367	<0.001	0.050	0.200	0.052	<0.001
P-value period*species	0.001 ***	0.007 **	0.001 ***	0.257	0.058	0.400	0.125
period*treatment	0.010 *	0.603	0.064	0.002 **	0.200	0.641	0.001***
species*treatment	0.182	0.062	0.689	0.063	0.086	0.684	0.269
period*species*treatment	0.007 **	0.074	0.196	0.115	0.387	0.344	0.577

data are presented as mean values ± SEM. SEM – standard error of the mean; [abc] – means within a column with different superscripts are significantly different at $P < 0.05$; ; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 5. Effect of silage treatments on metabolizable energy (ME) values and NH_3-N/Total N ratio by plant species and harvest period, dry matter, %

	ME - 1, kcal/kg DM	ME - 2, kcal/kg DM	ME - 3, kcal/kg DM	NH_3-N / Total N (%)
Period				
June	1899 ± 13.11	1782 ± 0.08^{b}	1770 ± 26.29^{b}	40.26 ± 2.39^{a}
August	1915 ± 13.31	1880 ± 0.08^{a}	1916 ± 26.69^{a}	13.94 ± 2.39^{b}
P-value	0.390	0.001	<0.001	<0.001
Species				
Phragmites australis	1712 ± 16.06^{C}	1748 ± 0.10^{b}	1862 ± 32.19^{a}	23.49 ± 2.93
Typha spp.	2136 ± 16.06^{a}	1882 ± 0.10^{a}	1748 ± 32.19^{b}	29.97 ± 2.93
Mix	1873 ± 16.42^{b}	1863 ± 0.10^{a}	1920 ± 32.93^{a}	27.84 ± 2.93
P-value	<0.001	<0.000	0.001	0.288
Treatment				
Control	1834 ± 18.54^{C}	1672 ± 0.11^{b}	1642 ± 37.17^{B}	47.86 ± 3.38^{a}
5% molasses	1969 ± 18.54^{a}	1920 ± 0.11^{a}	1941 ± 37.17^{a}	12.43 ± 3.38^{C}
5% barley crush	1934 ± 19.10^{ab}	1873 ± 0.12^{a}	1889 ± 38.30^{a}	26.50 ± 3.38^{B}
0.5% formic acid	1892 ± 8.54^{bc}	1858 ± 0.11^{a}	1901 ± 7.17^{a}	21.61 ± 3.38^{bc}
P-value	<0.001	<0.001	<0.001	<0.001
period*species	0.001 ***	0.032 *	0.002 **	0.001 ***
period*treatment	0.001 ***	0.012 *	0.008 **	0.001 ***
species*treatment	0.046 *	0.632	0.608	0.001 ***
period*species*treatment	0.278	0.158	0.416	0.001 ***

DM – dry matter; ME – 1, kcal/kg DM – 3309.50 – 35.64 × CF; ME – 2, kcal/kg DM = 238.85 × (14.70 – 0.15 x ADF); ME – 3, kcal/kg DM = 3464.70 – 58.10 × ADF + 27.99 × CF. CF – crude fibre, ADF – acid detergent fibre. Data are presented as mean values ± SEM. SEM – standard error of the mean; [abc] – means within a column with different superscripts are significantly different at $P < 0.05$; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Similarly to the present study, Beyzi et al. (2023) reported that the Flieg score of untreated *Phragmites* silage was very good, reaching a value of 95.55; however, they did not assess its interaction with the harvest period. In this study, the interaction between the harvest period and DM was found to be significant, suggesting that the period's effect on the Flieg score may be due to the difference in DM. Saaed et al. (2019) observed that the colour of *Phragmites* silage containing 1.5% formic acid changed from dark green to yellowishbrown, its odour resembled diluted fruit vinegar, and its pH decreased to 4.88. Nevertheless, the total DLG scores were not reported, making it impossible to compare results in this regard. In the present study, the pH of untreated silage was higher compared to maize silage reported by Yıldız et al. (2011), likely due to the higher total content of easily soluble carbohydrates in the plants. Asano et al. (2018) reported that glucosetreated common reed silage harvested in June and July had significantly higher lactic acid and lower propionic and butyric acid levels compared to silage harvested in May. The observed interactions between period and treatment in the current study align with the latter findings. The higher DM content in August may have contributed to increased propionic acid levels ($P \leq 0.006$) compared to June. This could also explain the lower levels of acetic acid observed in August ($P \leq 0.004$).

The effects of treatments on the ME values and NH_3-N/Total N ratio according to plant species and harvest period are shown in Table 5. Significant interactions were observed between harvest period and plant species, as well as between harvest period and treatment, for all ME calculations ($P \leq 0.032$). This could be due to the fact that the ADF content in silages was significantly affected by the period. Previous limited studies reporting ME values for *Typha* (John et al., 2022) and *Phragmites* (Beyzi et al., 2023) primarily compared the green plant material with the silage form, using different equations and without considering interactions between harvest period and treatment. In the current study, the effect of period, species and treatment on the NH_3-N/Total N ratio in silage were highly significant ($P \leq 0.001$). Interestingly, all treatments and the August period led to a reduction in the NH_3-N/Total N ratio in the silages. These findings are consistent with Saaed et al. (2019), who observed similar trends when increasing levels of formic acid were added to *Phragmites* silage. The results indicate that all the treatments effectively supported the silage production process. Although DM levels in August were higher than in June, it did not have a detrimental impact on silage quality. However, Asano et al. (2018) noted that the NH_3-N ratio of *Phragmites* harvested and ensiled without additives in Japan in July was significantly lower than in May. This variation could be attributed to the influence of different climatic conditions on plant growth in various regions.

The effects of silage treatments on total gas and methane production, ME content and OMD as a function of plant species and harvest period are summarised in Table 6. The combined effects of period, species, and treatment did not significantly influenced both gas and methane production, although period alone was affected. Species alone exerted an effect on the methane ratio; species, treatment, and their interactions significantly influenced energy values and organic matter digestibility, while period had no effect. The study found that the effect of interactions between period and species and period and treatment, determined by the gas production technique, on the ME value was similar with the results obtained using three other methods for ME assessment. In the study, 24 h pH values, as well as the interactions between period-species and period-treatment affected OMD in the silages. This may be due to the variation in plant leaf ratio and DM content. Research on these specific parameters is relatively scarce.

Table 6. Effects of silage treatments on total gas, methane, metabolizable energy (ME) and organic matter digestibility (OMD) by plant species and harvest period

Period	Species	Treatment	Gas, ml/0.2 g DM	Methane, %	Methane, ml/0.2 g DM	ME, MJ/kg DM	OMD, %	pH, 24 h on incubation
June	*Phragmites australis*	Control	34.26	14.26	4.87	7.28	49.26	7.01
		5% molasses	24.39	14.70	3.53	6.14	42.02	6.98
		5% barley crush	19.98	14.53	2.89	5.42	37.21	6.96
		0.5% formic acid	11.78	14.10	1.66	4.37	30.46	7.01
		Total	23.59	14.42	3.38	5.93	40.58	6.99
	Typha spp.	Control	37.76	14.46	5.59	7.70	51.93	6.98
		5% molasses	31.43	13.70	4.30	6.99	47.51	6.92
		5% barley crush	20.86	14.26	2.94	5.51	37.69	6.99
		0.5% formic acid	17.71	14.62	2.61	5.10	35.09	7.06
		Total	26.23	14.29	3.76	6.23	42.44	6.99
	Mix	Control	23.31	15.66	3.73	5.75	39.30	6.99
		5% molasses	30.39	13.70	4.17	6.91	47.21	6.97
		5% barley crush	16.56	16.50	2.73	4.93	34.00	7.03
		0.5% formic acid	13.10	15.83	2.08	4.44	30.78	7.01
		Total	20.84	15.42	3.17	5.51	37.82	7.00
August	*Phragmites australis*	Control	23.66	19.40	4.56	5.68	38.55	7.02
		5% molasses	14.05	16.10	2.25	4.43	30.50	7.02
		5% barley crush	26.93	18.50	4.99	6.24	42.45	7.01
		0.5% formic acid	37.85	18.50	7.00	7.67	51.75	6.98
		Total	25.62	18.12	4.70	6.00	40.81	7.01
	Typha spp	Control	15.81	14.93	2.34	4.81	33.05	7.03
		5% molasses	16.52	15.50	2.55	5.03	34.69	7.04
		5% barley crush	21.45	18.66	4.01	5.61	38.32	7.01
		0.5% formic acid	42.03	19.60	8.24	8.47	57.16	7.04
		Total	23.95	17.17	4.29	5.98	40.80	7.03
	Mix	Control	16.08	14.93	2.44	4.84	33.32	7.06
		5% molasses	19.02	19.26	3.64	5.24	36.09	7.00
		5% barley crush	23.40	16.96	3.92	5.92	40.70	7.01
		0.5% formic acid	37.27	20.50	7.64	7.73	52.40	6.98
		Total	23.94	17.91	4.41	5.93	40.63	7.01
SD			9.20	2.59	1.83	1.24	8.16	0.04
P-value	Period		0.263	0.000 ***	0.000 ***	0.459	0.514	0.002 **
	Species		0.030 *	0.201	0.461	0.023 *	0.031 *	0.503
	Treatment		0.001 ***	0.023 *	0.000 ***	0.002 **	0.003 **	0.034 *
	period * species		0.012 *	0.511	0.066	0.041 *	0.044 *	0.142
	period * treatment		0.000 ***	0.117	0.000 ***	0.000 ***	0.000 ***	0.001 ***
	species * treatment		0.001 ***	0.324	0.003 **	0.001 ***	0.001 ***	0.013 *
	period * species * treatment		0.410	0.025 *	0.220	0.353	0.351	0.188

DM – dry matter, SD – standard deviation, $P < 0.05$; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Beyzi et al. (2023) reported that for untreated *Phragmites* silage, gas production was 39 ml/0.2 g, methane production was 17.92%, and OMD was 62.10%. Although these findings are closer to the findings of the June harvest period of pragmites, they are higher compared to the present study. This may be due to the fact that the plants were harvested at different vegetation periods. The decrease in total gas production, ME and OMD levels for both plant species with 0.5% formic acid supplementation in the June period is consistent with the study of Kara et al. (2017) who reported that in vitro cumulative gas production, ME and OMD values and ammonia-N concentration were decreased with increasing levels of formic acid supplementation to the diet (1, 2, 4 or 8 ml/kg).

No AF were detected in the treated silages or in the control group. ZEN was detected in the control group of *Phragmites australis* harvested in June and in the molasses-treated *Phragmites* group. DON levels ranging from 195 to 292.5 mg/kg were found in six silage samples, while ZEN levels ranging from 100 to 215 µg/kg were detected in four samples. The frequency of contamination of silage samples was low. In comparison, previous research detected DON (0.01 – 0.1 mg/kg) and ZEN (2.84 – 40.64 µg/kg) in 23 of 60 maize silage samples from Burdur Province, Türkiye (Sahindokuyucu et al., 2010). The higher levels of DON (>195 mg/kg) and ZEN (>100 µg/kg) in this study could result from improper storage conditions.

Conclusions

When harvested in a controlled manner, wetland plants can serve as roughage in animal feed. The study confirms that common reed (*Phragmites australis*) and cattail (*Typha* spp.) plants shows substantial growth potential, and their silages possess nutritive qualities comparable to medium-grade forages. The interaction between harvest period, plant species, and treatment significantly influenced the crude protein content, Flieg score, NH_3-N/Total N ratio, and *in vitro* gas production of the silages. The June harvest of *Typha* treated with 5% molasses can be recommended as the most optimal silage from wetland plants. Moreover, the inclusion of 5% molasses had a positive impact on the nutrient content and Flieg score of both species from the August harvest.

Additional information

A part of this study was presented as an oral presentation at the 3rd International Animal Nutrition Congress, November 17 – 20, 2022, Titanic Beach Hotel Lara – Antalya / Türkiye.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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