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Fermentation Quality and Aerobic Stability of Silages from Forage Crops Mixed with Bamboo Silage in Southern Kyushu, Japan

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Abstract: Underutilization of bamboo contributes to the degradation of woody environments in southern Kyushu, Japan. A novel approach to using bamboo involves processing it to finely ground silage, although this product has shown inferior digestibility and palatability compared to traditional forage crops. This study evaluated the chemical composition, fermentation quality, and aerobic stability of silages produced from Italian ryegrass, maize, and Rhodes grass mixed with 10% (v/v) bamboo silage, ensiled for 30-40 days. One-day wilted first-cut Italian ryegrass and Rhodes grass and direct-cut maize were finely chopped, mixed with bamboo silage at a 9:1 volume ratio, and vacuum-sealed in polyethylene bags, with control silages produced without bamboo addition. Digestibility tended to decrease in bamboo-mixed maize and Rhodes grass silages. The addition of bamboo silage resulted in a significant decrease ($p<0.05$) in pH by 0.45 in Italian ryegrass, a trend toward lower pH in Rhodes grass, and a tendency for pH to increase in maize. Fermentation quality was improved by the addition of bamboo silage in Italian ryegrass and Rhodes grass, with significant increases ($p<0.05$) in contents of lactic acid by 2.91% Dry Matter (DM) and acetic acid by 1.03% DM, respectively, contrasting with a trend toward reduced lactic acid content in maize. Aerobic stability, measured as the time to reach a temperature increase of >2°C above ambient, was highest in Italian ryegrass silage with bamboo, which maintained stability for more than 6 days after air exposure, compared to significant temperature rises detected at 15 and 29 h in maize and Rhodes grass silages, respectively. Fermentation quality, including pH, lactic acid, and acetic acid contents, was maintained in Italian ryegrass silage mixed with bamboo for a week after air exposure, while rapid deterioration was observed in maize and Italian ryegrass silages with and without bamboo silage addition. It is concluded that wilted Italian ryegrass mixed with 10% bamboo silage may enhance the handling efficiency of silage after opening silos in early June for the site in southern Kyushu, Japan.

Keywords: Aerobic Stability, Italian Ryegrass, Maize, Rhodes Grass, Silage Fermentation

Introduction

Bamboo (*Phyllostachys edulis*) has traditionally been utilized for human consumption, as well as for architectural and artistic purposes (Takano *et al*., 2017). However, the increasing popularity of urbanized lifestyles and the availability of alternative plastic products have led to a decline in domestic bamboo use, compounded by the import of cheaper bamboo from other countries (Takano *et al*., 2017). Consequently, domestic bamboo production has decreased, resulting in the expansion of abandoned bamboo stands, which are encroaching upon upland fields and residential areas (Suzuki, 2015). Several initiatives have been proposed to utilize this abandoned bamboo, including its conversion to charcoal, pulp processing, and biomass fuel (Rathour *et al*., 2022). However, these measures have not provided a comprehensive solution to the problem of abandoned bamboo forests. One promising approach is processing bamboo leaves into silage, which has been shown to be of sufficient quality in terms of fermentation, nutrient content, and digestibility and comparable to self-sufficient forage crops in southern Kyushu, Japan (Nabenishi *et al*., 2013). Further, finely ground whole-crop bamboo silage has demonstrated excellent fermentation quality, particularly in terms of pH and lactic acid content, which can be improved by the addition of lactic acid bacteria (*Lactobacillus plantarum* and *L. lactis*), as reported by Kikukawa *et al*. (2020). In China, bamboo shoot shells (Liang *et al*., 2016), water bamboo sheath leaves (Li *et al*., 2023), and water bamboo shoot shells (Li *et al*., 2024) were utilized as the high-quality by-products for Fermented Total Mixed Rations (FTMR). The addition of lactic acid bacteria with natamycin prolonged aerobic stability, reaching 85.5 h after air exposure (Li *et al*., 2023). The most appropriate replacement level of bamboo shoot shell with whole-plant maize silage was 25% in the prolonged aerobic stability within 14 days after air exposure and high lactic acid content above 6% Dry Matter (DM) with low pH below 4.2 (Liang *et al*., 2016). Bamboo shows notable antibacterial characteristics, which are resistant to degradation by microorganisms in nature (Ramful *et al*., 2022). In our previous study, finely ground round-baled bamboo silage exhibited good aerobic stability, maintaining its fermentation quality for up to one month after exposure to air (Niimi *et al*., 2015).

Whole-crop maize (*Zea mays* L.) silage is a valuable nutritional source for ruminant roughage (Pang *et al*., 2024). However, during the feed-out stage, maize silage is prone to deterioration due to high environmental temperatures and humidity, which lead to increases in temperature and pH, indicative of aerobic spoilage (Pang *et al*., 2024). To improve aerobic stability in maize silage, several studies have explored the addition of essential oils and their blends (Aniceto *et al*., 2024), sweet orange essential oil (Silva *et al*., 2024), thermally treated ground shrimp shells (Meirelles *et al*., 2024) and bamboo shoot shell (Zhao *et al*., 2020-2021). In our previous research, maize silage mixed with finely ground bamboo silage at nil, 5, 10 and 20%, produced by Yamato Frontier Co. Ltd. (Miyakonojo, Miyazaki) as reported by MAFF (2020), demonstrated favorable fermentation quality, with a pH of 3.70-3.86, lactic acid content of 1.15-1.48% Fresh Matter (FM) and acetic acid content of 1.72−2.05% FM (Kurata *et al*., 2020). The moderate addition of 10% bamboo silage into maize silage led to the highest lactic and acetic acid contents, which was adopted as the present inclusion rate at 10% of bamboo silage. However, these studies did not examine aerobic stability, which remains a key potential benefit of mixing forage crops with finely ground bamboo silage.

In southern Kyushu, Japan, the typical cropping system involves the cultivation of winter Italian ryegrass (*Lolium multiflorum* Lam.), combined with summer maize (Li *et al*., 2019) or tropical Rhodes grass (*Chloris gayana* Kunth) for silage production (Komatsu and Shoji, 2005). The acreage of Italian ryegrass and maize occupies most of the summer and winter crops, respectively in the site of southern Kyushu. Therefore, the present study aimed to expand the utilization of bamboo and improve handling efficiency in typical forage silages by examining the chemical composition, fermentation quality, and aerobic stability of silages processed from Italian ryegrass, maize, and Rhodes grass mixed with bamboo silage after exposure to air.

Materials and Methods

Forage Species, Cultivation and Silage Processing

The forage crop species examined in this study included Italian ryegrass (cv. Goldblend, Kaneko Seed Co. Ltd.) as a typical winter forage, maize (cv. NS118 Super, Kaneko Seed Co. Ltd.) as a typical summer ensiling crop and Rhodes grass (cv. Callide, Kaneko Seed Co. Ltd.) as a typical summer grass. The crops were grown on sandy regosol soil, extending over 1 m in depth, at the Sumiyoshi Livestock Science Station (131.45°E, 31.99°N, 9.4 m meters above sea level). Italian ryegrass was sown on 25 September 2020 and harvested at the flowering stage on 9 May 2021. The harvested forage was allowed to wilt outdoors for one day, finely chopped, mixed with finely ground bamboo silage (produced by Yamato Frontier Co. Ltd.) at a 9:1 volume ratio, and vacuum-sealed in polyethylene bags containing 10 kg FM for storage at ambient room temperature for 30 days. Maize was sown on 16 April 2020 and harvested by a corn harvester at the milk-ripe stage on 29 July 2020. The maize was finely chopped to ensile herbage, mixed with finely ground bamboo silage at a 9:1 volume ratio, and vacuum-sealed in polyethylene bags containing 30 kg FM for storage at ambient room temperature for 40 days. Rhodes grass was sown on 14 May 2021 and harvested at the heading stage on 7 October 2021. The harvested forage was allowed to wilt outdoors for one day, finely chopped, mixed with finely ground bamboo silage at a 9:1 volume ratio, and vacuum-sealed in polyethylene bags containing 10 kg FM for storage at ambient room temperature for 31 days. Samples of each forage silage mixed with the bamboo silage were compared with control silages (i.e., silage without bamboo silage added) with three replicates for both bamboo-silage-added treatments and controls.

Aerobic Stability Test

For the aerobic stability test, 1,100 g FM of ensiled silages stored for 30 days (Italian ryegrass), 40 days (maize), and 31 days (Rhodes grass) were transferred to unsealed Styrofoam vessels (15×15×25 cm, 15 mm thick) and stored for a week. The inner temperature of the silages and the surrounding ambient temperature were measured at 15-minute intervals using a thermometer data logger (Ondotori TR-71Ui, T&D Corp., Matsumoto, Japan) using three replicates. Aerobic deterioration of the samples considered began when the silage temperature exceeded the ambient temperature by more than 2°C. Following exposure to air, samples were collected to assess silage fermentation quality at 1, 3, and 7 days for Italian ryegrass; 2, 5, and 7 days for maize; and 3, 5, and 7 days for Rhodes grass.

Chemical Analysis for Silage Quality and Fermentation Quality

To assess the quality attributes of the silages, 50 g of each silage sample was dried at 70°C for 18 h and ground with a cutting mill (Wiley 1029-B, Yoshida Seisakusho Co. Ltd., Tokyo, Japan) to determine the Dry Matter (DM) content. The moisture content of the dried samples was determined by the atmospheric heating drying method. The contents of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined using a fiber analyzer (ANKOM Technology Co., Ltd., Macedon, NY, USA). *In vitro* Dry Matter Digestibility (IVDMD) was evaluated using a pepsin-cellulase assay with a filter bag and an *in vitro* incubator (ANKOM Technology Co., Ltd.) by Goto and Minson (1977).

For silage fermentation quality, the Total Nitrogen (TN) content of FM was measured by the Kjeldahl method. Forty grams of each silage sample was cut into 1 cm pieces, soaked in 360 mL of distilled water for 12 h and the mixture was filtered with filter paper No. 5A (ADVANTEC MFS, INC., Dublin, CA, USA) to obtain the filtrate. The pH of the filtrate was measured with a pH meter (AS800, As One Corp., Osaka, Japan). The remaining samples were used to determine the contents of lactic acid and Volatile Fatty Acids (VFAs) such as acetic acid, propionic acid, butyric acid, and valeric acid. Lactic acid content was determined using a colorimetric method with a spectrophotometer (UVmini-1240, Shimadzu Corp., Kyoto, Japan) following the procedure described in Barker and Summerson (1941). Volatile Basic Nitrogen (VBN) was measured by steam distillation and titration (JGFFSA, 2009). The VFA content was analyzed by gas chromatography using a FAL-M column filler (Shimadzu Corp.).

Statistical Analysis

Statistical analyses were conducted to compare silage quality attributes, fermentation quality attributes, changes in temperature, and silage fermentation quality attributes after exposure to air in silages with and without bamboo silage added. Student's t-test at a 5% significance level was used, employing BellCurve for Excel (BellCurve, Tokyo, Japan).

Results

Chemical Composition and Fermentation Quality of Three Forages with Added Bamboo Silage

The chemical composition and fermentation quality of wilted Italian ryegrass and Rhodes grass, direct-cut maize, and finely ground bamboo silage before ensiling are shown in Table (1). The moisture contents of the bamboo silages were similar at 51% when mixed with Italian ryegrass, 51% with maize and 53% with Rhodes grass. Among the forages, direct-cut maize had the highest moisture content at 79%, followed by Rhodes grass at 69% and Italian ryegrass at 53% in their wilted states. The IVDMD values were comparable for Italian ryegrass at 57%, maize at 56% and Rhodes grass at 38%. Therefore, NDF and ADF contents per gram of DM were the lowest in maize at 64 and 37%, followed by Italian ryegrass at 71 and 41% and highest in Rhodes grass at 83 and 48%, respectively. However, in bamboo-supplemented silages, IVDMD values were low, ranging from 6.7-8.7%, while the NDF and ADF contents were very high, ranging from 95-99 and 49-68%, respectively.

The fermentation quality of silages before ensiling was characterized by a low pH, ranging from 4.3-4.6, with moderate acetic acid content between 0.06 and 2.47% DM and negligible butyric acid content, ranging from 0.00-0.04% DM. Among the forages, maize exhibited a lower pH of 4.4 due to moderate levels of lactic acid (1.30% DM) and acetic acid (0.92% DM), compared with the nearly neutral pH of 6.2 and 6.7 observed in wilted Italian ryegrass and Rhodes grass, respectively, which was due to the negligible production of lactic and acetic acids at the time of ensiling.

The chemical composition and fermentation quality of the three forage species with and without mixing with bamboo silage and ensiled for approximately one month are shown in Table (2). Regarding the chemical composition of the ensiled silages, IVDMD decreased with the addition of bamboo silage, except for Italian ryegrass silage. However, ADF and NDF contents remained relatively unchanged with a 9:1 volume ratio of forage to bamboo silage additives. Concerning the fermentation quality of the ensiled silages mixed with bamboo silage, pH decreased significantly ($p<0.05$) in the Italian ryegrass silage, no significant change was observed in maize silage and a decreasing tendency was observed in the Rhodes grass silage. These changes corresponded with a significant increase ($p<0.05$) in lactic acid content in Italian ryegrass silage and a significant increase ($p<0.05$) in acetic acid content in Rhodes grass silage, whereas no significant changes in fermentation attributes were found for maize silage. The ratio of VBN to TN (VBN/TN) did not change with the addition of bamboo silage in any of the forage species.

Table 1: Chemical composition and fermentation quality of forages in Italian ryegrass, maize, Rhodes grass, and finely-ground bamboo silage before ensiling

		Chemical composition†			Fermentation quality					
Species	Treatment	IVDMD (% DM)	NDF (% DM)	ADF (% DM)	Moisture (% FM)	pH	Lactic acid (% DM)	Acetic acid (% DM)	Butyric acid (% DM)	VBN/TN⁑ (%)
Italian ryegrass	Wilted forage	57.1	71.4	41.1	52.8	6.21	0.03	0.00	0.00	0.61
	Bamboo silage	6.7	95.3	68.2	51.0	4.26	0.17	0.06	0.04	5.11
Maize	Fresh forage	56.1	63.9	36.6	78.8	4.40	1.30	0.92	0.08	0.67
	Bamboo silage	8.7	98.8	49.2	50.7	4.44	0.01	2.47	0.00	18.43
Rhodes grass	Wilted forage	38.2	83.0	47.6	68.7	6.69	0.03	0.19	0.00	2.03
	Bamboo silage	8.6	98.8	66.6	52.5	4.58	0.30	1.21	0.00	6.72

† IVDMD: *in vitro* Dry Matter Digestibility, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber

⁑ VBN: Volatile Basic Nitrogen, TN: Total Nitrogen, VBN/TN: Ratio of VBN to TN

Table 2: Chemical composition and silage fermentation quality (mean ± standard error, n = 3) of Italian ryegrass, maize, and Rhodes grass with and without finely ground bamboo silage additives after ensiling for around a month

		Chemical composition†			Silage fermentation quality					
Species	Treatment	IVDMD (% DM)	NDF (% DM)	ADF (% DM)	Moisture (% FM)	pH	Lactic acid (% DM)	Acetic acid (% DM)	Butyric acid (% DM)	VBN/TN⁑ (%)
Italian ryegrass	Control (No additives)	50.1±2.56	77.0±1.59	46.7±0.95	58.9±1.52	4.48±0.12	0.34±0.02	0.11±0.04	0.05	3.66±0.29
	Bamboo silage additives	55.1±1.26	75.3±0.54	45.7±0.65	58.4±0.10	4.03±0.03	3.25±0.24	0.09±0.02	0.00	3.44±0.58
	Significance¶	ns	ns	ns	ns	*	*	ns	–	ns
Maize	Control (No additives)	56.7±2.84	66.2±7.24	39.5±5.44	77.7±1.20	3.55±0.15	5.89±0.29	3.61±0.14	0.00±0.00	4.36±0.06
	Bamboo silage additives	44.1±2.61	63.2±3.23	40.6±2.15	75.4±1.67	3.76±0.06	4.69±0.38	3.74±0.34	0.04±0.03	4.81±0.33
	Significance	*	ns	ns	ns	ns	*	ns	ns	ns
Rhodes grass	Control (No additives)	38.4±0.92	84.7±0.73	51.3±0.52	71.6±0.33	5.62±0.26	0.34±0.05	1.88± .19	0.06±0.03	11.82±0.99
	Bamboo silage additives	35.4±1.26	84.7±0.65	49.9±2.48	69.1±0.97	5.32±0.26	0.62±0.11	2.91±0.19	0.07±0.00	12.66±0.67
	Significance	*	ns	ns	ns	ns	*	*	ns	ns

† IVDMD: *in vitro* dry matter digestibility, NDF: neutral detergent fiber, ADF: acid detergent fiber

⁑ VBN: volatile basic nitrogen, TN: total nitrogen, VBN/TN: ratio of VBN to TN

¶ *: p<0.05; ns: p>0.05; –: Not determined

Aerobic Stability of Silages Exposed to Air

The changes in the temperature of forage silage with and without bamboo silage additives after silo opening are shown for Italian ryegrass, maize, and Rhodes grass in Fig. (1A-C), respectively. Italian ryegrass silage mixed with bamboo silage exhibited temperature changes that synchronized with the ambient air temperature for up to 6 days after exposure to air, in contrast to the rapid temperature rise observed within a day in the silage without bamboo silage additives. In maize (Fig. 1B) and Rhodes grass (Fig. 1C) silages, temperatures rose independently of changes in ambient air temperature approximately one day after exposure to air both in silage bamboo additives and in the control without additives.

Therefore, the time to onset of aerobic deterioration, defined as the point when the silage temperature exceeded the ambient room temperature by more than 2°C, was calculated and is shown in Table (3). In Italian ryegrass mixed with bamboo silage, aerobic deterioration did not occur within the monitoring period, indicating a duration of >144 h. In contrast, the no-additive Italian ryegrass silage, as well as the maize and Rhodes grass silages with and without bamboo silage, exhibited aerobic deterioration times of around or less than one day after exposure to air, with no significant differences observed between the treatments in either maize or Rhodes grass.

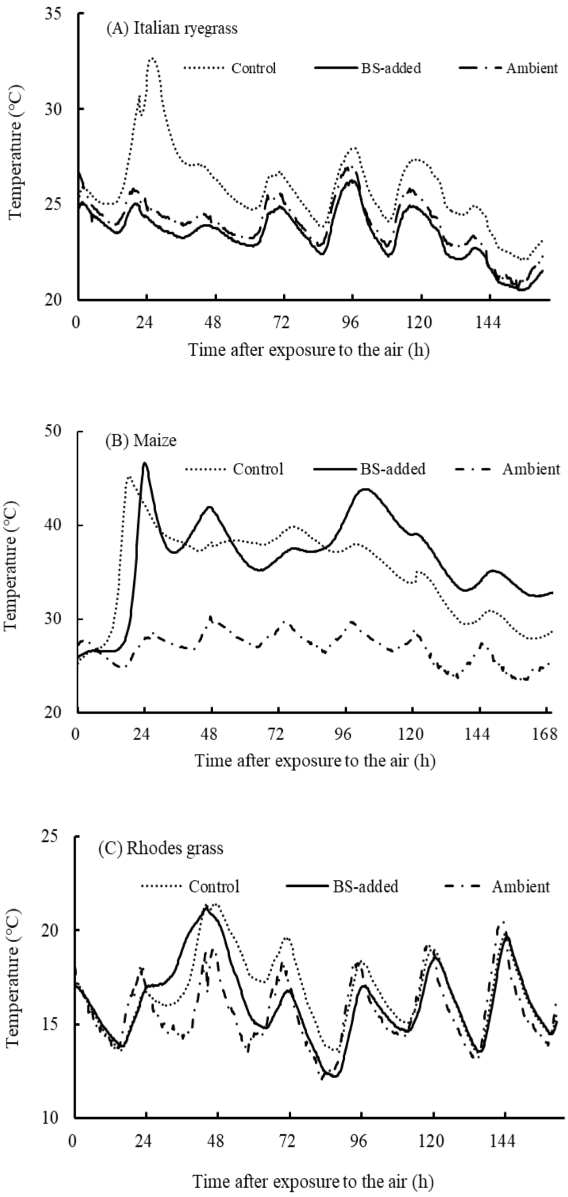

Fig. 1: Changes in silage temperature for (A) Italian ryegrass, (B) maize, and (C) Rhodes grass mixed with bamboo silage (BS) and without BS (control), exposed to air with ambient air temperature (Ambient)

Changes in silage fermentation quality of Italian ryegrass, maize, and Rhodes grass with and without finely ground bamboo silage additives after exposure to air are shown in Table (4). In Italian ryegrass silage ensiled with bamboo silage, even after exposure to air, the pH remained low at 4.0-4.1, the lactic acid content remained high at 3.0-3.6% DM, and the VBN/TN ratio remained low at 3.8-4.0 over the course of one week, suggesting maintained aerobic stability. In contrast, for the control Italian ryegrass silage without bamboo silage additives, pH values increased to 6.0 within a day and further to 7.2 and 7.5 at 3 and 7 days, respectively, after exposure to air. This increase was concurrent with a decline in lactic acid content to less than 0.1% DM within a week. These changes in fermentation quality attributes were closely correlated with the deterioration times shown in Table (3).

In maize silages, both with and without bamboo silage additives, the pH increased to 5.7-6.5 within 2 days and reached 7.6-7.7 within a week. These changes were correlated with the decrease in lactic and acetic acid contents to 0.1 and 0.3% DM, respectively, within a week, indicating the aerobic instability of maize silages when exposed to air.

In Rhodes grass silages ensiled with bamboo silage, pH increased above 7, reaching 7.4 within 3 days and 8.0 within one week after exposure to air. This increase in pH was correlated with a rapid decrease in lactic and acetic acid contents to 0.03 and 0.3% DM, respectively, by 3 days after air exposure. These trends in fermentation quality were similar to those observed in silage without bamboo silage, showing a rapid increase in pH and a decline in both lactic and acetic acid contents. Consequently, the aerobic stability of Rhodes grass silages almost disappeared 3 days after exposure to air, as indicated by the increase in silage temperature above 2°C within 29-35 h after air exposure (Table 3).

Table 3: Time to onset of aerobic deterioration of Italian ryegrass, maize, and Rhodes grass with and without finely ground bamboo silage additives

Species	Treatment	Time to onset of aerobic deterioration of silages (h)†
Italian ryegrass	Control (No additives)	18
	Bamboo silage additives	>144
	Significance	*
Maize	Control (No additives)	12
	Bamboo silage additives	15
	Significance	ns
Rhodes grass	Control(No additives)	35
	Bamboo silage additives	29
	Significance	ns

† Aerobic deterioration of silage was defined as an increase of silage temperature of more than 2°C surrounding ambient temperature

¶ *: $p<0.05$. ns: $p>0.05$

Table 4: Changes in silage fermentation quality (mean ± standard error, n = 3) of Italian ryegrass, maize, and Rhodes grass with and without finely ground bamboo silage additives after exposure to air

Species	Days after air exposure	Treatment†	Silage fermentation quality					
			Moisture (%FM)	pH	Lactic acid (%DM)	Acetic acid (%DM)	Butyric acid (%DM)	VBN/TN⁑ (%)
Italian ryegrass	1	Control	58.38±0.87	5.99±0.01	0.71±0.38	1.31	0.56±0.26	2.59±0.09
		BS added	58.49±0.72	4.11±0.00	3.54±0.08	1.16	0.20±0.12	3.75±0.30
		Significance¶	ns	*	*	–	ns	*
	3	Control	53.71±1.10	7.23±0.03	0.16±0.01	1.58±0.05	0.74±0.34	8.96±0.38
		BS added	57.97±0.44	4.10±0.02	2.98±0.05	1.48±0.19	0.62±0.14	3.99±0.20
		Significance	*	*	*	ns	ns	*
	7	Control	53.69±0.56	7.50±0.03	0.07±0.01	1.24±0.43	1.05±0.23	1.61±0.07
		BS added	55.21±1.55	4.03±0.00	3.58±0.14	1.57±0.16	0.45±0.11	3.90±0.22
		Significance	ns	*	*	ns	*	*
Maize	2	Control	77.86±0.38	6.53±0.24	0.23±0.01	0.33±0.01	0.33±	2.89±0.08
		BS added	74.70±0.74	5.68±0.35	0.83 ± 0.21	0.59±0.12	0.48±	3.05±0.22
		Significance	*	*	*	*		ns
	5	Control	76.77±0.65	7.10±0.06	0.38±0.04	0.64±0.08	0.50±	1.30±0.16
		BS added	73.61±1.02	6.20±0.08	0.24±0.04	0.59±0.05	0.48±	5.66±0.75
		Significance	*	*	*	ns		*
	7	Control	78.23±0.55	7.66±0.37	0.15±0.02	0.29±0.02	0.23±	2.07±0.10
		BS added	76.24±1.12	7.55±0.21	0.12±0.01	0.28±0.02	0.22±	2.50±0.17
		Significance	*	Ns	ns	ns		*
Rhodes grass	3	Control	68.65±0.42	7.68±0.06	0.09±0.01	0.50±0.06	0.00	12.38±3.12
		BS added	67.18±0.39	7.37±0.02	0.25±0.02	0.29±0.03	0.07±0.01	9.93±0.03
		Significance	ns	*	*	*	–	ns
	5	Control	67.09±0.38	7.98±0.03	0.04±0.01	0.38±0.01	0.00	10.33±0.45
		BS added	66.40±0.51	7.60±0.06	0.15±0.02	0.38±0.01	0.03±0.01	8.43±0.21
		Significance	ns	*	*	ns	–	*
	7	Control	66.74±0.49	8.28±0.06	0.02±0.00	0.34±0.03	0.00	10.90±0.61
		BS added	65.96±0.72	8.03±0.02	0.03±0.00	0.33±0.01	0.00	8.85±0.21
		Significance	ns	*	*	ns	–	*

† Control: No additives, BS added: Bamboo silage added

⁑ VBN: volatile basic nitrogen, TN: total nitrogen, VBN/TN: ratio of VBN to TN

*: <0.05; ns: p>0.05; –: Not determined

Discussion

Characteristics and Costs of Bamboo Silages

The finely ground bamboo silage used in the present study is produced by Yamato Frontier Co. Ltd., which sells it for 800 Japanese Yen (JPY) per 20 kg bag for forage use (MAFF, 2020). The cost of mixing the finely ground bamboo silage with 450 kg FM round-baled maize silage at a 9:1 ratio was calculated at 1,800 JPY. The cost of whole-crop maize silage (450 kg FM) ranged from 6,750 to 13,500 JPY (MAFF, 2022). Therefore, excluding labor costs for mixing bamboo silage with forages during ensiling, the addition of bamboo silage at ensilage is considered a cost-effective approach to enhancing handling efficiency after air exposure, particularly for Italian ryegrass silages. We need further evaluation on the additional labor costs to use bamboo silage at ensilage with harvested forage crops.

Effect of Examined Seasons on Forage Species

In this study, ensiling Italian ryegrass, maize, and Rhodes grass mixed with bamboo silage was conducted in early May, late July, and early October, respectively, which was the normal harvest season for each crop. The silages were then exposed to air after 30-40 days of ensiling in early June for Italian ryegrass, early September for maize, and early November for Rhodes grass. The ambient temperatures monitored for more than 6 days after air exposure were 24.0°C for Italian ryegrass, 26.9°C for maize, and 15.7°C for Rhodes grass. It is challenging to attribute the aerobic stability observed in Italian ryegrass ensiled with bamboo silage to a lower ambient temperature compared to the other forages (Wilkinson and Davies, 2013), as the ambient temperature was actually higher for Italian ryegrass at 24.0°C than for Rhodes grass at 15.7°C.

Superior Effectiveness of Italian Ryegrass Mixed with Bamboo Silage Compared to Other Forages in Aerobic Stability

Previous research on maize silage processing has demonstrated that the addition of bamboo shoot shell (Zhao *et al*., 2020-2022), essential oils (Aniceto *et al*., 2024), sweet orange essential oil (Silva *et al*., 2024), forage cactus in drylands (Sobral *et al*., 2024), lactic acid bacteria, or *Artemisia argyi* (Pang *et al*., 2024) can effectively stabilize silage fermentation quality and suppress the rise in silage temperature when exposed to air. However, the present study did not find a similar effect of bamboo silage addition on suppressing aerobic instability in maize silage, likely due to the silos being opened in early September when the ambient temperature averaged 26.9°C. To improve aerobic stability, double-cropped maize silage processing should be performed with harvesting in late autumn and silo opening after one month of ensiling in December, when ambient temperatures are below 15°C (Wilkinson and Davies, 2013). In the present study, Rhodes grass was ensiled in early October and exposed to air in early November when the ambient temperature was 15.7°C. However, aerobic instability began approximately one day after exposure to air (Table 3). In contrast, Italian ryegrass mixed with bamboo silage showed superior aerobic stability, maintaining stability for more than 6 days after air exposure compared to maize and Rhodes grass (Table 3).

The difference in aerobic stability of forages mixed with bamboo silage between Italian ryegrass and maize or Rhodes grass may be derived from the native lactic acid bacteria on leaves and available soluble carbohydrates for fermentation (Bao *et al*., 2023), combined with the antibacterial characteristics of bamboo silage (Ramful *et al*., 2022). These activities played a similar role to lactic acid bacterial inoculation and antimicrobial additives such as natamycin, which proved the successful fermentation quality and aerobic stability of herbage-based FTMR (Li *et al*., 2024). Therefore, Italian ryegrass silage mixed with bamboo silage should gain the favorable combination of soluble carbohydrates from Italian ryegrass and antibacterial activity from bamboo silage to exert prolonged aerobic stability. In contrast, plenty of soluble carbohydrates in maize silages should proliferate the activity of deteriorating microorganisms such as mold and *Pseudomonas* even under the antibacterial activity from bamboo silage, resulting in aerobic instability within 15 h. On the other hand, a shortage in soluble carbohydrates in Rhodes grass silage produced the lowest lactic acid content with the highest pH in Table (2), which should not be sufficient lactic acid bacteria to suppress the activity of deteriorating microorganisms.

However, we cannot neglect that the digestibility (IVDMD) decreased significantly ($p<0.05$) in maize and Rhodes grass silages mixed with the bamboo silage (Table 2). We cannot gain the true mechanism for the rapid deterioration of maize silage quality after opening silos, even inclusive of bamboo silage, which showed the opposite trends to the other research findings (Zhao *et al*., 2020-2022). In the future study, we are trying to detect the critical temperature of stabilizing silage fermentation quality after being exposed to air by comparing the double-crop maize silage cropping, harvested in November and faced air exposure in December.

Future Scope for Expanding Handling Efficiency in Typical Forage Silages After Silo Opening

In this study, the addition of bamboo silage was fixed at a single level of 10%, which resulted in decreased digestibility in maize and Rhodes grass (Table 2). Therefore, it is necessary to investigate the effects of varying levels of bamboo silage addition, even for Italian ryegrass silage, to optimize the costs of silage processing.

Conclusion

As a novel application of bamboo, the addition of finely ground bamboo silage at 10% (v/v) to one-day wilted Italian ryegrass was effective in maintaining aerobic stability for one week in early June at the examined site, suggesting potential for improvement in handling efficiency after opening silos of Italian ryegrass. The negative aspect of bamboo silage additives to other crops like maize and Rhodes grass should be examined in further study.

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Author's Contributions

Mitsuhiro Niimi: Conducted research, collected data, and manuscript preparation.

Mizuho Kurata: Conducted research, performed data analysis, and collected data.

Genki Ishigaki and Yasuyuki Ishii: Supervised research, revised and proofread manuscript.

Ethics

This article contains research results that have not yet been published. The corresponding author declares that there are no ethical issues and that the other authors have reviewed and approved the manuscript for publication.

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