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RESEARCH ARTICLE
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Ensilage Characteristics of Corn Silage Treated with Fermented Green Juice Prepared from Corn, Alfalfa or Timothy

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ABSTRACT

Fermented green juice (FGJ) has established its efficiency as a silage additive; its addition to alfalfa and timothy resulted in good fermentation-quality silage. Therefore, the fermentation quality of corn silage treated with FGJ was evaluated in a laboratory-scale experiment. Whole plant corn at the dough stage was harvested and treated with FGJ prepared from corn, alfalfa, or timothy. Comparing the fermentation characteristics of the three FGJs, the epiphytic lactic acid bacteria (LAB) predominantly grew in all FGJs, and its level reached 10^7 cfu/g in timothy and corn FGJs. While alfalfa FGJ contained the highest number of LAB 5.8×10^8 cfu/g. The highest lactic acid content was found in alfalfa FGJ, which subsequently resulted in the highest total acid content. However, alfalfa FGJ recorded the highest pH value, and this may be ascribed to the high buffering capacity of alfalfa. Aerobic bacteria and mold levels were decreased after 2 days of fermentation in all FGJs. On the contrary, yeast tended to increase in corn and alfalfa FGJs while it decreased only in timothy FGJ. Both control and FGJ-treated silages were well-preserved silages. The pH value and ammonia nitrogen (NH_3-N) content did not increase more than 3.79 and 4.9% TN, respectively. Corn FGJ-treated silage has a lower pH value, lower NH_3-N/TN, and higher Flieg's point and V-score than the control silage. Both molds and enterobacterial growth were depressed in all silages. Corn and alfalfa FGJs treated silages had lower aerobic bacterial count than the control. While the lowest level of yeast was detected in timothy FGJ-treated silage. In conclusion, data obtained in this trial suggested that adding FCJ to properly ensiled corn may be of questionable value.

Keywords: Corn silage, Fermentation, Fermented green juice, Nutrition, Timothy silage

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INTRODUCTION

Corn silage is one of the most popular forages fed to dairy cows because it has good agronomic characteristics and yields high concentrations of nutrients. It is recognized as an ideal crop for preservation by ensiling, but normally its aerobic instability is high once the silo is opened. Many kinds of additives are marketed for enhancing ensiling characteristics and quality of corn and other forage silages. Responses to inoculants as one of these additives are differing, because it is influenced by many variables such as bacterial species and their numbers, substrate moisture, maturity, and nutrient content in addition to the interaction caused by adding carbohydrates or chemicals with the bacteria at ensiling time (El Hag et al., 1982; Weiss et al., 2016; Nair et al., 2019; Zhang et al., 2019; Jiao et al., 2021; Wang et al., 2022; Khan et al., 2023). Burghardi et al. (1980) concluded that whole plant corn did not benefit from bacterial inoculant. The objectives of this study were to evaluate the effects of Fermented green juice (FGJ) addition on the fermentation quality of corn silage and to compare the efficiency of FGJ prepared from corn, alfalfa or timothy.

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MATERIALS & METHODS

Plant Materials

Whole plant corn at dough stage, alfalfa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.) both at vegetative stage were harvested for FGJ preparation. Two days later, the whole plant corn was harvested for silage preparation. The three crops were grown on the farm of Rakuno Gakuen University.

Preparation of Fermented Green Juice

FGJs were prepared from chopped alfalfa or timothy where approximately 100g of each fresh herbage was macerated with 300ml of water using a blender. The macerate was then filtrated through double cheesecloth, and each filtrate was diluted with 500ml distilled water to which 10g of glucose was added, then fitted with gas trap and kept at 30°C for 2 days (Ohshima et al., 1997a,b).

Preparation of Silage and Analytical Methods

Corn was chopped to 2-4cm lengths by a precision chop forage harvester and immediately transported to the silage-making site; then, one of these FGJs was sprayed at 5ml/500g. Silage without additives was also prepared. The chemical composition of the grasses and silages were determined using ground samples oven-dried at 60°C for 24h. Dry matter contents were determined by oven-drying of the samples at 135°C for 2 h. Crude protein (CP) was calculated by multiplying Kjeldal nitrogen by 6.25 (AOAC, 1990). Water soluble carbohydrate (WSC) content was estimated colorimetrically using anthrone. Data were subjected to analysis of variance and significance was declared at $P<0.05$ unless noted otherwise.

RESULTS

The chemical composition and epiphytic microorganisms on whole plant corn material are presented in Table 1. Dry matter averaged 27% with a crude protein (CP) content of 6.6% dry matter (DM), and WSC of 22% DM. Lactic acid bacterial count was 4.2×10^4 cfu/g.

Table 1: The chemical composition and viable counts of the ensiled corn

Growth stage	Dough stage
Moisture (%)	73
CP (% DM)	6.6
WSC (% DM)	22
LAB (cfu/g)	4.2×10^4
Aerobic bacteria (cfu/g)	1.1×10^6
Yeasts (cfu/g)	2.7×10^5
Molds (cfu/g)	3.3×10^4
E. Coli (cfu/g)	1.8×10^3
Enterobacteria (cfu/g)	1.1×10^5

CP, crude protein; WSC: water soluble carbohydrate; LAB, lactic acid bacteria

The changes of microbiological count of FGJ between the day of making and after 2 days of incubation are shown in Table 2. Lactic acid bacteria (LAB) level had greatly increased in all FGJs, from a level of 10^2 cfu/g at day 0 to a level of 10^7 cfu/g in corn and timothy FGJs, and to a level of 10^8 in alfalfa FGJ. Both molds and aerobic bacterial counts had decreased by the fermentation, while enterobacteria had greatly decreased from a level of 10^5 cfu/g to a level less than 10^2 cfu/g by the second day of incubation in all of the three FGJs. On the contrary, yeast tended to increase in both corn and alfalfa FGJs while it decreased in timothy FGJ. The pH value of all FGJs was low, but alfalfa FGJ had the highest pH value (3.9). Lactic and acetic acids were produced in all FGJs, and the highest total acid content was measured in alfalfa FGJ. Butyric acid was not detected in any of FGJs (Table 3), while a low level of 2, 3-butanediol was detected in all of them.

Table 2: The changes in viable counts of FGJ prepared from corn, alfalfa or timothy

	Corn		Alfalfa		Timothy	
	0[1)]	2	0	2	0	2
LAB (cfu/g)	4.0×10^2	4.1×10^7	5.0×10^2	5.8×10^8	1.2×10^4	2.7×10^7
Aerobic bacteria (cfu/g)	9.9×10^5	7.6×10^5	3.8×10^6	1.8×10^4	2.5×10^7	3.3×10^6
Molds (cfu/g)	2.6×10^4	1.2×10^3	6.8×10^4	9.0×10^2	1.2×10^5	1.5×10^3
Yeasts (cfu/g)	1.3×10^5	4.6×10^5	1.0×10^4	1.5×10^4	2.2×10^6	9.2×10^4
E. Coli (cfu/g)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Enterobacteria (cfu/g)	2.3×10^5	$< 10^2$	3.9×10^5	$< 10^2$	1.2×10^6	$< 10^2$

[1)] The days of incubation of in fermented juice. LAB, lactic acid bacteria

Table 3: The fermentation characteristics of FGJ prepared from corn, alfalfa or timothy

Parameters	Corn	Alfalfa	Timothy
pH	3.71	3.92	3.75
Lactic acid (%)	0.30	0.56	0.23
Acetic acid (%)	0.04	0.07	0.02
Butyric acid (%)	0.00	0.00	0.00
Total acid (%)	0.34	0.63	0.25
2,3 butanediol (%)	0.04	0.04	0.04
Flieg's point	100.00	100.00	100.00

Table 4 summarizes DM content, DM recovery, gas loss and CP percent to DM in the corn silage treated with FGJ prepared from corn, alfalfa or timothy. There was no significant difference in the chemical composition in any of FGJs treated silages than the control one. Both control and FGJs treated silages were well-preserved silages. All silages recorded low pH value and low portion of ammonia nitrogen (NH_3-N) to total nitrogen (TN) content, but only corn FGJ treated silage had lower pH value and NH_3-N % TN than the control silage. Corn FGJ treated silage had higher (*P<0.05*) lactic acid content than both alfalfa and timothy FGJ treated silages. Acetic acid content had decreased (*P<0.01*) by the addition of both corn and alfalfa FGJs, and the lowest level (*P<0.01*) was obtained in silage treated with alfalfa FGJ. Neither butyric nor propionic acid were detected in control and treated silages. All silages treated with FGJs recorded higher Flieg's point and V-score than those of the control (Table 5). At the end of ensiling period, identification and enumeration of microorganisms of corn silage were carried out (Table 6). Control silage had the highest LAB content. Corn FGJ treated silage had the lowest level of aerobic bacteria, while the lowest yeast number was detected in timothy FGJ treated silage. Both molds and enterobacteria were found in a very low level (less than 10 cfu/g) in all corn silages.

DISCUSSION

Comparing the fermentation end products of FGJs prepared from corn, alfalfa, or timothy, the highest lactic acid content was found in alfalfa FGJ. This subsequently resulted in the highest total acid content. However, alfalfa FGJ recorded the highest pH value, and this may be ascribed to the high buffering capacity of alfalfa.

Table 4: The chemical composition of corn silage treated with FGJ prepared from corn, alfalfa or timothy

Parameters	None	Corn FGJ	Alfalfa FGJ	Timothy FGJ	SE	P
DM (%)	23.30	24.00	23.70	23.80	0.39	0.68
DM recovery (%)	85.8	88.2	87.4	87.6	1.37	0.67
Gass loss (%)	1.89	2.69	2.20	1.90	0.26	0.25
CP (%DM)	6.9	7.5	7.1	6.3	0.51	0.52

SE; standard error; A,B: P<0.01; a,b,c P<0.05. DM, dry matter; CP, crude protein; FGJ, fermented green juice

Table 5: The fermentation characteristics of corn silage treated with FGJ prepared from corn, alfalfa or timothy

Parameters	None	Corn FGJ	Alfalfa FGJ	Timothy FGJ	SE	P
pH	3.79^{a}	3.70^{b}	3.76^{ab}	3.75^{ab}	0.02	0.08
NH_3-N (%TN)	4.89^{A}	3.46^{B}	4.06^{AB}	4.91^{A}	0.21	0.02
Lactic acid (%)	1.36^{ab}	1.61^{a}	1.21^{b}	1.28^{b}	0.07	0.06
Acetic acid (%)	0.44^{A}	0.29^{B}	0.15^{C}	0.38^{AB}	0.02	0.01
Butyric acid (%)	0.00	0.00	0.00	0.00	-	-
Total acid (%)	1.80^{a}	1.90^{a}	1.36^{b}	1.66^{ab}	0.09	0.04
Fleig's point	95.0^{Bc}	99.5^{Aa}	100^{Aa}	96.0^{Bb}	0.25	0.01
V-Score	98.1^{Bc}	99.4^{Ab}	100^{Aa}	98.4^{Bc}	0.13	0.01

SE: standard error; A,B: P<0.01; a,b,c P<0.05.

Table 6: The viable counts of corn silage treated with FGJ prepared from corn, alfalfa or timothy

Parameters	None	Corn FGJ	Alfalfa FGJ	Timothy FGJ
LAB (cfu/ g)	9.9×10^{7}	2.1×10^{6}	4.6×10^{6}	3.5×10^{7}
Aerobic bacteria (cfu/ g)	1.9×10^{4}	9.6×10^{3}	1.7×10^{4}	4.1×10^{4}
Molds (cfu/ g)	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$
Yeasts (cfu/ g)	1.8×10^{4}	5.9×10^{5}	2.3×10^{5}	7.0×10^{3}
E. Coli (cfu/ g)	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$
Enterobacteria (cfu/ g)	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$	$< 10^{1}$

By the second day of fermentation, the epiphytic LAB predominantly grew in all FGJs. Its level reached 10^7 cfu/g in timothy and corn FGJs. While alfalfa FGJ contained the highest number of LAB (5.8×10^8 cfu/g), which may explain the highest content of lactic acid in alfalfa FGJ. Molds level ranged from10^2 to 10^3cfu/g, and the level decreased by the second day of fermentation. Yeast level ranged from 10^4 to 10^5 cfu/g. The level decreased only in timothy FGJ in contrast with the results of Masuko et al. (2002) where the yeast's level in timothy FGJ was increased with the days of fermentation.

Both control and FGJs treated silages were well-preserved silages. The pH value and NH_3-N content did not increase more than 3.79, 4.9% TN, respectively. The efficient conservation of untreated corn silage was possibly attributed to the fact that the number of epiphytic LAB on whole plant corn before ensiling was relatively high. It was at a level of 4.2×10^4 cfu/g, which is higher than that reported in a survey by Speckman et al. (1981) of LAB on corn in 533 fields showed that 42, 63, 69 and 77% of samples had counts below 100, 500, 1000 and 2000 cfu/g of fresh material, respectively.

Variations in the numbers of LAB in crops could be due to some or all of the following; increased use of silage bacterial inoculants, climatic variations, and differences in counting media (Oladosu et al., 2016; Bernardes et al., 2018; Coblentz et al., 2018; Muck et al., 2018; Queiroz et al., 2018). The addition of FGJs during the ensiling process did not induce differences between the chemical composition of treated and untreated corn silage. The addition of FGJ prepared from corn resulted in a slight improvement of the fermentation characteristics of corn silage. This could be related to the fact that the fundamental substrates for epiphytic LAB were not different between corn and corn juice.

The Flieg's point and V-score of alfalfa FGJ-treated silage were higher than those of control silage. However, adding alfalfa FGJ did not improve the quality of corn silage. Moreover, the addition of FGJs to corn silage decreased the acetic acid concentration. These differences could be attributed to the fact that the efficiency of biological additives varies with the chemical and microbiological composition of the fresh crops and environmental conditions.

Speculated that inoculation of silage with LAB might improve aerobic stability via competitive suppression of yeasts; however, results with experiments with bacterial inoculants have indicated positive (Philips and Pendlum, 1984; Su et al., 2017; Kahyani et al., 2019a,b; Wang et al., 2021), negative (Rust et al., 1989; Wang et al., 2018; Coblentz et al., 2022), or no effects (Schaefer et al., 1989; Sanderson, 1993; Thomas et al., 2013) on aerobic stability of silage. However, FGJ should be viewed primarily as fermentation aids. In this study a little research has been done concerning the aerobic stability of corn silage. Enumeration of epiphytic microorganisms of corn silage after opining showed that; both molds and enterobacterial growth were depressed in all silages. Corn and alfalfa FGJs treated silages had lower aerobic bacterial count than that of the control, while the lowest level of yeast was detected in timothy FGJ treated silage.

In conclusion, comparing the fermentation characteristics of the three FGJs, the epiphytic LAB predominantly grew in all FGJs, and its level reached to 10^7 cfu/g in timothy and corn FGJs. While alfalfa FGJ contained the highest number of LAB 5.8×10^8 cfu/g. The highest lactic acid content was found in alfalfa FGJ, which subsequently resulted in the highest total acid content. However, alfalfa FGJ recorded the highest pH value, and this may be ascribed to the high buffering capacity of alfalfa. Aerobic bacteria and molds levels were decreased after 2 days of fermentation in all FGJs. On the contrary, yeast tended to increase in both corn and alfalfa FGJs while it decreased only in timothy FGJ. Both control and FGJs treated silages were well-preserved silages. The pH value and NH_3-N content did not increase more than 3.79, 4.9% TN, respectively. Corn FGJ treated silage has lower pH value, lower NH_3-N/TN, and higher Flieg's point and V-score than the control silage. Both molds and enterobacterial growth were depressed in all silages. Corn and alfalfa FGJs treated silages had lower aerobic bacterial count than that of the control. While the lowest level of yeast was detected in timothy FGJ treated silage. The data obtained in this trial suggested that the addition of FCJ to properly ensiled corn may be of questionable value. Therefore, data obtained in this trial suggested that the addition of FCJ to properly ensiled corn may be of questionable value.

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Conflicts of Interest Statement

The authors have no conflicts of interest to disclose.

Author Contributions

LS: concept, design and writing the manuscript draft. LS and KA: practical work. MT: revised and edited the manuscript draft. All authors revised and approved the final manuscript for publication.

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