Evaluation of Fermentation Characteristics and Nutritive Value of Green Tea Waste Ensiled with Byproducts Mixture for Ruminants

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ABSTRACT : In this study, the possibility of green tea waste (GTW) as a new ingredient of byproducts-mixed silage was investigated. Characteristics of GTW were low in dry matter (DM) content (20%), and high in crude protein (30 to 36%) and tannins (8.5%). The GTW was added to mixed silages composed of tofu cake, rice straw and rice bran that are locally available in Japan. In experiment 1, the effect of GTW addition to silage made from various patterns of byproducts mixture based on tofu cake was studied. In experiment 2, the effect of GTW addition and storage temperature on fermentation characteristics, nutrient contents and *in vitro* ruminal gas production of byproducts-mixed silages were examined. In experiment 1, GTW addition on tofu cake accelerated acetic, propionic and butyric acid accumulation in the silage. When rice straw was mixed with tofu cake, DM content was increased from 47 to 56%, lactic acid was the main acid and the pH was decreased below 4.2. In this case, GTW addition to those mixtures did not affect acid concentrations of the silage. In experiment 2, GTW addition to the byproducts mixture increased lactic acid concentration, decreased the pH and DM loss of the silages. In GTW treatments, tannin concentration was lower in the silage stored at 30°C than 15°C. Addition of GTW into the silage also increased *in vitro* ruminal gas production. It was concluded that addition of GTW into byproducts-mixed silage enhanced lactic acid fermentation when there were insufficient materials for lactic acid production. Utilization of GTW as an ingredient in mixed silages would be effective in enhancing fermentation characteristics, lowering tannin content and *in vitro* ruminal gas production. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 4 : 533-540)***

Key Words : Green Tea Waste, Byproducts, Mixed Silage, Lactic Acid, Tannin

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INTRODUCTION

Tea is one of the most popular beverages in the world (Graham, 1992), and more than 3 million tons of tea leaf were produced in 2003 (FAO, 2004). Consumption of ready-made tea drinks such as green tea has increased markedly in recent years in Southeast and East Asia. In Japan, beverage companies which manufacture various ready-made tea drinks produce about 100,000 tons of tea waste annually. Most of the waste is burned, dumped into landfills or used as compost. Tea leaves contain a variety of amino acids, proteins, vitamins, tannins and polyphenols (Yamamoto et al., 1997). After extraction of the tea drink, green tea waste (GTW) contains 22 to 35% of crude protein (CP) (Yang et al., 2003; Kondo et al., 2004a). These reports suggest that tea waste may have potential as a feedstuff. It might be utilized as an efficient feed-resource with environmental benefits, and the method should be developed.

GTW has a low dry matter (DM) content; therefore it deteriorates readily after being released as a byproduct from tea drink companies. Ensiling is a suitable method to preserve food industrial byproducts which contain low DM, such as tofu cake, orange pulp, wet brewers' grains, etc. (Niwa et al., 1995a, b; Megias et al., 1998; Mustafa et al., 2001; Nishino et al., 2003). However, making silages with low DM also has a risk of effluent production during ensiling. Therefore, silages made from a mixture of low DM byproducts with other dry feedstuffs such as rice straw and rice bran, would be an effective way to prevent loss of nutrients from byproducts. In addition, byproducts containing anti-nutritional factors and unpalatable components might be able to supplement mixed silages if their factors and odours were lessened during silage fermentation.

Our previous reports showed that GTW addition to forage ensiling enhanced lactic acid fermentation and lowered pH of the silage (Kondo et al., 2004b, c). These characteristics might be applied to food- and agro-industrial byproducts. However, there were few reports that showed the effects of GTW addition to several kinds of byproducts-mixed silage. Therefore, the objective of this study was to investigate the effects of GTW addition to mixed silages from food- and agro-industrial byproducts on fermentation characteristics and *in vitro* ruminal gas production.

MATERIALS AND METHODS

Silage preparation

Silages were made from locally available byproducts; tofu cake, rice straw, rice bran and GTW. Rice straw was chopped into lengths of about 2 to 3 cm by a forage chopper. Tofu cake and GTW used in both experiments were produced by tofu and tea companies, respectively, in the morning of the day of silage preparations. These byproducts

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Table 1. Ingredients of mixed silages (% FM)

Treatment	Tofu cake	Rice straw	Rice bran	GTW
Experiment 1				
TC	100	0	0	0
TC+GTW	90	0	0	10
TC+RB	90	0	10	0
TC+RB+GTW	80	0	10	10
TC+RS	60	40	0	0
TC+RS+GTW	50	40	0	10
TC+RB+RS	50	40	10	0
TC+RB+RS+GTW	40	40	10	10
Experiment 2				
Control silage	50	40	10	0
GTW silage	40	40	10	10

FM: fresh matter, TC: tofu cake, GTW: green tea waste, RB:rice bran, RS: rice straw.

were mixed thoroughly in the proportions shown in Table 1. The proportion of GTW in the silage was set to avoid a decrease of feed intake by ruminants (Kondo et al., 2004a). The mixed materials were packed into polyethylene bags (250 mm×350 mm×0.06 mm) in triplicate, and tied with a string after removing air by a vacuum pump. The ensiled amounts in experiment 1 and 2 were 500 g and 900 g, respectively. Storage temperature was set at 25°C in the experiment 1 to maintain steady fermentation as a preliminary trial, and at 15°C and 30°C in experiment 2. The former was assumed as an annual average temperature and the latter was set as a mean value of daily maximum temperature in summer. Silages were opened on day 30 in experiment 1, and on day 0, 5, 10 and 30 in experiment 2.

In vitro ruminal gas production

In experiment 2, *in vitro* ruminal gas production from mixed byproducts and their silages was determined according to Menke et al. (1979). Rumen fluid was collected from three castrated Japanese goats fed 720 g/d hay and 180 g/d commercial concentrates that were offered in equal proportions twice daily. Freeze-dried samples (200 mg) passed through a 1 mm screen were weighed into 100 ml calibrated glass syringe. Syringes were filled with 30 ml medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution, then incubated in a water bath at 39°C in triplicate and gas production was measured for 96 h.

Chemical analysis

DM content was determined by oven drying at 60°C for 48 h. Gross energy (GE) was measured by a bomb calorimeter (C-4A, Shimazu Co., Japan,). Buffering capacity of materials was measured by titration (Playne and McDonald, 1966) using the dried sample. Total nitrogen (N), CP and ether extract was determined by standard methods (AOAC, 1984). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as outlined by Van Soest et al. (1991). Water-soluble carbohydrate (WSC) concentrations were determined using the anthrone reaction rate assay (Koehler, 1952). In brief, carbohydrates were extracted using 80% aqueous ethanol. The extract was reacted with anthrone-sulfuric acid after the ethanol was removed by boiling and then deproteinized with 0.3 N of barium hydroxide and 5% zinc sulfate. Total extractable phenolics (TEPH), total extractable tannins (TET) and condensed tannins (CT) were analyzed by the methods of Makkar and Goodchild (1996) after extraction with 70% aqueous acetone. The concentration of TEPH was determined using the Folin Ciocalteu method and the regression equation for a tannic acid standard. TET was estimated indirectly after being absorbed to insoluble polyvinyl polypyrrolidone (PVPP). The concentration of TET was calculated by subtracting the TEPH remaining after PVPP treatment from the original TEPH estimation. CT were measured using 2% ferric ammonium sulphate in 2 N hydrochloric acid (HCl) and butanol-HCl (95:5, v/v) as described Makkar and Goodchild (1996). TEPH, TET and CT were measured colorimetrically using a spectrophotometer (UV-1200, Shimadzu Co., Japan). Viable counts of lactic acid bacteria (LAB) were measured using MRS agar (Difco, Sparks, MD, USA) plate as described by Nishino et al. (2004). Silage (20g) was macerated with 200 ml of distilled water. The macerate was filtered and the filtrate was used to determine pH, lactic acid, volatile fatty acids (VFA), and ammonia nitrogen (NH_3-N). The pH values were measured potentiometrically, and lactic acid concentrations colorimetrically (Barnett, 1951). VFA concentrations of silage were detected with a gas chromatograph (GC-12A, Shimadzu Co., Japan) using a FAL-M column (Shimadzu Co., Japan) after deproteinization with trichloroacetic acid (final concentration: 5%). NH_3-N of silage was determined by steam distillation and titration with sulphuric acid.

Statistical analysis

In experiment 1, data were analyzed by a one-way analysis of variance (ANOVA) and tested using Duncan's new multiple range test, performed with the Statistical Analysis System (1982). In experiment 2, the interaction and main effects of GTW and temperature, GTW and ensiling were analyzed by a two-way ANOVA and tested using Student's t-test, performed with the Statistical Analysis System (1982). Data of pH, lactic acid concentration and LAB counts during ensiling in experiment 2 were also analyzed by a two-way ANOVA and tested using Duncan's new multiple range test, performed with the Statistical Analysis System (1982).

RESULTS AND DISCUSSION

Chemical composition of byproducts and the mixture

Table 2 shows the chemical composition and LAB count of each byproduct. The characteristics of tofu cake and

Table 2. Chemical composition and lactic acid bacteria (LAB) counts of ingredients in mixed silages

	Tofu cake	Rice straw	Rice bran	GTW	Control silage	GTW silage
Experiment 1						
Dry matter (DM, %)	17.9	89.7	90.3	19.5		
Water-soluble carbohydrate (% DM)	0.9	6.3	10.4	0.1		
Buffering capacity (meq/kg DM)	336	173	340	206		
Crude protein (% DM)	26.0	6.4	15.2	29.2		
Lactic acid bacteria (log$_{10}$cfu g/FM)	9.55	-	-	8.21		
Experiment 2						
DM (%)	20.1	87.8	88.2	17.7	55.7	53.8
Water-soluble carbohydrate (% DM)	4.8	7.2	15.5	0.2	10.0	9.5
Buffering capacity (meq/kg DM)	376	179	388	205	310	303
Crude protein (% DM)	30.7	4.3	17.2	35.5	11.8	12.6
Ether extract (% DM)	12.9	1.7	22.3	6.5	8.8	8.8
NDF (% DM)	-	-	-	-	50.6	50.6
ADF (% DM)	-	-	-	-	29.3	29.1
TEPH (% DM)	0.35	0.54	0.92	9.73	0.51	0.87
TET (% DM)	0.22	0.25	0.24	8.50	0.10	0.37
CT (% DM)	ND	ND	ND	2.37	ND	0.11
Gross enegry (MJ/kg DM)	21.8	17.7	23.8	21.5	19.4	19.5
Lactic acid bacteria (log$_{10}$ cfu g/FM)	7.32			8.07		

GTW: green tea waste, FM: fresh matter, TEPH: total extractable phenolics, TET: total extractable tannins, CT: condensed tannins, -: not determined, ND: not detected.

Table 3. Fermentation characteristics of mixed silages stored for 30 days at 25°C in experiment 1

Treatment	DM	pH	Organic acid	Lactic acid	Acetic acid	Propionic acid	Butyric acid	NH$_3$-N
	%		--	% DM	---------------------------------			% TN
TC	16.6^{d}	4.64^{a}	5.29^{c}	0.16^{c}	2.39^{b}	0.47^{b}	2.28^{b}	0.93^{e}
TC+GTW	16.4^{d}	4.39^{b}	11.40^{a}	0.63^{c}	4.78^{a}	2.56^{a}	3.42^{a}	1.02^{e}
TC+RB	24.3^{c}	4.81^{a}	5.51^{c}	2.76^{b}	2.08^{bc}	0.20^{bc}	0.46^{d}	2.31^{c}
TC+RB+GTW	25.1^{c}	4.70^{a}	7.95^{b}	4.34^{a}	1.68^{bc}	0.15^{bc}	1.78^{c}	2.25^{c}
TC+RS	47.3^{b}	4.11^{c}	5.60^{c}	4.61^{a}	0.99^{c}	ND	ND	2.37^{c}
TC+RS+GTW	47.5^{b}	4.07^{c}	5.87^{c}	4.15^{a}	1.72^{bc}	ND	ND	1.94^{d}
TC+RB+RS	55.7^{a}	4.18^{c}	5.96^{c}	4.69^{a}	1.15^{bc}	0.04^{c}	0.08^{de}	3.28^{a}
TC+RB+RS+GTW	55.5^{a}	4.15^{c}	5.21^{c}	3.81^{a}	1.38^{bc}	0.02^{c}	0.01^{e}	2.84^{b}
SEM	0.4	0.06	0.61	0.30	0.40	0.12	0.14	0.08

TC: tofu cake, GTW: green tea waste, RB: rice bran, RS: rice straw, TN: total nitrogen, ND: not detected.

$^{a, b, c}$ Means with the same letter in a column are not significantly different.

GTW were low in DM and WSC, and high in CP and LAB counts. Buffering capacity of rice straw and GTW were relatively low compared with forage, while tofu cake and rice bran showed high buffering capacity (McDonald et al., 1991). The WSC content of tofu cake differed widely between experiments. These differences between experiments 1 and 2 were expected to have some effects on silage fermentation. Rice straw and rice bran could supply a large quantity of DM and WSC, respectively. Fermentable carbohydrates in a form of WSC are necessary in silage fermentation as a carbon source for microorganisms. Low DM and WSC in tofu cake and GTW could be compensated by adding rice straw and rice bran. Ether extract was high in tofu cake and rice bran, low in rice straw and GTW. As shown in Table 2, GTW contained high levels of TEPH, TET and CT; however, these tannin contents were diluted in the mixture. As a feed resource, it seems that GTW has several similar points (eg. high tannin and CP content) to tropical legume leaves, such as *Acacia spp*., *Gliricidia spp*., *Sesbania spp*. and so on (Rubanza et al., 2003; Evitayani et al., 2004; Osuga et al., 2005). In experiment 2, WSC, buffering capacity, CP, ether extract, NDF and ADF content were similar in both treatments before ensiling, but phenolic compounds were higher in the GTW treatment.

Fermentation characteristics of silage

Fermentation characteristics of the silages in experiment 1 are shown in Table 3. GTW addition to tofu cake + rice bran enhanced lactic acid production ($p<0.05$). This result supports our previous reports that GTW addition to forage ensiling increased lactic acid (Kondo et al., 2004b, c). The mixture of tofu cake and rice bran in this study and forage in our previous studies (Kondo et al., 2004b, c) had low DM and adequate amount of WSC in common. However,

Table 4. Fermentation characteristics and DM loss of mixed silages included with or without green tea waste (GTW) stored for 30 days at 15°C and 30°C in experiment 2

	15°C		30°C		SEM	GTW effect	Temp.effect	Interaction
	Control silage	GTW silage	Control silage	GTW silage				
Dry matter (DM, %)	51.8	51.7	51.9	51.7	0.6			
pH	5.09	4.12[1]	4.35[2]	4.11[1]	0.12	***	*	*
Lactic acid (% DM)	1.27	2.37[1]	2.25[2]	2.61[1]	0.08	***	***	**
Acetic acid (% DM)	0.45	1.13	0.89	1.56	0.11	***	**	
Propionic acid (% DM)	ND	ND	ND	ND				
Butyric acid (% DM)	ND	ND	ND	ND				
NH_3-N (% TN)	3.59	2.91	5.97	5.47	0.22	*	***	
DM loss (%)	8.84	5.51	10.50	7.44	2.12	*		

TN: total nitrogen, ND: not detected. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

[1] Control silage vs. GTW silage in the same temperature ($p<0.05$), [2] 15°C vs. 30°C in control silage or GTW silage ($p<0.05$).

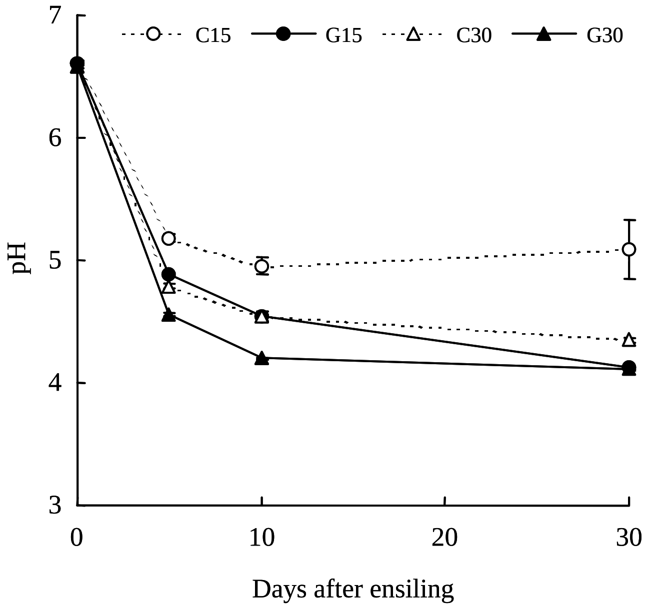

Figure 1. Changes of pH during the fermentation of mixed silages included with or without GTW. C15: Control silage stored at 15°C, G15: GTW silage stored at 15°C. C30: Control silage stored at 30°C, G15: GTW silage stored at 30°C.

GTW addition to tofu cake + rice bran silage showed higher butyric acid despite a high lactic acid content. This result was different to previous reports (Kondo et al., 2004b, c). When tofu cake was ensiled with rice straw, the pH was lower than 4.2 and there were no effects from addition of rice bran and GTW (Table 3). These results indicated that WSC supply was enough for lactic acid production and lowering pH by addition of rice straw in this experiment. Addition of GTW to tofu cake decreased pH compared to ensiled tofu cake only ($p<0.05$); this was due to higher acetic, propionic and butyric acid concentrations by GTW addition. It was expected that acetic acid production could be accelerated by GTW addition when ensiling materials lacked WSC and were low in DM. GTW addition to tofu cake, tofu cake+rice bran silage increased organic acid production (sum of lactic, acetic, propionic and butyric acids) ($p<0.05$); those silages had low DM contents (16 to 25%). However, acid production was not affected by GTW addition to high DM silage (47 to 56%), such as tofu cake + rice straw and tofu cake + rice straw + rice bran treatment ($p>0.05$). The differences in the ratio of NH_3-N to total nitrogen (% TN) in byproducts-mixed silages in this experiment were small (0.9 to 3.3% TN) and these values were relatively low compared to grass and legume silage (eg. 5 to 15% TN) (Zhu et al., 1999; Nguyen et al., 2004). This indicated that the rate of protein degradation of these silages was low in this experiment. Changes in the nitrogen fraction of grass silage were highly dependent on the DM content (Nguyen et al., 2005), that is, protein degradation was high in low DM silage, while low in high DM silage. However, the ratio of NH_3-N of byproduct-mixed silages in this experiment was not depending on their DM content. This is agreement with Nishino et al. (2003) who showed that silage of wet brewers' grains had a low NH_3-N content (about 1% TN). The low NH_3-N ratio of byproducts silage would be due to these byproducts containing less soluble and easily fermentable proteins for degradation by microorganisms during ensiling than fresh forages.

Fermentation characteristics and time course changes in pH, lactic acid and LAB counts in experiment 2, are given in Table 4 and Figures 1, 2 and 3, respectively. The pH values of control silage at 15°C declined slowly. On the other hand, the pH values of GTW-treated silage at 15°C and both control- and GTW-treated silages at 30°C decreased rapidly. Under both temperature conditions, GTW addition lowered pH compared to control silage at an early stage of the storage ($p<0.05$). From day 0 to 10 of ensiling, the higher temperature promoted lactic acid production enhanced by GTW addition and lowered the pH of silages, but not on day 30 (Figure 2). Lactic acid was the main fermentation product in both treatments, and its concentration was significantly higher in GTW silage than in the control ($p<0.05$) as shown in Table 4. However, the numbers of LAB at an early stage of the storage were similar in both treatments and lactic acid production was

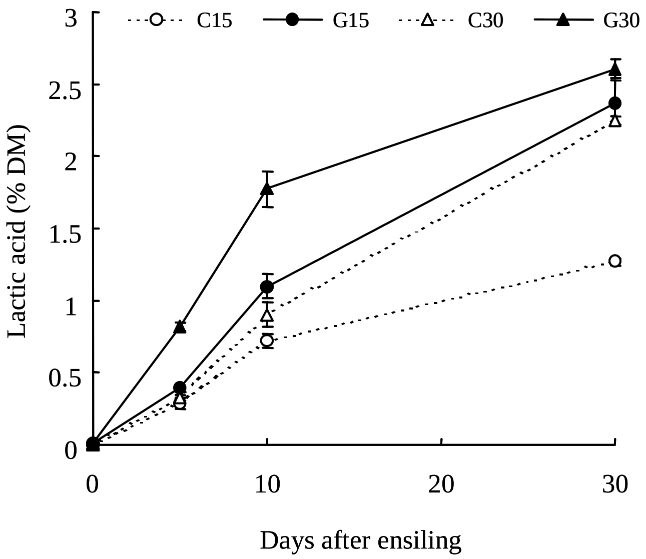

Figure 2. Changes of lactic acid concentration during the fermentation of mixed silages included with or without GTW. C15: Control silage stored at 15°C, G15: GTW silage stored at 15°C. C30: Control silage stored at 30°C, G15: GTW silage stored at 30°C.

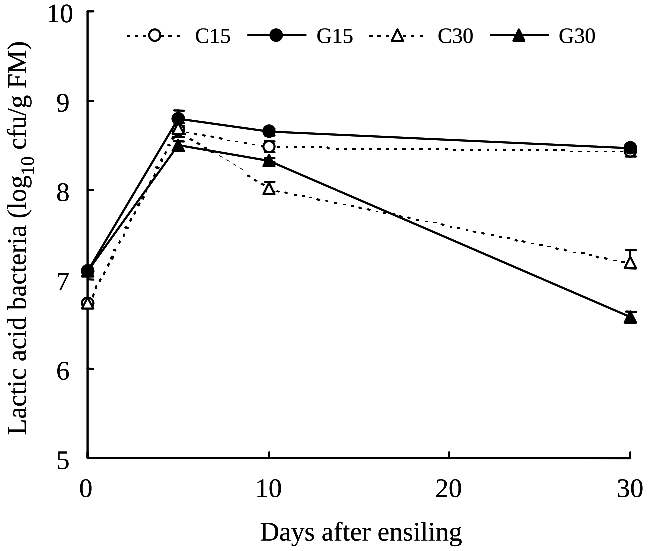

Figure 3. Changes of lactic acid bacteria counts ($\log_{10}$ cfu/g FM) during the fermentation of mixed silages included with or without GTW. C15: Control silage stored at 15°C, G15: GTW silage stored at 15°C. C30: Control silage stored at 30°C, G15: GTW silage stored at 30°C.

active throughout the silage fermentation period (Figures 2 and 3). Therefore, it is presumed that GTW addition affects not the number of LAB but the flora of microorganisms in this study. On day 30, the pH value and lactic acid production showed the interaction of GTW×temperature, that is, the temperature affected pH and lactic acid concentration of control silage, but not of GTW-treated silage as shown by the Student's t-test analysis in Table 4. This could be due to higher temperature promoting the activity of microorganisms and lactic acid production of control silage. Tamada et al. (1999) and Zahar et al. (2002) also made similar observation. However, when GTW was included in silage, the promotion by higher temperature was not effective on day 30 because GTW addition was sufficient to enhance lactic acid production and decrease pH even at lower temperature. Acetic acid concentrations were also increased by the addition of GTW ($p<0.05$) (Table 4). The results (see Figure 3) indicated that GTW addition could promote acid production such as lactic acid and acetic acid independently of the number of LAB. Green tea leaf is rich in polyphenols and manganese (Yamamoto et al., 1997). Hara (1997) and Ishihara et al. (2001) reported that animals fed a diet containing green tea polyphenols maintained high counts of *Lactobacillus spp.* in the intestinal microflora, but decreased the counts of clostridia. Fitzpatrick et al. (2001) reported that *Lactobacillus casei* converted sugars to lactic acid more quickly from whey permeate following manganese addition. These chemical characteristics may affect microflora and/or activated microbial metabolism that could promote acid production during silage fermentation. Butyric acid was not detected in all the treatments. Clostridia are known to produce butyric acid in silages (McDonald et al., 1991). In the present study, since DM of mixed silage was set at a level of 52%, growth of clostridia that are highly depending on moisture could be suppressed (McDonald et al., 1991). The ratio of NH_3-N was significantly higher at 30°C than 15°C in this study. This result could be due to enhanced microbial activity at 30°C. The DM loss during fermentation was decreased by addition of GTW and tended to be higher at 30°C storage than 15°C (Table 4). This is in agreement with Cai et al. (1998, 1999) who showed that lower pH and lower storage temperature suppressed DM loss of silages.

Chemical composition and ruminal gas production of silage in experiment 2

Table 5 shows the chemical composition of mixed silages after 30 days of storage in experiment 2. CP, EE, NDF and ADF contents were not affected by the addition of GTW and storage temperature ($p>0.05$). However, tannin contents, such as TEPH, TET, and CT, were higher in GTW silage than in the control ($p<0.05$). In GTW silages, TET content was significantly lower ($p<0.05$), and TEPH and CT contents tended to be lower ($p<0.065$) at 30°C storage than 15°C, indicating that tannins could be degraded by silage microorganisms which are activated at 30°C. Osawa et al. (2000) identified lactobacilli producing a tannin-degrading enzyme from fermented food. It is concluded that some lactobacilli in mixed silage in this experiment also produced a tannin-degrading enzyme which would have decreased

Table 5. Chemical composition of mixed silages included with or without green tea waste (GTW) stored for 30 days at 15°C and 30°C in experiment 2

	15°C		30°C		SEM	GTW effect	Temp.effect	Interaction
	Control silage	GTW silage	Control silage	GTW silage				
Crude protein (%DM)	12.5	12.7	12.8	13.1	0.45			
Ether extract (% DM)	9.1	9.2	9.7	9.3	0.33			
NDF (% DM)	55.2	54.4	52.5	54.4	0.97			
ADF (% DM)	33.0	32.4	31.5	32.4	0.59			
TEPH (% DM)	0.55	0.84	0.60	0.83	0.03	***	+	
TET (% DM)	0.15	0.34	0.13	0.23	0.03	***	*	
CT (% DM)	ND	0.08	ND	0.06	0.01		+	
Gross energy (MJ/kg DM)	19.1	19.1	19.7	19.8	0.18		**	

TEPH: total extractable phenolics, TET: total extractable tannins, CT: condensed tannins, ND: not detected.
+ p<0.065, * p<0.05, ** p<0.01, *** p<0.001.

Table 6. Measured cumulative *in vitro* gas production (ml/200 mg DM) of byproducts-mixed silage before and after storage at 15°C and 30°C in experiment 2

Treatment	Storage temperature	Hours of incubation							
		3	6	9	12	24	48	72	96
Before ensiling									
Control silage		9.9	13.9	17.5	20.4	25.6	31.5	35.1	37.0
GTW silage		11.7	17.2	20.0	22.8	28.9	34.2	37.7	39.6
30 days after ensiling									
Control silage	15°C	10.0	13.0	15.7	17.2	22.6	27.9	31.5	33.0
GTW silage	15°C	12.5	16.6	19.5	21.6	27.4	32.9	36.8	38.7
Control silage	30°C	9.1	13.1	14.8	16.8	21.4	29.0	31.4	32.5
GTW silage	30°C	10.4	14.4	18.1	20.6	25.8	31.7	34.8	36.7
SEM		0.7	0.8	1.0	1.3	1.4	1.8	1.9	1.7
Statistical significance (two-way ANOVA): p =									
Main effects									
GTW addition		0.016	0.003	0.004	0.015	0.004	0.054	0.059	0.026
Ensiling		0.730	0.140	0.007	0.047	0.034	0.133	0.120	0.062
Interactions		0.942	0.612	0.543	0.475	0.619	0.746	0.611	0.455
Statistical significance* (two-way ANOVA): p =									
Main effects									
GTW addition		0.007	0.011	0.004	0.013	0.005	0.089	0.071	0.047
Storage temperature		0.099	0.115	0.131	0.446	0.153	0.761	0.437	0.389
Interactions		0.286	0.206	0.856	0.868	0.916	0.602	0.735	0.804

* Two-way ANOVA was performed in the data set of after ensiling.

tannin concentration during ensiling. Table 6 shows *in vitro* gas production from mixed byproducts before and after ensiling; gas production tended to be higher before ensiling than after. This could be due to the loss of fermentable substrates for ruminal gas production during ensiling. In this study, gas production was higher in GTW-treated silage than control, which is in agreement with Kondo et al. (2004b). Since GTW silage maintained a lower pH from 5 days after ensiling than control silage it would be expected that GTW silage could preserve more fermentable substrates during ensiling than control silage. There are many reports that tannins suppress the activity of ruminal bacteria (Jones et al., 1994), and decrease ruminal gas production *in vitro* (Makkar et al., 1995, Tolera et al., 1997). However, the results in this study did not show any detrimental effect on gas production from GTW silage. It suggests that the amount of tannin in GTW-treated silage was too low to suppress the activity of rumen bacteria.

CONCLUSIONS

GTW addition to a byproduct-mixed silage could enhance acid production but the effect was dependent on the mixture of materials. When addition of rice straw to tofu cake ensiling was enough to improve lactic acid production, GTW did not show any effects on silage fermentation (experiment 1). On the other hand, when addition of rice straw and bran were insufficient for acid production, GTW enhanced acid production, and lowered pH and DM loss (experiment 2). It was also demonstrated that the decrease in tannin contents was dependent on storage temperature, that is, tannin content was lower in silage stored at 30°C than at 15°C. GTW addition to mixed silage also increased *in vitro* ruminal gas production. Judging from the fermentation characteristics, and *in vitro* ruminal gas production, utilization of GTW as an ingredient in mixed silage would be effective. Further research should be done to investigate the effects of GTW addition on feed intake and animal performance.

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