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Effects of Different Additives on Fermentation Characteristics and Protein Degradation of Green Tea Grounds Silage

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ABSTRACT : This study evaluated the fermentation characteristics and protein degradation dynamics of wet green tea grounds (WGTG) silage. The WGTG was ensiled with distilled water (control), or lactic acid bacteria (LAB), enzyme (E), formic acid (FA) and formaldehyde (FD) prior to ensiling. Three bag silos for each treatment were randomly opened at 0, 3, 7, 14, 28 and 60 days after anaerobic storage. For all the treatments, except for FA, there was a rapid decline in pH during the first 7 days of ensiling. LAB treatment had higher lactic acid content, lower ammonia-N (NH_3-N) and free-amino nitrogen (FAA-N) contents than other treatments ($p<0.05$). E treatment had higher lactic acid, water-soluble carbohydrates (WSC) and non-protein nitrogen (NPN) content than the control ($p<0.05$). FA treatment had higher NH_3-N and FAA-N content than the control ($p<0.05$). FD treatment had lower NPN and FAA-N content than the control, but it did not significantly inhibit the protein degradation when compared to LAB treatment ($p>0.05$). Results indicate that LAB treatment had the best effect on the fermentation characteristics and protein degradation of WGTG silage. (**Key Words :** Wet Green Tea Grounds, Additives, Microbial Population, Protein Degradation)

INTRODUCTION

In China, consumption of ready-made tea drinks in bottles, packs and cans has been increasing significantly over recent years (Hu, 2010). Consequently, a large mount of tea leaves grounds are released annually by beverage companies manufacturing various tea drinks. Although a small amount of tea grounds are converted into raw compost material, most are generally buried. There is increasing demand for efficient use of food by-products due to economic and environmental concerns.

Green tea grounds are usually rich in crude protein (CP), amino acids, catechins such as epigallocatechin-3-gallate, epicatechin, epigallocatechin and epicatechin gallate, and vitamins (Nishino et al., 2007; Xu et al., 2007). Therefore, wet green tea grounds (WGTG) may be a potential feed resource. However, there are some problems with the use of WGTG as feed, such as high moisture and CP and low water-soluble carbohydrates (WSC) all of which result in poor preservation. In order to solve such problems, many researchers focused on including different additives or adjusting moisture with dry feeds. Kondo et al. (2004b) investigated the effects of adding green tea grounds on the ensiling of forage. Xu et al. (2007) reported that total mixed ration silages in which WGTG was substituted for wet brewers' grains were well preserved. There are few reports evaluating microbial population changes (Kondo et al., 2004b; Nishino et al., 2007) while the protein degradation dynamics of wet green tea grounds has not been studied.

The purpose of this study was to evaluate the fermentation characteristics, microbial population changes, and protein degradation dynamics of high-moisture content WGTG silage treated with lactic acid bacteria (LAB), enzyme (E), formic acid (FA) and formaldehyde (FD).

MATERIALS AND METHODS

Silage preparation

Wet green tea grounds were obtained from a commercial beverage factory (Kinmalo Drink (Beijing) Co. Ltd., Beijing, China). Silages were prepared using a small-scale system of silage fermentation (Xu et al., 2008). Approximately 100 g WGTG were packed into plastic film bags (Hiryu KN type, 200 by 300 mm, Asahikasei) and the bags sealed with a vacuum sealer (BH950, Matsushita). The silage treatments were designed as follows: treated with distilled water (control); addition of lactobacillus plantarum Chikuso-1 (LAB, Snow Brand Seed Co. Ltd., Sapporo,

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Japan) at a rate of 5mg/kg to supply 1.0×10^5 colony forming units (cfu)/g of lactic acid bacterial per gram of fresh material; addition of enzyme E (a complex of cellulase and hemicellulase, Snow Brand Seed Co. Ltd., Sapporo, Japan) at a rate of 20 mg/kg of WGTG; addition of formic acid (FA, 99%, Beijing Chemical Industries., Beijing, China) at a rate of 5 g/kg of WGTG; addition of formaldehyde (FD, 99%, Beijing Chemical Industries., Beijing, China) at a rate of 1 g/kg of WGTG. The experiment was carried out in a complete randomized design. Total eighteen silos per treatment were prepared and stored at room temperature (20 to 25°C). Triplicate silos were randomly opened at 0, 3, 7, 14, 28 and 60 days during ensiling, respectively. They were used to measure fermentation quality, microbial population changes and protein degradation dynamics.

Chemical and microbial analysis

The fresh and ensiled green tea grounds were dried in a forced draught oven at 60°C for 48 h and ground to pass a 1mm screen with a Wiley mill. Dry matter (DM), CP, ether extract (EE) and organic matter (OM) were analyzed according to methods 934.01, 976.05, 920.39 and 942.05, respectively of AOAC (1990). Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were analyzed by the methods described by Van Soest et al. (1991). WSC concentration was determined using the method of McDonald and Henderson (1964).

Fermentation qualities were determined by measuring fermentation products in cold water extracts of the silage. Wet silage (10 g) randomly collected from the silo was homogenized with 90 ml of sterilized distilled water and stored at 4°C overnight (Xu et al., 2007). The pH was measured with a glass electrode pH meter (Model HI9024, Hanna Instruments Italia Srl, Italy). An aliquot of 5 ml (250 g/L, w/v) trichloroacetic acid (TCA) was added to 20 ml of the filtrate to precipitate protein (Guo et al., 2008). After centrifugation (18,000×g, 15 min, 4°C), the supernatant was analyzed for ammonia-N (NH_3-N) and free-amino nitrogen (FAA-N) by the method of Broderick and Kang (1980) and non-protein nitrogen (NPN) by the Kjeldahl method noted earlier. Peptide-N concentration was determined by the increase in AA-N in the TCA supernatant after digesting with 6 N of HCl for 21 h at 105°C under an N_2 atmosphere (Muck, 1987). The concentration of lactic, acetic, propionic and butyric acids was measured by HPLC (Xu et al., 2007). The silage filtrates were shaken with cation exchange resin, centrifuged at 12,000×g for 5 min, passed through a 0.45 μm filter under pressure, and then injected into an HPLC system. The analytical conditions were as follows: column, Hitachi GL C-610H; oven temperature 70°C; mobile phase, 0.2% H_3PO_4, 1.0 ml/min; detector, L-7420 S UV-Vis.

The numbers of microbes were counted from the plate count method (Cai et al., 1998). Colonies were counted from the plates at appropriate dilutions and the number of CFU was expressed per gram of fresh forage.

Statistical analysis

The present study was carried out in a complete randomized design. Silage data were analyzed as 5×6 factorial design (five additive treatments and six ensiling times) with three replications (mini-silos) using PROC MIXED procedure of SAS (SAS Institute Inc., 1997) and significance was declared at the 5% probability level. Additive treatment, ensiling time and interaction between additives and ensiling time were fixed effects. Data on the chemical composition, fermentation characteristics and protein degradation were analyzed for effects of additives, ensiling time and the additive×time interaction with the model:

$$Y_{ijk} = \mu + a_i + t_j + (at)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable under examination for additive i and time j; μ is the overall mean; a_i is the effect of the ith additive; t_j is the effect of the jth ensiling time; $(at)_{ij}$ is the interaction effect between a and t; and ε_{ijk} is the residual error.

RESULTS

Chemical compositions of materials

Table 1 shows the chemical compositions of WGTG. The WGTG had a low DM and WSC content of approximately 15.1% and 1.53% respectively, and a high CP content of 27.4% DM.

Fermentation quality and nutrient content

Figure 1 shows the changes of pH, lactic acid and WSC during ensiling. Silage pH was affected by additives, ensiling time and there was an additive×time interaction (Table 2). All added treatments had lower pH value than the

Table 1. Chemical composition (% dry matter) of wet green tea grounds (WGTG)

Item	WGCG
Dry matter (% fresh material)	15.1±0.8
Organic matter	95.9±0.1
Crude protein	27.4±0.5
Ether extract	2.32±0.2
Acid detergent fiber	36.7±3.1
Neutral detergent fiber	44.5±4.8
Water soluble carbohydrates	1.53±0.2

Means±SD, n = 3.

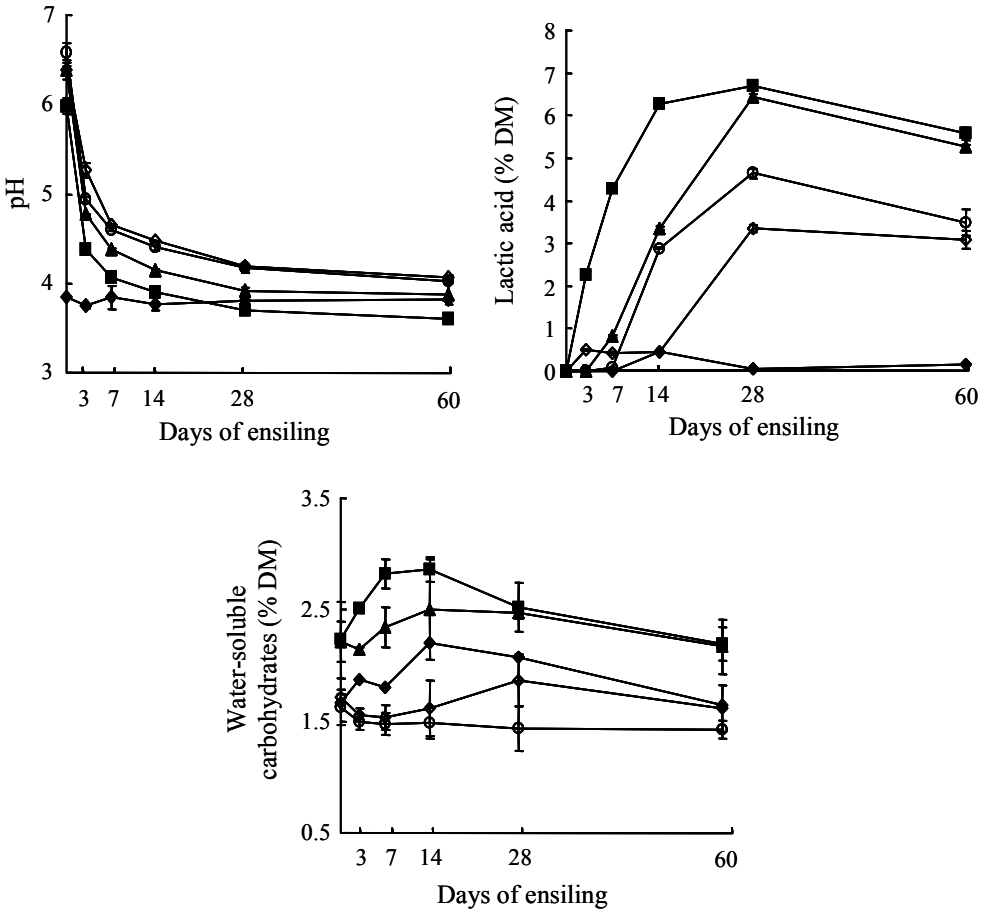

Figure 1. Changes in pH, lactic acid and water-soluble carbohydrates during ensiling of treated with lactic acid bacteria (LAB), enzyme (E), formic acid (FA), formaldehyde (FD) and the control. Control (○); LAB (■); E (▲); FA (♦); FD (◊).

control ($p<0.05$) except for the FD treatment which was higher than the control. There was a rapid decline in pH during the first 7 days for all the treatments then followed by a slow decline until day 60 except for FA where the PH kept relatively stable at about 3.8 at all ensiling periods. Overall, FA and LAB treatments had the lowest ($p<0.05$) pH.

Lactic acid concentration had similar trend during ensiling for all the treatments except for FA where the lactic acid concentration increased slowly for the first 14 days then decreased. LAB treatment had the highest ($p<0.05$) lactic acid content. FA and FD treatments had lower lactic acid concentration than the control at almost all ensiling periods. FA treatment had the lowest ($p<0.05$) lactic acid concentration.

The concentration of WSC increased from 0 to 14 days post-ensiling then decreased to day 60 for all silages except for the FD treated which increased up to day 28, then decreased and the control which decreased slowly to day 28 then kept stable. LAB and E treatments had higher ($p<0.05$) WSC concentration than the other treatments.

Table 2 shows the changes of organic acids during ensiling. The concentrations of acetic acid, propionic acid and butyric acid were affected by additives, ensiling time and their interaction. All the treatments decreased acetic and propionic acid contents at varying extent over the control. Overall, FA treatment had the lowest ($p<0.05$) acetic and propionic acid content and the highest ($p<0.05$) butyric acid content.

Changes in microbial population during the ensiling time

Figure 2 shows lactic acid bacteria, yeast and aerobic bacteria (log cfu/g fresh material) changes during ensiling of WGTG silage. The levels of lactic acid bacteria increased during the first 3 days for all silages except for the FA treated which increased up to day 7. Then the change differed between treatments. FD, LAB and control treatments kept relatively stable, while FA and E treated decreased rapidly to day 60 post-ensiling. LAB treatment had the highest ($p<0.05$) lactic acid bacteria population on day 60 post-ensiling. E and FA had the lowest ($p<0.05$) lactic acid bacteria population especially during day 28 and 60.

Both yeast and aerobic bacteria populations tended to decrease after 3 days of rapid increase for all the treatments.

Table 2. Effects of additive treatments and time of ensiling on chemical compositions and fermentation characteristics of wet green tea grounds (WGTG) silage

	Chemical composition (% DM)			Fermentation characteristics (% DM)				
	DM	CP	WSC	pH	LA	AA	PA	BA
Additives (A)[1]								
Control	15.5[c]	27.4[bc]	1.49[e]	4.79[b]	2.13[c]	0.79[a]	0.63[a]	0.75[b]
LAB	15.5[c]	27.9[a]	2.52[a]	4.27[d]	4.61[a]	0.60[b]	0.59[a]	0.54[e]
E	15.6[bc]	27.6[b]	2.30[b]	4.58[c]	2.64[b]	0.57[c]	0.54[a]	0.72[c]
FA	16.2[a]	26.2[d]	1.88[c]	3.81[e]	0.11[e]	0.24[e]	0.12[c]	0.87[a]
FD	15.7[b]	27.2[c]	1.66[d]	4.83[a]	1.48[d]	0.42[d]	0.30[b]	0.58[d]
SEM[x]	0.063	0.079	0.027	0.013	0.020	0.005	0.031	0.001
Ensiling time (T)[2]								
0	15.7[b]	26.9[c]	1.56[e]	5.83[a]	0.00[f]	0.00[f]	0.12[d]	0.07[f]
3	15.6[b]	26.7[c]	1.92[c]	4.62[b]	0.26[e]	0.19[e]	0.50[b]	0.25[e]
7	15.5[b]	27.2[b]	2.00[b]	4.31[c]	1.12[d]	0.45[d]	0.41[b]	0.51[d]
14	15.5[b]	27.3[b]	2.13[a]	4.14[d]	3.22[c]	0.58[c]	0.44[b]	1.02[b]
28	15.9[a]	27.4[b]	2.07[ab]	3.95[e]	4.23[a]	0.75[b]	0.30[c]	0.94[c]
60	16.0[a]	28.2[a]	1.81[d]	3.88[f]	3.81[b]	1.17[a]	0.85[a]	1.37[a]
SEM[y]	0.069	0.087	0.029	0.014	0.022	0.005	0.034	0.001
p-value								
A	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A×T	0.009	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-f] Means in the same column with different superscripts differ ($p<0.05$).

[1] LAB, E, FA and FD represent WGTG was treated with lactic acid bacteria, enzyme, formic acid and formaldehyde respectively. DM = Dry matter; CP = Crude protein; WSC = Water-soluble carbohydrates; LA = Lactic acid; AA = Acetic acid; PA = Propionic acid; BA = Butyric acid.

[2] 0, 3, 7, 14, 28 and 60 days represent the ensiling time of WGTG silage respectively; SEM = Standard error of the mean, n = 3; p = standard probability, effects of additives, ensiling time and the additive×time (A×T) interaction.

[x] Estimated for the least square means values of ensiling time between treatments.

[y] Estimated for least square means values of ensiling time across all treatments.

All tested treatments decreased the yeast population to a certain extent when compared to the control. E and FD treatments had the lowest ($p<0.05$) yeast population during the most ensiling periods. LAB and FA treatments had the lowest ($p<0.05$) aerobic bacteria population between the 7 and 28 days post-ensiling. E treatment had higher ($p<0.05$) aerobic bacteria population between 28 and 60 days than other added treatments.

Protein degradation of silage

Figure 3 shows the concentrations of total nitrogen (TN), NPN, NH_3-N, FAA-N and peptide-N during ensiling. The NPN content for all the treatments tended to increase during ensiling. LAB treatment had the lowest ($p<0.05$) NH_3-N content at all ensiling periods while FA treatment had higher ($p<0.05$) NH_3-N content than other treatments. The concentration of FAA-N displayed a rapid increase in the early days of ensiling and a sustained increase in later fermentation. E and FA treatments had higher ($p<0.05$) FAA-N content than the control between 3 and 28 days, while FD treatment had lower ($p<0.05$) FAA-N content than the control. Peptide-N concentration for all the treatments increased during the initial period of ensiling, followed by a decline and then increased for the duration of the fermentation. The largest changes in all the nitrogen fractions occurred within the first 28 days of ensiling and then the changes came slower.

DISCUSSION

The characteristics of a high-moisture content causes WGTG to deteriorate readily after being disposed as waste material from the beverage plant, while lack of WSC makes it difficult to silage alone. The technology of silage preparation for tea grounds was established using LAB and commercial acremonium cellulose with the nutritive value of the silage for sheep estimated by Xu et al. (2003) and Kondo et al. (2004a,b). Many studies revealed that FA (Barry et al., 1978; Nagel and Broderick, 1992) and FD (McDonald et al., 1991; Henderson, 1993) could effectively reduce NPN formation during ensiling. These previous studies mainly focused on the effects of the above additives on fermentation characteristics and nutritive value of WGTG in a totally mixed ration silage with the analysis of

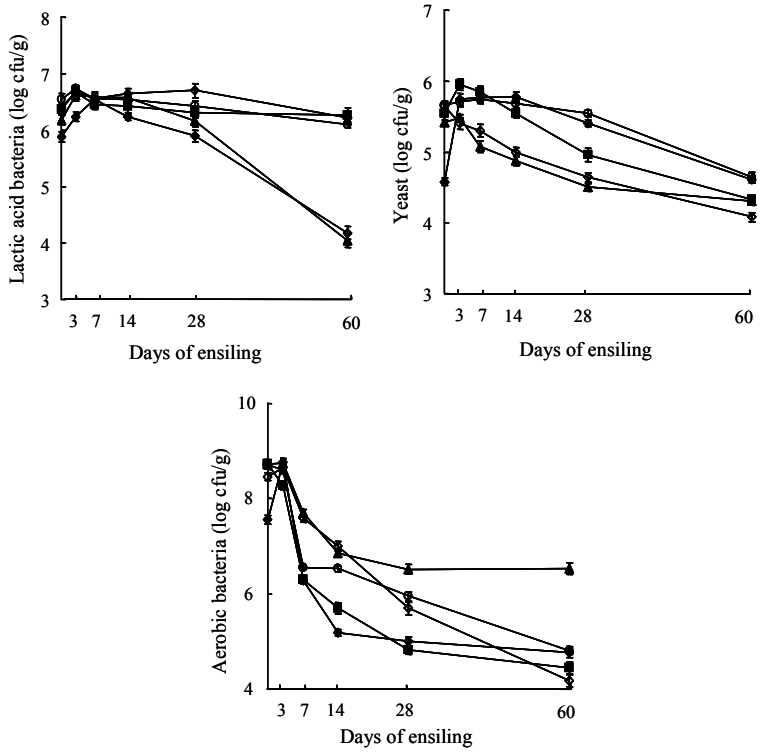

Figure 2. Changes in lactic acid bacteria, yeast and aerobic bacteria during ensiling of treated with lactic acid bacteria (LAB), enzyme (E), formic acid (FA), formaldehyde (FD) and the control. Control (○); LAB (■); E (▲); FA (♦); FD (◊).

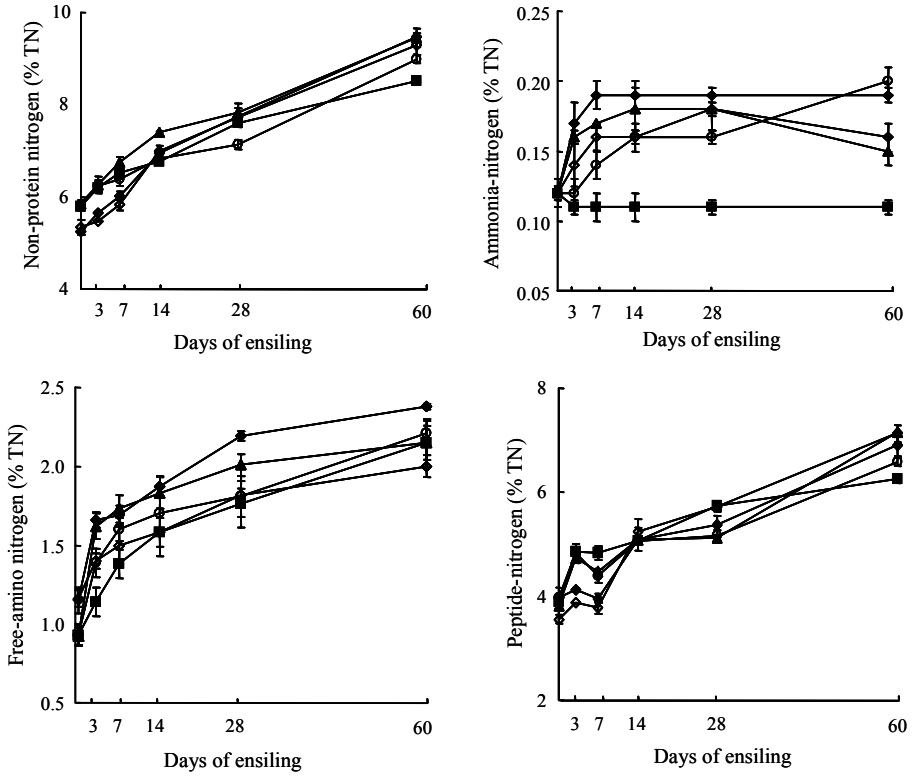

Figure 3. Changes in non-protein nitrogen, ammonia-nitrogen, free-amino nitrogen and peptide-nitrogen during ensiling of treated with lactic acid bacteria (LAB), enzyme (E), formic acid (FA), formaldehyde (FD) and the control. Control (○); LAB (■); E (▲); FA (♦); FD (◊).

protein in WGTG silage generally limited to determination of NPN constituents.

Silage pH is one of main factors that influences the extent of fermentation and silage quality of WGTG, as low pH ensures that the silage is retained in a stable form. In the present study, all tested treatments rapidly decreased pH to about 4.5 within the first 7 days ensiling except for FA, which kept relatively stable at about 3.8, creating a favorable condition for LAB. Of all the treatments tested, LAB treatment showed the greatest effect on the accumulation of lactic acid and the decline in pH which ensured rapid and vigorous fermentation. Many studies have shown the advantage of such inoculants (McDonald et al., 1991; Tengerdy et al., 1991). Besides the LAB treatment, E treatment had the highest WSC content owing to the degradation of cellulose and hemicellulose, which provided more substance for lactic acid bacteria fermentation (McDonald et al., 1991). FA treatment decreased silage pH to 3.8 at the initial period of ensiling, inhibiting the fermentation by disadvantageous microbes (Guo et al., 2008; Xu et al., 2008) and finally decreased the decomposition of WSC. FA and FD treatments had lower lactic acid and higher WSC content than the control. This could be attributed to the inhibition of most microbes as well as lactic acid bacteria which decreased the decomposition of organic matter (Guo et al., 2008). Overall, the above additives enhanced fermentation quality to a certain extent when compared with the control.

Of all tested treatments, the LAB treatment tended to have the best effect as evidenced by higher lactic acid bacteria population and lower aerobic bacteria population during the whole ensiling time. E treatment had higher aerobic bacteria population which may be attributed to the high WSC concentration encouraging the growth of aerobic bacteria (Xing et al., 2009). FA and FD treatments had a greater influence on inhibiting the growth of yeast and aerobic bacteria, but FA could also inhibit the lactic acid bacteria at some extent.

Proteolysis is an inevitable consequence of the ensiling process. Studies reveal that up to 75% of forage true protein can be converted to NPN during the first few days of ensiling by the action of plant proteases (Rooke and Hatfield, 2003; Hassanat et al., 2006). However, the efficiency of N utilization by ruminants fed silage with a high NPN content is low (Wilkinson et al., 1976; Buchanan-Smith, 1978). In addition, production of large quantities of NPN affects fermentation since amino acids, amines and ammonia counteract the desired rapid fall in silage pH (Voss, 1966). Consequently, it is necessary to reduce or inhibit proteolysis usually by creating a low pH environment that is unsuitable for the action of plant and microbial proteases (Rooke and Hatfield, 2003).

In the present study, NPN, NH_3-N and FAA-N content increased in the control and all added treatments. The proportion of peptide-N increased rapidly during the initial period of ensiling, followed by a decline, and then increased for the duration of the fermentation. This change was similar to that observed by Muck (1987) and Fairbairn et al. (1988) and suggests that extensive proteolysis exceeded the degradation of FAA during the early stages with the later decrease due to utilization of peptides by microorganisms. Of all the tested additives, LAB had the best effect. FA had the highest NH_3-N and FAA-N contents. FD treatment had lower FAA-N content than the control; this may be attributed to the combination of FD and protein which restrained the degradation of protein. Nevertheless, FD had no significant impact on inhibiting protein degradation when compared with the LAB; this result was inconsistent with previous trials (Guo et al., 2008; Wang et al., 2009).

Based on the fermentation characteristics, microbial population changes, and protein degradation dynamics of the WGTG silage, we conclude that all tested additives had some effect on improving improved WGTG silage. LAB had the best result, while FA and FD treatments had not achieved anticipated result in reducing protein degradation. Further research will be needed to attain a best addition rate of FA and FD for enhancing the fermentation characteristics and inhibiting protein degradation of WGTG silage.

CONCLUSIONS

The data presented here confirm that all the additives tested could improve fermentation quality of wet green tea grounds in varying degrees than the control. Overall, lactic acid bacteria treatment showed the greatest effect ($p<0.05$). Formic acid and formaldehyde treatments didn't reach the anticipated result in decreasing protein degradation.

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