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Ensiled or Oven-dried Green Tea By-product as Protein Feedstuffs: Effects of Tannin on Nutritive Value in Goats

Makoto Kondo[1,2,*], Kazumi Kita[2,3] and Hiro-omi Yokota[2]

[1]Sciences of Functional Foods (Integrated Department), Graduate School of Agriculture
Shinshu University, Minami-minowa, Kami-ina, Nagano, 399-4598, Japan

ABSTRACT : Ensiled or oven-dried green tea by-products (GTB) were evaluated in goats for their nutritive potential as protein feedstuffs based on *in vitro* and *in vivo* digestibility. To elucidate the effects of tea tannin on *in vitro* digestibility, polyethylene glycol (PEG) was used as a tannin binding agent. Both ensiled and dried GTB contained 31.9 to 32.6% of crude protein (CP) on a dry matter (DM) basis. Phenolics and tannins in soybean meal and alfalfa hay were low or not detected, but they were high in both ensiled and dried GTB (7.3-10.1% DM as total extractable tannins). *In vitro* protein digestibility in the rumen ranked: soybean meal>alfalfa hay cube>ensiled GTB = dried GTB. The protein digestibility post-ruminally of these feedstuffs showed a similar trend to that in the rumen, but the digestibility of ensiled GTB was significantly higher than that of dried GTB. Addition of PEG improved the *in vitro* protein digestibility of both kinds of GTB in the rumen and post-ruminally, indicating that tannins suppressed the potential protein digestibility of GTB. The increased protein digestibility by PEG addition was not significantly different between ensiled and dried GTB in the rumen, but the percentage increment of ensiled GTB was higher than dried GTB post-ruminally. In the *in vivo* digestibility trial, ensiled and dried GTB were offered to goats as partial substitutes for soybean meal and alfalfa hay cubes. Offering both GTB to goats as 5-10% on a DM basis did not affect nutrient digestibility, ruminal pH, volatile fatty acids, and ammonia concentration. However, the eating time of the GTB-incorporated diet was longer than that of the basal diet. It took 1.4 and 1.6 times longer than the control diet, to eat the diet completely when GTB silage was offered at 5 and 10% levels, respectively, of the total diet. These results show that ensiled and dried GTB are useful as partial substitutes for soybean meal and alfalfa hay cubes for goats with respect to nutritive value. Because of lessened palatability, it is recommended that GTB be incorporated into the diet at 5% on a DM basis. (**Key Words** : Green Tea By-product, *In vitro*, *In vivo*, Protein Digestibility, Tannin)

INTRODUCTION

Protein-rich commercial feedstuffs such as soybean meal and alfalfa hay are often used in livestock production, but they are generally expensive. The renewed interest in domestically produced protein-rich forage is partially a consequence of the BSE crisis and the fluctuations in soy product price resulting from global climatic aberration in the world and increasing demand from China and other factors. Green tea is a popular drink in eastern Asia, some parts of the Middle East and North Africa. Consumption of green tea in cans, packs and bottles has increased remarkably in recent years in eastern Asia and Southeast Asian countries, and particularly in Japan. Beverage companies manufacturing various tea drinks produce enormous quantities of tea leaf by-products. Tea leaves are rich in nitrogen compounds, amino acids, tannins, polyphenols and vitamins (Yamamoto et al., 1997), suggesting that tea leaf by-product might be useful as an animal feed. Reportedly, green tea leaves, their by-products, and tea polyphenols can be offered as an ingredient or as a supplement to broilers for reducing mortality (Cao et al., 2005) and to hens for improving laying performance (Uuganbayar et al., 2005; 2006) and for reducing cholesterol content of eggs. Our previous study showed that green tea by-product (GTB) silage can be used as a protein source in a total mixed ration for lactating cows. The GTB silage can be substituted for soybean meal and alfalfa at a level of about 25% of each on a DM basis without any detrimental effects on the performance of dairy cows.

* Corresponding Author: Makoto Kondo. Tel: +81-265-77-1623, Fax: +81-265-77-1700, E-mail: makotokondo526@hotmail.com
[2] Nagoya University Farm, Graduate School of Bio-agricultural Sciences, Nagoya University, Togo, Aichi, 470-0151, Japan.
[3] School of Agriculture, Iwate University, Morioka, Iwate, 020-8550, Japan.
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Furthermore, Nishida et al. (2006) reported that feeding GTB silage at 20% on a DM basis to Holstein steers had no negative impact on their ruminal fermentation, and increased their plasma antioxidative activity and concentration of vitamin E. Ishihara et al. (2001) also reported that offering green tea polyphenols to calves improved microflora balance and decreased the frequency of diarrhea.

The GTB might be a valuable protein source (22-35% of crude protein (CP)), while it contains high tannins (Yang et al., 2003; Kondo et al., 2004). Animals fed tannin-rich diets reduce feed intake (Silanikove et al., 1994), rumen degradability and feed digestibility (Tolera et al., 1997; Woodward and Reed, 1997). Therefore, attention must be given to tannins of GTB when it is used instead of commercial feed in livestock production. Effects of tannin on feed degradability have been studied *in sacco* and *in vitro*. Using these methods, polyethylene glycol (PEG) has been used as a tannin binding agent in the bioassay system to demonstrate the effects of tannin on decreased nutrient degradability in the digestive tract, especially in the rumen (Tolera et al., 1997).

The objective of this study was to evaluate the effects of tannin from ensiled and oven-dried GTB on *in vitro* digestibility. We also investigated the substitution of GTB for soybean meal and alfalfa hay cubes on *in vivo* digestibility.

MATERIALS AND METHODS

Green tea by-product preparation

The GTB used for this study was obtained from a local tea company. For the silage preparation, GTB was packed into polyethylene bags and tied with string to close the upper area of each bag after removing air using a vacuum pump. The GTB silage was stored at ambient temperature and used more than one month after ensiling. Oven-dried GTB was produced by drying in an air oven at 55°C for 48 h.

In vitro ruminal gas production and ammonia nitrogen concentration

In vitro ruminal gas production from feedstuffs was determined according to Menke et al. (1979). After passage through a 1 mm screen, 200 mg of feed sample was weighed into a 100 ml calibrated glass syringe. Rumen fluid was collected from three castrated Japanese goats fed 720 g hay and 180 g commercial concentrates that were offered in equal proportions twice a day. Syringes were filled with 30 ml medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution, then incubated in triplicate in a water bath at 39°C. Gas production was measured following incubation for 3, 6, 9, 12, 24, 48, and 72 h. The exponential equation proposed by Ørskov and McDonald (1979) was used to determine the characteristics of gas production using the Neway computer program (Macaulay Institute, Aberdeen, UK). The following model was applied to the data: $Y = a+b\ (1-e^{-ct})$, where Y is the volume of gas produced (ml/200 mg DM) with time (t), a is the intercept of the gas production curve, a+b is the asymptote of the exponential curve, which represents the potential extent of *in vitro* gas production, and c is the rate of gas production (%/h).

Feed samples (500 mg DM) and PEG (molecular weight: 6,000) (1,000 mg) were incubated in the syringes with 40 ml of the medium and the gas production was monitored for 24 h to determine the effect of tannin on ruminal degradation of feedstuffs (Getachew et al., 2000). In another incubation set, an isonitrogenous amount of sample (24.6 mg nitrogen (N)) with/without PEG (1000mg) was incubated similarly to determine the apparent ammonia production for 24 h in the rumen fluid *in vitro*. The medium was collected after 24 h incubation for measurement of ammonia nitrogen (NH_3-N) concentration.

In vitro three-step protein digestibility

Protein degradability in the rumen and digestibility post-ruminally were determined using the procedure of Calsamiglia and Stern (1995) with some modification as follows; ruminal incubation was done using the *in vitro* rumen technique instead of the nylon-bag method *in sacco*; residues after post-ruminal incubation were collected by filtration as described by Tilley and Terry (1963), instead of using trichloroacetic acid. Feed samples (500 mg DM) and PEG (1,000 mg) were weighed into test tubes. The buffered rumen fluid (10 ml of rumen fluid and 40 ml of artificial saliva) was added to the tubes which were then incubated in a 39°C water bath for 24 h. Rumen fluid was taken as described above. After the first step, the test tube contents were transferred to a centrifuge tube and centrifuged at 1,090×g for 10 min. After discarding the supernatant, 10 ml of 0.1 N HCl (pH 1.9) containing 1 g/L of pepsin (Sigma) was added. The mixture was vortexed and then incubated at 39°C for 1 h. After the incubation, 0.5 ml of 1 N NaOH and 13.5 ml of a pancreatin solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin (Sigma)) were added, vortexed, and incubated at 39°C for 24 h. Each residue at the first and the third step of incubation was obtained by filtration to determine the N content. For this test, six test tubes were prepared: three were used for N determination after the first incubation step to calculate protein degradability in rumen, the other three were used for N determination after the third step to calculate protein digestibility post-ruminally.

In vivo digestibility

Five castrated goats with mean body weight of 43.6±0.8 kg were used in a 5×5 Latin square design. The animals

Table 1. Chemical composition (% DM) of feedstuffs

	Timothy hay	Maize	Wheat bran	Soybean meal	Alfalfa hay cubes	GTB silage	Dried GTB
DM (%)	92.4	90.7	88.3	90.1	90.1	19.4	95.3
Ash	6.9	1.8	4.3	5.6	8.6	3.0	3.0
CP	7.6	6.4	15.2	48.3	16.6	32.6	31.9
BSP (% CP)	-	-	-	31.0	35.2	12.0	15.9
NDIP (% CP)	-	-	-	3.4	19.2	16.1	41.9
ADIP (% CP)	-	-	-	2.7	8.2	6.0	6.3
NDF	68.7	9.6	36.5	14.9	43.0	27.7	34.8
TEPH	1.29	0.19	0.29	0.28	0.59	12.81	8.26
TET	0.42	0.10	0.04	0.05	0.11	10.09	7.32
CT	0.12	ND	ND	ND	0.03	1.04	1.68
GP*	-	-	-	53.9[a]	44.0[b]	43.9[b]	46.3[b]
GP rate**	-	-	-	12.2[a]	10.2[b]	8.5[c]	7.2[c]

DM: dry matter, CP: crude protein, BSP: buffer soluble protein, NDIP: neutral detergent insoluble protein, ADIP: acid detergent insoluble protein, NDF: neutral detergent fiber, TEPH: total extractable phenolics, TET: total extractable tannins, CT: condensed tannins, ND: not detected, -: not determined.
* Potential gas production (ml/200 mg DM). ** Gas production rate constant (%/h).
[a,b,c] Means with different letters in the same row show statistical difference ($p<0.05$).

were kept individually in metabolism cages. The basal diet (Control), designed to satisfy the nutrient requirements of the goats (NRC, 1981), consisted of chopped timothy hay, corn, wheat bran, soybean meal, and alfalfa hay cubes. The GTB silage and dried GTB were incorporated at rates of 5% (GTBs5 and dGTB5, respectively) or 10% DM (GTBs10 and dGTB10, respectively) of the total diet on a DM base. Goats were fed twice daily at 09.00 and 17.00 h and had free access to water. As a preliminary step toward digestion trials, goats were fed control diets for 2 weeks in metabolism cages to become adapted to the diets and to the experimental environment. Each trial lasted for 16 days, which included an adaptation period of 10 days, the period of collection for feces and measurement of eating time of 5 days, and collection of rumen fluid for 1 day. Eating times were monitored in the morning and evening for 5 days, and the results were averaged per day per animal. Data were expressed as ratios of the time taken when each goat was fed a control diet. One-fifth of the feces collected daily were freeze-dried to determine CP and neutral detergent fiber (NDF) content. To determine the DM content, 100 g of feces was oven-dried at 60°C for 48 h. Rumen fluid was taken using a stomach tube at 0, 1, 2, 4, and 8 h after the morning feed. After measuring pH values, a few drops of saturated mercury chloride solution was added to the fluid which was then filtered through four layers of cheesecloth and the filtrate stored at -30°C until analysis.

Chemical analyses

Chemical composition of feedstuffs and feces (DM, ash, CP (N×6.25)) was analyzed using the methods of AOAC (1984). The NDF content was determined using detergent solutions (Van Soest et al., 1991). Protein fractions, buffer soluble protein (BSP), neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP), were determined using the methods of Licitra et al. (1996). Phenolics and tannins were analyzed using the methods of Makkar and Goodchild (1996). Approximately 200 mg DM of the ground sample were extracted in 10 ml of aqueous acetone (70:30 v/v) in an ultrasonicator. The concentration of total extractable phenolics (TEPH) was determined using Folin Ciocalteu reagent and the regression equation of a tannic acid standard. Total extractable tannins (TET) were estimated indirectly after being absorbed to insoluble polyvinyl polypyrrolidone (PVPP). The TET concentration was calculated by subtracting the TEPH remaining after PVPP treatment from the TEPH. Condensed tannins (CT) were measured using 2% ferric ammonium sulfate in 2N HCl and butanol-HCl (95:5, v/v). For *in vitro* and *in vivo* rumen fluid measurements, ammonia nitrogen (NH_3-N) was determined using the indophenol reaction (Weatherburn, 1967). The volatile fatty acid (VFA) concentrations in rumen fluid were measured using gas chromatography (GC-12A; Shimadzu Co., Japan.) with a FAL-M column (Shimadzu Co., Japan). The pH values were measured potentiometrically.

Statistical analyses

All data except PEG effects were analyzed using a one-way analysis of variance (ANOVA) and tested using Duncan's new multiple range test, performed using a Statistical Analysis System (1982). Data from PEG effects on *in vitro* experiments were analyzed using Student's *t*-test, which was performed using computer software (Statview for Windows; SAS Institute, 1992).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of feedstuffs used in *in vitro* and *in vivo* studies. Both ensiled and dried GTB were compared with soybean meal and alfalfa hay cubes as representative protein feedstuffs. The CP content

Table 2. Effect of polyethylene glycol (PEG) treatment on *in vitro* gas production and NH_3-N after 24 h incubation

	No PEG	+PEG	Increase	% Increase
Gas production (ml/500 mg DM)				
Soybean meal	48.4[a]			
Alfalfa hay cubes	39.2[b]	39.0	-0.1	-0.3
GTB silage	34.8[c]	36.5	1.7	4.9
Dried GTB	36.4[bc]	38.9	2.6	7.1
SEM	1.1		1.1	3.0
NH_3-N (mg/24.6 mg N/40 ml)				
Soybean meal	34.2[a]			
Alfalfa hay cubes	14.6[b]	14.0	-0.6[b]	-4.3[b]
GTB silage	13.7[bc]	16.5*	2.7[a]	16.7[a]
Dried GTB	10.5[c]	12.2*	1.8[a]	14.4[a]
SEM	1.0		0.4	2.8

[a, b, c] Means with different letters in the same line show statistical difference ($p<0.05$).
* Significant difference between No PEG ($p<0.05$).

Table 3. *In vitro* protein digestibility (%) of feedstuffs in the rumen and post-rumen

	No PEG	+PEG	Increase	% Increase
Protein degradability in rumen				
Soybean meal	80.9[a]			
Alfalfa hay cubes	59.4[b]	59.5	<0.1[b]	0.1[b]
GTB silage	43.5[c]	51.0*	7.5[a]	17.1[a]
dried GTB	45.3[c]	49.4+	4.1[ab]	9.0[ab]
SEM	0.8		1.8	2.9
Protein digestibility in rumen+post-rumen				
Soybean meal	98.2[a]			
Alfalfa hay cubes	93.1[b]	93.7	0.6[b]	0.6c
GTB silage	83.5[c]	89.8*	6.3[a]	7.6[b]
dried GTB	78.2[d]	87.3*	9.1[a]	11.6[a]
SEM	0.4		0.8	1.0
Rumen undegradable protein digestibility post-rumen				
Soybean meal	90.8[a]			
Alfalfa hay cubes	83.0[b]	84.4	1.4[b]	1.7[c]
GTB silage	70.8[c]	79.2*	8.4[ab]	11.8[b]
dried GTB	60.2[d]	74.8*	14.5[a]	24.1[a]
SEM	1.8		1.8	2.7

[a, b, c] Means with different letters in the same line show statistical difference ($p<0.05$).
* Significant difference between No PEG ($p<0.05$). +: Significant difference between No PEG ($p<0.10$).

of GTB was lower than that of soybean meal, but two times higher than alfalfa hay cubes. As a protein fraction, BSP content was lower in GTB than in soybean meal and alfalfa hay cubes. The neutral detergent insoluble fraction (NDIP and NDF) was high in oven-dried GTB, which might be attributable to binding of tannins in the GTB with protein and fiber by heat treatment (Balogum et al., 1998; Palmer et al., 2000). The ADIP content was similar in both GTB. Phenolics and tannins in feedstuffs other than GTB were quite low or not detected, but they were high in GTB. Potential gas production and the rate of gas production from soybean meal were the highest among the feedstuffs ($p<0.05$). Constant rates of gas production were higher in soybean meal and alfalfa hay cubes than in GTB. However, only soybean meal showed a higher level of potential production. In any case, ensiling or drying treatment did not affect gas production parameters.

Table 2 shows the effect of PEG treatment on *in vitro* gas production and NH_3-N at 24 h incubation from protein-rich supplements. Gas production was unaffected by PEG addition. However, ammonia concentration was increased by PEG addition to both types of GTB. *In vitro* gas production tests with PEG on tannin-rich forage have been a good tool to assess the effect of tannin on feed degradation in the rumen, because PEG binds tannins and deactivates the anti-nutritive activity (Makkar et al., 1995). Tannins in forages such as tropical legumes have anti-nutritive effects on ruminal degradation: the amount of gas production from the forage was increased by PEG addition, mainly after 24 h of incubation (Makkar et al., 1995; Getachew et al., 2000). Table 1 shows that, even though GTB contained high phenolics, tannins, and condensed tannins in similar amounts to tropical legumes, gas production was not increased by PEG. This phenomenon might be attributable to a lack of fermentable carbohydrate to N source (NH_3) for the rumen microorganisms (Getachew et al., 2000). Gas production is a result of feed fermentation in rumen. Proportions of fermentation products depend on feed composition, especially CP and carbohydrates. However, most CP is degraded by microorganisms and creates less gas. In contrast to gas production, PEG increased NH_3-N concentration from GTB incubation, which suggests that proteolysis of GTB in the rumen is suppressed by tannins.

In vitro protein degradability in the rumen was highest for soybean meal, followed by alfalfa hay cubes and lowest for GTB silage and dried GTB (Table 3). In this case, PEG addition did not affect the degradability of alfalfa hay cubes, but did so for GTB silage and dried GTB. No difference was found between ensiling and drying treatments. These results were closely related to NH_3-N concentration in the different incubation systems (Table 2). Protein digestibility in the rumen+post-ruminally and rumen-undegradable protein digestibility post-ruminally was also high in soybean meal, followed by alfalfa hay cubes, GTB silage and dried GTB. Protein digestibility post-ruminally of GTB was markedly affected by ensiling and drying treatment. Drying is known to modify the nutritive value of some tanniferous fodders. Results of this study showed high NDIP contents in dried GTB (Table 1). Oven-drying can produce tannin-protein complexes, increase fiber-bound proteins, and decrease *in vitro* N digestibility (Palmer et al., 2000). The increment of protein digestibility post-ruminally was significantly higher in dried GTB than GTB silage when PEG was applied. These differences indicated that tannin-protein binding would have occurred by oven-drying

Table 4. Diet formulation of digestibility trial in goats

	Diets				
	Control	GTBs5	GTBs10	dGTB5	dGTB10
Ingredients (% DM)					
Timothy hay	45.0	45.0	45.0	45.0	45.0
Maize	23.0	23.0	23.0	23.0	23.0
Wheat bran	11.0	11.0	11.0	11.0	11.0
Soybean meal	10.0	7.5	5.0	7.5	5.0
Alfalfa hay cubes	10.0	7.5	5.0	7.5	5.0
GTB silage/dried GTB	0.0	5.0	10.0	5.0	10.0
Mineral	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0
Analyzed chemical composition (% DM)					
DM (%)	90.5	76.4	66.1	90.8	91.0
CP	13.1	13.1	13.1	13.1	13.1
NDF	42.9	42.7	42.5	43.1	43.2
TEPH	0.74	1.36	1.97	1.13	1.52
TET	0.23	0.73	1.22	0.59	0.95
CT	0.06	0.11	0.16	0.14	0.22

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, TEPH: total extractable phenolics.
TET: total extractable tannins, CT: condensed tannins.

Table 5. Dry matter (DM) intake, apparent digestibility and eating time for goats fed the diets with GTB silage or oven-dried GTB

	Diets*					SEM
	Control	GTBs5	GTBs10	dGTB5	dGTB10	
DM intake (g/kg $M^{0.75}$)	44.0	44.5	44.5	44.7	44.5	-
Apparent digestibility						
Dry matter	73.4	73.1	73.1	73.3	73.5	0.82
Crude protein	72.3	70.9	70.3	71.3	70.7	0.97
Neutral detergent fiber	63.2	63.2	64.1	63.6	64.4	1.45
Eating time**	1.00	1.39	1.61	1.19	1.15	0.44

GTB: green tea by-product. $M^{0.75}$: metabolic body weight.
* See Table 4. ** Time spent to eat experimental diets. Averaged ratio to the control diet for each animal was expressed.

Table 6. Ruminal characteristics in goats fed the diets with GTB silage or dried GTB

	Diets*					SEM
	Control	GTBs5	GTBs10	dGTB5	dGTB10	
pH	6.49	6.46	6.38	6.50	6.39	0.07
Total VFA (mM)	91.0	90.9	90.0	88.8	92.9	1.9
NH_3-N (mg/dl)	18.3	16.9	16.8	15.8	17.1	1.3

GTB: green tea by-product, VFA: volatile fatty acid. * See Table 4.

at 55°C for 48 h, which was presumed to engender lower protein digestibility post-ruminally.

Eruden et al. (2006) reported, as a result of *in sacco* nylon bag tests, that protein degradability of GTB silage was 70.6%. The values in their report and this study were different, perhaps because of the different methods (*in vitro* vs. *in sacco*) and samples that were used. In this study, we specially examined the effect of tannin on feed degradation using PEG in *in vitro* gas production, NH_3-N concentration, and *in vitro* degradability and digestibility. The nylon bag technique and *in vitro* gas production method have been widely used to investigate the effects of tannin on rumen fermentation and to determine the nutritive value of feeds containing tannins (Khazaal et al., 1994; Makkar et al., 1995). Khazaal et al. (1994) compared these two methods and concluded that the gas production method showed phenolics-related anti-nutritive effects more clearly. Because the *in vitro* rumen environment is a closed system, the gas production method seems to be more reliable for detecting the effects of tannin (Khazaal et al., 1994). According to our results, measurement of gas production cannot fully assess the effects of tannin on feed degradation, especially high CP feeds. In addition to measurement of gas production, NH_3-N concentration is important for the tannin bioassay using *in vitro* tests.

Table 4 shows diet formulations used in the digestibility trial in goats. The GTB silage or dried GTB were offered as 5 and 10% on a DM basis in the diet; then the tannin concentration was diluted. Table 5 and 6 show the effects of feeding GTB as partial substitutes for goats. Nutrient

digestibilities (DM, CP and NDF) and averaged values of rumen fermentation parameters (pH, VFA, NH_3-N) were unaffected by including GTB silage or dried GTB. Tannins in forage have both negative and positive effects on nutritive value of forage and on livestock production (Reed, 1995). High amounts of tannins (>5.5% DM) reduce feed intake and digestibilities of protein and carbohydrates (Barry and Duncan, 1984; Barry and Manley, 1984). On the other hand, low or moderate amounts of tannins (2.0-4.5% DM) reportedly increase the flow of non-ammonia nitrogen and essential amino acids from the rumen (Barry and McNabb, 1999). Even though GTB treatments contained tannins in the ingested diets, no effects were apparent on nutrient digestibility and rumen NH_3-N concentration. Presumably, tannins in GTB can make tannin-protein complexes, but the binding would be weak *in vivo* in the digestive tract. Furthermore, offering GTB merely as one ingredient can dilute the effects of tannin. Tannin concentrations (TET and CT) in the GTB-incorporated diet were too low to show negative effects in the *in vivo* environment. On the other hand, the effects were not diluted in the *in vitro* incubation because GTB was incubated as the sole feed for the rumen *in vitro*. As described above, *in vitro* methods might reflect phenolics-related anti-nutritive effects more clearly because of the closed system compared to the dynamic environment *in vivo*. Mastication with saliva might also have lessened the negative effect of tannins during the *in vivo* digestibility trial. It has been reported that browsing animals, which normally ingest dietary tannin, produce tannin-binding proteins in their saliva (Robbins et al., 1987; Austin et al., 1989).

The eating time of the GTB-incorporated diet was longer than that of the control diet (Table 5). In comparison to ensiled and dried treatments, eating times for goats fed ensiled GTB tended to be longer than for dried GTB ($p = 0.11$). Animals took, respectively, 1.4 and 1.6 times longer to eat the 5 and 10% GTB silage diets completely, than the control diet. These results indicated that incorporating ensiled GTB into the basal diet lowered its palatability. Even though tannins are known to reduce feed intake, it is difficult to explain the effect of GTB tannin on eating time because the amount of tannins in GTB diets was low (Barry and McNabb, 1999). It was expected that lessened palatability of GTB silage was due to its smell and taste. The GTB silage smelled acidic and the aroma of green tea was strong, but dried GTB did not have those characteristics. After silage fermentation, ensiled GTB showed lower pH (3.80) and high amounts of acetate (4.8% DM) and lactate (2.5% DM) in this study. Although, it remains unclear why ensiled GTB reduced palatability, acidification of GTB by ensiling might have imparted some detrimental taste effects.

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