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Nutritive evaluation of spent green and black tea leaf silages by *in vitro* gas production characteristics, ruminal degradability and post-ruminal digestibility assessed with inhibitory activity of their tannins

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Abstract

Spent tea leaf contains high levels of crude protein, suggesting that it may be used as an alternative source for ruminant feeding. We assessed the nutritive characteristics of spent green tea leaf silage (GTS) and black tea leaf silages (BTS) in comparison with soybean meal (SBM) and alfalfa hay cube (AHC) using *in vitro* assay. The effects of tannin on the nutritive characteristics were also evaluated by adding polyethylene glycol (PEG) as a tannin-binding agent. The amount of gas production was greater for SBM, followed by AHC, GTS, and BTS. A significant improvement in gas production upon addition of PEG was observed only for BTS. Ruminal protein degradability and post-ruminal digestibility was higher for SBM, followed by AHC, GTS, and BTS. The presence of PEG significantly increased ruminal degradability and post-ruminal protein digestibility for GTS and BTS, but not for AHC. The increment of protein digestibility by PEG was much greater for BTS than for GTS, indicating that GTS tannins suppress protein digestibility slightly, whereas BTS tannins do so strongly. According to these results, GTS but not BTS has a potential as an alternative to AHC as a ruminant feedstuff.

KEYWORDS
post-ruminal digestibility, ruminal degradability, spent tea leaf, tannin

1 | INTRODUCTION

Ready-made tea is daily consumed worldwide. In Japan, beverage companies that manufacture tea-made drinks produce tons of spent tea leaf annually and most of them are composted. Spent tea leaf contain large amounts of crude protein (CP, 220 to 350 g/kg dry matter [DM]), and may, therefore, be considered a valuable protein source (Kondo, Kita, & Yokota, 2004a,b; Kondo, Hirano, & Ikai, et al., 2014; Ramdani, Chaudhry, & Seal, 2013). Those previous studies also showed that spent tea leaf contain tannins. Tannins have been known as anti-nutritive factors (Getachew, Makkar, & Becker, 2000). The depressive activity of tannins on ruminal feed degradability due to binding proteins and lowering microbial activity was observed in many kinds of leguminous leaves and fruit pods containing considerable amounts of the substances (Pal, Patra, Sahoo, & Kumawat, 2015; Saminathan, Sieo, Abdullah, Wong, & Ho, 2015). Among the agro-industrial by-products, spent tea leaf contains relatively high level of tannins, but their protein precipitating capacity (PPC) are different depending on the kinds of spent tea leaves (Kondo, Jayanegara, Uyeno, & Matsui, 2016). To evaluate the possibility of spent tea leaf as protein-rich feedstuffs for ruminant, studies are required to identify protein degradability in rumen and digestibility in post-rumen of spent green tea leaf silage (GTS) and black tea leaf silages (BTS) related to tannins.

Therefore, in the present study, we examined the nutritive characteristics of GTS and BTS compared with soybean meal (SBM) and

alfalfa hay cube (AHC) that are often used in ruminant diets. For these protein-rich feedstuffs, ruminal fermentation characteristics, protein degradability in the rumen and digestibility in the post-rumen were evaluated with regarding to tannins activity.

2 | MATERIALS AND METHODS

2.1 | Feed samples

Spent green and black tea leaves were obtained from a local beverage company, and 20 kg of each spent tea leaf were packed into four polyethylene bags and air-tied using a vacuum pump. Those silages were kept at ambient temperature for 1 month. After opening the silos, approximately 1 kg of the silages was taken from each bag and then those were homogenized and a composite sample was taken for the following experiments. These silages were freeze-dried and used for *in vitro* ruminal gas production, digestibilities and chemical analyzes except for the products of silage fermentation. As commercial protein-rich feedstuffs for cattle, SBM and AHC were purchased from a local feed company. These feedstuffs were milled to pass through 1-mm screen, and then used for following experiments.

2.2 | Preparation of rumen fluid for *in vitro* experiments

Rumen fluid was collected before morning feeding using a stomach tube from three castrated Japanese Shiba goats that had been fed two equal portions totaling 720 g of hay and 180 g of a commercial concentrate as fed basis. Collected rumen fluids from three goats were mixed and then filtrated with four layers of cheesecloth. The filtrated rumen fluid was used for following experiments. Animal care and handling procedure followed by guidelines for the ethical use of animals in research in Nagoya University.

2.3 | *In vitro* ruminal gas production kinetics

In vitro ruminal gas production from the feedstuffs (SBM, AHC, GTS and BTS) was determined according to the procedure of Menke and Stengass (1988). Two hundred mg of each feedstuff were weighed into 100 ml calibrated glass syringe (TOP Corporation, Tokyo, Japan). Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer and mineral solutions (Menke & Stengass, 1988), then incubated in a water bath at 39°C in triplicates and gas production was measured at various time point intervals, i.e. 3, 6, 9, 12, 24, 48 and 72 hr. The net gas production were fitted to according to Ørskov and McDonald (1979) to determine fermentation kinetics: $Y = b \times (1–e^{-ct})$, where '*Y*' is the cumulative gas volume (ml) at time '*t*' (hr), '*b*' represents asymptotic gas volume (ml), '*c*' represents rate of gas production (ml/h). The parameters *b* and *c* were determined using the nonlinear procedure of SAS (SAS Institute Inc.). The time when half of the asymptotic gas volume was calculated as: $t_{1/2} = \ln 2/c$.

2.4 | Tannin's activity on *in vitro* gas production and NH_3-N concentration

To assess the effect of tannin on ruminal degradation of feedstuffs, AHC, GTS and BTS samples (200 mg DM) with or without PEG (molecular weight: 6,000) (400 mg) were put into the syringes with 40 ml of the buffered-rumen fluid as mentioned above and gas production was monitored for 24 hr by following the protocol of Getachew et al. (2000) and Kondo, Kita, and Yokota (2007). In another incubation set, to observe the apparent ammonia production in the incubated medium for 24 hr, iso-nitrogenous amount of samples (24.6 mg N) with and without PEG (2 times as much as each samples on a DM basis) was incubated as the same way, and the incubated medium was taken 24 hr after incubation. Ammonia nitrogen (NH_3-N) concentration of the medium after incubation was measured.

2.5 | Tannin's activity on *in vitro* three-step protein digestibility

Protein degradability in the rumen and digestibility in the post-rumen were determined using the modified procedures of Tilley and Terry (1963) and Calsamiglia and Stern (1995), respectively. Feed samples prepared as mentioned above (500 mg DM) with or without PEG (1,000 mg) were weighed into test tubes. As the first step, the buffered rumen fluid (10 ml of rumen fluid and 40 ml of artificial saliva) was added into the tubes and incubated in a 39°C water bath for 24 hr. Rumen fluid was taken as the same way as described above. After the first step, the content of test tube was moved to centrifuge tube, then centrifuged $1{,}090 \times g$ for 10 min. After discarded the supernatant, 10 ml of a pH 1.9, 0.1 N HCl solution containing 1 g/L of peptidase from porcine gastric mucosa (pepsin, Sigma) was added, vortexed and incubated at 39°C for 1 hr as the second step. After the incubation, 0.5 ml of a 1 N NaOH solution and 13.5 ml of a solution containing 0.5 M KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of an enzyme mixture from porcine pancreas (pancreatin, Sigma) was added. The samples were incubated at 39°C for 24 hr as the third step. The residues from the first and the third steps were collected using a filter paper and used for the following N analysis. Six test tubes were prepared; three of them were used for N determination after first 24 hr incubation step to calculate protein degradability in rumen, and the others were used for N determination after the third step to calculate protein digestibility in post-rumen. Six tubes without any substrate containing only the same volume of buffered-rumen fluid were also incubated as blank to correct N amounts originated from rumen fluid in goats; three of them were used for rumen, and the others were for post-rumen.

2.6 | Disappearance of condensed tannins (CT) in GTS and BTS in *in vitro* ruminal incubation

The incubation and collection of residual incubated samples followed the procedure determined by Makkar, Blummel, and Becker (1995). Five hundred mg of GTS and BTS prepared as mentioned above

were incubated in triplicates in 40 ml of the buffered rumen fluid. At 6, 12 and 24 hr, the contents were centrifuged (1,090 × *g*) for 30 min. The pellet was washed with 0.85% of NaCl and re-centrifuged as above. The washed pellet was freeze-dried and measured for the CT content.

2.7 | Chemical analyses

Standard methods as described in AOAC International (2002) were used for determination of DM, organic matter (OM), crude ash, ether extract, CP. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as outlined by Van Soest, Robertson, and Lewis (1991) and both fiber contents were expressed as ash free forms. Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) was determined according to Licitra, Hernandez, and Van Soest (1996). Total extractable phenols (TEPH), total extractable tannins (TET), and CT were analyzed according to Makkar (2003). TEPH and TET were calculated as tannic acid equivalent, whereas CT was done as leucocyanidin equivalent. Protein precipitating capacity (PPC) of AHC, GTS and BTS was determined according to Makkar, Dawra, and Singh (1988). In brief, tannin fraction was extracted by 50% methanol and the extract was reacted with bovine serum albumin (BSA) at 20° for 20 min. The precipitated BSA was collected by centrifuge (5,000 × *g* for 20 min) and the protein amount was determined by ninhydrin reaction after hydrolyzed by NaOH. To determine the characteristics of silage fermentation, 20 g of GTS and BTS were macerated with 180 ml of distilled water, and then the water extracts were prepared. The pH, lactic acid, acetic acid, propionic acid, butyric acid and NH_3-N in the extracts were determined by the method described in Kondo et al. (2004a). The NH_3-N of *in vitro* rumen fluid was determined using the indophenol reaction (Weatherburn, 1967).

2.8 | Statistical analyses

Data from gas production, NH_3 concentration, protein degradability and digestibility except PEG effects were analyzed by analysis of variance and tested using Tukey's test. Data from PEG effects on *in vitro* experiments were analyzed by Student's *t*-test. Data from CT disappearance composed two factors; kinds of feedstuffs and incubation time were analyzed by two-way ANOVA and tested using least square means to compare the data of feedstuff at each incubation time. All experiments were done in triplicates and these analyses were performed with SAS 9.3 (SAS Institute Inc.).

3 | RESULTS AND DISCUSSION

3.1 | Chemical composition of protein-rich feedstuffs and fermentation characteristics of GTS and BTS

Table 1 shows the chemical compositions of SBM, AHC, GTS, and BTS. The CP content was greater in SBM, followed by GTS, and BTS, and AHC. Concerning the amount of NDICP and ADICP, BTS contained more NDICP and ADICP than did the other feedstuffs. The NDICP and ADICP contents of GTS and AHC were similar. Licitra et al. (1996) defined NDICP as being slowly degradable and ADICP as having insignificant biological availability. The greater NDICP and ADICP contents in BTS than those in GTS might be a consequence of processing after harvesting tea leaves. For green tea, the leaves are dried sooner after harvesting, and this process stops the activities of the enzymes in the leaves. While, to produce black tea, withered leaves are subjected to crushing, tearing, cutting or rolling operations to achieve faster oxidation. During this process, several enzymes such as polyphenol oxidase (PPO) and peptidase in the leaves are active (Tomins & Mashingaidze, 1997). PPO produces theaflavins and other large molecules of polyphenols from catechins monomers in natural leaves. The binding affinity of polyphenols to proteins is dependent on their molecular size (De Freitas & Mateus, 2001). Larger polyphenols like present in black tea are therefore more likely to bind leaf proteins during processing, and these bindings might increase detergent insoluble protein content in black tea leaves. Proteins in the leaves are also partly degraded by peptidase during black tea leaf processing (Tomins & Mashingaidze, 1997). As the results, proteins that are more difficult to be solubilized by detergent solutions might be remained in black

TABLE 1 Chemical composition, protein precipitation capacity and fermentation characteristics of feedstuffs

	SBM	AHC	GTS	BTS
Dry matter (DM, g/kg)	901	877	205	217
Organic matter (g/kg DM)	944	875	965	970
Crude protein (CP, g/kg DM)	483	213	356	260
NDICP (g/kg CP)	41	164	122	396
ADICP (g/kg CP)	19	56	39	69
Neutral detergent fiber (g/kg DM)	143	376	310	412
Acid detergent fiber (g/kg DM)	87	288	228	268
Ether extract	14	20	74	27
Total extractable phenolics (g/kg DM)	3.1	7.2	113.3	78.4
Total extractable tannins (g/kg DM)	ND	1.3	95.7	60.8
Condensed tannins (g/kg DM)	ND	ND	24.2	7.5
Protein precipitation capacity (mg BSA g/DM)		ND	16.6	138.0
pH			3.87	4.71
Lactic acid (g/kg DM)			19.1	ND
Acetic acid (g/kg DM)			51.7	4.3
Propionic acid (g/kg DM)			8.4	2.6
Butyric acid (g/kg DM)			4.4	ND
NH_3-N (g/kg N)			4.0	2.5

Notes. SBM: soybean meal; AHC: alfalfa hay cube; GTS: spent green tea leaf silage; BTS: spent black tea leaf silage; NDICP: neutral detergent insoluble CP; ADICP: acid detergent insoluble CP; BSA: bovine serum albumin; ND: not detected.

tea leaves. Silage fermentation of GTS and BTS might have small effects on protein fractions. According to our previous studies (Kondo, Hirano, Kita, Jayanegara, & Yokota, 2014), NDICP contents slightly decreased during ensiling only in the case of GTS, but ADICP contents both in GTS and in BTS were similar to those in the spent tea leaves before ensiling. Specific chemical compounds in GTS and BTS were phenolics and tannins. Kondo et al. (2016) also reported that spent tea leaf contained relatively higher amounts of phenolics than other agro- and food-industrial by-products. The amounts of TEPH, TET, and CT were greater in GTS than in BTS. In addition, AHC contained small amounts of TEPH and TET, whereas SBM did not contain TET. No CT was found in AHC. The PPC in GTS and BTS was clearly observed, but BSA was not precipitated from AHC. The PPC for BTS was almost sevenfold greater than that found for GTS, indicated that BTS bound proteins more strongly. Before ensiling, spent black tea leaves originally possess higher PPC than spent green tea leaves (Kondo et al., 2016). These different PPC between green and black tea leaves would be dependent on molecular structures of polyphenols as described above. The ensiled fermentation products and pH values of the GTS and BTS are also shown in Table 1. The pH value for GTS was less than that of BTS. Acetic acid was the main acid present in GTS with lactic acid being second most common. The amounts of propionic and butyric acids were minor in GTS. The higher pH value for BTS suggests that fermentation of BTS did not progress as far as that for GTS. The smaller NH_3-N values in GTS and BTS as silage indicates that proteolysis during ensiling were suppressed, that are in agreement with our previous report (Kondo, Hirano, & Kita et al., 2014). Lower NH_3-N values was also reported in other silage added with grape pomace containing high tannins (Li et al., 2017). During ensiling periods, any deterioration was found in both GTS and BTS, and they were preserved well.

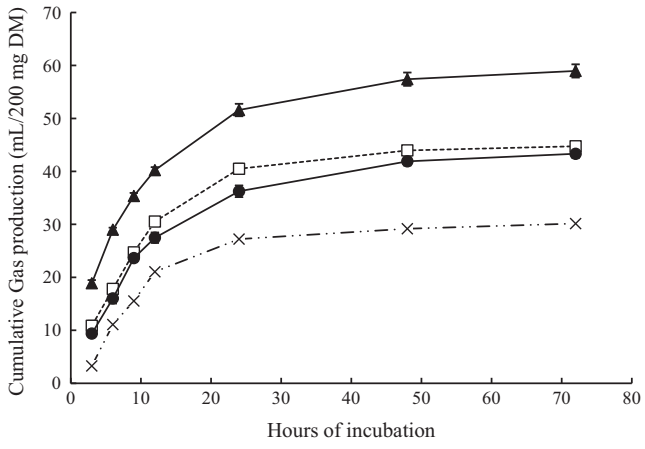

FIGURE 1 Cumulative gas production from soybean meal (▲), alfalfa hay cube (□), spent green tea leaf silage (●) and spent black tea leaf silage (×). Points indicate mean values of triplicates with standard errors represented by vertical bars.

3.2 | In vitro gas production kinetics

Cumulative gas production was shown in Figure 1. The gas production was higher in SBM, followed by AHC and GTS, and BTS. Based on the kinetics analyses (Table 2), potential gas production was significantly higher in SBM, and lower in BTS than others, but not different between in AHC and GTS. The volume of gas produced has been shown to be related to the fermentable organic matter content and available energy in the rumen (Menke & Stengass, 1988). Rate of gas production and half time of gas production from SBM were significantly higher and shorter, respectively, compared with the

TABLE 2 In vitro gas production kinetics of protein-rich feedstuffs

	b (ml/200 mg DM)	*c* (ml/h)	$t_{1/2}$
SBM	57.5a	0.109a	6.35b
AHC	44.9b	0.091b	7.65ab
GTS	42.9b	0.083b	8.36a
BTS	30.5c	0.082b	8.48a
SEM	1.1	0.003	0.32
p-value	<0.001	<0.001	0.005

Notes. b, potential gas production; *c*, rate constant of gas production; $t_{1/2}$, the half time of asymptotic gas production; SBM: soybean meal; AHC: alfalfa hay cube; GTS and BTS, spent green and black tea leaf silage, respectively; *SEM*: standard error of the mean.
a-c, Means with different letters in the same column are statistical different from each other.

TABLE 3 Effect of polyethylene glycol (PEG) treatment on *in vitro* gas production and NH_3-N concentration of protein-rich feedstuffs at 24 hr of incubation

	No PEG	+PEG	% Increase
Gas production (ml/200 mg DM)			
SBM	51.6a		
AHC	40.5b	39.3	−2.2b
GTS	36.2b	35.8	−1.2b
BTS	27.2c	32.1*	17.8a
SEM	1.1		1.6
p-value	<0.001		<0.001
NH_3-N (mg/40 ml)			
SBM	23.2a		
AHC	13.1b	12.5	−4.3c
GTS	10.8c	13.2*	22.7b
BTS	2.9d	5.9*	103.5a
SEM	0.1		5.9
p-value	<0.001		<0.001

Notes. a-d, Means with different letters in the same column are statistical different from each other.
SBM: soybean meal; AHC: alfalfa hay cube; GTS and BTS: spent green and black tea leaf silage, respectively; *SEM*: standard error of the mean.
*Significant difference found for the experiments performed in the absence and presence of PEG ($p < 0.05$).

TABLE 4 Effect of polyethylene glycol (PEG) treatment on *in vitro* ruminal protein degradability and post-ruminal digestibility of protein-rich feedstuffs

	No PEG	+PEG	% Increase
Ruminal protein degradability (g/kg)			
SBM	761a		
AHC	503b	486	−3.5c
GTS	340c	383*	12.8b
BTS	125d	219*	75.7a
SEM	9		3.1
p-value	<0.001		<0.001
Ruminal and post-ruminal protein digestibility (g/kg)			
SBM	964a		
AHC	846b	842	−0.6c
GTS	743c	808*	8.8b
BTS	288d	680*	135.8a
SEM	9		0.9
p-value	<0.001		<0.001
Post-ruminal protein digestibility of rumen undegradable protein (g/kg)			
SBM	849a		
AHC	690b	692	0.3c
GTS	610c	689*	13.0b
BTS	187d	590*	215.4a
SEM	15		1.6
p-value	<0.001		<0.001

Notes. a-d, Means with different letters in the same column are statistical different from each other.
SBM: soybean meal; AHC: alfalfa hay cube; GTS and BTS: spent green and black tea leaf silage, respectively; *SEM*: standard error of the mean.
*Significant difference found for the experiments performed in the absence and presence of PEG ($p < 0.05$).

other feedstuffs indicate that SBM contains more highly fermentable OM, whereas rate of OM fermentation of both spent tea leaf silages were similar to that of AHC.

3.3 | Tannin's activity on *in vitro* gas production and NH_3-N concentration

A significant improvement in gas production by addition of PEG was observed for only BTS ($p < 0.001$, Table 3). Previous studies have shown that PEG increased *in vitro* gas production in most tannin-containing feeds (Getachew et al., 2000; Rubanza et al., 2005; Tolera, Khazaal, & Ørskov, 1997). The extent to which PEG affects gas production is determined by the negative effects of tannins and that would depend on the type and amounts of the tannins in the feedstuffs. Although GTS contains large amounts of tannins, PEG did not affect the amount of gas produced. Since gas production is a result of fermentation of carbohydrates in the rumen mainly, it seems that the GTS tannins did not suppress carbohydrate fermentation. The NH_3-N concentration in *in vitro* ruminal fluid was higher for SBM, followed by those for AHC and GTS,

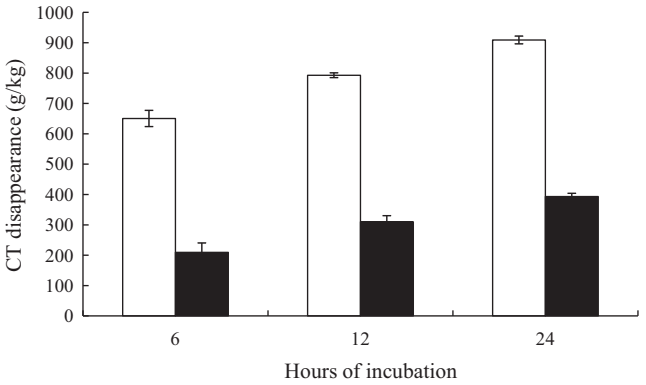

FIGURE 2 Disappearance of condensed tannins from spent green tea leaf silage (open bar) and black tea leaf silage (solid bar) at 6, 12, 24 hr of incubation

and BTS ($p < 0.001$). The percentage of NH_3-N increment by PEG addition was the greater for BTS than for GTS, although the differences were significant in both cases ($p < 0.001$). Conversely, no increase in NH_3-N concentration was observed for AHC when PEG was added. Increment of NH_3-N concentration by PEG addition indicates that PEG makes tannin-bound protein available to microbial degradation and metabolism. In other words, it can be estimated that the presence of tannins in GTS and BTS had suppressed their protein degradation in the rumen. This is consistent with our measured values of PPC for GTS and BTS (Table 1). As others have suggested, PPC measurements appear to be useful when assessing the biological activities of tannins (Jayanegara, Goel, Makkar, & Becker, 2015; Silanikove, Shinder, Gilboa, Eyal, & Nitsan, 1996; Singh, Sahoo, Sharma, & Bhat, 2005).

3.4 | Effect of polyethylene glycol (PEG) treatment on *in vitro* protein degradability in rumen and digestibility in post-rumen

In vitro ruminal protein degradability and post-ruminal digestibility were higher for SBM, followed by AHC and GTS, and BTS ($p < 0.001$, Table 4), which is consistent with the measured NH_3-N value. Because AHC does not contain much tannin, the PEG effect on protein digestibility was negligible as similar to the changes in gas production and NH_3-N from AHC (Table 3). The presence of PEG significantly increased ruminal protein degradability and post-ruminal digestibility for GTS and BTS ($p < 0.05$). However, the improvement in protein digestibility for GTS was relatively small, which is consistent with our finding for NH_3-N from GTS (Table 3). Conversely, the improvement in digestibility was relatively large for BTS, indicating that the tannins in BTS suppressed rumen protein degradation strongly. The trend of these suppressive activities in protein digestibility by tannins is consistent with PPC of GTS and BTS (Table 1). PEG promoted a greater increase in post-ruminal digestibility than in ruminal degradability for BTS. The suppressive effect of the tannins was more apparent using *in vitro* method, which is a completely closed system, than when an in sacco system was used

(Tolera et al., 1997). In the three-step method used in the present study, pepsin and pancreatic enzymes were the only added enzymes present during the digestion assays. If the spent tea leaf tannins bind these enzymes, then digestibility would be inhibited. Tannin-protein binding is pH dependent, and most plant tannins can bind to proteins at neutral pH, but not at acid pH (Oh & Hoff, 1987; Perez-Mal donado, Norton, & Kerven, 1995). The extent of binding is also dependent on the type of protein and tannin (Oh & Hoff, 1987; Perez-Maldonado et al., 1995). In the present experiment, the tannins in BTS may bind and deactivate enzymes in 2nd and 3rd step *in vitro*, that would be why a large improvement in protein digestibility is seen post-rumen when PEG is present. To understand in detail the pathway of post-ruminal GTS and BTS protein digestibility *in vivo*, a mobile-bag technique from intestine should be employed and the depressive effects of the feedstuffs in the digestive tract should be measured in the presence of PEG.

3.5 | Disappearance of condensed tannin in *in vitro* rumen

Figure 2 shows the CT disappearance for GTS and BTS when incubated the ruminal fluid. The CT disappearances were significantly different for the two types of spent tea leaf silages at all time points ($p < 0.001$) with that of GTS being greater. The interaction between feedstuff and incubation time was not significant ($p > 0.05$). Makkar et al. (1995) reported that although the rate of CT disappearance was dependent on the plant type, most tannins had disappeared from tannin-rich leaves by 24 hr with only a small amount remaining at 48 hr. However, most of the tannins found originally in BTS remained at the end of the incubation period. Various plant tissues contain CT, which accumulate in vacuoles or are bound to fibers and proteins (Lees, Suttill, & Gruber, 1993; Rubanza et al., 2005; Stewart, Mould, & Mueller-Harvey, 2000). Since BTS contained higher ADF and ADICP than those of GTS, it seems that the disappearance of CT from BTS was in lower magnitude compared with that from GTS. Tannins would be released from plant cells during mastication and in the rumen by microorganisms, allowing the tannins to bind digestive enzymes and ingested proteins (Min, Barry, Attwood, & McNabb, 2003). We found that most of the aqueous acetone-soluble CT was disappeared from GTS during the 24-hr incubation with the ruminal medium. In our ruminal degradation and post-ruminal digestion assay, any disappeared CT were discarded after centrifugation, therefore they could not affect the results of the post-ruminal portion of the assay. However, CT in BTS was slowly disappeared and they would be present during the post-ruminal assay, indicating that these CT might bind and deactivate pepsin and pancreatin used in this study, which would lead to the large PPC value observed for BTS. Therefore, the improved post-ruminal protein digestibility in the presence of PEG may be caused by PEG inactivating the suppressive activity of BTS tannins on the digestive enzymes (Table 4). It has been reported that hydrolysable tannins degraded to lower molecule phenolics in the rumen (Singh, Bhat, & Sharma, 2001). While, although the degradation of CT in the rumen was varied to plants, CT could be rarely or not degraded (Makkar et al., 1995). In this study, we did not examine the extent to which CT was degraded post-ruminal, but, given our data, it is possible that CT were still bound to the digestive enzymes. An *in vivo* feeding trial is necessary to assess the depressive effects of GTS and BTS tannins in each component of the ruminant digestive tract.

4 | CONCLUSIONS

The present study showed the nutritive values of GTS and BTS by *in vitro* method. As compared with AHC, GTS had the similar extent of fermentablity, but higher rumen undegradable protein due to tannins. On the other hand, BTS was low in ruminal gas production and protein digestibility because of high depressive activity of BTS tannins. Further studies are recommended on assessment of nutritive value in *in vivo*.

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