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# Ensiled green tea and black tea waste as protein supplement for goats

M. Kondo, M. Hidaka, K. Kita and H. Yokota

Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, 470-0151, Japan

Email: makotokondo526@hotmail.com

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**SUMMARY** – Tea waste, a by-product from beverage companies producing a ready-made tea drink, contains substantial concentrations of crude protein (CP, 25 ~ 30% of the DM), which may have value as a dietary supplement for goats. Tea waste also contains 6.1 ~ 9.6% total extractable tannins and 0.8 ~ 2.4% condensed tannins in the DM. In vitro gas production and in vivo digestibility of green tea waste silage (GTWs) and black tea waste silage (BTWs) were compared to lucerne hay cubes (LHC). In vitro gas production from BTWs was significantly lower than LHC and GTWs. Addition of polyethylene glycol as a tannin-binding agent to BTWs, increased gas production but did not affect LHC and GTWs. In vivo digestibility, nitrogen balance and ruminal NH<sub>3</sub>-N concentration were determined using four castrated Japanese goats fed diets of timothy hay + wheat bran (control), control + LHC, control + GTWs and control + BTWs, in a 4 x 4 Latin Square Design. Timothy hay and wheat bran were offered equally in all treatments, and LHC, GTWs and BTWs were supplied on a CP basis, contributing 15.6% of the total CP intake. DM, CP and NDF digestibility were not affected by the type of supplement ( $p = 0.40, 0.12, 0.81$ , respectively). Acid detergent insoluble N (ADIN) digestibility was 0.16 ~ 0.21 for control, LHC and GTWs diets, but -0.10 for BTWs treatment. Fecal N output from the BTWs treatment tended to be high compared with other treatments ( $p = 0.08$ ). Ruminal NH<sub>3</sub>-N concentration in BTWs treatment was significantly lower than other treatments at 1 ~ 4 hours after feeding. These results indicated that GTWs could substitute LHC as protein supplement; on the other hand, tannins in BTWs could bind protein in the digestive tract, lower N degradability in the rumen and increase fecal N output.

**Keywords:** Green tea waste, black tea waste, protein, tannin, digestibility.

**RESUME** – "L'ensilage de résidus de thé vert et de thé noir comme supplément protéique pour caprins". Les résidus de thé, un sous-produit des industries des boissons qui produisent une boisson à base de thé tout prêt, contiennent des concentrations substantielles de protéine brute (PB, 25 ~ 30% de la MS), qui pourraient être valorisées comme supplément à l'alimentation des caprins. Les résidus de thé contiennent également 6.1 ~ 9.6% de tannins libres totaux 0.8 ~ 2.4% de tannins condensés dans la MS. La production de gaz in vitro et la digestibilité in vivo de l'ensilage de résidus de thé vert (GTWs) et de l'ensilage de résidus de thé noir (BTWs) ont été comparés à des cubes de foin de luzerne (LHC). La production de gaz in vitro de BTWs était significativement inférieure à celle de LHC et GTWs. L'addition de polyéthylène glycol comme agent liant des tannins pour BTWs a augmenté la production de gaz mais ne l'a pas modifiée pour LHC et GTWs. La digestibilité in vivo, le bilan azoté et la concentration ruminale en NH<sub>3</sub>-N ont été déterminés en utilisant quatre caprins japonais castrés recevant un régime à base de foin de fléole des prés + son de blé (témoin), témoin + LHC, témoin + GTWs et témoin + BTWs, dans un dispositif en carré latin 4 x 4. Le foin de fléole des prés et le son de blé étaient offerts également dans tous les traitements, et du LHC, GTWs et BTWs étaient distribués en se basant sur la PB, représentant 15,6% de l'ingestion totale de PB. La digestibilité de la MS, PB et NDF n'étaient pas affectées par le type de supplément ( $p = 0,40, 0,12, 0,81$ , respectivement). La digestibilité de l'azote insoluble dans le détergent acide (ADIN) était de 0,16 ~ 0,21 pour les régimes témoin, LHC et GTWs, mais de -0,10 pour le traitement BTWs. L'excrétion d'azote fécal pour le traitement BTWs tendait à être élevée comparée aux autres traitements ( $p = 0,08$ ). La concentration ruminale en NH<sub>3</sub>-N du traitement BTWs était significativement inférieure aux autres traitements à 1 ~ 4 heures après la prise d'aliment. Ces résultats indiquent que GTWs pourrait remplacer LHC comme supplément protéique ; par ailleurs, les tannins de BTWs pourraient lier la protéine dans le tractus digestif, faire baisser la dégradabilité de l'azote dans le rumen et augmenter l'excrétion d'azote fécal.

**Mots-clés :** Résidus du thé vert, résidus du thé noir, protéine, tannin, digestibilité.

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## Introduction

Tea is one of the major drinks in the world. Consumption of ready-made tea, which packed into cans and bottles, has been increasing remarkably in recent years. Beverage companies manufacturing various tea drinks produce tons of tea-leaf waste annually, most of which is burned,

dumped into landfills or used as compost. Tea waste may be considered a valuable protein source consisting of 22 ~ 35% of crude protein (CP) (Kondo *et al.*, 2004). While tea waste contains high CP, it is known to contain a high proportion of tannins (Kondo *et al.*, 2004). Animals fed tannin-rich diets showed decreased their feed intake (Silanikove *et al.*, 1994), increased fecal N excretion (Núñez-Hernández *et al.*, 1991), reduced digestibility and less ruminal degradability (Woodward and Reed 1997; Tolera *et al.*, 1997). Thus, due attention must be given to tannin of tea wastes when it is used instead of commercial feed in livestock production. The objective of this study was to evaluate the effects of replacement of lucerne hay cube (LHC) with green tea waste or black tea waste. Both kinds of tea waste were offered to goats as silage in this study because tea wastes easily deteriorated after disposal from a beverage company. Two experiments were conducted in our investigation; the first aimed to test the gas production of LHC and the two kinds of ensiled tea wastes *in vitro*. The second experiment evaluated digestibility, N balance and ruminal fermentation characteristics *in vivo*.

## Material and methods

For *in vitro* gas production study, 200 mg of feed samples (green tea waste silage (GTWs), black tea waste silage (BTWs) and LHC) and 400 mg of PEG (polyethylene glycol), (MW:6000) were weighed into 100 ml glass syringes in triplicate before the incubation medium was added (Getachew *et al.*, 2000). The incubation medium was 10 ml of rumen fluid and 20 ml of buffer solution (Menke and Steingass, 1988). The syringes were set at 39°C for 24 hours and the amount of produced gas was measured. *In vivo* study was conducted in a 4 x 4 Latin square design using 4 castrated Japanese Shiba goats (mean body weight: 29.7 kg) in each phase of the experiment consisting of a 8-day adaptation period followed by a 5-day collection period for feces and urine, and a 1-day for rumen fluid. Feces and urine were collected once a day and rumen fluid was 0, 1, 2, 4, 8 h after morning feed on the last day. Goats fed chopped timothy hay + wheat bran (control), control + LHC, control + GTWs, or control + BTWs. Timothy hay and wheat bran were offered 580 and 40 g on a DM basis in all treatments, respectively, and LHC, GTWs and BTWs were supplied 27.4, 38.3 and 43.5 g on a DM basis, respectively. These supplements were on a CP basis equally. Timothy hay and wheat bran contribute 100% of CP requirement, while LHC, GTWs or BTWs were supplied 15.6% of the requirement. DM, N and CP content were determined by the method described in AOAC (1984). Neutral detergent fibre (NDF) and acid detergent insoluble N (ADIN) was analysed according to Van Soest *et al.* (1991) and Licitra *et al.* (1996), respectively. Total extractable phenolics (TEPH) were determined using Folin-Ciocalteu reagent. Total extractable tannins (TET) were estimated indirectly after being absorbed to insoluble polyvinyl polypyrrolidone (PVPP). Condensed tannins (CT) were measured using butanol-HCl method as described Makkar and Goodchild (1996). TEPH and TET were calculated as tannic acid equivalent, whereas CT was done as leucocyanidin equivalent. NH<sub>3</sub>-N of rumen fluid was measured colorimetrically (Weatherburn, 1967). The data were analysed using the General Linear Model Procedures (GLM; SAS/STAT, SAS Institute, Cary NC).

## Results and discussion

Table 1 shows chemical compositions of feedstuffs. As characteristics of tea wastes, CP content of GTWs and BTWs were 35.6 and 26.0% respectively, and total extractable phenolics, total extractable tannins and condensed tannins in GTWs and BTWs were higher than in other feedstuffs. *In vitro* gas production from the feed incubated with or without PEG is described in Fig 1. GTWs and LHC produced similar amounts of gas for 24 hours ( $p > 0.05$ ) but BTWs did significantly lower than these two feedstuffs ( $p < 0.05$ ). Addition of PEG to BTWs significantly increased gas production from 9 hours after incubation ( $p < 0.05$ ), however the volume of gas production was not affected in GTWs and LHC with PEG ( $p > 0.05$ ). These results indicated that ruminal degradability of GTWs and LHC was similar but BTWs was low. It seems that tannins in GTWs could not suppress its degradation in rumen, while tannins in BTWs might strongly bind protein and could suppress the ruminal degradation. According to these results, tannin concentration did not correspond to gas production and PEG response. PEG or polyvinyl pyrrolidone has high affinity for tannins (Makkar *et al.*, 1995a). Tolera *et al.* (1997) reported a significant correlation between CT and the PEG response of gas production, but Khazaal and Ørskov (1994) and Rubanza *et al.* (2003) show poor relation. Lower response in GTWs due to PEG treatment indicated relatively higher nutritive potential even if it contains higher tannins. Chemical composition could not explain perfectly the PEG response in gas production test.

Table 1. Chemical compositions (%DM) of feedstuffs

	Timothy Hay	Wheat	LHC bran	GTWs	BTWs
Dry matter (DM, %)	87.0	87.5	87.7	20.5	21.7
Crude protein	8.9	15.2	21.3	35.6	26.0
ADIN, % total N	9.1	3.3	5.6	3.9	6.9
Neutral detergent fibre	68.4	36.5	37.6	31.0	41.2
Total extractable phenolics	0.94	0.42	0.72	11.3	7.84
Total extractable tannins	0.26	ND	0.13	9.57	6.08
Condensed tannins	0.05	ND	ND	2.42	0.75

ND: not detected.

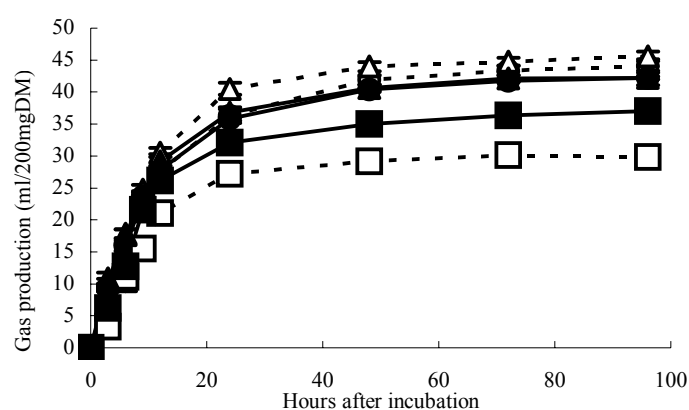


Fig. 1. Measured cumulative in vitro gas production (ml/200mg DM) for GTWs, BTWs, and LHC added with or without polyethylene glycol (PEG). ○: GTWs, ●: GTWs + PEG, □: BTWs, ■: BTWs + PEG, △: LHC, ▲: LHC + PEG.

DM, CP and NDF digestibility were not affected by type of supplement ( $p = 0.40, 0.12, 0.81$ , respectively) (Table 2). ADIN digestibility were 0.16 ~ 0.21 for control, LHC and GTWs diets, but -0.10 for BTWs treatment. In addition, fecal N output from the BTWs treatment tended to be high compared with other treatments ( $p = 0.08$ ) (data not shown). Makkar *et al.* (1995b) and Woodward and Reed (1997) also demonstrated similar results in *Acacia* species, which contains high tannins. Ruminal  $\text{NH}_3\text{-N}$  concentration in BTWs treatment was significantly lower than other groups at 1 ~ 4 hours after feeding (Fig. 2). These results indicated that supplemented GTWs and LHC would be digestible similar extent; on the other hand, degradability and digestibility of BTWs would be lower than other feedstuffs. In this study, BTWs supplementation showed the increment N output and decrement ruminal  $\text{NH}_3\text{-N}$  concentration even though BTWs contained lower tannin than GTWs. In temperate forages, such as birdsfoot trefoil, big trefoil, sainfoin and sulla, increasing CT concentration progressively increased the amount of undegraded feed protein flowing out of the rumen (Min *et al.*, 2003). These indicated CT binds feed proteins in the rumen and suppressed their protein degradation. However, these trends were different from our results. Tannins in BTWs could bind ingested proteins strongly in rumen and post-rumen even the tannin concentration was low. According to these in vitro and in vivo results, chemical reaction of Folin reagent and butanol-HCl with tea tannins (catechin, epicatechin gallate, epigallocatechin gallate, theaflavins and others) could not explain the biological reaction of feed degradation in rumen. Compared with chemical composition, in vitro and in vivo data, in vitro gas test with PEG must be suitable for assessment of antinutritive effect that occurred in digestive tract in vivo.

Table 2. Dry matter (DM) intake and nutrients digestibility of goats fed timothy hay and wheat bran supplemented with lucerne hay cube, green tea waste silage or black tea waste silage

	Control	+LHC	+GTWs	+BTWs	SEM
DM intake (g/kg W <sup>0.75</sup> )	48.3	50.7	50.2	51.5	-
Nutrients digestibility					
Dry matter	0.553	0.573	0.567	0.553	0.009
CP	0.599	0.646	0.641	0.602	0.017
ADIN	0.188 b*	0.213 b	0.158 b	-0.102 a	0.033
Neutral detergent fibre	0.532	0.543	0.543	0.533	0.011

\* Means with different letters within a row are significantly different (p<0.05).

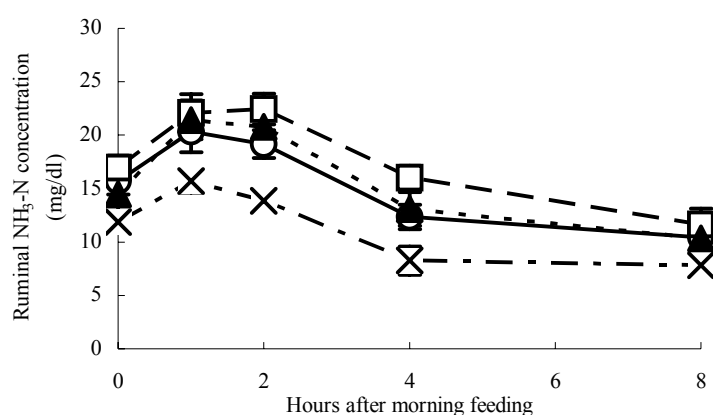


Fig. 2. Ruminal NH<sub>3</sub>-N concentration of goats fed timothy hay and wheat bran supplemented with LHC, GTWs or BTWs. Points indicate mean values of four replicates goats with standard errors represented by vertical bars. ○:control, □: +LHC, ▲: +GTWs, ×: +BTWs.

## Conclusions

GTWs could substitute LHC as protein supplement; on the other hand, tannins in BTWs suppressed N degradability in rumen and increased fecal N output which was due to making tannin-protein complex tightly in digestive tract.

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