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Research article

Effect of different levels of green tea waste supplementation on *in vitro* digestibility and ruminal fermentation

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Abstract

Importance of the work: The high nutritive value and multiple functional components of green tea waste (GTW) could be used as alternative feedstuff for ruminants.

Objectives: To investigate the effects of different levels of GTW supplementation on *in vitro* gas production, ruminal digestion and fermentation characteristics.

Materials & Methods: The experiment followed a completely randomized design with a 2×3 factorial arrangement of treatments with a control. The control treatment was total mixed ration (TMR) without any supplementation. Factor A was the type of GTW (containing fresh or dried GTW; FGTW and DGTW, respectively), and factor B was the level of GTW addition in TMR at rates of 5%, 10% or 15% on a dry matter (DM) basis.

Results: There was no interaction between the type and level of GTW supplementation on *in vitro* gas production, gas kinetics and ruminal fermentation end products, except for the *in vitro* digestibility of nutrients. Compared with the control, the addition of GTW resulted in significantly higher levels of *in vitro* gas production, *in vitro* digestibility of DM and organic matter (IVDMD and IVOMD), and total volatile fatty acid (VFA). Furthermore, the DGTW supplementation showed higher levels of gas production, IVDMD, IVOMD, ruminal NH_3-N and total VFA concentration compared with FGTW, particularly when 10–15% GTW was added.

Main finding: GTW could be effectively used as ruminant feed and the addition of DGTW in the TMR could enhance gas production, ruminal digestion and fermentation end products. Further *in vivo* study is needed to evaluate the use of GTW on animal performance.

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Introduction

Nowadays, much research on ruminant feed has focused on finding alternative feed ingredients that can replace protein-rich commercial feeds, such as soybean meal, to reduce feed costs especially in an intensive livestock production system (Lunsin, 2018; Lunsin et al., 2020). Local agricultural and food industrial by-products have received much attention as feed alternatives, it would be both economically and environmentally beneficial to use these by-products instead of commercial feedstuff (Kondo et al., 2004c; Seo et al., 2015), and this provide a more sustainable alternative feed for livestock production.

Green tea waste (GTW) is a food by-products of ready-made tea drinks, derived from tea leaves extracted using hot water to make tea drinks. GTW contains large amounts of protein, tannin, caffeine, beta-carotene and vitamin E, which could be used as feed resource or a nutrient supplement for animals (Wang and Xu, 2013). Other studies have reported that GTW usually comprises 15.1–9.6% dry matter (DM) with 25.2–33.2% crude protein (CP), 1.2–7.3% ether extract (EE), 29.9–44.5% neutral detergent fiber (NDF) and 26.1–36.7% acid detergent fiber (ADF) on a DM basis (Kondo et al., 2004a, b, c; Nishida et al., 2006; Theeraphaksirinont et al., 2009; Wang et al., 2011; Kondo et al., 2014). Ongoing research over decades had indicated that GTW could be a useful protein source for ruminants in a feeding system based on low quality feed areas. Kondo et al. (2004c) found that ensiled GTW can be used as a protein source in a total mixed ration (TMR) at a rate of 5% (DM basis) and 10% on a CP basis without any detrimental effects on the performance of lactating cows. Theeraphaksirinont et al. (2009) reported that GTW can be used as a source of protein at 5–10% (DM basis) in the diet of crossbred multiparous cows during their mid-lactation period without any deleterious effect on milk yield, nutrient digestibility and animal performance. Replacement of conventional ingredients with GTW in the diet for dairy cows produced a better net income than from using conventional feed. Additionally, Nishida et al. (2006) suggested that feeding diets containing 20% of dietary DM as GTW silage to Holstein steers had no negative impacts on their ruminal fermentation, while increasing their plasma antioxidative activity and the concentration of vitamin E, which would benefit their health, because high levels of antioxidants and vitamin E can protect cells and tissues from oxidative damage. Therefore, feeding GTW to animals is an option that is not only low cost but also provides a rich supply of nutrients, with multiple functional components; thus, it would be of benefit to both the farmer for the first benefit and to the animals for the other benefits.

Although extensive studies have been reported on feeding GTW to ruminants, it is still necessary to evaluate the use of GTW as a feed stuff form the viewpoints of *in vitro* ruminal digestion and fermentation. Using *in vitro* methods of feed evaluation has numerous advantages, such as being less expensive and less time-consuming and allowing incubation conditions to be maintained more precisely than for *in vivo*. Furthermore, *in vitro* techniques utilize small amounts of test feeds making them applicable to screening of feeds that are not available in sufficient quantity for *in vivo* experiments (Getachew et al., 2002). Therefore, the current study investigated *in vitro* gas production techniques to evaluate the effects of different levels of GTW supplementation on ruminal digestion and fermentation characteristics in Holstein-Friesian crossbred cows. It was hypothesized that the use of GTW as an alternative feed resource in the cows could enhance ruminal digestion and fermentation of end products.

Materials and Methods

Experimental design and dietary treatments

This experiment was conducted using an *in vitro* gas production technique, following a completely randomized design with a 2×3 factorial arrangement of treatments with a control. The two factors were: factor A was the type of green tea waste (GTW), being either fresh or dried GTW, while factor B was the level of GTW supplementation in the total mixed ration (TMR) at 5%, 10%, and 15% on a dry matter (DM) basis. The experimental diet in the control treatment was a TMR with a 40:60 roughage-to-concentrate ratio (DM basis). The concentrate was made from locally available feed ingredients, with rice straw used as a roughage source. Therefore, the experimental treatments were: T1 = control (TMR); T2 = 5% fresh GTW (5FGTW); T3 = 10% fresh GTW (10FGTW); T4 = 15% fresh GTW (15FGTW); T5 = 5% dried GTW (5DGTW); T6 = 10% dried GTW (10DGTW); and T7 = 15% dried GTW (15DGTW), with all amounts being as supplement for TMR on a DM basis.

Preparation of experimental treatments

The FGTW was obtained from a beverage shop at the Faculty of Agriculture, Ubon Ratchatani Ratchaphat University, Thailand. Samples of FGTW were dried in a hot-air oven at 60 °C for 48 h, before samples of FGTW and DGTW were ground to pass through a 1mm sieve and subsequently used for chemical analysis and as supplements in the different TMR diets. The feed ingredients and chemical compositions of the concentrate, rice straw, FGTW and DGTW are shown in Table 1.

The TMR diet was prepared using 40% rice straw and 60% concentrate on a DM basis (T1, control), then ground to pass through a 1 mm sieve for chemical analysis and mixing with the GTW treatments. As for the GTW treatments (T2–T7), either FGTW or DGTW was weighed and supplemented in the TMR at 5%, 10%, or 15% on a DM basis. Then, the experimental diets were used for chemical analysis and the *in vitro* gas production technique. Approximately 200 mg of experimental feeds on a DM basis were weighed into 50 mL serum bottle. The bottles were pre-warmed in a hot-air oven at 39 °C for 1 h prior to injection of 30 ml of rumen inoculum into each bottle, following the procedures of Menke and Steingass (1988). The chemical composition of the experimental diets is shown in Table 2.

Table 1 Feed ingredients and chemical composition of concentrate, rice straw, fresh green tea waste and dry green tea waste on dry matter basis

Ingredient (% DM)	Concentrate	Rice straw	FGTW	DGTW
Cassava chip	52.0	-	-	-
Palm kernel cake	15.0	-	-	-
Soybean meal	15.0	-	-	-
Fine rice bran	12.0	-	-	-
Urea	2.0	-	-	-
Molasses	2.3	-	-	-
Sulfur	0.2	-	-	-
Salt	0.5	-	-	-
Mineral mixture	0.5	-	-	-
Di-calcium phosphate	0.5	-	-	-
Total	100.0	-	-	-
Chemical composition (% DM basis)				
DM (%)	92.3	94.7	36.5	92.5
OM	94.8	92.8	98.4	96.1
CP	18.8	4.7	12.3	22.7
EE	3.8	0.4	1.1	1.2
NDF	23.7	82.8	31.1	45.7
ADF	12.0	45.3	20.4	28.8

FGTW = fresh green tea waste; DGTW = dry green tea waste; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber

Table 2 Chemical composition of experimental diets on dry matter (DM) basis

Items	Chemical compositions (%DM)					
	DM (%)	OM	CP	EE	NDF	ADF
T1 (Control)	92.8	93.3	13.4	2.4	46.9	24.4
T2 (5FGTW)	90.2	93.8	13.8	2.4	47.8	25.5
T3 (10FGTW)	86.9	95.6	14.1	2.5	49.7	27.0
T4 (15FGTW)	84.2	96.7	14.8	2.5	51.0	27.6
T5 (5DGTW)	93.0	94.5	14.3	2.5	49.3	26.5
T6 (10DGTW)	94.5	95.2	15.0	2.6	51.4	27.3
T7 (15DGTW)	93.9	96.0	15.9	2.6	53.6	28.4

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; Control = total mixed ration (TMR), containing 40:60 roughage-to-concentrate ratio; FGTW = fresh green tea waste; DGTW = dry green tea waste; 5FGTW = 5% FGTW supplementation in TMR; 10FGTW = 10% FGTW supplementation in TMR; 15FGTW = 15% FGTW supplementation in TMR; 5DGTW= 5% DGTW supplementation in TMR; 10DGTW = 10% DGTW supplementation in TMR; 15DGTW = 15% DGTW supplementation in TMR (all amounts of FGTW or DGTW being as supplement for TMR on a DM basis)

In vitro gas production technique

The *in vitro* fermentation was carried out according to the technique described by Menke et al. (1979). Two crossbred (75% Holstein-Friesian) lactating dairy cows used as rumen fluid donors were individually penned and provided with clean, fresh water and mineral-salt blocks *ad libitum*. The animals were fed with rice straw as roughage *ad libitum* and the concentrate was fed at a ratio of milk yield-to-concentrate of 2:1 for 14 d before the rumen fluid was collected. Rumen fluid was obtained from each animal before morning feeding through a stomach tube into a pre-warmed thermos flask and immediately brought to the laboratory. The rumen fluid was filtered through a four-layered cheese cloth into a conical flask and placed in a warm water bath with continuous flushing of CO_2 gas. Artificial saliva was prepared according to Menke and Steingas (1988). The artificial saliva and rumen fluid were mixed in a proportion of 2:1 to make the rumen inoculum at 39 °C under a CO_2 atmosphere. The rumen inoculum (30 mL) was transferred into each sample bottle containing 200 mg DM of substrate treatments. The dietary treatments were done in three replicates within the incubation, with three bottles containing only rumen inoculation mixture always included with each set of samples as a blank control. The sample bottles were prepared in three different sets: the first set of 21 sample bottles (7 treatments × 3 bottles per treatment) was used for the *in vitro* gas production test, the second set of 42 bottles (7 treatments × 3 bottles per treatment × 2 sampling times at 24 h and 48 h of incubation) were separately collected for *in vitro* ruminal pH, ammonia-nitrogen (NH_3-N) and volatile fatty acid (VFA), while final set of 42 bottles (7 treatments × 3 bottles per treatment × 2 sampling times at 24 h and 48 h of incubation) were used to evaluate *in vitro* DM and OM digestibility (IVDMD and IVOMD). All sample bottles were sealed with rubber stoppers and aluminum caps, then incubated in a hot-air oven at 39 °C.

Gas readings for the volumes of gas production were recorded over 96 h (2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h, 36 h, 48 h, 72 h and 96 h of incubation). At reading, the contents of the bottles were shaken gently. The cumulative gas production data recorded were fitted to the model of Ørskov and McDonald (1979) as shown in Equation 1:

$$y = a+b\,(1 - e^{ct}) \tag{1}$$

where y is the gas volume in milliliters at time t, a is the gas production from the immediately soluble fraction in milliliters, b is the gas production from the insoluble fraction in milliliters, $(a + b)$ is the potential extent of gas production in milliliters, c is the rate of gas production in milliliters per hour and t is the incubation time in hours.

Chemical analysis, digestibility of nutrient and rumen fermentation measurements

The concentrate, rice straw, FGTW, DGTW, TMR and experimental diets were analyzed for dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE), according to the methods of Association of Official Analytical Chemists (AOAC) (1998). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the protocol of Van Soest et al. (1991).

The *in vitro* digestibility of nutrients was elucidated at 24 h and 48 h of incubation, when the contents were filtered through pre-weighed Gooch crucibles and the residual DM was estimated. The weight loss was determined and defined as IVDMD. The dried feed sample and residue left above were ashed at 550 °C for determination of the IVOMD (Tilley and Terry, 1963).

The fermentation fluid was collected at 24 h and 48 h of incubation to investigate the ruminal pH, NH_3-N and VFA concentrations. The pH of the fermentation fluid was measured using a portable pH meter (HANNA Instruments; HI 8424 microcomputer, Singapore). Then, the fermentation fluid was filtered through four layers of cheesecloth, centrifuged at 16,000×g for 15 min and the supernatant was analyzed for NH_3-N using the micro-Kjeldahl method (AOAC, 1998) and for VFA using high-performance liquid chromatography (HPLC), according to Samuel et al. (1997).

Statistical analysis

All data were subjected to statistically analysis using a general linear model according to SAS (2006). The orthogonal contrast was used to investigate the effect of treatment response, while the following model was used for factorial comparison: $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$, where Y is the observations, μ is the overall mean, A_i is the effect of factor A (type of GTW, i = fresh or dried GTW), B_j = is the effect of factor B (level of GTW supplementation in TMR, j = 5%, 10%, or 15% DM, respectively), AB_{ij} is the interaction between factor A and B and ε_{ijk} is the residual effect. Differences among means were tested using Duncan's new multiple range test (Steel and Torrie, 1980) with $p < 0.05$ accepted as representing a statistically significant difference.

Ethics statements

All experimental procedures involving the animals were approved by the Ethics Committee of Ubon Ratchathani Rajabhat University, Thailand (Reference number: AN64005).

Results and Discussion

Chemical composition of experimental diets

The chemical compositions of the concentrate, rice straw, FGTW and DGTW are presented in Table 1. The concentrate had a CP content of 18.8% DM and the rice straw had 4.7% CP on a DM basis. The DGTW contained more DM and CP (92.5% and 22.3%, respectively on a DM basis) than FGTW (36.5% and 14.0%, respectively, on a DM basis), while the fiber fractions of NDF and ADF in FGTW were lower than in DGTW. The chemical composition of FGTW in this experiment was similar to that reported by Wang et al. (2011), except for their lower content of CP (12.4% on a DM basis). However, the chemical composition of GTW was inconsistent and probably differed due to factors such as the variety of green tea species, harvesting season, harvesting time, planting area and the processing of the tea drink.

Furthermore, the chemical compositions of the TMR and GTW treatments were slightly different among treatments (Table 2). The DM content of the TMR diet was 92.8%, while it was 84.2–90.2% for FGTW diets and 93.0–94.5% for DGTW diets, all on a DM basis. The FGTW diets had lower DM contents than the TMR and DGTW diets perhaps because FGTW contained high moisture levels (63.5%). The TMR (control) diet, which was comparable with the FGTW and DGTW diets, had lower CP and fibrous (NDF and ADF) contents, whereas the CP, NDF and ADF contents were lower in FGTW compared with DGTW. This was related to the DGTW containing higher CP, NDF, and ADF levels (22.7%, 45.7% and 28.8%, respectively, all on a DM basis) than FGTW (12.3%, 31.1% and 20.4%, respectively, all on a DM basis). Additionally, the OM and EE contents of the experimental diets were in the ranges range from 93.3–96.7% on an OM basis and 2.4–2.6% EE on a DM basis, respectively.

In vitro gas production and ruminal digestion

Gas production volumes, the kinetics of gas production and the *in vitro* digestibility of nutrients are illustrated in Tables 3 and 4, indicating that there no observed interaction between the type of GTW (FGTW or DGTW) and the level of GTW supplementation (5%, 10% or 15 % on a DM basis of TMR). The control treatment had highly significantly ($p < 0.01$) lower values of gas volumes, gas kinetics, potential gas production and *in vitro* digestibility of dry matter and organic matter (IVDMD and IVOMD) than those of GTW treatments, whereas gas production form the immediately soluble fraction and the gas production rate constant were not significantly ($p > 0.05$) different between the control and GTW treatments. For the GTW treatments, the highest gas volume, gas kinetics, IVDMD and IVOMD were observed in the DGTW diets. The gas volume, gas kinetics and *in vitro* digestibility of nutrients (IVDMD and IVOMD) were significantly increased with incremental GTW levels, being highest in 10% GTW supplementation in TMR, followed by 15% GTW and the lowest values were observed when 5% GTW was added. The addition of GTW in TMR increased *in vitro* gas production and nutrient digestibility, probably due to better fermentability of the GTW itself (Kondo et al., 2004a). Gas production on incubation of feeds in buffered rumen fluid is a result of feed fermentation, with the proportion of fermentation products depending on the feed, especially the CP and carbohydrate fractions (Kondo et al., 2014) The CP content of feed was positively correlated with total gas production (Kulivand and Kafilzadeh, 2015; Njidda et al., 2017), which was in agreement with the results of the current experiment. The GTW diets had a higher CP content, which resulted in higher gas production than the control and a high CP content was also found in DGTW compared with the FGTW treatment; thus, gas production was high when DGTW was added in TMR. Compared with the control, high values of IVDMD and IVOMD were observed in the GTW treatment, with DGTW having higher values for IVDMD and IVOMD than FGTW. This increase in the *in vitro* digestibility of nutrient was consistent with the observed high gas production (Lunsin et al., 2018), and agreed with Njidda (2011), who reported that there was a strong and positive correlation (r = 0.992) between gas production and IVOMD.

Table 3 Effects of green tea waste on rate of gas production and kinetics of gas production from *in vitro* fermentation

Treatment (T)	Gas kinetics				Cumulative gas production (96 h) (mL/0.2 g DM)
	a	b	c	a + b	
T1 (Control)	-5.9±0.17	59.6±0.48	0.049±0.0002	53.7±0.34	53.2±0.33
T2 (5FGTW)	-5.8±0.34	63.0±1.51	0.048±0.0004	57.2±1.20	56.6±1.20
T3 (10FGTW)	-5.8±0.31	64.1±1.59	0.048±0.0007	58.3±1.37	57.7±1.38
T4 (15FGTW)	-6.0±0.09	63.3±1.23	0.050±0.0014	57.3±1.14	56.8±1.18
T5 (5DGTW)	-5.9±0.19	65.1±0.58	0.048±0.0007	59.2±0.77	58.7±0.73
T6 (10DGTW)	-6.4±0.31	68.6±0.94	0.050±0.0013	62.2±1.25	61.7±1.19
T7 (15DGTW)	-6.2±0.11	66.9±0.51	0.050±0.002	60.7±0.47	60.2±0.46
SEM	0.33	0.52	0.38	0.52	0.52
Comparison					
Control versus Others					
Control	-5.9±0.17	59.6±0.48[b]	0.049±0.0002	53.7±0.34[b]	53.2±0.33[b]
Others	-6.0±0.33	65.2±2.29[a]	0.049±0.0014	59.1±2.07[a]	58.6±2.07[a]
p value	0.31	< 0.01	0.65	< 0.01	< 0.01
FGTW versus DGTW (A)					
FGTW	-5.9±0.25[b]	63.5±1.35[b]	0.049±0.0014[b]	57.6±1.20[b]	57.1±1.21[b]
DGTW	-6.2±0.30[a]	66.9±1.62[a]	0.050±0.0013[a]	60.7±1.48[a]	60.2±1.50[a]
p value	0.01	< 0.01	0.04	< 0.01	< 0.01
GTW levels, % DM (B)					
5%	-5.9±0.25	64.1±1.54[b]	0.04 ±0.0006[c]	58.2±1.44[b]	57.6±1.43[b]
10%	-6.1±0.45	66.4±2.72[a]	0.049±0.0016[b]	60.2±2.41[a]	59.7±2.43[a]
15%	-6.1±0.16	65.1±2.15[ab]	0.050±0.0009[a]	59.0±2.00[ab]	58.5±2.01[ab]
p value	0.09	0.02	< 0.01	0.02	0.02
Interaction A×B					
p value	0.14	0.22	0.10	0.36	0.33

a = gas production (in milliliters) from immediately soluble fraction; b= gas production (in milliliters) from the insoluble fraction; c = gas production rate constant (in milliliters per hour) for insoluble fraction; a + b = extent of gas production (in milliliters); FGTW = fresh green tea waste; DGTW = dry green tea waste; 5FGTW = 5% FGTW supplementation in TMR; 10FGTW = 10% FGTW supplementation in TMR; 15FGTW = 15% FGTW supplementation in TMR; 5DGTW = 5% DGTW supplementation in TMR; 10DGTW = 10% DGTW supplementation in TMR; 15DGTW = 15% DGTW supplementation in TMR (all amounts of FGTW or DGTW being as supplement for TMR on a DM basis). SEM = standard error of the mean
Mean values (±SD) within columns superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 4 Effect of green tea waste on *in vitro* digestibility of nutrients at 24 h and 48 h of incubation

Treatment (T)	IVDMD (%)		IVOMD (%)	
	24 h	48 h	24 h	48 h
T1 (Control)	44.5±1.38[c]	50.3±0.37[d]	69.1±0.79	72.1±0.09[e]
T2 (5FGTW)	46.2±0.86[c]	53.9±0.16[c]	69.0±0.64	72.3±0.38[e]
T3 (10FGTW)	45.8±0.93[c]	56.1±0.62[c]	68.6±0.76	73.7±0.30[d]
T4 (15FGTW)	46.0±0.84[c]	54.5±0.44[c]	68.8±0.83	72.2±0.36[e]
T5 (5DGTW)	55.5±1.20[b]	62.1±0.26[b]	70.4±0.73	74.6±0.37[c]
T6 (10DGTW)	60.1±0.31[a]	65.6±0.18[a]	73.8±0.70	76.5±0.50[a]
T7 (15DGTW)	59.0±0.45[a]	64.0±0.26[ab]	71.3±1.81	75.8±0.17[b]
SEM	0.57	0.55	0.56	0.56
Comparison				
Control versus Others				
Control	44.5±1.38[b]	50.3[b]	69.1±0.79[b]	72.1±0.09[b]
Others	52.1±6.50[a]	59.3[a]	70.3±4.85[a]	74.2±1.71[a]
p value	< 0.01	< 0.01	< 0.01	< 0.01

Table 4 Continued

Treatment (T)	IVDMD (%)		IVOMD (%)	
	24 h	48 h	24 h	48 h
FGTW versus DGTW (A)				
FGTW	46.0±0.78[b]	54.8[b]	68.8±1.19[b]	72.7±0.78[b]
DGTW	58.2±2.21[a]	63.9[a]	71.8±1.83[a]	75.6±0.90[a]
p value	< 0.01	< 0.01	< 0.01	< 0.01
GTW levels, % DM (B)				
5%	50.8±5.19[b]	58.0	69.7±4.54	73.4±1.30[c]
10%	53.0±7.88[a]	60.8	71.2±5.23	75.1±1.58[a]
15%	52.5±7.18[a]	59.2	70.0±5.18	74.0±2.02[b]
p value	0.01	0.15	0.11	0.01
Interaction A×B				
p value	< 0.01	< 0.01	0.69	0.02

IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; FGTW = fresh green tea waste; DGTW = dry green tea waste; 5FGTW= 5% FGTW supplementation in TMR; 10FGTW = 10% FGTW supplementation in TMR; 15FGTW = 15% FGTW supplementation in TMR; 5DGTW= 5% DGTW supplementation in TMR; 10DGTW = 10% DGTW supplementation in TMR; 15DGTW = 15% DGTW supplementation in TMR (all amounts of FGTW or DGTW being as supplement for TMR on a DM basis).
SEM = standard error of the mean
Mean (±SD) values within columns superscripted with different lowercase letters are significantly ($p < 0.05$) different.

In vitro ruminal fermentation characteristics

Compared with the control, no significant ($p > 0.05$) changes were observed in ruminal pH, NH_3-N and individual volatile fatty acids (VFA) concentration (Table 5), except for the total VFA concentration after 48 h of incubation, which was highly significantly ($p < 0.01$) different, while that for the GTW diets were higher. This result agreed with Nishida et al. (2006) who investigated the effects of supplementation at 20% DM of GTW silage in Holstein steers and reported no effect on individual VFA proportions, pH, or the concentration of NH_3-N; Thus, it would appear that feeding GTW silage at 20% DM in diets had no negative impact on ruminal fermentation in Holstein steers. In addition, Kondo et al. (2004c) reported that the ruminal pH, VFA concentration and molar proportion of VFA were not significantly different between the control and GTW treatments. Although there were no significant ($p < 0.05$) interactions of NH_3-N between the source of GTW (FGTW or DGTW) and the level of GTW supplementation (5%, 10% or 15% on a DM basis of TMR), higher NH_3-N values were observed in DGTW compared to FGTW, perhaps due to the supplementation of DGTW in the TMR diet, which contained a high protein level; thus, the DGTW diet introduced additional nitrogen to the ruminal fermentation and a resultant high NH_3-N concentration was observed. This agreed with Kondo et al. (2004a) who reported that the addition of GTW to oat silage increased CP digestibility, N retention and ruminal NH_3-N in goats. In addition, there were no significant ($p > 0.05$) interactions on individual VFA proportions and the total VFA concentration between the type of GTW and the level of GTW addition (Table 5), whereas, at 48 h of incubation, the total VFA concentration was highly significantly ($p < 0.01$) higher for the GTW diet than the control. There was a correlation between the digestibility of nutrient and VFA production as reported by Pazla et al. (2021); the production of VFA can be used as a benchmark for the level of feed digestibility where the higher level of feed digestibility, the greater VFA was produced. It was apparent from the relatively high IVDMD and IVOMD values that there was enhanced microbial access and degradation and fermentation (Ansah et al., 2021), resulting in enhancing the VFA production, especially in the DGTW treatment, as observed in the current experiment.

The results of the present study indicated that supplementation of GTW in the TMR diet enhanced the *in vitro* gas production, gas kinetics, digestibility of nutrients and total VFA, while ruminal pH, NH_3-N and the individual VFA concentration were not affected. The high CP content, *in vitro* gas volumes, digestibility of nutrients, ruminal NH_3-N, and total VFA concentration indicated the potential of DGTW as a protein supplement, with the addition of DGTW up to 10–15% on a DM basis of TMR. However, further research is required to better understand the utilization of GTW as protein supplements, compared with commercial protein-rich feedstuffs by conducting *in vivo* experiments in association with animal performance.

Table 5 Effect of green tea waste on *in vitro* ruminal ammonia nitrogen (NH_3-N) and volatile fatty acids at 24 h and 48 h of incubation

Treatment (T)	pH		NH_3-N (%)		Acetate (%)		Propionate (%)		Butyrate (%)		Acetate to Propionate ratio		Total VFA (mmol/L)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
T1 (Control)	6.63±0.01	6.61±0.01	15.6±0.08	16.3±0.18	68.2±0.56	67.4±0.93	19.5±1.01	21.0±0.48	12.3±0.46	11.6±0.45	3.5±0.21	3.2±0.12	86.9±3.10	93.2±0.14
T2 (5FGTW)	6.62±0.03	6.61±0.01	15.2±0.27	16.0±0.18	70.8±1.61	66.9±0.30	16.7±1.82	21.8±0.27	12.5±0.20	11.3±0.02	4.4±1.50	3.1±0.05	74.1±3.26	99.4±1.44
T3 (10FGTW)	6.67±0.01	6.64±0.03	15.3±0.09	16.0±0.28	67.0±2.29	66.8±1.32	20.7±0.78	21.3±1.66	12.3±2.93	11.9±0.34	3.2±0.06	3.1±0.04	87.7±1.81	100.5±1.99
T4 (15FGTW)	6.66±0.02	6.62±0.01	15.4±0.08	15.9±0.28	66.7±0.58	67.2±0.61	21.3±0.45	21.5±0.09	12.0±1.30	11.3±0.53	3.4±0.15	3.2±0.03	83.5±1.71	99.8±1.55
T5 (5DGTW)	6.64±0.03	6.61±0.01	16.1±0.24	16.7±0.33	67.5±0.60	67.4±0.38	20.3±0.15	21.7±0.19	12.2±0.45	11.0±0.19	3.3±0.05	3.1±0.04	93.8±5.03	100.0±1.17
T6 (10DGTW)	6.65±0.01	6.63±0.01	16.3±0.24	17.0±0.13	67.0±1.30	66.9±0.36	20.4±0.05	22.0±0.16	12.6±1.25	11.1±0.20	3.1±0.05	3.0±0.07	90.3±4.87	115.4±3.18
T7 (15DGTW)	6.67±0.01	6.64±0.01	16.6±0.35	17.4±0.08	66.4±1.23	66.4±0.26	21.2±0.41	22.4±0.81	12.4±0.82	11.2±0.55	3.3±0.10	3.1±0.03	97.8±0.76	109.3±1.30
SEM	0.34	0.32	0.52	0.53	0.24	0.17	0.32	0.19	0.02	0.31	0.29	0.34	0.25	0.51
Comparison														
Control vs Others														
Control	6.63±0.01	6.61±0.01	15.6±0.08	16.3±0.18	68.2±0.56	67.4±0.93	19.5±1.01	21.0±0.48	12.1±0.46	11.6±0.45	3.5±0.21	3.2±0.12	86.9±3.10	93.2±0.41[b]
Others	6.65±0.02	6.63±0.02	15.8±0.59	16.5±0.59	67.6±1.86	66.9±0.58	20.1±1.75	21.8±0.77	12.3±1.02	11.3±0.40	3.5±0.65	3.1±0.10	87.9±8.60	104.1±6.79[a]
p value	0.22	0.17	0.32	0.15	0.73	0.43	0.70	0.28	0.94	0.29	0.93	0.11	0.92	0.01
FGTW versus DGTW (A)														
FGTW	6.65±0.03	6.62±0.02	15.3±0.17[b]	16.0±0.17[b]	68.1±2.41	67.0±0.70	19.6±2.42	21.5±0.95	12.3±1.33	11.5±0.42	3.7±0.89	3.1±0.11	81.8±7.27	99.9±2.99[b]
DGTW	6.65±0.03	6.63±0.02	16.3±0.33[a]	17.0±0.33[a]	67.0±0.98	66.9±0.50	20.6±0.51	22.0±0.51	12.4±0.72	11.1±0.29	3.2±0.11	3.1±0.07	93.9±4.60	108.2±7.11[a]
p value	0.58	0.75	< 0.01	< 0.01	0.41	0.82	0.36	0.37	0.86	0.12	0.22	0.23	0.10	0.01
GTW levels, % DM (B)														
5%	6.63±0.03	6.61 ± 0.01	15.7±0.53	16.3±0.53	69.1±2.21	67.1±0.38	18.5±2.29	21.7±0.21	12.4±0.33	11.1±0.22	3.9±1.07	3.1±0.05	83.9±11.85	99.7±1.13
10%	6.66±0.01	6.64 ± 0.02	15.8±0.60	16.5±0.60	67.0±1.52	66.9±0.79	20.6±0.48	21.6±1.04	12.5±1.84	11.5±0.48	3.2±0.07	3.0±0.07	89.0±3.35	107.9±8.86
15%	6.66±0.02	6.63 ± 0.02	16.0±0.74	16.6±0.74	66.5±0.80	66.8±0.63	21.3±0.48	22.0±0.99	12.2±0.52	11.2±0.45	3.3±0.10	3.1±0.14	90.7±9.31	104.5±6.62
p value	0.05	0.14	0.06	0.08	0.16	0.55	0.07	0.71	0.84	0.78	0.21	0.53	0.43	0.16
Interaction A×B														
p value	0.46	0.77	0.56	0.09	0.62	0.41	0.36	0.71	0.93	0.48	0.46	0.31	0.61	0.17

VFA = volatile fatty acids; FGTW = fresh green tea waste; DGTW = dry green tea waste; 5FGTW= 5% FGTW supplementation in TMR; 10FGTW = 10% FGTW supplementation in TMR; 15FGTW = 15% FGTW supplementation in TMR; 5DGTW= 5% DGTW supplementation in TMR; 10DGTW = 10% DGTW supplementation in TMR; 15DGTW = 15% DGTW supplementation in TMR (all amounts of FGTW or DGTW being as supplement for TMR on a DM basis).

SEM = standard error of the mean

Mean (±SD) values within columns superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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