# Effect of Pre-treating Green Tea (*Camellia sinensis*) Powder with Graded Levels of Polyethylene Glycol on Chemical Composition and *In-vitro* Ruminal Fermentation

**Matabane, A.N.[1], Setou, B.A.[1], Tshukudu, J.L.[1], Nthinya, S.K.[1], Mahlake, S.K.[1], Mhlongo, G.[1], Mbhele, F.G.T.[1], and Mnisi, C.M.[1,2,*]**

[1]Department of Animal Science, School of Agricultural Science, North-West University, Mafikeng, South Africa
[2]Food Security and Safety Niche area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, South Africa

*Corresponding author: stevekgotlelelo@gmail.com

Received: 03 August 2021
Accepted: 25 February 2022

## Abstract

The study evaluated the effect of pre-treating green tea powder (GTP) with different concentrations of polyethylene glycol (PEG), a tannin-binding agent, on chemical composition and *in vitro* ruminal fermentation. The PEG (Mr 4400) was applied at 0 (PEG0), 25 (PEG25), 50 (PEG50), 75 (PEG75) and 100 (PEG100) g/kg (v/w). The PEG pre-treatment showed linear and quadratic trends for total soluble phenolics [$R^2$ = 0.510; P = 0.029], but not for dry matter, organic matter, soluble condensed tannins and acid detergent lignin of GTP. Crude protein [$R^2$ = 0.510, P < 0.0001] and neutral detergent fibre [$R^2$ = 0.580, P < 0.0001] showed a linear decrease, but acid detergent fibre [$R^2$ = 0.655, P < 0.0001] showed a linear increase with PEG levels. There were significant quadratic trends for fractions *b* [$R^2$ = 0.475, P = 0.023] and *c* [$R^2$ = 0.446, P = 0.001] as well as effective degradability [$R^2$ = 0.474, P = 0.023] in response to PEG concentrations. PEG0 had similar (P > 0.05) potential degradability as PEG50, PEG75 and PEG100. The PEG25 treatment had the higher effective gas production (73.1 ml/g OM) than PEG75 and PEG100, which did not differ (P > 0.05). Organic matter degradability (OMD) linearly increased at 24-h [$R^2$ = 0.344; P = 0.003], but declined at 36-h [$R^2$ = 0.190; P = 0.190] post-incubation as PEG levels increased. Significant linear and quadratic trends were observed for 12-h [$R^2$ = 0.389; P = 0.032] and 24-h [$R^2$ = 0.637; P = 0.040] partitioning factors as PEG concentrations increased. It can be concluded that PEG pre-treatment of GTP reduced total soluble phenolics, crude protein and neutral detergent fibre, and improved fermentation efficiency. Based on the quadratic responses for the degradation kinetics and partitioning factors, the optimum PEG treatment for maximum ruminal fermentation efficiency of green tea powder was determined to be 50 g/kg.

**Keywords:** Condensed tannins, Feed additives, Nutritive value, Phytogenic plants, Ruminants

## Introduction

The use of phytogenic plant products with bioactive compounds to optimise ruminant production systems for sustainable intensification has gained worldwide research attention. Green tea powder (GTP) is one such phytogenic plant product that has nutraceutical properties with growth-stimulating, antioxidant and antimicrobial

activities (Hara-Kudo *et al*., 2005). Green tea powder is an excellent source of polyphenols and catechins mainly epigallocatechin gallate, which acts as a potent antioxidant (Yang *et al*., 2003). Sarker *et al*. (2010) reported that the presence of polyphenols in GTP improved the feed efficiency and weight gains in Korean Hanwoo calves compared to the control.

The supplementation with GTP was reported to maintain microflora balance while enhancing antimicrobial activities against pathogenic bacteria (Hara-Kudo *et al*., 2005). Earlier studies have shown that GTP flavonoids, including catechins, have a wide range of biological activities that promote animal health and prevent diseases (Dufresne and Farnworth, 2001). In addition, GTP is a rich source of protein (22 - 35%), essential amino acids, carbohydrates, lipids, minerals and vitamins (Yang *et al*., 2003; Georgiev *et al*., 2014), suggesting that the powder may be a potential nutrient source for ruminants.

However, GTP also has high concentrations of condensed tannins (CT), ranging between 3 and 5% (w/w) (Georgiev *et al*., 2014), which reduce its utilisation as a functional feed ingredient in ruminant rations. Condensed tannins are plant secondary metabolites that interfere with the digestibility of dietary protein and carbohydrates by binding these molecules and form complexes that are resistant to microbial fermentation (Makkar *et al*., 1995). This consequently reduces the efficiency of nitrogen utilisation by rumen microbes. McDonald *et al*. (2010) reported that large amounts of dietary CT cause digestive upsets, haemorrhages and toxicities in ruminants. Thus, polyethylene glycol (PEG), a tannin-binding compound, can be used to negate the deleterious effects of CT in GTP. The PEG is a non-nutritive synthetic polymer that is safe for use in animal diets and presents a potential strategy to negate the effects of CT (D'souza and Shegokar, 2016). Makkar *et al*. (1995) reported that PEG has a high affinity to CT, and can be utilised to increase rumen degradable protein by preventing tannin-protein complexes. However, this phenomenon has not yet been tested using GTP produced in South Africa. This study, therefore, investigated the effect of graded levels of PEG on the chemical composition, *in vitro* ruminal fermentation kinetics, organic matter degradability and partitioning factors of GTP. It was hypothesized that pre-treatment of GTP with PEG improves its nutritive value as measured by proximate constituents and *in vitro* ruminal fermentation.

## Materials and methods

*Study area and treatment of green tea powder*

The study was carried out at Molelwane Farm (25°40.459′ S; 26°10.563′ E; Altitude: 1225 m above sea level) of the North-West University (North West, South Africa). The green tea leaves were purchased from Mountain Herb Estate (25°43′27.6″ S; 27°57′54.8″ E) situated in Kameeldrift-West (Pretoria, South Africa). The tea plants are grown on sandy soil, under mild climatic conditions with an average annual temperature of 19.0°C, and an average rainfall of 675 mm per annum.

Fresh green tea leaves were air-dried to constant weight and then milled (1-mm; Polymix-MFC0 90D, Kinematica AG, Switzerland) prior to the application of PEG. Polyethylene glycol granules (PEG Mr 4400) were acquired from Associated Chemical Enterprises (LTD) (Gauteng, South Africa). Green tea powder (100 g per sample bottle) was weighed into 30 labelled sample bottles, which were designated as experimental units. Four PEG solutions were prepared by dissolving 2.5, 5, 7.5 and 10 g of PEG in 100 ml of distilled water. The solutions were then mixed individually with 100 g of GTP samples in a half-open foil plate to produce PEG treatment rates of 25 (PEG25), 50 (PEG50), 75 (PEG75) and 100 g/kg (v/w) (PEG100). The control GTP samples were only mixed with 100 ml of distilled water

(PEG0). Each of the five treatments was replicated six times, with each replicate GTP sample being treated independently of each other. The PEG and GTP mixture was left to react for 24 h and then dried in an oven (60°C) to a constant weight. The treated GTP samples were thereafter re-milled (1-mm) in preparation for chemical analyses and the *in vitro* fermentation experiments. The PEG treatment concentrations were selected in an attempt to completely inactivate the CT by ensuring a 1:1 ratio of CT to PEG.

*Analyses of chemical constituents*

The PEG-treated GTP samples were analysed using the Official Analytical Chemists Methods (AOAC, 2005) for dry matter (DM), organic matter (OM) and crude protein (CP). An ANKOM$^{2000}$ Fibre Analyser (ANKOM Technology, NY, USA) was used to analyse fibre fractions (neutral detergent fibre (NDF) and acid detergent fibre (ADF)) according to Van Soest *et al*. (1991). Acid detergent lignin (ADL) was analysed by subjecting the ADF residue to 72% sulphuric acid. Total soluble phenolics (TSPh) were determined following the Folin-Ciocalteau method and absorbance was measured using a T60 UV-Visible Spectrophotometer (PG Instruments Limited, UK) after 40 min at 725 nm wavelength as described by Makkar (2003). Soluble condensed tannins (SCT) were determined following the butanol-HCL method described by Makkar (2003) and absorbance was taken at 550 nm wavelength using the afore-mentioned spectrophotometer. Tannic acid (Sigma-Aldrich, South Africa) was used to develop a calibration curve that was used to estimate the concentration of TSPh in PEG-treated GTP samples and expressed as tannic acid equivalents (g TAE/kg DM).

*In vitro dry matter degradability*

The procedures for handling and care of the cannulated donor cow were approved by the Animal Care Research Ethics Committee (approval no. NWU-00126-13-A9) of the North-West University, South Africa. The dry matter degradability (DMD) of the PEG-treated GTP samples was determined using the ANKOM Daisy$^{II}$ incubator following the ANKOM Technology method no. 3 for the *in vitro* true digestibility (ANKOM Technology Corp., Fairport, NY). About 0.45 - 0.5 g of PEG-treated GTP samples were weighed in ANKOM F57 filter bags and placed in ANKOM Daisy$^{II}$ jars to which 1600 ml of ANKOM buffer solution was added. The jars were placed in the incubator to warm (39°C) overnight. The following morning prior to feeding, rumen inoculum was collected from the cannulated Bonsmara cow (~600 kg) and instantly taken to a laboratory for blending until it passed through a two-layer muslin cloth while being purged with a stream of carbon dioxide to simulate the anaerobic rumen conditions. Exactly 400 ml of the processed rumen liquor was added to the pre-warmed 1600 ml buffer solution in each Daisy$^{II}$ incubator jar. The pre-weighed ANKOM F57 filter bags containing the GTP were then incubated, and the bags were periodically withdrawn at 2, 4, 8, 12, 24, 36, 48 and 72 hours. After every withdrawal, the bags were washed with tap water for 15 minutes and then dried in an oven set at 105°C for 12 h to determine DMD. The 0-hour filter bags were not incubated but were washed and dried similarly to the incubated bags. To determine degradation kinetics, the DMD data was fitted using Datafit 9.0 (Oakdale Engineering) into the Ørskov and McDonald (1979) non-linear model: $y = a + b(1 - e^{-c(t)})$, where $a$ = the immediately degradable fraction, $b$ = the slowly degradable fraction, $c$ = the rate constant from the slowly degradable fraction $b$. Potential degradability (PDeg) was calculated as the sum of fractions $a$ and $b$, while effective degradability (EDeg) was calculated following the formula:

$EDeg = a + \frac{b \times c}{K+c}$, where *K* is the assumed 2% rumen outflow rate per hour.

*Gas production parameters*

The Reading Pressure Technique (RPT) developed by Mauricio *et al*. (1999) was used to evaluate *in vitro* ruminal gas production parameters of PEG-treated GTP samples. Samples weighing 1 g were added into individual RPT bottles (size: 125 ml) to which an ANKOM buffer solution (90 ml per bottle) was added to the bottles and sealed with rubber stoppers. The sealed bottles were then transferred into a laboratory-fitted incubator set at 39°C for 12 h prior to inoculation with rumen liquor. The rumen liquor used for the RPT was collected and processed as described for the DMD experiment above. The bottles containing the samples were inoculated with 10 ml of the processed rumen fluid under a stream of $CO_2$ gas and incubated at 39°C. A pressure transducer device (PX4200-015GI, Omega Engineering Inc., Canada) was used to measure headspace gas pressure at 2, 4, 8, 12, 24, 36, 48, 72 and 96 hours post-inoculation. The pressure readings (psi) were converted into gas volume (ml) using the following equation: $y = 0.034\ x^2 + 6.23\ x + 1.81$, set for the RPT (Mhlongo *et al*., 2021). To determine fermentation kinetics, data for cumulative gas production was fitted (Datafit 9.0, Oakdale Engineering) into the Ørskov and McDonald (1979) non-linear model: $y = a + b\left(1 - e^{-c(t-lt)}\right)$, where $a$ = the gas produced from the immediately fermentable fraction, $b$ = gas produced from the slowly fermentable fraction, $c$ = the rate of gas production from fraction $b$, and lt = lag time. Potential gas production (PGas) and effective gas production (Egas) were calculated as stated for PDeg and EDeg above. *In vitro* organic matter degradability (OMD) was determined by incinerating the ANKOM F57 filter bags used to measure DMD as described by Mhlongo *et al*. (2021). Partitioning factors (PF) were calculated as a ratio of cumulative gas production to OMD at 12, 24, 36 and 48 h post-inoculation to determine fermentation efficiency.

*Statistical analyses*

Chemical composition and *in vitro* ruminal fermentation parameters data were evaluated for linear and quadratic effects using polynomial regression analysis (PROC RSREG; SAS, 2010). Quadratic responses were used to determine the optimum PEG treatment level, according to the equation: $y = c + bx + ax^2$, where $y$ = the response variable; $x$ = PEG treatment level (g/kg); $a$ and $b$ = the coefficients of the quadratic equation; and $c$ = intercept. Optimal $x$ values were calculated as: $-\frac{b}{2a}$. In a completely randomized design, one-way ANOVA (PROC GLM; SAS, 2010) was employed to analyse the data for chemical composition and *in vitro* ruminal fermentation parameters, with PEG treatment as the main factor. Least square means were separated using the probability of difference in SAS and significance was declared at $P < 0.05$.

## Results and discussion

*Chemical composition*

Regression results show that there were no linear and quadratic effects ($P > 0.05$) for DM, OM, ADL and SCT in response to PEG levels (Table 1). However, linear and quadratic responses were observed for TSPh [$y = 0.001$ ($\pm 0.001$) $x^2$ - 0.181 ($\pm 0.053$) $x$ + 12.6 ($\pm 1.12$); $R^2 = 0.510$; $P = 0.029$] as PEG levels increased. A significant linear decline was observed for CP [$y = 292.7$ ($\pm 4.905$) - 0.163 ($\pm 0.232$) $x$; $R^2 = 0.510$, $P < 0.0001$] and NDF [$y = 192.6$ ($\pm 1.55$) – 0.187 ($\pm 0.073$) $x$; $R^2 = 0.580$, $P < 0.0001$], while a linear increase was recorded for ADF [$y = 0.017$ ($\pm 0.138$) $x$ + 140.7 ($\pm 2.903$); $R^2 = 0.655$, $P < 0.0001$] in response to increasing PEG levels.

Table 1. Chemical composition (g/kg DM, unless stated otherwise) of green tea powder pre-treated with graded levels of polyethylene glycol.

| [2]Parameters | [1]Treatments | | | | | [3]SEM | P value | |
|---|---|---|---|---|---|---|---|---|
| | PEG0 | PEG25 | PEG50 | PEG75 | PEG100 | | Linear | Quadratic |
| Dry matter (g/kg) | 971.8 | 976.5 | 971.9 | 975.9 | 972.2 | 3.266 | 0.338 | 0.333 |
| Organic matter | 923.0 | 929.0 | 927.4 | 930.8 | 928.2 | 3.372 | 0.205 | 0.368 |
| Crude protein | 289.8$^{b}$ | 295.3$^{c}$ | 275.7$^{abc}$ | 271.3$^{ab}$ | 262.7$^{a}$ | 5.017 | 0.0001 | 0.509 |
| NDF | 192.9$^{b}$ | 187.5$^{ab}$ | 185.8$^{a}$ | 182.6$^{a}$ | 181.2$^{a}$ | 1.706 | 0.0001 | 0.305 |
| ADF | 142.6$^{a}$ | 138.5$^{a}$ | 144.6$^{a}$ | 159.4$^{b}$ | 162.2$^{b}$ | 2.789 | 0.0001 | 0.064 |
| ADL | 55.2 | 47.3 | 45.5 | 45.3 | 46.9 | 4.510 | 0.183 | 0.216 |
| SCT ($AU_{550/200mg}$) | 1.42 | 1.96 | 1.39 | 1.30 | 2.18 | 0.424 | 0.174 | 0.718 |
| TSPh (g TAE/kg DM) | 13.1$^{b}$ | 7.7$^{a}$ | 7.2$^{a}$ | 5.9$^{a}$ | 6.2$^{a}$ | 1.199 | 0.0001 | 0.029 |

[a,b,c] Means in a row with different superscripts significantly differ ($P < 0.05$).
[1]Treatments: PEG0 = untreated green tea powder; PEG25 = green tea powder treated with 25 g/kg of polyethylene glycol; PEG50 = green tea powder treated with 50 g/kg of polyethylene glycol; PEG75 = green tea powder treated with 75 g/kg of polyethylene glycol; PEG100 = green tea powder treated with 100 g/kg of polyethylene glycol.
[2]Parameters: NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; SCT = soluble condensed tannins; TSPh = total soluble phenolics.
[3]SEM = standard error of the mean.

Treating GTP with PEG significantly influenced CP, ADF, NDF and TSPh, but not ($P > 0.05$) DM, OM, SCT and ADL. PEG25 had the highest CP content (295.3 g/kg) and the lowest CP content was from PEG100 (262.7 g/kg). PEG0 had the highest NDF (192.9 g/kg DM) than PEG50, PEG75 and PEG100, which were statistically similar. PEG75 and PEG100 had higher ($P < 0.05$) ADF content compared to the other treatments. PEG0 had higher TSPh (13.1 g TAE/kg DM) than the other PEG treatments, which were similar ($P > 0.05$).

The presence of condensed tannins in green tea powder could reduce protein and energy utilisation by binding protein and carbohydrates and form ruminal undegradable complexes (Makkar *et al*., 1995). Dey *et al*. (2008) found that concentrations beyond 50 g/kg DM of dietary CT have adverse effects on feed utilisation efficiency, palatability and nutrient digestibility, which subsequently reduce animal performance. As such, PEG was used in this study to ameliorate the negative effects of CT in GTP. Surprisingly, PEG treatment had no effect on the SCT content of GTP, which was not expected given that PEG has a high affinity to CT (Makkar *et al*., 1995; Dentinho *et al*., 2018). Contrary to these findings, Dentinho *et al*. (2018) reported positive effects of PEG against harmful effects of CT in leguminous leaf meals. The treatment of GTP with PEG up to a rate of 100 g/kg reduced the concentration of TSPh indicating the effectiveness of PEG against some polyphenolic acids. Indeed, a decrease in total phenolics of rockrose (*Cistus landanifer L*.) leaves and stems with increasing concentrations (0, 25, 50 and 75 g/kg) of PEG was reported (Dentinho *et al*., 2018).

The CP values reported in this study ranged from 260 to 290 g/kg DM, which was consistent with the CP range (220 – 350 g/kg) reported by Yang *et al*. (2003). Although these variations could be due to different growth sites, the reported values indicate that the powder can be used as a potential protein source for ruminants in different production stages (NRC, 2001). However, PEG treatment of GTP at the rate of 50 – 100 g/kg reduced the CP content of GTP from 295.3 to 262.7 g/kg DM. Similar findings were observed by Dentinho *et al*. (2018), who reported a decrease in CP concentrations of *Cistus ladanifer* L. from 70.4 to 64.9 g/kg DM with incremental concentrations of PEG treatment. This was inconsistent with the report by Besharati and Taghizadeh (2011) who found that the use of PEG increases the availability of plant proteins by inactivating CT. The decrease in CP values with PEG treatment could be due to the inefficiency of the PEG to deactivate the CT content of the GTP used in this study. The NDF content of the treatments showed a linear decrease in PEG concentrations, which corroborates the report by Yisehak *et al*. (2014) that PEG is capable of breaking tannin-fibre complexes, making them more digestible by microbial enzymes. Treatment with PEG at 75 and 100 g/kg increased the ADF content of GTP, which indicates that higher PEG concentrations could, to a certain extent, have negative effects on the digestibility of GTP. This is because CT binds carbohydrates and forms insoluble complexes, which results in high fibre values.

*In vitro dry matter degradability parameters*

Table 2 indicates that there were significant quadratic trends for fractions *b* [$R^2$ = 0.475, P = 0.023] and *c* [$R^2$ = 0.446, P = 0.001] as well as EDeg [$R^2$ = 0.474, P = 0.023] in response to PEG concentrations. No linear trends (P > 0.05) were observed for all the degradation kinetics as PEG concentrations increased. PEG treatment of GTP affected (P > 0.05) all the other parameters, with the exception of the immediately degradable fraction (*a*). PEG0 (508.3 g/kg) had the lowest (P < 0.05) fraction *b* while the highest (P < 0.05) was from PEG25 (574.7 g/kg). PEG100 had a higher (P < 0.05) fraction *c* than PEG75 but were both statistically similar (P > 0.05) to the other treatments. PEG0 had similar (P > 0.05) PDeg as PEG50, PEG75 and PEG100. However, PEG50 (597.9.6 g/kg) had the lowest (P < 0.05) PDeg while the highest was from PEG25 (645.6 g/kg). PEG25 (574.7 g/kg) had the highest EDeg compared to PEG0 and PEG100, which were statistically similar (P > 0.05).

Table 2. The effect of polyethylene glycol-treated green tea powder on *in vitro* ruminal dry matter degradability kinetics (g/kg DM, unless stated otherwise).

| [2]Parameters | [1]Treatments | | | | | [3]SEM | P value | |
|---|---|---|---|---|---|---|---|---|
| | PEG0 | PEG25 | PEG50 | PEG75 | PEG100 | | Linear | Quadratic |
| *a* | 963.9 | 715.2 | 666.9 | 684.2 | 908.2 | 167.4 | 0.728 | 0.075 |
| *b* | 508.3$^{a}$ | 574.7$^{c}$ | 531.2$^{abc}$ | 571.2$^{bc}$ | 513.0$^{ab}$ | 14.85 | 0.802 | 0.023 |
| *c* (%/h) | 0.106$^{ab}$ | 0.075$^{ab}$ | 0.072$^{ab}$ | 0.055$^{a}$ | 0.111$^{b}$ | 0.011 | 0.712 | 0.001 |
| PDeg | 604.7 | 645.6 | 597.9 | 639.6 | 603.8 | 9.77 | 0.958 | 0.208 |
| EDeg | 510.4$^{a}$ | 575.0$^{c}$ | 531.2$^{abc}$ | 571.2$^{bc}$ | 513.1$^{ab}$ | 14.85 | 0.802 | 0.023 |

$^{a,b,c}$ Means in a row with different superscripts significantly differ ($P < 0.05$).

[1]Treatments: PEG0 = untreated green tea powder; PEG25 = green tea powder treated with 25 g/kg of polyethylene glycol; PEG50 = green tea powder treated with 50 g/kg of polyethylene glycol; PEG75 = green tea powder treated with 75 g/kg of polyethylene glycol; PEG100 = green tea powder treated with 100 g/kg of polyethylene glycol.

[2]Parameters: *a* = the immediately degradable fraction; *b* = the slowly degradable fraction; *c* = rate of degradation of the slowly degradable fraction *b*; PDeg = Potential degradability; EDeg= Effective degradability.

[3]SEM = standard error of the mean.

*In vitro* ruminal dry matter degradation indicates the rate and extent of fermentation and absorption of nutrients in the rumen as well as the energy partition estimation of potential feedstuffs (Baba *et al*., 2002). This is because when fibrous substrates are degraded by rumen microbes, glucose molecules and volatile fatty acids, which are major energy sources for ruminants, are released for absorption by the host animal (Dijkstra, 1994). However, high concentrations of CT interfere with the utilisation of carbohydrates and thus reduce energy utilisation (Rubanza *et al*., 2003). In the present study, the immediately degradable fraction (*a*) was not influenced by PEG treatment indicating that PEG does not improve the solubility of feed substrates. However, the effect of PEG was more pronounced on fractions *b* and *c* as well as EDeg, which could have been influenced by the increasing ADF content and the lack of effect on ADL content of GTP, since these parameters are related to the type of fibre and fibre content of feeds (Rubanza *et al*., 2003). Indeed, high fibre fractions have been reported to reduce nutrient digestibility and prolong ruminal fermentation (Njidda *et al*., 2013). In addition, the inability of PEG to bind CT could be the reason why the control treatment had similar dry matter degradability as the PEG treatments, with the exception of treatment concentrations of 25 and 75 g/kg that had higher fractions *b*, EDeg and PDeg values. High degradability values could be attributed to the linear decrease observed for TSPh, which according to Rubanza *et al*. (2003) could be related to their reduced anti-nutritive activity. These results were consistent with those by Dentinho *et al*. (2018) who reported an improvement in EDeg and factional rate of degradation when rockrose leaves were treated with PEG at 25 - 75 g/kg DM in an *in situ* studies.

*Gas production parameters*

No significant linear or quadratic trends were recorded for fraction *a*, lag time, PGas and EGas as PEG concentrations increased (Table 3). Fraction *b* quadratically [$R^2$ = 0.335; P = 0.007] responded to increasing PEG concentrations. PEG treatment of GTP

only affected (P > 0.05) fractions *a* and Egas. PEG75 (5.65 ml/g OM) had the lowest fraction *a* while PEG25 had the highest (9.05 ml/g OM). PEG25 had the highest Egas (73.1 ml/g OM) compared to PEG75 and PEG100, which did not differ (P > 0.05). The control treatment PEG0 had similar (P > 0.05) Egas as the other PEG treatments.

Table 3. *In vitro* ruminal gas production parameters (ml/g OM) of green tea powder treated with graded levels of polyethylene glycol.

| | [1]Treatments | | | | | | P value | |
|---|---|---|---|---|---|---|---|---|
| [2]Parameters | PEG0 | PEG25 | PEG50 | PEG75 | PEG100 | [3]SEM | Linear | Quadratic |
| *a* | 7.55[d] | 9.05[e] | 7.31[c] | 5.65[a] | 6.61[b] | 0.589 | 0.144 | 0.613 |
| *b* | 124.6 | 124.0 | 117.9 | 123.8 | 117.0 | 3.294 | 0.288 | 0.007 |
| *c* (%/h) | 0.020 | 0.021 | 0.020 | 0.017 | 0.020 | 0.001 | 0.305 | 0.847 |
| Lag time (h) | 3.40 | 3.06 | 3.13 | 3.76 | 3.36 | 0.237 | 0.065 | 0.105 |
| PGas | 132.2 | 133.0 | 125.2 | 129.5 | 123.7 | 3.438 | 0.401 | 0.822 |
| EGas | 70.2[ab] | 73.1[b] | 66.5[ab] | 62.8[a] | 65.2[a] | 2.079 | 0.163 | 0.078 |

[a,b,c,d] Means in a row with different superscripts significantly differ (P < 0.05).

[1]Treatments: PEG0 = untreated green tea powder; PEG25 = green tea powder treated with 25 g/kg of polyethylene glycol; PEG50 = green tea powder treated with 50 g/kg of polyethylene glycol; PEG75 = green tea powder treated with 75 g/kg of polyethylene glycol; PEG100 = green tea powder treated with 100 g/kg of polyethylene glycol.

[2]Parameters: *a* = the immediately fermentable fraction; *b* = the slowly fermentable fraction; *c* = fermentation rate of fraction *b*; PGas = potential gas production; EGas = effective gas production.

[3]SEM = standard error of the mean.

Table 4 shows that there was a linear increase for OMD24 [$R^2$ = 0.344; P = 0.003], and a linear decrease for OMD36 [$R^2$ = 0.190; P = 0.190] as PEG concentrations increased. No significant linear or quadratic trends were observed OMD12 and OMD48. PEG25 (377.5 g/kg OM) had the lowest OMD24 than PEG50 and PEG100, which did not differ (P > 0.05). PEG0 had similar (P > 0.05) OMD24 as all the other PEG treatment. PEG25 (431.2 g/kg OM) had the highest OMD36 than PEG50, PEG75 and PEG100, which were significantly similar.

Table 4. *In vitro* ruminal organic matter degradability (g/kg OM) and partitioning factors (mL/mg OM) of green tea powder pre-treated with graded levels of polyethylene glycol.

| | [1]Treatments | | | | | | P value | |
|---|---|---|---|---|---|---|---|---|
| [2]Parameters | PEG0 | PEG25 | PEG50 | PEG75 | PEG100 | [3]SEM | Linear | Quadratic |
| OMD12 | 548.6 | 607.3 | 572.8 | 590.7 | 602.8 | 16.34 | 0.175 | 0.725 |
| OMD24 | 436.4$^{ab}$ | 377.5$^{a}$ | 468.3$^{b}$ | 454.1$^{ab}$ | 512.8$^{b}$ | 19.69 | 0.003 | 0.794 |
| OMD36 | 415.4$^{ab}$ | 431.2$^{b}$ | 392.3$^{a}$ | 382.2$^{a}$ | 378.6$^{a}$ | 9.306 | 0.038 | 0.900 |
| OMD48 | 386.9 | 368.1 | 387.1 | 342.2 | 389.7 | 25.54 | 0.409 | 0.879 |
| PF12 | 0.051$^{b}$ | 0.044$^{ab}$ | 0.055$^{b}$ | 0.048$^{ab}$ | 0.038$^{a}$ | 0.003 | 0.010 | 0.032 |
| PF24 | 0.111$^{b}$ | 0.116$^{b}$ | 0.111$^{b}$ | 0.105$^{b}$ | 0.075$^{a}$ | 0.006 | 0.0001 | 0.040 |
| PF36 | 0.163 | 0.147 | 0.183 | 0.167 | 0.152 | 0.009 | 0.449 | 0.089 |
| PF48 | 0.218 | 0.212 | 0.228 | 0.254 | 0.195 | 0.022 | 0.150 | 0.681 |

[a,b,c] Means in a row with different superscripts significantly differ ($P < 0.05$).

[1]Treatments: PEG0 = untreated green tea powder; PEG25 = green tea powder treated with 25 g/kg of polyethylene glycol; PEG50 = green tea powder treated with 50 g/kg of polyethylene glycol; PEG75 = green tea powder treated with 75 g/kg of polyethylene glycol; PEG100 = green tea powder treated with 100 g/kg of polyethylene glycol.

[2]Parameters: OMD12 = *in vitro* organic matter degradability at 12 h after inoculation; OMD24 = *in vitro* organic matter degradability at 24 h after inoculation; OMD36 = *in vitro* organic matter degradability at 36 h after inoculation; OMD48 = *in vitro* organic matter degradability at 48 h after inoculation; PF12 = partitioning factors at 12 h post-incubation; PF24 = partitioning factors at 24 h post-incubation; PF36 = partitioning factors at 36 h post-incubation; PF48 = partitioning factors at 48 h post-incubation.

[3]SEM = standard error of the mean.

Significant linear and quadratic responses were observed for PF12 [$R^2 = 0.389$; $P = 0.032$] and PF24 [$R^2 = 0.637$; $P = 0.040$] as PEG concentrations increased. Neither linear nor quadratic effects ($P > 0.05$) were observed for PF36 and PF48 in response to PEG concentrations. PEG100 (0.038 mL/mg OM) had the lowest PF12 than PEG0 and PEG50. Similarly, PEG100 (0.075 mL/mg OM) promoted the least PF24 than all the other treatments, which were significantly similar.

Treatment of GTP with PEG had no pronounced effect on fermentation kinetics (fermentation rate *c*, lag time, PGas and EGas), which confirms that the PEG did not deactivate the antinutrient activity of CT. This could be the reason why PEG treatment concentrations (50 – 100 g/kg) had low gas produced from the immediately fermentable fraction *a*. Moreover, a negative quadratic trend was observed for the slowly fermentable fraction *b* as PEG concentrations increased, indicating the extent to which structural cell components of GTP are fermented. Contrarily, Besharati and Taghizadeh (2011) reported an increase in fraction *b* of PEG-treated grape by-products, which could be due to the plant's genotypic traits and the nature of tannins' activities on fermentation. The lack of effect on lag time shows that PEG treatment does not enhance the rapid proliferation of rumen microbes to colonize substrates and initiate fermentation. Treatment with PEG had no effect on PGas but significantly reduced EGas, which further confirms the inability of PEG to deactivate CT, which are known to reduce gas production. Makkar *et al*. (1995) reported that CT has the potential to reduce enteric gas production by binding dietary nutrients such as protein and carbohydrates and inhibiting the activities of fibrolytic and

proteolytic enzymes, and reduce ruminal fermentation efficiency.

Treating GTP with PEG enhanced OMD, which could be a result of the linear decline in NDF content of GTP. Low NDF values reveal high cell contents that may be due to soluble polyphenolic compounds, which subsequently leads to an overestimation of digestibility (Rubanza *et al*., 2003). However, this would require further research to verify the effect of PEG treatment on fibre fractions (NDF and ADF) since ADF values increased in response to PEG treatment. Thus, the linear increase for 24-h OMD and linear decrease at 36-h OMD post-inoculation could be explained by the partial reduction of the cell wall component (NDF) upon PEG treatment, which enhanced microbial fermentation. Similar findings were reported by Arhab *et al*. (2009), who observed an increase in OMD of browse tree leaves and tannin-containing feedstuff with PEG concentrations, respectively. Partitioning factors are usually used to measure ruminal fermentation efficiency because a higher PF value indicates a higher proportion of true degradable organic matter that is used for the synthesis of microbial biomass (Baba *et al*., 2002). The quadratic responses observed for PF12 and PF24 indicate that higher concentrations of PEG may compromise ruminal fermentation efficiency. Based on the non-linear equations generated for the dry matter degradability kinetics and partitioning factors in this study, PEG treatment concentrations beyond 50 g/kg may have a negative impact on ruminal fermentation efficiency.

## Conclusion

Pre-treatment of green tea powder with polyethylene glycol reduced the concentration of total soluble phenolics, crude protein and neutral detergent fibre, and showed a positive impact on partitioning factors, a measure of *in vitro* ruminal fermentation efficiency. Based on the quadratic responses for the degradation kinetics (*b*, *c* and Edeg) and partitioning factors, the optimum PEG treatment for maximum ruminal fermentation efficiency of green tea powder was determined to be 50 g/kg.

## References

Arhab, R., Macheboeuf, D., Aggoun, M., Boudisseboua, H., Viala, D. and Besle, J.M. 2009. Effect of polyethylene glycol on *in vitro* gas production and digestibility of tannin-containing feedstuffs from North African arid zone. *Trop. Subtrop. Agroecosystem*. 10: 475–486.

AOAC. 2005. *Official Methods of Analysis of AOAC International*, 16th ed, Arlington, VA, USA.

Baba, A.S., Castro, F.B. and Ørskov, E.R. 2002. Partitioning of energy and degradability of browse plants in vitro and the implications of blocking the effects of tannin by the edition of polyethylene glycol. *Anim. Feed. Sci. Technol.* 95: 93–104.

Besharati, M. and Taghizadeh, A. 2011. Effect of tannin-binding agents (Polyethylene glycol and polyvinylprrolidone) supplementation in *in vitro* gas production kinetics of some grape yield by-products. *Vet. Sci.* 780540.

D'souza, A. and Shegokar, R. 2016. Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. *Expert. Opin. Drug. Deliv.* 13(9): 1257–1275.

Dentinho, M.T.P., Moreira, O.C. and Bessa, R.J.B. 2018. The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in *Cistus ladanifer L*. *For. Syst*. 27(1): 12.

Dey, A., Dutta, N., Sharma, K. and Pattanaik, A.K. 2008. Effect of dietary inclusion of *Ficus infectoria* leaves as a protectant of proteins on the performance of lambs. *Small Rumin Res*. 75: 105–114.

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. *Livest. Prod. Sci.* 39(1): 61–69.

Dufresne, C.J. and Farnworth, E.R. 2001. A review of latest research findings on the health promotion properties of tea. *J. Nutr. Biochem.* 12: 404–421.

Georgiev, K., Zhelev, I. and Georgieva, S. 2014. Total phenolic compounds and tannins content of *Bancha* green tea (*Camellia sinensis*) depending on extraction conditions. *Sci. Pharmacol.* 1: 48–51.

Hara-Kudo, Y., Yamasaki, A., Sasaki, M., Okubo, T., Minai, Y., Haga, M., Kondo, K. and Sugita-Konishi, Y. 2005. Antibacterial action on pathogenic bacterial spore by green tea catechins. *J. Sci. Food Agr.* 85: 2354–2361.

Makkar, H.P.S., Blümmel, M. and Becker, K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins and their implication in gas production and true digestibility in *in vitro* techniques. *Brit. J. Nutr.* 73: 897–913.

Makkar, H.P.S. 2003. Quantification of tannins in tree and shrub foliage. *A Laboratory Manual*. Vienna, Austria. pp. 43–54.

Mauricio, M.R., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. and Theodorou, M.K. 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.* 79: 321–330.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. and Wilkinson, R.G. 2010. *Animal nutrition*. Pearson, Harlow, England.

Mhlongo, G., Mnisi, C.M. and Mlambo, V. 2021. Cultivating oyster mushrooms on red grape pomace waste enhances potential nutritional value of the spent substrate for ruminants. *Plos One.* 16(2): e0246992.

Njidda, A.A., Olatunji, E.A. and Garba, M.G. 2013. *In sacco* and *in vitro* organic matter degradability (OMD) of selected semi-arid browse forages. J. Agr. Vet. Sci. 3(2): 9–16.

NRC. 2001. *Nutrient Requirement Council of Domestic Animals*. Seventh revised Ed. The National Academic Press: Washington DC, USA.

Ørskov, E.R. and McDonald, J. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. *J. Agr. Sci*. 92: 499–503.

Rubanza, C.D.K., Shem, M.N., Otsyina, R., Nishino, N., Ichinohe, T. and Fujihara, T. 2003. Content of phenolics and tannins in leaves and pods of some *Acacia* and *Dichrostachys* species and effects on *in vitro* digestibility. *J. Anim. Feed Sci.* 12(3): 645–663.

Sarker, M.S.K., Ko, S.Y., Lee, S.M., Kim, G.M., Choi, J.K. and Yang, C.J. 2010. Effect of different feed additives on growth performance and blood profiles of Korean Hanwoo calves. *Asian-Australas. J. Anim. Sci.* 23: 52–60.

SAS. 2010. Users Guide Version 9.4 Statistical Analyses System Institute Inc.: Cary, NC, USA.

Van Soest, P.J. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 358–359.

Yang, C.J., Yang, I.Y., Oh, D.H., Bae, I.H., Cho, S.G., Kong, I.G., Uuganbayar, D., Nou, I.S. and Choi, K.S. 2003. Effect of green tea by-product on performance and body composition in broiler chicks. *Asian-Australas. J. Anim. Sci.* 16: 867–872.

Yisehak, K., De Boever, J.L. and Janssens, G.P.J. 2014. The effect of supplementing leaves of four tannin-rich plant species with polyethylene glycol on digestibility and zootechnical performance of zebu bulls (*Bos indicus*). *J. Anim. Physiol. Anim. Nutr.* 98: 417–423.