



## Ruminal Gas Production Response of Diets Supplemented with Green Tea



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### Abstract

The current study was carried out to investigate the effect of green tea as feed additive in diets on gas production and DM degradation using batch culture technique. Three experimental diets were used as follow: Control diet, GT1; control diet supplemented with 2% green tea, GT2; control diet supplemented with 4% green tea, and GT3; control diet supplemented with 8% green. The results showed no significant differences between control diet and other groups on rumen pH values and DM degradation. While, adding green tea as 2% of DM significantly declined total gas production and methane parameters as compared with other experimental groups. The results of the current study suggested that low supplementing level of green tea (2% of DM) had a potential positive impact in ruminant diet.

**Keywords:** green tea; rumen; methane; diet; degradation.

### 1. Introduction

With the increasing population in the world to reach 9.7 billion people in 2050[1], which requires the provision of the necessary food needs, and with limited resources, it is necessary to work on increasing the productivity of the production unit to cover those needs while taking into account the environmental impacts of the production process because of the world's suffering severe environmental crises which require the search for sustainable production strategies.

Animal production is one of the main pillars of agricultural production and food security, and ruminants are distinguished by their unique ability to benefit from cellulosic materials that other mammals cannot benefit from in feeding, due to ruminants having a unique gut channel that contains rumen and its microbial clan that can crushing and digesting a hill; Cellulosic compounds and their conversion into energy compounds through anaerobic fermentation[2]. One of the disadvantages of anaerobic fermentation in rumen is the production of methane as a by-product of metabolism, as it is considered a type of lost energy, which is estimated at about 12% of the gross energy that is lost through the belching process, and it is understood that approximately

37% of global agricultural CH<sub>4</sub> arise from direct animal and manure emissions.[3]

The environmental impact of ruminants has won the attention of many researchers in recent decades by searching for effective strategies to reduce the amount of methane produced without negatively affecting production, and the use of natural feed additives was one of the most important of these strategies[4].

Green tea is perceived to be one of the healthiest drinks in the world. The beneficial properties of green tea are due to the abundant polyphenolic compounds (catechins), and these catechins include catechin, (2)-epicat-echin, (2)-epigallocatechin, (2)-EC-3-gallate, and (2)-EGC-3-gallate [5]. Many researchers have found potential antioxidant and cancer prevention activities in caffeine, catechins and theaflavins but there is a limited information on their potentials as dietary supplementation[6]. Adding polyphenols such as tannins in ruminant diets may increase the availability of by-pass protein [7] and non-ammonia nitrogen supply to be absorbed in small intestine due to their ability to bind to plant proteins reducing their breakdown and thus reducing ammonia production during rumen degradation of the feeds [8][9,10].

The current study hypothesized that supplementing

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ruminant diets with green tea could improve environmental impacts of ruminant through reducing methane produced in the rumen and enhance the rumen activity and performance.

## 2. Experimental

### 2.1. Experimental diets

The chemical composition of experimental diet presented in table (1). Four experimental diets were as follow: Control diet (50% Concentrate feed mixture : 50% hay) (Control), control diet supplemented with 2% green tea (Matcha green tea) (GT1), control diet supplemented with 4% green tea (GT2), and control diet supplemented with 8% green tea (GT3).

### 2.2. In-vitro batch culture

In-vitro incubation experiments was carried out according to Menke and Steingass [11] as described by Khattab et al [12]. Rumen fluid was collected before morning feeding from two fistulated Friesian cows. The collected rumen fluid was mixed and squeezed through 4 layers cheesecloth under continuous flushing with CO<sub>2</sub> and immediately transported to laboratory at 39°C where it was used as a source of inoculum.

Each treatment was tested in eight replicates and repeated twice accompanied by blank vessels (no substrate). 400 mg of milled substrate was added to the incubation vessels of 120 mL capacity. Each vessel was filled with 45 mL of the incubation medium (292 mg K<sub>2</sub>HPO<sub>4</sub>, 240 mg KH<sub>2</sub>PO<sub>4</sub>, 480 mg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 480 mg NaCl, 100 mg MgSO<sub>4</sub>.7H<sub>2</sub>O, 64 mg CaCl<sub>2</sub>.2H<sub>2</sub>O, 4 mg Na<sub>2</sub>CO<sub>3</sub> and 600 mg cysteine hydrochloride) per 1 liter of distilled water (dH<sub>2</sub>O) and dispensed anaerobically in the 1:2 (v/v) ratio. Then the treatments were incubated at 39°C for 24h.

After 24 h of incubation, gas production (GP) was recorded from each bottle using the pressure reading technique, bottles were uncapped, pH was measured using Beckman pH meter, and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate. The samples were transferred from each bottle into test tubes and centrifuge for 1h in order to obtain the residues. The dry residues were weighed and degradability calculated using the equation as follows [2]

$$\text{IVDMD (\%)} = \frac{[(\text{initial DM input} - (\text{DM residue} - \text{Blank})) / \text{initial DM input}] * 100}{1}$$

### 2.3. Samples analysis

Substrates and substrate residues after 24 h of incubation were dried at 70°C and analyzed for the amount of DM (DM degradability) according to

AOAC[13], Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by Ankom200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest et al.[14]. Methane concentrations were determined as described by Lateef et al.[15]. Gas sample was taken with air-tight micro syringe. The composition was determined using a Shimadzu gas chromatograph (GC-14C) equipped with a thermal conductivity detector (stainless column and Porapak Q packing). The operational temperatures of injector port, column, and the detector were 220, 150, and 220 °C, respectively. Argon was the carrier gas at a flow rate of 50 mL/min.

### 2.4. Statistical analysis

Data were statistically analyzed using ANOVA procedure of SAS software (Version 9.4). data normality (Shapiro-Wilk at 5% probability) was estimated. Significant differences between means of treatments were carried out by the Duncan's test and the significance threshold was set at  $p < 0.05$ .

**Table (1): chemical composition of experimental diet**

Item	Chemical composition (g/Kg DM)
Dry matter (DM)	843.75
Organic matter (OM)	929.1
Crude protein (CP)	182.55
Ether extract (EE)	39.1
Neutral detergent fibre (NDF)	480.9
Acid detergent fibre (ADF)	214.05
Acid detergent lignin (ADL)	37.6

## 3. Results and discussion

### 3.1. Ruminal pH

Supplementing diet with different levels of green tea on ruminal pH values were investigated. Fig (1) showed that different supplementing levels of green tea had no significant ( $p > 0.05$ ) effect on Ruminal pH values as compared with control (GT1, 6.46; GT2, 6.46; GT3, 6.40; and control 6.45, respectively) while adding green tea as 8% of DM (GT3) significantly ( $p > 0.05$ ) decreased pH value than those recorded with other supplementing levels (GT1 and GT2). The ruminal pH results agreed with those obtained by Nishida [16] who recorded non-significant changes in rumen pH values as affected by feeding cattle diets containing green tea silage. The ruminal pH, which ranged from 6.40 (GT3) to 6.64 (GT2), was above the stipulated value (5.6) for the degradation of fiber [17].

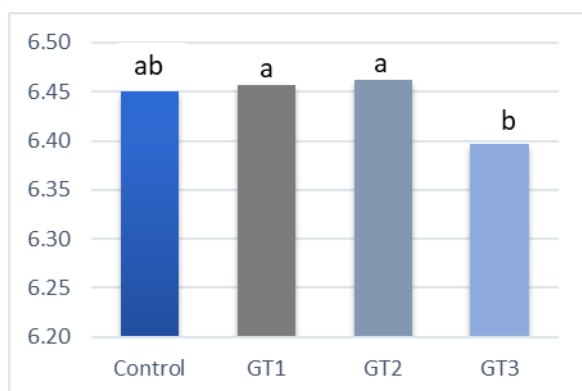


Fig. (1): effect of different supplemented diets with green tea on ruminal pH.

The current results showed that ruminal pH values were in ideal range for fibrolytic bacterial activity in rumen which ranging between 5.7 – 7 as optimum condition for growth and activity [18]

### 3.2. Ruminal gas production

Ruminal gas production results are shown in table (2). Data showed that supplementing diets with low level of green tea (2% of DM) (GT1) significantly ( $p < 0.05$ ) decreased total gas production (TGP), gas production per each gram of dry matter (TGP/g DM), gas production per each

gram of degraded dry matter (TGP/g dDM), gas production per each gram of NDF (TGP/g NDF) as compared with other experimental groups (Control, GT2, and GT3).

Also, methane production was the same trend within the experimental groups, GT1 recorded the lowest methane production value as total methane (TCH<sub>4</sub>), total methane per each gram dry matter (TCH<sub>4</sub>/g DM), total methane per each gram degraded dry matter (TCH<sub>4</sub>/g dDM), and methane per each gram NDF (TCH<sub>4</sub>/g NDF). While, other supplementing group (GT2, and GT3) did not significantly change the methane parameters as compared with control.

Since gas production is a result of fermentation of carbohydrates in the rumen mainly, it seems that low supplementing level of green tea suppressed gas production which related to the fermentable organic matter content and available energy in the rumen [19].

One of the explanations of gas production reduction could be due the binding green tea phenols takes place via H bonding, therefore reduce the available hydrogen for production methane and gas production [20].

Table (2): effect of different supplementing diets with green tea on ruminal gas production

Item	Control	GT1	GT2	GT3	±SE	P-value
Total GP (ml)	121.20 <sup>a</sup>	102.40 <sup>b</sup>	122.20 <sup>a</sup>	127.80 <sup>a</sup>	4.466	0.006
TGP/ g DM (ml)	297.56 <sup>a</sup>	255.05 <sup>b</sup>	301.08 <sup>a</sup>	314.87 <sup>a</sup>	11.596	0.013
TGP/ g dDM (ml)	439.8	363.1	454.5	470.9	13.234	0.0080
TGP / g NDF (ml)	726.10 <sup>a</sup>	622.53 <sup>b</sup>	735.71 <sup>a</sup>	767.99 <sup>a</sup>	28.495	0.013
Total CH <sub>4</sub> (ml)	7.13 <sup>a</sup>	6.28 <sup>b</sup>	7.49 <sup>a</sup>	7.92 <sup>a</sup>	0.260	0.003
TCH <sub>4</sub> / g DM (ml)	17.50 <sup>ab</sup>	15.66 <sup>b</sup>	18.46 <sup>a</sup>	19.50 <sup>a</sup>	0.678	0.007
TCH <sub>4</sub> / g dDM (ml)	25.9	22.3	27.9	29.2	0.797	0.0038
TCH <sub>4</sub> / g NDF (ml)	42.70 <sup>ab</sup>	38.21 <sup>b</sup>	45.09 <sup>a</sup>	47.57 <sup>a</sup>	1.656	0.007

GT1: control diet + 2% of DM green tea; GT2: control diet + 4% of DM green tea; GT3: control diet + 8% of DM green tea.

a, b Means in the same row with different superscripts differ,  $P < 0.05$ .

P-value is the observed significance level of the F-test for treatment;

±SE = standard error of the mean

### 3.3. Ruminal dry matter digestion

Fig. (2) Showed the effect of different experimental diets on ruminal DM digestion. The results cleared that supplementing diets with rather 2, 4, or 8% of green tea (67.66, 70.38, 66.64 and 66.86% for control, GT1, GT2 and GT3 respectively) had no adverse effect on DM degradability in the rumen. It can also be expected that phenolic compounds present in green tea could have no negative biological activity in the rumen [21].

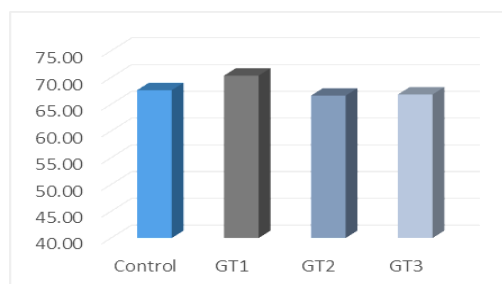


Fig. (2): effect of different supplemented diets with green tea on ruminal DM digestion.

#### 4. Conclusions

The current results showed a potential positive effects of supplementing ruminant animal diets with green tea as feed additive to improve the environmental impacts of ruminant animals. It could be concluded that supplementing diets with green tea (2% of DM) significantly decreased methane production in the rumen without negative effect on dry mater degradation in the rumen. We suggested that using green tea as feed additive in ruminant diet need more investigations to clear its effects on productive performance of ruminant.

#### Conflicts of interest

The author report no conflict of interest.

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