

# Effect of Tannin in Green Tea By-Product in Combination with Bio-Char Supplemented into Basal Beef Cattle Diet on Nutrient Digestibility, Methane Production and Animal Performance

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## Abstract

The experiment was conducted by using 20 bulls of Lai Sind cattle (a cross breed between Red Sindhi and local Vietnam yellow cattle) from 15 - 16 months of age, weighed approximately 154 - 156 kg, housed individually with provision of adequate feed trough and free access to water to evaluate the effect of tannin sourced from green tea by-product and addition of bio-char on methane production and animal performance. The experimental diets were formulated by replacing elephant grass in basal diet by bio-char and green tea by-product with the replacement of 0.5 - 1 bio-char and 3% - 5% green tea by-products, respectively to make the content of tannin in the diet of 7.3 g and 12.5 g tannin per kg dry matter. Results from this study revealed that bio-char and green tea by-product included into the diet with different levels had significantly affected on the feed intake, as the increasing level of tannin in the diet tended to increase the feed intake. The inclusion of green tea by-product in the diet which resulted in the content of tannin in the diet of 12.5 g/kg DM had significantly reduced methane emission without altering animal performance, thus the inclusion of 0.5% bio-char and 5% of green tea by-product can be applied in order to mitigate methane emission, thus contributed to environment protection.

## Keywords

Green Tea By-Product, Bio-Char, Tannin, Methane Emission, Animal Performance

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## 1. Introduction

Methane production in ruminant can be reduced from 12% - 37% by altering ruminant diets [1] [2] [3]. It has been reported that the inclusion of tannin into ruminant diet could reduce rumen methane production in both *in vitro* and *in vivo* conditions. Tannin directly affects the formation of methane or indirectly altered the number of protozoa and microbial cellulose [4]. Therefore, tannin, in combination with bio-char presented in the diet was believed to effectively reduce rumen methane production [5] without lowering nutrient digestibility.

The source of tannin in the diet materials plays an important role on methane production in both *in vitro* and *in vivo* so that tannin could be presented in the diet up to 15% depended on its source [6] [7].

Bio-char was known as an antidote additive and it has been extensively studied in ruminant nutrition [8] [9]. Thank to its absorption capacity, bio-char played as gas absorptive agent in the rumen [10].

Currently, Thai Nguyen—Vietnam has a large area of tea production, thanks to the suitable natural conditions. Annually, tea production left over tons of green tea by-products (matured leaves and branches). This could be the important source for animal feed especially it can be used for ruminant supplement diet.

This tannin source, in combination with bio-char, would improve feed intake, thus improve animal productivity [11]. Therefore, in this study, green tea by-product was the source of tannin tested in order to reduce methane emission without altering rumen microbial system and thus the diet digestibility.

## 2. Materials and Methods

### 2.1. Animals and Housing

The experiment was conducted by using 20 bulls of Lai Sind cattle (a cross breed between Red Sindhi and local Vietnam yellow cattle) from 15 - 16 months of age, weighed approximately 154 - 156 kg, housed individually with provision of adequate feed trough and free access to water. Each pen was equipped with respiratory chamber for methane emission evaluation, the bulls was diet acclimatized for 15 days before any measurements taken place. All experimental animals were vaccinated, de-wormed and were cared to comply with Vietnamese standard for experimental animals. The experiment was conducted in 90 days at the Experimental Unit of the Center for Animal production in the Northern Mountainous region—Vietnam.

### 2.2. Experimental Diets

The basal diet was formulated from elephant grass, maize bran, cassava by-product, and the experimental diets were formulated by replacing elephant grass by bio-char and green tea by-product with the replacement of 0.5 - 1 bio-char and 3% - 5% green tea by-products, respectively.

The basal diet was prepared to comply with NRC (1996) for growing cattle (10

- 11MJ ME/kg DM and 12% - 14% crude protein contents). The diets composition is presented in **Table 1**.

Bulls were assigned randomly to access either 1 of 5 diets twice daily (8:00 AM and 4:00 PM) with free access to water, the refusal was weighed twice daily in accordance to feeding schedule.

### 2.3. Monitoring

Nutrients intake includes gross energy, metabolism energy, dry matter, and organic matter, crude protein, NDF, ADF, crude fibre, total ash was recorded and calculated.

Animals were weighed monthly interval to determine production performance.

For methane emission measurement, each month, each bull was entered respiratory chamber for 24 hours, where total methane emission (liter/animal/day) was recorded based on the different of air inlet and outlet automatic recorder (20 minutes interval for inlet air and 5 minutes interval for outlet air). Air sample was collected and analyzed automatically at the beginning of the experiment (the diet acclimatize period) and at the end of the experimental period.

### 2.4. Statistical Analysis

Data analysis was performed followed equation:  $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ .

Where: ( $ij$ ) observation from diets, ( $i$ ) replication, ( $\mu$ ) Overall mean, ( $T_i$ ) mean of each formula, ( $\varepsilon_{ij}$ ) random errors. The comparison was done following Duncan's New Multiple Range Test (DMRT) [12].

**Table 1.** The composition of experimental diets.

Ingredients	Composition (% DM)				
	Control	DIET1	DIET2	DIET3	DIET4
1) Elephant grass	89.0	85.5	85.0	83.5	83.0
2) Cassava	1.8	1.8	1.8	1.8	1.8
3) Soybean meal	3.9	3.9	3.9	3.9	3.9
4) Maize bran	2.5	2.5	2.5	2.5	2.5
5) Rice bran	2.8	2.8	2.8	2.8	2.8
6) Green tea by-product	0	3	3	5	5
7) Bio-char	0	0.5	1	0.5	1
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
Dry matter	25.2	25.5	25.7	25.4	25.2
CP	13.0	13.02	13.05	13.1	13.05
ME	10.30 (MJ/kg)	10.32 (MJ/kg)	10.32 (MJ/kg)	10.35 (MJ/kg)	10.35 (MJ/kg)
Tannin		0.73	0.73	1.25	1.25

### 3. Results and Discussion

#### 3.1. Effect of Green Tea By-Product and Bio-Char Supplement on Nutrients Intake

The effect of green tea by-product and bio-char supplement on feed and nutrients intake is presented in **Table 2**.

It can be seen from the Table that the different levels of green tea by-product which led to the difference of tannin content in the diets had no effect on voluntary feed intake as the increasing levels of tannin and bio-char the feed intake also did not tend to increase ( $p > 0.05$ ). This could be explained that the tannin and bio-char contents in the diets did not affect the palatability of the diets as in our trial, the tannin content was 7.3 to 12.5 g/kg DM, which was by far medium tannin content of <50 g/kg DM that could reduce voluntary feed intake as reported by Barry & Manle [13], Barry, Manley, & Duncan [14], Waghorn, Shelton, & McNabb [15]. The increasing level of bio-char content also did not affect the voluntary feed intake of the tested animals as it was supported by findings from Leng *et al.* [16], even though, in our experiment the supplement of bio-char was 0.4% higher than their report. The addition of bio-char into diet had been used widely in order to improve the fermentation kinetic of the diets in the rumen. Bio-char has been utilized as feed additive to increase microbial habitats [17], thus bio-char may improve microbial growth efficiency [16], and thus improved rumen digestibility and then voluntary intake was compensated.

#### 3.2. Effect of Green Tea By-Product and Bio-Char Supplement on the Growth of Cattle

Data of the effect of green tea by-product and bio-char supplement on the animal grows is presented in **Table 3**.

Data present in **Table 3** showed that after 30 days of experimental period there was no significant difference in animal bodyweight. After 60 days of experimental period, there was an increase in BW of animals fed with diets containing higher levels of green tea by-product (higher containing tannin diet) compared to the Control. Animals fed with diets containing 3% green tea by-product (or 0.73% tannin) and 0.5% bio-char content had the similar bodyweight compare to those in the Control ( $p > 0.05$ ).

**Table 2.** Feed and nutrients intake of Lai Sind bulls.

Categories	Control		DIET1		DIET2		DIET3		DIET4	
	Mean	Se	Mean	Se	Mean	Se	Mean	Se	Mean	Se
DM intake per day (kg)	2.86	0.008	2.86	0.011	2.88	0.006	2.89	0.006	2.90	0.009
CP (g/day)	371	1.2	371	1.4	374	1.2	375	1.2	377	1.4
ME (MJ/day)	29.45	0.08	29.51	0.11	29.72	0.06	29.91	0.07	30.01	0.09

Note: The different subscript letters in the same row are significant different ( $p < 0.05$ ).

**Table 3.** The effect of green tea by-product and bio-char on animal growth.

Category	Control		DIET1		DIET2		DIET3		DIET4		P (Sig.)
	Mean	Se	Mean	Se	Mean	Se	Mean	Se	Mean	Se	
Initial BW	154.6	0.24	153.7	0.75	153.0	1.09	153.2	0.43	154.2	1.11	0.954
After 30days	174.6	0.99	174,5	0.65	175.8	0.77	175.0	0.41	174.0	0.91	0.138
After 60days	190.3 <sup>d</sup>	0.55	193.7 <sup>cd</sup>	1.09	197.5 <sup>b</sup>	1.40	200.2 <sup>a</sup>	0.85	194.6 <sup>c</sup>	0.55	0.000
After 90day	211.0 <sup>c</sup>	0.41	213.2 <sup>b</sup>	1.74	218.0 <sup>a</sup>	0.74	221.1 <sup>a</sup>	0.43	214.8 <sup>b</sup>	0.59	0.000

Note: The different subscript letters in the same row are significant different ( $p < 0.05$ ).

After 90 days of the experimental period, this difference was significant among those fed with diet containing 3% green tea by-product (or 0.73% tannin) and 1% bio-char; and those fed with 5% green tea by-product (or 1.25% tannin) and 0.5% bio-char. It can be inferred that the increasing of tannin content with 0.5% bio-char in the diet would give the best growth in cattle.

### 3.3. The Effect of Green Tea By-Product and Bio-Char Supplement on Methane Emission

The data of methane emission from experimental animals fed with different diets containing green tea by-product and bio-char is presented in **Table 4**.

It can be inferred from **Table 4** that methane emission from bulls fed with diets containing green tea by-product and bio-char was lowered than that compares to those fed with control diet ( $p < 0.05$ ). Especially those fed with diet containing 5% green tea by-product (or diet containing 1.25% tannin) and 1% bio-char had the lowest methane emission which was reduced by 12.6 l/animal/day (by 6.96%). It can be explained that tannin content of green tea by-product that brought up the tannin content in the diet up to 1.25% has impaired methane formation due to the reduction of rumen degradation [18]. On the other hand, tannin content in the diet also prevent the development of protozoa and other hydrogenesis bacteria [18] [19], and thus reduced hydrogen source for methane formation in the rumen.

Our findings was also supported by the findings of Tiemann *et al.* [5], when tannin from *Calliandra calothyrsus* and *Flemingi amacrophyll* were added into Swiss white sheep diet at 1.11% - 1.75% DM which reduced methan emission by 3% - 21%.

Similarly, Hu *et al.* [20], reported that the inclusion of 1%, 2%, 3% and 4% of tannin into diet containing corn meal and grass meal (ratio of 50/50) had resulted in methane emission declined from 13%, 22%, 25% and 26%, respectively, and they also found that the protozoa population declined from 19%, 25%, 45% and 79%, respectively, after 24 hours of incubation with rumen juice.

### 3.4. Nutrients Intake, Growth and Methane Emission Intensity

The data of nutrients intake, growth and methane emission are presented in **Table 5**. It can be seen that there was the lower methane emission in accordant

**Table 4.** Methane and Carbon dioxide emission from experimental animals.

Category	Control		DIET1		DIET2		DIET3		DIET4		P (Sig.)
	Mean	Se	Mean	Se	Mean	Se	Mean	Se	Mean	Se	
CH <sub>4</sub> (l/day)	180.88 <sup>a</sup>	0.90	177.50 <sup>ab</sup>	0.74	176.05 <sup>b</sup>	0.95	172.28 <sup>cd</sup>	1.44	168.28 <sup>d</sup>	0.48	0.000
Heat production (MJ)	20.14 <sup>a</sup>	0.57	19.74 <sup>abc</sup>	0.55	18.53 <sup>bc</sup>	0.35	18.44 <sup>c</sup>	0.15	19.75 <sup>ac</sup>	0.20	0.005
CO <sub>2</sub> (l/day)	925.92 <sup>a</sup>	2.65	907.66 <sup>abc</sup>	25.66	852.22 <sup>bc</sup>	16.35	848.15 <sup>c</sup>	7.33	908.23 <sup>ac</sup>	9.33	0.005
CH <sub>4</sub> /CO <sub>2</sub>	0.195 <sup>abc</sup>	0.001	0.196 <sup>abc</sup>	0.006	0.207 <sup>ac</sup>	0.005	0.203 <sup>c</sup>	0.003	0.185 <sup>b</sup>	0.002	0.012

Note: The different subscript letters in the same row are significant different ( $p < 0.05$ ).

**Table 5.** Nutrients intake, growth and methane emission intensity.

Categories	Control		Diet 1		Diet 2		Diet 3		Diet 4		P (Sig.)
	Mean	Se	Mean	Se	Mean	Se	Mean	Se	Mean	Se	
CH <sub>4</sub> /DM (l/kg/day)	64.47 <sup>a</sup>	0.40	62.22 <sup>ab</sup>	0.46	61.22 <sup>b</sup>	0.40	59.35 <sup>cd</sup>	0.41	57.40 <sup>d</sup>	0.22	0.000
CH <sub>4</sub> /DM (g/kg/day)	45.77 <sup>a</sup>	0.28	44.18 <sup>ab</sup>	0.33	43.47 <sup>b</sup>	0.28	42.14 <sup>cd</sup>	0.29	40.76 <sup>d</sup>	0.16	0.000
CH <sub>4</sub> /NDF (l/kg/day)	105.18 <sup>a</sup>	0.65	102.52 <sup>ab</sup>	0.76	101.59 <sup>b</sup>	0.67	97.98 <sup>cd</sup>	0.68	95.45 <sup>d</sup>	0.37	0.000
CH <sub>4</sub> /ADF (l/kg/day)	199.59 <sup>a</sup>	1.23	194.44 <sup>ab</sup>	1.45	192.63 <sup>b</sup>	1.26	185.72 <sup>cd</sup>	1.29	180.91 <sup>d</sup>	0.71	0.000
CH <sub>4</sub> /kg gain (l/kg gain)	283.81 <sup>a</sup>	3.16	269.38 <sup>a</sup>	8.77	244.52 <sup>bd</sup>	4.45	230.60 <sup>cd</sup>	2.05	246.10 <sup>d</sup>	5.51	0.000

Note: The different subscript letters in the same row are significant different ( $p < 0.05$ ).

with the increase level of tannin content in the diet ( $P < 0.05$ ). When there was no tannin present in the diet (the control), methane emission was the highest 64.47 l/kg DM/day, or 45.77 g/kg DM/day. When there was a presence of bio-char at 0.5% and tannin at 1.25% in the diet this emission was significantly the lowest at 57.40 l/kg DM/day or 40.76 g/kg DM/day ( $p < 0.05$ ).

The monitoring data of methane emission based on bodyweight gain during 0 - 90 days of experimental period revealed that diet without tannin content (control) had the largest methane emission capacity (283 l/kg BW gain). This emission of diet 3 (with 0.5% bio-char and 5% green tea by-product) was the lowest (230.60 l/kg BW gain) ( $p < 0.05$ ). When compares among the experimental diets with addition of green tea by-product and bio-char, the higher level of supplement, the amount of methane emission per each kg BW gain was reduced. However, when bio-char was added at 1% and green tea by-product at 5%, this emission was increased significantly ( $p < 0.05$ ).

According to O'Mara *et al.* [21] the better growth in animals was obtained via better nutrition, so that energy requirement for maintenance was calculated base on total energy requirement, so when energy loss via CH<sub>4</sub> was reduced, the amount of methane emission per each kg of milk or meat produced was also reduced. Therefore, when 0.5% of bio-char and 5% of green tea by-product were

**Table 6.** Methane emission based on total digestible nutrients.

Category	Control		Diet 1		Diet 2		Diet 3		Diet 4		P (Sig.)
	Mean	Se	Mean	Se	Mean	Se	Mean	Se	Mean	Se	
CH <sub>4</sub> /DM (l/kg/day)	91.57 <sup>a</sup>	0.91	83.82 <sup>bc</sup>	0.71	82.58 <sup>c</sup>	0.91	78.08 <sup>de</sup>	0.76	78.34 <sup>e</sup>	0.62	0.000
CH <sub>4</sub> /OM (l/kg/day)	229.19 <sup>a</sup>	1.49	219.25 <sup>b</sup>	2.12	215.71 <sup>ce</sup>	0.83	201.49 <sup>d</sup>	2.14	211.67 <sup>e</sup>	1.09	0.000
CH <sub>4</sub> /NDF (l/kg/day)	147.17 <sup>a</sup>	0.43	138.81 <sup>bc</sup>	1.26	137.44 <sup>c</sup>	0.83	126.54 <sup>d</sup>	1.10	131.70 <sup>e</sup>	0.82	0.000
CH <sub>4</sub> /ADF (l/kg/day)	315.98 <sup>a</sup>	2.30	285.22 <sup>ce</sup>	0.94	287.09 <sup>c</sup>	2.78	260.36 <sup>d</sup>	0.59	276.65 <sup>e</sup>	2.06	0.000

Note: The different subscript letters in the same row are significant different ( $p < 0.05$ ).

added into diet there was a better in nutrients utilization efficiency compare to others.

### 3.5. Methane Emission Based on Total Digestible Nutrients

The data on the effect of bio-char and green tea by-product inclusion into diet on methane emission based on the total digestible nutrients is presented in **Table 6**.

The total digestible nutrients included dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF), data presented in **Table 6** showed that methane emission tend to be lowered when difference bio-char and green tea by-product were added into diet, as in the control, this emission was 91.57 l/kg digestible DM and 229.19 l/kg digestible OM; whereas, with inclusion of diet 3 (0.5% bio-char and 5% green tea by-product), this emission was significantly ( $p < 0.05$ ) the lowest (78.08 l/kg digestible DM and 201.49 l/kg digestible OM).

This can be explained that when the nutrients digestibility was improved it increased the nutrients uptake and increased ingesta passage rate thus decreased fermentation time that resulted in reduction of methane production [22].

## 4. Conclusion

Bio-char and green tea by-product included into diet with different levels had significantly affected on the feed intake, as the increased level of tannin level in the diet tended to increase the feed intake. The inclusion of green tea by-product in the diet which resulted in the content of tannin in the diet of 12.5 g/kg DM had significantly reduced methane emission without altering animal performance, thus the inclusion of 0.5% bio-char and 5% of green tea by-product can be applied in order to mitigate methane emission, thus contributed to environment protection.

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