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USE OF GREEN TEA BY PRODUCTS AS A TANNIN SUPPLEMENTAL SOURCE IN BEEF CATTLE DIETS TO MITIGATE ENTERIC METHANE EMISSION

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LIST OF PUBLICATIONS

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Lê Tuấn An, Chu Mạnh Thắng, Lê Đình Phùng, Trần Hiệp (2020). Ảnh hưởng của bổ sung phụ phẩm chè xanh đến tỷ lệ tiêu hóa các chất dinh dưỡng của khẩu phần trong môi trường dạ cỏ. Tạp chí Khoa học kỹ thuật chăn nuôi, 256 (4):26-34.

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INTRODUCTION

1.1 Problem statement

In Vietnam, cattle breeding play an important role in many people's livelihoods. The national cattle population reached 5.09 million in 2015 and increased to 6.1 million by 2020 (GSO, 2020) and is expected to continue to increase in the coming years. Several studies have shown that tannin is the most promising compounds for reducing CH4 emissions from rumen. Tannin inhibits the activity of CH4 gas-producing groups, inhibits protozoa activity and thus minimizes CH4 emissions from ruminant (Huang et al., 2010; Mao et al., 2010; Puchala et al., 2012). For dairy cows, Tran Hiep et al. (2016) reported that tannin supplementation at 0.3% and 0.5% improved milk yield and did not affect digestibility. Pham Quang Ngoc (2019) commented that by adding dry acacia leaves at 0.3% tannin significantly reduced the amount of CH4 from the rumen and improved the growth rate of beef cattle.

Tea plant (*Camellia sinensis*) is a popular industrial plant grown in Vietnam. Tea stems and leaves are not only high in crude protein (24% VCK) but also rich in secondary plant compounds such as tannin (17.6% VCK) (Ramdani et al., 2013). In Vietnam, tea processing by-products are quite popular, can be found in Thai Nguyen, Son La, PhuTho, Bac Kan ....

Currently, the research on the potential of using tea processing by-products as supplementary feed in the cow's diet has not been fully and systematically studied. Therefore, the implementation of research to evaluate the potential and effects of tea processing by-products as supplementary feed for beef cattle to improve livestock efficiency and reduce CH4 emissions from beef rumen is very important and necessary.

1.2. Objectives

+ To evaluate the potential source of green tea by products as supplemental feed for beef cattle in the North of Vietnam.

+ To determine the effect of supplementation of green tea by products on in vitro gas production and in sacco digestibility.

+ To study the effect of supplementation of green tea by products on in vivo digestibility and performance of fattening cattle.

+ To investigate the effect of supplementation of green tea by products in the diet for beef cattle on enteric methane emissions

+ To develop equations of predicting enteric methane emissions from beef cattle fed green tea by products as a supplemental tannin in the diet.

1.3. Significance/Innovation of the dissertation

This dissertation is about a scientific work that has been relatively fully and systematically evaluated on the potential of green tea by-products as supplementary food, improving productivity, and reducing CH4 emissions, contributing to the development of beef production towards environmental friendly.

This dissertation determines the appropriate level of tannin supplementation from tea processing by-products (0.3% and 0.5%, calculated by dry matter) in the diets for fattening crossbred cows to reduce the intensity of gas emissions. CH4 (7.9% - 26.2%) and improved mass growth rate (2.2-8.1%).

The dissertation has setup four equations to estimate CH4 gas emissions with high accuracy and reliability. The equation helps to quickly assess the CH4 emissions from the cow's diet, thereby helping to adjust the cow's diet in accordance with the goals of productivity and environmental protection.

1.4. Practical significance of study

The dissertation is a systematic study, providing sufficient information and scientific basis on the potential and valuable of green tea by-products as a supplemental tannin for beef cattle.

This dissertation setup a numerous equations to predicting CH_4 emissions from tannin-rich diets, contributing to consolidating and completing data for calculating GHG emission factors in Vietnam.

The research results of the study serve as a scientific basis to contribute to the sustainable and environmentally friendly development of cattle breeding. The research results of the study can be used as reference for further research, and as teaching materials for training institutions.

The study contributes to the efficient exploitation of green tea by-products, increasing net income for poor farmers in the Northern part of Vietnam.

CHAPTER I: LITERATURE REVIEW

Many studies on the use of tea by-products (Spent tea leaf, STL) as silage to feed ruminants (Kondo et al., 2004a, 2007). Kondo et al., 2004; Kondo et al. (2018) show the chemical composition and criteria assessed by in vitro and *in vivo* experiments between dry or brewed tea processing by-products. The results showed that both STL annealed and dried had similar CP. Although the concentrated tannin (CT) content of the dry STL was higher, the total tannin (TT) was lower than that of the dry STL. There was no difference in total gas, ammonia (NH3) content and protein digestibility between STL incubated and dried in vitro experiments. Similarly, in vivo study results on goats showed that, using STL incubated and dried at the rate of up to 10% in mixed diets with DM intake, digestibility of DM, CP and NDF, rumen pH, total fatty acid volatility (VFA) and NH3 were equivalent.

STL inclusion in the silage diet increases total phenol (TP), TT, CT and lactic acid but reduces pH and NH3 (Kondo et al., 2018). STL provides significantly higher levels of TP, TT and CT than other conventional foods, and can increase the plant's consumption of secondary metabolites. Interestingly, the plant's consumption of secondary metabolites increased and subsequently decreased pH due to more lactic acid production, decreased NH3 concentrations due to protein breakdown in the rumen lower due to the formation of complexes associated with tannin proteins.

The addition of STL to the silage mixed diets gave mixed results in the digestibility of nutrients. Adding 5% STL to replace soybean meal and alfalfa did not affect dry matter intake but decreased CP digestibility and decreased NH3 production (Kondo et al., 2004b). This variation shows that each type of food has its own nutritional characteristics and when mixed together they give different reactions depending on their potential nutritional interactions (Ramdani, 2014). This leads to changes in the complex interactions between many species of microorganisms during rumen digestion (Demeyer, 1981), which in turn leads to changes in digestion, fermentation and performance. use of nutrients.

Some scientists confirmed that tannin is an anti-nutritional compound because tannin combines with protein of food and with gastrointestinal enzymes reduces digestibility of food protein, reduces food intake, and reduces growth, reducing livestock production (Duong Thanh Liem, 2008) and it is necessary to overcome the harmful effects of tannin with alkaline treatment (urea supplementation) or with combining tannin-containing foods with iron sulfate or polyethylene glycol - 4000 (PEG-4000) (Vu DuyGiang, 2001). But on the contrary, according to Nguyen Xuan Trach (2003), a low level of tannin addition to ruminant diets (20-40 g / kg feed dry matter) increases the protein efficiency of the ruminant. To achieve the two goals of CH4 minimization and maintain diet digestibility, we need to identify the source of tannin as well as the appropriate rate of addition to the ruminant diet.

Tran Hiep et al. (2016) reported that the addition of 0.3% and 0.5% of tannin from tea processing by-products increased ME and CP intake on dairy cows, but did not affect digestibility, but the additional 0.7% level decreased the rate of digestion of nutrients. Tannin supplementation increased milk mass and yield, simultaneously reduced CH4 emissions (7.47% to 22.77%) and decreased CH4 emission intensity in terms of DM intake (8 , 40% to 24.06%) and FCM (20.70% to 31.58%).

According to Pham Quang Ngoc (2019), the addition of 19.1% of acacia leaves (by dry matter) to the diet of Sind crossbred cows (equivalent to 0.3% of tannin) significantly reduces the amount of CH4 production. weight gain (g) / kg increase in weight compared to the group of cows fed the control diet (165.0 versus 214.8), at the same time achieved the highest weight gain of 683g / head / day, the efficiency of using feed is good. most (6.14 kg CK / kg weight gain). The author also said that the general tendency is that the tannin content increased from 1 to 6 g / kg VCK of the experimental diet, the gas generated at the time and the gas accumulated at 96 o'clock decreased gradually compared to the amount of gas generated. In the control diet (variable gas volume but no rules), although there was a difference in the amount of gas generated at the same time between diets at the same tannin level ($P < 0.05$). However, when the tannin content increased to 6 g / kg VCK of the experimental diet, the gas generated at the time and the gas accumulated at 96 o'clock was greatly affected and decreased sharply compared to the gas generated in the diet. controls and diets with lower tannin ($P < 0.05$).

HYPOTHESES

The estimated amount of green tea by-products of three provinces will be considered as potential supplement feed source for beef production

Use the level of tea tannin supplementation at 0.3% and 0.5% will not be effect on the total gas produced but reduced methane production and will be improved OM digestibility and *in sacco* DM, CP, NDF, ADF degradation.

The performance of beef cattle will be improved when feeding green tea by-products at level of 0,3 to 0.5% tannin.

Some prediction equations will be used to develop in practical to estimate the enteric methane emissions for beef cattle in Vietnam based on nutrient intake (DM, ME, CP) and levels of tannin supplement with acceptable precision and accuracy.

CHAPTER II: MATERIALS AND METHODS

2.1. Animal and materials

Tannin in tea processing by-products (crushed tea powder - by-product of dry tea processing establishments).

Two fistulated crossbred Sindhi cattle (200 kg/head) in Exp. 2 and twenty four Brahman crossbred beef cattle in Exp. 3 and 4 were used.

2.2. Contents

2.2.1. Survey on the potential source of green tea by products as supplemental feed for beef cattle in the North of Vietnam

2.2.2. Effects of different tannin levels from green tea by products on in vitro gas production and in sacco digestibility.

2.2.3. Effects of different tannin levels from green tea by products on in vivo digestibility, growth rate and enteric methane emissions of fattening beef cattle.

2.2.4. Setting the prediction equations of enteric methane emissions from beef cattle fed green tea by products as supplemental tannin in the diet.

2.3. Methodology

2.3.1. Survey on the potential source of green tea by products as supplemental feed for beef cattle in the North of Vietnam

2.3.1.1. Location

- Location: at Vietnam Cooperative Union, farms of tea production at 3 provinces (Thai Nguyen, Bac Kan and Phu Tho).
- The Exp. was lasted from January 2018 to July 2018.

2.3.1.2. Study area and farm selection

A total of 270 samples of green tea by products (180 tea-growing households and 90 cooperatives) at three provinces (Thai Nguyen, Bac Kan and Phu Tho) were took samples according to the area of tea production. The samples were collected based on processing methods (traditional and machinery processing).

2.3.1.3. Measurement and sample collection

+ Tea varieties yield, processing methods, types of tea by-products, quantity of by-products...

+ Chemical composition and nutrient value of green tea by-products was analyzed.

+ The ratio of green tea by-products and nutritional characteristics of tea processing by-products.

2.3.1.4. Chemical analysis

DM, CP, EE, CF, Ash were analyzed according to procedured by TCVN 5613: 2007; TCVN 4328-1: 2007; TCVN 4331: 2001; TCVN 5714: 2007; TCVN 5611: 2007; NDF, ADF by Van Soest et al. (1991); total tannin by AOAC method 952.03 (2000).

2.3.1.4. Estimated amount of green tea by-products

The amount green tea by-products was calculated based on the plantation area (ha) of each province, the tea products (tons/ ha) and percentage of the total amount of tea products.

2.3.2. Effects of different tannin levels from green tea by products on in vitro gas production and in sacco digestibility

2.3.2.1. Location

The Exp. was conducted from January 1, 2018 to August 8, 2018 at the Mountainous Livestock Research and Development Center, Binh Son Commune, Song Cong City, Thai Nguyen Province and the Center for Science, Technology and Environment (Giang Vo, Dong Da, Hanoi).

2.3.2.2. Materials and methods

Exp. of *in vitro* gas production:

The experiment was designed according to the completely randomized design (CRD) with three replicates. The animal was fed a based diet and supply with different level of tannin from green tea by-products or pure tannin (Table 2. 1). The treatments were 0%; 0.3%; 0.5%; 0.7% in T0.0; T0.3; T0.5 and T0.7, respectively. TK0.5: 0.5% pure tannin.

Table 2. 1. Experimental design

Diet	**T0.0**	**T0.3**	**T0.5**	**T0.7**	**TK0.5**
Based diet	Corn silage: 30%; natural grass: 10%, cornstarch: 15%; Cassava residue: 10%; TĂHH: 35%				
Tannin levels	0%	0.3%	0.5%	0.7%	0.5%

Note: T0.0: Control diet; T0.3: supplement with 0.3% of tannin from tea by-products (% VCK); T0.5: supplement with 0.5% tannin from tea by-products (% VCK); T0.7: supplement with 0.7% tannin from tea by-products (% VCK); TK0.5: supplement with 0.5% tannin from pure tannin.

The chemical composition and nutrient value of the experimental feed was showed in Table 2. 2.

Table 2. 2. Chemical composition of experimental feed

Items	DM (%)	% as DM basis						
		CP	NDF	ADF	CF	EE	Ash	Total tannins
Natural grass	25.68	11.20	65.36	33.18	29.12	2.96	7.60	NA
Corn silage	28.12	8.61	65.77	38.12	35.39	2.26	8.62	NA
Tapioca	23.18	3.63	62.22	39.12	8.78	0.11	1.83	NA
Cornstarch	90.28	10.41	35.99	10.88	2.72	4.93	1.54	NA
Cargill	90.81	18.50	56.52	12.86	9.28	1.62	9.86	NA
Tea processing PP	90.67	22.88	32.45	21.13	18.33	2.08	6.36	25.22
Pure tannin	-	-	-	-	-	-	-	90

Note: DM: dry matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: acid detergent fiber; NA: not analysis.

In vitro gas production: The feed samples were incubated in vitro with rumen fluid in calibrated glass syringes as described by Menke and Steingass (1988), modified by Makkar et al. (1995). The procedure was followed by weighing 200 mg substrate into each numbered syringe placing them in an incubator at 39°C. The blanks, i.e. rumen fluid/artificial saliva mixture on its own, were included at the beginning, in the middle of the set, and at the end. Samples were done in triplicate.

The gas volume was recorded at 3, 6, 12, 24, 48, 72 and 96 hours of incubation.

The results of gas volume reading (means of triplicates) at different times of incubation were fitted to the exponential equation of the form: P = a+b(1-e-ct) (Orskov and McDonald, 1979), where P represents gas production at t time, (a+b) the potential gas production, c the rate of gas production and a, b and c are constants in the exponential equation.

The organic matter digestibility (OMD), ME energy and short-chain fatty acids (SCFA) were calculated based on the gas produced at 24h of incubation and data of chemistry composition following OMDinv (%) = 14.88 + 0.889 x GP24 + 0.45 x CP ; ME (MJ / kg VCK) = 3.78 - 0.0614GP24 + 0.168CP + 0.789EE + 0.227 Ash (R2 = 0.819) (Dinh Van Muoi, 2011) and SCFA (mmol / 200gVCK) = 0.0239 * GP24 - 0.0601. In which: GP24 is the total gas produced at 24 hours of incubation; CP (%) is the crude protein, EE is crude fat, Ash is the total mineral.

Table 2. 3. The experimental diet (as fresh basis)

Resources	T0.0	T0.3	T0.5	T0.7	TK0.5
Natural grass	38.94	38.94	38.94	38.94	38.94
Corn silage	106.69	106.69	106.69	106.69	106.69
Tapioca	43.14	43.14	43.14	43.14	43.14
Corn meal	16.61	16.61	16.61	16.61	16.61
Cargill commercial feed	38.54	38.54	38.54	38.54	38.54
Green tea by products	-	1.19	1.98	2.78	-
Pure tannin	-	-	-	-	0.56

In sacco DM degradation: The experiment was conducted following the procedure introduced by Orskov et al. (1980). The course of degradation of the feed was described by fitting DM loss values to the exponential equation of Orskov and McDonald (1979): P = a+b(1-e-ct). The degradation characteristics of the samples are defined as: A = washing loss (representing the soluble fraction of the feed); B = (a+b)-A (representing the insoluble but fermentable materials); c = the rate of degradation of B (Orskov et al., 1980)

2.3.2.3. Analysis: According to section 2.3.1.4.

2.3.2.4. Statistical analysis

The data were analyzed as a Completely Randomized design (CRD) using the general linear model and pair-wise comparison in Minitab software version 13.31, following the statistical model below:

In vitro gas production: $Yik = \mu + Ti + \varepsilon ik$.

In sacco experiment: $Yij = \mu + Ti + Bj + \varepsilon ijk$.

2.3.3. Effects of different tannin levels from green tea by products on in vivo digestibility, growth rate and enteric methane emissions of fattening beef cattle

2.3.3.1 Location

The experiment was conducted at Mr. Nguyen Van Son farm - Dong Chi village, Le Chi commune, Gia Lam district, Hanoi city.

2.3.3.2. Animal and experimental feed:

Total of 24 Brahman crossbred fattening cattleat the age of 16-18 months (323.10 ± 30.45 kg).

The experimental diet included the basal diet and supply with different levels of tannin from green tea by products powder. green tea by products powder collected from tea by products.

2.3.3.3. Experimental Design

The animals were fed the basal diet and supplement with different level of tannin from green tea by products. The treatment is 0.0%; 0.3%, 0.5% and 0.7% in KP0.0; KP0.3; KP0.5 and KP0.7, respectively (Table 2.4). According to the treatment, tea by-products was supplemented at level of 0%, 1.19%, 1.98%, and 2.78% in KP0.0; KP0.3; KP0.5 and KP0.7, respectively (percentage of the diet).

Table 2.4. Layout of the experiment

Items	KP0.0	KP0.3	KP0.5	KP0.7
N_0 of cattle	6	6	6	6
Initial weight (kg)	321.62± 27.92	326.21± 30.36	319.18± 32.94	325.38± 30.33
Adaptation (days)	15	15	15	15
Experimental period (days)	90	90	90	90
Basal diet	Corn silage: 30%; Nature grass: 10%; cornstarch: 15%; Cassava residue: 10%; Cargill (*): 35%			
Tannin level (% DM basis)	0	0.3	0.5	0.7

In the last 7 days of experiment, the faeces and urine excreted by individual animals were collected and measured daily. During the collection time the faeces was sampled and frozen and stored for future analysis.

2.3.3.5. Measurements and analysis

The feeds offered and refused were recorded daily for individual animals and weighed before new feed was added.

Samples of feeds, refusals and faeces were analysed for DM, ash, CP, ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to item 2.3.1.4.

At the start and the end of the growth trial, all animals were weighed individually for two consecutive days in the morning before feeding, and the mean taken as the initial and final weight. During the growth study, the LW was recorded every 15 days with the same procedure. Growth rate of each animal was calculated from the average daily live weight gain (LWG) at each 15 days measurement change over the time of experiment.

The data were analyzed statistically as a Completely Randomized design (CRD) by variance analysis (ANOVA) using the general linear model (GLM) procedure of Minitab software version 14.0 (Minitab 2003). The statistical model: $Yij = \mu + Ti + Bj + \varepsilon ij$. Where (Yij) is the mean, (Ti) is the effect of the experimental diet (i = 1,2,3,4,5); Bj is the effect of the block (j = 1,2,3,4); (εij) is the random error.

2.3.4. Effect of tannin supplement levels in tea processing by-products on levels and intensity of CH4 emissions from fattened beef rumen

2.3.4.1. Experimental Design

The experiment was carried out as following in the item 2.3.3.4

2.3.4.2. Measurements

Gas samples were collected 2-3 continuous days at the beginning and the end of experiment. The total methane emission was estimated for each cow using the equation developed by Madsen et al. (2010) as follows: CH4 produced (l/d) = a * (b-d)/(c-e). Where; a is CO_2 produced by the animal, l/day; b is the concentration of CH_4 in air mix, ppm; c is the concentration of CO_2 in air mix, ppm; d is the concentration of CH_4 in background air, ppm; e is the concentration of CO_2 in background air, ppm.

2.3.4.3. Setting the prediction equation to estimate the enteric methane emission base on the DM intake and the tannin consume

Using algorithm "Best Subset" to determine the variables that correlate closely to the total amount of CH_4 emitted. Then use the algorithm "General Regression" to construct diagnostic equations for the amount of CH4 emitted. The computations are calculated using Minitab 16 software.

$$MAB = \frac{\sum_{i=1}^{n} |O_i - P_i|}{n} \quad RAB = \frac{MAB}{\bar{O}} * 100 \quad R^2 = 1 - \frac{SS\ Error}{SS\ Total}$$

Statistical parameters that evaluate the accuracy, precision of according to the equations:

$$SEC,\ SEP = \sqrt{\frac{\sum_{i=1}^{n} (O_i - P_i)^2}{n}} \quad RSEC,\ RSEP = \sqrt{\frac{\sum_{i=1}^{n} (O_i - P_i)^2}{\sum_{i=1}^{n} O_i^2}} x100$$

$$\text{Predicted } R^2 = 1 - \frac{PRESS}{SS\ Total}$$

CHAPTER III: RESULTS AND DISCUSION

3.1. Survey on green tea by-product potential

3.1.1. Quantity of green tea by-products of various green tea variaties

The results are shown in Table 3. 1. The results show that, each variety of tea has its own characteristics, according to which there will be varieties of productivity. However, compared to other tea varieties, LPD1 and Trung Du tea have a high rate of by-productss, which are a plentiful source of by-productss as supplementary food for cattle.

Table 3. 1. Effects of tea varieties on the by-products proportion

Tea varities	Total quantity of fresh tea *(ton)*	Total quantity of dried tea *(ton)*	Total quantity of tea by-products *(ton)*	Percentage of by-products *(%)*
Trung du	2245.29	449.06	55.49	12.36
LPD1	3184.27	636.85	88.32	13.87
Kim Tuyen	450.89	90.18	6.05	6.71
Phuc Van Tien	572.95	114.59	6.75	5.89
PH1	1129.35	225.87	25.58	11.33
LPD2	918.37	183.67	16.07	8.75
Tri 777	431.00	86.20	11.19	12.98
Bat Tien	318.00	63.60	2.84	4.46
Total	**9250.11**	**1850.02**	**212.30**	**11.48**

3.1.2. Quantity of green tea by-products by locality

Results of survey in three provinces (Thai Nguyen, Phu Tho, and Bac Kan) on the same tea variety and cultivated area are shown in Table 3. 2.

Table 3. 2. Effects of locality on green tea by-products

Varieties	Provinces	Total quantity of fresh tea *(ton)*	Total quantity of dried tea *(ton)*	Total quantity of tea by-products *(ton)*	Percentage of by-products *(%)*
Trung du	Thai Nguyen	1128.96	225.79	28.45	12.60
	Phu Tho	243.51	48.70	7.00	14.38
	Bac Kan	872.82	174.56	20.04	11.48
	Total	*2245.29*	*449.06*	*55.49*	*12.36*
LPD1	Thai Nguyen	1481.49	296.30	39.59	13.36
	Phu Tho	960.48	192.10	29.08	15.14
	Bac Kan	13.99	148.46	19.66	13.24
	Total	*2455.95*	*636.85*	*88.32*	*13.87*
Phuc Van Tien	Thai Nguyen	198.63	39.73	3.29	8.28
	Phu Tho	90.68	18.14	1.10	6.06
	Bac Kan	283.65	56.73	2.36	4.16
	Total	*572.95*	*114.59*	*6.75*	*5.89*

The average of the portion of tea by-products are 12.36%, 13.87% and 5.89% respectively with Trung Du, LPD1 and Phuc Van Tien tea in Thai Nguyen, Phu Tho and Bac Kan. Total quantity of tea by-products in Thai Nguyen province is highest, followed by Phu Tho province, and then Bac Kan province.

3.1.3. Effects of processing method on green tea by-products proportion

The results of the study on the effects of manual processing and industrial processing are shown in Table 3. 3. The manual method gives the percentage of tea by-productss of 17.81%, which is twice as high as that of industrial processing, although the total amount of dried tea when processed by manual method is almost half lower than that of industrial processing. The percentage of tea by-productss when processed by two methods is 11.49% of the total dried tea being producted.

Table 3. 3. Effects of green tea production processing on the rate of by-products

Criteria	Processing method		Total
	Manual	Industrial	
Total quantity of dried tea (tons/year)	623.46	1226.57	1850.02
Total quantity of tea by-products (tons/year)	111.06	101.56	212.62
Percentage of by-products (%)	17.81	8.28	11.49

3.1.4. Estimation of green tea by-products in surveyed provinces

The total amount of tea by-products of the three provinces is 9.5 thousand tons / year.

Table 3. 4. Estimation of green tea by-products in surveyed provinces

Province	**Area** *(ha)*	**Quantity of fresh tea** *(ton/ha)*	**Quantity of dried tea** *(thousand ton/year)*	**Quantity of by-products** *(thousand ton/year)*
Thai Nguyen	16.726	12.5	41.7	4.8
Phu Tho	15.600	11.0	34.5	4.0
Bac Kan	2.800	12.2	6.8	0.8
Total	**35.126**		**83.0**	**9.6**

3.1.5. Some factors affecting the chemical composition of green tea by-products

The results on chemical composition analysis of 4 varieties of tea namely LPD1,Trung du, TRI777 and PH1 have been shown in Table 3.5

Table 3.5. Effects of tea varieties on chemical composition of green tea by-products

Tea varieties	**DM**	**CP**	**NDF**	**ADF**	**CF**	**Ash**	**EE**	**Tannin**
	(%)	*% DM*						
LPD1	35.4	18.4	28.2	21.3	18.5	4.6	2.9	29.8
Trung du	36.6	17.6	26.5	19.3	18.4	4.6	3.9	27.1
TRI777	33.7	19.9	27.2	20.3	21.7	4.5	2.5	28.8
PH1	35.3	16.7	28.2	20.9	19.5	4.4	4.2	28.5

The quantity of tea by-products of the three provinces is plentiful, estimated at 9.6 thousand tons / year, equivalent to 11.49% of the total dry tea quantity. Thai Nguyen has the largest amount of by-products, followed by Phu Tho, and the Bac Kan with 4.8, 4.0 and 0.8 thousand tons respectively. Tea processing by industrial machines will give lower quantity of tea by-product than that processing by manual method, which are 8.28% and 17.81% respectively. Criterias such as CP, NDF and tannin of surveyed tea varieties ranged from 16.7-19.9; 26.5-28.2 and 27.1-29.8% dry matter.

3.2. *Effects of tannin from green tea by-products to in vitro* gas production and *in sacco* degradation

3.2.1. In vitro gas production

The results on the *in vitro* gas production of experimental diets have described in Table 3.6

Table 3. 6. Amount of gas accumulated during fermentation of the experimental diets (ml)

Time	T0.0	T0.3	T0.5	T0.7	TK0.5	SEM	P -value
3h	4.33^{a}	3.92^{ab}	3.62^{b}	3.25^{b}	3.55^{b}	0.26	0.047
6h	7.83^{a}	6.48^{b}	6.33^{bc}	5.50^{c}	6.22^{bc}	0.44	0.042
9h	12.00^{a}	10.46^{b}	10.04^{bc}	8.50^{c}	9.86^{bc}	0.69	0.035
12h	19.67^{a}	17.53^{a}	16.11^{b}	14.41^{b}	15.81^{b}	1.05	0.032
24h	36.67^{a}	32.07^{b}	30.73^{bc}	27.57^{c}	30.18^{bc}	2.17	0.022
48h	49.67^{a}	43.51^{b}	42.25^{b}	38.13^{b}	41.50^{b}	2.98	0.020
72h	53.33^{a}	47.39^{a}	45.10^{b}	40.59^{b}	44.30^{b}	3.19	0.026
96h	57.33^{a}	50.45^{b}	47.82^{bc}	43.17^{c}	46.96^{bc}	3.40	0.019

Note: a,b Mean within rows with different superscripts are significantly different ($P<0.05$)

The results in the study also showed that supplementing with a level of 0.7% tannin from tea by-products or supplementing with 0.5% pure tannin significantly reduced the potential of gas production while there was no difference in this value between T0.3; T0.5 and T0.0.

3.2.2. Metabolizable energy and in vitro disgestibility of experimental diets

The estimated value of ME, SCFA and OMD of experimental diets has illustrated in Table 3.7

Table 3. 1. Digestibility and energy value of diets

Criteria	**T0.0**	**T0.3**	**T0.5**	**T0.7**	**TK0.5**	SEM	P-value
ME, *MJ/kgDM*	8,47^{a}	8,32^{ab}	8,28^{ab}	7,88^{b}	8,10^{ab}	0,28	0,016
SCFA, *mmol/200gDM*	0,91^{a}	0,89^{ab}	0,88^{ab}	0,82^{b}	0,84^{ab}	0,03	0,048
OMD_{inv}, *%*	56,8^{a}	55,86^{a}	55,56^{ab}	53,06^{b}	55,24^{ab}	1,26	0,021

Note: SCFA: Short Chain Fatty Acids; OMDinv: Organic Matter in vitro digestibility; a,b Mean within rows with different superscripts are significantly different ($P<0.05$)

3.2.3. Degradation degree of dry matter

The degradation degree *in sacco* dry matter of experimental diets being supplement tannin from green tea by-products is illustrated in Table 3.8

Table 3. 8. Dry matter degradation in feed diets

Incubation time	**T0.0**	**T0.3**	**T0.5**	**T0.7**	**TK0.5**	SEM	P-value
3h	18.37	17.78	17.74	15.12	17.09	1.78	0.147
6h	25.11	26.23	24.58	23.66	24.29	1.77	0.051
9h	39.04^{a}	38.04^{ab}	38.37^{ab}	34.14^{b}	37.66^{ab}	2.47	0.012
12h	45.20^{a}	44.65^{ab}	44.12^{ab}	42.45^{b}	43.9^{ab}	1.27	0.015
24h	62.5^{a}	61.66^{a}	61.68^{a}	58.27^{b}	59.37^{ab}	1.66	0.024
48h	73.13^{a}	72.80^{a}	70.94^{ab}	66.11^{b}	69.85^{ab}	3.20	0.002
72h	79.89^{a}	77.80^{ab}	78.16^{ab}	73.90^{b}	75.59^{ab}	3.23	0.023

Note: a,b Mean within rows with different superscripts are significantly different ($P<0.05$)

The result of DM degradation in experimental feed diets is illustrated in Table 3.9. Using supplement level of 0.3% and 0.5% tannin from green tea by-productss in feed diets does not decrease the *in vitro* gas production, the degree of DM, CP, NDF, ADF *in sacco* degradation and nutriment digestibility. However, if using the level of 0.7%, all above mentioned criterias will decrease. Supplementation of 0.5% of tannin from green tea by-productss tended to improve the digestibility of organic matter and *in sacco* degradation of nutriment in feed diets compared with supplementation at the same level from pure tannin.

Table 3. 9. DM degradation of experimental diet

Items	T0.0	T0.3	T0.5	T0.7	TK0.5	SEM	P -value
A (%)	4.7^{a}	4.4^{ab}	4.0^{b}	4.5^{ab}	4.4^{ab}	0.71	0.05
B (%)	74.2^{a}	72.9^{ab}	72.8^{ab}	47.5^{c}	70.1^{b}	2.00	0.03
A + B (%)	78.9^{a}	77.3^{ab}	76.8^{ab}	52^{c}	74.5^{b}	2.01	0.03
c	0.063	0.065	0.065	0.065	0.066	0.08	0.12
Lag phase (fraction/h)	0	0	0.1	0.15	0.1	0.05	0.09

Note: a,b Mean within rows with different superscripts are significantly different (P<0.05) A = washing loss (representing the soluble fraction of the feed); B = (a+b)-A (representing the insoluble but fermentable materials); c = the rate of degradation of B, Lag phase

3.3. Effects of tannin from green tea by-products on *in vivo* digestibility and productivity of beef cattle at fattening stage

3.3.1. Effects of tannin supplementation on feed intake and *in vivo* digestibility of nutrients.

As can be seen in Table 3.10, the dry matter intake in the experiments varied from 2.48% to 2.63% BW.

Table 3. 10. Nutrients intake

Items	ĐC	KP0.3	KP0.5	KP0.7	SEM	P-value
DM (kg/a/day)	9.34	10.02	9.83	9.35	0.17	0.021
DM, % BW	2.51^{ab}	2.63^{a}	2.62^{a}	2.48^{b}	0.06	0.001
ME, Kcal/a/day	25866^{b}	27748^{a}	27220^{a}	25896^{b}	425.81	0.001
OM, kg/a/day	8.74^{b}	9.38^{a}	9.20^{ab}	8.75^{b}	0.14	0.001
CP, kg/a/day	1.03^{b}	1.12^{a}	1.11^{ab}	1.06^{b}	0.02	0.001
NDF, kg/a/day	5.15^{b}	5.50^{a}	5.38^{ab}	5.10^{b}	0.09	0.001
ADF, kg/a/day	2.18^{b}	2.34^{a}	2.29^{a}	2.18^{b}	0.04	0.001

Ghi chú: a,b Mean within rows with different superscripts are signifcantly different (P<0.05)

This result is also consistent with the research of Thang and his colleagues (2010) which reported that there is no significant differences in DM intake when growing beefs are fed with tannin (from Cassava leaf and *Stylosanthes)* at the level of 22.5 to 33.5 g/kg DM.

3.3.2. In vivo nutrients digestibility

The results show that the digestibility rate tended to decrease when the supplementation of tannin from green tea by-productss increase. However, the digestibility of DM, OM and CP in the tannin supplementation level of 0.3-0.5% is not statistically different from that of DC (P>0.05)

Table 3. 11. Nutrients digestibility rate (%)

Items	Control	KP0.3	KP0.5	KP0.7	SEM	P-value
DM	76,9	75,04	74,13	70,88	4,211	0,052
OM	77,74^{a}	75,98^{a}	74,88^{ab}	71,01^{b}	4,242	0,025
CP	79,16^{a}	77,73a	75,52^{ab}	72,86^{b}	4,338	0,033
NDF	76,43^{a}	76,40^{a}	73,99^{b}	70,51^{c}	4,222	0,036
ADF	70,93^{a}	69,36^{a}	66,97^{b}	63,33^{c}	3,827	0,035

Note: a,b Mean within rows with different superscripts are signifcantly different (P<0.05). DM: dry matter; OM: organic matter; CP. Crude protein, NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

3.3.3. Effects of tannin on body weight gain

It can be seen in Table 3.12 that beefs being fed with 0.3%-0.5% tannin from green tea by-productss improve their ability to gain weight compared to the control group (increase by 2.2-8.1%)

Table 3. 12. Effects of tannin supplementation on body weight gain

Items	Control	KP0.3	KP0.5	KP0.7	SEM	P-value
Initial weight, *kg*	321,62	326,21	319,18	325,38	6,21	0,522
Final weight, *kg*	422,99^{b}	435,95^{a}	431,24^{ab}	428,74^{b}	7,29	0,001
BWG, *g/day*	1126,31^{b}	1219,3^{b}	1245,13^{a}	1148,39^{a}	18,66	0,003

Note: a,b Mean within rows with different superscripts are signifcantly different (P<0.05)

3.3.4. Feed conversion ratio and feed costs

In terms of feed costs, in order to gain 1 kg of weight, supplementation of tannin at 0.5% and 0.7% reduced feed cost by 4.33% and 1.37% respectively. This illustrates the most appropriate level of tannin supplementation for cattle is 0.5% (Table 3.13)

Table 3. 13. Effects of tannin supplementation on feed conversion efficiency

Items	Control	KP0.3	KP0.5	KP0.7	SEM	P-value
Feed price (VND/kg DM)	5926	5940	5950	5959	-	-
Feed conversion ratio	8,30^{a}	8,22^{ab}	7,90^{c}	8,14^{b}	0,12	0,001
Feed cost (VND/kg kg BWG)	49,182	48,828	47,003	48,507	-	-
Comparison (%)	100	99,28	95,57	98,63	-	-

Note: a,b Mean within rows with different superscripts are signifcantly different (P<0.05)

When the supplementation level of tannin from green tea by-productss increased to 0.3 or 0.5%, it improved the DM intake by 4.8% compared with the control group without supplement. Using additional 0.3-0.5% tannin from green tea by-productss improved the ability to increase weight compared to the control group (increase by 2.2-8.1%). The level of weight gaining was highest when the supplementation is provided at 0.5% , but there was no difference in this criteria when tannin supplementation increased to 0.7%.

3.4. Effects of tannin from green tea by-products on total enteric methane emssion and enteric methane emssion of fattening beef cattle

The results in this experiment showed that the lowest reduction value was observed when adding 0.5% tannin from green tea by-products. The intensity of CH4 emissions calculated according to L CH4 / kg DM tended to decrease gradually as tannin supplementation was increased, decreased by 15.9%-20.1%, however, when tannin from green tea by-products increase to 0.7%, there is no significantly reduce (5.9%) compared with the control group (P> 0.05) (Table 3. 14 and Figure 3.1).

Based on the intake, digestibility of nutrients, breeding productivity and CH4 emission intensity, the results showed that the tannin supplementation level at 0.3 - 0.5% (corresponding to the level of green tea by-products supplementation at 1.19% - 1.98%, according to the dry matter of the ration), will bring the highest breeding efficiency and environmental efficiency.

Table 3. 14. Total enteric methane emssion and enteric methane emssion

Items	Control	KP0.3	KP0.5	KP0.7	SEM	P-value
L CH4/kg DMI	32,73[a]	28,24[b]	27,26[b]	30,88[ab]	0,74	<0,001
g CH4/kg DMI	23,24[a]	20,05[b]	19,36[b]	21,93[b]	0,54	<0,001
L CH4/kg OMI	34,98[a]	30,18[b]	29,14[b]	33,01[a]	0,81	<0,001
L CH4/kg NDFi	59,34[a]	51,45[c]	49,83[c]	56,62[b]	1,36	<0,001
L CH4/kg ADFi	140,24[a]	121,14[c]	117,03[c]	132,66[b]	3,39	<0,001
L CH4/kg BW	271,55[a]	232,12[c]	215,24[c]	251,46[b]	5,61	<0,001

Note: a,b Mean within rows with different superscripts are signifcantly different (P<0.05) *DMI: Dry Matter Intake; OMI: Organic Matter Intake NDFi: NDF intake ; ADFi: ADF intake; BW: body weight*

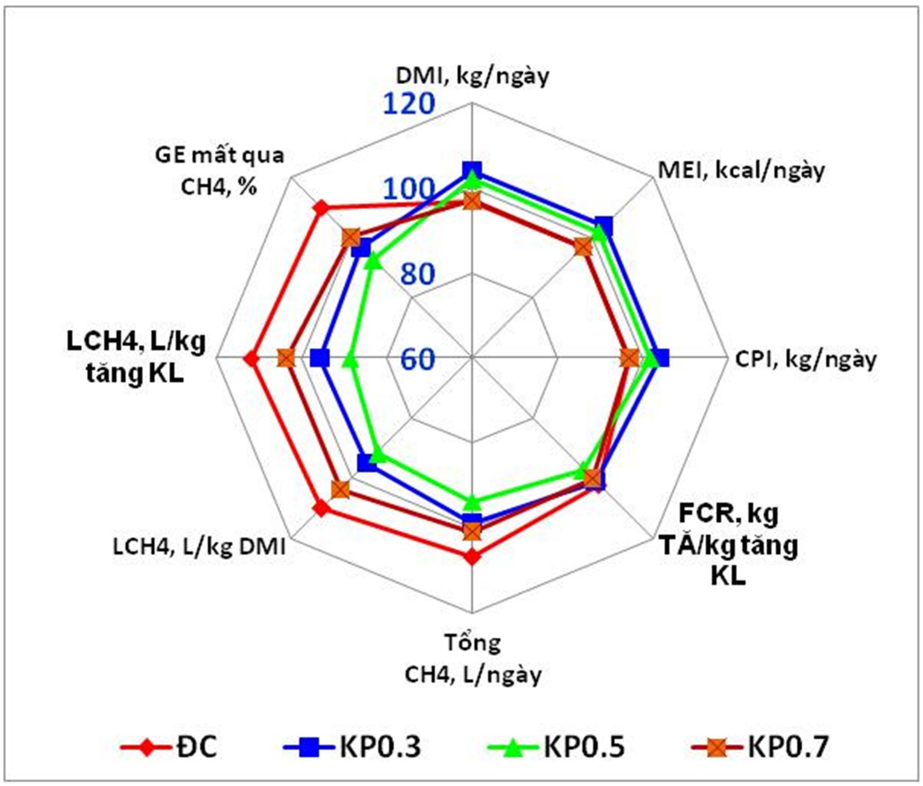

Figure 3. 1. Animal performance and environmental efficiency

3.4.3. Analysis of optimal tannin supplement level

Figure 3. 2 and Figure 3. 3 show that, when the tannin level increased from 0.3% to 0.5%, the weight was gained and the intensity of CH4 emissions was reduced. However, when analyzing through the optimal function (Solver Analysis), the results showed that the best increase in beef weight was when the tannin level was 0.38%, the emission intensity was the lowest when the tannin level reached 0.41%. Thus, to meet the expectation in breeding productivity and environmental efficiency at the same time, the tannin level should be 0.38-0.41%.

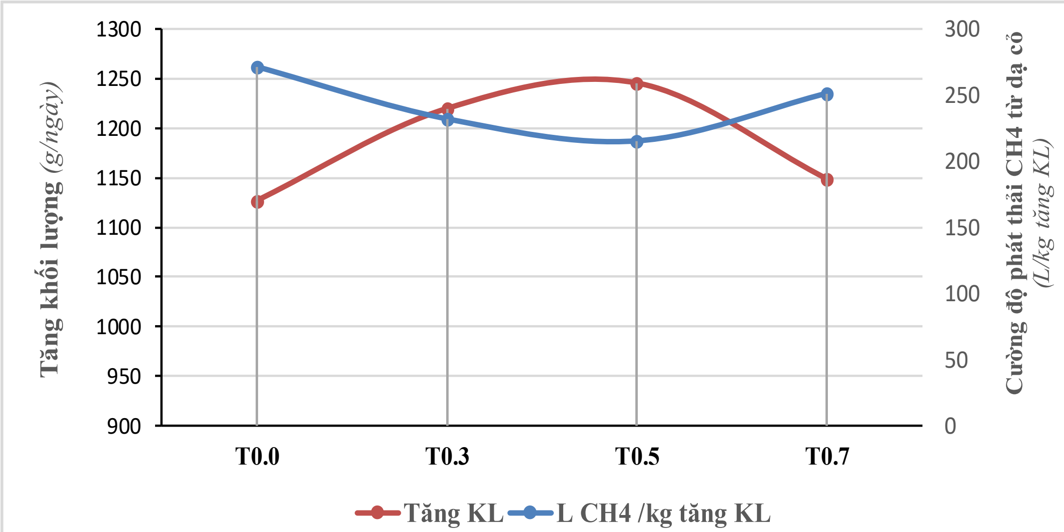

Figure 3. 2. Correlation between body weight gain and CH4 emission intensity of experimental feed diets

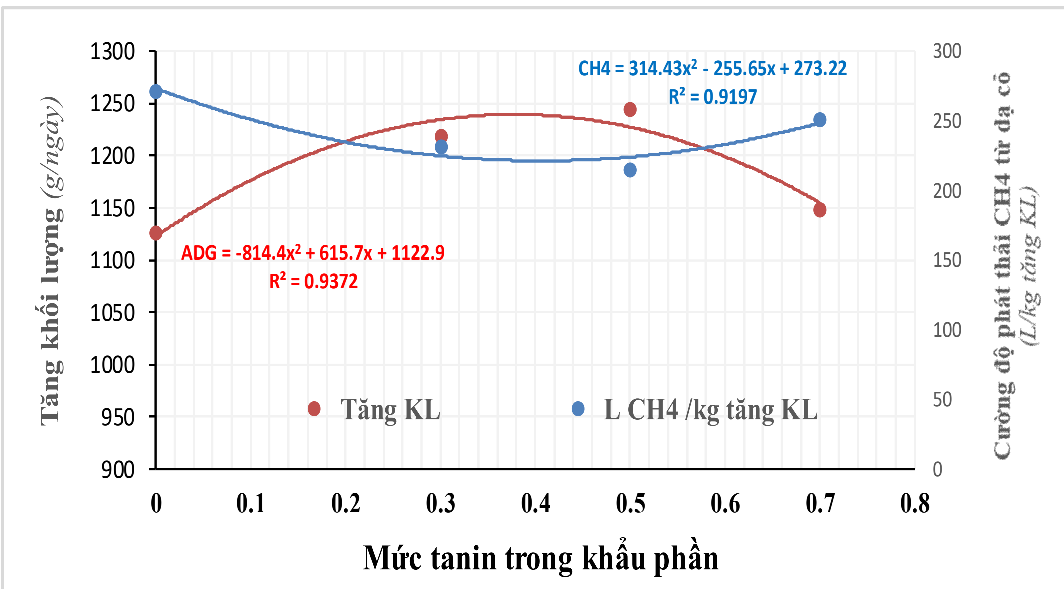

Figure 3. 3. Correlation between weight gain and CH_4 emission intensity at different tannin levels

In this study, we do not analyze the correlation between breeding productivity, feed costs and environmental efficiency because feed costs may vary by season, region, etc…

3.5. Development of prediction equation for estimating methane emission

Dataset:

To build a regression equation to estimate the amount of CH4 emission from the independent variables namely cattle weight, nutrient intake, the database must have a sufficiently large degree of variation (Table 3.16). Thus, it is possible to formulate regression equations to diagnose methane emissions from independent variables related to cattle and nutrient intake.

Table 3. 16. Statistical parameters for database description

Parameters	**Abbreviate**	**Min**	**Max**
Cattle information			
Body weight at the time measure of CH_4, kg	*BW*	**339.11**	441.23
Body weight gian, g/a/day	*ADG*	1047.5	1265.10
Diets Intake information			
Tannin level, % DMI	Tannin	0.00	0.70
DM intake, kg/a/day	*DMI*	7.88	13.53
DM intake, % of BW	*DMI%BW*	2.27	3.08
DM intake, g/kg BW	*DMIP0.75*	99.717	140.54
ME intake, kcal/a/day	*MEI*	21744	36731
CP intake, kg/c/day	*CPI*	0.72	1.21
CH_4 emissions			
Total CH_4 emissions, l/a/day	*TotalCH_4*	208.10	335.59

Development of prediction equation

Results in Table 3.17 show that the coefficient of determination (R^2) ranges from 67.49% to 81.63% with an error of 10.33 to 13.69 L (the average was 11.56 liters CH4 or 5.19%). The equations are used for statistic (P <0.001). When analyzing each variable, the results also show that the variables have significance in statistic when being used to estimate methane emissions (P <0.05), in which the variables of DMI% BW, DMI, MEI have significance in statistic at P <0.01 and P <0.001.

The results illustrate the estimated equation has a high value of R^2, reaching over 80%, which are equation 3,4,5 and 6 when using 2-3 variables: DMI% BW, DMI, MEI, Tannin with average error 10.5 liters (4.5%).

Table 3. 17. Development of CH4 emission prediction equations

No.	Equations	Summary of statistical parameters
PT1	Total CH_4 = 63,8326 + 16,3228 DMI - 4,27216 Tannin	S = 13.6858 R-Sq = 67.58% P < 0.001 Term Coef SE Coef T P Constant 63.8326 20.9108 3.05262 0.005 DMI 16.3228 2.0997 7.77370 0.000 Tannin -4.2722 9.3535 -0.45675 0.048
PT2	Total CH_4= 66,5123 + 0,00592195 MEI - 10,7196 Tannin	S = 13.6858 R-Sq = 67.49% Term Coef SE Coef T P Constant 66.5123 20.6122 3.22684 0.003 MEI 0.0059 0.0008 7.75887 0.000 Tannin -10.7196 9.4407 -1.13546 0.045
PT3	Total CH_4= 547,996 - 174,133 DMI%BW + 18,6016 DMI	S = 10.3394 R-Sq = 81.45 P < 0.001 Term Coef SE Coef T P Constant 547.996 104.556 5.2412 0.000 DMI%BW -174.133 37.089 -4.6950 0.000 DMI 18.602 1.662 11.1921 0.000
PT4	Total CH_4= 548,222 - 173,844 DMI%BW + 18,6373 DMI - 3,78877 Tannin	S = 10.4703 R-Sq = 81.63% P < 0.001 Term Coef SE Coef T P Constant 548.222 105.880 5.1778 0.000 DMI%BW -173.844 37.562 -4.6282 0.000 DMI 18.637 1.684 11.0645 0.000 Tannin -3.789 7.166 -0.5287 0.046
PT5	Total CH_4 = 549,028 - 173,387 DMI%BW + 0,00664103 MEI	S = 10.7287 R-Sq = 80.02% P < 0.001 Term Coef SE Coef T P Constant 549.028 108.530 5.0588 0.000 DMI%BW -173.387 38.495 -4.5041 0.000 MEI 0.007 0.001 10.6897 0.000
PT6	Total CH_4 = 552,045 - 174,142 DMI%BW + 0,00676566 MEI - 11,1555 Tannin	S = 10.4823 R-Sq = 81.59% P < 0.001 Term Coef SE Coef T P Constant 552.045 106.055 5.2053 0.000 DMI%BW -174.142 37.614 -4.6297 0.000 MEI 0.007 0.001 11.0489 0.000 Tannin -11.155 7.232 -1.5426 0.034

Note: Total CH_4: total amount of CH4 emissions (L/beef/day); DMI%BW: Dry Matter Intake (% beef weight); DMI: Dry Matter Intake (kg/beef/day); MEI: Metabolizable Energy Intake; Tannin: level of tannin supplementation (% dry matter of diets).

Evaluation of equation precision

The results in Table 3.18 show that the adjusted determination coefficient (Adj-R2) is from 65.25% to 80.17% with absolute error ranging from 6.98 - 9.72 liters (error 3.14% - 4, 36%). This shows that the equation has quite good accuracy.

Table 3. 2. Precision of prediction equations

Equations	Variables	Absolute bias		$Adj\text{-}R^2$
		MAB	RAB	
1	DMI, Tannin	9,717	4,36	65,34
2	MEI, Tannin	9,719	4,37	65,25
3	DMI%BW, DMI	7,083	3,18	80,17
4	DMI%BW, DMI, Tannin	6,983	3,14	79,66
5	DMI%BW, MEI	7,621	3,42	78,64
6	DMI%BW, MEI, Tannin	7,038	3,16	79,61

Evaluation of equation accuracy

Table 3.19 shows that diagnostic error (SEP) ranged from 9.8-13.01 liter, estimated relative error (RSEP) from 4.40 to 5.85% and Pre-R2. from 57.85 - 76.07%.

Table 3. 3. Aaccuracy of prediction equations

Equation	Variables	Diagnostic error			$Pre\text{-}R^2$
		PRESS	SEP	RSEP	
PT 1	DMI, Tannin	7012,22	13,012	5,84	58,03
PT 2	MEI, Tannin	7042,69	13,028	5,85	57,85
PT 3	DMI%BW, DMI	3998,55	9,843	4,42	76,07
PT 4	DMI%BW, DMI, Tannin	4196,87	9,794	4,40	74,88
PT 5	DMI%BW, MEI	4265,39	10,213	4,59	74,47
PT 6	DMI%BW, MEI, Tannin	4210,30	9,805	4,40	74,80

CHAPTER IV. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

The green tea by-products accounts for 11.49% of the total amount of dry tea. The quantity of tea by-products of the three provinces (Thai Nguyen, Phu Tho, and Bac Kan) is plentiful, estimated at 9.6 thousand tons/year. Tea varieties LPD1 and Trungdu have a high rate of by-products. The tea by-products ratio from manual method was two times higher than industrial processing. The rate of tea by-products of the tea varieties grown in Phu Tho province had the highest rate, and Bac Kan province had the lowest one. The highest tannin content was in LPD1 tea (29.8%), and lowest in Trung Du tea (27.1%).

The supplementation of 0.3% and 0.5% tannin from tea by-products in the diet did not affect the rate of *in vitro* gas production, *in sacco* DM, CP, NDF, ADF degradation and OM disgestibility.

The supplementation at level of 0.3% and 0.5% tannin from tea by-products in the diet did not affect the DM intake and not lead to the decrease *in vivo* nutrient digestibilities.

The supplementation at level of 0.3% and 0.5% tannin from tea by-products in the diet improved daily eight gain compared with the control group (increased by 2.2-8.1%). The highest level of gaining weight reached when the supplementation of tannin is 0.5%.

The supplementation of tannin reduced the level and intensity of CH_4 emission by the DM intake (decreased by 5.9% - 20.1%) and by the daily weight gain (decreased by 7.9% - 26.2%)

The optimal level of tannin supplementation to maintain both animal performance and environmental efficiency is 0.4% (0.38-0.41% tannin) (as DM basis).

Development of four equations to predict CH_4 emission from weight, intake tannin supplementation level variables.

4.2. Recommendations

The supplemental levels of 0,4% tannin from green tea by products (or 1.59% in DM basis) in the diets for fattening Brahman crossbred cattle were recommended.