



Combined Effects of Cottonseed Oil and Tannin from Tea By-Products (*Camellia sinensis*) Supplementation on Nutrient Digestibility, Performance and Enteric Methane Production of Fattening Cattle

CHU MANH THANG^{1*}, TRAN HIEP²

¹National Institute of Animal Science (NIAS), Hanoi, Vietnam; ²Vietnam National University of Agriculture (VNUA), Hanoi, Vietnam.

Abstract | The combined effect of cottonseed oil and tannin from tea by-products (*Camellia sinensis*) supplementation on feed intake, digestibility, growth performance and enteric methane production was studied using twenty-four fattening crossbred cattle (Brahman x Laisind) in a completely randomized block design (CRBD) experiment with six replications in each. All animals were fed a basal diet consisting of ensiled maize stover, elephant grass; concentrate; corn mill and cassava pulp. The treatments were four supplements of 1.5% oil plus 0.3% tannins (O1.5T0.3), 1.5% oil plus 0.5% tannins (O1.5T0.5), 3.0% oil plus 0.3% tannins (O3.0T0.3) và 3.0% oil plus 0.5% tannins (O3.0T0.5) (%DM intake). The results showed that the supplement of cottonseed oil and tea by-products containing tannins, respectively at 1.5% oil and 0.5% tannins provided higher nutrient digestibility, increased CP intake and daily gain, reducer FCR comparing to these other groups. This combination at these levels also reduced from 4.8 to 12.1% methane emission intensity (CH₄/kg LWG) of fattening cattle. It is likely that supplementation of cottonseed oil combined with tannin from tea by-products increased available energy to cattle in the fattening period, thus increasing weight gain and methane reduction. The lowest enteric methane production (CH₄/kg LWG) found in the group fed 1.5% cottonseed oil combined with 0.5% tannins from tea by-products.

Keywords | Methane, Fattening cattle, Tea by-products, Tannins, Cottonseed oil

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***Correspondence** | Chu Manh Thang, National Institute of Animal Science (NIAS), Hanoi, Vietnam; **Email:** thangslu@gmail.com

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INTRODUCTION

Global warming in recent years is an immerging issue, attracting attention of human because of its effect on ecological balance. It is expected that the earth surface temperature will increase from 1.1 to 6.4 °C by 2100. Global warming is mainly due to the greenhouse gas effect but the main cause is the emission of CO₂, CH₄, NO₂ etc. from agriculture and livestock production (IPCC, 2007). Methane (CH₄) production from ruminant has impact on greenhouse gas (GHG) emission (Wanapat et al., 2015). Steinfeld et al. (2006) estimated GHG emissions arise from livestock farming mostly related CH₄ emission from digestive fermentation in herbivorous animals and animal waste. Reducing enteric CH₄ emissions decreases

greenhouse gases and improves the efficiency of converting plant material into milk and meat.

Numerous methods of reducing enteric CH₄ emissions from ruminants, many studies have reported that some feed additives such as condensed tannins can reduce enteric CH₄ emissions from ruminants. Tannins may inhibit the methanogenesis directly and also via inhibition of protozoal growth (Patra and Saxena, 2010). Essential oils, organosulphur compounds and flavonoids appear to have direct effects against methanogens, and a reduction of protozoa associated methanogenesis probably plays a minor role for these metabolites. In Vietnam, some foliage containing tannins in diet has reduced methane production in beef cattle (Chu et al., 2016) and in dairy cattle (Tran

et al., 2016b). However, tannins exert an anti-microbial action on microbial growth including cellulolytic bacteria and fungi (Patra and Saxena, 2009), which may adversely affect fiber utilization. Macheboeuf et al. (2008) studied in detail on the dose–response effects of different essential oil on methane inhibition and VFA production. Cotton seed oil supplement in diet for lactating cows increased milk yield by 5.4–12.2% and reduced methane emission intensity calculated as L/kg FCM by 18.8–37.9% (Tran et al., 2016a). In an *in vitro* experiment, Suharti et al. (2019) concluded that the combination of *Sapindus rarak* extract containing tannins and canola oils in the concentrate ration decreased protozoa population, increased *Anaerovibrio lipolytica* growth, NH_3 concentration, dry matter and organic matter digestibility.

The combined effect of cottonseed oil and tannin from tea by-products supplementation on both CH_4 emission and animal performance in fattening cattle is not well documented. Thus, the study aimed to improve animal performance and to mitigate enteric CH_4 emission by mean of oil from cottonseed oil and tannin from tea by-products supplement in diet for fattening cattle.

MATERIALS AND METHODS

LOCATION

The experiment were conducted at the cattle farm in DongChi village, LeChi commune, GiaLam district, Hanoi, Northern part of Vietnam.

EXPERIMENTAL ANIMALS AND FEEDS

Twenty four fattening crossbred cattle (Brahman x Laisind) were used in the experiments. The animals were collected from 2 farms and had the same breed, at around 17 months of age with an average live weight of 324.1 kg (SD= 41.2) and were individually identified by numbered ear tags. The animals were kept in individual pens with roofing and concrete floor. Before the adaptation period, all the experimental animals were treated against intestinal parasites using DeplinB™ (4ml/100 kg LW) and were vaccinated against pasteurellosis and 15 days later for Foot and Mouth Disease. The animals were weighed after the adaptation period when the feed intake was stable.

The feeds used in the experiments were elephant grass, maize stover silage, maize meal, cassava pulp, concentrate and supplemental feed. The re-growth of elephant grass (*pennisetum purpureum*) at an age of 45 days was harvested daily in the morning. It was chopped into 10–15 cm length before feeding. The maize stover ensilaged with 0.5% salt for 60 days before feeding. The tea by-products (*Camellia sinensis*) was collected from tea processing factory at Thai Nguyen province, northern part of Vietnam. Based on the tannins amount supplementing in each treatment, the tea

by-products (25.2% tannin on DM, Table 1) was calculated and mixed with concentrate before feeding to animals. In the basal diet, all animal were fed the diet consisting of maize stover (25%), elephant grass (10%); concentrate (30%); corn mill (25%) and cassava pulp (10%). The concentrate consisted of ground corn, ground cassava, ground soybean cake, ground palm cake, mineral-vitamin premix, NaHCO_3 , lime, and common salt (no hormone, antibiotic or growth promotor). The chemical composition and nutrition value of feeds using in experiment were presented in Table 1.

EXPERIMENTAL DESIGN AND TREATMENTS

The daily rations for the animal in each treatment was offered a basal diet and one of the following supplements with 1.5% oil plus 0.3% tannins; 1.5% oil plus 0.5% tannins; 3.0% oil plus 0.3% tannins and 3.0% oil plus 0.5% tannins (%DM intake), corresponding to four treatments O1.5T0.3; O1.5T0.5; O3.0T0.3 and O3.0T0.5. Oil and tannins is from cottonseed oil and tea by-products, respectively.

FEEDING AND MANAGEMENT

The feeds were offered twice per day, in the morning (07.30 h) and afternoon (16.30 h). At each feeding occasion, the concentrate, maize meal and cassava pulp mixed with cottonseed oil and tea by-products in a separate bucket was supplied first to the animals, and then elephant grass, maize stover silage were offered. Each animal had free access to clean drinking water and a mineral lick block containing Ca 90 g, P 90 g, Na 150 g, Mg 5 g, Fe 10 g, Mn 6000 mg, Cu 800 mg, Co 400 mg, I 50 mg and Se 100 mg per 1 kg block. The experimental period lasted for 90 days including 15 days of adaptation.

DATA COLLECTION

FEED INTAKE

The daily feed consumption was recorded and refusals collected for individual animals in morning of the next day. The intake of concentrate, supplemental feeds and forages were measured daily, based on the amount of feeds offered and refused. The total feed intake was calculated as the sum of the intake of the feed components.

LIVE WEIGHT

After the adaptation period the animals were weighed at 06.00 h before feeding on two consecutive days by using an electronic scales (Rud weight equipment scales models KWB-WB-600), and at 15 day intervals. Feed conversion ratio (FCR) was calculated as kg feed consumed /kg live weight gain (LWG).

The temperature and humidity in the animal house were measured three times per day at 07.00 h, 14.00 h, 21.00 h to investigate the effect of the environment on feed intake and performance of the animals.

Table 1: Chemical composition and nutrition value of experimental feeds.

Ingredients	DM (%)	ME (kcal/kg DM)	CP (% DM)	NDF (% DM)	ADF (% DM)	CF (% DM)	EE (% DM)	Ash (% DM)	Total tannins (% DM)
Elephant grass	22.10	1896.00	12.80	73.50	43.20	36.00	3.23	9.66	NA
Maize stover silage	27.13	2281.22	9.22	65.43	39.96	35.40	2.55	8.51	NA
Cassava pulp	88.00	2766.67	3.60	61.82	42.81	8.78	0.11	1.83	NA
Maize meal	90.28	3213.00	10.41	35.99	10.88	2.72	4.93	1.54	NA
Concentrate ^(*)	90.81	3075.00	16.00	56.52	12.86	9.28	1.62	9.86	NA
Cottonseed oil	95.00	7521.00	-	-	-	-	-	-	-
Tea by-products (<i>Camellia sinensis</i>)	90.67	2816.00	22.88	32.45	21.13	18.33	2.08	6.36	25.22

Note: NA: not analysis; ^(*) The concentrate consisted of ground corn, ground cassava, ground soybean cake, ground palm cake, mineral-vitamin premix, NaHCO₃, lime, and common salt (no hormone, antibiotic or growth promotor).

Table 2: Feed intake, digestibility, and efficiency of treatments.

Items	O1.5T0.3	O1.5T0.5	O3.0T0.3	O3.0T0.5	SEM	P-value
Intake						
DM, kg/head/day	8.39 ^c	9.43 ^a	9.49 ^a	8.68 ^b	0.16	0.033
DM, %BW	2.22 ^b	2.45 ^a	2.46 ^a	2.26 ^b	0.05	0.001
ME, Kcal/day	24009.61 ^c	26998.44 ^a	27800.84 ^a	25421.67 ^b	414.68	0.001
OM, kg/ day	7.86 ^c	8.84 ^a	8.90 ^a	8.14 ^b	0.13	0.001
CP, kg/ day	0.93 ^c	1.06 ^a	1.04 ^a	0.96 ^b	0.02	0.001
NDF, kg/ day	4.53 ^b	5.08 ^a	5.06 ^a	4.61 ^b	0.08	0.001
ADF, kg/ day	2.00 ^b	2.25 ^a	2.23 ^a	2.04 ^b	0.04	0.001
Digestibility (%)						
DM	75.35 ^{ab}	76.85 ^a	74.77 ^b	73.92 ^c	1.51	0.052
OM	76.18 ^{ab}	77.80 ^a	75.52 ^{bc}	74.05 ^c	1.87	0.001
CP	77.60 ^b	80.55 ^a	76.16 ^b	75.90 ^b	2.00	0.039
NDF	74.87 ^b	78.21 ^a	74.63 ^{bc}	73.55 ^c	1.29	0.022
ADF	69.37 ^b	72.18 ^a	67.60 ^{bc}	66.37 ^c	1.10	0.041
Nutrient utilization efficiency						
Initial weight, kg	321.36	325.61	323.33	326.12	8.41	0.521
Final weight, kg	431.67 ^{bc}	452.61 ^a	448.28 ^{ab}	440.32 ^b	10.59	0.002
LWG, g/head/day	1225.66 ^b	1411.16 ^a	1388.32 ^a	1268.91 ^b	42.39	0.046
FCR, kg DM/kg LWG	6.82	6.76	6.84	6.83	0.10	0.056

Note: ^{a,b} Mean within rows with different superscripts are significantly different (P<0.05). FCR: Feed conversion ratio, LWG: live weight gain.

CHEMICAL ANALYSIS

The feeds offered and individual feed refusals were sampled daily and pooled to a sample for each fifteen days. Samples of feeds, refusals were analyzed for DM, CP, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash. The DM (ID 930.15), CP (ID 976.05), and ash (ID 942.05) were analyzed according to the standard methods of AOAC (2000). The EE was analyzed by ISO-6492 (1999) and NDF and ADF concentrations were determined according to the procedure of (Van Soest et al., 1991).

GAS SAMPLING AND METHANE MEASUREMENT

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:

$$CH_4 \text{ produced (l/d)} = a * (b-d)/(c-e)$$

Where;
 a is CO₂ produced by the animal, l/day; b is the concentration of CH₄ in air mix, ppm; c is the concentration of CO₂ in air mix, ppm; d is the concentration of CH₄ in background air, ppm; e is the concentration of CO₂ in background air, ppm.

The CH₄ and CO₂ concentration in the air sample were analyzed by GASMET portable analyzer. Total CO₂ is the quotient of total HP divided by 21.75 and HP (kj) = ME intake (kj) – kg body gain x 20,000 (kj). 1 little CH₄ is equivalent to 0.71 g CH₄ (Brouwer, 1965).

STATISTICAL ANALYSIS

The data were analyzed statistically as a Randomized Completely Block Design (RCBD) by variance analysis (ANOVA) using the general linear model (GLM) procedure of Minitab software version 14.0 (Minitab, 2003). The treatment least square means showing significant differences at the probability level of P<0.05 were compared using Tukey’s pairwise comparison procedure.

The statistical model used in the trial was $Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$ where Y_{ij} is the dependent variable, μ is the overall mean, α_i is effect of treatment i , β_j is effect of block (initial animal weight) and ϵ_{ij} is a random error.

RESULTS

Feed intake, nutrient digestibility and growth performance
The data on feed intake of the four diets is shown in Table 2. Among these treatments, the animals fed higher level of oil from cottonseed oil or higher level of tannins from tea by-products in the combination treatment resulted in the highest value of DM intake in (%BW). The difference in DMI between the O1.5T0.5 và O3.0T0.3 was not significant (P>0.05). At the same higher level of tannins (0.5%), the nutrients digestibility was significantly decreased with increasing supplemental level of oil from 1.5% to 3.0%. In contract to the lower level of tannins (0.3%) when combined with increasing level of oil. The highest value of DM, OM, NDF and ADF digestibility was observed in group fed level at O1.5T0.5. The LWG was significantly higher in the group fed higher level of either oil or tannins in combination treatment than for these other groups given a both lower or higher level. The feed conversion ratio (FCR) was slightly lower in the group fed O1.5T0.5 but treatments did not differ significantly.

METHANE EMISSION

Table 3 showed the combined effect of feeding cottonseed oil and tannin from tea by-products in the diets on methane production. At the same lower level of oil (1.5%), the methane production (g CH₄/kg DMI, and /kg LWG) was significantly decreased with increasing supplemental level of tannins from 0.3% to 0.5% while the group fed higher level of oil (3.0%) did not reduced significantly. The lowest value of methane emission rate was found in group fed level at O1.5T0.5.

Figure 1 summarize the combined effect of cottonseed oil and tannin from tea by-products on diet intake, feed

efficiency and methane emission. When considering both animal performance and environmental impact, it should be supplement at level of O1.5T0.5 in the diet for fattening cattle. The Figure 1 showed that the animal fed O1.5T0.5 and O3.0T0.3 treatments have higher nutrient intake (DM, ME, CP) while resulted in higher value of methane emission efficiency (lower in methane produced per kg DMI or per kg gain).

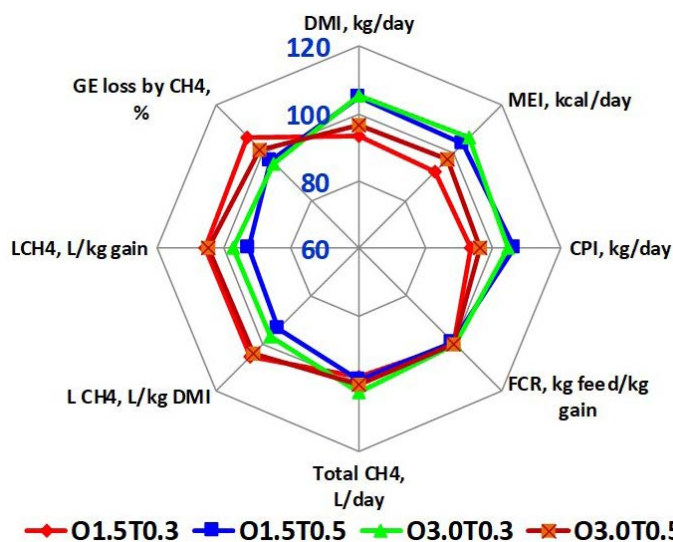


Figure 1: Effect of supplemental levels in diet on feed intake, efficiency and methane emission.

DISCUSSION

Generally, dietary tannins reduce feed intake by decreasing palatability or affecting digestion (Landau et al., 2000). However, other factors such as the source of tannins, animal species and diet composition may also influence the maximum effect of tannins. In the current experiment, the effect of higher level of 0.5% tannins from tea by-products on total feed intake was not substantial, which may be partly attributed to the combination with different level of oil in the treatments. This discrepancy could be related to the fact that the effects of fat supplementation on feed intake are influenced by degree of fatty acid saturation, feeding level, palatability, fatty acid chain length, and form of fat (Bu et al., 2007). According to Aprianita et al. (2014) reported that cottonseed oil contains a high concentration of linoleic acid. It is likely that supplementation of cottonseed oil increased available energy to microbial protein synthesis in the rumen. In this study, the positive effects of supplementation in combination of higher level of tannins (0.5%) and lower level of oil (1.5%) on nutrients digestibility and live weight gain of fattening cattle at the age of 17-18 months. The CP content of tea by-products is 22.9% DM found in this study (Table 1). Min et al. (2003) suggested that tannins is binding and protecting protein, fiber, and carbohydrates from degradation in the rumen when the animals fed the diet containing tannins.

Table 3: The effect of supplemental levels in diets on methane emission.

Parameters	O1.5T0.3	O1.5T0.5	O3.0T0.3	O3.0T0.5	SEM	P-value
Calculated methane emission						
Total HP, Kj/day	75586.51	85929.11	88555.11	80739.42	2320.84	0.005
Total CO ₂ , L/day	3475.24	3950.76	4071.50	3712.16	125.65	0.030
CH ₄ /CO ₂ ratio	0.075	0.067	0.067	0.072	0.00	0.001
CH ₄ emission, L/day	261.21 ^b	264.35 ^{ab}	273.21 ^a	267.74 ^{ab}	6.87	0.001
Total CH ₄ , kg/day	0.185 ^b	0.188 ^{ab}	0.194 ^a	0.190 ^{ab}	0.004	0.001
Emission intensity						
L CH ₄ /kg DM	31.25 ^a	27.73 ^b	28.79 ^b	30.91 ^{ab}	0.77	0.001
g CH ₄ /kg DM	22.19 ^a	19.69 ^b	20.44 ^b	21.95 ^{ab}	0.56	0.001
L CH ₄ /kg OM	33.37	29.61	30.71	32.98	0.77	0.001
L CH ₄ /kg NDF	57.82	51.46	54.04	58.21	1.45	0.001
L CH ₄ /kg ADF	130.92	116.27	122.36	131.50	3.46	0.001
L CH ₄ /kg LWG	213.11 ^a	187.33 ^b	196.79 ^b	211.00 ^{ab}	4.91	0.001

Note: ^{a,b} Mean within rows with different superscripts are significantly different (P<0.05).

These compounds form complexes with protein in the rumen, then dissociate under the acidic conditions of the abomasum, allowing the protein to be digested and absorbed in the small intestine (Barry et al., 2001). In fattening cattle, high protein level to encourage greater intake and in order to slaughter animals earlier (Dung et al., 2019).

Enteric methane emission of crossbred cattle (Brahman x Sindhi) in the present study ranged from 0.18 to 0.19 kg/animal/day, these results were higher than the 0.12 kg/day reported by Dung et al. (2019), which found in the local Yellow cattle (at the initial live weight of 145-150 kg) in the central of Vietnam. In the study, enteric methane production was significantly decreased with increasing supplemental level of tannins from tea by-products with lower level of oil supplement. However, the combination with higher level of oil from cottonseed oil did not significantly reduced methane. This could be due to the different individual responses to levels of feeding of tannins or oil in combination in the diet for fattening cattle. While our results suggest a synergistic effect on methane reduction at lower level of cottonseed oil supplement with lower levels of tannins from tea by-products. Patra and Saxena (2010) suggested that tannins may inhibit the methanogenesis directly and also via inhibition of protozoal growth while some essential oils or some organosulphur compounds occurred direct effects against methanogens, and a reduction of protozoa associated methanogenesis probably. Patra and Yu (2015) suggested that binary combination of anti-methanogenic inhibitors with complementary mechanisms of actions on methanogenesis may alter the archaeal communities and may decrease methane production additively without negatively impacting upon rumen fermentation and degradability. Among the treatments, group fed O1.5T0.5 supplement in diet resulted in lower enteric methane production (CH₄/kg

LWG) by 12.1, 11.2 and 4.8% comparing to other groups fed O1.5T0.3, O3.0T0.5 and O3.0T0.3, respectively.

CONCLUSIONS

This study showed that inclusion of 1.5% cottonseed oil and 0.5% tannins from tea by-products into fattening cattle diet could positive affect on feed intake, and nutrients digestibility. It is likely that supplementation of cottonseed oil increased available energy to cattle in the fattening period, thus resulting in additive effect on the increasing weight gain and methane reduction. The lowest enteric methane production (CH₄/kg LWG) found in the group fed 1.5% cottonseed oil combined with 0.5% tannins from tea by-products.

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AUTHORS CONTRIBUTION

Both authors contributed to the work, discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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