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USING SEVERAL PLANT LEAVES CONTAIN TANNIN IN THE DIET FOR MINIMIZE METHANE EMISSION IN BEEF CATTLE

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PUBLISHED PAPERS RELATED TO THE THESIS

1. **Phạm Quang Ngọc, Nguyễn Viết Đôn, Lưu Thị Thi và Vũ Chí Cương (2013).** *Effect of levels and sources of tannins from Leucaena leucocephala, Manihot esculenta Crantz, Camellia sinensis leaves and pure tannin added to the basal diet to fermentation, rumen digestion and methane emissions from rumen under vitro conditions.* Journal of animal science and technology, vol. 42 Jun 2013, pp 36-60.
2. **Phạm Quang Ngọc, Lưu Thị Thi, Vũ Chí Cương, Cấn Thị Thanh Huyền và Phạm Kim Cương (2014)**. *Effect of levels and sources of tannins from Camellia sinensis, Acacia mangium, Acacia auriculiformis leaves and pure tannin added to the basal diet to fermentation, rumen digestion and methane emissions from rumen under vitro conditions.* Journal of animal science and technology – Vol. 47 Apr 2014, pp 48-70.
3. **Phạm Quang Ngọc, Phạm Kim Cương, Lê Văn Hùng, Lưu Thị Thi, Nguyễn Thiện Trường Giang, Bùi Thị Thu Huyền.** *Effect of supplementation of Leucaena leucocephala leaves on diets on methane emissions, live weight and feed conversion efficiency of growing Red Sindhy crossbred*. Journal of animal science and technology. Vol 103 Sept 2019, pp. 74-87.

INTRODUCTORY

1. The necessary of the thesis

The source of methane emissions into the atmosphere from ruminant livestock production accounts for 12-41% from agricultural production (Afzalani et al. 2015). Methane occurs in the fermentation of plant organic matter mainly in the rumen, so the amount emitted is closely related to the amount of feeds intake and digested.

The strategy of changing ruminant diets to reduce methane emissions has been widely considered (Grainger et al., 2011). A number of *in vitro* and *in vivo* studies have been conducted to reduce CH_4 emissions, including the supplementation of concentrate feeds (Bhatta et al., 2009), lipids (Carulla et al., 2005), organic acids (Chadwick et al., 2011; D'Mello, 2000), essential oils (Evans and Martin, 2000) probitics and prebiotics (Fuller and Johnson, 1981; Carulla et al., 2005). Antimicrobial compounds such as monensin and lasalocid have also been used to reduce CH_4 production (Goel et al., 2008).

So far, the potential for research and use of natural compounds in plants such as tannins, saponins and essential oils is of interest. Tannins, also known as polyphenols, are capable of acting as anti-methanogenic substances in the rumen. The effect of tannin use depends largely on the type and dosage of tannin. Low molecular weight tannins inhibit methanogen bacteria more effectively than higher molecular weight tannins, as they form stronger bonds with microbial enzymes (Ningrat et al., 2017).

The cattle population of nearly 6 million cows in Vietnam of which mostly beef cattle, also contribute significantly to methane emissions and the greenhouse effect, while understanding the impact to reduce methane emissions in beef cattle is still limited, so it is necessary to study the use of plant leaves contain tannin in diets to minimize methane emissions in beef cattle production.

2. Objectives

– Determine the effects of the source and the level of supplementation of several types of leaves contained tannin on the substrate to the rate and characteristics of *in vitro* gas production, amount of methane production, in vitro digestibility, energy values and short-chain fatty acids.

– Determine the effect of dietary supplementation of leaves containing tannin on methane emissions, digestibility and nitrogen accumulation of crossbred beef cattle

– Formulate a diet with supplementary leaves containing tannin to reduce methane emissions into the environment while providing good live weight gain and high feed efficiency in crossbred beef cattle.

3. Scientific and practical meanings of thesis

– The thesis has identified the chemical composition, nutritional value of several tannin-fed leaf tops common in Vietnam and the effects of the source and the level of each type of plant to the substrate on the rate and *in vitro* gas characterization, methane production, *in vitro* digestibility, energy value (ME) and short-chain fatty acid content.

– Determined the effect of dietary supplementation of Acacia tree leaves to diets on methane emissions, digestibility, nitrogen accumulation, live weight gain and feed conversion efficiency of growing Red Sindhy crossbred.

– Formulate a diet supplemented with the tip of Acacia tree leaves at a reasonable rate to reduce methane emissions into the environment but still increase the live weight and better feed convert rate of growing Red Sind crossbred in production.

– The research results of the thesis are references for the next research, teaching materials for training institutions, agricultural extension office and farmers.

4. New contributions of the thesis

– Have determined the effect of the level of *Leucaena leucocephala* leaf and shoot supplementation into the cattle diet on methane emission, digestibility, nitrogen retention, weight gain and feed conversion efficiency of growing crossbred red sindhi beef.

– Have formulated a diet with the optimal supplementation level of the leaf and shoot of *Leucaena leucocephala* for minimizing the amount of methane released into the environment, increase the weight and feed conversion of growing crossbred red sindhi beef.

– Have developed a regression equation that represented the relationship between the two methods of methane determination (by gas chromatography (GC) and by the volumetric method with NaOH solution) and made recommendations on the usage of the volumetric method with NaOH solution to determine methane emission in the absence of expensive GC equipment.

5. Thesis structure

The entire thesis consists of 166 pages, 4 chapters, 27 tables, 17 figures, referencing 345 Vietnamese and foreign language scientific papers and articles and 2 scientific research papers related to the published thesis and the appendix.

CHAPTER I: LITERATURE REVIEW

1.1. BACKGROWN OF TANNIN

Tannins are secondary polyphenolic plant compounds, which have been demonstrated to influence microbial activity during fermentation, protein breakdown, methane production and minimize pathogen transmission via food products. Tannins are a group of chemical compounds produced by several broadleaf plant groups, which can bind to proteins. Greater tannin levels are usually found in broadleaf plants in warm climates (Jennifer et al., 2013). Tannins are oligomeric compounds with many structural units with free phenolic groups. Tannins are generally divided into two groups: hydrolyzable tannins (HT) and concentrated tannins (Condensed tannins - CT) (Athanasiadou et al., 2000).

1.2. EFFECTS OF TANNIN SOURCES IN THE DIET ON RUMEN FERMENTATION AND RUMINANT PERFORMANCE

1.2.1. Feed intake

Most researchers believed that tannin in the diet reduced the amount of feed intake. Feeding the diet with high concentration of condensed tannin (>50g/kg dry matter) significantly reduced the feed intake of cattle, while the diets with concentration of condensed tannin less than 50g/kg dry matter are unlikely this parameter (Waghorn et al., 1994a).

There are three main mechanisms for explaining the negative effects of high condensed tannin levels on feed intake: (i) reducing feed palatability; (ii) slowing down the digestive process and (iii) increasing the conditional reactions. The appetite of cattle reduced through a reaction between tannin and saliva mucoprotein, or by a direct reaction to the taste receptors, causing an astringent sensation (McLeod, 1974).

1.2.2. Digestibility of diet

Tannins mainly affected protein, but also other components of feed at the different level (Kumar and Singh, 1984). Their main effect on protein is based on their ability to form stable hydrogen bonds within the pH level from 3.5 to approximately 8. These complexes are stable at rumen pH dropping down below 3 (such as in abomasum, pH 2.5-3) or greater than 8 (eg, duodenum, pH 8), this may explain more about the activity of tannin in the gastrointestinal tract (Hagerman and cs., 1992).

1.2.3. Rumen Fermentation

The obvious effect of tannin is to reduce the process of rumen protein degradation (Hagerman et al., 1992). The affinity of tannin with the very large protein molecules combined with the pH of the rumen environment has spurred the formation of tannin-protein complexes. In general, in the case of a decrease in the level of proteolysis involved in the production of lower ammonia nitrogen and greater amounts of non-ammonia nitrogen to the duodenum (Waghorn, 1996). Basically, the effect of tannin on the rapid reduction of proteolysis is to immediately reduce the rate and speed of degradation of structural fragments

(Hervás et al., 2001). Although tannins primarily affect proteins, they also have an impact on carbohydrates, especially hemicellulose, cellulose, starch and pectin (Leinmüller et al., 1991), and therefore the effects of tannin on fiber degradation are considered as secondary anti-nutritional factors.

1.2.4. Positive effects of tannin

1.2.4.1. Rumen bypass

Tannin-proteins complexes are formed in the rumen pH environment and protects proteins from the degradation of microbial enzymes. These complexes are unstable at the acidic pH of abomasum and are digested here (Barry and Manley, (1984); Jones and Manganese, (1977).

1.2.4.2. Urea regeneration

Tannins can increase the efficiency of urea regeneration in rumen walls. Tannin lowers the rate of proteolysis and amino reduction in rumen resulted in lowering N-NH3. Plasma urea nitrogen, rumen $N\text{-}NH_3$ and N lost in urine were lower if sheep and goats were fed tannin-containing legumes (Woodward, 1989). Tannins can increase glycoprotein content and salivary secretion, whereby more N is regenerated in the rumen wall (Robbins et al., 1987).

1.2.4.3. Effective with microorganisms

Tannins increase the ability to produce microorganisms per unit of digestible organic matter. Some researchers have observed that the amount of non-ammonia nitrogen (non-ammonia nitrogen-NAN) to the duodenum is greater than the N content of tannin-containing legumes. Therefore, N is not produced in the rumen, a part of the increased NAN is from endogenous sources integrated into microorganisms. The amount of nitrogen in the duodenum is greater than the common amount of N in a low-N diet (<l%), but the legumes with N>2%, the N content in the duodenum is usually lower than the amount of N (Barry and Manley, 1984).

1.2.4.4. Nutrition

The beneficial effects of tannin on sheep are associated with greater amino acid bypass and absorption, particularly for sheep fed feed containing tannin ratios ranging from 2-4% (Wang et al., 1996b ; Min et al., 1999). The increase in growth rate, animal performance and nutritional value of milk in sheep may be due to an increase in essential amino acids (Wang et al., 1994). Increasing the amount of sulfur-containing amino acids as the main precursor to wool production may contribute to increased wool production (McNabb et al., 1993). Tannins form complexes with proteins that prevent the level of protein degradation in the rumen, thereby increasing the amount of protein reaching the intestine (McNabb et al., 1996).

1.2.4.5. Parasite control

Recent reports on ruminants suggested that tannins contained feed reduced the adverse effects of gastrointestinal parasites by killing larvae and adult worms (Athanasiadou et al., 2000). Tannins binding to proteins in the gastrointestinal tract reduced the availability of nutrients and caused the hunger for larvae and worms resulted in the parasite death (Athanasiadou et al., 2001). In addition, tannin also bound to the epidermis of larvae containing high levels of glycoproteins, thereby killing the larvae (Thompson and Geary, 1995).

1.2.4.6. Reducing Proteolytic enzyme activity and the growth of rumen microorganisms

Condensed tannin (CT) significantly reduced the activity of proteolytic enzymes and the growth of bacteria in sheep rumen (Jones et al., 1993). CT forms complexes with polymers that cover the cell of bacteria and their protein degraded enzymes and therefore, allowed proteins to escape through the rumen. These complexes will release proteins under the acidic conditions of abomasum. These protein molecules are hydrolyzed by enzymes in the small intestine leading to increase the amount of absorbed amino acids (Jones and Mangan, 1977; Martin and Martin, 1983; McNabb et al., 1998).

1.2.5. The effect of tannin in livestock

Tannin-containing diets may affect the feed intake, digestibility and performance of cattle. In general, high tannin content has a markedly negative effect on productivity; nutrients are reduced due to complexes forming between tannin and some macromolecules, reduced feed intake and digestibility and the digestive physiology of animals and mucosa may be impaired. However, studies showed that the moderate tannins content in some types of forage provided a beneficial effect (Min et al., 2003; Waghorn and McNabb, 2003). Consumption of less than 50 g CT / kg dry matter (10-40 g / kg DM) improved feed efficiency in ruminants, mainly due to a decrease in rumen protein degradation and therefore, more protein supplied to the host animals (mainly essential amino acids are absorbed in the small intestine (Schwab, 1995; Barry and McNabb, 1999; Min et al., 2003).

CHAPTER 2: MATERIALS AND METHODS

2.1. MATERIALS, TIME AND LOCATION OF THE STUDY

2.1.1. Materials

– Fistulated Sindhi cross-bred beefs and growing Sindhi cross-bred beefs

– Six species of tannin containing plants include: the leaf and shoot of *Leucaena leucocephala*, the leaf and shoot of *Acacia auriculiformis*, the leaf and shoot of *Acacia mangium*, the leaf of *Camellia sinensis*, the leaf and shoot of *Manihot esculenta Crantz*, the leaf of *Trichanthera gigantea*. Pure tannin.

2.1.2. Location: Department of Nutrition and Animal Feed, Center for Animal Experiments and Conservation (National Institute of Animal Science). Institute of Natural Products Chemistry (University of Technology)

2.1.3. Time: from 2013 to 2019

2.2. RESEARCH CONTENTS

– Determine the chemical composition and nutritional value of leaf and shoot of some tannin containing plants for ruminant

– Determine the effect of the source and the supplementation level of leaf and shoot of some tannin containing plants into substrate on the speed and characteristics of in vitro gas production, amount of methane production, in vitro digestibility, ME value and short-chain fatty acids

– Determine the effect of the supplementation level of leaf and shoot of some tannin containing plants on methane emissions, digestibility and nitrogen retention of growing Sind crossbred beef.

– Determine the effect of the supplementation level of leaf and shoot of some tannin containing plants on methane emissions, weight change and feed conversion efficiency of growing Sindhi crossbred beefs.

2.3. METHODS

2.3.1. Determine the chemical composition and nutritional value of leaf and shoot of some tannin containing plants for ruminants

Six (6) plants with high tannin content were collected at households in Ba Vi district - Hanoi. Each sample was taken at 5 positions and then chopped (2-3 cm), mixed well and each type took about 2 kg of fresh as a representative sample. The representative samples were weighed and divided into 2 parts of drying 450C (3 days), then a part was taken to weigh and grind passed through 1mm sieve. The remaining part continued to dry at 1050C (2 days) for determining dry matter of each plant feed. The grinding part will be divided into two parts, (i) chemical composition analysis and (ii) in vitro gas production experiments (Menke and Steingass, 1988).

Monitoring parameters and identification methods

– Fermentation dynamic: the amount of gas produced at 0; 3; 6; 12; 24; 48; 72 and 96 hours after the start of the incubation are recorded to determine the fermentation dynamic of each type of high tannin containing feed.

– Organic matter digestibility (OMD) and metabolisable energy value (ME): The amount of gas generated at 24 hours after incubation, combined with chemical composition were used to estimate organic matter digestibility and metabolisable energy value according to the equations of Menke and Steingass (1988):

OMD (%) = 14.88 + 0.889 * GP24 + 0.45 * CP + Ash

ME (MJ / kg DM) = 2.20 + 0.113 * GP_{24} + 0.057 * CP + 0.0029 * CP^2

– Methane concentration: The total amount of gas generated in each cylinder after 96 hours of sample incubation will be collected in a separate glass vial that has been vacuumed and analyzed by gas chromatography to determine the amount of methane using machine 17A Shimadzu (Japan). CH_4 content of fermentation is calculated according to Jayanegara et al. (2009).

– Analysis of the chemical composition of samples: Dry matter; crude protein; crude fat; crude fiber and total minerals according to TCVN 4326-2007, TCVN 4328-2007, TCVN 4321-2007, TCVN 4329-2007, TCVN 4327-2007, particularly NDF, ADF were determined according to Goering and Van Soest (1970). All parameters were analyzed at the Department of analysis of feed and livestock products, National Institute of Animal Science. Total tannin (% of DM) analyzed by AOAC (1975) at the Institute of Natural Products Chemistry (Vietnam Academy of Science and Technology)

2.3.2. Determine the effect of the source and the supplementation level of leaf and shoot of some tannin containing plants into substrate on the speed and characteristics of in vitro gas production, amount of methane production, in vitro digestibility, ME value and short-chain fatty acids

a / Basal diet - substrates

Table 2.1. The composition and proportion of the basal diet - substrate

Ingredients	Proportion (% DM)
1. Elephant grass	89
2. Cassava powder	1,8
3. Soybeans	3,9
4. Maize bran	2,5
5. Rice bran	2,8
Nutrition composition	
Dry matter	25,2
Crude protein	13
ME (MJ/kg)	10,3

b/ Diet balance

The experiment was organized according to a completely randomized design. Six species of plants with high tannin content and pure tannin are used in this content as a dietary supplement.

Each tannin containing plant or pure tannin was added to the basal diet at different levels: 0% (control), 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6% as % tannin total/dry matter. These mixtures were called the substrate. Therefore, there were 43 substrates (1 control sample and 7 plants x 6 mixing ratio). After that, the samples were divided into two parts: (i) chemical composition analysis; (ii) do in vitro gas production experiments. This is a two-factor experiment (tannin source and tannin supplementation rate) arranged in a completely randomized model as Table 2.2.

Table 2.2. Experimental design

Total tanin/DM of Diets	Khẩu phần thí nghiệm						
	Leucaena leucocephala	*Manihot esculenta Crantz*	*Trichanthera gigantea*	*Camellia sinensis*	*Acacia mangium*	*Acacia auriculiformis*	Pure tannin
	KD	***LS***	***CĐ***	***LC***	KTT	(KLT)	***TN***
Control: 0,0%							
0,1%	KD1	LS1	CD1	LC1	KTT 1	KLT 1	TN1
0,2%	KD2	LS2	CD2	LC 2	KTT 2	KLT 2	TN2
0,3%	KD3	LS3	CD3	LC 3	KTT 3	KLT 3	TN3
0,4%	KD4	LS4	CD4	LC 4	KTT 4	KLT 4	TN4
0,5%	KD5	LS5	CD5	LC 5	KTT 5	KLT 5	TN5
0,6%	KD6	LS6	CD6	LC 6	KTT 6	KLT 6	TN6

c / In vitro gas production experiments: according to Menke and Steingass (1988).

d / Chemical composition of feed: analytical criteria as in the content 1.

Monitoring parameters and identification methods

– Speed and characteristics of in vitro fermentation

– The total volume of gas production of all rations at 0; 3; 6; 12; 24; 48; 72 and 96 hours

– The gas dynamics were calculated using Chen's NEWAY software, (1995) to estimate rumen degradation and characteristics of gras production, following the nonlinear equation of McDonald (1981): $Y = a + b (1 - e^{-c\,(t-L)})$

In vitro organic and dry matter digestibility of diets were determined at 48 hours after fermentation and exhaust. After exhausting the gas, samples in cylinders are filtered with Whatman No.4 absorbent paper. The filtered samples are poured into porcelain beakers (weighted) and dried at 105 °C for 24 hours to determine *in* *vitro* dry matter digestibility. Thereafter, the sample was burn in a combustion chamber at 550^{0}C for 4 hours to determine the in vitro organic matter digestibility.

– Metabolisable energy value (ME): equation of Menke and Steingass (1988): $ME\ (MJ / kg\ DM) = 2.20 + 0.136 * GP_{24} + 0.057 * CP + 0.0029 * CP^2$

– Short chain fatty acid content (mmol/200gVCK): equation of Getachew et al. (1998): $SCFA\ (mmol/200gVCK) = 0.0239 * GP_{24} - 0.0601$

– Determine the amount of methane concentration by two methods: (i) The method for determining the percentage volume of CH_4 by NaOH solution (10M); (ii) Method of determining the percentage volume of CH_4 by Gas Chromatography (GC)

2.3.3. Determine the effect of the supplementation level of leaf and shoot of some tannin containing plants on methane emissions, digestibility and nitrogen retention of growing Sind crossbred beef

Four Sindhi crossbred bulls with an average weight of 160 kg / head was allocated in a latin square design. The bulls were dewormed before the experiment and kept individually in the crate with free access to feed and clean water. Each experimental period lasted for 15 days including 7 days of adaptation and 7 days of continuous sample collection (Table 2.3).

Table 2.3. Percentage of feed ingredients and nutritional value of in vivo experimental diets (% of DM)

Ingredients	DIET1	DIET2	DIET3	DIET4
Wet season rice straw (%)	42,4	40,2	41,1	35,9
Elephant grass silage (%)	13,6	10,7	10,3	10,3
Maize (%)	16,1	9,6	6,7	6,7
Rice bran (%)	20,8	14,3	10,5	10,5
Molasses (%)	5,2	5,3	5,3	5,3
Leucaena leucocephala dried leaf-stem (%)		19,1	25,9	31,5
Urea (%)	1,9	0,9	0,3	
Minerals (licking block)	*Ad lib*	*Ad lib*	*Ad lib*	*Ad lib*
Metabolisable energy ME (MJ/kg DM)	*9,5*	*9,9*	*10,1*	*10,3*
Crude protein (g/kg DM)	*145,3*	*154,1*	*148,9*	*154,4*
DM (%)	61,6	66,5	67,5	67,7

Note: DIET1: Control - 0% dried rooster leaves; DIET2: 20% of dried rooster leaves; DIET3: 25% of dried rooster leaves; DIET4: 30% of dried rooster leaves

Table 2.4. In vivo experimental diagram

Periods	Bull tag			
	A	B	C	D

1	DIET1	DIET2	DIET3	DIET4
2	DIET2	DIET1	DIET4	DIET3
3	DIET3	DIET4	DIET1	DIET2
4	DIET4	DIET3	DIET2	DIET1

Monitoring parameters and calculation methods

– Feed intake: weigh and record daily offered feed and leftovers for each individual bull to calculate feed intake.

– The amount of methane production: (L / head / day) is determined through the methane analysis system attached to the respiratory chamber.

– *In vivo* digestibility of diets: calculated from the amount of ingested nutrients in feed intake and excreted nutrients in the feces as a percentage of the intake by the method of Cochran and Galyean (1994), Burns and Pond (1994).

– Nitơ Amount of nitrogen accumulated: calculated by the formula

– Nitrogen retention = [(Intake nitrogen- (Fecal nitrogen + Urine nitrogen)] / (Intake nitrogen)*100

2.3.4. Determine the effect of the supplementation level of leaf and shoot of some tannin containing plants on methane emissions, weight change and feed conversion efficiency of growing Sindhi crossbred beefs

Twenty growing Sind crossbred bulls (15-18 months of age, weight from 157-159 kg) were assigned into the completely randomized design (Complete rendomized design - CRD). Experimental bulls were divided into 4 groups (5 bulls per a group). The experimental groups as follows: control group (DIET1) without *Leucaena leucocephala* supplementation; 3 *Leucaena leucocephala* supplemented groups: (DIET2), (DIET3) and (DIET4) with the *Leucaena leucocephala* supplementation of 20, 25 and 30% of DM in the diet; equivalent to the additional tannin ratio of 0.3; 0.4 and 0.5%. The value of ME and crude protein were similar among the diets. The proportion of feed ingredients is similar among the diets (Table 2.3). All bulls were dewormed and adapted for a period of 15 days with experimental diets before entering the experimental period. The bulls were kept individually in crate and had free access to feed and clean water.

Monitoring parameters and calculation methods

– Amount of food intake

– Weight

– Absolute weight gain

– Relative weight gain

– Feed conversion

– Methane emission

- The amount of methane production per kg weight gain
- The ability of minimizing the amount of methane production per kg weight gain
- Economic efficiency

Methods of data processing

Two mathematical models are used to analyze the experimental data.

* Model 1: Used for experiment with 1 factor: $X_{ij} = \mu + \alpha_i + e_{ij}$

Which Xij: observed value j of experimental factor i; μ : mean; α_i: effect of i factor and e_{ij}: random error.

If the variance gives a significant effect, use the t-student test to compare the errors between pairs of means.

* Model 2: Used for experiments with 2 factors: $X_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk} + (\alpha\beta)_{ij}$

Which: Xijk: observed value k of experimental factors i and j; μ: mean; α_i: effect of experimental factor i; β_j: effect of experimental factor j; eijk: random error; (αβ) ij: interaction of factors i and j.

All data were statistically processed on computers using Minitab 14.0 (2005) and SAS software, (1998).

CHAPTER 3: RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION AND NUTRITIONAL VALUES OF LEAF AND SHOOT OF SOME TANIN CONTAINING PLANTS

Table 3.1. Chemical composition of leaf and shoot of some tanin containing plants (% according to DM)

	DM (%)	Crude Pro.	Lipit	Crude fiber	NDF	ADF	Ash
Leucaena leucocephala	22,65	31,19	2,54	18,38	32,60	21,90	7,87
Cassava leaf	18,41	26,16	3,81	17,84	33,99	22,85	9,45
Trichanthera gigantea	12,73	14,33	1,47	14,37	34,69	25,46	8,59
Camellia sinensis	30,15	19,04	2,44	18,17	34,91	21,22	5,66
Acacia mangium	35,76	15,02	2,93	24,23	43,49	30,64	5,31
Acacia auriculiformis	32,52	16,12	2,34	32,13	52,77	37,8	5,9

Note: DM: dry matter, Ash: Total minerals

The results showed that the chemical composition of the leaf and shoot of *Leucaena leucocephala* including crude protein, crude lipid, crude fiber, NDF, ADF and

Ash were 31.19; 2.54; 18.38; 32.60; 21,90 and 7.87 % of dry matter, respectively. In general, some parameters of the chemical composition of the leaf and shoot of *Leucaena leucocephala* in this study are similar to previous studies, which were in the range of fluctuations as crude protein: 10,3 -27,8; Ash: 8.4 -17.96; NDF 48.1-59.49; ADF 21.3-50.8% (Njiadda and Nasiru. 2010; Babayemi et al, 2009; Chumpawadee and Pimpa, 2008, Khanum et al, 2007). Therefore, the leaf and shoot of *Leucaena leucocephala* with high protein content and low fiber, NDF and ADF content really a valuable source of protein supplementation for ruminants.

For cassava leaf tops, some parameters of the chemical composition in this study were the same as previous studies of Yves Froehlich and Thai Van Hung, (2001). However, the crude protein content of cassava leaf in this study was slightly higher (26.16%) than the result of Wanapat (2001) of 23.4%.

The chemical composition of *Camellia sinensis* leaf in this study was basically similar to the results of Pascal Leteme (2005). Despite this, the crude protein content of *Trichanthera gigantea* leaf in our study (14.33%) is lower than this content of *Trichanthera gigantea* leaf (16.6%) in the study of Pascal Leteme (2005).

The chemical composition of *Camellia sinensis* leaves in this study showed that the parameters were similar to and in the range of previous studies. For example, the crude protein content of *Camellia sinensis* leaf was 19.04%, which ranged from 18.2 to 30.7% (Chu and Juneja, 1997). With *Acacia mangium* and *Acacia auriculiformis*, the chemical composition parameters were also within the fluctuation range of published Acacia tree studies. According to Abdulrazak et al. (2000) reported that leaf of Acacia plants in Nigeria had a crude protein content ranging from 13.4 to 21.3% of DM; NDF and ADF ranging from 15.4 to 30.8% and 11.4 to 25.1%, respectively. Thus, with a relatively high protein content, fiber, NDF and ADF content being not too high, *Camellia sinensis* leaf, *Acacia mangium* and *Acacia auriculiformis* leaf, if carefully studied, can be a valuable source of protein supplementation for ruminants.

In general, compared with other research results, the chemical composition of the studied feeds here was fluctuated and varied considerably. This fluctuation is natural, completely reasonable and caused by many elements. The important elements include: variety, cutting age or harvest age, growth stage of the tree, grass, environment and care and management of plants, grasses, crops, fertilizers, irrigation water, preservasion and processing methods of feed (Zinash et al, 1995; Daniel, 1996; Mei-Ju Lee et al, 2000; Tesema et al, 2002; Adane, 2003; Bayble et al, 2007).

Table 3.2. In vitro gas production rate, energy value and organic matter digestibility of leaf and shoot of some tannin containing plants

	Gas production speed (ml)				OMD (%)	ME (MJ)
	*24^h^*	*48^h^*	*72^h^*	*96^h^*		
Leucaena leucocephala	28,2	34,1	37,3	39,5	60,9	9,2
Cassava leaf	26,4	32,3	34,1	34,0	57,5	8,2
Trichanthera gigantea	18,1	26,2	28,9	30,9	53,4	6,8
Camellia sinensis	16,2	22,4	24,2	25,2	43,1	6,1
Acacia mangium	8,7	14,9	16,4	17,3	39,3	5,7
Acacia auriculiformis	7,9	13,8	14,6	15,6	37,6	5,4

The data in Table 3.2 showed the in vitro gas production speed, all incubation samples had the fastest gas production speed in the first stage of 96 hours incubation. At the same time in the incubation process, the amount of gas production from different samples is not the same in which the *Leucaena leucocephala* sample is the highest and lowest in the *Acacia auriculiformis*.

Regarding the value of metabolisable energy (ME) and the organic matter digestibility (OMD) showed that the leaf of *Leucaena leucocephala* had ME value (9.2MJ / kg dry matter) and OMD (60.9%) was greatest, meanwhile ME and OMD values of *Acacia auriculiformis* (5.4MJ / kg dry matter and 37.6%, respectively) and *Acacia mangium* (5.7MJ / kg dry matter and 39.3%, respectively) were very low in vitro conditions.

Table 3.3. Tannin content and methane concentration produced after 96 hours incubation of leaf and shoot of some tannin containing plants in in vitro condition

	DM (%)	Tannin (g/kg DM)	CH_4 at 96^h	
			%	*ml*
Leucaena leucocephala	22,65	14,98	23,0	9,1
Cassava leaf	18,41	14,16	23,3	7,9
Trichanthera gigantea	12,73	8,98	25,7	8,0
Camellia sinensis	30,15	48,37	20,6	5,2
Acacia mangium	35,76	42,22	23,0	4,0
Acacia auriculiformis	32,52	27,19	22,9	3,6

The results in Table 3.3 showed that the tannin content of 6 types of leaves here ranged from: 8.98g to 48.37g / kg dry matter equivalent to 0.88 to 4.84% dry matter. The tannin content of *Camellia sinensis* leaf, *Acacia mangium* and *Acacia auriculiformis* here was quite high (2.72 to 4.84%) compared to this content in *Camellia sinensis* leaf and *Trichanthera gigantea* leaf (0.88 to 1.5%) and would probably affect diet digestibility and rumen fermentation, if these tannin sources was supplemented in diets, and may also reduce methane production.

Methane concentration after 96 hours incubation was lowest in the *Camellia sinensis* sample (20.6%) and highest in *Trichanthera gigantea* (25.7%). However, because of the total amount of gas production in each type of feed is very different and therefore the amount of methane emissions is not the same among samples; the highest volume in the sample of *Leucaena leucocephala* (9.1ml) and the lowest volume in the sample of *Acacia auriculiformis* (3.6ml)

3.2. EFFECTS OF SOURCES AND SUPPLEMENTATION LEVELS OF OF LEAF AND SHOOT OF SOME TANNIN CONTAINING PLANTS INTO SUBSTRATE ON THE SPEED AND CHARACTERISTICS OF IN VITRO GAS PRODUCTION, METHANE EMISSION, IN VITRO DIGESTIBILITY, ENERGY VALUE OF ME AND SHORT CHAIN FATTY ACIDS.

3.2.1. Chemical composition of experimental diets

Data of 43 diets showed that, compared with the control diet, adding the leaves of *Leucaena leucocephala* and *Cassava*, the crude protein content of the experimental diets increased, except for the addition of *Trichanthera gigantea, Camellia sinensis, Acacia mangium and Acacia auriculiformis* leaves, crude protein did not increase signifcantly in stead of the high protein content of the above types of leaves due to low supplementation rate. The addition of pure tannin, crude protein did not increase.

In the experimental diet with *Leucaena leucocephala* supplementation, crude protein content increased from 14.23 to 19.23% while in the experimental diet with cassava leaves, the crude protein content increased from 13.35 to 18.30%; with *Camellia sinensis* leaves, the crude protein content increased from 13.16 to 13.67%. Furthermore, the experimental diet had *Acacia mangium and Acacia auriculiformis* leaves, the crude protein content increased from 12.90 to 13.19% and from 13.02 to 13.27%, respectively.

The supplementation of *Leucaena leucocephala* and *Cassava, Trichanthera gigantea, Camellia sinensis, Acacia mangium and Acacia auriculiformis* and pure tanin, the tannin content of the experimental diets increased significantly. In the diet with leaf and shoot of *Leucaena leucocephala* the tannin content increased from 0.97 to 6.01 g tannin / kg DM. Similarly, in the experimental diets supplemented with cassava, *Trichanthera gigantea, Camellia sinensis, Acacia mangium and Acacia auriculiformis* leaves and pure tannin, the tannin content increased from: 0.93 to 6 , 07; 1.05 to 6.02; 1.01 to 6.06; 1.13 to 6.12; 1.05 to 6.07 and 1.03 to 6.14 g of tannin/kg DM, respectively.

Compared to the control diet, the content of lipid, crude fiber, NDF, ADF, Ash of the experimental diets did not change significantly. In all 7 experimental diets, the highest tannin content reached over 6.14 g tannin/kg DM, while the highest crude protein content achieved in these diets was about 18-19%.

3.2.2. Effect of supplemented leaf types and tannin supplementation levels on the accumulated gas production of experimental diets (ml).

The results showed that: The amount of generated gas increased rapidly from 3h to 12h after incubation and soared from 12h – 48h after incubation, then from 48h to 96h after incubation the amount of generated gas gradually decreased. This gas production results reflects a common pattern in in vitro fermentation with three stages: in the first stage, the gas is formed by the fermentation of the soluble fraction; in the second phase gas is generated by fermentation of insoluble part and in the third stage gas is generated by decomposition of microbial populations in the experimental environment (Cone et al, 1996; Cone et al., 1998).

Due to the different experimental diets in terms of chemical composition (mostly protein content) and the amount of added tannin and supplemented leaf types, the accumulated gas production of different diets was different at different incubation times.

The general trend was that the tannin content increased from 1 to 6 g/kg DM of the experimental diet, the amount of gas production at different times and accumulated gas production at 96 hours gradually decreased compared to the amount of gas production in the control diet (The amount of gas fluctuates but there is no rule), although there is a significant difference in the amount of gas production at the same time among diets at the same tannin level ($P < 0.05$). However, when the tannin content increased to 6g/kgVCK of the experimental diet, the amount of gas production at times and accumulated gas production at 96 hours was greatly affected and decreased sharply compared to the amount of gas production of the control diet and the diets with lower tannin content ($P < 0.05$).

The reason for the differences in the amount of gas production at the same time among the diets with the same tannin level from 1 to 6g/kg DM was quite complicated and cannot be explained by only a reason. According to Pellikaan et al. (2011), the amount of *in vitro* gas production and the amount of CH_4 emission depended on the properties of tannin such as tannin types (condensed or ellagitanins or gallotanins), the solubility of tannin. The effects of tannin were conditional and depended on their composition (Waghorn, 2008; Goel and Makkar, 2012). In addition, many factors may have regulated the amount of gas production during the fermentation, such as type and level of fiber, the presence of secondary metabolites such as saponins (Babayemi et al., 2009). , crude protein content of diet, other anti-nutritional ingredients (Njiadda and Nasiru, 2010). The nature of carbohydrates also had an effect on the amount of gas production (Sallam et al., 2007; Blummel et al, 1997; Menke and Steingass, 1988) and Chenost et al., 1997).

3.2.3. Effect of several type of leaves and level of tannin supplementation on charateristics of *in vitro* gasproduction of diets

The results showed that: Because the experimental diets differed in chemical composition (mainly protein content) and the amount of added tannin, supplemented leaf type so that the parameters of a, b, (| a | + b)), c and L are different in the diets.

The general trend is that the tannin content increased from 1 to 5 g/kg DM of the diets the parameters of a, b, (| a | + b), c and L were not significantly affected compared to these parameters in the control diet (parameters that fluctuate but have no rules), although there is a difference in values of a, b, (| a | + b), c and L between the diets at the same tannin level ($P<0.05$). However, when the tannin content increased to 6 g/kgDM of diets, the parameters a, b, (| a | + b), c and L were greatly affected and decreased sharply compared to these parameters in the control diet and diets with lower tannin supplemented ($P <0.05$).

The reasons for the differences in the parameters a, b, (| a | + b), c and L among the diets at the same tannin levels from 1 to 5 g/kg of DM are quite complicated and cannot be caused by only one cause. These causes are similar to the total amount of gas generated at different times and may include: tannin type, solubility of tannin (Pellikaan et al, 2011; Waghorn, 2008; Goel and Makkar, 2012), type and fiber level, the presence of secondary metabolites (Babayemi et al, 2009), crude protein content of the diet, other anti-nutritional ingredients (Njiadda and Nasiru, 2010), the nature of carbohydrates (Sallam et al, 2007; Blummel et al, 1997; Menke and Steingass, 1988 and Chenost et al, 1997). The characteristics of gas production depend on the relative proportions of the soluble and insoluble parts of the feed (Sallam et al., 2007).

3.2.4. Effect of several type of leaves and tannin supplementation on *in vitro* digestibility, ME and SCFA of diets

The results showed that: Because the experimental diets differed in chemical composition (mainly protein content) and the amount of added tannin, supplemented leaf type so that the digestibility of dry matter and organic matter *in vitro*, ME, SCFA are different in the diets.

The general trend is that the tannin content increased from 1 to 5 g/kgDM of the experimental diet, the percentage of dry matter and organic matter digestibility *in vitro*, ME, SCFA increased not much compared to the these value of control diet although there was a difference between the diets at the same tannin level ($P <0.05$). However, when the tannin content increased to 6 g/kgDM of the experimental diet, the *in vitro* digestibility of dry matter and organic matter, ME, SCFA of experimental diets was greatly affected and decreased sharply compared with these of the control diet and also the diets with lower level of tannin (P <0.05).

The reasons for the variation of *in vitro* dry matter and organic matter digestibility, ME and SCFA are due to the effect of type of leaf supplement and the amount of tannin that is the main cause to make a fluctuation in the amount of *in vitro* gas produced as discussed above.

3.2.5. Effect of several type of leaves and tannin supplementation on methane production after 96 hours of incubation of diets

The results showed that: Because the experimental diets differed in chemical composition (mainly protein content) and the amount of added tannin, supplemented leaf type so that the concentration and volume of methane were different in the diets.

The volume of methane produced in diets KD6, LS6, CD6, LC6, KTT6, KLC6 and TN6 (with the same tannin content of 6g/kg DM of experimental diet) significantly different (P<0,05) such as 7.1; 7,8; 5,9; 5.5; 5.3; 6.5 and 5.4 ml/200mg DM of the diet respectively. Compared to the control diets of KD6, LS6, CD6, LC6, KTT6, KLC6 and TN6 reduced the amount of methane produced in the rumen such as 36.0; 29.7; 46.8; 50.45; 52.25; 41.4 and 51.4% respectively.

Differences in the concentration and volume of methane in diets with different sources of tannin and even with the same tannin source but with different tannin content are caused by different causes. Firstly, the difference in the methane volume produced by the diets has the same tannin content but the source difference is due to the effect of the tannin types in the diet. According to Goel and Makkar (2012), the methane reduction that effect of the type of tannin (chemical structure) and is positively correlated with the number of hydroxyl groups in its structure. Overall hydrolyzed tannin tends to directly inhibit rumen-producing methane bacteria while condensed tannin affects rumen methane formation through inhibition of fiber digestion (Goel and Makkar, 2012).

As the tannin content of the experimental diets increased, the methane volume decreased that compared to the methane volume in the control diets that have been confirmed by many studies. Meta-analysis of *in vivo* studies with tannins by Jayanegara et al (2011) showed a strong relationship between tannin concentration and the amount of methane produced per unit of organic digestion. Woodward et al. (2001), supplemented 0.4% of tannin from Lotus seed husks to diets for sheep so that reduced methane emissions by 28.5%. Tannins as supplements or feeds have the potential to reduce methane from rumen fermentation by up to 20% (Beauchemin et al., 2006).

To analyze the effects of various factors on *in vitro* methane production, we have builded up regression equations describing the relationship between methane production and *in vitro* fermentation diets.

Equation 1 has the following type:

$CH_4(ml) = 20,2 – 0,631*Tanin + 0,474*CF – 0,666*NDF + 0,177*(|a|+b);$ R^2(adj) = 84.0%, (P<0,01).

When investigating the relationship between methane production, tannin content, NDF and gas generated after 96 hours of incubation (gas 96h), we have equation 2 that as a first-order linear regression equation of type:

$CH_4(ml) = 11,5 - 0,561*Tanin - 0,213*NDF + 0,216*G_{96};$ R^2(adj) = 94,3%; (P<0,01).

Thus, the amount of methane generated is influenced by many factors. While tannin and NDF have a negative effect on methane production *in vitro*, NDF and parametter of (| a | + b) and generated gas (96h gas) positive affect methane produce. Therefore, it is not possible to explain the amount of methane produced *in vitro* only by a single variable or a single factor. According to Jayanegara et al (2011): Total tannin is very closely related to CH_4/digestible organic matter (r = - 0.74; P <0.001), negatively correlated with CH_4 (-0.66) and negative correlation with CH_4/Total gas volume (- 0.77)

** Relationship between two methods of determining methane by GC (Gas chromatography) and the method of NaOH volume*

Using GC method to analyze methane concentration is a modern method with high accuracy, but the disadvantage is that it requires expensive equipment, while using the method for NaOH 10M to determine the methane concentration is simple, cheap and does not require expensive tools and machines, and the results are quite accurate (Fievez et al., 2005). In order to replace the GC method with the NaOH volumetric method, build a regression equation between the two methods.

In the case of linear regression, the equation has the type:

$CH_4\ (GC\text{-}\ ml) = -\ 0,1802 + 0,8792*CH_4\ (NaOH\text{-}ml)$; R^2(adj) = 80,9%; P<0,01.

In the quadratic case, the equation takes the type:

$CH_4\ (GC\text{-}\ ml) = 3.783+ 0,2587*CH_4\ (NaOH\text{-}ml)+ 0,02236*CH_4\ (NaOH\text{-}ml)^2$; R^2(adj) = 81.7%; P<0.01.

The results show that the relationship between the two methods is best represented by a linear equation (P = 0.001). This relationship can also be expressed at an acceptable level using a quadratic equation (P = 0.030).

3.2.6. Effects of supplemented type of leaves (tannin sources) on experimental

diets on *in vitro* methane production, rumen fermentation and digestion

The results showed that: *in vitro* methane reduction effect of tannin in giant tea, tea leaves and pure tannin was stronger than this effect in *Leucaena*, *cassava* leaves, *Acacia mangium* and *A. auriculiformis* (31,8; 31,38 and 28.65 vs. 17.39; 13.51; 25.38 and 24.17%) but there was no significantly difference (P>0.05). However, tannin in giant tea leave has the most negative effect and reduces *in vitro* DMD, OMD, gas generated after 96 h incubation, parameters a, b, (| a | + b), ME and SCFA (P <0.05). Pure tannin also has a less negative effect and only reduces the *in vitro* gas produced after 96 h of incubation, parameters a, b, (| a | + b, c, ME and SCFA (P<0.05).

Thus, tannin source has a very different influence on the amount of methane produced and fermented, rumen digestibility under *in vitro* conditions. Overall, when considering both the amount of *in vitro* methane produced and fermentation, rumen digestion that the tannin from the Acacia leaves and cassava leaves is better than the pure tannin, tannin from the giant tea leaves has the lowest overall efficiency.

3.2.7. Effect of tannin level in diets on *in vitro* methane production, fermentation, rumen digestion

The results showed that from the tannin dose of 4-5g/kg DM of diet, the amount of methane decreased significantly (20.5 and 22.3% compared to the control diet). However, dose of 6.0g/kgDM of diet has the strongest methane reduction effect. But at this tannin dose the DMD and OMD, amount of gas generated after 96 hours, parameters of b and (| a |+ b) also decreased sharply (P<0.05), indicating that fermentation and digestion of feed were affected strongly. As a result, the tannin level of 6.0g/kgVCK rations, the value of ME and SCFA decreased significantly (P<0.05).

Thus, the amount of tannin supplementation has a very different effect on the amount of methane generated and fermented, digestibility of rumen under *in vitro* conditions. Overall, when considering both the amount of *in vitro* methane produced and the fermentation and rumen digestion, the 5% tannin level is the best for the efficiency.

3.3. EFFECTS OF LEVEL OF *LEUCAENA LEUCOCEPHALA* LEAVE SUPPLEMENTED TO DIET ON DIGESTIBILITY, NITROGEN ACCUMULATION AND METHNE EMMISION OF GROWING RAD SINDHY CROSSED BREED

3.3.1. The *in vivo* digestibility nutrient of diet

Table 3.15. Effect of dietary supplement on tannin levels of Leucaena leucocephala leaves on in vivo digestibility (%)

Items	KP1 (ĐC)	KP2	KP3	KP4	SEM

Items	KP1 (ĐC)	KP2	KP3	KP4	SEM
Dry matter	61,23[a]	60,86[a]	57,18[b]	58,56[ab]	1,41
Protein	69,8[a]	71,99[a]	59,76[b]	60,48[b]	1,17
Fat	69,29[a]	73,27[a]	54,74[b]	49,23[b]	1,80
Crude fiber	57,37	58,30	56,12	58,55	1,49
NDF	43,78	42,76	45,41	42,09	2,19
ADF	53,54	53,55	49,98	49,13	5,58
Ash	40,11	39,68	43,36	43,46	2,36
Organic matter	63,44[a]	63,05[a]	58,79[b]	60,34[b]	1,33

The mean value in the same row with different letters is significantly different (P<0.05).

The data in Table 3.15 show that the digestibility of dry matter, crude protein, crude fat and organic matter have statistical differences (P <0.05) when comparing between the control diets KP1; KP2 with KP3 and KP4. Meanwhile, the digestibility of crude fiber, NDF, ADF and total minerals did not show any statistically significant difference (P>0.05) when comparing the control diet KP1; KP2 with KP3 and KP4.

Studied *in vivo* conditions of Grainger et al (2009a) with the addition of two tannin levels (8.6 and 14.6 g/kg of DM intake) extracted from *Acacia mearnsii* into the diet of grazing dairy cows intake 4.5 kg of concentrate feed showed that this supplementation reduced 11.5 and 28% of methane emissions, but also reduced the digestibility of the diet. Several other studies suggest that tannin extract and tannin-containing plants reduce digestibility in both *in vitro* (Bhatta et al, 2009; Patra et al, 2006) and *in vivo* (Animut et al, 2008).

However, according to research results of Jetana et al (2011) showed that supplementation crude protein of *Leucaena leucocephala* leaves increases fiber digestion in cows. The reason may be that the crude protein in *Leucaena leucocephala* leaves has affected the activity of microorganisms and the digestion of nitrogen in the rumen. Traore et al. (2010) suggested that the digestibility of feed and activity of rumen microorganisms was improved when supplementing crude protein in rumen, increasing the digestion and digestibility of feed.

3.3.2. Nitrogen balance in cows fed experimental diets

Bảng 3.16. Effect of dietary supplement on tannin levels from the leaves of the Leucaena leucocephala on nitrogen balance (g/day)

Items	KP1 (ĐC)	KP2	KP3	KP4	SEM
Nitrogen intake	83,86[b]	92,24[a]	88,48[ab]	85,14[ab]	1,01
– *Ratio (%)*	*100*	*100*	*100*	*100*	
Nitrogen evacuate	28,02[b]	29,38[b]	39,38[a]	36,34[a]	0,83
– *Ratio (%)*	*33.4*	*31.9*	*44.5*	*42.7*	

Items	KP1 (ĐC)	KP2	KP3	KP4	SEM
Nitrogen accumulation	55,86[ab]	62,88[a]	49,06[b]	48,8[b]	1,05
– *Ratio (%)*	*66.6*	*68.2*	*55.4*	*57.3*	

Note: The mean value in the same row with different letters is significantly different (P<0.05).

The amount of nitrogen intake ranged from 83.86 to 92.24g, the highest in the fed on KP2 and the lowest in animal group fed on KP1 and there was a significant difference between the experimental groups (P<0.05). In general, the amount of nitrogen ingested and evacuated in cattle fed the diets supplemented with *Leucaena leucocephala* leaves tended to be higher than those fed the control diets. However, the highest nitrogen accumulation was found in cattle fed KP2 of 62.88 g (accounting for 68.2% of the intake), followed by cattle fed on KP1 of this value of 55.86 g/day (accounting for 66.6% of the intake), while the accumulated nitrogen in cattle fed KP3 and KP4 reached only 49.06 and 48.8g, accounting for 55.4 and 57.3% of the nitrogen intake, respectively.

In this experiment, animal fed on KP3 and KP4 with higher tannin content could cause nutritional resistance that affects their uptake capacity, thereby affecting the nitrogen accumulation value. The efficiency of using nutritional components and the N balance tends to improve when animals are fed a diet supplemented with tannin dosage from *Leucaena leucocephala* leaves suitable for the rate of 0.3% corresponding to 20% DM of the diet.

3.3.3. Methane emminsion

The data showed that the amount of methane produced (l/head/day) ranges from 114.5 to 149.6 liters depending on the diet and there is a statistically significant difference (P<0.05) between cattle fed on diet KP3; KP4 compared to the cattle fed on diet KP1 and KP2. In general, volume of methane tend to decrease significantly when supplemented tannins from *Leucaena leucocephala* leaves, which is also seen when methane production per 100 kg of and over 1 kg of metabolisable body weight.

3.4. EFFECTS OF LEVEL OF *LEUCAENA LEUCOCEPHALA* LEAVE SUPPLEMENTED TO DIET ON LIVE WEIGHT GAIN, EFFICIENCY OF FEED, METHANE EMISSION AND ECONOMIC EFFICIENCY OF THE GROWING RED SINDHY CROSSBRED

3.4.1. Feed intake

Bảng 3.18. Feed intake of cattle fed on diets supplemented with tannin levels from Leucaena leucocephala leaves

Item	KP1	KP2	KP3	KP4	SEM
DM intake (kg/head/day)	4,12	4,21	4,13	4,11	0,04

Item	KP1	KP2	KP3	KP4	SEM
DM (kg/$BW^{0.75}$)	8,23	8,30	8,05	8,20	0,07
DM (kg/100kg BW)	2,24	2,24	2,17	2,23	0,02

Results showed that the amount of DM intake ranged from 4.11 to 4.21 kg, decreasing with increasing percentage of *Leucaena leucocephala* leaves in the diet, although there was no statistical difference (P>0.05). The amount of DM/100 kg of body weight ranges from 2.17-2.24 kg lower than the research results of Preston and Willis (1967) on heifers body weight 200 kg (2.8-3 kg). Makkar et al. (1995) reported that a reduction in feed intake could be due to three reasons: (i) an unappetizing food due to the combination of tannin and saliva protein; (ii) tannin astringent or irritation, resulting from reduced dry matter digestibility and (iii) tannin adhesion to the small intestine leading to hormone response. According to research results of Makkar (2003), cattle reduced feed intake when tannin content in diets ≥ 3%. In this study, tannin levels were 0.3-0.5% of diet ingredients and this ratio did not affect dry matter intake.

3.4.2. Live weight gain

Bảng 3.19. Effect of dietary supplement of tannin levels from Leucaena leucocephala leaves on live weight gain of cattle

Items	KP1	KP2	KP3	KP4	SEM
Initial weight (kg)	157,1	157,7	157,2	158,9	2,19
1st month live weight (kg)	177,3	178,0	172,6	173,8	2,58
ADG1 (g/head/day)	*673,26^{a}*	*677,74^{a}*	*513,64^{b}*	*497,48^{b}*	*16,59*
2ndmonth live weight (kg)	198,0^{ab}	200,4^{a}	192,4^{b}	193,8^{ab}	3,32
ADG2 (g/head/day)	*690,14^{ab}*	*747,38^{a}*	*660,62^{b}*	*667,94^{b}*	*27,42*
3rd month live weight (kg)	216,7^{ab}	219,2^{a}	208,9^{b}	210,5^{b}	3,53
ADG3 (g/head/day)	*623,14^{b}*	*627,88^{a}*	*550,02^{b}*	*557,42^{b}*	*9,37*
ADG whole period (g/head/day)	*662,52^{a}*	*683,34^{a}*	*574,44^{b}*	*573,94^{b}*	*12,11*

Note: The mean value in the same row with different letters is significantly different (P<0.05). Average daily gain: ADG.

The results in Table 3.19 show that: Average daily gain after a month of experiment reached from 497 to 677g/head/day, the highest was in the group of cattle fed the 19,1% *Leucaena leucocephala* leaves (KP2) and the lowest in the animal fed on diet supplemented with 31,5% of *Leucaena leucocephala* leaves (KP4) and there was a significant difference (P <0.05). By the 2nd and 3rd month, the highest ADG was still the cattle fed on diet (KP2) reaching 747 and 627g/head/day, respectively, and with significant difference (P<0.05) compared to this value in cattle fed other diets.

For the whole experimental period, the ADG was highest in the group of cattle fed on the diet KP2 (683g/head/day) while this value in cattle fed the diets KP1 (control); KP3 and KP4 are 662; 574 and 573g/head/day and there was a statistically significant difference (P <0.05).

3.4.3. Feed convertion efficiency

Feed consumption from 6.14 to 7.40 kg VCK/kg live weight gain, lowest group of cattle fed on diet KP2 and highest found in cattle fed on diet KP3 (P<0.05). Feed consumption of DM/kg live weight gain of this study within the standard range of ARC (1980); NRC (1984); INRA (1989); Rajan (1990); Perry (1990) and AFRC (1993) ranged from 7.1 to 8.8 kg DM/kg live weight gain. Therefore, the amount of DM intake of cattle was in the range of diet standards

3.4.4. Methane emmision

Bảng 3.21. Effect of dietary supplement of tannin levels from Leucaena leucocephala leaves on methane emmision of cattle

Items	KP1	KP2	KP3	KP4	SEM
V_{CH4} (L/head/day)	150,8^{a}	134,8^{a}	113,6^{b}	132,1^{ab}	2,89
L_{CH4}/100 kg BW	93,24^{a}	79,16^{ab}	77,20^{b}	70,84^{b}	4,94
V_{CH4} (L/kg $BW^{0.75}$)	3,42^{a}	2,98^{ab}	2,68^{b}	2,92^{ab}	0,08
L_{CH4}/kg LW gain	299,58^{a}	230,10^{b}	197,68^{b}	203,56^{b}	9,12
gCH_4/100 kg BW	66,86^{a}	56,76^{ab}	55,36^{b}	50,80^{b}	3,54
gCH_4/kg $BW^{0.75}$	2,38^{a}	2,05^{ab}	2,00^{b}	1,80^{b}	0,12
gCH_4/kg LW gain	214,80^{a}	165,0^{b}	141,72^{b}	145,94^{b}	6,53

Note: The mean value in the same row with different letters is significantly different (P<0.05).

The results of the table show that the amount of CH_4 produced (L/head/day) ranges from 113.6 to 150.8 liters depending on the diet and there is a statistically significant difference (P<0.05) between cattle fed on diet KP4 compared to cattle fed on diet KP1 and diet KP2.

Similar to *in vivo* experiments, the amount of CH_4 tended to decrease significantly when supplementation of tannin, which was also observed when methane production per 100 kg of body weight and per 1 kg of metabolism live weight. The amount of CH_4 produced (g) per kg of weight increased from 141.7 to 214.8 g, the highest in the cattle fed on KP1 (control group) while this value in cattle fed on diet KP2; KP3 and KP4 supplemented tannin from *Leucaena leucocephala* leaves of 141.7; 145.9 and 165g respectively, and there were significant differences (P<0.05).

3.4.5. Preliminary economic eficiency

This result shows that, depending on the diets, the average income was 540,300 - 871,200 VND/head/month, the highest was in the cattle fed on the diet KP1 followed by the cattle fed on diet KP2. However, the difference is not much and considering combining with other factors such as live weight gain and methane emmision into the environment, the cattle fed on diet KP2 was supplemented with 19,1% *Leucaena leucocephala* leaves/kg DM the most effective part. The income per head of cattle in this experiment is higher than the figures from some other studies that may be due to price fluctuations of inputs and outputs at the beginning and end of the experiment.

CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

1. Conclusion

– The source of tannin has a very different effect on the amount of methane produced in in vitro fermentation. Overall, when considering both the amount of methane produced and fermented under *in vitro* conditions, the tannin *Leucaena leucocephala* leaves and *Manihot esculenta* Crantz leaves is better than the pure tannin, the tannin from the tea leaves is the least effective.

– The levels of tannin supplementation have a very different effect on the amount of methane produced in an *in vitro* fermentation. Overall, when considering both the amount of methane produced and fermented under *in vitro* conditions, the 5% tannin level is the best for the effect.

– The *Leucaena leucocephala* leaves have the highest digestibility of organic matter (60.9%) and metabolism energy value (9.2 MJ) compared to other leaves in the study and get a low methane emissions

– The appropriate supplementation of dried *Leucaena leucocephala leaves* to growing Red Sind crossbred with level of 19.1% DM of diet rations (equivalent to 0.3% tannin) that significantly reducing the amount of CH_4 produced (g)/kg increased weight ($P<0.05$) compared to the control group (165.0 vs. 214.8 L) and achieved the highest weight gain of 683g/head/day, the best feed efficiency (6.14 kgDM/kg live weight gain.

2. Recommendations

– Apply a supplement of 0.3% tannin (equivalent of 19.1% DM of diet) from the dried *Leucaena leucocephala* leaves for rearing Red Sindhy crossbred cattle.

– Continuing to study the effect of supplementation of tannin leaf feed in fattening diets on productivity, feed efficiency and methane emission in crossbred cattle