JACKFRUIT LEAVES SILAGE GREATLY REDUCES METHANE PRODUCTION WITHOUT AFFECTING RUMINAL FERMENTATION AND DIGESTIBILITY

Tran Thi Thuy Hang[2], Vo Thi Phuong Tien[1], Tang Que Phuong[1], Lam Phuoc Thanh[1,*]

[1] *College of Agriculture, Can Tho University*

[2] *College of Rural Development, Can Tho University*

[*] *Email: phuocthanh@ctu.edu.vn*

ABSTRACT

An *in vitro* research was conducted to evaluate the effect of replacing Napier grass (NG) silage with jackfruit leaves (JL) silage in a concentrate-containing diet on nutrient digestibility, ruminal fermentation and methane (CH_4) production. The experiment was assigned as a completely randomized design with 5 treatments using separate ruminal fluid from 4 dairy goats as statistical replicates. In all treatments, the concentrate (35% DM) was persistent. The experimental treatments were developed by the replacement of JL silage for NG silage at levels of 0%, 16.2%, 32.5%, 48.8% and 65% DM, corresponding to treatments JLS0, JLS16.2, JLS32.5, JLS48.8, and JLS65. The results showed that NH_3-N and VFA levels were not altered ($P > 0.05$) across treatments after 72 h of incubation. The digestibility of DM and OM remained unchanged when using up to 48.8% of JL silage; however, it decreased ($P < 0.05$) at 65% of JL usage, compared to the replacement level at 16.2%. The use of JL silage did not affect total gas production, but CH_4 production in the JLS48.8 and JLS65 tended to reduce by 26.5 and 27.4%, respectively, compared to JLS0 ($P < 0.1$). In conclusion, replacing the proportion of jackfruit leaves silage for Napier grass silage up to 48.8% in the diet containing 35% concentrate greatly reduces CH_4 emissions without negative impact on nutrient digestibility and ruminal fermentation.

Keywords: *digestibility, goat, jackfruit leaves silage, ruminal fermentation, methane.*

Received: 8 October 2024; revised: 6 November 2024; accepted: 29 November 2024.

1. INTRODUCTION

In recent years, ruminant production in Vietnam has grown due to increased demand for meat and milk, especially in goat farming. In 2021, the national ruminant population reached 11.5 million, with goats accounting for 2.68 million (23.2%), including approximately 4.34 thousand in the Mekong Delta [1]. Goats are an important livestock species in developing countries, particularly in Asia and Africa. They have a diverse diet, including grasses, tree leaves (e.g., jackfruit leaves), and agricultural by-products.

In the aspect of the environment, global livestock produces 14.5% of total greenhouse gases (GHG) emissions; of which, 47% of CH_4 emissions originate from ruminants [2]. Climate change caused by greenhouse gases (GHG) is currently having a stronger impact on human life as well as the living environment and health of farmed animals. Tannin-contained feed, which is abundant in tropical countries including Vietnam, has been shown to have a high potential to mitigate CH_4 emission from ruminants [3].

Jackfruit is widely grown in Vietnam, especially in the Mekong Delta, with 72,200 hectares cultivated in 2021. Jackfruit leaves, often abundant after annual pruning, are an untapped feed resource. Previous studies have shown fresh jackfruit leaves are the favorite feed for ruminants [4, 5, 6]. A study by Thanh *et al.* (2021) [6] showed that jackfruit leaves contained up to 11.6% total tannins, highlighting their potential as a feed

source to reduce CH_4 emissions from ruminants. However, the research on using jackfruit leaves silage (JLS) as a feed ingredient for goats is still scarce. Thus, this study aimed to evaluate the impact of replacing Napier grass silage with jackfruit leaves silage on *in vitro* digestibility, ruminal fermentation, and methane emission using inoculum from dairy goats.

2. MATERIALS AND METHODS

2.1. Time and location of the experiment

Time: from December 2023 to April 2024.

Location: The study was performed at the Laboratory of Ruminant Production Techniques, Faculty of Animal Sciences, College of Agriculture, Can Tho University, Vietnam. All procedures were performed according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law on Livestock Production (No. 32/2018/QH14).

2.2. Experimental substrates

After harvesting, jackfruit leaves (JL) and Napier grass (NG) were transported to the laboratory and chopped into small pieces measuring 0.5 - 1 cm for ensiling. Each kg of both JL and NG was ensiled with 30 g of sugarcane molasses. After 3 weeks, the quality of the ensiled samples was assessed. The bag that exhibited the best quality, characterized by a pleasant odor, absence of mold growth, and a pH range between 3.8 - 4.2, was selected for the subsequent *in vitro* experiments.

The concentrate was formulated from soybean meal (20.3%), coconut meal (5.74%), corn DDGS (28.5), fine rice bran (33.5%), de-oiled rice bran (7.49), NaCl (0.71%), limestone (2.86%) and premix (0.86%). In 1 kg of premix (commercial name Calphovit) contains 2,000,000 IU of vitamin A, 400,000 IU of vitamin D3, 2,000 mg vitamin E, 6,300 - 7,700 mg zinc, 5,400 - 6,600 mg iron, 4,500-5,500 mg magnesium, 5,400 - 6,600 mg manganese, 360 - 440 mg copper, 109 C FU *Bacillus subtilis*, 106 CFU *Pediococcus*, 99,750 IU phytase and calcium carbonate to make up 1 kg.

2.3. Experimental design and diets

The research was divided into 2 experiments: the first used 100 mL and 50 mL incubation bottles to evaluate *in vitro* digestibility and ruminal fermentation, respectively, while the second employed 500 mL incubation bottles to determine methane (CH_4) emission. Both experiments were assigned as completely randomized designs with 5 treatments and 4 replicates. The concentrate proportion was fixed at 35% (DM basis) in all treatments. The experimental treatments were developed by replacing Napier grass silage with jackfruit leaves silage at 0, 16.2, 32.5, 48.8 and 65% (DM basis) in the diet, corresponding to treatments JLS0, JLS16.2, JLS32.5, JLS48.8 and JLS65, respectively. The control diet was formulated based on NRC (1981) [7] standards for goats, with a CP content of 14% and ME of 9.8 MJ/kg DM. The chemical composition of treatments is presented in table 1.

Table 1. Feed ingredients and chemical composition

Item[1]	Treatment[2]				
	JLS0	JLS16.2	JLS32.5	JLS48.8	JLS65
Feed ingredients, % DM					
Concentrate	35.0	35.0	35.0	35	35.0
Napier grass silage	65.0	48.8	32.5	16.2	0
Jackfruit leaves silage	0	16.2	32.5	48.8	65.0
Total	100	100	100	100	100
Chemical composition, % DM					
DM	46.1	48.4	50.8	53.1	55.4
Ash	9.60	11.1	12.6	14.1	15.6

Item[1]	Treatment[2]				
	JLS0	JLS16.2	JLS32.5	JLS48.8	JLS65
OM	90.4	88.9	87.4	85.9	84.4
CP	14.0	15.0	15.9	16.8	17.8
EE	4.69	5.02	5.34	5.66	5.99
CF	22.9	20.9	18.9	16.9	14.9
NDF	48.0	44.5	41.0	37.4	33.9
NFE	48.8	48.0	47.3	46.5	45.7
ME, MJ/kg DM	9.79	9.83	9.87	9.91	9.95

[1]DM: Dry matter, OM: organic matter, Ash: Total minerals, NDF: Neutral detergent fiber, CF: Crude fiber, EE: Ether extract, ME: Metabolizable energy.

[2]JLS0, JLS16.2, JLS32.5, JLS48.8, JLS65: replacement of jackfruit leaves silage for Napier grass silage at 0, 16.2, 32.5, 48.8 and 65%, respectively.

2.4. Inoculum and medium solution

Ruminal fluid was collected from 4 crossbred Saanen goats. Goats were fed the basal diet consisting of EG and 18% CP concentrate (roughage:concentrate was 70: 30, wt/wt on DM basis) twice a day at 7: 00 and 17: 00 for 1 week before sampling. To ensure uniformity in the inoculum, as suggested by previous research [8], the donor animals were offered a similar diet to minimize variability and ensure that observed differences in the study were due to the experimental treatments during the incubation. Feeding the experimental diet before the inoculum collection was unnecessary and cost - and time - consuming. Approximately 300 ml of ruminal fluid was collected from each goat 3 h after the morning feeding using a stomach tube passed through the mouth. Ruminal fluid was filtered through a metal sieve with a pore size of 1 mm under a stream of CO_2 at 39°C. Donor fluid from each goat was kept separately and served as a statistical replicate during the *in vitro* experiments. Ruminal inocula were diluted (1: 4, vol/vol) in the medium solution prepared according to the method of Menke and Steingass (1988) [9].

2.5. *In vitro* incubation

In Experiment 1, 0.6250 and 0.3125 g DM of substrates were weighed into each 100 ml and 50 ml bottle, respectively. Thereafter, 50 ml mixture of ruminal fluid and medium solution (1: 4 vol/vol) was added into a 100 ml bottle while 25 ml of the same mixture was added into a 50 ml bottle. Bottles were incubated in a shaking incubator (ISS - 4075R, Jeiotech, Korea) at 120 rpm, 39°C for 72 h. The fermentation reaction was stopped by placing the bottles in the ice, and the pH value of the fermentation solution was measured immediately (HI - 5522, Hanna Instruments Inc., USA). The fermented liquor was then filtered through four layers of cheesecloth, acidified with H_2SO_4 1M (10: 1, vol/vol), and centrifuged at 10,000 g for 15 min. The supernatant was obtained and stored at -20°C for further analysis of NH_3-N and VFA. The *in vitro* digestibility of DM, OM, CP and NDF was determined according to Van Soest and Robertson (1985) [10].

In Experiment 2, 6.25 g DM of substrates was weighed into 500 ml glass bottles, then 400 ml of a mixture consisting of ruminal fluid and medium solution at a 4: 1 ratio was added. Bottles were connected to multi-layer foil gas sampling bags (22952, Restek, USA) and incubated in a shaking incubator (ISF - 7200R, Jeiotech, Korea) at 120 rpm, 39°C for 72 h. Total gas production was measured at 24, 48 and 72 h of incubation. At each time point of collection, the gas production was recorded, transferred into another respective gas sampling bag, and stored until the determination of gas composition.

2.6. Chemical analysis and calculation

Feed samples were analyzed DM, OM, CP, CF and EE following the methods outlined by AOAC (1990) [11]; NDF was analyzed according to the method of Van Soest *et al.* (1991) [12]. Metabolizable energy (ME) was calculated according to Abate and Mayer (1997) [13]. The chemical composition was calculated and presented on a DM basis.

NH_3-N content in ruminal fluid was analyzed using a micro - Kjeldahl method [10], individual VFA concentrations were determined by gas chromatography (Trace 13010, Thermo Scientific, USA) and concentrations of produced gases were measured using a gas analyzer (GeoTech GA5000, Queensway, UK).

The digestibility of DM, OM, CP, and NDF was calculated using the formula 100 x (nutrient content of the substrates before incubation – nutrient content of the substrate after incubation)/nutrient content of the substrates before incubation.

2.7. Statistical analysis

Data were statistically analyzed with the GLM procedure for a completely randomized design using SAS OnDemand for Academics. The statistical model was $Y_{ij} = \mu + D_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, D_i = the diet effect and ε_{ij} = the random residual error. Significant differences among diet means were statistically compared using Tukey's test. The significant effect of diet was declared at $P < 0.05$, and the tendency was noted at $0.05 \leq P < 0.1$.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feed ingredients

JL silage had DM and CP contents that were 1.64 and 1.77 times higher than those of NG silage (Table 2). The DM content of the JL silage used in the experiment was consistent with the results from Keir *et al.* (1997) [14], which was 36%. The CP content in NG silage in the current study was in accordant to the CP content of NG reported by Kha *et al.* (2020) [15], which was 7.27%. In the previous study, Thanh *et al.* (2022) [16] found the DM content of JL was 38.5%, which was slightly higher than the DM content of JL silage in this study. NDF and CF levels of NG silage were 1.52 and 1.64 times higher than those of JL silage. However, JL silage had higher EE content compared to NG silage (4.21% vs. 2.21%). The ME content of NG silage and JL was similar (8.33 and 8.58 MJ/kg DM, respectively).

Table 2. Chemical composition of feed ingredients

Item	Chemical composition (% DM)								
	DM	Ash	OM	CP	EE	CF	NDF	NFE	ME*
Concentrate	89.8	11.8	88.2	26.0	9.30	7.15	19.2	45.8	12.5
NG silage	22.5	8.42	91.6	7.55	2.21	31.4	63.5	50.5	8.33
JL silage	36.9	17.7	82.3	13.4	4.21	19.1	41.9	45.6	8.58

DM: Dry matter, OM: Organic matter, Ash: Total minerals, NDF: Neutral detergent fiber, CF: Crude fiber, EE: Ether extract, ME: Metabolizable energy (MJ/kg DM), NG: Napier grass, JL: Jackfruit leaves

3.2. Ruminal fermentation patterns

After 72 h of incubation, ruminal pH was increased ($P < 0.01$, Table 3) when NG silage was replaced by JL silage in the diet. pH value was lowest in JLS0, while it was highest in JLS65. NH_3-N concentration was not different ($P > 0.05$) among treatments, which ranged from 4.66 - 6.09 mg/dL. Similarly, there was no discrepancy ($P > 0.05$) found in the total VFA content, which was 85.9 - 117 mM. Noteworthy, despite the lack of difference in NH_3-N and VFA, the proportion of acetic acid in JLS65 was 5.1% higher ($P<0.05$) when compared to JLS0. Besides, propionic acid concentration tended ($P < 0.1$) to be lower in JL silage-containing treatments, compared to the diet containing only NG silage. As a result, the C2/C3 ratio tended to increase ($P < 0.1$) when the substitution of JL silage increased up to 65% in the diet.

Table 3. Ruminal fermentation patterns after 72 h incubation

Item	Treatment[1]					SEM	P
	JLS0	JLS16.2	JLS32.5	JLS48.8	JLS65		
pH	6.92^{b}	7.29^{ab}	7.12^{ab}	7.11^{ab}	7.47^{a}	0.09	0.009
NH_3-N, mg/dL	5.67	6.09	5.32	4.83	4.66	0.77	0.677
Total VFA, mM	104	85.9	117	92.7	96.4	12.4	0.490
Acetic acid, %	72.6^{b}	74.7^{ab}	74.0^{ab}	77.5^{ab}	77.7^{a}	1.30	0.027
Propionic acid, %	13.3	12.0	12.8	11.5	10.7	1.96	0.089
Iso-butyric acid, %	1.94	1.81	1.96	1.92	1.59	0.18	0.469
Butyric acid, %	6.11	5.41	5.09	4.14	4.65	0.67	0.210
Iso-valeric acid, %	2.72	2.64	2.58	2.09	2.25	0.31	0.417
Valeric acid, %	3.35	4.43	3.57	2.84	3.14	0.37	0.580
C2/C3	5.55	6.26	5.77	6.85	7.46	0.52	0.059

[1]JLS0, JLS16.2, JLS32.5, JLS48.8, JLS65: Replacement of jackfruit leaves silage for Napier grass silage at 0, 16.2, 32.5, 48.8 and 65%, respectively; [a, b]Means within a row with different superscripts are significantly different (P < 0.05).

Ruminal pH, NH_3-N and total VFA were not different among JLS0, JLS16.2, JLS32.5, and JLS48.8 suggesting that the JL silage can replace NG silage without impact on ruminal fermentation. The rise in acetate levels in treatments with JL silage could be due to the increased acetogenesis, where acetate is produced through CO_2 reduction [17]. The decrease in propionate levels observed in this study was consistent with the findings of Guerreiro *et al.* (2021) [18], who reported a similar pattern when increasing the levels of *Cistus ladanifer L.* condensed tannin extract from 0 - 10% DM in the diet. In contrast, the inclusion of 100 - 200 g/kg DM of acacia and quebracho tannins resulted in a propionate increase from 9.27 - 13.2%, without affecting acetate levels after 24 h of incubation. Likewise, the addition of hydrolysable tannins from chestnut (100 - 200 g/kg DM) and valonea (20 - 200 g/kg DM) increased acetate levels, while propionate remained unchanged [19]. The similarity in proportions of iso - butyrate, butyrate, iso-valerate, and valerate observed among treatments in this study could indicate that protein degradation was not influenced by the replacement of JL silage for NG silage in the diet.

3.3. Nutrient digestibility

DM digestibility was improved (P < 0.05, Table 4) by 11.2% when 16.2% JL silage was used in the diet, but then sharply decreased when the substitution increased up to 65%. A similar outcome (P < 0.05) was also observed in OM digestibility, which was highest in JLS16.2 and lowest in JLS65. On the other hand, CP and NDF digestibility were not different (P > 0.05) among treatments, which ranged from 64.5 - 74.9% and 56.6 - 65.4, respectively.

The reductions in DM and OM digestibility observed in this study were in line with previous studies using tanniferous plants. *Peltrophorum Africanum* and *Rhus lancea*, for instance, were reported to deeply decrease OM digestibility after 72 h of incubation using ruminal fluid from cannulated South African mutton Merino sheep [20]. The inclusion of *A. mearnsii* at 20 and 40% (DM basis) did reduce *in vitro* DM and OM digestibility at 20.3 - 43.6% and 20.9 - 40.1%, respectively, compared to the control diet [21]. The effect of tanniferous plants on ruminal fermentation and digestibility is mainly due to the

presence of phenolic compounds, which form tannin-carbohydrate and tannin - protein complexes, leading to the reduction in degradable and fermentable ability or due to the inhibition of rumen microbes [22].

Table 4. Nutrient digestibility after 72 h incubation

Digestibility, %	Treatment[1]					SEM	P
	JLS0	JLS16.2	JLS32.5	JLS48.8	JLS65		
DM	45.4^{ab}	56.6^{a}	39.4^{ab}	37.5^{ab}	20.2^{b}	6.98	0.030
OM	47.4^{ab}	58.6^{a}	40.7^{ab}	40.5^{ab}	23.5^{b}	6.84	0.034
CP	66.6	74.9	70.8	66.2	64.5	4.60	0.521
NDF	58.5	57.9	65.4	62.4	56.6	3.68	0.445

[1]JLS0, JLS16.2, JLS32.5, JLS48.8, JLS65: replacement of jackfruit leaves silage for Napier grass silage at 0, 16.2, 32.5, 48.8 and 65%, respectively; [a,b]Means within a row with different superscripts are significantly different (P < 0.05).

3.4. Gas production

After 72 h of incubation, no difference (P > 0.05, Table 5) in total gas production was detected among the treatments, ranging from 805 - 1027 ml. Neither CH_4 nor CO_2 concentration was influenced (P > 0.05) JL silage replaced NG silage in the diet. Noteworthy, their production tended to decrease (P < 0.1) when the level of replacement was at 48.8 and 65% in the diet. CH_4 production in JLS48.8 and JLS65 was 26.5 and 27.4% lower, while CO_2 production decreased by 25.3 and 27.2% lower, respectively, when compared to JLS0.

Table 5. Gas production after 72 h incubation

Item	Treatment[1]					SEM	P
	JLS0	JLS16.2	JLS32.5	JLS48.8	JLS65		
Total gas, ml	1,027	987	995	840	805	69.2	0.154
CH_4, %	13.2	13.5	13.9	11.7	12.4	0.55	0.105
CO_2, %	57.8	57.2	57.3	52.8	54.2	1.75	0.248
O_2, %	3.73	4.17	3.70	4.67	5.10	1.09	0.864
H_2S, ppm	7,476	7,338	7,523	7,101	6,165	543	0.426
CO, ppm	100	84.7	78.3	69.3	63.3	9.08	0.109
CH_4, ml	136	133	138	100	98.7	11.0	0.055
CO_2, ml	596	564	572	445	434	45.2	0.080
O_2, ml	38.0	41.0	35.2	38.6	42.5	10.1	0.987
H_2S, ml	7.69	7.25	7.54	6.04	4.87	0.78	0.121
CO, ml	0.10	0.08	0.07	0.06	0.05	0.01	0.050

[1]JLS0, JLS16.2, JLS32.5, JLS48.8, JLS65: Replacement of jackfruit leaves silage for Napier grass silage at 0, 16.2, 32.5, 48.8 and 65%, respectively; [a,b]Means within a row with different superscripts are significantly different (P<0.05).

Similar to this study, the utilization of tannin-rich plants such as *Gliricidia sepium, Leucaena leucocephala,* and *Manihot esculenta* at 0, 25, 50, 75 and 100% in sheep diets gradually reduced total gas and CH_4 emission [23]. Using sulla and big trefoil extract at 30 g/kg DM reduced up to 15%

CH_4 production but it also decreased feed digestibility. In contrast, tannin extracts from birdsfoot trefoil and salad burnet decreased 12% of CH_4 production without influence on gas production and digestibility [24]. The anti-methanogenic mode of action of tannins is well-documented, acting either directly on ruminal archaea or indirectly by reducing hydrogen production [25]. In the current study, despite an increase in acetic acid and a reduction in propionic acid proportion, the methane mitigation effect of JL silage may be due to its direct influence on methanogens, rather than an alteration of the hydrogen sink. Differences in substrate composition, tannin doses, the mechanisms of various tannin sources, and animal species used across studies likely contribute to the variability observed in ruminal fermentation, total gas production, and methane emissions.

4. CONCLUSION

Replacing Napier grass silage with jackfruit leaves silage at 48.8% in goat diet containing 35% concentrate could be an ideal approach to reduce CH_4 emissions without negative impact on nutrient digestibility and ruminal fermentation.

REFERENCES

1. General Statistics Office (2021). Vietnam livestock statistics January 1, 2021 on the number of heads and livestock and poultry products.

2. Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., and Tempio, G. (2013). Tackling climate change through livestock: A Global assessment of emissions and mitigation opportunities. *Food and Agriculture Organization of the United Nations* (FAO): Québec City, QC, Canada.

3. Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J. and Wang, M. (2020). Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal*, 14(S1), s2 - s16.

4. Islam, M., Chowdhury, S. and Alam, C. (1997). The effect of supplementation of jackfruit leaves (*Artocarpus heterophyllus*) and mashkalai (*Vigna mungo*) bran to common grass on the performance of goats. *Asian-Australasian Journal of Animal Sciences,* 10(2), 206 - 209.

5. Van, D. T. T., Mui, N. T. and Ledin, I. (2005). Tropical foliages: effect of presentation method and species on intake by goats. *Animal Feed Science and Technology,* 118(1-2), 1 - 17.

6. Thanh, L. P., Kha, P. T. T., Tinh, P. V. T. and Hang, T. T. T. (2021). Effect of jackfruit leaves on feed utilization and ruminal fermentation of growing goats. *Livestock Research for Rural Development*, 33, 104.

7. NRC. (1981). Nutrient Requirements of Domestic animals. National Academy Press. Washington, D. C., USA.

8. Mould, F. L., Kliem, K. E., Morgan, R. and Mauricio, R. M. (2005). In vitro microbial inoculum: A review of its function and properties. *Animal Feed Science and Technology*, 123, pp. 31 - 50.

9. Menke, K. H. and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research Development*, 28, 7 - 55.

10. Van Soest, P. J. and Robertson, J. (1985). Analysis of forages and fibrous foods. Cornell University.

11. AOAC. (1990). Official methods of analysis. 15th edition. Association of official analytical chemists 15th edition. Arlington, USA.

12. Van Soest, P. V., Robertson, J. B. and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583 - 3597.

13. Abate, A. and Mayer, M. (1997). Prediction of the useful energy in tropical feeds from proximate composition and in vivo derived energetic contents 1. Metabolisable energy. *Small Ruminant Research*, 25(1), 51 - 59.

14. Keir, B., Van Bien, D., Preston, T. R. and Ørskov, E. R. (1997). Nutritive value leaves from tropical trees and shrubs: 2. Intake, growth and

digestibility studies with goats. *Livestock Research for Rural Development*, 9(4), 31 - 35.

15. Kha, P. T. T., Tham, H. T, Hang, T. T. T. and Thanh, L. P. (2020). Effects of oils and condensed tannins on ruminal fermentation and methane emission in dairy cows, *The 3rd International Conference on Sustainable Agriculture and Environment*. Ho Chi Minh National University Publishing House. Pp. 182 - 191.

16. Thanh, L. P., Kha, P. T. T. and Hang, T. T. T. (2022). Jackfruit leaves can totally replace traditional grass in the diet of lactating dairy goats. *Journal of Applied Animal Research,* 50(1), 97 - 102.

17. Tan, H., Sieo, C., Abdullah, N., Liang, J., Huang, X. and Ho, Y. (2011). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. *Animal feed science and technology,* 169(3 - 4), 185 - 193.

18. Guerreiro, O., Alves, S. P., Costa, M., Duarte, M. F., Jerónimo, E. and Bessa, R. J. (2021). Effects of increasing doses of condensed tannins extract from *Cistus Ladanifer L.* on *in vitro* ruminal fermentation and biohydrogenation. *Animals,* 11(3), 761.

19. Hassanat, F. and Benchaar, C. (2013). Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. *Journal of the Science of Food and Agriculture*, 93(2), 332 - 339.

20. Gemeda, B. S. and Hassen, A. (2015). Effect of tannin and species variation on in vitro digestibility, gas and methane production of tropical browse plants. *Asian - Australasian Journal of Animal Sciences,* 28(2), 188.

21. Vargas-Ortiz, L., Chavez-Garcia, D., Barros-Rodríguez, M., Andrade-Yucailla, V., Lima-Orozco, R., Macías - Rodríguez, E., Guishca-Cunuhay, C. and Zeidan Mohamed Salem, A. (2022). Rumen function and *in vitro* gas production of diets influenced by two levels of tannin-rich forage. *Fermentation,* 8(11), 607.

22. Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., Enishi, O. and Kurihara, M. (2009). Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science*, 92(11), 5512 - 5522.

23. Rira, M., Morgavi, D., Archimède, H., Marie-Magdeleine, C., Popova, M., Bousseboua, H. and Doreau, M. (2015). Potential of tannin-rich plants for modulating ruminal microbes and ruminal fermentation in sheep. *Journal of Animal Science*, 93(1), 334 - 347.

24. Verma, S., Akpensuen, T. T., Wolffram, S., Salminen, J. P., Taube, F., Blank, R., Klub, C. and Malisch, C. S. (2024). Investigating the efficacy of purified tannin extracts from underutilized temperate forages in reducing enteric methane emissions *in vitro*. *Scientific Reports,* 14(1): 12578.

25. Tavendale, M. H., Meagher, L. P., Pacheco, D., Walker, N., Attwood, G. T. and Sivakumaran, S. (2005). Methane production from in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology*, 123, 403 - 419.