American Journal of Animal and Veterinary Sciences

Original Research Paper

In-vitro Gas Production and Digestibility of Indian Marsh Fleabane (*Pluchea indica L.*) and Portia Tree Leaves (*Thespesia populnea*)

[1]Thaintip Kraiprom, [1]Sitthisak Jantarat, [2]Santi Madman, [3]Suphawadee Yaemkong and [4]Tossaporn Incharoen

[1]Faculty of Science and Technology, Prince of Songkla University, Songkla, Thailand
[2]Faculty of Agricultural Technology, Songkhla Rajabhat University, Songkla, Thailand
[3]Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Phitsanulok, Thailand
[4]Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand

Article history
Received: 20-09-2021
Revised: 18-12-2021
Accepted: 22-12-2021

Corresponding Author:
Tossaporn Incharoen
Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand
Email: tossaporni@nu.ac.th

Abstract: The use of plant leaves as an alternative roughage is an effective feeding strategy under a limited supply or lack of feed resources for small ruminants. Thus, this study aimed to investigate the potential of Indian Marsh Fleabane (*Pluchea indica L.*) Leaf (IMFL) and Portia Tree (*Thespesia populnea*) Leaf (PTL) as roughage sources. This experiment was carried out to determine the effects of different ratios of IMFL or PTL to concentrate on degradability and *in-vitro* gas production. A completely randomized design with 5 replicates per treatment was used to determine the effect of different ratios of IMFL or PTL to concentrate (12.66% CP) as Dry Matter (DM) basis. The tested treatments of IMFL were 100:0 (T1), 60:40 (T2) and 50:50 (T3) and of PTL were 100:0 (T4), 60:40 (T5) and 50:50 (T6). The results showed that gas production from soluble fractions, gas production from insoluble fractions, the potential extent of gas production and the gas production rate among all treatments were not significantly different. *In-vitro* gas production at 4, 8, 12, 24, 48, 72 and 96 h. and metabolized energy in the T1 group was significantly higher ($P<0.05$) than in other groups. However, NH_3-N and *In-vitro* True Digestibility (IVTD) were not significant among treatments. Compared to other treatments, *In-vitro* Organic Matter Digestibility (IVOMD) was significantly the lowest in the T4 group ($P<0.05$). The obtained results indicated that the optimal ratios of IMFL or PTL to concentrate were 60:40 and 50:50 on a DM basis. Therefore, we concluded that IMFL and PTL had the potential to be used as alternative roughage sources for ruminants without negative impact on gas production, *In-vitro* digestibility and NH_3-N assessment.

Keywords: Indian Marsh Fleabane, Portia Tree, Digestibility, *In-vitro* Gas Production

Introduction

Ruminant animals have a unique system involving the slow, pre-gastric fermentation of plant fibers by bacteria, protozoa and fungi, all of which provide the host animal with nutrients (volatile fatty acids, microbial protein and B vitamins). High-quality forage plants are crucial for the proper feeding of ruminant animals since they provide energy, proteins and minerals (McSweeney *et al*., 1999). When small ruminants browse, they may find the leaves of different trees. Fodder trees may also be considered as a leguminous fodder crop (Akram *et al*., 1989). Tree leaves play an important role in the nutrition of grazing animals (Meuret *et al*., 1991). Trees forage may be used as a source of protein and energy by small ruminants (Singh *et al*., 1989). Fodder tree leaves are an alternative source of livestock feed and tree leaves have

the potential to alleviate some of the feed shortages and nutritional deficiencies in small ruminants and thus may be an important component of goat and sheep diets (Kamalak *et al*., 2004). Indian marsh fleabane (*Pluchea indica L.*), in the *Asteraceae* family, is a shrub plant that naturally grows in the littoral zones of many Asian and Pacific countries and is a source of phytochemicals and antioxidants, which can prevent cell damage resulting from the oxidative effects of free radicals (Seifried *et al*., 2007). Indian marsh fleabane has been used in traditional medicine to treat respiratory disease, fever, rheumatism, ulcers, tuberculosis; it also has potential anti-ophidian properties (Cho *et al*., 2012). Indian Marsh Fleabane Leaf (IMFL) was observed to contain powerful antioxidants that can be found in a wide variety of herbal tea products (Srimoon and Ngiewthaisong, 2015). Portia tree (*Thespesia populnea*) has been planted and is naturalized in tropical climates throughout the world. It is a typical coastal species in Southeast Asia, Africa and various Pacific Islands (Sujanapal and Sankaran, 2016). It tolerates occasional tidal inundation and saline soils (Iqbal *et al*., 2002). Kraiprom and Samae (2015) studied plants used in sheep husbandry in the South of Thailand. The results showed that many farmers used the cut-and-carry method to feed their sheep Portia Tree Leaf (PTL). The Dry Matter (DM), crude protein, neutral detergent fiber, acid detergent lignin and ash of the PTL were 28.64, 17.67, 26.05, 19.73 and 7.90%, respectively. Furthermore, Kedaree *et al*. (2019) reported that goats fed PTL with paddy straw in a ratio of 30:70 had optimum production nutrient digestibility, ADG and FCR. In Thailand, green forage and the leaves of some trees, such as Leucaena (*Leucaena leucocephala*), Acacia (*Acacia mangium*), Gliricidia (*Gliricidia sepium*) and Jackfruit (*Artocarpus heterophyllus Lam.*) are occasionally supplemented to goats via the cut-and-carry feeding system (Kraiprom and Samae, 2015).

The *in-vitro* gas production technique is used to measure the rate and extent of nutrient degradation in ruminants (Cone *et al*., 1997; Menke *et al*., 1979). Feed substrates are incubated in cultures of mixed rumen microorganisms; fermentation end-products are accumulated in the medium and can be measured after a given incubation time (Rahman *et al*., 2013). In addition, the *in-vitro* gas production technique is inexpensive (Getachew *et al*., 2004) and has easy determination and evaluation means (Khazaal *et al*., 1993). Consequently, it is suitable for use in developing countries (Blummel and Becker, 1997). The study aimed to investigate the potential of IMFL and PTL as a roughage source. This study was carried out to determine the effects of different ratios of IMFL or PTL to concentrate on degradability and *in-vitro* gas production.

Materials and Methods

Diet and Management

The present assessment was conducted using the *in-vitro* gas production technique as described by Menke and Steingass (1988). A completely randomized design with 5 replicates per treatment was used to determine the effect of different ratios of IMFL or PTL to concentrate (12.66% CP) as a DM basis. The tested treatments with different ratios of IMFL to concentrate were 100:0 (T1), 60:40 (T2) and 50:50 (T3) and PTL to concentrate were 100:0 (T4), 60:40 (T5) and 50:50 (T6). The chemical compositions (% DM basis) of diets used in the experiment were shown in Table 1.

Sample Preparation

IMFL, PTL and concentrate were dried at 70°C until constant for DM determination. Then, the samples were ground until they passed through a 1-mM sieve. Then, 200 mg of each treatment was placed in separate serum bottles. After being weighed, each bottle was placed in an incubator at 39°C. Ruminal fluid was collected via suction from 5 male goats (50% Thai Native x 50% Anglo Nubian cross-breed) weighing 15 kg, which had been kept for an adaptation period of 14 days in a metabolic cage and accessed for rumen fluid collection. Goats with rumen cannula were fed ad libitum with a diet containing rice straw (60%) and concentrate (40%) to meet their maintenance and maintain adequate activities of cellulolytic microorganisms throughout the experiment. They were also supplemented with 2.5% of a mineral and vitamin premix. The rumen donor goats was fed twice daily at 06.00 A.M. and 06.00 P.M. and had free access to clean drinking water. Rumen fluid obtained from the goat through a suction tube before the morning feed was put into a thermal flask that had been pre-warmed to a temperature of 39°C (Babayemi and Bamikole, 2006). The rumen fluid was then filtered through four layers of cheesecloth into plastic bottles and pre-warmed in thermal flasks. Artificial saliva was prepared according to the method of Menke and Steingass (1988), which involved adding distilled water, buffer solution, macro-mineral solution and resazurin solution to a flask and warming it to 39ºC. Then, a reducing solution was added and the final solution was placed in a magnetic flask. CO_2 was gently bubbled into this solution until it turned blue, pink and then clear. The rumen fluid was then poured into the artificial saliva using a ratio of saliva to rumen fluid of 2:1. The rumen liquor (rumen fluid + artificial saliva) was then dispensed into serum bottles. A sample of 30 mL solution was added to each bottle using a dispenser. The bottles were then placed in an incubator at 39ºC.

Chemical Analysis

The substrates comprised of IMFL, PTL and concentrate were analyzed for DM, Ether Extract (EE), crude ash and Crude Protein (CP) content according to the methods of the AOAC (1990). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined using the method of Goering and Van Soest (1970).

Gas Production Recording

During incubation, gas production was recorded at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h. The cumulative gas production data were fitted to the model used by Ørskov and McDonald (1979) and shown in Eq. 1:

$$Y = a + b\left(1 - e^{-ct}\right)$$

where, *Y* is the volume of gas production (mL) at a time, t (hr), a is the gas production from the immediate solution fraction (mL), b is the gas production from the insoluble fraction (mL), c is the gas production rate constant for the insoluble fraction (mL/hr), t is the incubation time (hr).

The Metabolizable Energy (ME) content of the leaves was estimated according to the equations below, used for forages (Eq. 1):

ME, MJ/kg DM= $2.20+0.136GP+0.057CP+0.002859EE^2$ (Menke *et al*., 1979)

where, GP is 24 h net gas production (ml/200mg DM), CP is a crude protein (%), EE is ether extract (%)

Volatile Fatty Acid and NH_3-N

At 4 h post-inoculation, the samples were analyzed for Volatile Fatty Acids (VFAs) and NH_3-N. Random samples of 20 mL were placed in glass bottles, to which 1M sulfuric acid was added; this was then centrifuged at 16,000 g for 15 min. Then 15 mL of the supernatant was sampled and frozen at -20°C. The samples were analyzed for NH_3-N by the method according to Bremner and Keeney (1965). The levels of acetic acid, propionic acid and butyrate were analyzed according to Mathew *et al*. (1997).

Determination of In-vitro True Digestibility

After an incubation time of 48 h, the *In-vitro* True Digestibility (IVTD) was determined via the method reported by Van Soest and Robertson (1985). Samples from the whole treatment were transferred quantitatively to a spoutless beaker by repeated washings with 100 mL of neutral detergent solution. The content was refluxed for 60 min., filtered through pre-weighed crucibles and then rinsed with 25 mL acetone. Then, each sample was dried at 100°C for 5 h and the final weight was recorded. The crucible was placed in a furnace at 600°C for 2 h. The DM of the residue was weighed and the IVTD was calculated using Eq. 1:

True digestibility = (DM of feed taken for incubation-NDF residue x 100)/DM of feed taken for incubation

Where DM is the dry matter (g), NDF (% of dry matter) is the neutral detergent fiber.

The *In-vitro* Organic Matter True Digestibility (IVOMD) was obtained by incinerating the dried residues at 600°C for 2 h. IVOMD of the samples was calculated via the method described by Close and Menke (1986) using Eq. 1:

IVOMD = (14.88+0.889GP+0.045CP+0.065CA)/100

where, GP is the number of milliliters produced at 72 h, CP (g/kg dry matter) is the crude protein (g/kg dry matter), EE (g/kg dry matter) is the ether extract, CA (g/kg dry matter) is the crude ash.

Statistical Analysis

All data obtained from the experiment were measured using Analysis of Variance (ANOVA) with a completely randomized design using the SAS software for statistical analysis (SAS Institute Inc., NC, USA). Duncan's new multiple range tests were used to examine differences between treatment means. Differences between means with values of $P<0.05$ were considered statistically significant. The statistical model and experimental design were as follows:

$$Yij = \mu + Mi + \varepsilon ij$$

where, *Yij* denotes the observation variable, μ denotes the overall mean, *M* denotes the effect of treatments and εij denotes the residual effect.

Results and Discussion

The nutrient contents of IMFL and PTL with or without concentrate are presented in Table 1. The study indicated that DM, organic matter, crude protein, ether extract, crude ash, crude fiber, NDF, lignocellulose and lignin of PTL had higher values compared to IMFL. The chemical composition of PTL in this study was higher than those reported by Kraiprom and Samae (2015), who found that DM, crude protein, NDF and ADL were 28.64, 17.67, 26.00,19.73%, respectively. However, the present results were similar to data reported by Kedaree *et al*. (2019) who reported that DM, organic matter, crude protein, ether extract, crude fiber and NFE of PTL were 34.18, 92.57, 8.49, 7.63, 16.51 and 49.94%, respectively. It may be inferred that the variations observed were due to differences in varieties and species, the characteristics of the soil in which the plants were grown, the time of harvest, fertilization, the drying process, leaf-branch ratio, the climate, etc.

Table 1: Chemical compositions of concentrate and experimental diets (as DM basis)

		Experimental treatments					
Item (%)	Concentrate	T1	T2	T3	T4	T5	T6
DM	89.69	17.21	45.19	53.44	25.21	50.98	57.12
Organic matter	91.83	78.97	86.42	83.41	89.11	90.18	90.87
Crude protein	12.66	20.02	15.29	16.21	23.22	18.89	17.94
Ether extract	5.13	1.27	3.45	3.15	2.63	3.55	3.88
Crude ash	8.17	21.03	12.32	14.52	10.89	9.56	9.40
Crude fiber	9.43	14.07	11.01	11.74	15.51	13.07	12.42
NDF	39.64	42.92	40.65	41.21	46.51	43.28	42.82
Lignocellulose	24.87	30.33	27.85	27.41	34.83	30.83	29.84
Lignin	6.67	29.55	15.87	17.77	30.42	20.97	18.54

T1 = 100% Indian marsh fleabane leaf, T2 = 60% Indian marsh fleabane leaf + 40% concentrate, T3 = 50% Indian marsh fleabane leaf + 50% concentrate, T4 = 100% Portia tree leaf, T5 = 60% Portia tree leaf + 40% concentrate, T6 = 50% Portia tree leaf + 50% concentrate, DM = Dry matter, NDF = Neutral detergent fiber

Table 2: Gas volume and values of the kinetic parameter from the fermentation of the experimental treatments

	Experimental treatments						
Item							
Gas volume (mL/200 mg dry matter)	T1	T2	T3	T4	T5	T6	Pooled SEM
4 h	48.94^{a}	17.07^{c}	19.43^{c}	31.49^{b}	17.76^{c}	16.06^{c}	0.80
8 h	58.23^{a}	22.47^{c}	24.66^{c}	34.80^{b}	22.53^{c}	19.42^{c}	0.82
12 h	60.36^{a}	23.34^{c}	27.27^{c}	36.66^{b}	26.12^{c}	20.11^{c}	1.27
24 h	67.32^{a}	24.92^{c}	30.51^{c}	37.70^{b}	30.89^{c}	22.14^{c}	1.66
48 h	79.32^{a}	29.89^{c}	34.56^{c}	45.39^{b}	37.22^{c}	25.56^{c}	1.42
72 h	88.61^{a}	33.64^{c}	38.73^{c}	50.15^{b}	41.01^{c}	27.71^{c}	1.70
96 h	91.45^{a}	34.51^{c}	39.45^{b}	50.42^{b}	41.53^{b}	28.65^{c}	2.20
Gas production parameter							
a (mL)	18.58^{a}	4.49^{b}	6.54^{b}	16.12^{a}	6.42^{b}	3.99^{b}	1.87
b (mL)	62.57^{a}	24.91^{b}	28.28^{b}	34.21^{b}	31.97^{b}	21.22^{b}	1.93
a+b (mL)	81.15^{a}	29.27^{b}	34.83^{b}	44.83^{b}	38.39^{b}	25.21^{b}	2.74
c (per h)	0.17^{b}	0.15^{b}	0.12^{b}	0.28^{b}	0.13^{b}	0.43^{a}	0.06
ME (MJ/kg DM)	12.53^{a}	6.15^{c}	7.32^{bc}	8.60^{b}	7.53^{bc}	6.30^{c}	0.22

T1 = 100% Indian marsh fleabane leaf, T2 = 60% Indian marsh fleabane leaf + 40% concentrate, T3 = 50% Indian marsh fleabane leaf + 50% concentrate, T4 = 100% Portia tree leaf, T5 = 60% Portia tree leaf + 40% concentrate, T6 = 50% Portia tree leaf + 50% concentrate, SEM = Standard error of mean, ME = Metabolizable energy as calculated from 2.20 + 0.136*GP + 0.057*CP + 0.0029*CF according to method of Menke *et al*. (1979)
$^{a-c}$Mean values in the same row with different superscripts show a significant difference at P<0.05

Table 3: *In-vitro* true digestibility, *In-vitro* organic matter digestibility, volatile fatty acids and ammonia nitrogen of the experimental treatments

	Experimental treatments						
Item	T1	T2	T3	T4	T5	T6	Pooled SEM
In-vitro true digestibility (%)	50.49	46.75	55.05	31.22	38.78	37.96	3.23
In-vitro organic matter digestibility (%)	53.98^{a}	67.12^{a}	63.54^{a}	21.38^{b}	45.56^{a}	34.93^{a}	0.65
The molar proportion of VFAs (%)							
Acetic acid (C2)							
4 h	68.65	67.33	66.95	68.51	68.23	67.33	0.17
8 h	67.36	69.89	67.76	67.36	69.75	68.17	0.06
12 h	68.89	68.41	66.93	70.18	68.65	68.05	0.11
Propionic acid (C3)							
4 h	22.52	22.14	22.50	21.01	21.57	22.11	0.05
8 h	25.82	20.19	21.39	22.89	20.65	21.93	0.31
12 h	19.47	20.30	21.47	19.44	20.37	20.78	0.05
Butyric acid (C4)							
4 h	9.56	9.76	9.45	12.37	10.11	9.49	0.78
8 h	10.64	10.47	10.48	12.15	10.21	10.15	0.28
12 h	9.88	9.92	10.15	9.98	9.68	9.89	0.87
NH_3-N (mg/dL)	14.50	12.45	13.74	14.36	12.35	13.85	1.29

T1 = 100% Indian marsh fleabane leaf, T2 = 60% Indian marsh fleabane leaf + 40% concentrate, T3 = 50% Indian marsh fleabane leaf + 50% concentrate, T4 = 100% Portia tree leaf, T5 = 60% Portia tree leaf + 40% concentrate, T6 = 50% Portia tree leaf + 50% concentrate, SEM = Standard error of mean, VFAs = Volatile fatty acids
^{ab}Mean values in the same row with different superscripts show a significant difference at P<0.05

These results demonstrate their high nutritive value and positive effects on rumen function, microbial yields and metabolism (Kamalak *et al*., 2004). However, the use of plant leaves as an alternative roughage is an effective feeding strategy under a limited supply or lack of feed resources for small ruminants. Several studies suggested that various plant leaves can be used as forages sources for browsing ruminants (Aderinboye *et al*., 2016; Lopez *et al*., 2016; Olfaz *et al*., 2018; Khejornsart *et al*., 2021).

The gas production characteristics are presented in Table 2 and Fig. 1. The results showed that gas volumes at 4, 8, 12, 24, 48, 72 and 96 h after incubation were significantly (P<0.05) different among treatments. The microbes in the rumen fluid in the treatment group with 100 IMFL and 100% PTL (T1 and T4) produced high levels of gas (Fig. 1). The gas levels from treatments with only plant leaf (IMFL and PTL) were higher than those with plant leaf and concentrate. The forage materials are fermentable and have readily degradable cell wall fractions, which increases the substrates available to cellulolytic microbes, with a consequent increase in the population of these microorganisms (Van Soest, 1982). Similarly, the presence of these microbes influences the extent and rate of substrate degradation, which is related to the gas volumes produced (Blummel *et al*., 1997).

The concentrate supplement was formulated to provide readily digestible energy from locally available concentrates, but also protein and non-protein N. Ruminants fed low-quality forages are often provided protein or non-protein N supplements (Egan and Doyle, 1985). The *in-vitro* gas production levels at 12, 24, 48, 72 and 96 h were higher in the present study than those reported for tree leaves such as *Olea europaea L.* (29.51, 34.82, 38.87, 42.06 and 43.64 mL/200mg), *Morus alba L.* (20.58, 37.18, 45.20, 50.24 and 54.29 mL/200 mg) and *Citrus aurantium L.* (20.43, 25.37, 29.25, 31.07 and 31.80 mL/200 mg) (Olfaz *et al*., 2018).

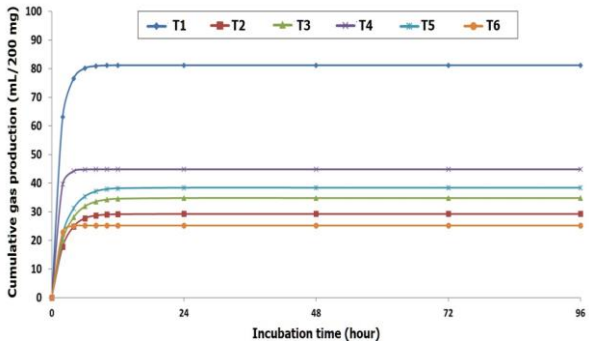

Fig. 1: The gas volume produced versus incubation time for various treatments

The absolute value for (a) in Eq. 1 can be described as the ideal fermentation of the soluble fraction. In the present study, the absolute gas production rates in treatments T1 and T4 were greater (P<0.05) than those in T3, T5 and T2, respectively. The gas volume at the asymptote (b) describing the fermentation of the insoluble fraction and the potential extent of gas production (a+b) was the highest (P<0.05) in T1. Shakeri *et al*. (2017) reported the *in-vitro* gas production of olive leaves as 49-49.4 mL/200 mg DM. These values are similar to those for PTL (a + b = 44.83 mL/200 mg DM). The rates of gas production (c) in T6 were significantly the highest (P<0.05). Increasing the gas production rate found in the current study merely implies an alteration in the microbial population in the rumen in response to a high level of ether extract in the T6 diet (Table 1) rather than a direct impact on microbial activity. The highest values of gas production in IMFL are at least partly caused by the addition of rapidly fermentable carbohydrates and the higher degradability of the insoluble fraction. The results of the *In-vitro* gas production in this study could provide an estimate of Metabolizable Energy (ME). Among the treatment groups, IMFL was significantly highest (P<0.05). The findings of this study are similar to those of Karabulut *et al*. (2007), who found that legume hays ranged widely from 9.09 to 11.12 MJ/kg DM and those of Olfaz *et al*. (2018), who noted that the ME resulting from the *In-vitro* gas production technique in olive, mulberry and sour orange tree leaves had a range of 6.06 to 8.11 MJ/kg DM together.

IVTD was not significantly different among treatments (Table 3). However, IVOMD was significantly lowest (P<0.05) in the T4 group (100% PTL). The value of IVOMD was similar to the results reported by Olfaz *et al*. (2018), who reported that the IVOMD of olive, mulberry and sour orange tree leaves had a range of 40.91 to 55.38%. The molar proportion of VFAs (acetic acid, propionic acid and butyric acid) at 4, 8 and 12 h was not significant among treatments. Degradation of fibrous or cellulosic materials is likely to produce a higher molar proportion of acetate and a lower proportion of propionate (Moss *et al*., 2000). Gas is produced mainly when feed ingredients are fermented to acetate and butyrate, with propionate yielding gas-only due to the buffering of acid (Getachew *et al*., 2004). High levels of acetate usually occur in animals fed rations containing large amounts of roughage, whereas lower levels are associated with concentrate diets (Madrid *et al*., 2002). In the current study, NH_3-N was not significant among treatments. However, this finding was similar to Illius (1989) who reported that an appropriate concentration of rumen NH_3-N to enhance the growth of microorganisms and digestion efficiency ranges from 5 to 25 mg/dL. While

Weakley *et al*. (1983) noted that ruminal NH_3-N from 9.34 to 11.23 mg/dL is suitable for rumen bacterial growth and metabolism.

Conclusion

The obtained results indicated that the optimal ratios of IMFL or PTL to concentrate were 60:40 and 50:50 on a DM basis. Therefore, IMFL and PTL had the potential to be used as an alternative roughage source for ruminants without negative impact on gas production, *In-vitro* digestibility and NH_3-N assessment.

Acknowledgment

The authors would like to acknowledge the financial support for the experiment from a Government Budget Grant and thank the Faculty of Sciences and Technology, Prince of Songkla University, for providing support in facilities.

Author's Contributions

Thaintip Kraiprom: Participated in all experiments, designed the experiment, performed the data analysis and wrote the study.

Sitthisak Jantarat: Collected the data, contributed data or analysis tools, performed the laboratory analysis and prepared the data for writing the manuscript.

Santi Madman: Performed the laboratory analysis, collected the data and prepared the data for writing the manuscript.

Suphawadee Yaemkong: Performed the laboratory analysis, contributed data or analysis tools and prepared the data for writing the manuscript.

Tossaporn Incharoen: Participated in all experiments, conceived and designed the analysis, designed the overall experiment, contributed data or analysis tools and wrote the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Aderinboye, R. Y., Akinlolu, A. O., Adeleke, M. A., Najeem, G. O., Ojo, V. O. A., Isah, O. A., & Babayemi, O. J. (2016). In vitro gas production and dry matter degradation of four browse leaves using cattle, sheep and goat inocula. Slovak Journal of Animal Science, 49(1), 32-43. https://sjas.ojs.sk/sjas/article/view/159

Akram, M., Hanjra, S. H., Qazi, M. A., & Bhatti, J. A. (1989, July). Availability and use of shrub and tree fodder in Pakistan. Proceed. In Workshop, Denpasar, Indoneshia (pp. 24-29). ISBN: 0-88936-556-3

AOAC. (1990). Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Babayemi, O. J., & Bamikole, M. A. (2006). Effects of Tephrosia candida DC leaf and its mixtures with Guinea grass on in vitro fermentation changes as feed for ruminants in Nigeria. Pakistan Journal of Nutrition, 5(1), 14-18. doi.org/10.3923/pjn.2006.14.18

Blummel, M., & Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by in vitro gas production and their relationship to voluntary feed intake. British Journal of Nutrition, 77(5), 757-768. doi.org/10.1079/bjn19970073

Bremner, J. M., & Keeney, D. R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. Analytica chimica acta, 32, 485-495. doi.org/10.1016/S0003-2670(00)88973-4

Cho, J. J., Cho, C. L., Kao, C. L., Chen, C. M., Tseng, C. N., Lee, Y. Z., ... & Hong, Y. R. (2012). Crude aqueous extracts of Pluchea indica (L.) Less. inhibit proliferation and migration of cancer cells through induction of p53-dependent cell death. BMC complementary and alternative medicine, 12(1), 1-11. doi.org/10.1186/1472-6882-12-265

Close, H., & Menke, K.H. (1986). Selected topics in animal nutrition. In A Manual Prepared for the 3rd Hohenheim Course on Animal Nutrition in the Tropics and Semi-tropics, 2nd Edn, University of Hohenheim, Stuttgart, Germany."

Cone, J. W., van Gelder, A. H., & Driehuis, F. (1997). Description of gas production profiles with a three-phasic model. Animal Feed Science and Technology, 66(1-4),31-45. doi.org/10.1016/S0377-8401(96)01147-9

Egan, J. K., & Doyle, P. T. (1985). Effect of intraruminal infusion of urea on the response in voluntary food intake by sheep. Australian Journal of Agricultural Research, 36(3), 483-495. doi.org/10.1071/AR9850483

Getachew, G., Robinson, P. H., DePeters, E. J., & Taylor, S. J. (2004). Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Animal Feed Science and Technology, 111(1-4), 57-71. doi.org/10.1016/S0377-8401(03)00217-7

Goering, H. K., & Van Soest, P. J. (1970). Forage fiber analyses (apparatus, reagents, procedures and some applications) (No. 379). US Agricultural Research Service.

Iqbal, M. Z., Yasmin, N., & Shafiq, M. (2002). Salt tolerance variation in some common trees. Acta Botanica Hungarica, 44(1-2), 67-74. doi.org/10.1556/ABot.44.2002.1-2.5

Illius, A. W. (1989). Matching ruminant production systems with available resources in the tropics and sub-tropics: TR Preston & RA Leng. Renambul Books, Armidale, NSW, 1987. ISBN 0-9588290-12. Agricultural Systems, 30(2), 200-201. doi.org/10.1016/0308-521X(89)90048-6.

Kamalak, A., Canbolat, O., Gurbuz, Y. A. V. U. Z., Ozay, O., Ozkan, C. O., & Sakarya, M. (2004). Chemical composition and in vitro gas production characteristics of several tannin containing tree leaves. Livestock research for rural development, 16(6),44. http://www.lrrd.org/lrrd16/6/kama16044.htm

Karabulut, A., Canbolat, O., Kalkan, H., Gurbuzol, F., Sucu, E., & Filya, I. (2007). Comparison of in vitro gas production, metabolizable energy, organic matter digestibility and microbial protein production of some legume hays. Asian-Australasian Journal of Animal Sciences, 20(4),517-522. doi.org/10.5713/ajas.2007.517

Kedaree, V. C., Khirari, P. B., & Jadhav, S. M. (2019). Performance of growing goats fed paddy straw supplemented with graded levels of Thespesia Populnea. Journal of Pharmacognosy and Phytochemistry, 8(2), 2425-2428 E-ISSN: 22784136. P-ISSN: 2349-8234

Khazaal, K., Dentinho, M. T., Ribeiro, J. M., & Ørskov, E. R. (1993). A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of the apparent digestibility in vivo and the voluntary intake of hays. Animal Science, 57(1), 105-112. doi.org/10.1017/S0003356100006668

Khejornsart, P., Cherdthong, A., & Wanapat, M. (2021). In Vitro Screening of Plant Materials to Reduce Ruminal Protozoal Population and Mitigate Ammonia and Methane Emissions. Fermentation, 7(3), 166. doi.org/10.3390/fermentation7030166

Kraiprom, T., & Samae, A. (2015). Survey of sheep production situation and nutritive value study of plants for sheep raising in Pattani province. Journal of Agriculture (Thailand). https://agris.fao.org/agris-search/search.do?recordID=TH2019000111

Lopez, D., Vázquez-Armijo, J. F., López-Villalobos, N., Lee-Rangel, H. A., Salem, A. Z. M., Borquez-Gastelum, J. L., ... & Rojo-Rubio, R. (2016). In vitro gas production of foliage from three browse tree species treated with different dose levels of exogenous fibrolytic enzymes. Journal of Animal Physiology and Animal Nutrition, 100(5), 920-928. doi.org/10.1111/jpn.12467

Madrid, J., Megías, M. D., & Hernández, F. (2002). In vitro determination of ruminal dry matter and cell wall degradation and production of fermentation end-products of various by-products. Animal Research, 51(3), 189-199. doi.org/10.1051/animres:2002018

McSweeney, C. S., Dalrymple, B. P., Gobius, K. S., Kennedy, P. M., Krause, D. O., Mackie, R. I., & Xue, G. P. (1999). The application of rumen biotechnology to improve the nutritive value of fibrous feedstuffs: Pre-and post-ingestion. Livestock Production Science, 59(2-3), 265-283. doi.org/10.1016/S0301-6226(99)00032-9

Menke, K.H., & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development, 28, 7-55. https://ci.nii.ac.jp/naid/10025840911/

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. The Journal of Agricultural Science, 93(1), 217-222. doi.org/10.1017/S0021859600086305

Menke, W., West, M., Brandsdóttir, B., & Sparks, D. (1998). Compressional and shear velocity structure of the lithosphere in northern Iceland. Bulletin of the Seismological Society of America, 88(6), 1561-1571. ISBN: 0037-1106

Meuret, M., Boza, J., Narjisse, H., & Nastis, A. (1991). Evaluation and utilization of rangeland feeds by goats. Goat nutrition, 46, 160. ISBN: 90-220-1009-0

Moss, A. R., Jouany, J. P., & Newbold, J. (2000, May). Methane production by ruminants: Its contribution to global warming. In Annales de zootechnie (Vol. 49, No. 3, pp. 231-253). EDP Sciences. doi.org/10.1051/animres:2000119

Olfaz, M., Kilic, U., Boga, M., & Abdi, A. M. (2018). Determination of the *in vitro* gas production and potential feed value of olive, mulberry and sour orange tree leaves. Open life sciences, 13(1), 269-78. doi.org/10.1515/biol-2018-0033

Ørskov, E. R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science, 92(2), 499-503. doi.org/10.1017/S0021859600063048

Rahman, M. M., Salleh, M. A. M., Sultana, N., Kim, M. J., & Ra, C. S. (2013). Estimation of total Volatile Fatty Acid (VFA) from Total Organic Carbons (TOCs) assessment through *in vitro* fermentation of livestock feeds. African Journal of Microbiology Research, 7(15), 1378-1384. doi.org/10.5897/AJMR12.1694

Srimoon, R., & Ngiewthaisong, S. (2015). Antioxidant and antibacterial activities of Indian marsh fleabane (*Pluchea indica* (L.) Less). Asia-Pacific Journal of Science and Technology, 20(2), 144-154.

Mathew, S., Sagathevan, S., Thomas, J., & Mathen, G. (1997). An hplc method for estimation ofvolatile fatty acids in ruminal fluid. Indian Journal of Animal Sciences, 67(9), 805-807.

Seifried, H. E., Anderson, D. E., Fisher, E. I., & Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of nutritional biochemistry, 18(9), 567-579. doi.org/10.1016/j.jnutbio.2006.10.007

Shakeri, P., Durmic, Z., Vadhanabhuti, J., & Vercoe, P. E. (2017). Products derived from olive leaves and fruits can alter in vitro ruminal fermentation and methane production. Journal of the Science of Food and Agriculture, 97(4), 1367-1372. doi.org/10.1002/jsfa.7876

Singh, R. B., Bannerjee, G. C., & Gupta, B. N. (1989). Chemical composition and nutritive value of Gogun (*Saurauia napalensis*) tree leaves. Indian Journal of Animal Nutrition, 6(2), 174-176. Print ISSN: 0970-3209. Online ISSN: 2231-6744

Sujanapal, P., & Sankaran, K. V. (2016). Common plants of Maldives. Bangkok: Food and Agriculture Organization of the United Nations and Kerala Forest Research Institute. ISBN-10: 978-92-5-109295-8, pp: 271

Van Soest, P. J. (1982). Nutritional ecology of the ruminant. O & B Books. Inc., Corvallis, OR, 374. ISBN-10: 09-601-5860X, pp: 374

Van Soest, P.J., & Robertson, J.B. (1985) Analysis of Forages and Fibrous Foods. In A Laboratory Manual for Animal Science 613. Cornell University, Ithaca, NY, USA.

Weakley, D. C., Stern, M. D., & Satter, L. D. (1983). Factors affecting disappearance of feedstuffs from bags suspended in the rumen. Journal of Animal Science, 56(2), 493-507. doi.org/0.2527/jas1983.562493x