



UNIVERSITI PUTRA MALAYSIA

***THE EFFECT OF SELECTED LOCAL PLANTS USED IN GOATS DIET IN
MALAYSIA ON IN VITRO DEGRADABILITY***

MUHAMMAD AZROLHARITH BIN RASHID

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MALAYSIA ON *IN VITRO* DEGRADABILITY**

The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. At the top left, the letters 'UPM' are written in white on a red rectangular background. In the center, there is a stylized red and white graphic that resembles a book or a pair of wings. The shield is divided into several sections by white lines. The text 'MUHAMMAD AZROLHARITH BIN RASHID' is centered over the logo.

MUHAMMAD AZROLHARITH BIN RASHID

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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CERTIFICATION

It is hereby certified that I have read this project paper entitled “The Effect of Selected Local Plants Used in Goats Diet in Malaysia on *In Vitro* Degradability”, by Muhammad Azrolharith bin Rashid and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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Finally, a special gratitude to those who were directly or indirectly involved in the completion of this project.

DEDICATION

This project is lovely dedicated to:

My parents

Rashid bin Omar & Kalsoom binti Husin

My only big Brother & his wife

Mohd Ikram bin Rashid & Syarifah Syafafwati binti Syed Alawi

My sweet nephew and niece

Nur Imanina Qaireen & Muhammad Ilham Mukhlis

My future wife

Vetman Production

Relatives & Friends

Thank you for loving me
unconditionally..

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LIST OF ABBREVIATIONS

BA	<i>Mallothus spp.</i> (Balik Angin)
LD	<i>Macaranga spp.</i> (Lebar Daun)
MX	50% Lebar Daun & 50% Balik Angin
MW	20% Lebar Daun, 20% Balik Angin & 60% Napier
N	Napier
NDF	Neutral Detergent Fiber
VFA	Volatile Fatty Acids

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Ilmiah Tahun Akhir.

KESAN TUMBUHAN TEMPATAN TERPILIH YANG DIGUNAKAN DALAM DIET KAMBING DI MALAYSIA TERHADAP FERMENTASI RUMEN *IN VITRO*

Oleh

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2015

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Makanan ternakan merupakan salah satu penyumbang kos terbesar dalam pengeluaran kambing yang mencapai kira-kira 70% daripada jumlah kos pengeluaran. Disebabkan oleh kekurangan ruang untuk penanaman rumput dan juga persaingan

makanan antara manusia dan ternakan, beberapa cara alternatif telah digunakan oleh penternak dengan menggunakan tumbuh-tumbuhan tempatan untuk dijadikan sebagai makanan haiwan. Oleh itu, pemilihan bahan makanan ternakan yang bersesuaian dan berkhasiat adalah penting untuk menghasilkan nilai tenaga dan pengeluaran gas yang dapat membantu meningkatkan fermentasi pada rumen kambing. Dalam kajian ini, dua tumbuhan tempatan telah dipilih iaitu *Macaranga spp.* (Lebar Daun) dan *Macrostachyus spp.* (Balik Angin) untuk mengkaji pengeluaran gas serta pengeluaran asid lemak meruap yang terdiri daripada asetat, propionat, dan butirat dengan mengaplikasikan teknik inkubasi *in vitro* selama 24 jam, menggunakan cecair rumen daripada kambing yang telah difistulasi. Terdapat 5 kumpulan diet eksperimen yang telah dilabelkan sebagai LD (Lebar Daun), BA (Balik Angin), N (Napier), MX (50% Lebar Daun & 50% Balik Angin), dan MW (20% Lebar Daun, 20% Balik Angin & 60% Napier). Keputusan menunjukkan pengeluaran asid lemak meruap sebanyak 772.46 mM/ML daripada Balik Angin adalah yang tertinggi berbanding dengan kumpulan diet lain. Lebar daun pula menghasilkan kepekatan tertinggi asetat (94.30%) berbanding rawatan lain. Campuran kedua-dua tumbuhan tempatan (MX) menghasilkan kepekatan tertinggi untuk butirat (give the value here) dan propionat (give the value here). Selain itu, Napier menghasilkan gas (give the value here) yang paling tinggi berbanding dengan tumbuhan-tumbuhan tempatan, Walau bagaimanapun kadar pengeluaran gas dan asid lemak meruap antara kumpulan diet tidak memberi terlalu banyak perbezaan ($P > 0.05$). Oleh itu, tidak ada perbezaan yang signifikan antara sampel-sampel kumpulan diet tersebut ($P > 0.05$). Daripada kajian ini, keterdegradasian rumen *in vitro* tumbuhan tempatan terpilih adalah sama atau kurang daripada sumber serat lain, iaitu Napier yang kebiasaannya digunakan sebagai

makanan kambing,yang jelas menyatakan bahawa kedua-dua tumbuhan tempatan terpilih ini sesuai untuk digunakan sebagai sumber serat lain sebagai makanan kambing.

Kata Kunci: tumbuhan tempatan, fermentasi rumen, asid lemak meruap, penghasilan gas, *Mallothus spp.*, *Macaranga spp.*



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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement of the VPD 4999 – Final Year Project.

THE EFFECT OF SELECTED LOCAL PLANTS USED IN GOATS DIET IN MALAYSIA IN IN VITRO RUMEN DEGRADABILITY

By

Muhammad Azrolharith bin Rashid

2015

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Livestock feeds are considered as one of the largest cost associated with goat production, typically accounting around 70% of total production costs. Due to lack of space for grass planting and animal-human feed competition, farmers have come out with alternatives ways to use local plants as animal feed. Thus, it is important to choose the appropriate and nutritive feedstuffs that can compromise the energy value and essential nutrients in the goats which will improve rumen degradability. In this study,

two of selected local plants which are *Macaranga spp.* (Lebar Daun) and *Mallothus spp.* (Balik Angin) were selected to assess the *in vitro* ruminal fermentation with respects on the gas production and production of volatile fatty acids (VFA) which are acetate, propionate, and butyrate. The *in vitro* incubation was done for 24 hours, using rumen fluid from fistulated goats. There are 5 different experimental designs which are LD (Lebar Daun), BA (Balik Angin), N (Napier), MX (50% Lebar Daun & 50% Balik Angin), and MW (20% Lebar Daun, 20% Balik Angin & 60% Napier). From the results, the production of total VFA 772.46 mM/ml from Balik Angin was the highest compared to other treatment groups. Lebar Daun produced the highest acetate production (94.30%) compared to others. Mixture of both selected local plants produces highest concentration for butyrate and propionate compared to other treatments. However, there is no significant different between the treatments ($P < 0.05$). Meanwhile, the production of total gas from selected local plants when compared with the control plant which is Napier grass, did not show significant different. Indeed, there was no significant difference among the treatment samples for gas production ($P < 0.05$). From this study, the *in vitro* ruminal degradability of selected local plants are equal or less than other fibre source, which is Napier grass that commonly used in goat's diet in Malaysia. This represent that these two selected local plants can be used as fibre sources for goats feedstuffs without adverse effect of rumen fermentation.

Keywords: local plants, rumen fermentation, volatile fatty acids, gas production, *Mallothus spp.* and *Macaranga spp.*

1.0 INTRODUCTION

Animal nutrition is facing an increasing sustainability dilemma related to directly covering requirements by vegetal sources in diets, particularly when derived from a livestock system depending on crops that can be consumed by humans (Kayouli, 2007). Growing concerns about this problem have prompted researchers to search ways to promote the efficient utilization of non-competitive feed resources such as agricultural by-products and local plants that may supply essential nutrients (e.g. fibres) to the animals (Nguyen, 1998).

There are a variety of options among farmers concerning feeds for livestock production. However, the utilisation of conventional options, such as maize and soy bean, for livestock feeding is not feasible as the maize and soy bean are also widely used for human consumption. Hence, the price of the commodities would be high. As such, there is great interest in the utilisation of unconventional materials as livestock feed.

In this era of globalisation, suitable feed choices have become one of the main factor in diversify the nutrients contents for animal requirement especially for farm animals. Indeed, Diego (1994) has suggested that an ideal nutrition program should be able to support optimum production, minimizes related problems and in the same time is efficient and economical. As to create or produce well balance feed, several ways have been specialized to ensure the good nutrition supplements for the animals. In Malaysia, most of the local plants which subjected as higher plants, wild shrubs, palms, climbers that grown in the wild such as *Mallothus spp.* (Balik Angin), *Acacia*

spp. (Akasia), *Codiaeum spp.* (Puding), *Passiflora spp.* (Daun Letup) and *Macaranga spp.* (Macaranga) are widely being utilized in goat diets as source of roughage.

Although the local plants are abundantly available and use in goats diet, concerns about their role to enhance rumen degradability are of interest. Hence, this study is aim to assess the effect of some selected local plants *Mallothus spp.* (Balik Angin) and *Macaranga spp.* (Lebar Daun) that are being fed to goats on *in vitro* rumen degradability. This study was done in a farm under Ladang Angkat, Fakulti Perubatan Veterinar, Universiti Putra Malaysia. A comparison of rumen degradability parameters (total gas production and volatile fatty acids) of these local plants with other fibre source (Napier) that commonly used in goats diet was done to evaluate its potential to be included in total mixed ration.

2.0 LITERATURE REVIEW

2.1 Species of Local Plants

In Malaysia, there are abundant of local plants which grow wild in the forest. Interestingly, some of the species of local plants can be used to replace the feedstuffs for the goats, which chemically tested to have balance nutrition with other feedstuffs if given in accurate ratio. In this study, two of local plants which are *Macaranga spp.* (Lebar Daun) and *Mallothus spp.* (Balik Angin), have been chosen since these plants have been given with other ration of feedstuffs in some goat farms and interestingly, there is no presence of harmful effects towards the goat. However, study for nutrients contents of these species are still limited.

- *Macaranga spp.*(Lebar Daun)

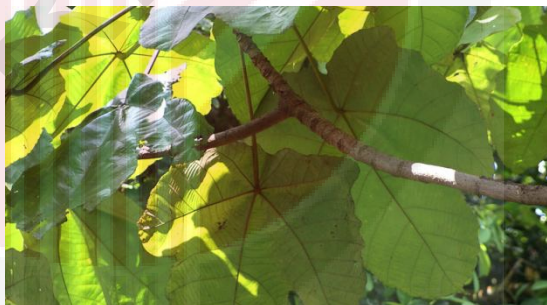


Figure 1.0: Picture of *Macaranga spp.* (Lebar Daun).

Sources from <http://www.plantsofasia.com/>

Macaranga or also known as “Kubin”, “Mahang Gajah”, and “Lebar Daun” (Figure 1.0) is one the plants that categorised as wild local plants. According to Hanum (2004), this plant is one of the pioneer species which have the characteristics of components of secondary forests. It grows and form pure stands for other plants in the forests. The tree usually 15 m tall with the designs

of spreading-domed crowned. The plants also been used for temporary construction by human, light framing and packing cases. Moreover, some people use the leaves to wrap food since it has wider shape area.

- *Mallothus spp.* (Balik Angin)



Figure 2.0: Picture of *Mallothus spp.* (Balik Angin)

Sources from <http://www.tfbc.frim.gov.my>

Mallothus spp. which also formally known as “Balik Angin” (Figure 2.0) is also one the local plants that growth wildly in Malaysia. This plant have been categorised as higher plants among the local plants species. Hanum (2004) also stated that the ecology of this plant species usually grow in lowlands of secondary forest and open areas to 1200m. This plant is a small tree and can grow for 18m tall. The characteristics of the leaves are sub-opposite and ovale shape. Apart from that, this plant fruit a capsule, globule around 2cm across and have woolly and hanging spikes of the fruits. This plant usually been used for wood carvings since it has soft wood. Apart from that, it also been utilized as traditional medicine to treat chronic hepatitis and enteritis (Hoai *et al.*, 2009).

2.2 Rumen and Fermentation

Ruminants have very complex digestive system which consist of four compartments which are rumen, reticulum, omasum, and abomasum. From these parts, rumen is the largest compartment and apparently covers 65% of the cow stomach organ. Rumen is very unique since it takes place for rumination to occur. There were abundance of microbes-anaerobic and facultative anaerobes, that help to digest feedstuffs since rumen provide favourable anaerobic environment for the microbes. Remarkably, these microbes will cause fermentation of food processes and enable to convert feedstuffs which consist of carbohydrates into volatile fatty acids (VFA). The dietary carbohydrates also been fermented as to produce ATP (Fellner, 2009). Volatile fatty acids are very important since it provide 70% of daily energy requirement in ruminants (Bergman, 1990). From the fermentation process, there will be production of three types of fatty acids which are acetate, propionate and butyrate. Fellner (2009) also stated that the final product for fermentation process may be changes by the microbes depending to the type of substrate, pH and rate of passage from the rumen.

2.3 *In Vitro* Gas Production Method

In vitro gas production technique is a technique which performed under laboratory conditions. According to Getachew et al. (2004), *in vitro* techniques need to be done by introducing the normal microorganisms which are allocated in the rumen towards feed and feedstuffs, as to mimic the real rumen fermentation process in the ruminant. The feed samples which coordinate as treatment was incubated with buffered rumen fluid at 39°C and the gas produced from the fermentation process will act as an indirect indicator of fermentation kinetics. As for the microbes, the rumen content was taken from different donors as to get more miscellaneous of rumen microbes for fermentation process (Rymer et al., 2005). Compared to *in vivo* process, *in vitro* methods consume less time to be done in which the process usually takes 24-48 hours to complete. While *in vivo* methods, the time consumed for the whole process to be finished may lengthen from 1-4 weeks. Moreover, by *in vitro* methods, the treatment samples can be done towards hundreds samples compared to *in vivo* which have limit sample numbers to be experimental in one time. Apart from that, the process also required cheap apparatus and materials. Finally, the physiological, microbial activities and environment for *in vitro* methods can be fixed well according to the requirement, and also will penetrate as to get the accurate results. Therefore, from all of these advantages, *in vitro* methods have been chosen as the methodology of this study.

3.0 MATERIALS AND METHOD

3.1 Sample Preparation

This study was conducted at a farm under Ladang Angkat, Faculty of Veterinary Medicine, UPM, located at Negeri Sembilan. In this study, two types of local plants; *Mallothus spp.* (Balik Angin) and *Macaranga spp.* (Lebar Daun) were selected. The sample of other fibre source such as Napier was used as the control. All samples (local plants and Napier) were dried and grounded for further analysis.

4 different treatments were prepared as follow;

1. *Mallothus spp.* (Balik Angin) = BA
2. *Macaranga spp.* (Lebar Daun) = LD
3. Mixture of 50% *Mallothus spp.* (Balik Angin) and 50% *Macaranga spp.* (Lebar Daun) = MX
4. Mixture of 20% *Mallothus spp.* (Balik Angin), 20% *Macaranga spp.* (Lebar Daun) and 60% Napier grass = MW

The mixture was done according to the common ratio that has been practiced by the farmers in the goat diets.

3.2 *In Vitro* Incubation and Analysis

In vitro incubation was performed according to the method of Hassim et al. (2012). 0.25g of samples were added into gas tight 100 mL plastic syringes. Before that, the plunger of the syringes have been lubricated with Vaseline to ensure the plunger can easily move during incubation process. Phosphate buffer and bicarbonate buffer were prepared for 150mL each. The phosphate (contained 5.184g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.098 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 0.252 g NH_4Cl) and bicarbonate buffer (contained 7.056 g of NaHCO_3) were prepared. The pH of the phosphate-bicarbonate buffer was adjusted to 6.9-7.0 and flushed with CO_2 for 1.5 h.

Rumen fluid was collected from the fistulated goats before the animal were being fed in the morning. The rumen fluid was kept in the pre-warmed thermo flask during transportation to the laboratory. Later, the rumen fluid was filtered with four layers of cheesecloth. The mixture of rumen fluid and buffer; 30mL was added to each of the plastic syringe and shake well to ensure the complete mixing of incubated contents. The air was expelled from each syringe and the tips were closed by using three way stopper. All syringes were incubated in an incubator (temp 39°C) for 24 hours. The volume of the gas produced at 0, 2, 4, 6, 8, 10, 12 and 24h of incubation period were recorded.

According to Soliva and Hess (2007), the amount of gas produced during the fermentation process is subtracted from the amount of gas volume recorded for tested samples as to obtain the total gas production and net gas production.

3.3 Volatile Fatty Acids Analysis

Volatile fatty acids was measured by using gas liquid chromatography. After the pH of the samples were measured, the rumen liquor was fixed with metaphosphoric acid with ratio 4:1 in the test tubes. Next, the samples were centrifuged at 3000 rpm for 10 minutes to obtain the supernatant. Then, 0.5 mL of the supernatant was collected into vials and mixed with 0.5mL 20mM methyl-n-valeric acid. The separation was done on Quadrex 007 Series (Quadrex Corporation, New Haven, CT 06525 USA) bonded phase fused silica capillary column (15m, 0.32mm ID, 0.25 µm film thickness) in a 6890A HP Gas Liquid Chromatograph which equipped with flame ionisation detector. By setting the injector at 220/230°C, and the column temperature was set on 70-150°C as to facilitate optimal separation. By the time, the temperature will increase 7°C/min. Finally, the identification of the peaks was made by compare the authentic standards of acetic, propionic, butyric, and 4- methyl-n-valeric acid.

3.4 Statistical Analysis

The data were analysed using SAS programme to compare the *in vitro* rumen degradability parameters of selected local plants with other fibre source which are Napier that commonly used in goat's diets. The analysis was done by performing Duncan's Multiple Range Test.

4.0 RESULTS

4.1 Total Gas Production

Total gas production was obtained during *in vitro* incubation for 24 hours. The Table 1.0 shows the rate and total gas production for different treatments of this study.

Treatment	Rate of gas production	Total gas production
Balik Angin (BA)	1.94 ± 0.27^a	23.33 ± 3.29
Lebar Daun (LD)	2.39 ± 0.38^b	28.67 ± 4.56
Napier (N)	$2.58 \pm 0.22^{a, b}$	31.00 ± 2.65
MX (mix of BA & LD)	$2.35 \pm 0.11^{a, b}$	28.17 ± 1.35
MW (mix of BA, LD & N)	$2.38 \pm 0.20^{a, b}$	28.50 ± 2.40

^{a,b} Means with different letters within columns have significant difference.

Table 1.0 The rate and total gas production of different treatments after 24 hours *in vitro* incubation

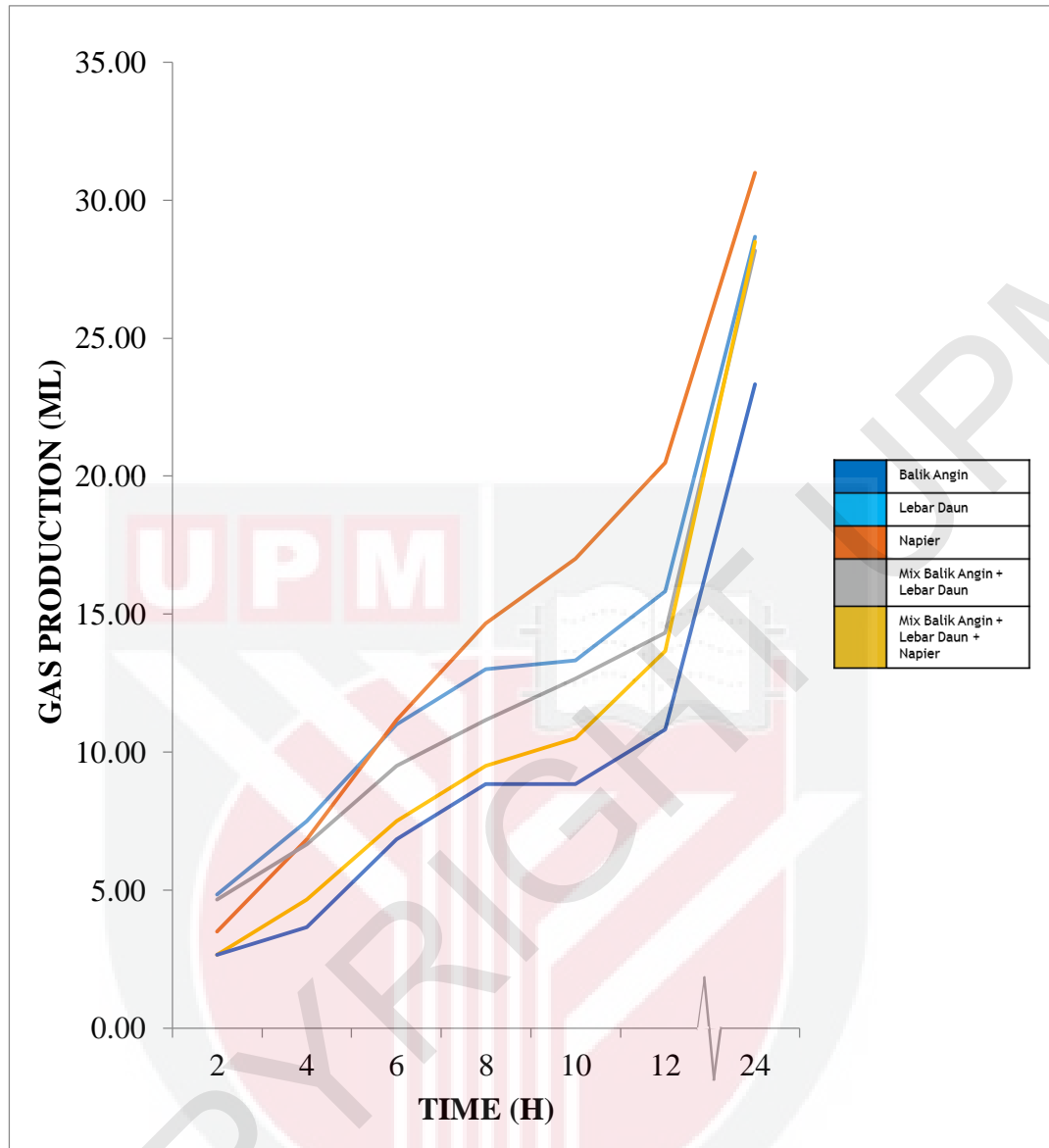


Figure 3.0 Total rate of gas production for different treatments after 24 hours of *in vitro* incubation

This graph (Figure 3.0) presents the rate of gas production between 2 hours until 24 hours. The gas production was increasing from the start of incubation (0 h) until the end of incubation period (24 h) for all treatments. This indicates that the degradation process was taken place over the incubation period. The rate of gas production, at first 4 h showed that there were significant difference between LD and

BA whereas during 10 and 12 hours, there were significance difference between N and BA. However, there were no significant differences between all treatments after 24 hours ($P < 0.05$).

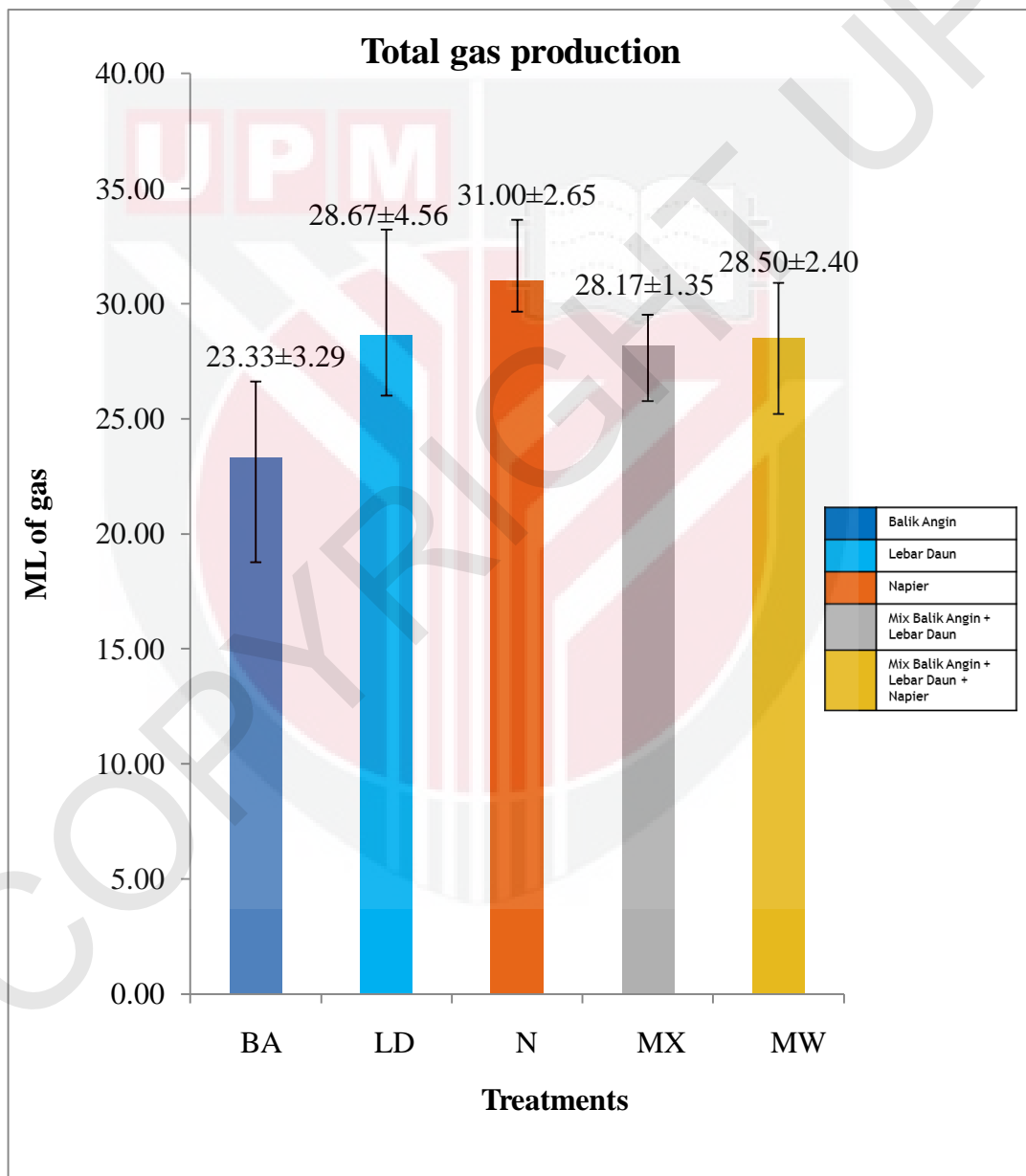


Figure 4.0 Total gas production for different treatments after 24 hours

In other parts, the total gas production was shown in Figure 4.0. Among the treatments, Napier shown the highest total gas production, followed by LD, MW, MX, and BA.

4.2 The Production of Volatile Fatty Acids

Treatments	Acetate	Propionate	Butyrate	Total VFA
Balik Angin (BA)	93.83 ± 1.69	4.33 ± 0.930	1.83 ± 0.783	772.46 ± 133.986
Lebar Daun (LD)	94.30 ± 0.324	3.88 ± 0.133	1.83 ± 0.226	635.27 ± 71.006
Napier(N)	94.23 ± 0.37	3.90 ± 0.188	1.87 ± 0.205	641.32 ± 78.802
MX (mix) BD & LD)	93.21 ± 0.179	4.34 ± 0.063	2.46 ± 0.138	709.07 ± 83.951
MW (mix) BD,LD & N)	93.96 ± 0.179	3.94 ± 0.063	2.10 ± 0.138	768.59 ± 83.951

Table 2.0 The table shows different treatments with production of volatile fatty acids

Volatile fatty acids (VFA) are measured after the incubation process for 24 hours. There are three types of VFA produced which are acetate, propionate and butyrate. In this study, these VFA were measured to see the relationship the VFA with the degradability.

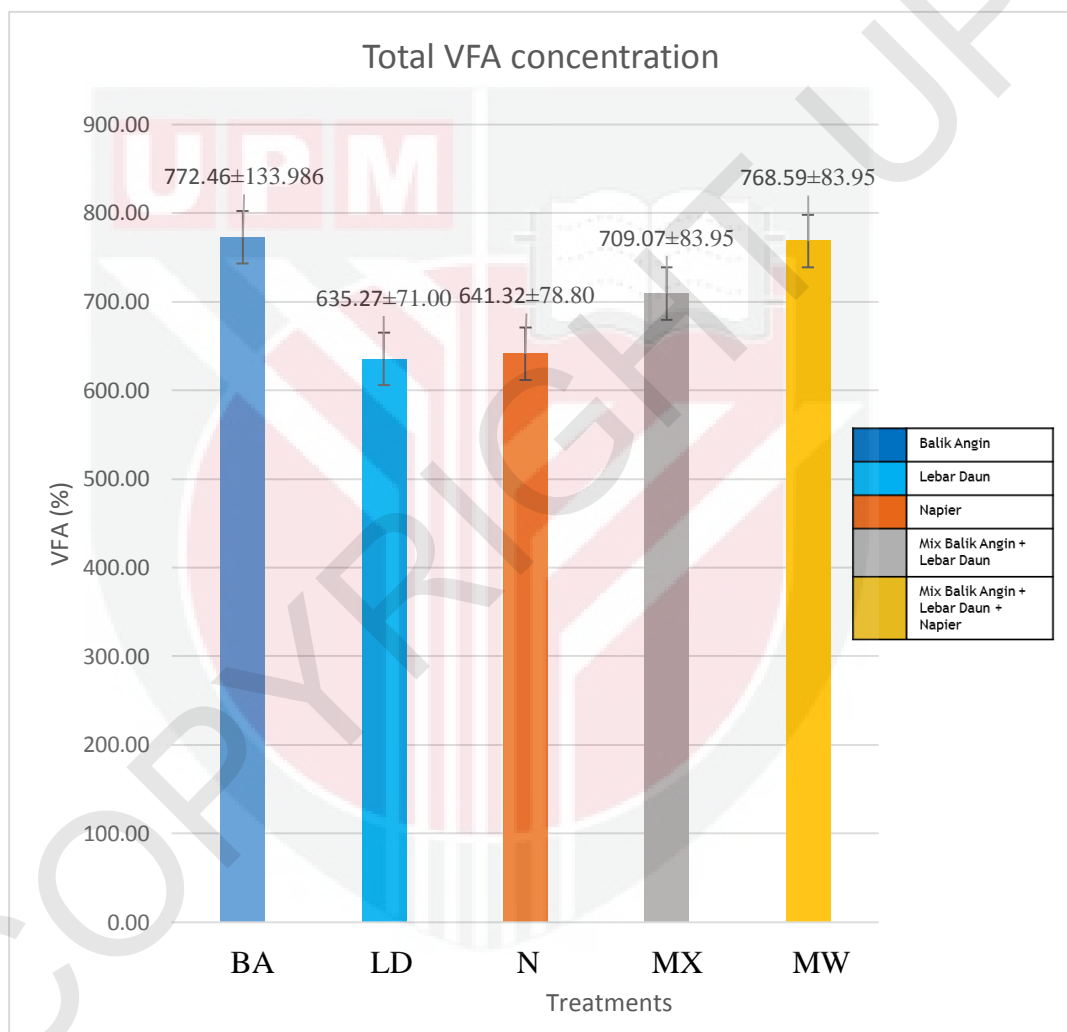


Figure 5.0 Total volatile fatty acids production for different treatments after 24 hours

Figure 5.0 shows the total VFA production for different treatments after 24 hours. From the graph, BA and MW shows the highest production of total VFA. However, there was no significant different between the treatments ($P < 0.05$).

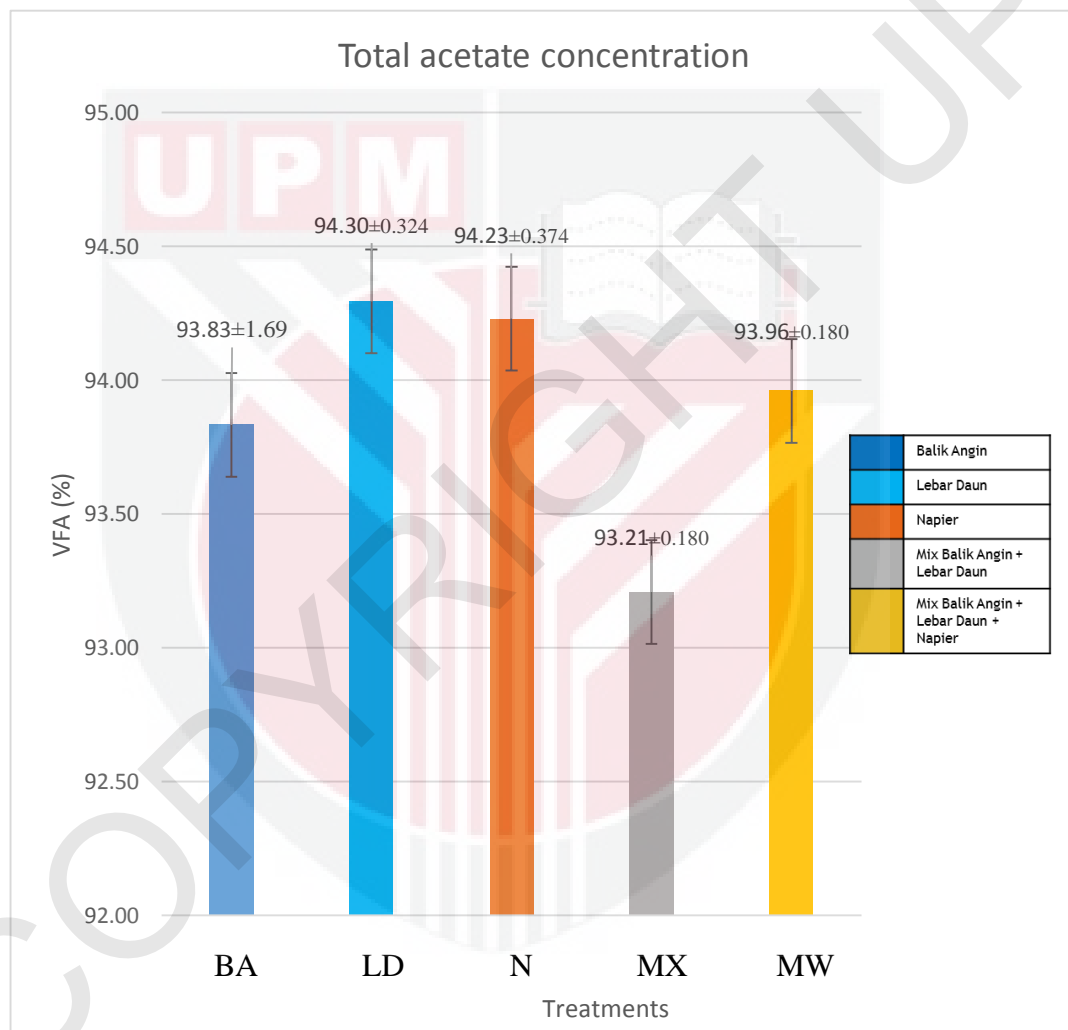


Figure 6.0 Total acetate concentration for different treatments after 24 hours

Figure 6.0 shows the total acetate concentration for different treatments after 24 hours. Indeed, from the graph shown, LD and N contain the highest concentration

for acetate. However, there was no significant different between the samples ($P < 0.05$).

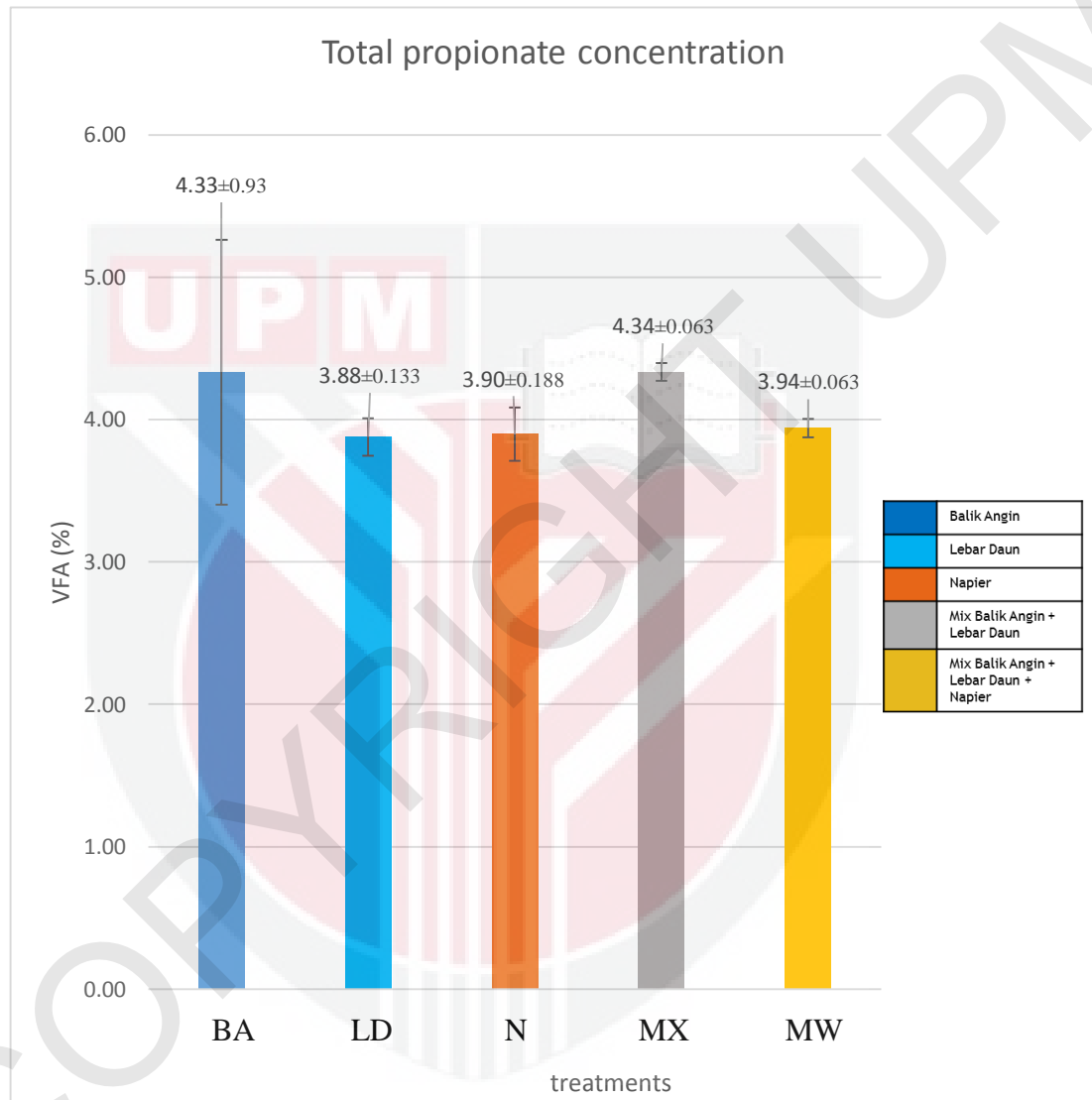


Figure 7.0 Total propionate concentration for different treatments after 24 hours

Figure 7.0 shows the total propionate for the different treatments after 24 hours. From the results, the treatments are almost similar. However, there was no significant different between the samples ($P < 0.05$).

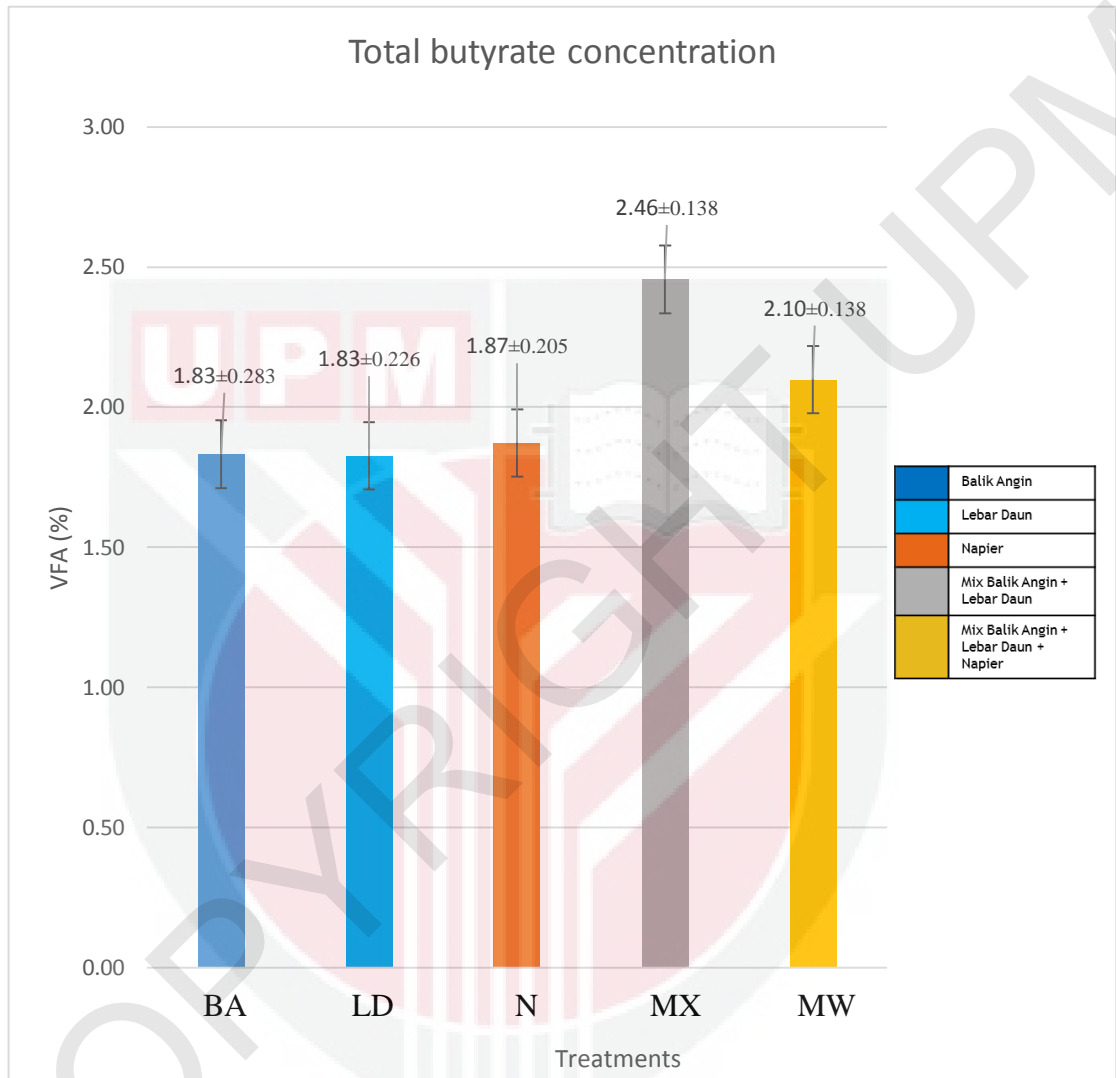


Figure 8.0 Total butyrate concentration for different treatments after 24 hours

Figure 8.0 illustrates the results for the total mean of butyrate concentration after 24 hours. Most of the treatments samples are similar except MX (mixture of Balik Angin and Lebar Daun) which shows the highest production of butyrate concentration. In this study, there was a significant different ($P < 0.05$) between Balik Angin with MX and MW. There was also significant different between LD and N with MX.

5.0 DISCUSSION

5.1 Total Gas Production

In this study, the *in vitro* incubation technique was done to evaluate the total gas and VFA production for the treatment samples. Indeed, the rate of gas production reflects the degradability of the feed. Apart from that, the total gas production also involves production of gases such as nitrogen, oxygen and carbon dioxide (Getachew, 2004).

The total gas production showed that the Napier produced the highest gas production among the treatment samples, however, there was no significant different among the samples. The higher the rate of gas production, the more the samples become digested. Thus, since there were no significant differences between Napier with the local plants, it can be concluded that the local plants also have similar digestibility roughage with the control plants.

On top of that, the degradability of the plants also affected by the total silica contents in the feed samples. According to Drake *et al.* (2002), the degradability, which is total gas production of the plants, may be affected due to the silica content. This is supported by other studies that show Napier has high silica content compared to the local plants (Norliza et al, 2014).

5.2 Volatile Fatty Acids

Volatile fatty acids (VFA) are the energy source produced by the fermentation process of microbial in the rumen. VFA usually consist of acetate, propionate and butyrate. According to Rasmussen and Harrison (2011), the acetate production might be higher than propionate due to high level of Neutral Detergent Fiber (NDF). NDF which is made up of silica contents, cellulose, hemicellulose, and lignin may represent that the relationship of total gas production and VFA production which affect the degradability.

The VFA and gas production of Napier from this study are slightly lowered compared to the degradability analysis of standard VFA (Norliza et al, 2014), which proved that the Napier that have been used in this study has poor quality. However, the fermentation process from the mixture of local plants, the mixture shows better fermentation pattern with standard Napier and may indicate the potential of using this mixture in total mixed ration. Moreover, the fermentation patterns also were comparable with other roughage sources such as *Setaria spp.*, *Brachiaria spp.* and others.

6.0 CONCLUSION

The results represent that the plant cell wall characteristics which are silica and NDF as well as nutrient contents affect the ruminant degradability. These two selected local plants can be used as fibre sources for goats feedstuffs without adverse effect of rumen fermentation. Apart from that, all of the selected local plants which are Lebar Daun and Balik Angin, represent similar rate of fermentation pattern with the control plant which means that these two species is reasonable to be used for goats feedstuffs. Thus, these mixture of local plants have high potential to be used as source of goat feeds and to enhance rumen degradability.

7.0 RECOMMENDATIONS

Feeding trial can be done by combination of selected local plants with other feedstuffs, in *in vivo* studies as to look the performance, meat quality, and milk quality. Furthermore, it is also advisable to extend the research for these local plants on nutritional profile and degradability with the concentrate, such as soy husk and palm kernel cake, as to prepare a well balanced nutrition for the goats. Moreover, it is also recommended to do more research on other local plants or wild shrubs that can replace for animal's source of diet which may help to be use as source of diet.

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