UNIVERSITI PUTRA MALAYSIA

EFFECT OF PAPAYA SEED SUPPLEMENTATION ON GAS PRODUCTION ACTIVITY IN *IN VITRO* FERMENTATION BY RUMEN FLUID

MOHD ZHARIF RUSLI

FP 2016 106

EFFECT OF PAPAYA SEED SUPPLEMENTATION ON GAS PRODUCTION ACTIVITY IN *IN VITRO* FERMENTATION BY RUMEN FLUID

BY

MOHD ZHARIF BIN RUSLI

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of SHW4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural (Animal Science)

Faculty of Agriculture

Universiti Putra Malaysia

2015/2016

CERTIFICATION FORM

This project report entitled "EFFECT OF PAPAYA SEED SUPPLEMENTATION ON GAS PRODUCTION ACTIVITY IN *IN VITRO* FERMENTATION BY RUMEN FLUID" was prepared by Mohd Zharif Bin Rusli and submitted to the Faculty of Agriculture in fulfillment of the requirement of SHW4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural (Animal Science)

Student's name: Student's signature:

Mohd Zharif Bin Rusli

…… …..……..…

Certified by:

…….

(DR. ISWAN BUDY BIN HJ. SUYUB)

Department of Animal Science,

Faculty of Agriculture

Date:

ACKNOWLEDGEMENT

First of all I want to show my gratitude and appreciation to all those who had gave me the strength and help through completing this final year project. Alhamdulillah, all praises to Allah S.W.T for the strength and His blessing upon on completion of this project report.

I would like to give a special appreciation to my supervisor, Dr. Iswan Budy bin Hj. Suyub for his valuable guidance, advices, help and support throughout this project. Furthermore, my fullest gratitude to the entire laboratory staffs of the Department of Animal Sciences for their kindly cooperation towards the preparation of my study and provide me the necessary facilities.

Not to forget, my deepest gratitude and appreciation to my family for their support, encouragement, understanding and endless love for me while in completing this final year project.

Sincerely thanks to all of my friends especially Ahsanuddin Shah Bin Suhaimi and Syahira Binti Ahmad Daud for their kindness and moral support during my study. Thanks for the encouragement, friendship and memories.

TABLES OF CONTENTS

CERTIFICATION FORM	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLE	vi
LIST OF FIGURE	vii
LIST OF ABBREVIATION	viii
ABSTRACT	ix
ABSTRAK	xi
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	4
2.1 *In vitro* methods	4
2.2 Papaya (*Carica papaya*)	6
2.3 Papaya seed	8
2.4 Napier grass	10
2.5 In Vitro Dry Matter Digestibility (IVDMD)	12

CHAPTER 3: MATERIALS METHODS 13

3.1 Study's location 13

3.2 Experimental design 13

3.3 *In vitro* gas production 14

3.3.1 Syringes for incubation 14

3.4 Buffer solution 15

3.5 Calculations 16

3.6 *In vitro* dry matter digestibility 16

3.7 Chemical analysis 17

3.7.1 Dry matter (DM) 17

3.7.2 Ash (Organic Matter) 18

3.7.3 Crude protein (CP) 18

3.7.4 Neutral detergent fibre (NDF) 19

3.7.5 NDF solution 20

3.7.6 Acid detergent fibre (ADF) 20

3.7.7 ADF solution 21

3.7.8 Acid detergent lignin (ADL) 21

3.8 Statistical analysis 22

CHAPTER 4: RESULTS AND DISCUSSION 23

4.1 Chemical analysis 23

4.1.1 Napier grass and percentage of papaya seed 23

4.2 Digestibility 27

4.2.1 Napier grass and papaya seed percentage 27

4.3 *In vitro* dry matter digestibility 29

4.4 Discussion 31

4.4.1 Chemical analysis 31

4.4.2 Digestibility 32

4.4.3 *In vitro* dry matter digestibility (IVDMD) 34

CHAPTER 5: CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH 35

REFERENCES 36

APPENDICES 42

LIST OF TABLES

TABLE	TITLE	PAGE
2.1	Raw papaya nutritional value per 100g	7
2.2	*Carica papaya* L. is a pack of enzymes	8
2.3	Constituents of different parts of the papaya tree	9
3.1	Treatments used in this experiment	13
3.2	Compositions of solutions used	15
4.1	Mean of Nutritive Value between Napier Grass and Papaya Seed Percentage	25
4.2	Mean Nutritive Values between Napier Grass (100%) and Papaya Seed (100%)	26

LIST OF FIGURE/GRAPH

FIGURE/GRAPH	TITLE	PAGE
4.1	Mean Nutritive Values between Napier Grass and Papaya seed Percentage in ash, CP and DM	23
4.2	Mean Nutritive Values between Napier Grass and Papaya Seed Percentage in NDF, ADF and ADL	24
4.3	Mean Nutritive Values between Napier Grass (100%) and Papaya Seed (100%)	26
4.4	Cumulative Gas Production of Napier Grass and Papaya Seed in 48 Hours	27
4.5	Mean of Total Gas Production between Napier Grass (100%) and Papaya Seed (100%)	28
4.6	Mean of Total Gas Production between Napier Grass and Papaya Seed Percentage	29
4.7	Percentage of In Vitro Dry Matter Digestibility (IVDMD) between Napier Grass (100%) and Papaya Seed (100%)	30
4.8	Percentage of In Vitro Dry Matter Digestibility (IVDMD) between Napier Grass and Papaya Seed Percentage	30

LIST OF ABBREVIATION

ADF	acid detergent fibre
ADL	acid detergent lignin
ANOVA	Analysis of Variance
CTab	cetyl trimethylammonium bromide
CP	crude protein
CRD	complete randomized design
DM	dry matter
DPS	Dried Pawpaw Seed
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization of United Nation
IVGPT	*In Vitro* Gas Production Technique
IVDMD	In Vitro Dry Matter Digestibility
ME	metabolizable energy
mg	Milligrams
ml	Millilitres
NDF	neutral detergent fibre
SCFA	short chain fatty acids
SPSS	Statistical Package for the Social Sciences

ABSTRACT

Papaya is commonly used as dessert and ingredient in the cooking. However, only the flesh has been consumed and the skin and seed will be discarded. The parts that did not consume will become waste and can attribute to pollution. Papaya seed is not being fully utilized by farmers. This study evaluates the comparative outcome of the Napier grass (*Pennisetum purpureum)* and papaya seed as supplement. These treatments are done for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), *in vitro* gas production and *in vitro* dry matter digestibility (IVDMD) by using various parameters. 15 samples were collected randomly for this study including the control. Napier grass is used in this experiment acting as the basal diet for the ruminants. First sample is Treatment 1 = 100% Napier grass (n=3) followed by Treatment 2 = 99% Napier grass + 1% papaya seed (n=3), Treatment 3 = 98% Napier grass + 2% papaya seed (n=3) and Treatment 4 = 97% Napier grass + 3% papaya seed (n=3). Napier grass had been cut at week 6, dried in the oven at 105 °C until it reach the constant weight and the dried Napier grass was grinded into small pieces. Meanwhile papaya seed was collected at the stall nearby in Seri Serdang and dried it in the oven at 105 °C. Then, the papaya seed was grinded into small pieces. In the proximate analysis that had been done, the dry matter (DM), ash and neutral detergent fibre (NDF) had higher ($P<0.05$) than acid detergent fibre (ADF), acid detergent lignin (ADL), crude protein (CP) and crude fibre (CF). The DM, ash and NDF had significant difference between Treatment 1, Treatment 2, Treatment 3 and Treatment 4. From *in*

vitro gas production analysis, it shows that the gas production had significance difference ($p > 0.05$) between the treatments. However, *in vitro* dry matter digestibility did not have significance difference between others treatments. For the recommendation, the researchers or farmers can exploit more about papaya seed because there are still more parameters that can be studied in the future.

ABSTRAK

Betik biasanya digunakan sebagai pencuci mulut dan ramuan dalam masakan. Walau bagaimanapun, hanya isi betik sahaja yang digunakan dan kulit dan benih akan dibuang. Bahagian-bahagian yang tidak diambil akan menjadi sisa dan boleh membuat pencemaran. Biji betik tidak digunakan sepenuhnya oleh petani. Satu kajian telah dijalankan untuk membandingkan rumput Napier (Pennisetum purpureum) dan biji betik sebagai makanan tambahan. Rawatan ini dilakukan untuk bahan kering (DM), abu, protein kasar (CP), gentian neutral detergen (NDF), gentian asid detergen (ADF), penentuan lignin detergen (ADL), penghasilan gas in vitro dan penghadaman kering in vitro (IVDMD) dengan menggunakan pelbagai parameter. 15 sampel diambil secara rawak untuk kajian ini termasuk rawatan kawalan. Rumput Napier yang digunakan dalam eksperimen ini bertindak sebagai diet asas untuk ruminan. Sampel pertama adalah Rawatan 1 = 100% rumput Napier (n = 3) diikuti dengan Rawatan 2 = 99% rumput Napier + 1% biji betik (n = 3), Rawatan 3 = 98% rumput Napier + 2% biji betik (n = 3) dan Rawatan 4 = 97% rumput Napier + 3% biji betik (n = 3). Rumput Napier telah dipotong pada minggu 6, dikeringkan di dalam ketuhar pada suhu 105 ° C sehingga ia mencapai berat badan yang malar dan rumput napier yang kering telah dikisar ke dalam kepingan kecil. Sementara itu, biji betik dikumpulkan di gerai yang berhampiran di Seri Serdang dan dikeringkan di dalam ketuhar pada suhu 105 ° C. Kemudian, biji betik itu dikisar kepada kepingan kecil. Dalam analisis proksimat yang telah dijalankan, bahan kering (DM), abu dan serat detergen neutral (NDF) mempunyai nilai lebih tinggi (P

<0.05) daripada asid serat bahan pencuci (ADF), asid pencuci lignin (ADL), protein mentah (CP) dan serat kasar (CF). DM, abu dan NDF mempunyai perbezaan yang ketara antara Rawatan 1, Rawatan 2, 3 dan Rawatan Rawatan 4. Dari analisis penghasilan gas in vitro, ia menunjukkan bahawa gas pengeluaran mempunyai perbezaan yang ketara (p> 0.05) antara rawatan. Tambahan pula, nilai p bahan kering penghadaman in vitro tidak mempunyai perbezaan yang ketara antara rawatan. Untuk cadangan, penyelidik atau petani boleh mengeksploitasi lebih lanjut mengenai biji betik kerana masih terdapat banyak parameter yang boleh dikaji pada masa hadapan.

Chapter 1

Introduction

Productivity of ruminants depends on sufficient nutrition with sense to composition and quality of feedstuffs, which is reflected in voluntary intake and digestibility. For animal, it is necessary to provide a nutritional balanced ration by evaluation of feedstuffs and their capability to supply nutrients. Evaluation is also important from an environmental perspective, since excretion of undigested feed nutrients, as well as emission of gases, affects the environment (Getachew, DePeters, Robinson, & Fadel, 2005).

Despite of all scientific technologies that have been developed and implemented over the past few decades, the world is still searching for better ways to feed livestock and human in terms of finding new potential feed, as well as, accurate methods of improvement and evaluation of their nutritive value (Marimo, *et al*., 2010). In Malaysia, there are a lot of fruit wastes that have been disposed after the main part of fruit was taken which is the flesh. The other parts such as skin and seed will be discarded to the environment. However, inappropriate management of landfill will result in emissions of methane and carbon dioxide (Qdais, Abdulla, & Qrenawi, 2010), and incineration involves the subsequent formation and releases of pollutants and secondary wastes such as dioxins, furans, acid gases as well as particulates (Buekens & Huang, 1998), which pose serious environmental and health risks. To avoid this, the government including the farmer has made further effort to use the waste as the feed for the livestock or bio-compost for the plants.

One of the fruit wastes is derive from papaya. Papaya is one of the famous fruits in Malaysia and served as a dessert or appetizer. But only the flesh was consumed but the skin and seed were discarded, whereas seeds constitute 22% of the waste from papaya puree plant. Papaya seeds are recently gaining importance due to its medicinal value, since it recently had been used in curing sickle cell diseases, poisoning renal disorder and as anti- helmithes (Samia, Rabab, & Abd El-Ghany, 2012). For example in livestock industries, the papaya seeds have been used as a method of elimination of goats' parasitic passengers. Led by Adegbola Adesogan, a study by Adesogan *et al.* (2013) examined the effect of natural food supplements on reducing intestinal worms in goats. Papaya seeds were found to be the most effective treatment, significantly reducing parasite egg and adult counts by adding 10 grams of ground papaya seed to a base diet of Bahia grass removed 78 percent of adult parasites and 72 percent of their eggs.

1.1. Objectives

1.1.1. General objective

- To evaluate the papaya seed as supplement in the feed for small ruminants via *in vitro* rumen digestion.

1.1.2. Specific objectives

- To evaluate the nutrient composition of papaya seed by proximate analysis.

- To investigate the effect on papaya seed as supplement in the Napier grass (*Pennisetum purpureum*) fermentation by measuring *in vitro* gas production as indicator of feed digestibility.
- To measure the *in vitro* dry matter digestibility (IVDMD) of feed sample of post *in vitro* gas production.

References

Adebiyi, A., Adaikan, G. P., & Prasad, R. N. (2002). Papaya (*Carica* papaya) consumption is unsafe in pregnancy: fact or fable? Scientific evaluation of a common belief in some part of Asia using a rat model. *British Journal of Nutrition, , 88*, 199-203.

Aganga, A. A., Omphile, U. J., Thema, T., & Baitshotlhi, J. C. (2005). Chemical composition of Napier grass (*Pennisetum purpureum*) at differant stages of growth and Napier grass silages with additives. *Journal of Biological Sciences 5*(4), 493-496.

Ansah, T., Osafo, E. L. K., & Hansen Hanne, H. (2010). Herbage yield and chemical composition of four varieties of Napier (*Pennisetum purpureum*) grass harvested at three different days after planting. *Agriculture Biology Journal of North America, 1*(5), 923-929.

Anuara, N. S., Zaharia, S. S., Taiba, I. A., & Rahman, M. T. (2008). Effect of green and ripe *Carica* papaya epicarp extracts on wound healing and during pregnancy. *Food and Chemical Toxicology, 46*(7), 5.

Aravind, G., Debjit, B., Duraivel, S., & Harish, G. (2013). Traditional and medicinal uses of *Carica* papaya. *Journal of Medicinal Plants Studies, 1*(1), 15.

Bolu, S. A. O., Sola-Ojo, F. E., Olorunsanya, O. A., & Idris, K. (2009). Effect of graded levels of dried Pawpaw (*Carica papaya*) seed on the performance, haematology, serum biochemistry and carcass evaluation of chicken broilers. *International Journal of Poultry Science 8*(9), 905-909.

Boshra, V., & Tajul, A. (2013). Papaya - An innovative raw material for food and pharmaceutical processing industry. *Health and the Environment Journal, 4*(1).

Buekens, A., & Huang, H. (1998). Comparative evaluation of techniques for controlling the formation and emission of chlorinated dioxins furans in municipal waste incineration. *J. Hazard Mater 62*, 1-33.

Chinoy, N. J., Dilip, T., & Harsha, J. (2006). Effect of *Carica* papaya seed extract on female rat ovaries and uteri. *Phytotherapy Research, 9*, 165-169.

Expert Committee on Animal Nutrition (1986). Laboratory evaluation of farm grown forage. . *In: Proc. Third ECAN Workshop. Winnipeg, Man., Agriculture Canada*, 24-27.

FAO (2015). A searchable catalogue of grass and forage legumes. *Grassland Index*.

Fenwick, G. R., Heaney, R. K., Mullin, W. J., & Van Etten, C. H. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Review of Food Sci. Nutr., 18*, 123-201.

Getachew, G., DePeters, E. J., Robinson, P. H., & Fadel, J. G. (2005). Use of an *in vitro* rumen gas production technique to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. *Animal Feed Science and Technology*, 547–559.

Grant, R. J., Van Soest, P. J., & McDowell, R. E. (1974). Influence of rumen fluid source and fermentation time on in vitro true dry matter digestibility. *J. Dairy Sci. , 57*, 275.

Grant, R. J., & Weidner, S. J. (1992). Digestion kinetics of fiber: Influence of *in vitro* buffer pH varied within observed physiological range. *J. Dairy Sci. , 75*, 1060.

Jenkins, T. C., & Palmquist, D. L. (1982). Effect of added fat and calcium on *in vitro* formation of insoluble fatty acid soaps and cell wall digestibility. *J. Anim. Sci., 55*, 957.

Kermanshai, R., McCarry, B. E., Rosenfeld, J., Summers, P. S., Weretilnyk, E. A., & Sorger, G. J. (2001). Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. . *Phytochem, 57*, 427-435.

Krishna, K., & Paridhavi, M. (2008). Review on nutritional, medicinal and pharmacological properties of papaya (*Carica* papaya Linn.). *Nat Prod Radian, 7*, 364-373.

Mahyuddin, P. (2008). Relationship between chemical component and *in vitro* digestibility of tropical grasses. *Journal of Biosciences, 15*, 85-89.

Makkar, H. P. S. (2004). Applications of the *in vitro* gas method in the evluation of feed resources, and enhancement of nutritional value of Tannin-rich tree/browse leaves and agro-industrial by-products. *Animal Production and Health Section*.

Marimo, C. T., Hector, B., Rodgrigues, P. H. M., Borgatti, L. M., Meyer, P. M., Alves da Silva, E. J., et al. (2010). Characterization of vegetables and fruit potential as ruminant feed by *in vitro* gas production technique. *Livestock Research for Rural Development, 22*, 168.

Marinucci, M. T., Dehority, B. A., & Loerch, S. C. (1992). *In vitro* and *in vivo* studies of factors affecting digestion of feeds in synthetic fiber bags. . *J. Anim. Sci., 70*, 296.

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*. *J. agric. Sci., 93*, 217-222.

Milind, P., & Gurditta (2011). Basketful benefits of papaya. *International Research Journal of Pharmacy, 2*(7), 6-12.

Nadkarni, K. M., & Nadkarni, A. (1954). Indian Materia Medica: With Ayurvedic, Unani-tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies. *Popular Book Depot*

Oderinde, O., Noronha, C., Oremosu, A., Kusemiju, T., & Okanlawon, O. A. (2002). Abortifacient properties of aqueous extract of *Carica* papaya (Linn) seeds on female Sprague-Dawley rats. *Nigerian Postgraduate Medical Journal, 9*, 95-98.

Panse, T. B., & Paranjpe, A. S. (1943). Isolation of carpasemine from papaya seeds. *Proc. Indian Acad. Sci. , 18A*, 140.

Qdais, H. A., Abdulla, F., & Qrenawi, L. (2010). Solid waste landfills as a source of green energy: Case study of Al Akeeder landfill. *Jordan J. Mech. Ind. Eng, 4*, 69-74.

Quicke, G. V., Bentley, G., Scott, H. W., & Moxon. A. L. (1959). Cellulose digestion *in vitro* as a measure of the digestibility of forage cellulose in ruminants. *J. Anim. Sci. , 18*, 275.

Rehman, R., & Israr, M. (2003). In vitro regeneration of witloof chicory (Cichoriumintybus L.) from leaf explants and accumulation of esculin. *In Vitro Cellular & Developmental Biology-Plant 39*(2), 142-146.

Ronoredjo, E. P., & Bastiaensen, P. X. M. (1995). The use of indigeneous papaya (*Carica papaya*) as an anthelmintic for the treatment of gastro-intestinal nematodes in naturally infected calves in Suriname. *De Surinaamse Landbouw.*

Samia, E.-S., F., , Rabab, S., H.,, & Abd El-Ghany, M. E. (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein *World Journal of Dairy & Food Sciences, 7*(1), 59-65.

Satrija, F., Retnani, E. B., Ridwan, Y., & Tiuria, R. (2001). Potential use of herbal anthelmintics as alternative anti-parasitic drugs for smallholder farms in developing countries. *Proceedings of the 10th Conference of the Association of Institutions for Tropical Veterinary Medicine, Copenhagen, Denmark.*

Satrija, F., Ridwan, Y., Tiuria, R., & Retnani, E. B. (1999). The impact of repeated doses of papaya latex against sheep infected with *Haemonchus contortus. . Hemera Zoa. , 81*(9-15).

Singh, S. P., & Sudhakar Rao, D. V. (2011). Papaya (Carica papaya L.). *Postharvest Biology and Technology of Tropical and Subtropical Fruits*

Woodhead Publishing Series in Food Science, Technology and Nutrition.

Tedeschi, Schofield, L. O. P., & Pell, A. N. (2008). Determining feed quality for ruminants using *in vitro* gas production technique. *Workshop on Modeling in Ruminant Nutrition: Application of the Gas Production Technique,, 4*, 15.

Tekletsadik, T., Tusari, S., Juntakool, S., & Prasanpanich, S. (2004). Effect of dry season cutting managementon subsequent forage yield and quality of Ruzi (*Brachiaria ruziziensis*) and Dwarf napier (*Pennisetum purpureum*. *L*) in Thailand. *Kastsart J. (Nat. Sci.), 38*(457-467).

Topps, J. H. (1996). Assessment of forage legumes as protein-rich supplement in ruminant production systems in Zimbabwe. In: Sustainable Feed Production and Utilisation for Smallholder Livestock Enterprises in Sub-Saharan Africa,. *Proceedings of the Second African Feed Resources Network (AFRNET).* , 201.

Urbano, M. Z. (2012). Effects of supplementation with tropical plants on the performance and parasite burden of goats. *Journal of Animal Sciences, Graduate School of the University Of Florida.*, 1-102.

Van Soest, P. J. (1994). The nutritional ecology of the ruminant. *2nd edition. Ithaca: Cornell Univ Pr.*, 476.

Weiss, P. W. (1994). Estimation of digestibility of forages by laboratory methods. In: Forage Quality, Evaluation and Utilization. *Madison, American Society of Agronomy Inc., Crop Science of America Inc. and Soil Science Society of America Inc.*, 644-682.

Wijitphan, S., Lorwilai, P., & Arkaseang, C. (2009). Effect of cuting heights on productivity and quality of King Napier grass (*Pennisetum purpureum* cv. King Grass) under irrigation. *Pakistan Journal of Nutrition. , 8*(8), 1244-1250.

Williams, B. A. (2000). Cumulative gas-production techniques for forage evaluation. *Forage Evaluation in Ruminant Nutrition*, 189-213.

Williams, B. A., van der Poel, A., Boer, H., & Tamminga, S. (1997). The effect of extrusion conditions on the fermentability of wheat straw and corn silage. *Journal of the Science of Food and Agriculture, 74*, 117-124.

Woodard, K. R., & Prine, G. M. (1991). Forage yield and nutritive value of elephant grass as affected by harvest frequency and genotype. *Argon. J. , 83*, 541-546.

Yamaguchi, T. (1980). Mutagenicity of isothiocyanates, isocyanates and thioureas on *Salmonella typhimurium*. *Agric. Biol. Chem., 44*, 3017-3018.

Yokota, H., Okajima, T., & Ohshima, M. (1992). Nutritive value of Napier grass (*Pennisetum purpureum* schum.) silage ensiled with molasses by goats. . *AJAS, 5*(1), 33-37.