THE EFFECT OF ANTHOCYANIN FROM PURPLE NAPIER GRASS (*PENNISETUM PURPUREUM* "PRINCE") ON RUMEN FERMENTATION, MILK YIELD, MILK COMPOSITION AND BLOOD ANTIOXIDANT ACTIVITY IN DAIRY GOATS

Narawich Onjai-uea

มหาวิทยาลัยเทคโนโลยีสุรนารี

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Production Technology

Suranaree University of Technology

Academic Year 2020

ผลของแอนโทไซยานินจากหญ้าเนเปียร์สีม่วง (*PENNISETUM PURPUREUM* “PRINCE”) ต่อกระบวนการหมักในกระเพาะรูเมน ผลผลิตน้ำนม องค์ประกอบน้ำนม และฤทธิ์ต้านอนุมูลอิสระในเลือดของแพะนม

นายณราวิชญ์ อ่อนใจเอื้อ

มหาวิทยาลัยเทคโนโลยีสุรนารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2563

THE EFFECT OF ANTHOCYANIN FROM PURPLE NAPIER GRASS (*PENNISETUM PURPUREUM* "PRINCE") ON RUMEN FERMENTATION, MILK YIELD, MILK COMPOSITION AND BLOOD ANTIOXIDANT ACTIVITY IN DAIRY GOATS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

Pakanit Kupittayanant

(Asst. Prof. Dr. Pakanit Kupittayanant)

Chairperson

Pramote Paen

(Assoc. Prof. Dr. Pramote Paengkoum)

Member (Thesis Advisor)

A.

(Assoc. Prof. Dr. Amonrat Molee)

Member

P. Lounglawan

(Asst. Prof. Dr. Pipat Lounglawan)

Member

Opart Pimpa

(Assoc. Prof. Dr. Opart Pimpa)

Member

Siwaporn Paengkoum

(Dr. Siwaporn Paengkoum)

Member

(Assoc. Prof. Flt. Lt. Dr. Kontorn Chamniprasart)
Vice Rector for Academic Affairs and Internationalization

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

มหาวิทยาลัยเทคโนโลยีสุรนารี

ณราวิชญ์ อ่อนใจเอื้อ : ผลของแอนโทไซยานินจากหญ้าเนเปียร์สีม่วง (*PENNISETUM PURPUREUM* “Prince”) ต่อกระบวนการหมักในกระเพาะรูเมน ผลผลิตน้ำนม องค์ประกอบน้ำนม และฤทธิ์ต้านอนุมูลอิสระในเลือดของแพะนม. (THE EFFECT OF ANTHOCYANIN FROM PURPLE NAPIER GRASS (*PENNISETUM PURPUREUM* “PRINCE”) ON RUMEN FERMENTATION, MILK YIELD, MILK COMPOSITION AND BLOOD ANTIOXIDANT ACTIVITY IN DAIRY GOATS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 178 หน้า.

วิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อ ศึกษาผลของแอนโทไซยานินจากหญ้าเนเปียร์สีม่วง ต่อกระบวนการหมักในกระเพาะรูเมน ผลผลิตน้ำนม องค์ประกอบน้ำนม และฤทธิ์ต้านอนุมูลอิสระในเลือดของแพะนม การศึกษาครั้งนี้ประกอบด้วย 3 การทดลอง

การทดลองที่ 1 เป็นการศึกษาผลของสายพันธุ์ ระยะปลูก และอายุการเก็บเกี่ยวหญ้าเนเปียร์ต่อผลผลิตหญ้า ลักษณะทางสัณฐานวิทยา องค์ประกอบทางเคมี และองค์ประกอบของแอนโทไซยานินอิทธิพลร่วมของหญ้า 2 สายพันธุ์ คือ หญ้าเนเปียร์ปากช่อง 1 และหญ้าเนเปียร์สีม่วง ระยะปลูก 3 ช่วง คือ 50×50 50×75 และ 75×75 ซม. และอายุการเก็บเกี่ยว 3 ระยะ คือ 45 60 และ 75 วัน หลังการเจริญเติบโตใหม่ ผลการทดลองพบว่า หญ้าเนเปียร์สีม่วงปลูกระยะ 75×75 ซม. โดยมีอายุการเก็บเกี่ยว 45 วัน จะมีจำนวนต้นหญ้า/กอ ค่า LSR (2.0) องค์ประกอบโปรตีน (12.06%) เหมาะสมสำหรับสัตว์เคี้ยวเอื้อง รวมถึงผลผลิต ether extract (EE) (kg/ha), crude fiber (CF) (kg/ha), Neutral Detergent Fiber (NDF) (kg/ha), hemicellulose (kg/ha) และองค์ประกอบแอนโทไซยานินสูงสุด

การทดลองที่ 2 เป็นการศึกษาผลของหญ้าเนเปียร์แบบสด และแบบหมักต่อการผลิตแก๊สในหลอดทดลอง สมรรถภาพการเจริญเติบโต กระบวนการหมักในกระเพาะรูเมน และประชากรจุลินทรีย์ในกระเพาะรูเมนของแพะ แพะเพศผู้จำนวน 24 ตัว สายพันธุ์ลูกผสมพื้นเมืองของประเทศไทย×แองโกลนูเบียน น้ำหนักตัวประมาณ 18.50±2.06 กก. การทดลองมี 4 ทรีทเมนต์คอมบิเนชัน ได้แก่ T1 = หญ้าเนเปียร์ปากช่อง 1 แบบสด T2 = หญ้าเนเปียร์ปากช่อง 1 แบบหมัก T3 = หญ้าเนเปียร์สีม่วงแบบสด T4 = หญ้าเนเปียร์สีม่วงแบบหมัก ผลการทดลองพบว่า หญ้าเนเปียร์สีม่วงแบบสด เพิ่มค่าพารามิเตอร์ของการผลิตก๊าซในหลอดทดลอง ความสามารถในการย่อยได้ของอาหารในหลอดทดลอง ปริมาณ NH_3-N ในกระเพาะรูเมน (ruminal NH_3-N) กรดโพรพิโอนิกในกระเพาะรูเมน (ruminal C_3) ปริมาณกรดไขมันระเหยได้ทั้งหมด (total VFA) สมรรถภาพการเจริญเติบโต การใช้ประโยชน์ของไนโตรเจน ยูเรียไนโตรเจนในเลือด (BUN) และจำนวนประชากร *Butyrivibrio fibrisolvens* ให้มีประสิทธิภาพและปริมาณมากขึ้น ในขณะที่ผลผลิตแก๊สมีเทน (CH_4) จำนวนประชากร methanogen และโปรโตซัวลดลงในแพะที่กินหญ้าเนเปียร์สีม่วงแบบสด

การทดลองที่ 3 เป็นการศึกษาผลของแอนโทไซยานินจากหญ้าเนเปียร์สีม่วงแบบหมักต่อผลผลิตน้ำนม องค์ประกอบของน้ำนม และฤทธิ์ต้านอนุมูลอิสระในเลือดของแพะรีดนม แพะสายพันธุ์ลูกผสมซาเนนเพศเมียจำนวน 18 ตัว น้ำหนักตัวโดยประมาณ 52.34±2.86 กก. การทดลองมี 3 ทรีทเมนต์ ได้แก่ กลุ่มควบคุม = หญ้าเนเปียร์ปากช่อง 1 แบบหมัก 100%; PNS 50% = การทดแทนกลุ่มควบคุมด้วยหญ้าเนเปียร์สีม่วงแบบหมัก 50% และ PNS 100% = หญ้าเนเปียร์สีม่วงแบบหมัก 100% ผลการทดลองพบว่า หญ้าเนเปียร์สีม่วงแบบหมัก 100% ช่วยเพิ่มองค์ประกอบของน้ำนม (น้ำตาลแลคโตส) ความสามารถในการเป็นสารต่อต้านออกซิเดชัน DPPH ความสามารถโดยรวมในการต่อต้านอนุมูลอิสระ (TAC) เอนไซม์ SOD และเอนไซม์ GST ในพลาสมาหลังการให้อาหารและน้ำนม ในขณะที่ระดับสาร MDA ในพลาสมาและน้ำนมลดลง นอกจากนี้ยังพบว่ามีองค์ประกอบของสารแอนโทไซยานินสูงสุดในน้ำนมของแพะนมที่เลี้ยงด้วยหญ้าเนเปียร์สีม่วงแบบหมัก 100%

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา ณราวิชญ์ อ่อนใจเมือ

ปีการศึกษา 2563 ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

NARAWICH ONJAI-UEA : THE EFFECT OF ANTHOCYANIN FROM PURPLE NAPIER GRASS (*PENNISETUM PURPUREUM* "PRINCE") ON RUMEN FERMENTATION, MILK YIELD, MILK COMPOSITION AND BLOOD ANTIOXIDANT ACTIVITY IN DAIRY GOATS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 178 PP.

ANTHOCYANIN/PURPLE NAPIER GRASS (*PENNISETUM PURPUREUM* "PRINCE")/RUMEN FERMENTATION/MILK YIELD/MILK COMPOSITION/ BLOOD ANTIOXIDANT ACTIVITY/DAIRY GOATS.

The objectives of this thesis were to investigate the effects of anthocyanin from Purple Napier grass on rumen fermentation, milk yield, milk composition and blood antioxidant activity in dairy goats.

Experiment I: This experiment investigated the effects of cultivars, plant spacing and harvesting age of Napier grass on forage yield, morphological characteristics, chemical composition and anthocyanin composition. Combinations of two cultivars of grasses: Napier Pakchong-1 and Purple Napier grass and three plant spacings: 50×50, 50×75 and 75×75 cm and three harvesting ages: 45, 60 and 75 days after regrowth cuttings. The results showed that Purple Napier grass planted 75×75 cm with harvesting at 45 days would contain the proper number tillers per plant, LSR value (2.0), crude protein composition (12.06%) for ruminants including the highest of ether extract (EE) (kg/ha), crude fiber (CF) (kg/ha), Neutral Detergent Fiber (NDF) (kg/ha) and hemicellulose (kg/ha) and anthocyanin composition.

Experiment II: This experiment investigated the effects of fresh and silage of Napier

grass on *in vitro* gas production, growth performance, rumen fermentation and microbial population in goat's rumen. Twenty-four male goats, crossbred Thai native ×Anglo-Nubian approximately 18.50±2.06 kg body weight. There were four treatment combinations: T1 = Fresh Napier Pakchong-1 grass, T2 = Napier Pakchong-1 grass silage, T3 = Fresh Purple Napier grass and T4 = Purple Napier grass silage. The results showed that fresh Purple Napier grass improved *in vitro* gas production parameters, *in vitro* digestibility, ruminal NH_3-N, ruminal propionic acid (C_3), total VFA, growth performance, nitrogen utilization, blood urea nitrogen (BUN) and *Butyrivibrio fibrisolvens*, while methane gas production (CH_4), methanogen and protozoa population decreased in goats fed fresh Purple Napier grass.

Experiment III: This experiment investigated the effects of anthocyanin from Purple Napier grass silage on milk yield, milk composition and blood antioxidant activity in lactating dairy goats. Eighteen female crossbred Saanen lactating goats approximately 52.34±2.86 kg in body weight. There were three treatments: control = Napier Pakchong-1 grass silage 100%, PNS 50% = control replaced with Purple Napier grass silage 50% and PNS 100% = Purple Napier grass silage 100%. The results showed that Purple Napier grass silage 100% enhanced milk composition (lactose), DPPH scavenging activity, TAC, SOD and GST enzymes in plasma after feeding and milk, while the level of MDA in plasma and milk decreased. Moreover, it was also found that the milk of dairy goats fed Purple Napier grass silage 100% treatment had highest anthocyanin composition.

School of Animal Technology and Innovation Student's Signature Navarich Onjai-uea

Academic Year 2020 Advisor's Signature

Co-advisor's Signature

ACKNOWLEDGEMENTS

First of all, I am deeply grateful to my advisor, Associate Professor Dr. Pramote Paengkoum, School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology for his constant encouragement, invaluable suggestion, and skilled technical assistance. He guided these experiments from the beginning and made correction as needed, include revision English in the manuscript and for providing the research funds for my thesis.

I would like to express gratitude to my co-advisor, Dr. Siwaporn Paengkoum, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University for their kind advice, suggestions, and comments for my thesis. Moreover that I would like to express gratitude to my committee. I would like to express my sincere thanks to Professor Juan J. Loor, the Department of Animal Sciences and the Division of Nutritional Sciences at the University of Illinois, Urbana-Champaign for gave me a big opportunity to been there.

My research and education would not have been possible without members of the small ruminant group (especially for Alisa Pinsuntear, Jiravan Khotsakdee, Anan Petlum, Bhutharit Vittayaphattananurak Raksasiri, Krung Wilachai, Kanokwan Kamkajon, Sorasak Thongpea, Ngo Thi Minh Suong, Tian Xingzhou, Rayudika Aprilia Patindra Purba, Thansamay Vorlaphim, Chao Ban and Nittaya Taethaisong)

I would like to thank undergraduate student (On-anong Puangklang, Kunakorn Viset, Chawakorn Khamfan, Duangporn Khuenpho, Sirirot Khotsalee and Thittiya Yothaphan) and PhD student of School of Animal Technology and Innovation,

Institute of Agricultural Technology, Suranaree University of Technology. Financial support from the Thailand Science Research and Innovation Fund through the Royal Golden Jubilee PhD Program (Grant no. PHD/0085/2557 to Narawich Onjai-uea and Assoc. Prof. Dr. Pramote Paengkoum) is gratefully acknowledged.

Finally, I would like to express my deepest gratitude to my father (Pachoen Onjai-uea), my mother (Ranu Onjai-uea) and my older brother (Assistant Professor Dr. Nawitcha Onjai-uea) for their understanding and permission for me to study in Suranaree University of Technology. Last but not least, I wish to express my sincere appreciation to all other who helped me to complete this thesis, but whose names do not appear here.

Narawich Onjai-uea

มหาวิทยาลัยเทคโนโลยีสุรนารี

CONTENTS

Page

CONTENTS (Continued)

Page

มหาวิทยาลัยเทคโนโลยีสุรนารี

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

มหาวิทยาลัยเทคโนโลยีสุรนารี

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

มหาวิทยาลัยเทคโนโลยีสุรนารี

CONTENTS (Continued)

Page

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF ABBREVIATIONS

a + *b*	=	Potential extent of gas production
a	=	Gas production from the immediately soluble fraction
ADF	=	Acid detergent fiber
ADG	=	Average daily gain
AOAC	=	Association of Official Analytical Chemists
b	=	Gas production from the insoluble fraction
BUN	=	Blood urea nitrogen
BW	=	Body weight
c	=	Gas production rate constant
C3G	=	Cyanidin-3-Glucoside
CF	=	Crude fiber
CP	=	Crude protein
Cya	=	Cyanidin
DE	=	Digestible energy
Del	=	Delphinidin
DM	=	Dry matter
DMI	=	Dry matter intake
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl
ECM	=	Energy-corrected milk
ED	=	Effective degradability

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF ABBREVIATIONS (Continued)

EE	=	Ether extract
FCM	=	Fat-corrected milk

LIST OF ABBREVIATIONS (Continued)

g	=	gram
GC	=	Gas chromatography
GE	=	Gross energy
GLM	=	General Linear Model
GST	=	Glutathione S-transferase
kcal	=	Kilocalorie
kJ	=	Kilojoules
M3G	=	Malvidin-3-o-glucoside
Mal	=	Malvidin
MDA	=	Malondialdehyde
ME	=	Metabolizable energy
MEC	=	Milk energy content
MEO	=	Milk energy output
NDF	=	Neutral detergent fiber
NH_3-N	=	Ammonia nitrogen
NP-1	=	Napier Pakchong-1 grass
NRC	=	National Research Council
OM	=	Organic matter
OMD	=	Organic matter digestibility
P3G	=	Pelargonidin-3-glucoside
Pel	=	Pelargonidin
Peo3G	=	Peonidin-3-o-glucoside
PN	=	Purple Napier grass

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF ABBREVIATIONS (Continued)

Real-time PCR	=	Real-time Polymerase chain reaction
SCC	=	Somatic cell count
SEM	=	Standard error of mean
SNF	=	Solid not fat
SOD	=	Superoxide dismutase
TAC	=	Total antioxidant capacity
TMR	=	Total mixed ration
TS	=	Total solids
TVFA	=	Total volatile fatty acid
VFA	=	Volatile fatty acid

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER I

INTRODUCTION

The body of an organism with a natural balance between the formation of free radicals caused by normal metabolic processes of cells with anti-oxidants inside the cell of the animal to act to prevent the accumulation and damage cells. Animals such as milk yield and during pregnancy are more prone to stress caused by oxidation. This situation is even worse when combined with environmental factors such as diet and physiological conditions (Castillo et al., 2005). When the level of free radicals exceeds the antioxidant within the cell, such as vitamins (C and E) and a group of enzymes that act as antioxidants, etc., resulting in cell damage by free radicals. This condition will cause a negative effect on milk yield and milk composition of dairy goat. It is necessary to get the source of antioxidants.

Antioxidants are found naturally in many foods can be divided into two groups. The first group consists of vitamins (A, B, C and E) and minerals (Zn, Cu and Se) compounds. The second group consists of phenols and flavonoids these secondary metabolites contained in the plant (Castaneda-Ovando, 2009). The anthocyanin (pigments) can be found in the flower and fruit of plants. Anthocyanin is classified in the group of flavonoids are also found in agricultural crops such as grapes, strawberries, berries, corn and grass, etc. Anthocyanin has many colors such as purple, red, and blue etc. that soluble in the water. This anthocyanin has been the focus of many researchers because it has antioxidant properties and such properties

make anthocyanin role to prevent chronic diseases such as heart disease (Cardiovascular disease), cancer and diabetes (Konczak and Zhang, 2004; Lule and Xia, 2005). The use of antioxidants may be an alternative way to balance the oxidation process and yield performance of the animals.

This research aims to study the use of Purple Napier grass in dairy goats. The planting of grass to measure the productivity of grass and concentration of anthocyanin. Evaluation of nutrient digestibility fermentation in the rumen and change of the enzyme in plasma is indicative of the effect of antioxidants of Purple Napier grass on yield and quality of milk.

1.1 Research hypotheses

1.1.1 Purple Napier grass (*Pennisetum purpureum* "Prince") harvest different plant spacing and harvesting age have different influences on forage yield, morphological characteristics, chemical composition and anthocyanin composition.

1.1.2 Purple Napier grass forms can improve *in vitro* gas production, growth performance, rumen fermentation and microbial population in goat's rumen.

1.1.3 Anthocyanin from Purple Napier grass have different influences on milk yield, milk composition and blood antioxidant activity in dairy goats.

1.2 Research objectives

1.2.1 To investigate the forage yield, morphological characteristics, chemical composition and anthocyanin composition of Purple Napier grass harvest under different plant spacing and harvesting age.

1.2.2 To examine the effect of fresh and silage forms of Purple Napier grass on *in vitro* gas production, growth performance, rumen fermentation and microbial population in goat's rumen.

1.2.3 To evaluate effect of anthocyanin from Purple Napier grass on milk yield, milk composition and blood antioxidant activity in dairy goats.

1.3 Scope and limitation of the study

This study was focused on the influence of Purple Napier grass (*Pennisetum purpureum* "Prince"), plant spacing, harvesting age (days after regrowth) in Korat soil series and forms of Purple Napier grass (fresh and silage) on their yield, chemical composition, anthocyanin content, rumen fermentation, nutrient digestibility, milk yield, milk composition and blood antioxidant activity in female crossbred Saanen lactating goats.

1.4 Expected results

1.4.1 Grass planting strategy using plant spacing and harvesting age to improve forage yield, morphological characteristics, chemical composition and anthocyanin composition of Purple Napier grass.

1.4.2 Characteristics of anthocyanin content in Purple Napier grass.

1.4.3 Nutrition strategy using Purple Napier grass to improve goat performance, rumen fermentation, milk yield, milk composition and antioxidant activity in plasma of dairy goat.

1.5 References

Castaneda-Ovando, A., Pacheco-Hernandez, M., Paez-Hernandez, M., Rodriguez, J., and Galan-Vidal, G. (2009). Chemical studies of anthocyanins: a review. **Food Chemistry**. 113(4): 859-871.

Castillo, C., Hernandez, J., Bravo, A., Lopez-Alonso, M., Pereira, V., and Benedito, J. L. (2005). Oxidative status during late pregnancy and early lactation in dairy cows. **The Veterinary Journal**. 169(2): 286-292.

Konczak, I., and Zhang, W. (2004). Anthocyanins-more than natures colours. **Journal of Biomedicine and Biotechnology**. 2004(5): 239-240.

Lule, S., and Xia, W. (2005). Food Phenolics, Pros and Cons: A Review. **Food Reviews International**. 21(4): 367-388.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER II

REVIEW OF LITERATURE

2. 1 Purple Napier grass (Hanna and Ruter, 2008)

Classification:

Botanical: "Prince" is a cultivar of *Pennisetum purpureum*.

Parentage: Seed from unknown Napier grass accessions.

Propagation: Vegetatively by stem cuttings.

The plant is perennial in USDA hardiness Zones 8410, and can be grown as a vigorous annual in more northern zones. The height of the plant ranged from 94 to 200 cm under different environmental conditions, with an average height of 159 cm. The base circumference ranged from 66 to 259 cm, with an average of 157 cm, with 20 to 91 tillers, averaging 52. The top canopy spread, or diameter of arching leaves at the top of the plant ranged from 138 to 259 cm, with an average of 186 cm. The plant is quite vigorous, and produced 40 tillers in one year under non-irrigated conditions, and twice that number of tillers under irrigated conditions. Two or three node cuttings with foliage removed root well in 8.3 cm liner pots, with rooting percentages in excess of 90%. Hard pruning to control plant size results in good regrowth.

Initial leaves on "Prince" emerge with a purple midrib and a mottled mixture of purple and green blade. Later, the leaves emerge purple on both the adaxial and abaxial sides from the whorls. The leaf blade is flat, narrow, pointed at the end, sessile to the stem, and connected to the stem internode via the leaf sheath. The inside of the

leaf collar is lined with abundant 2 mm long trichomes. There is bloom on the stems, abundant trichomes (1 to 2 mm long) on the sheath, trichomes (up to 4 mm long) 12 cm up the margin of the blade from the collar, and sparse trichomes (1 mm long) on the adaxial leaf surface. Margins of leaf blades have prominent trichomes (0.2 mm long). The abaxial leaf surface is smooth. Leaf color of both the adaxial and abaxial leaf surfaces best fits the greyed-purple. The length of leaf ranges from 84 to 86 cm under different environmental conditions, with an average length of 84 cm. The leaf width ranges from 29 to 35 mm, with an average width of 31 mm.

2. 2 Oxidative stress

In the body there is a natural balance between the formation of free radicals during the normal metabolism of the cells and the endogenous antioxidant capacity of the animal that would prevent free radicals from accumulating and harming the cells. Weiss (1998) reported that the level of free radicals can exceed the antioxidant capacity of the animal leading to oxidative stress. High producing dairy animal are tend to oxidative stress and the situation can be increased negative effect under certain environmental, physiological and dietary conditions (Castillo et al., 2005).

Many condition of physiological affects the productivity of animals. The levels of free radical can be higher than the endogenous antioxidant and resulting uncomfortable condition, this condition will give negative effect to the productivity of dairy goats especially on milk yield and milk quality. Oxidative stress in a living organism is a result of an imbalance between reactive oxygen metabolites (ROM) production and neutralizing capacity of antioxidant mechanisms (Sies, 1991). Oxidative stress lead to the modification physiological and metabolic functions that

could alter the physiology and could cause pathologies (Miller and Brezeinska-Slebodizinska, 1993).

2. 3 Use of antioxidants in oxidative stress

In terms of nutrition, the use of antioxidants has been proven as a successful technique. Various studies have presented evidence that *in vitro* blastocyst formation is enhanced by the addition of vitamin E, the most common lipid-soluble antioxidant in animal cells, and by other extracellular antioxidants such as anthocyanins when embryos are exposed to heat stress (Olson and Seidel, 2000; Sakatani, et al., 2007). Natural antioxidant found in many kinds of food, the first group of nutrients consisting of vitamins (A, B, C and E) and minerals (Zn, Cu and Se), the second group of non-nutritional food consisting of phenol compounds, flavonoids, steroids, alkaloids, terpenoids, tannins and saponins.

Antioxidant may be an alternative to improve lipid metabolism and oxidative status. Vázquez-Añón et al. (2008) reported that antioxidant cloud be effective to improve oxidative balance and performance in lactating cows by improving rumen metabolism but dietary that source of antioxidant did not affect on milk compositions from cows such as protein, fat, lactose, total solids and non-fat solids (Wang et al., 2010).

Natural antioxidant present in foods, especially fruit and vegetables consisting three main groups, vitamins, phenolics and carotenoids. Antioxidants present in food or as supplements such as vitamin E, vitamin C, ß-carotene, flavonoids could improve health protection against free radicals as an attempt to prevent damage caused by

oxidation process. It encourages more exploration of natural materials as source of antioxidants.

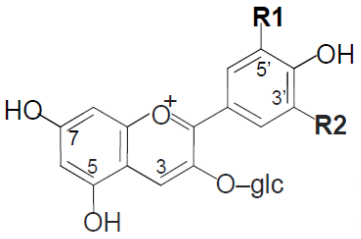
Sources of antioxidants could be found in many vegetables, fruits, herbs and spices (Howard et al., 2002). Antioxidant activity found in flavonoids and polyphenols (Panovskai et al., 2005), together with vitamin C and carotenoids protect the tissue from oxidative stress (Scalbert and Williamson, 2000). The benefits exhibited by the use of antioxidants to treat oxidative stress are due to their ability to scavenge free radicals, reducing the total number of reactive oxygen species (Lykkesfeldt and Svendsen, 2007). Sakatani et al. (2007) reported that extracellular treatment with antioxidants can also aid in increasing intracellular antioxidants already present, such as glutathione, which further helps to protect cells from reactive oxygen species.

2. 4 Anthocyanins

Anthocyanins are natural pigments responsible for the blue, purple, red and orange colors of many fruits and vegetables. More than 500 different anthocyanins have been described in the literature. Anthocyanins are food bioactive compounds with a double interest, one technological, due to their impact on the sensorial characteristics of food products, and the other for their health related properties throw different biological activities, one of them being their implication on cardiovascular disease risk protection (Pascual-Teresa and Sanchez-Ballesta, 2008).

Anthocyanins are mainly present in nature in the form of heterosides. The aglycone form of the anthocyanins, so-called anthocyanidin, is structurally based on the flavilium or 2-phenylbenzopyrilium cation, with hydroxyl and methoxyl groups present at different positions of the basic structure. Depending on the number and

position of the hydroxyl and methoxyl groups as substituents, different anthocyanins have been described, and six of them are commonly found in fruits and vegetables: pelargonidin, cyanidin, delphinidin, petunidin, peonidin and malvidin (Figure 2.1) (USDA, 2003; Pascual-Teresa et al., 2000).



R1	R2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH_3	Petunidin
OCH_3	H	Peonidin
OCH_3	OCH_3	Malvidin

Figure 2.1 Anthocyanin Structure (Anthocyanidin) (Castaneda-Ovando et al., 2009).

Anthocyanidins are unstable to changes in temperature, pH, light and water-insoluble which several factors contribute to the stability of anthocyanins including storage temperature, pH, concentration, light, chemical structure, oxygen, solvents, proteins, metallic ions, flavonoids and presence of enzymes (Castaneda-Ovando et al., 2009) so that they do not occur usually in their free state. Instead, they are present in the cell vacuole linked to sugars, which provide stability and water solubility. These glycosides are called anthocyanins. Some time ago, the known glycosidic variation among these pigments was restricted to four main types: 3-monoglycosides, 3-diglycosides, 3,5-diglycosides and 3-diglycoside-5-monoglyco-sides. The most common sugar present was glucose, but rhamnose, xylose and galactose were also encountered. More recent research has revealed many more complex structures. There are many anthocyanins with acyl substituents linked to sugars, aliphatic acids (such as malonic, succinic, malic and acetic acid), cinnamic acids (such as *p*-coumaric, ferulic, or

sinapic acid) and pigments with both aliphatic and aromatic substituents. A further complexity in some anthocyanins results from the presence of several acylated sugars in the structure. These anthocyanins have sometimes been designated as polyglycosides (Harborne, 1993; USDA, 2003; Pascual-Teresa et al., 2000). The main dietary sources of anthocyanins are red fruits like berries and red grapes, red wine, cereals and purple corn, as well as some vegetables such as red cabbage (Table 2.1). Daily consumption of total anthocyanins has been estimated to be between 3 and 215 mg/day (Wu et al., 2006; Frankel et al., 1995; Kuhnau, 1976; Chun et al., 2007).

Table 2.1 Anthocyanin contents in food and plant.

Food	Content (mg/100 g fresh weight)	References
Apple	0-60	Koponen et al. (2007); Wojdylo et al. (2008)
Black bean	24.1-44.5	Macz-Pop et al. (2006)
Black currant	130-476	Timberlake (1988); Wu et al. (2006)
Blackberry	82.5-325.9	Torre and Barritt (1977); Wang and Lin (2000)
Blueberry	61.8-299.6	Prior et al. (1998); Wang et al. (2008)
Cherry	2-450	Mazza and Miniati (1993); Gao and Mazza (1995)
Cranberry	67-140	Wu et al. (2006); Koponen et al. (2007)
Pomegranate	15-252	Alighourchi et al. (2008)
Raspberry	20-687	Wang and Lin (2000); Wu et al. (2006)
Saskatoon berry	234	Koponen et al. (2007)
Strawberry	19-55	Lopes-da-Silva et al. (2002)

The average intake of dietary flavonoids is estimated at about 23 mg/day for Holland (Lou et al., 1999) and 650 mg/day for USA (Monagas et al., 2007; Maatta-Riihinen et al., 2005). The daily intake of anthocyanins in humans has been estimated at 180-215 mg/day in USA (Monagas et al., 2007). This value is considerably higher than the intake of other flavonoids such as flavones and flavonols in the Dutch diet (23 mg/day, measured as aglycones) (Lou et al., 1999). Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. Servings of 100 g of berries can provide up to 500 mg of anthocyanins (Harborne and Baxter, 1999).

2. 5 Metabolism of anthocyanin

The bioavailability of anthocyanins has been examined in humans. Charron et al. (2007) examined the concentration of anthocyanins being excreted in the urine of twelve, healthy volunteers. They were fed an anthocyanin free diet and then one of three treatments of red cabbage, 100, 200, or 300 g of cooked red cabbage. The results showed a linear response, with those eating higher levels of cabbage having greater excretion of anthocyanins, but the levels seen in the urine from the 200 or 300 g diet were not two and three times higher than the 100 g treatment group (Charron et al., 2007). It was also found that non-acylated anthocyanins were excreted at a significantly higher level in the urine.

Another study by Kurilich et al. (2005) also used twelve, healthy volunteers who were fed an anthocyanin free diet and then placed in treatment groups and fed 250 g raw purple carrots, 250 g cooked purple carrots, or 500 g of cooked purple carrots. This study measured blood levels of anthocyanins rather than urine

concentrations. All three treatment groups showed very similar levels of anthocyanins in the blood (Kurilich et al., 2005). Although there are different forms of anthocyanins that may not have been measured using these tests, they do demonstrate that anthocyanins have a low bioavailability.

Factors affecting bioavailability of anthocyanins include acylation and structure (Charron et al., 2007; Yi et al., 2006). When anthocyanins are absorbed into the bloodstream, the process of absorption and excretion is fairly rapid. Both the liver and kidney modify anthocyanins by the processes of methylation and also mono-glucuronidation. This is done more by the liver than the kidneys (Talavera et al., 2005). Excretion begins around 20 minutes after absorption, with bloodstream levels reaching their peak between 30 minutes and 2 hours. By entering the blood steam, anthocyanins are circulated throughout the entire body and levels can be measured in other tissues (Prior and Wu, 2006; Talavera et al., 2005). Although anthocyanins are absorbed to an extent, they are excreted to a large extent unmetabolized. The amount of anthocyanins seen in the urine compared to the amount ingested demonstrates the low levels of absorption (Cao et al., 2000).

The anthocyanins are passing through the body unabsorbed. The bioavailability of the various anthocyanins differs dependent upon the structure of the anthocyanin. The specific anthocyanins are absorbed and excreted to differing degrees (McGhie et al., 2003). This is true in both rat and human models. If the bioavailability of anthocyanins is relatively low, it follows that the colon would have a relatively high concentration of undigested anthocyanins present after ingestion of an anthocyanin rich food. Using the colon cancer cell model replicates these conditions, however the form that ingested anthocyanins are in when they reach the colon is still unclear.

Several studies have indicated that, when ingested orally, anthocyanins are absorbed by the stomach and gut of monogastrics and are eventually detected, structurally intact, in the blood (Passamonti et al., 2003; Miyazawa et al., 1999; Mazza et al., 2002). Very little research can be found which directly utilizes anthocyanins in ruminant animals. One such study used anthocyanin-rich corn to evaluate the effects of rumen fermentation on anthocyanins. The results of this study indicate that anthocyanins in the corn remain very stable under rumen fermentation (Hosoda et al., 2009). This evidence, coupled with the fact that the abomasum and gut of ruminants have similar digestive and absorptive functions as the stomach and gut of monogastrics (Dijsktra et al., 2005), would lead to the assumption that, if anthocyanins are able to pass through the rumen of an animal without being degraded, they should be absorbed by the abomasum and gut.

2. 6 Anthocyanin and rumen fermentation

The changes in the rumen fermentation from anthocyanin studies in animals (*in vivo*) and laboratory (*in vitro*) (Table 2.2). Hosoda et al. (2012b) studied milking cows receiving maize containing anthocyanin found that the concentration of acetic acid (C_2), propionic acid (C_3), butyric acid (C_4) and total volatile fatty acid (TVFA) in rumen fluid did not differ ($P>0.05$).

Leatherwood (2014) studied the effect of anthocyanin extracts from Purple-fleshed sweetpotatoes (PFSP) in aqueous extract and powder. *In vitro* experiment found that purple-fleshed sweetpotato extract (PE) has concentrations of volatile fatty acid higher than diluted purple-fleshed sweet potato extract of 50 percent (DE). The level of volatile fatty acids of purple-fleshed sweetpotato powder 1 g (GP (1 g)) higher

than purple-fleshed sweet potato powder 5 g (GP (5 g)). It was also found that purple-fleshed sweet potato extract (PE) and purple-fleshed sweetpotato powder 1 g (GP (1 g)) have the highest concentrations of propionic acid.

Table 2.2 Effect of anthocyanin on pH and volatile fatty acids in rumen fluid.

Treatment	pH	Oraganic acid				References
		C_2(%)	C_3(%)	C_4(%)	TVFA (mmol)	
Control	6.57	63.20	21.00	11.60	107.50	Hosoda et al. (2012b) (*In vivo*)
AR	6.48	62.30	21.20	12.30	109.20	
PE	5.90	52.18	36.54[a]	11.86	15.60	Leatherwood (2014) (*In vitro*)
DE	6.10	59.49	30.19[ab]	10.45	7.85	
GP (1g)	4.4	67.22	27.52[a]	5.41	47.90	
GP (5g)	4.4	80.25	14.89[b]	3.98	62.80	

[a–b]Means within a column with no common superscripts differ significantly (P<0.05); AR = Anthocyanin rich-corn; PE= 1 ml Purple-Fleshed Sweetpotato Extract; DE= 1 ml 50% Diluted Purple-Fleshed Sweetpotato Extract; GP (1 g)= 1 g Purple-Fleshed Sweetpotato Powder; GP (5 g)= 5 g Purple-Fleshed Sweetpotato Powder.

2. 7 Anthocyanin and plasma antioxidant enzyme

The body has an extensive system of antioxidant enzymes to safely remove free radicals formed by metabolism and the immune system. These enzymes protect the body's cells from reactive oxygen species (ROS), reactive nitrogen species (RNS) and any other environmental free radicals by directly inhibiting the formation of, or

metabolize, free radicals. Many of these antioxidant enzymes function together (Thomas, 2006). Antioxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR). Superoxide anions, one type of free radical, are neutralized by superoxide dismutase into hydrogen peroxide and oxygen. Catalase converts hydrogen peroxide to water (Thomas, 2006). GR, GST, and GPx are found primarily in the cytosol of cells.

These enzymes function individually, but also together, to remove pro-oxidants from the cell. GPx, like catalase, converts hydrogen peroxide to water (Paglia and Valentine, 1967). It uses reduced glutathione to reduce hydrogen peroxide to water. GR catalyzes the reduction of oxidized glutathione and NADPH back to reduced glutathione (Carlberg and Mannervik, 1975; Xia et al., 1985). The reduced glutathione can be used to chelate metals or again as a substrate for GPx. GST is a conjugation enzyme that transfers glutathione to other molecules. Glutathione is itself a reducing agent. It makes molecules water-soluble, which allows them to be easily excreted. Making the molecules water-soluble is one way for the body to expel mutagens and carcinogens (Habig et al., 1974).

GST and GPx both remove peroxides from the body (Paglia and Valentine, 1967; Shih et al., 2007). Thus GST is considered to be both a phase 2 enzyme and anantioxidant enzyme. The antioxidant response element (ARE) reacts to chemical stress in the cell and induces GST and NADPH: quinone oxidoreductase, another antioxidant enzyme. It has been determined that ARE responds to increased ROS levels or decreased antioxidant levels, such as a decrease in glutathione. Activation of the ARE is primarily controlled by nuclear factor E2-related factor 2 (Nrf2). Nrf2 is

normally bound to Kelch-like erythroid CNC homologue (ECH)-associated protein 1 (Keap1).

ROS react with cysteine bonds on the Keap1 protein and as a result the Nrf2 disassociates with Keap1. The Nrf2 is then free to move into the nucleus where is activates ARE, so ARE can in turn activate antioxidant enzymes. When the ARE is activated, it directs the formation of messenger-RNA to code for peptides and proteins to make antioxidant enzymes. Nrf2 is sensitive to levels of ROS and other pro-oxidants, but also antioxidants, including phenolic compounds, like anthocyanins. Therefore, the Nrf2/ARE pathway could be one more way anthocyanins increase the activities of GST, GR and GPx in the cells (Shih et al., 2007; Nguyen et al., 2009).

The benefits of feeding anthocyanin-rich corn to dairy cattle reported by Hosoda et al. (2012a) (Table 2.2). Anthocyanin-rich corn diet had significantly lower aspartate aminotransferase in holstein cattle, this lower levels indicates a healthier liver. Dairy cow that received the anthocyanin-rich corn diet had also had significantly higher levels of this superoxide dismutase.

Hosoda et al. (2012b) reported that the effect of the supplementation of purple pigment from anthocyanin-rich corn on blood antioxidant activities and oxidation resistance in sheep, sheep that received the anthocyanin-rich corn diet had a significant increase in the SOD activity of the plasma, although the total antioxidant capacity and glutathione concentration in the plasma of both treatment groups were similar. Hosoda et al. (2012c) found that the feeding of the purple rice silage led to an elevation in plasma activity of superoxide dismutase in sheep, which is an important antioxidant enzyme. Evidence from these, suggest that purple forages may become a source of anthocyanin to improve oxidative status in ruminants (Hosoda et al., 2012c).

Table 2.3 Plasma metabolite concentrations and enzyme and antioxidant activities in ruminants fed a diet containing control or anthocyanin forages.

Animals	Treatment	TAC (μmol/L)	SOD (U/ml)	GSH (μ/ml)	AST (IU/L)	MDA (μmol/L)	8-OHdG (μg/d)	References
Dairy cow	Control	1,060	2.4[b]	-	97	-	-	Hosoda et al. (2012a)
	AR corn silages	1,040	3.9[a]	-	86	-	-	
Sheep	Control	453.8	164.2[b]	5.1	-	0.16	2.8	Hosoda et al. (2012b)
	Purple corn pigment	458.8	184.4[a]	5.9	-	0.16	2.3	
Sheep	Control	463.2	318.7[b]	4.2	-	0.16	261.9	Hosoda et al. (2012c)
	Purple rice silage	470.2	360.2[a]	4.0	-	0.16	247.3	

[a–b]Means within a column with no common superscripts differ significantly ($P<0.05$).
TAC = total antioxidant capacity; SOD = superoxide dismutase; GSH = glutathione; AST = aspartate aminotransferase; MDA = malondialdehyde; 8-OHdG = 8-hydroxy-2′-deoxyguanosine.

2. 8 Anthocyanin with milk yield and compositions

Anthocyanin in purple corn (*Zea mays* L.) has been reported to show several functional and biological attributes, displaying antioxidant, antiobesity and antidiabetic effects in monogastric animals. Hosoda et al. (2012a) reported that the effect of feeding anthocyanin-rich corn (*Zea mays* L., Choko C922) silage on milk yield and compositions in lactating dairy cows (Table 2.4).

Table 2.4 Effect of anthocyanin on milk yield and composition.

Item	Treatment		SEM	*P*-value
	Control	Anthocyanin-rich		
Milk yield (kg/day)	28.50	27.10	0.30	0.094
FCM yield (kg/day)	27.00	26.10	0.20	0.073
Composition (%)				
Fat	3.66	3.76	0.05	-
Protein	3.36	3.38	0.02	-
Lactose	4.64	4.58	0.01	0.083
Total solids	12.67	12.73	0.06	-
Solids-not-fat	9.00	8.96	0.01	0.086

SEM = Standard error of the mean.

Source: adapted from Hosoda et al. (2012a).

The cows were fed diets based on the control corn compare with the anthocyanin-rich corn silage (AR treatment). The average milk yield and the yield of 4% fat corrected milk in the AR-treatment cows tended ($P<0.10$) to be reduced in comparison to those in the control cows. No significant difference was detected between the treatments in the fat, protein and total solid contents of the milk. The milk lactose composition of cows receiving the AR treatment tended ($P<0.10$) to be lower than that of the control cows, which led to a decrease in solids-not-fat composition in the AR treatment (Hosoda et al., 2012a).

2.9 Reference

Cao, G., Muccitelli, H., Sanchez-Moreno, C., and Prior, R. (2000). Anthocyanins are absorbed in glycated forms in elder women: a pharmacokinetic study. **The American Journal of Clinical Nutrition**. 73(5): 920-926.

Carlberg, I., and Mannervik, B. (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. **Journal of Biological Chemistry**. 250(14): 5475-5480.

Castaneda-Ovando, A., Pacheco-Hernandez, M., Paez-Hernandez, M., Rodriguez, J., and Galan-Vidal, G. (2009). Chemical studies of anthocyanins: a review. **Food Chemistry**. 113(4): 859-871.

Castillo, C., Hernandez, J., Bravo, A., Lopez-Alonso, M., Pereira, V., and Benedito, J. L. (2005). Oxidative status during late pregnancy and early lactation in dairy cows. **The Veterinary Journal**. 169(2): 286-292.

Charron, C., Clevidence, B., Britz, S., and Novotny, J. (2007). Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage. **Journal of Agricultural and Food Chemistry**. 55(13): 5354-5362.

Chun, O. K., Chung, S. J., and Song, W. O. (2007). Estimated dietary flavonoid intake and major food sources of U.S. adults. **Journal of Nutrition**. 137(5): 1244-1252.

Dijsktra, J., Forbes, J., and France, J. (2005). **Quantitative aspects of ruminant digestion and metabolism.** Wallingford, UK: CABI Publishing. pp. 1-10.

Frankel, E. N., Waterhouse, A. L., and Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. **Journal of Agricultural and Food Chemistry**. 43(4): 890-894.

Gao, L., and Mazza, G. (1995). Characterization, quantification and distribution of anthocyanins and colourless phenolics in sweet cherry. **Journal of Agricultural and Food Chemistry**. 43(2): 343-346.

Habig, W., Pabst, M., and Jakoby, W. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. **Journal of Biological Chemistry**. 249(22): 7130-7139.

Hanna, W. W., and Ruter, J. M. (2008). *Pennisetum purpureum* 'Prince'. **United States Plant Patent**. US PP18,509 P3. Available source: https://patentimages. storage.googleapis.com/pdfs/USPP18509.pdf.

Harborne, J. B. (1993). **The Flavonoids: Advances in Research since 1986**; Chapman and Hall: London, UK.

Harborne, J. B., and Baxter, H. (1999). **The Handbook of Natural Flavonoids Volume 2**. Wiley: West Sussex, UK.

Hosoda, K., Eruden, B., Matsuyama, H., and Shioya, S. (2009). Silage fermenative quality and characteristics of anthocyanin stability of anthocyanin-rich corn (*Zea mays* L.). **Asian-Australasian Journal of Animal Sciences**. 22(4): 528-533.

Hosoda, K., Eruden, B., Matsuyama, H., and Shioya, S. (2012a). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Journal of Animal Science**. 83(6): 453-459.

Hosoda, K., Matsuo, M., Miyaji, M., Matsuyama, H., Maeda, H., Ohta, H., Kato H., and Nonaka, K. (2012b). Fermentative quality of purple rice (*Oryza sativa* L.) silage and its effects on digestibility, ruminal fermentation and oxidative status markers in sheep: A preliminary study. **Grass and Forage Science**. 58(3): 161-169.

Hosoda, K., Miyaji, M., Matsuyama, H., Haga, S., Ishizaki, H., and Nonaka, K. (2012c). Effect of supplementation of purple pigment from anthocyanin-rich corn (*Zea mays* L.) on blood antioxidant activity and oxidation resistance in sheep. **Journal of Livestock Science**. 145(1-3): 266-270.

Howard, L. R., Pandjaitan, N., Morelock, T., and Gil, M. I. (2002). Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. **Journal of Agricultural and Food Chemistry**. 50(21): 5891-5896.

Koponen, J. M., Happonen, A. M., Mattila, P. H., and Torronen, A. R. (2007). Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. **Journal of Agricultural and Food Chemistry**. 55(4): 1612-1619.

Kuhnau, J. (1976). The flavonoids. A class of semi-essential food components: Their role in human nutrition. **World Review of Nutrition and Dietetics**. 24: 117-191.

Kurilich, A., Clevidence, B., Britz, S., Simon, P., and Novotny, J. (2005). Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. **Journal of Agricultural and Food Chemistry**. 53(16): 6537-6542.

Leatherwood, W. L. (2014). **Effect of anthocyanins from Purple-fleshed Sweetpotatoes on *in vitro* fermentation by rumen microbial cultures**. Animal Science. North Carolina State University.

Lopes-da-Silva, F., de Pascual-Teresa, S., Rivas-Gonzalo, J., and Santos-Buelga, C. (2002). Identification of anthocyanin pigments in strawberry (cv Camarosa) by LC using DAD and ESI-MS detection. **European Food Research and Technology**. 214(3): 248-253.

Lou, H. X., Yamazaki, Y., Sasaki, T., Uchida, M., Tanaka, H., and Oka, S. (1999). A-

type proanthocyanidins from peanut skins. **Phytochemistry**. 51(2): 297-308.

Lykkesfeldt, J., and Svendsen, O. (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals. **The Veterinary Journal**. 173(3): 502-511.

Maatta-Riihinen, K. R., Kahkonen, M. P., Torronen, A. R., and Heinonen, I. M. (2005). Catechins and procyanidins in berries of Vaccinium species and their antioxidant activity. **Journal of Agricultural and Food Chemistry**. 53(22): 8485-8491.

Macz-Pop, G. A., Rivas-Gonzalo, J. C., Perez-Alonso, J., and Gonzalez-Paramas, A. M. (2006). Natural occurrence of free anthocyanin aglycones in beans (*Phaseolus vulgaris* L.). **Food Chemistry**. 94(3): 448-456.

Mazza, G., and Miniati, E. (1993). **Anthocyanins in Fruits, Vegetables, and Grains**; CRC Press: Boca Raton, FL, USA.

Mazza, G., Kay, C., Cottrell, T., and Holub, B. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. **Journal of Agricultural and Food Chemistry**. 50(26): 7731-7737.

McGhie, T., Ainge, G., Barnett, L., Cooney, J., and Jensen, D. (2003). Anthocyanin glucosides from berry fruit are absorbed and excreted unmetabolized by both human and rats. **Journal of Agricultural and Food Chemistry**. 51(16): 4539-4548.

Miller, J. K., and Brezeinska-Slebodizinska, E. (1993). Oxidative stress, antioxidants, and animal function. **Journal of Dairy Science**. 76(9): 2812-2823.

Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., and Someya, K. (1999). Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. **Journal of Agricultural and Food Chemistry**. 47(3): 1083-1091.

Monagas, M., Garrido, I., Lebron-Aguilar, R., Bartolome, B., and Gomez-Cordoves, C. (2007). Almond (Prunus dulcis (Mill.) D.A. Webb) skins as a potential source of bioactive polyphenols. **Journal of Agricultural and Food Chemistry**. 55(21): 8498-8507.

Nguyen, T., Nioi, P., and Pickett, C. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. **Journal of Biological Chemistry**. 284(20): 13291-13295.

Olson, S., and Seidel Jr, G. (2000). Culture of *in vitro*-produced bovine embryos with vitamin E improves development development *in vitro* and after transfer to recipients. **Biology of Reproduction**. 62(2): 248-252.

Paglia, D. E., and Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. **The Journal of Laboratory and Clinical Medicine**. 70(1): 158-169.

Panovskai, T. K., Kulevanova, S., and Stefova, M. (2005). *In vitro* antioxidant activity of some Teucrium species Lamiaceae. **Acta Pharmaceutica**. 55(2): 207-214.

Pascual-Teresa, S., and Sanchez-Ballesta, M. T. (2008). Anthocyanins: from plant to health. **Phytochemistry Reviews**. 7(2): 281-299.

Pascual-Teresa, S., Santos-Buelga, C., and Rivas-Gonzalo, J. C. (2000). Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. **Journal of Agricultural and Food Chemistry**. 48(11): 5331-5337.

Passamonti, S., Vrhovsek, U., Vanzo, A., and Mattivi, F. (2003). The stomach as a site for anthocyanins absorption from food. **FEBS Lett.** 544(1-3): 210-213.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., and Mainland, C. M. (1998). Antioxidant

capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. **Journal of Agricultural and Food Chemistry**. 46(7): 2686-2693.

Sakatani, M., Suda, I., Oki, T., Kobayashi, S., Kobayashi, S., and Takahashi, M. (2007). Effects of Purple-Fleshed sweet potato anthocyanins on development and intracellular redox status of bovine preimplantation embryos exposed to heat shock. **Journal of Reproduction and Development**. 53(3): 605-614.

Scalbert, A., and Williamson, G. (2000). Dietary intake and bioavaibility of polyphenols. **Journal of Nutrition**. 130(8): 2073S-2085S.

Shih, P. H., Yeh, C. T., and Yen, G. C. (2007). Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. **Journal of Agricultural and Food Chemistry**. 55(23): 9427-9435.

Sies, H. (1991). Oxidative stress: introduction. In **Oxidative Stress: Oxidants and Antioxidants**. ed. SIES, H., pp. xv-xxii. Academic Press, London.

Talavera, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison, J., and Remesy, C. (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney and brain. **Journal of Agricultural and Food Chemistry**. 53(10): 3902-3908.

Thomas, J. A. (2006). Oxidant defense in oxidative and nitrosative stress. In: **Modern Nutrition in Health and Disease**. 10 ed. (Shils ME, Shike M, Ross AC, Caballero B and Cousins RJ, eds.) Hagerstown: Lippincott Williams & Wilkins. pp. 685-694.

Timberlake, C. F. (1988). The biological properties of anthocyanin compounds. **Natural**

food colours. 1: 4-15.

Torre, L. C., and Barritt, B. H. (1977). Quantitative evaluation of Rubus fruit anthocyanin pigments. **Journal of Food Science**. 42(2): 488-490.

USDA (U.S. Department of Agriculture). (2003). **USDA Database for the Flavonoid Content of Selected Foods-2003**. 176 pp.

Vázquez-Añón, J. N., Bowman, G., Hampton, T., Atwell, C., Vazquez, P., and Jenkins, T. (2008). Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. **Journal of Dairy Science**. 90(9): 4361-4867.

Wang, S. Y., and Lin, H. S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. **Journal of Agricultural and Food Chemistry**. 48(2): 140-146.

Wang, S. Y., Chen, C. T., Sciarappa, W., Wang, C. Y., and Camp, M. J. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. **Journal of Agricultural and Food Chemistry**. 56(14): 5788-5794.

Wang, Y. M., Wang, J. H., Wang, C., Wang, J. K., Chen, B., Liu, J. X., Cao, H., and Guo, F. C. (2010). Effect of dietary antioxidant and energy density on performance and antioxidative status of transition cows. **Asian-Australasian Journal of Animal Sciences**. 23(10): 1299-1307.

Weiss, W. P. (1998). Requirements of fat-soluble vitamins for dairy cows: A review. **Journal of Dairy Science**. 81(9): 2493-2501.

Wojdylo, A., Oszmianski, J., and Laskowski, P. (2008). Polyphenolic compounds and antioxidant activity of new and old apple varieties. **Journal of Agricultural and**

Food Chemistry. 56(15): 6520-6530.

Wu, X. L., Beecher, G. R. Holden, J. M. Haytowitz, D. B. Gebhardt, S. E., and Prior, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. **Journal of Agricultural and Food Chemistry.** 54(11): 4069-4075.

Xia, Y., Hill, K., and Burk, R. (1985). Effect of selenium deficiency on hydoperoxide-induced glutathione release from the isolated perfused rat heart. **Journal of Nutrition**. 115(6): 733-742.

Yi, W., Akoh, C. C., Fischer, J., and Krewer, G. (2006). Absorption of anthocyanins from blueberry extracts by Caco-2 human intestinal cell monolayers. **Journal of Agricultural and Food Chemistry**. 54(15): 5651-5658.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER III

EXPERIMENT I

EFFECT OF CULTIVARS, PLANT SPACING AND HARVESTING AGE OF NAPIER GRASS ON FORAGE YIELD, MORPHOLOGICAL CHARACTERISTICS, CHEMICAL COMPOSITION AND ANTHOCYANIN COMPOSITION

3.1 Abstract

The objective of this experiment was to investigate effect of cultivars, plant spacing and harvesting age of Napier grass on forage yield, morphological characteristics, chemical composition and anthocyanin composition. Combinations of two cultivars of grasses: Napier Pakchong-1 (*Pennisetum purpureum* cv. pakchong 1) and Purple Napier grass (*Pennisetum purpureum* 'Prince') and three plant spacing: 50 ×50, 50×75 and 75×75 cm and three harvesting age: 45, 60 and 75 days were planted in experimental plots (5×5 m^2) and harvested after re-growth cutting. The study design was 2×3×3 factorial arrangements in randomized completed design (CRD) for Napier grass planting.

The results showed that forage yield and morphological characteristics, the highest dry matter yield was influenced by plant spacing ($P<0.05$), the grass planted 75×75 cm. The highest dry matter yield was influenced by interaction between

cultivars and age (cultivars×age) (P<0.05) such as Napier Pakchong-1 grass with the harvesting age 75 and 60 days, while Purple Napier grass with the harvesting age 75 days. The plant height parameter of Napier grass planted 50×50 cm with the harvesting age 75 days was higher (P<0.05) than other treatment combinations. Purple Napier grass had number of tillers per plant higher (P<0.05) than Napier Pakchong-1 grass and Napier grass planted 75×75 cm had the highest (P<0.05) number of tillers per plant value, while the harvesting age 60 days had the highest (P<0.05) number of tillers per plant value. For LSR (leaf per stem ratio) of Napier grass, Purple Napier grass planted 75×75 cm with the harvesting age 45 days had the highest (P<0.05) LSR value.

Chemical composition, the DM content of Napier grass planted 75×75 cm with the harvesting age 75 days had the highest (P<0.05) percentage of DM. The OM content of Purple Napier grass planted 50×50 cm had the highest (P<0.05) OM content and Napier grass with the harvesting age 45 days was higher (P<0.05) than 60 and 75 days. The CP and EE content of Napier grass planted 75 × 75 cm at harvesting age 45 days had higher (P<0.05) than other treatment combinations. The CP content of Purple Napier grass was significantly higher (P<0.05) than Napier Pakchong-1 grass.

NDF, ADF, CF and hemicellulose content of Napier grass planted 75×75 cm with harvesting age 75 days were higher (P<0.05) than other treatment combinations. Lignin content of Napier grass planted 50×75 cm and 75×75 cm with harvesting age 60 and 75 days had the highest (P<0.05) value. The ADF and cellulose content of Napier Pakchong-1 grass was higher (P<0.05) than Purple Napier grass. The cellulose content of Napier grass with harvesting age at 75 days was significantly higher (P<0.05) than other treatment combinations.

For Anthocyanin composition, Purple Napier grass planted 75×75 cm had seven anthocyanin compositions (C3G, P3G, Del, Peo3G, CYA, Pel and Mal) and total anthocyanin composition higher ($P<0.05$) than other treatment combinations. The significant influences by interaction of cultivars×age, Purple Napier grass with harvesting age 45 days had six anthocyanin compositions (C3G, Del, Peo3G, CYA, Pel and Mal) and total anthocyanin composition higher ($P<0.05$) than other treatment combinations. The significant influences by interaction of space×age, Napier grass planted 75×75 cm with harvesting age 45 days had four anthocyanin compositions (C3G, Peo3G, Pel and Mal) and total anthocyanin composition higher ($P<0.05$) than other treatment combinations. Moreover, The M3G had significant influences by interaction of cultivars×space×age ($P<0.05$), Purple Napier grass planted 75×75 cm with the harvesting age 45 days had the highest M3G content.

Based on the evidence in the present study, it concluded that Purple Napier grass planted 75×75 cm with harvesting day at 45 days would contain proper number tillers per plant, LSR value, chemical composition for ruminants and highest anthocyanin composition.

Key words: cultivars, plant spacing, harvesting age, Napier grass, forage yield, morphological characteristics, chemical composition, anthocyanin composition.

3.2 Introduction

Napier grass is the principal forage in humid tropical countries with the capability to develop dry matter per unit area especially in comparison to several other grass crops. Napier grass was already introduced to all tropical countries and widely

cultivated throughout Southeast Asia, where average rainfall is estimated 1000 mm (Mannetje and Jones, 1992a). Napier grass can survive in soil where many plant species are relatively crucial and do not grow excellently (Sanderson and Paul, 2008). Napier grass is vegetative propagation and can survive repeated cutting and rapidly regrow the production of grasses that is favorable to leafy cattle (Maria et al., 2010).

Even though cultivars are strongly related with each other, there are differences among cultivars such as various types, such as high and dwarf. Variations of cultivars cause differences in morphological characteristics. Grasses with higher growth rates usually have lower nutrient quality because relatively high DM yield enhances the need to form more structural carbohydrate (Wilson and Minson, 1980), while grasses with greater growth performance actually have higher nutrient quality (Cid et al., 2008). The morphological characteristics, the production and the quality of the cultivar differed. The exact timing of defoliation in order to obtain optimum yield and quality is generally related to age of the plant and provide recommendations vary. An interval of 70 days for grass defoliation or when the plant achieves 120-150 cm for optimal results and forage quality (Zahid et al., 1999). Moreover, Mwebaze (2002) suggests grass defoliation at 56-84 days of age, whilst also Moran (2005) noticed that grass can be harvested after 25-30 days in the rainy season or 50-60 days in the dry season. The result from these research have shown that the growing age of plant has also increased the yield of dry matter, while the quality has decreased.

In addition, study on the morphological characteristics of Napier grass cultivars on yield and quality has been widely applied (Nyambati et al., 2007), while there was little study in Purple Napier grass cultivars, so the effect of plant spacing and harvesting age of Purple Napier grass on forage yield, morphological characteristics,

chemical composition, and anthocyanin composition remains unclear. Therefore, the aim of this study was to investigate the effect of cultivars, plant spacing and harvesting age of napier grass on forage yield, morphological characteristics, chemical composition, and anthocyanin composition.

3.3 Objective

The objective of this experiment was to investigate the effect of cultivars, plant spacing and harvesting age of Napier grass on forage yield, morphological characteristics, chemical composition, and anthocyanin composition.

3.4 Materials and methods

3.4.1 Forage crops and planting

Planting of Napier grass was done by dividing its old clumps into rooted slips each containing two tillers 10 cm long along with rooted. These slips were planted by digging a hole with small hand hoe, into the soil. Combinations of two cultivars of grasses: Napier Pakchong-1 (*Pennisetum purpureum* cv. Pakchong 1) and Purple Napier grass (*Pennisetum purpureum* 'Prince') and three plant spacing: 50×50, 50×75 and 75×75 cm and three harvesting age: 45, 60 and 75 days were planted in experimental plots (5×5 m^2) and harvested after re-growth cutting.

Approximately 312.5 kg/ha of NPK 15-15-15 fertilizer and approximately 62.5 kg/ha of NPK 46-0-0 fertilizer were applied to all grass plots before planting and after each cutting interval respectively. Irrigation was managed by sprinkler every 5 days interval or when necessary to ensure optimal soil moisture conditions for pasture growth (Prasanpanich, 2002).

3.4.2 Data collection and sampling

Morphological characteristic measurements included plant height, number of tillers/plant and leaf stem ratio (LSR). Plant height, number of tillers/plant and leaf stem ratio (LSR) were measured at the time of cutting. Grasses were cut close to the soil surface and first cuttings were at day-120 after planting. Forage yields were measured by using quadrat technique (size 50×50 cm^2) then converted to kilogram per hectare (kg/ha).

3.4.3 Laboratory analyses

3.4.3.1 Chemical composition

For forage yields of each cutting interval, the forage yield was measured in the area of 0.25 m^2 and then hand-clipped and weighed. Each subsample was dried in a hot-air oven at 105°C for 48 hours to determine dry matter (DM) content. For nutritive value analysis, the forages were dried in a hot-air oven at 60°C for 72 hours, then ground to pass through a 1 mm^2 mesh screen and analyzed for chemical compositions. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, ether extract (EE) and ash contents were quantified by AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) estimated by the methods described by Van Soest et al. (1991).

3.4.3.2 Anthocyanin composition

Grass samples were modified to pH 4 with 1% hydrochloric acid and pre-treated with acetone/chloroform liquid-liquid extraction (70:30, v/v) and afterwards centrifuged 10,000 r/min at 4°C for 15 minutes after incubation at room temperature for 4 hours, the supernatant was collected for anthocyanin composition.

The analysis of the specimen has been performed with the HPLC and Diode Array Detector (DAD). Anthocyanin content extraction was achieved on the column C_{18} Symmetry (mobile phase: A, acetonitrile (CH_3CN); B, 10% acetic acid/5% CH_3CN/1% phosphoric acid in deionized water). The time period was 30 minutes, followed by a delay of 5 minutes before the next injection. Another conditions were determined sample temperature at 4°C and injection volume of 20 µL, flow rate of 0.8 mL/min, column temperature of 25°C and DAD wavelength of 520 nm.

3.4.4 Statistical analysis

All statistical calculations were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System 9.1.3 (SAS, 1990) according to 2×3×3 factorial in Completely Randomized Design (CRD). Significant differences ($P<0.05$) among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1980). The statistical model for the analysis of data was:

$$Y_{ijk} = \mu + C_i + S_j + A_k + C_i*S_j + C_i*A_k + S_j*A_k + C_i*S_j*A_k + \varepsilon_{ijkl}$$

where: Y_{ij} = all dependent variables

μ = the overall mean

C_i = the effect of i^{th} cultivars of Napier grass (NP-1 and PN)

S_j = the effect of j^{th} plant spacing of Napier grass (50×50, 50×75 and 75×75 cm)

A_k = the effect of k^{th} harvesting age of Napier grass (45, 60 and 75 days)

C_i*S_j = the interaction of cultivars and plant spacing

C_i*A_k = the interaction of cultivars and harvesting age

S_j*A_k = the interaction of plant spacing and harvesting age

$C_i*S_j*A_k$ = the interaction of cultivars, plant spacing and harvesting age

ε_{ijkl} = residual

3.4.5 Experimental location

The experiment was conducted at Suranaree University of Technology (SUT) goat farm, Nakhon Ratchasima, Thailand (14°53'37.9"N, 102°01'22.0"E).

3.4.6 Experimental period

The experiment was from June 2016 to June 2017.

3.5 Results

3.5.1 Morphological characteristics, chemical composition and yield

Morphological characteristics and yield of two Napier cultivars including Napier Pakchong-1 and Purple Napier has shown in Table 3.1 and 3.4. The yield of Napier grass was significant influences by interaction of cultivars×space×age ($P<0.05$).

The plant height was significant influences of cultivars, space, age and interaction of space×age ($P<0.05$). The plant height parameter of Napier grass was influenced by interaction of space×age ($P<0.05$), the plant spacing 50×50 cm with the harvesting age 75 days (176.54-190.75 cm) had the highest of plant height value. The plant height parameter of Napier grass was influenced by cultivars ($P<0.05$), Napier Pakchong-1 grass had plant height value (120.67-190.75 cm) higher than Purple Napier grass (109.43-176.54 cm).

The number of tillers per plant had significant influences of cultivars, space and age ($P<0.05$). The number of tillers per plant parameter of Napier grass was influenced by cultivars ($P<0.05$), Purple Napier grass had number of tillers per plant

(17.08-41.00 tillers/plant) higher than Napier Pakchong-1 grass (15.23-39.44 tillers/plant). The number of tillers per plant value of Napier grass was influenced by space ($P<0.05$), the plant spacing 75×75 cm (21.03-39.44 tillers/plant) had the highest number of tillers per plant value. The number of tillers per plant parameter of Napier grass was influenced by age ($P<0.05$), the harvesting age 60 days (28.35-41.00 tillers/plant) had the highest number of tillers per plant value. For LSR (leaf per stem ratio) of Napier grass, there were presented influence of cultivars×space×age ($P<0.05$). The LSR value of Napier grass was influenced by cultivars×space×age ($P<0.05$) in Purple Napier grass planted 75×75 cm with the harvesting age 45 days (2.00) had the highest LSR value.

The results of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and lignin has shown in Table 3.2. The DM, ash, CP, EE, NDF, hemicellulose and lignin were significantly affected by main effect and the interaction of cultivars, space and age. The DM content had significant influences by interaction of space×age, the Napier grass planted 75×75 cm with the harvesting age 75 days (24.44-24.58%) had the highest ($P<0.05$) percentage of DM.

The ash content had significant influences by interaction of cultivars× space×age ($P<0.05$). Interaction effect of cultivars×space×age, Napier Pakchong-1 grass planted 75×75 cm (15.44%) had the highest ($P<0.05$) percentage of ash. The CP content had significant influences by cultivars and interaction of space×age. Interaction effect of space×age, Napier grass planted 75×75 cm with harvesting age 45 days (11.37-94.73%) had the highest ($P<0.05$) percentage of CP. Purple Napier grass

(3.98-12.06%) had CP content significantly higher (P<0.05) than Napier Pakchong-1 grass (3.86-11.37%).

The EE content in the Napier grass was significant influences by interaction of space×age, Napier grass planted 75×75 cm with harvesting age 45 days (3.57-3.63%) had the highest (P<0.05) percentage of EE. The NDF content in the Napier grass was significant influences by interaction of space × age (P<0.05), the grass planted 75×75 cm with harvesting age 75 days (82.05-82.32%) had the highest percentage of NDF. The hemicellulose content in the Napier grass was significant influences by interaction of space×age (P<0.05), the grass planted 75×75 cm with harvesting age 75 days (32.20-34.11%) had the highest percentage of hemicellulose. The lignin content in the Napier grass was significant influences by interaction of space×age (P<0.05), the grass planted 50×75 cm and 75×75 cm with harvesting age 60 and 75 days (3.46-3.69%) had the highest percentage of lignin.

For CF, ADF and cellulose content had significant influences of main effect without interactions of cultivars, space and age. The CF content was significant influences of space and age, the plant spacing 75×75 cm (34.43-39.04%) was significantly higher (P<0.05) than 50×75 cm (33.28-38.61%) and 50×50 cm (31.79-36.75%), respectively and the harvesting age 75 days (36.40-39.04%) was significantly higher (P<0.05) than 60 days (35.22-38.61%) and 45 days (31.79-34.82%), respectively.

The ADF content was significant influences by cultivars, space and age, Napier Pakchong-1 grass (43.98-50.12%) was significantly higher (P<0.05) than Purple Napier grass (43.03-48.27%) and the plant spacing 75×75 cm (43.44-50.12%) was significantly higher (P<0.05) than 50×75 cm (43.03-48.27%) and 50×50 cm

(43.05-47.94%), respectively and the harvesting age 75 days (46.35-50.12%) was significantly higher (P<0.05) than 60 days (44.55-46.73%) and 45 days (43.03-45.95%), respectively. The cellulose content was significant influences by cultivars and age, the cellulose content was significantly higher (P<0.05) in Napier Pakchong-1 grass (41.67-46.67%) than Purple Napier grass (40.40-44.94%) and the harvesting age 75 days (42.85-46.67%) was significantly higher (P<0.05) than 60 days (41.26-43.42%) and 45 days (40.40-43.22%), respectively.

The highest yield of DM, CP, EE, CF, ash, NDF, ADF, hemicellulose, cellulose and lignin of Napier Pakchong-1 grass planted 75×75 cm with the harvesting age at 45, 60 and 75 days (P<0.05). The highest yield of EE, CF, NDF, hemicellulose of Purple Napier grass planted 75×75 cm with the harvesting age at 75 days (P<0.05).

3.5.2 Anthocyanin composition

The results of anthocyanin composition including cyanidin-3-glucoside (C3G), pelargonidin-3-glucoside (P3G), delphinidin (Del), peonidin-3-O-glucoside (Peo3G), malvidin-3-O-glucoside (M3G), cyanidin (CYA), pelargonidin (Pel), malvidin (Mal) and total anthocyanin were significantly affected by main effect and the interaction of cultivars, space and age (Table 3.3). The C3G composition had significant influences by interaction of cultivars×space, cultivars×age and space×age (P<0.05), respectively without of cultivars×space×age. These treatment combinations including Purple Napier grass planted 75×75 cm (0.53-2.24 mg/g dry weight), Purple Napier grass harvested at 45 days (1.54-2.24 mg/g dry weight) and the grass planted 75×75 cm with harvesting age 45 days (0.87-2.24 mg/g dry weight), respectively had the highest (P<0.05) C3G composition.

The P3G composition had significant influences by interaction of cultivars×space×age ($P<0.05$), Purple Napier grass planted 75×75 cm (0.46 mg/g dry weight) had the highest P3G composition. The Del composition had significant influences by interaction of cultivars × space and cultivars×age ($P<0.05$), Purple Napier grass planted 75×75 cm (0.09-0.24 mg/g dry weight) and Purple Napier grass with harvesting age 45 days (0.18-0.24 mg/g dry weight) had the highest Del composition.

The Peo3G had significant influences by interaction of cultivars×space, cultivars×age and space×age ($P<0.05$), respectively without of cultivars×space×age, Purple Napier grass planted 75×75 cm (0.18-0.64 mg/g dry weight), Purple Napier grass harvested at 45 days (0.43-0.64 mg/g dry weight), and Plant spacing 75×75 cm with harvesting age 45 days (0.25-0.64 mg/g dry weight), respectively had the highest Peo3G composition. The M3G composition had significant influences by interaction of cultivars×space×age ($P<0.05$), Purple Napier grass planted 75×75 cm with the harvesting age 45 days (0.27 mg/g dry weight) had the highest M3G composition.

The CYA composition had significant influences by interaction of cultivars×space and cultivars×age ($P<0.05$), Purple Napier grass planted 75×75 cm (0.11-0.28 mg/g dry weight) and Purple Napier grass harvested at 45 days (0.22-0.28 mg/g dry weight) had the highest CYA composition. The Pel composition had significant influences by interaction of cultivars×space, cultivars×age and space×age ($P<0.05$), respectively without of cultivars×space×age. Purple Napier grass planted 75 ×75 cm (0.08-0.26 mg/g dry weight), Purple Napier grass harvested at 45 days (0.20-0.26 mg/g dry weight) and Plant spacing 75×75 cm with harvesting age 45 days (0.10-0.26 mg/g dry weight), respectively had the highest Pel composition.

The Mal composition had significant influences by interaction of cultivars×space, cultivars×age and space×age ($P<0.05$), respectively without of cultivars×space×age. Purple Napier grass planted 75×75 cm (0.54-0.98 mg/g dry weight), Purple Napier grass harvested at 45 days (0.71-0.98 mg/g dry weight) and Plant spacing 75×75 cm with harvesting age 45 days (0.38-0.98 mg/g dry weight), respectively had the highest Mal composition.

For total anthocyanin composition had significant influences by interaction of cultivars×space, cultivars×age and space×age ($P<0.05$), respectively without of cultivars×space×age. Purple Napier grass planted 75×75 cm (1.65-5.37 mg/g dry weight), Purple Napier grass harvested at 45 days (3.62-5.37 mg/g dry weight) and Plant spacing 75×75 cm with harvesting age 45 days (2.09-5.37 mg/g dry weight), respectively had the highest total anthocyanin composition.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.1 Overall average value of morphological characteristics in fresh Napier Pakchong-1 and Purple Napier grass as affected by cultivars, plant spacing (cm×cm) and harvesting age (day).

Cultivars[1]	Space	Age	Parameters		
			Plant height (cm)	Number of tillers/plant	LSR
NP-1	50 × 50	45	131.45^{ef}	15.23^{e}	1.72^{cd}
		60	156.07^{cd}	28.08^{bc}	1.44^{k}
		75	190.75^{a}	28.35^{bc}	0.96^{m}
	50 × 75	45	134.00^{ef}	20.19^{e}	1.78^{b}
		60	147.67^{de}	30.83^{b}	1.51^{ij}
		75	163.68^{ab}	34.85^{a}	0.99^{lm}
	75 × 75	45	120.67^{ef}	21.03^{e}	1.94^{a}
		60	129.00^{ef}	33.89^{ab}	1.64^{ef}
		75	163.32^{ab}	39.44^{a}	1.53^{hi}
PN	50 × 50	45	115.70^{ef}	17.08^{e}	1.74^{bc}
		60	141.11^{de}	30.26^{b}	1.45^{jk}
		75	176.54^{a}	33.34^{b}	0.98^{lm}
	50 × 75	45	118.43^{ef}	22.44^{d}	1.79^{b}
		60	136.22^{de}	36.11^{a}	1.59^{fg}
		75	160.32^{ab}	41.00^{a}	1.03^{l}
	75 × 75	45	109.43^{f}	25.56^{c}	2.00^{a}
		60	116.22^{ef}	34.67^{a}	1.67^{de}
		75	158.68^{bc}	39.22^{a}	1.58^{gh}
Cultivars			<.0001	0.0004	<.0001
Space			<.0001	<.0001	<.0001
Age			<.0001	<.0001	<.0001
Cultivars × Space			0.499	0.346	0.093
Cultivars × Age			0.652	0.822	0.753
Space × Age			0.011	0.078	<.0001
Cultivars × Space × Age			0.865	0.344	0.043

[a, b, c,…,m] Means followed by a different letter within the same column are significantly different (P<0.05) and SEM = standard error of mean.

[1] Cultivars = cultivars of Napier grass; Space = plant spacing (cm×cm); Age = harvesting age (days); NP-1 = Napier Pakchong-1 grass; PN = Purple Napier grass.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.2 Overall average value of chemical composition (%) in fresh Napier grass as affected by cultivars, plant spacing (cm×cm) and harvesting age (days).

Cultivars[1]	Space	Age	%DM[2]	Chemical compositions (% Dry matter)								
				CP	EE	CF	Ash	NDF	ADF	Hemicellulose	Cellulose	Lignin
NP-1	50×50	45	19.81^{h}	9.50^{cde}	3.42^{bc}	32.91^{f}	9.41^{fg}	62.80^{c}	43.98^{f}	18.82^{c}	41.67^{e}	2.31^{d}
		60	21.37^{f}	7.84^{hi}	2.93^{d}	36.75^{c}	9.76^{ef}	73.90^{b}	45.02^{e}	28.88^{b}	42.50^{d}	2.52^{cd}
		75	22.96^{c}	3.86^{j}	2.32^{f}	36.44^{d}	10.08^{e}	73.50^{b}	47.94^{b}	25.57^{b}	44.73^{c}	3.21^{b}
	5 ×75	45	20.23^{g}	9.87^{cd}	3.54^{ab}	34.33^{e}	10.81^{d}	65.08^{c}	44.45^{f}	20.63^{c}	41.69^{e}	2.76^{c}
		60	21.60^{ef}	8.23^{gh}	3.25^{c}	37.73^{b}	11.20^{d}	73.99^{b}	45.45^{e}	28.54^{b}	42.17^{e}	3.28^{b}
		75	22.32^{d}	4.09^{j}	3.37^{c}	38.61^{a}	12.57^{c}	76.37^{b}	46.54^{c}	29.82^{ab}	42.85^{d}	3.69^{a}
	7 ×75	45	21.67^{e}	^{b}11.37	3.63^{a}	34.82^{c}	12.93^{c}	64.20^{c}	45.95^{d}	18.25^{c}	43.22^{d}	2.73^{c}
		60	22.32^{d}	9.05^{ef}	3.37^{c}	38.61^{a}	13.65^{b}	76.37^{b}	46.54^{c}	29.82^{ab}	42.85^{d}	3.46^{a}
		75	24.58^{a}	7.36^{i}	2.75^{e}	39.04^{a}	15.44^{a}	82.32^{a}	50.12^{a}	32.20^{a}	46.67^{a}	3.69^{a}
PN	50×50	45	19.71^{h}	9.66^{cde}	3.37^{bc}	31.79^{g}	6.03^{k}	62.17^{c}	43.05^{g}	19.75^{c}	40.75^{f}	2.30^{d}
		60	21.18^{f}	7.90^{hi}	2.90^{d}	35.22^{c}	6.75^{j}	72.19^{b}	44.55^{e}	27.64^{b}	41.95^{e}	2.60^{cd}
		75	22.90^{c}	3.98^{j}	2.31^{f}	36.40^{d}	6.95^{j}	72.63^{b}	46.35^{d}	26.29^{b}	43.12^{d}	3.23^{b}
	5×75	45	20.09^{g}	9.95^{c}	3.45^{ab}	33.28^{f}	7.09^{j}	63.15^{c}	43.03^{g}	20.12^{c}	40.40^{g}	2.63^{c}
		60	21.36^{ef}	8.73^{fg}	3.24^{c}	36.22^{d}	8.03^{i}	73.70^{b}	46.73^{c}	26.97^{b}	43.42^{d}	3.31^{b}

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.2 (Continue).

Cultivars[1]	Space	Age	%DM[2]	Chemical compositions (% Dry matter)								
				CP	EE	CF	Ash	NDF	ADF	Hemicellulose	Cellulose	Lignin
		75	23.64^{b}	4.28^{j}	2.62^{e}	37.28^{c}	8.69^{h}	73.36^{b}	48.27^{b}	25.09^{b}	44.94^{c}	3.33^{ab}
	75×75	45	21.53^{e}	^{a}12.06	3.57^{a}	34.43^{e}	8.85^{gh}	62.55^{c}	43.44^{g}	19.10^{c}	40.65^{f}	2.79^{c}
		60	22.19^{d}	9.25^{def}	3.31^{c}	37.81^{b}	8.91^{gh}	74.08^{b}	44.72^{e}	29.37^{ab}	41.26^{f}	3.34^{ab}
		75	24.44^{a}	7.65^{hi}	2.73^{e}	37.89^{b}	9.05^{gh}	82.05^{a}	47.94^{b}	34.11^{a}	44.60^{c}	3.45^{ab}
SEM			0.202	0.335	0.058	0.306	0.345	0.897	0.326	0.706	0.294	0.059
P-value												
Cultivars			0.840	<0.0001	0.105	0.187	<0.0001	0.051	<0.01	0.969	<0.01	0.434
Space			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.05	<0.01	0.247	<0.0001
Age			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivars × Space			0.111	0.169	0.078	0.088	<0.0001	0.802	0.173	0.398	0.207	0.392
Cultivars × Age			0.233	0.744	0.417	0.089	<0.0001	0.955	0.318	0.470	0.311	0.965
Space × Age			<0.0001	<0.0001	<0.0001	0.945	<0.0001	<0.01	0.633	<0.0001	0.372	<0.0001
Cultivars × Space × Age			0.571	0.157	0.710	0.614	<0.0001	0.693	0.846	0.947	0.875	0.300

$^{a, b, c,\ldots,h}$ Means followed by a different letter within the same column are significantly different (P<0.05) and SEM = standard error of mean.

[1] Cultivars = cultivars of Napier grass; Space = plant spacing (cm×cm); Age = harvesting age (days); NP-1 = Napier Pakchong-1 grass; PN = Purple Napier grass.

[2] DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.3 Overall average value of anthocyanin composition (mg/ g dry weight) in fresh Napier grass as affected by cultivars of grass, plant spacing (cm×cm) and harvesting age (days).

Cultivars[1]	Space	Age	Anthocyanin composition (mg/g dry weight)[2]								
			C3G	P3G	Del	Peo3G	M3G	CYA	Pel	Mal	Total
NP-1	50×50	45	0.59^{fg}	0.08^{def}	0.07^{fgh}	0.17^{fgh}	0.06^{fgh}	0.08^{efghi}	0.08^{efg}	0.28^{ghi}	1.41^{ghi}
		60	0.24^{hij}	0.04^{fghij}	0.04^{hij}	0.08^{ij}	0.03^{hijk}	0.05^{ijk}	0.04^{ghi}	0.22^{hi}	0.75^{jklm}
		75	0.03^{k}	0.01^{j}	0.02^{j}	0.02^{j}	0.01^{k}	0.03^{k}	0.03^{i}	0.18^{i}	0.32^{m}
	50×75	45	0.76^{ef}	0.10^{de}	0.08^{efg}	0.19^{ef}	0.07^{ef}	0.09^{efgh}	0.09^{ef}	0.33^{gh}	1.71^{fg}
		60	0.31^{hi}	0.07^{efgh}	0.05^{ghij}	0.12^{ghi}	0.04^{hijk}	0.06^{hijk}	0.06^{fghi}	0.23^{hi}	0.93^{ijkl}
		75	0.09^{jk}	0.01^{j}	0.02^{j}	0.05^{j}	0.02^{jk}	0.04^{k}	0.03^{i}	0.20^{i}	0.45^{lm}
	75×75	45	0.87^{de}	0.18^{c}	0.09^{ef}	0.25^{de}	0.10^{d}	0.11^{def}	0.10^{ef}	0.38^{fg}	2.09^{ef}
		60	0.42^{gh}	0.07^{defg}	0.06^{fghi}	0.15^{fgh}	0.04^{hij}	0.07^{ghijk}	0.07^{efgh}	0.27^{ghi}	1.16^{hijk}
		75	0.21^{ijk}	0.02^{hij}	0.04^{ij}	0.07^{ij}	0.03^{ijk}	0.04^{jk}	0.03^{hi}	0.22^{hi}	0.66^{klm}
PN	50×50	45	1.54^{c}	0.20^{c}	0.18^{b}	0.43^{c}	0.14^{c}	0.22^{b}	0.20^{bc}	0.71^{bc}	3.62^{c}
		60	0.61^{fg}	0.12^{d}	0.11^{de}	0.19^{f}	0.08^{def}	0.12^{de}	0.11^{e}	0.54^{de}	1.88^{efg}
		75	0.06^{jk}	0.02^{ij}	0.05^{ghij}	0.05^{j}	0.03^{hijk}	0.08^{fghij}	0.07^{efgh}	0.47^{ef}	0.82^{jklm}
	50×75	45	1.90^{b}	0.26^{b}	0.20^{b}	0.50^{b}	0.19^{b}	0.24^{b}	0.24^{ab}	0.82^{b}	4.34^{b}
		60	0.78^{ef}	0.17^{c}	0.13^{d}	0.30^{d}	0.10^{de}	0.14^{cd}	0.16^{d}	0.56^{de}	2.33^{e}

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.3 (Continue).

Cultivars[1]	Space	Age	Anthocyanin composition (mg/g dry weight)[2]								
			C3G	P3G	Del	Peo3G	M3G	CYA	Pel	Mal	Total
		75	0.24^{hij}	0.04^{ghij}	0.06^{fghi}	0.12^{hi}	0.05^{ghi}	0.09^{efghi}	0.08^{efg}	0.51^{e}	1.18^{hij}
	75 × 75	45	2.24^{a}	0.46^{a}	0.24^{a}	0.64^{a}	0.27^{a}	0.28^{a}	0.26^{a}	0.98^{a}	5.37^{a}
		60	1.06^{d}	0.18^{c}	0.16^{c}	0.39^{c}	0.10^{d}	0.17^{c}	0.18^{cd}	0.66^{cd}	2.90^{d}
		75	0.53^{g}	0.06^{efghi}	0.09^{def}	0.18^{fg}	0.07^{efg}	0.11^{defg}	0.08^{efg}	0.54^{de}	1.65^{fgh}
SEM			0.074	0.013	0.008	0020.	0.008	0.009	0.008	0.028	0.162
			P-value								
Cultivars			<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Space			<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Age			<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Cultivars × Space			<.0001	<.0001	0.0003	<.0001	<.0001	0.013	0.010	0.005	<.0001
Cultivars × Age			<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Space × Age			0.005	<.0001	0.943	0.0009	<.0001	0.360	0.005	0.004	0.0007
Cultivars × Space × Age			0.653	<.0001	0.958	0.419	0.0004	0.938	0.569	0.357	0.319

$^{a, b, c,..., m}$ Means followed by a different letter within the same column are significantly different (P<0.05).

[1] Cultivars = cultivars of Napier grass; Space = plant spacing (cm×cm); Age = harvesting age (days); NP-1 = Napier Pakchong-1 grass;

[2] C3G = cyanidin-3-glucoside; P3G = pelargonidin-3-glucoside; Del = delphinidin; Peo3G = peonidin-3-O-glucoside; M3G = malvidin-3-O-glucoside; Cya = cyanidin; Pel = pelargonidin; Mal = malvidin.

Table 3.4 Overall average value of yield (kg/ha) in fresh Napier grass as affected by cultivars, plant spacing (cm×cm) and harvesting age (days).

Cultivars[1]	Space	Age	Yield (kg/ha)[2]									
			DM	CP	EE	CF	Ash	NDF	ADF	Hemicellulose	Cellulose	Lignin
NP-1	50×50	45	14,499.04^{hijk}	1,377.12^{de}	335.01^{h}	4,609.97^{hi}	1,364.32^{gh}	9,014.21^{klm}	6,373.54^{ijk}	2,640.68^{ij}	6,373.54^{ijk}	363.93^{kl}
		60	15,413.77^{fghi}	1,362.30^{de}	446.49^{e}	5,664.97^{def}	1,503.62^{fg}	11,390.62^{def}	6,939.92^{fghij}	4,450.70^{cd}	6,939.92^{fghij}	406.99^{ij}
		75	15,570.01^{fgh}	912.48^{fg}	504.47^{c}	5,667.78^{def}	1,569.39^{ef}	11,443.80^{def}	7,463.57^{def}	3,980.24^{de}	7,463.57^{def}	565.25^{cde}
	50×75	45	15,696.13^{efgh}	1,627.58^{bc}	431.40^{ef}	5,389.11^{defg}	1,696.58^{de}	10,215.29^{ghij}	6,977.05^{fghij}	3,238.24^{fgh}	6,977.05^{fghij}	451.57^{ghi}
		60	16,107.98^{cdef}	1,486.71^{cd}	568.58^{b}	6,076.25^{bcd}	1,804.34^{d}	11,918.18^{cd}	7,320.85^{efg}	4,597.34^{c}	7,320.85^{efg}	509.92^{bc}
		75	16,794.22^{bcde}	1022.17^{f}	579.99^{b}	6,462.17^{abc}	2,111.75^{c}	12,736.05^{bc}	8,269.07^{bc}	4,466.98^{cd}	8,269.07^{bc}	556.65^{cd}
	75×75	45	16,944.79^{abcd}	2,011.34^{a}	445.64^{e}	5,834.10^{cde}	2,190.60^{c}	10,877.83^{defg}	7,786.39^{cde}	3,091.45^{ghi}	7,786.39^{cde}	439.39^{efgh}
		60	17,303.25^{abc}	1,739.65^{b}	583.11^{b}	6,677.04^{ab}	2,362.60^{b}	13,210.26^{b}	8,052.77^{bcd}	5,157.49^{b}	8,052.77^{bcd}	525.84^{a}
		75	18,051.11^{a}	1,690.22^{b}	665.65^{a}	6,837.57^{a}	2,787.97^{a}	14,857.38^{a}	9,045.86^{a}	5,811.52^{a}	9,045.86^{a}	619.43^{a}
PN	50×50	45	12,735.33^{m}	1,230.51^{e}	295.49^{i}	4,189.16^{i}	768.12^{k}	7,990.24^{m}	5,478.18^{l}	2,512.06^{j}	5,478.18^{l}	316.38^{l}
		60	13,280.33^{lm}	1,248.79^{e}	388.67^{g}	4,677.68^{ghi}	896.43^{jk}	9,587.46^{ijkl}	5,916.70^{kl}	3,670.76^{ef}	5,916.70^{kl}	393.30^{jkl}
		75	13,538.92^{klm}	810.06^{g}	448.56^{de}	4,932.65^{fgh}	941.51^{j}	9,831.17^{hijk}	6,277.08^{jk}	3,554.09^{efg}	6,277.08^{jk}	472.14^{fghi}
	50×75	45	13,733.33^{jklm}	1,508.38^{cd}	375.52^{g}	4,582.89^{hi}	976.63^{j}	8,698.65^{lm}	5,926.35^{kl}	2,772.31^{hij}	5,926.35^{kl}	358.60^{jk}
		60	14,224.44^{ijkl}	1,454.76^{d}	486.52^{cd}	5,151.48^{efgh}	1,142.70^{i}	10,482.20^{fghi}	6,648.65^{ghij}	3,833.55^{e}	6,648.65^{ghij}	469.90^{efg}
		75	14,727.78^{ghijk}	925.30^{fg}	496.84^{c}	5,490.24^{def}	1,280.10^{hi}	10,803.83^{efgh}	7,108.88^{efgh}	3,694.95^{ef}	7,108.88^{efgh}	561.74^{def}
	75×75	45	14,969.11^{fghij}	1,780.38^{b}	393.06^{fg}	5,213.42^{efgh}	1,334.38^{h}	9,362.68^{jkl}	6,503.03^{hijk}	2,859.65^{hij}	6,503.03^{hijk}	374.20^{hi}
		60	15,804.44^{defg}	1,699.46^{b}	514.24^{c}	5,975.17^{bcd}	1,399.35^{gh}	11,708.55^{cde}	7,066.91^{fghi}	4,641.64^{c}	7,066.91^{fghi}	487.90^{c}

ลิขสิทธิ์มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 3.4 (Continue).

Cultivars[1]	Space	Age	Yield (kg/ha)									
			DM	**CP**	**EE**	**CF**	**Ash**	**NDF**	**ADF**	**Hemicellulose**	**Cellulose**	**Lignin**
		75	17,982.22^{ab}	1,736.30^{b}	639.92^{a}	7,033.34^{a}	1,627.68^{ef}	14,728.50^{a}	8,609.94^{ab}	6,118.56^{a}	8,609.94^{ab}	508.79^{ab}
SEM			216.80	46.05	13.60	113.63	73.30	261.03	132.93	142.16	132.93	13.68
			P-value									
Cultivars			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Space			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivars × Space			0.006	0.357	0.006	0.008	<0.0001	0.040	0.692	0.0001	0.692	0.413
Cultivars × Age			0.129	0.003	0.047	0.077	0.0002	0.296	0.463	0.001	0.463	0.429
Space × Age			0.002	<0.0001	<0.0001	0.065	<0.0001	<0.0001	0.001	<0.0001	0.001	<0.0001
Cultivars × Space × Age			0.011	0.026	0.024	0.033	0.023	0.003	0.019	0.007	0.019	0.022

[a, b, c,..., m] Means followed by a different letter within the same column are significantly different (P<0.05) and SEM = standard error of mean.

[1] Cultivars = cultivars of Napier grass; Space = plant spacing (cm × cm); Age = harvesting age (days); NP-1 = Napier Pakchong-1 grass; PN = Purple Napier grass.

[2] Yield (kg/ha); DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber.

3.6 Discussion

3.6.1 Morphological characteristics, chemical composition and yield

The DM yield of Napier grass was significantly affected by cultivars, plant spacing and harvesting age. The DM yield, morphological characteristics and chemical composition in wider plant spacing at longer harvesting age, these characteristics would contribute to increased photosynthetic activity and hence higher DM yield and plant height, more tillers per plant, more leaves per plant and higher value of chemical composition (Tilahun et al., 2017).

The Napier Pakchong-1 and Purple Napier grass were classified into tall grass cultivars and short grass cultivars (dwarf Napier). The average of DM yield, plant height, CF, NDF, ADF, cellulose and lignin for Napier Pakchong-1 grass were higher than Purple Napier grass caused by the growth rate of Napier Pakchong-1 grass faster so accumulate more dry matter compared to Purple Napier grass (Tilahun et al., 2017). In addition, the plant height of Napier Pakchong-1 grass was higher than Purple Napier grass caused higher DM yield. Obok et al. (2012) reported that there is a high correlation between plant height and DM yield and the significantly bigger tillers in Purple Napier grass highlight the vigorous growth and its adaptation to the hot and humid climate (Tilahun et al., 2017).

Purple Napier grass was the leafier grass where the leaf fraction was heavier than the stem fraction. The leafier structure of short cultivars contributed to high nutritive value than tall cultivars (Ansah et al., 2010). The Purple Napier (dwarf grass) had higher LSR than Napier Pakchong-1 (tall grass). The difference between Napier Pakchong-1 and Purple Napier grass caused the differences establishment and

growth of new cells that occurs in intercalary meristem located at the base of internode (Trlica, 1999).

Length of internode difference between dwarf with the high type of Napier grass is caused by differences in the rate of cell elongation (Roodrigues et al., 1987). Sollenberger (2008) reported that the internode of Mott cultivar (dwarf Napier) shorter (2.54 cm) compared with the high type of Napier grass (10.16 to 15.25 cm). The leaf to stem ratio (LSR) is one of the criterion in evaluating the quality of the pasture grass because the higher proportion of leaves compared to stem indicate a better nutritive value.

The effect of plant spacing is also an important cultural practice that affects crop productivity. Optimum spacing is therefore necessary for effective growth, yield and quality of Napier grass. Mannetje et al. (1992b) found that if seedlings are widely scattered (spaced) Rhodes grass can quickly produce a dense stands that means that close spacing produces thin, slow growth and weaker sword. The average for plant height was greater with narrow spacing than wider spacing but lower DM yield, tillers per plant, LSR and chemical composition. The narrow spacing in Napier grass increased interplant competition, causing individual plants to grow taller with longer internodes, plus slender, thin and weak stalks due to poor light exposure and hence poor photosynthetic output (Yasin et al., 2003).

The number of tillers per plant increased as plant spacing increased. The wider spacing, light can easily penetrate to the base of the plant and this may have stimulated tiller development that resulting in higher DM yield, LSR and chemical composition. Moreover, under wider spacing competition for nutrients is less, so individual plants can support more tillers (Melkie, 2005). The capacity of Napier grass

to increase the number of tillers per plant with the variation among the different plant spacing being ascribed to variable nutritional areas and access to light when sufficient space is available (Yasin et al., 2003).

The lower tiller counts at narrow plant spacing may be due to high plant competition for resources, namely light, space and nutrients. The increasing of competition for light causes reduced the capacity of growth and tillering. The competition of interplant in Napier grass causes rapid and exhaustive height increments that overcrowding results in neighboring plants producing weak tillers.

Therefore, the competitor plants are forced to grow upright to dominate other tillers produced on the same plant rather than expanding laterally by bearing more tillers (Boonman, 1993). If the density is maintained above the optimum, there will be a higher overall requirement for resources resulting in plant stress condition. The wider plant spacing requires less planting material and enables greater grazing capacity in forage grasses, but the possibility of weed invasion results in an increase and it may lead to additional weeding costs (Trenbath, 1986).

The effect of harvesting day was closely related to the higher DM, plant height, fiber contents (ADF and NDF), CF and lignin content, whereas the number of tillers per plant, LSR, CP, and EE content was decreased. The high DM yield, plant height, fiber compounds (ADF and NDF), CF and lignin content were harvested at longer harvesting age due to the growing maturity of Napier grass due to the biomass increase resulting in weight gain from biomass production (Sitompul and Guritno, 1995). The plant height was higher in 75 days than 60 and 45 days, which could be referred to a longer photoperiod for better physiological development due to absorption of more sunlight, moisture and mineral nutrients (Bhatti et al., 1985).

At the harvesting age 75 days, the number of tillers per plant decreased. Defoliation and activation of basal buds could increase the number of tillers per plant to the removal of apical dominance (Jones, 1985). The number of tillers per plant was lower in the long harvesting age has been led to higher mortality of tillers under reduced cutting frequency because increasing the number of tillers per plant is probably an adaptive feature to tolerate frequent defoliation by re-establishing lost photosynthetic area and maintaining the basal area (Clavero, 1997). High production of number of tillers per plant indicates not only stable productivity but it also improved persistence after adverse environmental conditions (Mukhtar, 2006; Assuero and Tognetti, 2010). The production of tillers to be such a key factor in grassland resistance to aging deterioration (Lafarge and Loiseau, 2002).

The LSR of Napier grass decreased as the harvesting age increased. The leaves fraction was decreased significantly from 45 days to 75 days. The result showed that Napier Pakchong-1 grass had a higher proportion of stem compared to leaves with the LSR lower than Purple Napier grass. Therefore, Napier Pakchong-1 grass can be classified as high yielding and stemmy cultivars than Purple Napier grass. Purple Napier grass was the leafier cultivar (high LSR value) and this has a significant implication in nutritive quality as the leaves contain higher level of nutrient and less fibrous compared to stems fraction (Trlica, 1999).

Percentage of CP at 60 and 75 days harvesting age after planting were lower than 45 days harvesting age. This is caused different of stage maturity. The plant has changed from the vegetative phase to the reproductive phase, the stage of reproductive proportion of more stem increases cause increased fiber content but crude protein content decreased, quality will be reduced due to the increased maturity

(Rayburn, 1993). Wong (1996) reported that the crude protein content of Napier grass King and Mott cultivars decreased with increasing defoliation interval. Adjei and Fianu (1985) reported that the average CP content in leaves and stems for forage legumes decreased from 22.5 to 17.5% and from 11.9 to 9.4 and the corresponding rise in the average CF content in leaves and stems from 20.0 to 26.8% and 27.1 to 31.9% with a longer harvesting age (60, 90 and 120 days).

A short harvesting day of 45 days seriously reduced dry matter yields while percentage of protein was very high, this would scarcely compensate for the greatly reduced forage production (Tessema et al., 2010). A harvesting age of 45 days appears optimal for the hot humid tropical climate. This is supported by results revealing high LSR value and acceptable percentage of CP at 45 days harvesting age. Napier grass harvested at young age in this study had excellent nutrient value, particularly high percentage of CP, a limiting nutrient in tropical forages.

However, most of Napier grass harvested at 60-75 days had CP content well above 7%, which is the level below which voluntary intake of ruminants might be depressed. All of the forage produced would provide sufficient energy and protein to support some level of production above a maintenance level. Although, harvesting at the early stage had great value of nutrient composition but resulted in low DM yield (Tilahun et al., 2017). The grass to grow until 75 days harvesting age resulted in much higher DM yield without a great reduction in quality despite some reduction in CP content and increase in NDF content. In any pasture situation, compromises between quality and yield must be made when deciding at what stage to harvest or graze a crop or pasture (Tilahun et al., 2017).

For the current study that the studied the Napier Pakchong-1 contain high NDF and low CP content while the Purple Napier grass contain low NDF and high CP content. The Napier Pakchong-1 grass was better than Purple Napier grass for giving higher DM yield. Therefore, two very different types of Napier grasses would be selected for ruminants, but the balance among DM yield and chemical composition would be regarded as the best possible forage properties for ruminants under the circumstances of the current study.

3.6.2 Anthocyanin composition

The anthocyanin composition of Napier grass was significantly affected by cultivars, plant spacing and harvesting age. Most of the anthocyanin composition was higher in Purple Napier grass when cultivated in a wider plant spacing at a shorter harvesting age. Purple Napier grass had anthocyanin composition higher than Napier Pakchong-1 grass because the anthocyanin content in red leaves and stems were significantly greater than that in green leaves and stems, and the highest anthocyanin content was detected in red leaves and then the anthocyanin content in leaves was more than stems (Zhang et al., 2019).

Leaf color is an important external indicator of plant quality, and it is determined by various pigments, including chlorophylls, carotenoids, and flavonoids (Vimolmangkang et al., 2014). The high expression of chalcone synthase (CHS) helps the production of anthocyanins and other flavonoids, however, CHS expression was significantly lower in green leaves compared with Purple leaves and light Purple leaves of Napier grass (Zhou et al., 2019).

Purple Napier grass was the leafy grass in which the leaf fraction was heavier than the stem fraction and the leafy structure of short cultivars contributed to

high leaf to stem ratio (LSR) (Ansah et al., 2010). The higher proportion of the leaves (high LSR) compared to the stem provides a positive anthocyanin composition. Napier grass planted under wider plant spacing could be referred to an absorption of more sunlight, moisture and mineral nutrients, and the shorter harvesting age had a higher percentage of CP than the longer harvesting age leading to higher anthocyanin composition.

The increased consumption of potassium resulted in increased absorption of nitrogen, that was reflected in increased CP content and there was an increasing amount of anthocyanins with increased fertilizer nitrogen rates. (Shaikh et al., 2008). An increase in nitrogen (N) and potassium (K) to certain levels were required to encourage vegetative growth and to delay leaf senescence. Nitrogen fertilizer interacted with potassium fertilizer in stimulating plant regrowth through increasing the activities of cytokinin as well as other phytohormones that influencing plant growth (Kanzikwera et al., 2001).

Protein content increased with the enhance of N and K fertilizer at the optimum harvest intervals. It establishes that N and K are the fundamental components of the protein synthesis. Nitrogen applied to plant roots will be absorbed and metabolized into amino acids, which formed peptide bonds to synthesize proteins, while potassium has important functions in the activation of the enzyme and in the formation of peptide bonds during the protein synthesis process (Campbell and Farrel, 2006).

Phenylalanine and tyrosine are key players in producing specialized metabolites of the huge and varied family of phenylpropanoid. Phenylpropanoids play a major role in the adaptation of plants to changing environmental conditions and in their

protection against pathogens (Dixon and Paiva, 1995). Both of amino acids are products of the pathways of shikimate and aromatic amino acids, and are the source of phenylpropanoids, primarily associated with specialized metabolic pathways. The phenylpropanoid pathways include the flavonoid and anthocyanins, benzenoid, stilbenoid, and lignin pathways (Bentley, 1990; Tzin and Galili, 2010; Maeda and Dudareva, 2012).

The different pathways of phenylpropanoid resulting from phenylalanine and tyrosine catabolism are differentially induced, based on plant, developmental and environmental conditions, and both biotic and abiotic stresses (Castellarin et al., 2007; Naoumkina et al., 2010; Dai et al., 2013; Degu et al., 2014). Whereas total concentrations of anthocyanins were not significantly affected by overproduction of phenylalanine and tyrosine (Manela et al., 2015). Longer harvest age stimulated earlier flowering and fruit development, thus the assimilates will be divided into many sinks. This partitioning might also have decreased the total nitrogen content and protein synthesis in the leaves, as longer harvesting age might lead to earlier ripening, decrease biological properties, reduce overall nitrogen and lignification in the leaves resulting in decrease protein synthesis (Manyawu et al., 2003; Sarwar et al., 2006).

Moreover, Anthocyanin synthesis is enhanced by sunlight and high temperature (Tyas et al., 1998; Niu et al., 2017). Solar radiation is the most important external factor regulating the synthesis of anthocyanins (Saure, 1990; Lancaster, 1992). The red color of a skin in a lot of plants, several flavonoid genes required for anthocyanin synthesis, were transcribed in a coordinated manner in response to light exposure (Takos et al., 2006). Anthocyanin synthesis is expected to increase in response to high temperature stress, which may increase the amount of ROS in mitochondria by

respiration. High temperatures can increase the respiratory rate and the production of ethylene, it was assumed that high temperatures could also increase gene expression and the production of anthocyanins (Niu et al., 2017).

3.7 Conclusion

Napier Pakchong-1 and Purple Napier grass differentiated in terms of forage yield, morphology, chemical composition and anthocyanin composition. Purple Napier grass was greater than Napier Pakchong-1 grass in terms of number of tillers per plant, leafiness (high LSR), high CP content and anthocyanin composition, but Napier Pakchong-1 grass had higher DM yield, plant height and NDF, ADF, CF, cellulose and lignin content with developed forage maturity. The wider plant spacing of Napier grass had a higher yield of DM, number of tillers per plant, LSR, chemical and anthocyanin composition, while a plant height reduced with developed maturity. Longer harvesting age had reduced quality, particularly decreased CP content and increased DM yield, NDF and ADF content at advanced maturity. The Purple Napier grass planted 75×75 cm with harvesting day at 45 days would contain proper number tillers per plant, LSR value, chemical composition for ruminants and highest anthocyanin composition.

3.6 References

Adjei, M. B., and Fianu, F. K. (1985). The effect of cutting interval on the yield and nutritive value of some tropical legumes on the coastal grassland of Ghana. **Tropical Grasslands**. 19(4): 164-171.

Ansah, T., Osafo, E. L. K., and Hansen, H. H. (2010). Herbage yield and chemical composition of four varieties of Napier (*Pennisetum purpureum*) grass harvested at three different days after planting. **Agriculture and Biology Journal of North America**. 1(5): 923-930.

AOAC. (1995). Official Methods of Analysis, 16th ed. **Association of official analytical chemists**. Washington DC, USA.

Assuero, S. G., and Tognetti, J. A. (2010). Tillering regulation by endogenous and environmental factors and its agricultural management. **American Journal of Plant Science Biotechnology**. 4: 35-48.

Bentley, R. (1990). The shikimate pathway: a metabolic tree with many branches. **Critical Reviews in Biochemistry and Molecular Biology**. 25(5): 307-384.

Bhatti, M. B., Sartaj, D. M., and Sultani, M. I. (1985). Effect of different inter-and intra-rowspacings on forage yield and quality in elephant grass. **Pakistan Journal of Agricultural Research**. 6(2): 107-112.

Boonman, J. G. (1993). **East Africa's grasses and fodders: Their ecology and husbandry**. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Campbell, M. K., and Farrell, S.O. (2006). **Biochemistry. 5th Ed**. Thomson Learning, Inc.

Castellarin, S. D., Matthews, M. A., Gaspero, G. D., and Gambetta, G. A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. **Planta**. 227(1): 101-112.

Cid, M. S., Ferri, C. M., Brizuela, M. A., and Sala, O. (2008). Structural heterogeneity and productivity of a tall fescue pasture grazed rotationally by cattle at four stocking densities. **Grassland Science**. 54(1): 9-16.

Clavero, L. T. (1997). Tiller dynamics of dwarf elephant grass (*Pennisetum purpureum* cv. Mott) under defoliation. In: **Proceedings of the XVIII International Grassland Congress**, Winnipeg and Saskatoon, Canada. 22: 31-32.

Dai, Z. W., Leon, C., Fell, R., Lunn, J. E., Delrot, S., and Gomes, E. (2013). Metabolic profiling reveals coordinated switches in primary carbohydrate metabolism in grape berry (*Vitis vinifera* L.), a non-climacteric flesh fruit. **Journal of Experimental Botany**. 64(5): 1345-1355.

Degu, A., Hochberg, U., Sikron, N., Venturini, L., Buson, G., Ghan, R., Plaschkes, I., Batushansky, A., Chalifa-Caspi, V., Mattivi, F., Delledonne, M., Pezzotti, M., Rachmilevitch, S., Cramer, G. R., and Fait, A. (2014). Metabolite and transcript profiling of berry skin during fruit development elucidates differential regulation between Cabrernet Sauvignon and Shiraz cultivars at branching points in the polyphenol pathway. **BMC Plant Biology**. 14: 188.

Dixon, R. A., and Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. **Plant Cell**. 7(7): 1085-1097.

Jones, C. A. (1985). **C_4 grasses and cereals: Growth, development and stress response**. Wiley and Sons, New York, USA.

Kanzikwera, C. R., Tenywa, J. S., Osiru, D. S. O., Adapala, E., and Bhagsari, A. S. (2001). Interactive effect of nitrogen and potassium on dry matter and nutrient partitioning in true potato seed mother plants. **African Crop Science Journal**. 9(1): 127-146.

Lafarge, M., and Loiseau, P. (2002). Tiller density and stand structure of tall fescue swards differing in age and nitrogen level. **European Journal of Agronomy**. 17(3): 209-219.

Lancaster, J. E. (1992). Regulation of skin color in apples. **Critical Reviews in Plant Sciences.** 10: 487-502.

Maeda, H., and Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. **Annual Review of Plant Biology**. 63: 73-105.

Manela, N., Oliva, M., Ovadia, R., Sikron-Persi, N., Ayenew, B., Fait, A., Galili, G., Perl, A., Weiss, D., and Oren-Shamir, M. (2015). Phenylalanine and tyrosine levels are rate-limiting factors in production of health promoting metabolites in *Vitis vinifera* cv. Gamay Red cell suspension. **Frontiers in Plant Science**. 6: 538.

Mannetje, L., and Jones, R. M. (1992a). **Plant Resources of South-East Asia**. No. 4. Forages. Pudoc Scientific Publishers, Wageningen.

Mannetje, T. L., Kersten, S. M. M., and Cloris, G. K. (1992b). **Plant Resource of East-Asia**, in Mannetje TL, Jones RM (Eds).

Manyawu, G. J., Chakoma, C. Sibanda, S., Mutisi, C., and Chakoma, I. C. (2003). The effect of harvesting interval on herbage yield and nutritive value of Napier grass and hybrid *Pennisetum*. **Asian-Australasian Journal of Animal Sciences**. 16: 996-1002.

Maria, M., Rego, T., Neuman, J., do Rego, A. C. M., Candido, M. D. J., de Sousa Carneiro, M. S., and Lobo, R. N. B. (2010). Chemical and bromatological characteristics of elephant grass silages containing a mango by-product. **Revista Brasileira de Zootecnia**. 39(1): 81-87.

Melkie, B. (2005). **Effect of planting patterns and harvesting days on yield and quality of Bana grass (*Pennisetum purpureum* x *Pennisetum americanum*)**. M.Sc. Thesis. Haramaya University, Harar, Ethiopia.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Moran, J. (2005). Growing quality forages. In: **Tropical dairy farming : feeding management. for small holder dairy farmers in the humid tropics**. Department of Primary Industries.

Mukhtar, M. (2006). Dry matter productivity of the dwarf and normal elephant grasses as affected by the planting density and cutting frequency. **Indonesian Journal of Animal and Veterinary Science**. 11: 198-205.

Mwebaze, S. (2002). **Pasture improvement technologies based on an on-farm study in Uganda**. Regional Land Management Unit (RELMA). Department of Animal Production and Marketing, MAAIF, P.O. Box 513, Entebbe Uganda.

Naoumkina, M. A., Zhao, Q., Gallego-Giraldo, L., Dai, X., Zhao, P. X., and Dixon, R. A. (2010). Genome-wide analysis of phenylpropanoid defence pathways. **Molecular Plant Pathology**. 11(6): 829-846.

Niu, J., Zhang, G., Zhang, W., Goltsev, V., Sun, S., Wang, J., Li1, P., and Ma, F. (2017). Anthocyanin concentration depends on the counterbalance between its synthesis and degradation in plum fruit at high temperature. **Scientific Reports**. 7(1): 7684.

Nyambati, E. M., Muyekho, F. N., Luwesti, C. M., and Ongonjo, E. (2007). Production, characterization and nutritional quality of *Pennisetum purpureum* (Schum) cultivars in western Kenya. **Proceedings, The 8th African Crop Science Society Conference**, El-Minia Egypt, October 27-31, 2007. 185-188.

Obok, E. E., Aken'Ova, M. E., and Iwo, G. A. (2012). Forage potentials of interspecific hybrids between elephant grass selections and cultivated pearl millet genotypes of Nigerian origin. **Journal of Plant Breeding and Crop Science**. 4(9): 136-143.

Prasanpanich, S., Sukpituksakul, P., Tudsri, S., Mikled, C., Thwaites, C. J., and Vajrabukka, C. (2002). Milk production and eating patterns of lactating cows under grazing and indoor conditions in central Thailand. **Tropical Grasslands**. 36(2): 107-115.

Rayburn, E. B. (1993). Plant growth and development as the basis of forage management. **West Virginia University Extension Service**.

Rodrigues, L. R. D. E. A., Mott, G. O., Veiga, J. B., and Ocumpaugh, W. R. (1987). Tillering and morphological characteristics of dwarf elephant grass under grazing. **Pesquisa Agropecuaria Brasileira (Bra.)**. 21(11): 1209-18.

Sanderson, M. A., and Paul, R. A. (2008). Perennial forages as second generation bioenergy crops. **International Journal of Molecular Sciences**. 9(5): 768-788.

Sarwar, M., Nisa, M., Khan, M. A., and Mushtaque, M. (2006). Chemical composition, herbage yield and nutritive value of *Panicum antidole* and *Pennisetum orientale* for *Nili* buffaloes at different clipping intervals. **Asian-Australasian Journal of Animal Sciences**. 19(2): 176-180.

SAS. (1990). SAS **User's guide: statistics**. Version 6. 14th ed. SAS Inst., Carry, NC.

Saure, M. C. (1990). External control of anthocyanin formation in apple. **Scientia Horticulturae (SCI HORTIC-AMSTERDAM)**. 42(3): 181-218.

Shaikh, N. P., Adjei, M. B., and Scholberg, J. M. (2008). Interactive effect of phosphorus and nitrogen on leaf anthocyanins, tissue nutrient concentrations, and dry-matter yield of *Floralta Limpograss* during short day length. **Communications in Soil Science and Plant Analysis**. 39(7-8): 1006-1015.

Sitompul, S. M., and Guritno, B. (1995). **Analisis Pertumbuhan Tanaman**. Gadjah Mada University Press.

Sollenberger, L. E. (2008). **Mott elephant grass**. University of Florida, IFAS, Florida A. & M. University Cooperative Extension Program. SS-AGR-58.

Steel, R. G. D., and Torrie, J. N. (1980). **Principles and Procedures of Statistics**. 2nd ed. McGraw-Hill. Book C, New York.

Takos, A. M., Robinson, S. P., and Walker, A. R. (2006). Transcriptional regulation of the flavonoid pathway in the skin of dark-grown ‘‘Cripps’ Red’’ apples in response to sunlight. **Journal of Horticultural Science and Biotechnology**. 81(4): 735-744.

Tessema, Z. K., Mihret, J., and Solomon, M. (2010). Effect of defoliation frequency and cutting height on growth, dry-matter yield and nutritive value of Napier grass (*Pennisetum purpureum* (L.) Schumach). **Grass Forage Science.** 65(4): 421-430.

Tilahun, G., Asmare, B., and Mekuriaw, Y. (2017). Effects of harvesting age and spacing on plant characteristics, chemical composition and yield of desho grass (*Pennisetum pedicellatum* Trin.) in the highlands of Ethiopia. *Tropical Grasslands.* 5(2): 77-84.

Trenbath, B. R. (1986). **Resource use by intercrops**. In: Francis CA, ed. Multiple cropping. Macmillan Publishing Company, New York, USA. p. 57-81.

Trlica, M. J. (1999). Grass growth and response to grazing. Colorado State University. Cooperative Extension Range. **Natural Resource Series**. 6: 108 pp.

Tyas, J. A., Hofman, P. J. Underhill, S. J. R., and Bell, K. L. (1998). Fruit canopy position and panicle bagging affects yield and quality of ‘‘Tai So’’ lychee. **Scientia**

Horticulturae (SCI HORTIC-AMSTERDAM). 72(3): 203-213.

Tzin, V., and Galili, G. (2010). New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. **Molecular Plant**. 3(6): 956-972.

Van Soest, P. J., 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.

Vimolmangkang, S., Zheng, D., Han, Y., Khan, M. A., Soria-Guerra, R. E., and Korban, S. S. (2014). Transcriptome analysis of the exocarp of apple fruit identifies light-induced genes involved in red color pigmentation. **Gene**. 534(1): 78-87.

Walaiphan, C., Chittamart, N., Tawornpruek, S., Aramrak, S., and Fujii, K. (2019). Studies on microbial biomass carbon and nitrogen turnover derived from sugarcane residues incorporated into a sandy loam soil. **Proceedings, The 45th Congress on Science and Technology of Thailand (STT45) Seedling Innovation for Sustainable**, Mae Fah Luang University, Chiang Rai, Thailand.

Wilson, J. R., and Minson, D. J. (1980). Prospects for improving the digestibility and intake of tropical grasses. **Tropical Grasslands**. 14(3): 253-259.

Wong, C. C. (1996). Productivity and sustainability of some selected tropical fodder grasses for smallholders. **Fifth Proceedings**: Forage Regional Working Group of East Asia.

Yasin, M., Malik, M. A., and Nazir, M. S. (2003). Effect of different spatial arrangements on forage yield, yield components and quality of Mott Elephant grass. **Pakistan Journal of Agricultural**. 2(1): 52-58.

Zahid, M. S., Mufti, M. U., Bhatti, M. B., and Ghafoor, A. (1999). Nitrogen fertilizer requirement of elephant grass cv. Mott grown in Pothwar area. **Science, Technology**

and Development. 18(3): 25-30.

Zhang, Q., Zhai, J., Shao, L., Lin, W., and Peng, C. (2019). Accumulation of anthocyanins: an adaptation strategy of Mikania micrantha to low temperature in winter. **Frontiers in Plant Science**. 10: 1049.

Zhoua, S., Chena, J., Laib, Y., Yinc, G., Chena, P., Pennermanc, K. K., Yand, H., Wua, B., Zhanga, H., Yie, X., Wanga, C., Fuf, M., Zhanga, X., Huanga, L., Maa, X., Penga, Y., Yana, Y., Niea, G., and Liua, L. (2019). Integrative analysis of metabolome and transcriptome reveals anthocyanins biosynthesis regulation in grass species *Pennisetum purpureum*. **Industrial Crops and Products**. 138: 111470.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER IV

EXPERIMENT II

EFFECT OF FRESH AND SILAGE OF NAPIER GRASS ON *IN VITRO* GAS PRODUCTION, GROWTH PERFORMANCE, RUMEN FERMENTATION AND MICROBIAL POPULATION IN GOAT'S RUMEN

4.1 Abstract

The objective of this experiment was to investigate the effect of fresh and silage of Napier grass on *in vitro* gas production, growth performance, rumen fermentation and microbial population in goat's rumen. Twenty four male goats, crossbred Thai native× Anglo-Nubian (approximately 18.50±2.06 kg body weight; mean±standard deviation (SD)). The growing goats were divided six goats per treatment in a 2×2 factorial arrangements in randomized completed design (CRD). There were four treatment combinations: Treatment 1 = Fresh Napier Pakchong-1 grass; Treatment 2 = Napier Pakchong-1 grass silage; Treatment 3 = Fresh Purple Napier grass; Treatment 4 = Purple Napier grass silage.

The results showed that *in vitro* gas production characteristics after incubation differed significantly ($P<0.05$). The fresh Purple Napier grass had highest ($P<0.05$) factor *a* value (soluble fraction). The rate of gas production *c*, the fresh form of grass had higher ($P<0.05$) than silage form. The highest ($P<0.05$) of CH_4 production was

observed in fresh Napier Pakchong-1 grass at 6, 12, and 24 hours after incubation, while fresh Purple Napier grass had lowest ($P<0.05$) of CH_4 production. *In vitro* digestibility (%), fresh Purple Napier grass had highest ($P<0.05$) value of DMD. The grass silage treatment had the value of OMD higher ($P<0.05$) than fresh grass treatment but the ED value of silage grass was lower ($P<0.05$) than fresh grass treatment. The fresh Purple Napier grass had highest ($P<0.05$) ruminal NH_3-N and C_3 at 6, 12 and 24 hours after incubation. The fresh of Purple Napier and Napier Pakchong-1 grass had the highest percentage ($P<0.05$) of TVFA at 6 and 12 hours.

Final weight, weigh change and ADG were highest ($P<0.05$) in the goats fed fresh Purple Napier grass treatment. Dry matter intake of roughage and total dry matter intake were highest ($P<0.05$) for the goats fed on fresh Purple Napier grass treatment as g/day, %BW and g/kg $BW^{0.75}$, respectively. Nutrient intake, the CP and anthocyanin intake were highest ($P<0.05$) in the goats fed fresh Purple Napier grass treatment. Appearance digestibility of OM and CP were highest ($P<0.05$) in the goats fed fresh Purple Napier grass. Goats fed fresh Purple Napier grass had higher levels ($P<0.05$) of nitrogen utilization than other treatments. The ruminal NH_3-N, BUN, ruminal C_3 and ruminal TVFA after feeding of goats fed fresh Purple Napier grass were higher ($P<0.05$) than other treatments, while the ratio of C_2/C_3 value after feeding were lowest ($P<0.05$) in goats fed fresh Purple Napier grass. There were no different of total bacteria in goat's rumen ($P>0.05$). The *B. fibrisolvens* in rumen of goats after feeding with fresh Purple Napier grass was higher ($P<0.05$) than other treatments, whereas the methanogen and protozoa in rumen of goats after feeding with fresh Purple Napier grass were lower ($P<0.05$) than other treatments. Based on the evidence in the present study, It concluded that fresh Purple Napier grass can improve *in vitro* gas production, growth performance, rumen fermentation and microbial population in the goat's rumen.

Key words: fresh Napier grass, Napier grass silage, *in vitro* gas production, growth performance, rumen fermentation, microbial population, goat's rumen

4.2 Introduction

Feed is the important factor and the major cost of livestock production (65-70%). When farmers can discover high quality and cheap animal feed for their livestock, this means that farmers can lead to higher profitability of livestock production. Tropical livestock production is limited by acute shortages of high quality animal feed (Hove et al., 2001; Kanani et al., 2006). Poor nutrition results in a low quantity and quality of animal productivity. Hence, the animals must receive high quality animal feed throughout the period of production.

Napier Pakchong-1 grass is one of the most promising grasses available for ruminant production due to very high yield and nutritional value (Cherdthong et al., 2015d). Nevertheless, during the rainy season, forage is plentiful, which grows excellently and there is more than enough for cattle, while it is rare in the dry season. Preserving the yield of forage throughout the rainy season should be made in silage form. Silage was a good choice for the supply of ruminants, offering nutritional value for the production of ruminants during the dry season.

Silage is forage products of fermentation forage full of water inside a plant in an anaerobic container to prevent oxygen until microbial activity was already preserved during the fermentation process and the forage remains a plant value. In the fermentation process, lactic acid bacteria (LAB) are used to convert water-soluble carbohydrate (WSC) in to the organic acids. Fermentation processes can lead to an accumulation of lactic acid, decreasing pH value and then working with microorganisms, as with yeast

and fungi, which will lead to stopped working of plant fermentation spoilage (Woolford, 1990).

Information of digestibility values of each feedstuff is essential as asset feed information. The value could be obtained by *in vivo*, *in sacco*, or *in vitro* procedures. Even though the *in vitro* method produce faster, less cost, and need small quantity of sample to get more numbers of feed but the *in vivo* procedure produce more accurate information (Adesogan, 2004; Damiran et al., 2007; Mahadevamma et al., 2004; Williams, 2000). In addition, both of *in vitro* and *in vivo* experiments on Napier Pakchong-1 grass in ruminants were reported widely by many researchers, while there was little study in providing Purple Napier grass with ruminant animals, so the effect of feeding Purple Napier grass to ruminant animals remains unclear.

Therefore, the aim of this study was to investigate the effect of fresh and silage of Napier grass on *in vitro* gas production, growth performance, rumen fermentation, and microbial population in goat's rumen.

4.3 Objective

The objective of this experiment was to investigate the effect of fresh and silage of Napier grass on *in vitro* gas production, growth performance, rumen fermentation, and microbial population in goat's rumen.

4.4 Materials and methods

4.4.1 Plant materials

The Napier grass used in the current study were Napier Pakchong-1 grass (*Pennisetum purpureum* cv. pakchong 1) and Purple Napier grass (*Pennisetum purpureum*

'Prince'). Two grasses cultivars were planted 75×75 cm in experimental plots (5×5 m^2) and harvested at 60 days for fresh and grass silage after re-growth, which were selected from experiment I for plant spacing and harvesting age. Each forage comprised 9 plots giving a total of 2×9 =18 plots; two grasses were Napier Pakchong-1 (*Pennisetum purpureum* cv. pakchong 1) and Purple Napier grass (*Pennisetum purpureum* 'Prince'). The soil is a sandy loam, Korat soil series (Oxic Paleustals).

Estimated 312.5 kg/ha of NPK 15-15-15 fertilizer and estimated 62.5 kg/ha of NPK 46-0-0 fertilizer were applied to all grass plots prior to planting and at each cutting interval, respectively. Sprinkler watering was managed every 5 days or, if necessary, to ensure optimal soil moisture conditions for pasture growth (Prasanpanich, 2002). First cuttings of Napier grass was at 120 days after planting.

4.4.2 Silage making

Napier grass was harvested at 60 days of re-growth and chopped with a forage cutter into pieces of 2-3 cm. The chopped forages were treated with no additives. Afterwards, the experimental forages were packed tightly in large plastic bags. Oxygen was removed from the plastic bags by ensures of a vacuum sealer. The experimental forages were also packed tightly in 200-litre plastic round drums with clamp lid and stored until the start of the feeding trial. The plastic containers were stored at room temperature (27-30°C).

4.4.3 *In vitro* gas technique (Menke and Steingass, 1988)

4.4.3.1 Buffer preparation

- Ammonium bicarbonate (NH_4HCO_3) 4 g
- Sodium bicarbonate ($NaHCO_3$) 35 g
- Dissolve in water and bring up to 1 L in volumetric flask.
- Increase volume of buffer solution as required.

4.4.3.2 Macro-mineral preparation

- Sodium hydrogen phosphate, dibasic (Na_2HPO_4) 5.7 g
- Potassium phosphate, monobasic (KH_2PO_4) 6.0 g
- Magnesium sulfate, heptahydrate ($MgSO_4 \cdot 7H_2O$) 0.6 g
- Dissolve in water and bring up to 1 L in volumetric flask.
- Increase volume of buffer solution as required.

NOTE: Buffer and Macromineral solution can be stored refrigerated for up to 3 months and at room temperature for up to 1 month.

4.4.3.3 Micro-mineral preparation

- Calcium chloride, dehydrate ($CaCl_2 \cdot 2H_2O$) 13.2 g
- Manganese chloride, tetrahydrate ($MnCl_2 \cdot 4H_2O$) 10.0 g
- Cobalt chloride, hexahydrate ($CoCl_2 \cdot 6H_2O$) 1.0 g
- Ferric chloride, hexahydrate ($FeCl_2 \cdot 6H_2O$) 8.0 g
- Dissolve in water and bring up to 100 mL in volumetric flask.

NOTE: Micro-mineral solution can be stored refrigerated for up to 12 months.

4.4.3.4 0.1% (wt/vol) Resazurin

- Dissolve 0.1 g of resazurin 100 mL water.
- Store in dark (amber coloured) bottle at 4°C (infridge).

4.4.3.5 Substrate preparation

Substrates were dry at 60°C until dry (72 hours) and ground with mill through 1 mm screen after that weigh 0.5 g of substrate into each syringe.

4.4.3.6 Medium preparation

This recipe is for 1 L, increase volume as required

- Weigh out 2.5 g tryptone and dissolve completely in 500 mL water

- Add 0.125 mL micromineral solution

- Add 250 mL buffer solution and 250 mL macromineral solution

- Add 1.25 mL 0.1% resazurin solution

Place container with medium in water bath (39°C) and flushed with CO_2 through solution for 45 minutes. Put in 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide and add directly to medium and flushed with CO_2 through solution for another 15 minutes or until solution turns grey to clear. A Purple/pink color indicates the presence of oxygen. Keeping the medium in water bath and head space saturated with CO_2 until medium+inoculums, then transfer to incubation syringe. At this point rumen fluid can be collected.

4.4.3.7 Donors of rumen fluid for *in vitro* incubations

Inoculum for the batch culture was obtained from four matured rumen fistulated Saanen male goats (about 25 kg weighs) fed with 2.5% of body weight (% BW) DM/day containing dried-ground Pangola (*Digitaria eriantha*) and commercial concentrate (14% of Crude protein) (60:40) will be used as donors of rumen fluid. Rumen fluid was collected from different sites within the rumen approximately 2 hours after the morning feeding, strained through 4 layers of cheesecloth into a flask and flushed with oxygen free CO_2. Rumen fluid was transported in insulated flasks to the laboratory within less than 1 hours of collection. Added rumen fluid to medium in a ratio of 1:4 (rumen fluid:medium). Anaerobic buffer medium 20 mL, (Goering and Van Soest, 1970) containing tryptone, buffer, macro and micro mineral solution, resazurin, and water.

Twenty five milliliters of rewarmed media and 5 mL of inoculum were added anaerobically to the 100 mL syringes by flushed with oxygen free CO_2, after that incubated at 39^{o}C for 96 hours. Blanks (rumen fluid plus anaerobic buffer medium) were also incubated using 12 replications for correction of gas production and disappearance, respectively.

4.4.4 Animals, treatments, and experimental design

Twenty-four male goats, crossbred Thai native×Anglo-Nubian (approximately 18.50±2.06 kg body weight; mean ± standard deviation (SD)), were used in 2×2 factorial arrangements in CRD. The forages were fed ad libitum and clean drinking water was provided. Period length was last for 60 days of which the first 7 days used as adjustment period to the experimental diets. The goats were randomly allocated to 4 treatment combinations of 6 each. The experimental treatments were;

Treatment 1 = Fresh Napier Pakchong-1 grass

Treatment 2 = Napier Pakchong-1 grass silage

Treatment 3 = Fresh Purple Napier grass

Treatment 4 = Purple Napier grass silage

The goats in the experimental treatments fed grass by cut-and-carry feeding regime. Animals were housed in clean individual pens with free access to water (H_2O) and were fed diet with a ratio of roughage:concentrate at 60:40. Ration was offered in equal amounts twice daily at 07:00 and 16:00 for ad libitum intake and allowed for approximately 10% refusal. The diets were formulated to meet requirements for growing goats having 18 kg of BW according to the NRC (1981).

4.4.5 Data collection and sampling

4.4.5.1 *In vitro* gas technique sampling

At pre-determined time points, head space gas production (GP) were measured at 3, 6, 9, 12, 24, 48, 60, 72 and 96 hours post incubation, using *in vitro* gas production of (Ørskov and McDonald, 1979). GP was calculated according to the following equation by Ørskov and McDonald (1979):

$$y = a + b\ (1- e^{-ct})$$

where y denotes the volume of gas produced at time t, a describes the immediately soluble fraction (mL), b is the insoluble fraction (mL), c is the rate constant for the insoluble fraction b (%/h), t expresses incubation time (h), and a + b represents the potential extent of gas production (mL). The fermentation was stopped by submerging the syringe into ice-cold water, and then the pH of the rumen fluid was immediately measured using the portable pH meter. In the meantime, 20 mL of fermentation liquid and 5 mL of HCl (6 mol/L) were kept in a container after being mixing together, then stored in a refrigerator at -20°C until the samples were analyzed for NH_3-N and individual volatile fatty acid (VFA) content. Acetic acid (C_2), propionic acid (C_3) and butyric acid (C_4) were determined using HPLC. The total VFA (TVFA) value was calculated from the following equation:

$$TVFA = C_2 + C_3 + C_4$$

Each sample had three replications and three control replications. In addition, organic matter digestibility (OMD), metabolizable energy (ME) and

effective degradability (ED) were calculated using the following formulas by Menke and Steingass (1988); Ørskov and McDonald(1979), respectively:

$$OMD\ (\%) = 0.986 \times GP\ (24\ h) + 0.0606 \times CP + 11.03$$

$$ME\ (MJ/kg) = -0.20 + 0.1410 \times OMD$$

$$ED\ (\%) = a + b \times c/(k + c).$$

where k is ruminal outflow rate and the value sets as 0.031 hours.

4.4.5.2 Feed, fecal and urine sampling

Data were obtained for continuously monitored DMI in each day. At the conclusion of each period, feed ingredients and feed refusal composites were dried in the oven at 65°C for 72 hours, and then ground and passed through a 1-mm sieve and kept at 4°C until further analysis. The metabolic cages were specially designed with a facility for separate collection of feces and urine. The animals were kept in metabolic cages for 3 days, prior to actual collection of 7 days to acclimatize the animals to the new surroundings. Fecal (around 100 g) and urine (approximately 30 mL) samples were collected. Fecal samples were dried in the oven at 65°C for 72 hours and ground to pass through a 1-mm sieve after grinding and kept 4°C until further analysis. Fecal and urine samples were also composited by each period. Daily collection of urine of each animal was acidified with 20% sulphuric acid (H_2SO_4) to keep the pH<3, and then stored at -20°C until the analysis of chemical composition.

4.4.5.3 Rumen fermentation and blood sampling

After 60 days of the experiment, the rumen contents was collected at 0 hour before feeding, 2 and 4 hours after feeding using a stomach tube attached to a suction pump, pH measured immediately using a glass electrode pH meter. After recording pH, aliquot of the samples were strained through 4 layers of cheese

cloth. The rumen fluid was then acidified with H_2SO_4 (10%, v/v) and stored at -20°C for subsequently quantifying NH_3-N and volatile fatty acids (VFAs) concentration.

For DNA extraction samples, the rumen content of each animal was collected at 0 hour before feeding, 2 and 4 hours after feeding and were strained through 4 layers of cheese cloth and stored only rumen fluid (no additive solution) at -20°C used for DNA extraction of methanogen, protozoa, *Butyrivibrio fibrisolvens* (*B. fibrisolvens*), *Fibrobacter succinogenes* (*F. succinogenes*) *Ruminococcus flavefaciens* (*R. flavefaciens*) and total bacteria, respectively using real-time PCR technique, (LightCycler® Nano System version 1.0.1, Roche).

The NH_3-N were determined using distillation method according to the Kjeldahl method. The acetic acid (C_2), propionic acid (C_3), butyric acid (C_4) and total VFAs were determined by high performance liquid chromatography (HPLC, Shim-pack SCR-102H, 300×8.0 mm i.d.; column temperature, 40°C; flow rate, 0.8 ml/min, Shimadzu Co. Ltd., Kyoto, Japan). Blood samples were taken from the jugular vein at 0 hour before feeding, 2 and 4 hours after feeding. Then, the blood samples were prior to plasma separation by centrifugation (3,000×g for 15 min) and plasma samples were then stored at -20°C for determining blood urea nitrogen (BUN) concentration.

4.4.6 Laboratory analyses

4.4.6.1 Chemical analysis

Samples were immediately dried in an air forced oven at 100°C with a constant weight to evaluate the dry matter content (DM) before being ground over a 1 mm screen using a Wiley hammer mill. Ash content was evaluated by burning at 550°C for 3 hours in a muffle furnace. Neutral detergent fiber (NDF) was analyzed by a modified method of Van Soest et al. (1991) with addition of a heat stable amylase, and acid detergent fiber (ADF) were analyzed by the method of Goering and Van Soest

(1970). The content of hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. Nitrogen content was measured by AOAC (1995) and the crude protein (CP) content was calculated as N×6.25.

Community DNA was extracted from 1.5 ml aliquots of rumen fluid and digesta by the RBB+C method (Yu and Morrison, 2004). The cell lysis is achieved by bead-beating in the presence of 4% (w/v) sodium dodecyl sulfate (SDS), 500 mM NaCl, and 50 mM EDTA. The buffer should also protect the released DNA from degradation by DNases, which are very active in the rumen and gastrointestinal sample. After bead-beating, most of the impurities and the SDS are removed by precipitation with ammonium acetate and then the nucleic acids are removed by precipitation with isopropanol. Genomic DNA can then purified via sequential digestion with RNase A and proteinase K, and the DNA are purified according to QIAmp PowerFecal DNA kit and PCR condition. Real-time PCR, Species specific PCR primers used to amplify 16S rDNA regions (target DNA) were chosen from Denman et al. (2007) for methanogen, Sylvester et al. (2004) for protozoa, Klieve et al. (2003) for *B. fibrisolvens*, Koike et al. (2001) for *F. succinogenes*, *R. flavefaciens* and total bacteria. Real-time PCR amplification and detection were performed using a LigthCycler Nano (LightCycler® Nano System version 1.0.1, Roche).

4.4.6.2 Anthocyanin composition

Grass samples (50 g) were modified to pH 4 with 1% hydrochloric acid and pre-treated with acetone/chloroform liquid-liquid extraction (70:30, v/v) and afterwards centrifuged 10,000 r/min at 4°C for 15 minutes after incubation at room temperature for 4 hours, the supernatant was collected for anthocyanin composition. The analysis of the specimen has been performed with the HPLC and Diode Array Detector

(DAD). Anthocyanin content extraction was achieved on the column C_{18} Symmetry (mobile phase: A, acetonitrile (CH_3CN); B, 10% acetic acid/5% CH_3CN/1% phosphoric acid in deionized water). The time period was 30 minutes, followed by a delay of 5 minutes before the next injection. Another conditions were determined sample temperature at 4°C and injection volume of 20 µL, flow rate of 0.8 mL/min, column temperature of 25°C and DAD wavelength of 520 nm.

4.4.7 Statistical analysis

All statistical calculations were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System 9.1.3 (SAS, 1990) according to 2×2 factorial in Completely Randomized Design (CRD). Significant differences ($P<0.05$) among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1980). The statistical model for the analysis of data was:

$$Y_{ijk} = \mu + C_i + F_j + C_i*F_j + \varepsilon_{ijk}$$

where: Y_{ij} = all dependent variables

μ = the overall mean

C_i = the effect of i^{th} cultivars of Napier grass (NP-1 and PN)

F_j = the effect of j^{th} form of Napier grass (fresh and silage)

C_i*F_j = the interaction of cultivars and form

ε_{ij} = residual

4.4.8 Experimental location

The experiment was conducted at Suranaree University of Technology (SUT) goat farm, Nakhon Ratchasima, Thailand (14°53'37.9"N, 102°01'22.0"E).

4.4.9 Experimental period

The experiment was from July 2018 to September 2018.

4.5 Results

4.5.1 Feed chemical composition

The chemical composition and anthocyanin content of Napier Pakchong-1 and Purple Napier grass in the experimental treatments were demonstrated in the Table 4.1. Chemical composition, which was similar to each other for the major compositions (DM, OM, CP, NDF, ADF and EE) and the anthocyanin profile mainly contained C3G, P3G, Del, Peo3G, M3G, Cya, Pel and Mal in experimental I. Furthermore, the major compositions (DM, OM, CP, NDF and ADF) and the anthocyanin composition in grass silage form were lower than fresh grass form.

4.5.2 Gas production kinetics and *In vitro* digestibility

Gas production from the fermentation of Napier Pakchong-1 and Purple Napier grass with fresh and silage form were measured at 3, 6, 12, 24, 48, 72 and 96 hours using *in vitro* gas production of Ørskov and McDonald (1979) method. The *in vitro* gas production characteristics of treatments are presented in Table 4.2. The results showed that *in vitro* gas production characteristics after incubation differed significantly ($P<0.05$) for all the parameters on different grass forms (fresh and silage) especially factor *a* had interaction between cultivars and forms. The fresh Purple Napier grass had the highest ($P<0.05$) factor *a* value. The rate of gas production (*c*) had significant influences by form of grass ($P<0.05$), the fresh form of grass had higher than silage form.

Total gas production was influenced by form of grass. At 6, 12 hours and the mean of total gas production, fresh form of grass had higher ($P<0.05$) than silage form along with gas cumulative at different incubation times (Figure 3.1). Methane (CH_4) production was influenced by interaction of cultivars×form. The highest ($P<0.05$) CH_4 production was observed in fresh Napier Pakchong-1 treatment at 6, 12, 24 hours

and the mean value of CH_4 production, while fresh Purple Napier grass had the lowest ($P<0.05$) of CH_4 production. The ratio of CH_4/total gas at 6 hours was influenced by form of grass, the grass silage had higher ($P<0.05$) than the fresh grass.

For *in vitro* digestibility (%), dry matter digestibility (DMD) was influenced by interaction of cultivars×form, while the organic matter digestibility (OMD) and effective degradability (ED) were influenced by form of grass. The highest ($P<0.05$) value of DMD was observed in fresh Napier Pakchong-1 grass treatment. The grass silage treatment had the value of OMD higher ($P<0.05$) than fresh grass treatment but the value of ED was lower ($P<0.05$) than fresh grass treatment.

4.5.3 *In vitro* rumen characteristics

The effects of dietary treatment on *in vitro* fermentation of rumen have been shown in Table 4.3. No effect of experimental treatments on pH ($P>0.05$) was observed at 6, 12, 24 hours and pH mean, respectively. Ammonia nitrogen (NH_3-N) and propionic acid (C_3) were affected by the interaction of cultivars×form. The NH_3-N at 6 hours was highest ($P<0.05$) in fresh Purple and Napier Pakchong-1 grass tretament. The NH_3-N at 12, 24 hours and the mean value of NH_3-N, respectively with the percentage of C_3 at 6, 12, 24 hours and the mean value of C_3, respectively were highest ($P<0.05$) in fresh Purple Napier grass.

Acetic acid (C_2) was affected by cultivars × form interaction, fresh Napier Pakchong-1 grass had the highest ($P<0.05$) the percentage of C_2 at 6, 12, 24 hours and the mean of C_2, respectively. The butyric acid (C_4) was affected by grass form, the silage form of grass had the highest ($P<0.05$) percentage of C_4 at 6, 12, 24 hours and average of C_4, respectively. Total volatile fatty acid (TVFA) at 6 and 12 hours was affected by the interaction of cultivars × form, the fresh of Purple Napier and Napier Pakchong-1 grass had the highest percentage ($P<0.05$) of TVFA.

The percentage of TVFA at 24 hours and the mean of TVFA was affected by grass form, the fresh form of grass had the highest ($P<0.05$) percentage of TVFA and the mean of TVFA among all treatments. The ratio of acetic/propionic acid (C_2/C_3) was influenced by the interaction of cultivars × form. The Napier Pakchong-1 grass silage, fresh Napier Pakchong-1 and Purple Napier grass silage at 6 and 24 hours had the highest ($P<0.05$) C_2/C_3 ratio. The ratio of C_2/C_3 at 12 hours and the mean of C_2/C_3 was highest ($P<0.05$) in Napier Pakchong-1 grass silage treatment.

4.5.4 Growth performance, feed intake and nutrient digestibility

The result in Table 4.4, there was significant difference of interaction of cultivars × form on final weight, weigh change, average daily gain (ADG), DM intake dry matter of roughage, total DM intake, nutrient intake (CP, NDF and ADF), anthocyanin intake and apparent nutrient digestibility (OM, CP, NDF and ADF). Final weight, weigh change and ADG were highest ($P<0.05$) for the goats fed on fresh Purple Napier grass treatment. Dry matter intake of roughage and total dry matter intake were highest ($P<0.05$) for the goats fed on fresh Purple Napier grass, fresh Napier Pakchong-1 grass and Purple grass silage treatment as g/day, %BW and g/kg $BW^{0.75}$, respectively.

Nutrient intake, the CP and anthocyanin intake were highest ($P<0.05$) for the goats fed on fresh Purple Napier grass and the NDF intake was highest ($P<0.05$) for the goats fed on fresh Napier Pakchong-1 grass, whereas the ADF intake was highest ($P<0.05$) for the goats fed on fresh Napier Pakchong-1 and Purple Napier grass. Appearance digestibility of OM and CP were highest ($P<0.05$) for the goats fed on fresh Purple Napier grass and the NDF and ADF digestibility were highest ($P<0.05$) for the goats fed on fresh Napier Pakchong-1 and Purple Napier grass. Various effects of grass form were observed for OM intake, EE intake, EE digestibility and ME digestibility that

were higher (P<0.05) in the goats fed on fresh grass treatment than grass silage treatment.

4.5.5 Nitrogen utilization

As a result, there was a significant difference in the interaction between cultivars×form of nitrogen utilization parameters in Table 4.5. Fresh Purple Napier grass had the highest levels (P<0.05) of N intake (g/d), N excretion from feces (g/d), N excretion from urine (g/d), total N excretion (g/d), N absorption (g/d), N absorption (%), N retention (g/d) and N retention (%), respectively among all the treatments.

4.5.6 Rumen characteristics and blood urea nitrogen of goats

The result in Table 4.6 has shown the rumen characteristics and blood urea nitrogen of goats. There was significant difference of interaction between cultivars×form of ammonia-nitrogen (NH_3-N), blood urea nitrogen (BUN), propionic acid (C_3), total volatile fatty acid (TVFA) and ratio of C_2/C_3, respectively. The NH_3-N, BUN, C_3 and TVFA after feeding (2 to 4 hours) and the mean value were highest (P<0.05) in fresh Purple Napier grass treatment. The ratio of C_2/C_3 value at 2 hours after feeding was highest (P<0.05) in fresh Napier Pakchong-1 and Napier Pakchong-1 grass silage treatment and the ratio of C_2/C_3 value at 4 hours after feeding and the mean value were highest (P<0.05) in Napier Pakchong-1 grass silage treatment, while the ratio of C_2/C_3 value after feeding (2 and 4 hours) and the mean value were the lowest (P<0.05) in fresh Purple Napier grass treatment.

There were significant difference effect of cultivars and form with no interaction effect in the percentage of acetic acid (C_2) and butyric acid (C_4). The C_2 at 2 and 4 hours after feeding and the mean value were higher (P<0.05) in Napier Pakchong-1 cultivars treatment than Purple Napier cultivars treatment and the C_4 at 4 hours after feeding of Purple Napier cultivars treatment was higher (P<0.05) than Napier Pakchong-

1 cultivars treatment. For the effect of grass form, The C_2 at 2 and 4 hours after feeding and the mean value of fresh grass form treatment were higher ($P<0.05$) than grass silage form treatment, while the C_4 at 2 and 4 hours after feeding and the mean value of silage grass form treatment were higher ($P<0.05$) than fresh grass form treatment.

4.5.7 Microbial population in goat's rumen

The microbial population in the goat's rumen has been shown in Table 4.7. There were no different of total bacteria in goat's rumen ($P>0.05$). The methanogen, protozoa, *Butyrivibrio fibrisolvens* (*B. fibrisolvens*) and *Ruminococcus flavefaciens* (*R. flavefaciens*), respectively were influenced by the interaction between cultivars × form of grass. The rumen methanogen and *R. flavefaciens* at 2, 4 hours after feeding and the mean value were highest ($P<0.05$) in fresh Napier Pakchong-1 grass treatment, while protozoa at 2 and 4 hours after feeding were highest ($P<0.05$) in Napier Pakchong-1 grass silage treatment and the mean value of rumen protozoa was highest ($P<0.05$) in fresh and silage form of Napier Pakchong-1 grass.

The *B. fibrisolvens* at 2 and 4 hours after feeding were highest ($P<0.05$) in fresh Purple Napier grass treatment. There was found effect of grass cultivars, the mean value of *B. fibrisolvens* was higher in Purple Napier grass cultivars and the *Fibrobacter succinogenes* (*F. succinogenes*) at 2 and 4 hours after feeding and the mean value were highest ($P<0.05$) in Napier Pakchong-1 cultivars. There was found effect of grass form, the mean value of *B. fibrisolvens* was higher ($P<0.05$) in fresh grass form than grass silage form treatment and the *F. succinogenes* at 2 and 4 hours after feeding and the mean value were higher ($P<0.05$) in fresh grass form than grass silage form treatment.

Table 4.1 The chemical composition in experiment diets.

Item[1]	NP-1		PN[2]		Concentrate
	Fresh	Silage	Fresh	Silage	
DM (%)	24.29	22.03	23.92	22.94	90.56
	---------- % on dry matter basis ----------				
OM	90.02	85.95	91.98	88.83	91.93
CP	10.38	9.08	11.77	9.34	16.08
NDF	74.57	63.55	72.30	62.19	59.35
ADF	46.74	38.85	40.71	37.78	43.47
EE	3.27	2.50	3.20	2.56	5.13
Ash	9.98	14.05	8.02	11.17	8.07
GE (kcal/kg)	3,740.80	3,718.60	3,765.40	3,728.00	4,018.30
Anthocyanin composition (mg/ g DM)[3]					
C3G	0.82	0.46	2.23	1.07	-
P3G	0.18	0.07	0.46	0.18	-
Del	0.01	0.06	0.02	0.02	-
Peo3G	0.25	0.02	0.64	0.39	-
M3G	0.01	0.004	0.03	0.01	-
CYA	0.01	0.01	0.03	0.02	-
Pel	0.01	0.01	0.03	0.02	-
Mal	0.38	0.27	0.98	0.66	-
Total	1.67	0.89	4.42	2.36	-

[1] DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; GE = gross energy.

[2] NP-1 = Napier Pakchong-1 grass; PN = Purple Napier grass.

[3] C3G = cyanidin-3-glucoside; P3G = pelargonidin-3-glucoside; Del = delphinidin; Peo3G = peonidin-3-O-glucoside; M3G = malvidin-3-O-glucoside; Cya = cyanidin; Pel = pelargonidin; Mal = malvidin.

Table 4.2 Comparative of gas production kinetics and *In vitro* digestibility of two Napier grass cultivars with fresh and silage form.

Items[1]	NP-1		PN[2]		SEM	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Gas kinetics								
a (mL)	1.44[b]	0.95[c]	2.43[a]	0.85[c]	0.118	<.0001	<.0001	<.0001
b (mL)	82.38	77.22	81.42	79.11	1.988	0.372	0.732	0.911
a + *b* (mL)	83.82	78.17	83.85	79.96	1.987	0.444	0.555	0.826
c (% h)	0.04[a]	0.03[b]	0.04[a]	0.03[b]	0.001	0.363	0.023	0.883
Total gas production (mL/0.2 g DM of substrate)								
6-h	28.22[a]	23.28[b]	27.36[a]	22.88[b]	0.995	0.706	0.020	0.888
12-h	35.26[a]	30.93[b]	33.46[a]	28.81[b]	0.982	0.297	0.022	0.932
24-h	55.06	49.83	52.11	46.51	1.412	0.259	0.057	0.946
Mean	39.56[a]	34.68[b]	37.64[a]	32.73[b]	1.272	0.301	0.029	0.951
Methane (CH_4) production (mL/0.2 g DM of substrate)								
6-h	20.81[a]	19.97[b]	19.44[c]	19.59[bc]	0.165	<.0001	0.0077	0.0009
12-h	20.07[a]	19.85[ab]	19.31[c]	19.71[b]	0.088	0.0002	0.265	0.003
24-h	19.88[a]	19.02[b]	18.82[b]	18.92[b]	0.144	0.007	0.051	0.018
Mean	20.25[a]	19.62[b]	19.19[b]	19.41[b]	0.126	0.0002	0.059	0.002
CH_4/total gas ratio (v/v)								
6-h	0.73[b]	0.85[a]	0.71[b]	0.87[a]	0.030	0.997	0.024	0.704
12-h	0.58	0.66	0.60	0.72	0.029	0.521	0.099	0.763
24-h	0.37	0.40	0.38	0.43	0.013	0.427	0.121	0.694
Mean	0.56	0.66	0.60	0.72	0.028	0.379	0.054	0.880

Table 4.2 (Continue).

Items[1]	NP-1		PN[2]		SEM	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
***In vitro* digestibility (%)**								
DMD (%)	74.16[a]	67.35[c]	72.29[b]	63.53[d]	0.976	<.0001	<.0001	0.030
OMD (%)	61.15[b]	67.02[a]	64.18[b]	69.15[a]	1.272	0.296	0.034	0.854
ME (MJ/kg)	9.39	8.60	9.78	9.05	0.193	0.270	0.050	0.931
ED%	48.64[a]	43.01[b]	49.69[a]	44.75[b]	1.215	0.554	0.032	0.884

[a, b, c] Means with different superscripts in the same row differ significantly (P<0.05)

[1] *a* = gas production from the immediately soluble fraction; *b* = gas production from the insoluble fraction; *c* = gas production rate constant; *a* + *b* = potential extent of gas production; DMD = dry matter digestibility; OMD = organic matter digestibility; ME = metabolizable energy; ED = effective degradability.

[2] NP-1 = Napier Pakchong-1 grass; PN = Purple Napier grass.

[3] C = effect of Napier grass cultivars; F = effect of grass form.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.3 *In vitro* rumen characteristics of pH, NH_3-N, and VFAs values of two Napier grass cultivars with fresh and silage form.

Time (h)	NP-1		PN		SEM	P-value		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Ruminal pH								
6	6.75	6.88	6.74	6.73	0.036	0.303	0.443	0.328
12	6.54	6.60	6.56	6.55	0.025	0.723	0.696	0.565
24	6.46	6.36	6.44	6.49	0.024	0.228	0.641	0.112
Mean	6.55	6.60	6.56	6.55	0.027	0.746	0.733	0.708
NH_3-N (mg/dL)								
6	21.23^{a}	16.82^{b}	21.58^{a}	17.62^{b}	0.476	0.569	0.151	<.0001
12	16.40^{b}	11.48^{c}	18.32^{a}	15.40^{b}	0.561	0.037	<.0001	<.0001
24	6.86^{b}	5.13^{c}	8.12^{a}	5.81^{bc}	0.266	0.285	0.002	<.0001
Mean	16.40^{b}	11.48^{c}	18.32^{a}	15.40^{b}	0.561	<.0001	<.0001	0.037
Acetic acid (C_2), %Molar								
6	62.16^{a}	57.92^{b}	58.61^{b}	56.90^{b}	0.637	0.003	0.001	0.044
12	63.14^{a}	60.13^{bc}	61.08^{b}	59.34^{c}	0.443	0.001	<.0001	0.044
24	61.50^{a}	58.83^{b}	58.51^{b}	58.61^{b}	0.436	0.016	0.041	0.030
Mean	62.16^{a}	58.83^{b}	59.26^{b}	58.61^{b}	0.486	0.018	0.005	0.034
Propionic acid (C_3), %Molar								
6	28.15^{b}	25.90^{c}	31.36^{a}	26.87^{bc}	0.648	0.001	<.0001	0.034
12	26.67^{b}	21.92^{d}	28.59^{a}	23.29^{c}	0.800	<.0001	<.0001	0.042
24	21.77^{b}	20.55^{b}	24.82^{a}	20.56^{b}	0.563	0.013	0.001	0.013
Mean	26.34^{b}	22.18^{d}	28.70^{a}	23.28^{c}	0.780	<.0001	<.0001	0.023

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.3 (Continue).

Time (h)	NP-1		PN		SEM	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Butyric acid (C_4), %Molar								
6	9.70[b]	16.17[a]	10.03[b]	16.23[a]	0.994	0.768	<.0001	0.835
12	10.19[b]	17.96[a]	10.33[b]	17.37[a]	1.121	0.284	<.0001	0.106
24	16.73[b]	20.61[a]	16.68[b]	20.83[a]	0.640	0.862	<.0001	0.786
Mean	12.21[b]	18.25[a]	12.34[b]	18.14[a]	0.903	0.956	<.0001	0.710
TVFA, mmol/L								
6	168.53[b]	170.49[b]	173.88[a]	167.91[b]	0.749	0.054	0.011	0.0002
12	190.89[a]	179.72[b]	184.64[b]	180.33[b]	1.427	0.035	0.0001	0.015
24	115.20[a]	88.14[ab]	114.74[a]	76.39[b]	5.761	0.364	0.001	0.399
Mean	158.21[a]	146.11[b]	157.75[a]	141.54[b]	2.401	0.310	0.0003	0.400
TVFA, mmol/L								
6	168.53[b]	170.49[b]	173.88[a]	167.91[b]	0.749	0.054	0.011	0.0002
12	190.89[a]	179.72[b]	184.64[b]	180.33[b]	1.427	0.035	0.0001	0.015
24	115.20[a]	88.14[ab]	114.74[a]	76.39[b]	5.761	0.364	0.001	0.399
Mean	158.21[a]	146.11[b]	157.75[a]	141.54[b]	2.401	0.310	0.0003	0.400
Ratio of C_2/C_3								
6	2.21[a]	2.24[a]	1.87[b]	2.12[a]	0.045	<.0001	0.001	0.004
12	2.37[c]	2.74[a]	2.14[d]	2.55[b]	0.068	<.0001	<.0001	0.064
24	2.83[a]	2.86[a]	2.36[b]	2.86[a]	0.070	0.006	0.003	0.007
Mean	2.47[b]	2.61[a]	2.12[c]	2.51[b]	0.057	<.0001	<.0001	0.0007

[a, b, c, d] Means with different superscripts in the same row differ significantly ($P < 0.05$); Time (h) = hour (s) after feeding experimental diets; NH_3-N = ammonia nitrogen; TVFA = total volatile fatty acid; NP-1 = Napier Pakchong-1; PN = Purple Napier; C = effect of grass cultivars; F = effect of grass form; C × F = interaction effect of grass cultivars and form.

มหาวิทยาลัยเทคโนโลยีสุรนารี

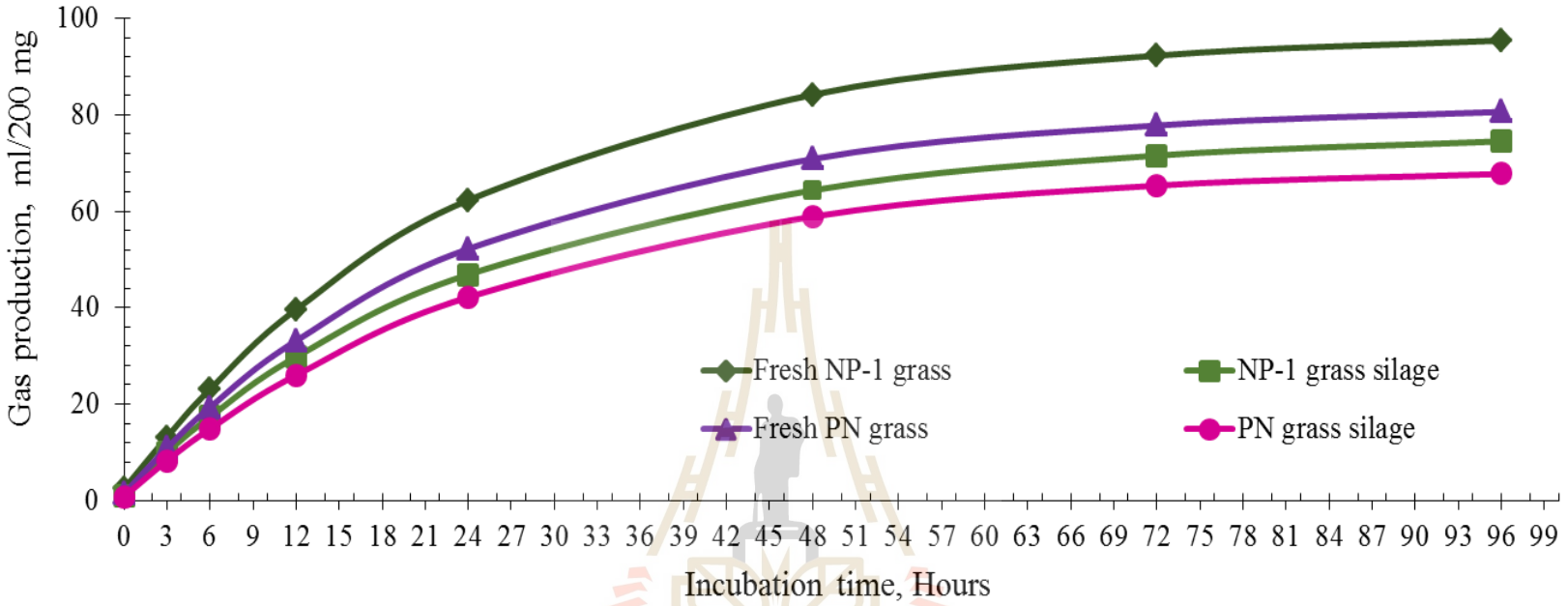


Figure 3.1 Gas cumulative of treatment combinations including Fresh NP-1 grass = fresh Napier Pakchong-1 grass, NP-1 grass silage = Napier Pakchong-1 grass silage, Fresh PN = fresh Purple Napier grass and PN grass silage = Purple Napier grass silage at different incubation times.

Table 4.4 Effect of Napier grass fresh and silage on BW, feed intake and nutrient digestibility of the experimental goats.

Item	NP-1		PN[1]		SEM[2]	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Initial BW, kg	18.46	18.58	18.47	18.47	0.025	0.367	0.264	0.283
Final BW, kg	22.82[b]	22.15[c]	23.11[a]	22.41[c]	0.099	0.0004	<.0001	0.007
Weigh change, kg	4.36[b]	3.56[b]	4.64[a]	3.93[b]	0.113	0.003	<.0001	0.006
ADG, g/d	72.59[b]	59.40[d]	77.36[a]	65.51[c]	1.769	<.0001	<.0001	0.0297
Dry matter intake Roughage								
g/day	527.77[a]	465.26[b]	536.73[a]	511.63[a]	7.905	0.004	0.0001	0.032
%kg BW	2.31[a]	2.10[b]	2.32[a]	2.28[a]	0.023	0.021	0.004	0.032
g/kg $BW^{0.75}$	50.56[a]	45.58[b]	50.91[a]	49.68[a]	0.649	0.013	0.002	0.032
Concentrate								
g/day	346.25	339.55	347.81	340.31	12.464	0.967	0.803	0.989
%kg BW	1.51	1.53	1.50	1.52	0.055	0.914	0.904	0.996
g/kg $BW^{0.75}$	33.17	33.27	32.99	33.05	1.204	0.943	0.978	0.995
Total								
g/day	874.02[a]	804.81[b]	884.54[a]	851.94[a]	8.046	<.0001	<.0001	0.0001
%kg BW	3.83[a]	3.63[b]	3.82[a]	3.80[a]	0.023	0.0011	<.0001	0.001
g/kg $BW^{0.75}$	83.73[a]	78.84[b]	83.91[a]	82.73[a]	0.548	<.0001	<.0001	<.0001

Table 4.4 (Continue).

Item	NP-1		PN		SEM	P-value		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Nutrient intake (g/day)								
OM	793.41[a]	712.03[b]	813.42[a]	767.32[b]	15.709	0.195	0.038	0.532
CP	110.53[b]	93.36[d]	119.22[a]	105.79[c]	2.428	<.0001	<.0001	0.011
EE	35.01[a]	29.05[b]	35.02[a]	30.56[b]	0.956	0.622	0.004	0.621
NDF	623.10[a]	521.50[c]	570.16[b]	496.02[d]	12.794	0.030	<.0001	<.0001
ADF	395.81[a]	372.05[b]	393.33[a]	357.18[c]	4.294	0.001	<.0001	0.043
Anthocyanin (mg/day)	882.42[c]	415.87[d]	2371.33[a]	1208.43[b]	186.596	<.0001	<.0001	<.0001
Appearance digestibility, %								
OM	60.44[ab]	52.33[c]	63.30[a]	54.74[b]	1.131	<.0001	<.0001	0.019
CP	74.36[ab]	61.96[c]	75.57[a]	65.33[b]	1.509	0.0002	<.0001	0.025
EE	75.83[a]	61.94[b]	76.09[a]	63.58[b]	1.767	0.356	<.0001	0.501
NDF	78.59[a]	71.97[b]	77.99[a]	70.34[c]	0.939	<.0001	<.0001	0.009
ADF	74.68[a]	67.52[c]	72.73[a]	66.10[d]	0.921	<.0001	<.0001	0.017
ME (Mcal/kg DM)	2.17[a]	1.98[b]	2.19[a]	2.03[b]	0.027	0.783	0.0001	0.280

[a, b, c, d] Means with different superscripts in the same row differ significantly (P<0.05); SEM = standard error of mean; NP-1 = Napier Pakchong-1; PN = Purple Napier; C = effect of Napier grass cultivars; F = effect of grass form (fresh and silage); C×F = interaction effect of Napier grass cultivars and grass form.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.5 Effect of Napier grass fresh and silage on nitrogen utilization of the experimental goats.

Item	NP-1		PN[1]		SEM[2]	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
N intake (g/d)	17.56[b]	14.80[d]	19.50[a]	16.18[c]	0.451	<.0001	<.0001	0.004
N excretion from feces (g/d)	5.14[b]	4.88[d]	5.36[a]	5.08[c]	0.044	<.0001	<.0001	0.007
N excretion from urine (g/d)	4.12[b]	3.20[d]	4.52[a]	3.63[c]	0.128	<.0001	<.0001	0.006
Total N excretion (g/d)	9.26[b]	8.08[d]	9.88[a]	8.71[c]	0.171	<.0001	<.0001	0.031
N absorption (g/d)	12.43[b]	9.92[d]	14.14[a]	11.09[c]	0.408	<.0001	<.0001	0.004
N absorption (%)	70.76[b]	67.01[d]	72.52[a]	68.57[c]	0.541	<.0001	<.0001	0.030
N retention (g/d)	8.31[b]	6.71[d]	9.62[a]	7.46[c]	0.279	<.0001	<.0001	<.0001
N retention (%)	47.29[b]	45.37[d]	49.35[a]	46.13[c]	0.398	<.0001	<.0001	0.009

[a, b, c, d] Means with different superscripts in the same row differ significantly (P<0.05).

[1] NP-1 = Napier Pakchong-1; PN = Purple Napier. [2] SEM = standard error of mean.

[3] C = effect of Napier grass cultivars; F = effect of grass form (fresh and silage); C × F = interaction effect of Napier grass cultivars and grass form.

Table 4.6 Effect of Napier grass fresh and silage on rumen characteristics and blood urea nitrogen in plasma of feeding trial experiment.

Time (h)	NP-1		PN		SEM	P-value		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Ruminal pH								
0	6.98	6.96	7.00	6.99	0.166	0.925	0.945	0.979
2	6.75	6.76	6.78	6.79	0.137	0.722	0.919	1.000
4	6.86	6.87	6.82	6.85	0.159	0.786	0.874	0.964
Mean	6.86	6.86	6.87	6.88	0.139	0.975	0.992	0.990
NH_3-N, mg/dL								
0	14.98	15.00	15.07	15.05	0.077	0.686	0.983	0.904
2	22.24^{b}	21.08^{d}	23.13^{a}	21.79^{c}	0.192	<.0001	<.0001	0.026
4	18.59^{b}	16.11^{d}	19.94^{a}	17.54^{c}	0.362	<.0001	<.0001	0.027
Mean	18.60^{b}	16.12^{d}	19.95^{a}	17.55^{c}	0.362	0.0001	<.0001	0.036
BUN, mg/dL								
0	12.06	11.98	12.08	12.01	0.121	0.942	0.787	0.988
2	18.74^{b}	17.08^{d}	19.13^{a}	17.79^{c}	0.208	<.0001	<.0001	0.0002
4	18.00^{b}	15.51^{d}	19.00^{a}	17.02^{c}	0.333	<.0001	<.0001	<.0001
Mean	16.27^{b}	14.86^{d}	16.74^{a}	15.60^{c}	0.185	<.0001	<.0001	0.035
Acetic acid (C_2), %Molar								
0	66.43	66.06	66.39	66.05	1.075	0.991	0.891	0.995
2	70.99^{a}	68.56^{c}	69.86^{b}	67.62^{c}	0.400	0.003	<.0001	0.717
4	72.17^{a}	69.52^{b}	70.97^{a}	68.38^{b}	0.447	0.003	0.0002	0.917
Mean	69.86^{a}	68.04^{b}	69.07^{a}	67.35^{b}	0.300	0.004	<.0001	0.814

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.6 (Continue).

Time (h)	NP-1		PN		SEM	P-value		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Propionic acid (C_3), %Molar								
0	17.34	17.28	17.46	17.30	0.074	0.681	0.530	0.770
2	19.33^{b}	18.44^{c}	20.72^{a}	18.96^{bc}	0.265	0.001	<.0001	0.040
4	19.07^{b}	17.91^{d}	19.56^{a}	18.26^{c}	0.196	<.0001	<.0001	0.043
Mean	18.58^{b}	17.88^{d}	19.25^{a}	18.18^{c}	0.156	<.0001	<.0001	0.009
Butyric acid (C_4),% Molar								
0	16.23	16.66	16.15	16.65	0.374	0.853	0.068	0.876
2	9.68^{b}	13.00^{a}	9.42^{b}	13.42^{a}	0.580	0.838	<.0001	0.427
4	8.77^{b}	12.57^{a}	9.47^{b}	13.35^{a}	0.603	0.034	<.0001	0.894
Mean	11.56^{b}	14.07^{a}	11.68^{b}	14.47^{ab}	0.417	0.326	<.0001	0.599
TVFA, mmol/L								
0	48.41	45.72	48.89	46.09	1.045	0.856	0.256	0.981
2	95.04^{b}	80.46^{d}	105.10^{a}	85.82^{c}	2.828	<.0001	<.0001	<.0001
4	77.45^{b}	64.01^{d}	84.76^{a}	71.72^{c}	2.296	<.0001	<.0001	0.001
Mean	73.64^{b}	63.39^{d}	79.58^{a}	67.87^{c}	1.839	<.0001	<.0001	0.024
Ratio of C_2/C_3								
0	3.83	3.82	3.80	3.82	0.017	0.674	0.928	0.789
2	3.67^{b}	3.72^{a}	3.37^{c}	3.57^{b}	0.041	<.0001	0.001	0.010
4	3.79^{b}	3.88^{a}	3.63^{d}	3.75^{c}	0.027	<.0001	<.0001	0.040
Mean	3.76^{b}	3.80^{a}	3.60^{d}	3.71^{c}	0.023	<.0001	<.0001	0.008

$^{a, b, c, d}$ Means with different superscripts in the same row differ significantly (P<0.05); SEM = standard error of mean; Time (h) = hour (s) after feeding experimental diets; NH_3-N = ammonia nitrogen; BUN = blood urea nitrogen; TVFA = total volatile fatty acid; NP-1 = Napier Pakchong-1; PN = Purple Napier; C = effect of grass cultivars; F = effect of grass form; C×F = interaction effect of grass cultivars and form.

Table 4.7 Effect of Napier grass fresh and silage on microbial population in rumen fluid of the feeding trial experiment by Real-time PCR method.

Time (h)	NP-1		PN[1]		SEM[2]	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Total bacteria (log10 copies/mL)								
0	10.63	10.56	10.24	10.27	0.086	0.070	0.757	0.934
2	10.84	11.00	10.38	10.80	0.117	0.178	0.580	0.228
4	11.32	10.99	10.60	10.81	0.135	0.118	0.333	0.801
Mean	10.93	10.85	10.41	10.63	0.108	0.112	0.498	0.747
Methanogen (log10 copies/mL)								
0	5.59	4.77	4.33	4.71	0.206	0.100	0.132	0.564
2	6.57^{a}	5.52^{b}	4.42^{c}	4.86^{c}	0.249	<.0001	0.0002	0.031
4	6.81^{a}	6.31^{b}	4.63^{d}	5.75^{c}	0.248	<.0001	<.0001	0.008
Mean	6.32^{a}	5.54^{b}	4.46^{d}	5.11^{c}	0.205	<.0001	<.0001	0.043
Protozoa (log10 copies/mL)								
0	6.74	6.76	6.78	6.75	0.055	0.927	0.856	0.980
2	6.94^{b}	7.07^{a}	6.42^{d}	6.54^{c}	0.082	<.0001	0.994	0.0004
4	7.17^{b}	7.27^{a}	6.97^{c}	7.08^{b}	0.035	<.0001	0.990	0.002
Mean	6.95^{a}	7.03^{a}	6.72^{b}	6.79^{b}	0.039	<.0001	0.757	0.016
Butyrivibrio fibrisolvens (log10 copies/mL)								
0	8.32	7.41	8.50	7.98	0.213	0.384	0.111	0.633
2	9.54^{b}	8.08^{d}	9.85^{a}	9.24^{c}	0.203	<.0001	<.0001	0.0001
4	10.11^{b}	9.65^{c}	10.57^{a}	9.86^{c}	0.106	0.0001	<.0001	0.031
Mean	9.32^{ab}	8.38^{c}	9.64^{a}	9.03^{b}	0.150	0.005	0.0002	0.221

Table 4.7 (Continue).

Time (h)	NP-1		PN[1]		SEM[2]	P-value[3]		
	Fresh	Silage	Fresh	Silage		C	F	C × F
Fibrobacter succinogenes (log10 copies/mL)								
0	8.10	7.47	7.59	6.92	0.182	0.125	0.065	0.959
2	8.60[a]	8.24[bc]	8.40[b]	8.10[c]	0.059	0.004	<.0001	0.483
4	9.15[a]	8.29[b]	8.43[ab]	7.99[b]	0.147	0.015	0.004	0.250
Mean	8.62[a]	8.00[b]	8.14[ab]	7.67[b]	0.113	0.006	0.001	0.517
Ruminococcus flavefaciens (log10 copies/mL)								
0	8.58	8.47	8.37	8.60	0.058	0.653	0.190	0.752
2	9.37[a]	8.77[b]	8.86[ab]	8.62[b]	0.097	0.005	0.141	0.018
4	11.18[a]	9.55[bc]	10.13[b]	8.83[c]	0.272	<.0001	0.431	0.002
Mean	9.71[a]	8.93[b]	9.12[b]	8.68[b]	0.125	0.0008	0.176	0.007

[a, b, c, d] Means with different superscripts in the same row differ significantly (P<0.05); SEM = standard error of mean; Time (h) = hour(s) after feeding experimental diets; NP-1 = Napier Pakchong-1; PN = Purple Napier; C = effect of grass cultivars; F = effect of grass form; C × F = interaction effect of grass cultivars and form.

4.6 Discussions

4.6.1 Feed chemical composition

The chemical composition of fresh Napier Pakchong-1 and Purple Napier grass was close to each other for the major compositions (DM, OM, CP, NDF, ADF, EE and ash) and the anthocyanin profile contained mainly C3G, P3G, Del, Peo3G, M3G, Cya, Pel and Mal in experimental I. The some of major compositions of Napier

Pakchong-1 and Purple Napier grass silage was lower than fresh grass, especially CP content. The some of major compositions and anthocyanin may occur during ensiling due to most of the reduction occurred during the first 21 days of fermentation. On the other hand, a rapid lactic acid synthesis in the absence of oxygen had a positive correlation with nutrients and anthocyanin composition, which means that a rapid pH decrease below 4 under anoxic conditions is beneficial to the conservation of nutrients and anthocyanin composition as found in well-preserved silages (Bureenok et al., 2006a; Bureenok et al., 2011b; Virtanen, 1933).

4.6.2 Gas production kinetics and *In vitro* digestibility

The production from gas kinetics including soluble fraction (*a*), gas production from insoluble fraction (*b*), potential gas production (*a*+*b*) and gas production rate (*c*) were higher in fresh Napier Pakchong-1 and Purple Napier grass compared with the grass silage treatment. Soluble fraction (*a*) was inversely correspondent to the ash content (Kariuki et al., 2001). The degradation rates in this study, the fresh grass treatment (0.04 h^{-1}) was slightly higher the degradation rates in grass silage treatment due to ensiling process (Kis et al., 2005). This reflected by the low fraction of easy fermentable carbohydrates that loss during ensiling period. These higher values of digestibility fractions in the fresh grass treatment indicated that gas volumes were significantly higher at 6, 12, 24 hours and the mean of total gas production.

The gas produced by the fresh Napier grass within 24 h incubation period was more than the grass silage because of the variation in values of gas production was a direct result of dry matter and organic matter digestibility (Danielsson, 2016). This indicated that most of fermentable carbohydrates fraction available in the fresh grass treatment was fermented into volatile fatty acids (acetic, butyric and propionic acids)

within 24 hours. This is because the gas yielded predominately by fermentation of carbohydrates (340-370 ml gas/g substrate) followed by protein (130 ml gas/g substrate) and negligible in fat fermentation (1-2 ml gas/g substrate) (Cone and Gelder, 1999; Menke and Steingass, 1988).

For CH_4 production was highest in the fresh Napier Pakchong-1 grass and the CH_4/total gas ratio was higher in the grass silage treatment than the fresh grass treatment. There is usually a high correlation between digestibility and type of diet. The CH_4 production is positively correlated with intake of DM and hence high intake means more feed to ferment. Type of diet has been shown to affect microbial community composition and microbial diversity, which in turn can impact on CH_4 production.

The primary fermenters in the rumen, such as bacteria, fungi and protozoa, start to digest the feed macromolecules into monomers such as simple sugars and carbon skeletons. The effective degradation of fibrous feed in the rumen is due to fibrolytic enzymes produced by bacteria, protozoa and fungi, which include cellulases, xylanases, β-glucanases and pectinases. The amounts of the different end-products vary depending on diet composition, but when considering VFA, CO_2 and CH_4 as sole fermentation end-products (Wolin, 1979b). Depending on the amount and proportions of different VFAs produced, different amounts of CH_4 and CO_2 are also produced. When acetate is produced, reoxidation of NADH occurs by production of H_2 that can be further used by methanogenic Archaea (methanogens) to reduce CO_2 to CH_4 (Wolin, 1960a).

Moreover, the Purple Napier grass treatment, especially fresh Purple Napier grass had the lowest CH_4 production due to the Purple Napier grass had high anthocyanin composition, which could inhibit methanogens with antibacteria property and CH_4 synthesis by changing hydrogen from the CH_4 pathway to propionic acid (C_3)

form (Cherdthong et al., 2019c; Patra and Saxena, 2010). An alternative electron sink for a metabolic route to dispose the reduced power has to happen. Newbold et al. (2005) proposed that the succinate-propionate pathway could possibly lead to propionic acid production.

Based on the results, the IVDMD of fresh grass form had higher than grass silage due to grass silage lost of soluble content during ensiling process. the IVDMD of Napier cultivars varied from 63-74%. The dry matter digestibility of Napier grass was reported in several studies, ranging from 53-80% (Budiman et al., 2012; Wijitphan et al., 2009). Fresh Napier Pakchong-1 and Purple Napier grass had high digestibility above 65% IVDMD. Mugeriwa et al. (1973) found that the IVDMD values were greater than 65% indicating good feeding value and values below this threshold level would result in reduced dry matter intake due to lowered digestibility.

The IVOMD of grass silage form had higher than fresh grass due to the structural carbohydrate improved by microorganism during ensiling process resulting in easily to digestion. The IVOMD of Napier cultivars ranged from 61-69%. These values were relatively higher compared to Evitayani et al. (2004) who reported the organic matter digestibility of tropical grasses ranging from 51% (*P. purpuphoides*) to 64.4% (*P. purpureum*). In general, the digestibility of Napier grass can be reflected in a number of factors, such as the selection and management practices of cultivars including harvesting age, cutting interval and cutting height (Lounglawan et al., 2014; Zailan et al., 2015). In addition, high ambient temperatures could inhibit digestibility due to an increase in lignification of the plant cell wall because the increase in structural carbohydrate (cell wall) reduces the cellular content of the pool, such as crude protein and water-soluble carbohydrate (Van Soest, 1991).

The OMD, ED, and ME also appear to be highest fresh grass treatment. Enhanced OMD could be responsible for the breakdown of the CF and ADF content of grass (Kinfemi et al., 2009) and it may also be caused by the breakdown of cell wall bonds during the fermentation of grass (Akinfemi, 2012; Call and Mineke, 1997). The production of gas represents degradable carbohydrates and also that the amount of gas produced depends on the nature of the carbohydrate (Bummel and Becker, 1997).

4.6.3 *In vitro* rumen characteristics

The concentration of NH_3-N in the rumen fluid was affected by CP content, the fresh Purple Napier grass treatment was highest NH_3-N value. The concentration of NH_3-N decreased rapidly after 24 hours, the decrease in the concentration of NH_3-N in rumen could be described by the lower intake of CP and the digestibility caused by fugal decomposition of the lignin bond in the growth of its own cell.

The basic anaerobic end-product of microbial in carbohydrate fermentation in the rumen of ruminant has been observed to be volatile fatty acids (VFAs), carbon dioxide and methane (Camero and Franco, 2001). The C_2 values was highest in the fresh Napier Pakchong-1 grass due to the fresh Napier Pakchong-1 had high fiber content and the fermentation of high fibre content in a diet will result in higher proportion of C_2 concentration and CO_2 released (Widiawati and Thalib, 2009). The high concentration of C_3 and C_4 obtained in the fresh Purple Napier grass treatment and the grass silage treatment might be as a result of high organic matter degradation and the percentage of C_4 almost followed the same pattern of variation as observed in C_3.

In ruminants, the C_3 was reported to be the major glycogenic fatty acid. This observed volatile fatty acid pattern of variation between the Napier grass cultivars and the form of grass could probably be an indication of the rapid rate of fermentation

from water soluble fractions containing starch and soluble carbohydrates together with the more easily accessible and degradable cellulose (Widiawati and Thalib, 2009). The grass silage treatment lowered the CH_4 output to total VFA, while the fresh grass treatment, especially Napier Pakchong-1 grass increased the CH_4 output to total VFA. Reduced production of methane is common to enhanced feed consumption but it is also common with such a decrease in the C_2/C_3 ratio (Russell, 1998).

The level of C_2 resulting from carbohydrate fermentation in the rumen may be decreased, whereas the concentration of C_3 is increased, and the energy of the ruminant ration may be more efficiently utilized. This shift in the ratio of C_2/C_3 in favor of C_3 decreases the energy losses caused in metabolism at the cellular level (Kariuki et al., 2001; Widiawati and Thalib, 2009). These results indicate that the fresh Purple Napier grass could be a beneficial and efficient feed and would be a better choice.

4.6.4 Growth performance, feed intake and nutrient digestibility

Growth performance of the goats, the final body weight, weigh change, and ADG were relatively high in goats fed fresh Purple Napier grass diets. The significant difference in final body weight, weight change and ADG of goat in the treatment of fresh Purple Napier grass could be due to the relatively good quality of the basal (Napier grass) diet. Due to the relatively high intake DM of roughage, total intake per day and CP content of the forage (Goetsch et al., 2011; Pralomkarn et al., 1995; Sahlu et al., 2004) at an early stage of growth, the goat were able to meet their nutrient requirements for a reasonable final body weight, weigh change, and ADG on fresh Purple Napier grass diet. The feeding experiment demonstrated good growth performance throughout the experimental period, indicating that the fresh Purple Napier grass diets had nutritional

composition above the goat's maintenance threshold requirement base on nutrient requirement recommended for goat by NRC (1981).

Intake of roughage and total intake per day were highest in fresh Purple Napier grass, fresh Napier Pakchong-1 grass and Purple grass silage, which had no difference in concentrate intake between treatments due to high DM content. The nutrient intake and digestibility of feed will certainly be dependent on its nutrient composition and digestive characteristics. Purple Napier grass and fresh grass treatment had higher DM intake and chemical composition as a result of increased intake and digestibility of OM, CP, EE and ME, respectively, although ADF and NDF were not higher (Hosoda et al., 2012). Paengkoum (2010a) reported the protein requirement for the maintenance of male Thai native cattle fed with Pangola hay as roughage and found that the crude protein digestibility increased with increasing levels of CP in diets.

As a result, the increase in grass intake can be explained by its increased degradability in the rumen and the increase in the outflow of grass cell walls to the abomasum (Trach et al., 2001; Wanapat et al., 2013). The passage of the particle is predicted to reduce with greater NDF intake. The intake is also anticipated to be inversely linked to the fiber content of the forage, as further intake is limited as the slower digestive fraction becomes greater in relation to the volume of the digestive tract (Van Soest, 1965).

4.6.5 Nitrogen utilization

All Nitrogen utilization parameters as N intake, N excretion from feces and urine, total N excretion, N absorption and N retention in goats fed with fresh Purple Napier grass treatment were higher than the other treatment groups. This might be because the high CP content and carbohydrates in fresh Purple Napier grass have the

potential to improve the ruminal fermentation toward maximizing microbial protein production (Anantasook et al., 2016).

Brooker et al. (1995) reported that total N excretion followed the same pattern as N excretion from urine due to when feed is high in soluble plant protein, large amounts of (NH_3-N) were also generated in excess of the requirements of rumen microorganisms, and excess ammonia is converted to urea by animals and excreted in urine. This means that more NH_3-N has been produced in rumen with fresh Purple Napier grass, that has increased urinary nitrogen. In addition, Cherdthong et al. (2019c) reported that anthocyanins could support a synchronized release of nitrogen and carbohydrates from Purple field corn stover, which is responsible for microbial efficiency enhancement. Further explanations might be that protozoa engulf other microbes as their primary source of nutrients and as a result, a definition might be more effective in photosynthesis to enhance the duodenal microbe mass flow (Williams and Coleman, 1992).

All treatment showed the positive N balance, which confirms that the nutrient each treatment adequately encountered the protein maintenance requirements in the goats. Paengkoum et al. (2013c) found that the crude protein intake, N excretion in urine and N balance increased ($P<0.05$) with the increase CP content in the diet of maintenance in swamp buffalo calves. Paengkoum and Bunnakit (2009b) reported the levels of Caspurea (urea gelatinizes) replacement for soybean meal in concentrate found that increasing the level of Caspurea, nitrogen absorption and nitrogen retention were decreased, while BUN was increased.

4.6.6 Rumen characteristics and blood urea nitrogen of goats

Rumen characteristics and blood urea nitrogen, the ruminal pH post-feeding was not different among treatments. The pH decreased quantitatively for all

treatments as post-feeding time increased. The decreased ruminal pH is related to the accumulation of VFA and lactic acid (Owens et al., 1998). The ruminant animal will act to avoid acid accumulation by discarding these from the rumen wall (González et al., 2012). The rumen pH had average values ranged from 6.75 to 7.00, which is optimal for microbial digestion in rumen (Cherdthong et al., 2018b). However, the ruminal pH was slightly dropped in 2 hours post feeding. This result indicates the possibility that the microbial digestion of feed could decrease pH value in the rumen. The ranges of rumen pH were decreased at 2-4 hours after morning feeding (Cherdthong et al., 2014a).

No interaction effects on NH_3-N were observed at 0 hours for all treatments and there was a significant effect of fresh Purple Napier grass treatment on NH_3-N at 2-4 h after feeding. It may be due to the high intake of CP when the fresh Purple Napier grass was fed, resulting in a high amount of CP available from grass for microbial breakdown to NH_3-N in rumen. Ruminal NH_3-N is the main product of protein digestion that would be used by rumen microorganisms in rumen. In this study the NH_3-N concentration was 14.98-23.13 mg/dL. These results show that the ruminal NH_3-N content for all treatments was sufficient to maintain microbial growth (5 mg/dL) (NRC, 1981) and was within or above the optimal level of 12-20 mg/dL at 2 to 4 hours post-feeding (Islam et al., 2000). Thereby, the additional levels of CP in the feed may provide for the accessibility of NH_3-N rumen. Hristov et al. (2004) indicated that protein could be degraded to NH_3-N by microbial extracellular enzymes and deamination. In addition, the high concentration of NH_3-N in the fresh Purple Napier grass was likely affected by the high level of CP, that is a supplemental source of nitrogen. Furthermore, fresh Purple Napier grass is consisted of CP, and the goats are likely to receive additional protein.

The BUN concentration in plasma was no differ among the experimental groups at 0 hours and there was a significant effect of fresh Purple Napier grass treatment on BUN at 2-4 h after feeding. The ruminal concentration of ammonia is generally affected by CP content in a diet (Hristov et al., 2004; McDonald et al., 2002). Furthermore, urea is synthesized in the liver from ammonia absorbed from the digestive organs, and so a BUN concentration in the plasma is positively associated with ammonia concentration in ruminal fluid (Davidson et al., 2003; Lewis, 1957; Petit and Flipot, 1992). The increasing of ruminal ammonia and BUN concentrations in the goats fed fresh Purple Napier grass were probably caused by higher CP content of the fresh Purple Napier grass followed by higher concentration of ruminal ammonia. The concentration of BUN in plasma also reflected the balance of the animal feed between the CP and energy content. The high amount of BUN in plasma demonstrated ineffective use of the diet (O'Doherty and Crosby, 1998). In this study, the BUN concentration in plasma was 11.03-19.65 mg/dL. These results show that the BUN concentration in plasma for all treatments was within or above the optimal level of 10-20 mg/dL at 2 to 4 hours post-feeding (Kaneko, 1989). It can be indicated that the fresh Purple Napier grass did not cause too much change the balance between protein and energy content.

The C_2 concentration was higher in the goats fed Napier Pakchong-1 cultivars and fresh grass treatment at 2-4 hours after feeding. This is likely due to higher content and digestibility of NDF and ADF in Napier Pakchong-1 cultivars and fresh grass treatment. However, the concentration and proportion of VFAs also depend on the type of feed and the time of sampling after feeding. Concentration of VFAs were also variable and the C_2 in this experiment was 66.12-72.07%. These results show that the C_2 in rumen

fluid for all treatments was within or above the optimal level of 60-70% at 2 to 4 hours post-feeding (Leng et al., 1966).

The rumen C_3 concentration was higher in the goats fed fresh Purple Napier grass treatment at 2-4 hours after feeding. Ruminal bacteria ferment soluble carbohydrates and break down into simple sugars. The microbes use these sugars as an energy source for their own growth and produce C_3 (Moran, 2005; Varga, 2014). The water soluble carbohydrate (WSC) in fresh grass are rapidly degradable in the rumen, which can then be used as a substrate for VFA synthesis (Holden et al., 1994). The goats fed fresh Purple Napier grass had greater C_3, which supports Oba (2011) summarized that feeding high sugar content forage to animals increased the microbial nitrogen flow and efficiency of microbes in the rumen, resulting in improved digestibility of nutrients.

Berthiaume et al. (2010) reported that high non-structural carbohydrate (NSC) alfalfa increased microbial synthesis and improved digestibility of DM and OM, but did not affect the digestibility of NDF and ADF in continuous culture. Increased C_3 production in the rumen is related to increased insulin secretion, resulting in increased fat deposition and protein synthesis while inhibiting lipolysis and protein breakdown (Zhou et al., 2014). The results in this study show that the C_3 in rumen fluid for all treatments was within or above the optimal level of 18-20% at 2 to 4 hours post-feeding (Leng et al., 1966). In addition, the most widespread anthocyanin is cyanidin 3-glucoside was highest in Purple Napier grass cultivars.

The chemical structure of anthocyanin in Purple Napier grass cultivars containing rather complicated acylation patterns attached on different sugar moieties. The most common sugar moieties glycosylating aglycones are glucose, galactose, rhamnose, xylose, arabinose, as mono-, di-, and tri-glycosides (Andersen and Fossen, 1995a;

Andersen et al., 1995b; Giusti et al., 1998; Torskangerpoll et al., 2005). These sugars may be acylated with aromatic acids, such as p-coumaric, caffeic, ferulic, sinapic, gallic or phydroxybenzoic acids or aliphatic acids, such as malonic, acetic, malic, succinic or oxalic acids (Robbins, 2003). It can be indicated that these sugar moieties are the precursors for ruminal C_3 synthesis.

The percentage of C_4 was greater in the goats fed Purple Napier grass treatment than Napier Pakchong-1 grass treatment at 4 hours after feeding and greater in grass silage treatment than fresh grass treatment at 2 and 4 hours after feeding. The increased ruminal proportion of C_2 and C_4 of fresh Napier Pakchong-1 grass and Napier Pakchong-1 grass silage treatment indicate that the source of roughage and preservation method affected ruminal VFA profile and these diets promoted acetogenic fermentation. The C_4 also is primarily used as a source of energy for the host (McDonald et al., 2002). In this study, high level of C_4 was observed when Napier Pakchong-1 grass silage diets are given in high quantity to the goats.

The higher level of C_4 in Napier Pakchong-1 grass silage diet probably could be related to population of protozoa because rumen protozoa produce C_4 as their metabolic end-product (Carberry et al., 2012). When C_4 is absorbed through the rumen wall, it is mostly converted to β-hydroxy-butyric acid, resulting in low levels of butyric acid in the blood circulation, which the C_4 is used as an energy source through oxidation and synthesized as fat in the liver (Fahey and Berger, 1988). The results in this study show that the C_4 in rumen fluid for all treatments was within or above the optimal level of 10% at 2 to 4 hours post-feeding (Leng et al., 1966).

The total VFA was higher in rumen fluid of the goats fed fresh Purple Napier grass due to the superior quality of roughage supplied more nutrients to rumen

microbes for growth and resulted in higher microbial and rumen fermentation end-products demonstrated in total VFA and C_3 concentrations. The level of total VFA concentrations was similar to the results of ruminal NH_3-N and BUN concentrations. Firkins et al. (2006) reported the proportion of VFA produced in the rumen depends largely on the composition of diets consumed by the ruminant in particular the fractions contained in the feed. The production of VFA in rumen is influenced by a number of factors, such as the composition of substrates and the availability of specific types of rumen microbes to degrade the diet received (Dijkstra, 1994).

The ratio of C_2/C_3 was higher in the goats fed Napier Pakchong-1 grass silage treatment, whereas fresh Purple Napier grass treatment was lower value. In this experiment, the ratio of C_2/C_3 in rumen fluid for all treatments was within the normal range of 1-4% (Preston and Leng, 1987). The lower ratio of C_2/C_3 increases energy retention because C_3 synthesis provides energy efficiency. Theoretically, the production of methane can be reduced by the reduction of CO_2 with H atom resulting from the synthesis of C_2 and C_4. On the other hand, the synthesis of C_3 does not produce methane production (Preston and Leng, 1987).

Therefore, the higher synthesis of C_3 results in lower of methane production. In contrast, the higher synthesis of C_2 and C_4 also produces large amounts of methane production, which is a loss of energy in addition to the heat produced by fermentation (Preston and Leng, 1987). Kariuki et al. (2001) and Widiawati and Thalib (2009) reported that if the level of C_3 is increased, the energy of the ruminant ration will be used more efficiently. The rise of C_2/C_3 ratio in favor of C_3 has decreased the energy losses caused during cell metabolism. Potter et al. (1976) confirmed that a higher proportion of C_3 was much more energy efficient in beef cattle.

4.6.7 Microbial population in goat's rumen

The average value concentration of total bacteria population in the rumen fluid of the goats in this study was not affected by cultivars and grass form. In this study, the highest methanogen was detected in rumen fluid of the goats fed fresh Napier Pakchong-1 grass treatment and the lowest was observed in the fresh Purple Napier grass treatment. The high fiber content of fresh Napier Pakchong-1 grass treatment generally produced more hydrogen gas production due to cellulolytic bacteria produces hydrogen for their fermentation end-products. Hydrogen atom does not accumulate in the rumen because it is immediately utilized by hydrogen utilizing archea (methanogen) that present in the complex rumen microbial ecosystem (Bunglavan, 2014).

The goats fed high fiber resulted in more rumen C_2 production together with high hydrogen production and leading to an increase methanogenic archaea (Adeyosoye et al., 2010). The increase in the proportion of starch changes the proportion of rumen VFA in such a way that it decreases C_2 and increases the proportion of C_3 and the supply of hydrogen for methanogenesis is rather restricted (Iqbal et al., 2008). Therefore, the high production of C_3 in rumen fluid of the goats fed fresh Purple Napier grass treatment that the rumen methanogen reduces the uses of hydrogen availability and inhibits their population activity (Hook et al., 2011; Martin et al., 2010).

The rumen protozoa are important hydrogen producers and reduction of these microbes, affects the transfer of hydrogen between the protozoa and the methanogen (Martin et al., 2010; Morgavi et al., 2012). The rumen protozoa engulf the starch particle and attacked amylolytic bacteria, thus regulates the rate of starch fermentation in the rumen fluid (Carberry et al., 2012). The highest protozoa was detected in rumen fluid of the goats fed Napier Pakchong-1 grass silage treatment and the lowest

was observed in the fresh Purple Napier grass treatment. Dennis et al. (1983) reported that the number of rumen protozoa population was increased when the level of starch was incorporated into the diets. The low rumen protozoa population detected in fresh Purple Napier grass treatment might be due to a toxic effect of the high C_3 level (Lettat, 2011).

Carberry et al. (2012) reported that an increase of corn silage proportion in the diet (i.e., increasing starch supply) has decrease the cellulolytic bacteria population but increased the number of total bacteria and amylolytic bacteria species which favoured the C_3 production. Furthermore, the high anthocyanins in fresh Purple Napier grass treatment can inhibit rumen methanogen and protozoa population due to anthocyanins have high antimicrobial activity. The inhibitory mechanisms, for 2 hours after treated with anthocyanins, the nucleic acid leakage and protein release would be increased, while the whole protein content, enzyme activity and the TCA cycle were decreased, reducing the energy transfer of microbial, which demonstrated that anthocyanins could destroy the cell membrane and inhibiting the growth and reproduction of microbial (Sun et al., 2018).

The highest *Butyrivibrio fibrisolvens* (*B. fibrisolvens*) has been detected in rumen fluid of the goats fed fresh Purple Napier grass treatment. Ruminal protein degradation is a composite of several microbial processes, including protein hydrolysis, peptide degradation, amino acid deamination and the fermentation of amino acid carbon skeletons (Brock et al., 1982). The one of important proteolytic species are *B. fibrisolvens* species (Blackburn and Hobson, 1962). Proteolytic species that use proteins as a sole source of carbon and energy are usually facultative anaerobes which are numerically in significant in the rumen (Blackburn and Hobson, 1962; Hunt and Moore, 1958). It could be indicated that *B. fibrisolvens* species was highest in rumen fluid of the goats fed fresh

Purple Napier grass treatment because the highest of CP content and concentration of ruminal NH_3-N.

The primary rumen cellulolytic bacteria are *F. succinogenes, R. flavefaciens* (Hobson and Stewart, 1997) have a higher ability to degrade fiber than other species of cellulolytic bacteria (Carberry et al., 2012). In this study, The high quantity of *R. flavefaciens* has been detected in rumen fluid for all treatments compared to *F. succinogenes*. The highest rumen *F. succinogenes* and *R. flavefaciens* in the goats fed Napier Pakchong-1 grass and fresh grass form treatment, while the lowest were observed in Purple Napier grass and grass silage form treatment due to low structural carbohydrate content in the Purple Napier grass and grass silage form treatment.

The rumen cellulolytic bacteria was required to break down the hemicellulose and a cellulose fraction of the Napier grass. Collings and Yokoyama (1980) reported the hemicellulose content was degraded by *R. flavefaciens* and *F. succinogen* with the same degradation pattern. *R. flavefaciens* degraded slightly more effective in wheat straw, corn silage and kentucky bluegrass degradation, whereas *F. succinogen* degrades more effectively in the hemicellulose degradation of alfalfa. Khaing et al. (2016) reported the reduction of the cellulolytic bacteria could be substituted by increased of amylolytic bacterial numbers due to availability of more readily fermentable carbohydrates (i.e., starch) in whole corn plant silage diet. Furthermore, the total bacteria population in this experiment was not significant among the treatments, although the cellulolytic bacteria population was low in the Purple Napier cultivars and grass silage form.

4.7 Conclusion

The results in this study, it can be concluded that fresh Purple Napier grass in the goat diets improve efficiency in terms of a high *in vitro* gas production parameters, *in vitro* digestibility, ruminal NH_3-N, ruminal propionic acid (C_3), total VFA, growth performance, nitrogen utilization, blood urea nitrogen (BUN) and *Butyrivibrio fibrisolvens*, respectively and low ruminal acetic acid (C_2), cellulolytic bacteria population although the ruminal pH value and total microbial population were not significantly different by dietary treatments. In addition, it was found that the lowest methane gas production (CH_4), methanogen and protozoa population, respectively were observed in goats fed fresh Purple Napier grass. Based on the information in the current study, it can be concluded that fresh Purple Napier grass can be used as a source of roughage for feeding goats.

4.8 References

Adesogan, A. T. (2004). Effect of bag type on the apparent digestibility of feeds in ANKOM DaisyII incubators. **Animal Feed Science and Technology**. 119(3-4): 333-344.

Adeyosoye, O. I., Adesokan, I. A., Afolabi, K. D., and Ekeocha, A. H. (2010). Estimation of proximate composition and biogas production from *in vitro* gas fermentation of sweet potato (*Ipomea batatas*) and wild cocoyam (*Colocasia esculenta*) peels. **African Journal of Environmental Science and Technology**. 4(6): 388-391.

Akinfemi, A. (2012). Chemical composition and nutritive value of fungal treated each straw. **Agricultural Science and Technology**. 4(2): 120-124.

Anantasook, N., Wanapat, M., Gunun, P., and Cherdthong, A. (2016). Reducing methane production by supplementation of *Terminalia chebula* RETZ. containing tannins and saponins. **Animal Science Journal**. 87(6): 783-790.

Andersen, Ø. M., and Fossen, T. (1995a). Anthocyanins with an unusual acylation pattern from stem of Allium victorialis. **Phytochemistry**. 40(6): 1809-1812.

Andersen, Ø. M., Viksund, R. I., and Pedersen, A. T. (1995b). Malvidin 3-(6-acetylglucoside)-5-glucoside and other anthocyanins from flowers of Geranium sylvaticum. **Phytochemistry**. 38(6): 1513-1517.

AOAC. (1995). Official Methods of Analysis, 16th ed. **Association of official analytical chemists**. Washington DC, USA.

Berthiaume, R., Benchaar, C., Chaves, A. V., Tremblay, G. F., Castonguay, Y., Bertrand, A., Bélanger, G., Michaud, R., Lafrenière, C., McAllister, T. A., and Brito, A. F. (2010). Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture. **Journal of Dairy Science**. 93(2): 693-700.

Blackburn, T. H., and Hobson, P. N. (1962). Further studies on the isolation of proteolytic bacteria from the sheep rumen. **Journal of General Microbiology**. 29: 69-81.

Blummel, M., and Becker, K. (1997). The degradability characteristics of fifty-four roughages and rough neutral detergent fiber as described *in vitro* gas production and their relation to voluntary intake. **British Journal of Nutrition**. 77(5): 757-768.

Brock, F. M., Forsberg, C. W., and Buchanan-Smith, J. G. (1982). Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. **Applied and Environmental Microbiology**. 44(3): 561-569.

Brooker, J. D., Lum, D. K., Miller, S., Skene, I., and O' Donovan, L. (1995). Rumen microorganisms as providers of high quality protein. **Livestock Research for Rural Development**. 6(3).

Budiman, B., Soetrisno, R. D., Budhi, S. P. S., and Indrianto, A. (2012). Morphological characteristics, productivity and quality of three Napier grass (*Pennisetum purpureum Schum*) cultivars harvested at different ages. **Journal of the Indonesian Tropical Animal Agriculture**. 37(4): 294-301.

Bunglavan, S. J. (2014). Methanogenesis and recent techniques for mitigation of methanogenesis inruminants. **Journal of Livestock Science**. 5: 35-48.

Bureenok, S., Namihira, T., Mizumachi, S., Kawamoto, Y., and Nakada, T. (2006a). The effect of epiphytic lactic acid bacteria with or without different by-product from defatted rice bran and green tea waste on Napier grass (*Pennisetum purpureum* Schumach) silage. **Journal of the Science of Food and Agriculture**. 86(7): 1073-1077.

Bureenok, S., Sisaath, K., Homsai, W., Wongsuthavas, S., Yuangklang, C., and Vesupen, K. (2011b). Fermentation quality and nutritive value of Purple guinea grass and legumes silages. **Khon Kaen Agriculture Journal**. 39: 137-146.

Call, H. P., and Mineke, I. (1997). History, overview and applications of mediated lignolytic systems, especially laccase-mediator systems (Lignozym(R)-process). **Journal of Biotechnology**. 53(2-3): 163-202.

Camero, A., and Franco, M. (2001). Improving rumen fermentation and milk production with legume-tree fodder in the tropics. **Agroforestry Systems**. 51(2): 157-166.

Carberry, C. A., Kenny, D. A., Han, S., McCabe, M. S., and Waters, S. M. (2012). Effect of phenotypic residual feed intake and dietary forage content on the rumen

microbial community of beef cattle. **Applied and Environmental Microbiology**. 78(14): 4949-4958.

Cherdthong, A., and Wanapat, M. (2014a). *In vitro* gas production in rumen fluid of buffalo as affected by urea-calcium mixture in high quality feed block. **Animal Science Journal**. 85(4): 420-426.

Cherdthong, A., Khonkhaeng, B., Seankamsorn, A., Supapong, C., Wanapat, M., Gunun, N., Gunun, P., Chanjula, P., and Polyorach, S. (2018b). Effects of feeding fresh cassava root with high-sulfur feed block on feed utilization, rumen fermentation, and blood metabolites in Thai native cattle. **Tropical Animal Health and Production**. 50(6): 1365-1371.

Cherdthong, A., Prachumchai, R., Wanapat, M., Foiklang, S., and Chanjula, P. (2019c). Effects of supplementation with royal poinciana seed meal (*Delonix regia*) on ruminal fermentation pattern, microbial protein synthesis, blood metabolites and mitigation of methane emissions in native Thai beef cattle. **Animals**. 9(9): 625.

Cherdthong, A., Rakwongrit, D., Wachirapakorn, C., Haitook, T., Khantharin, S., Tangmutthapattharakun, G., and Saising, T. (2015d). Effect of leucaena silage and napier Pakchong 1 silage supplementation on feed intake, rumen ecology and growth performance in Thai native cattle. **Khon Kaen Agriculture Journal**. 43(1): 484-490.

Collings, G. F., and Yokoyama, M. T. (1980). Gas liquid chromatography for evaluating polysaccharide degradation by *Ruminococcus flavefaciens* C94 and *Bacteroides succinogenes* S85. **Applied and Environmental Microbiology**. 39(3): 566-571.

Cone, J. W., and Gelder, A. H. V. (1999). Influence of protein fermentation on gas production profiles. **Animal Feed Science and Technology**. 76(3-4): 251-264.

Cotta, M. A., and Hespell, R. B. (1986). Proteolytic activity of the ruminal bacterium *Butyrivibrio fibrisolvens*. **Applied and environmental microbiology**. 52(1): 51-58.

Damiran, D., DelCurto, T., Bohnert, D. W., and Findholt, S. L. (2008). Comparison of techniques and grinding sizeto estimate digestibility of forage based ruminant diets. **Animal Feed Science and Technology**. 141(1-2): 15-35.

Danielsson, R. (2016). **Methane Production in Dairy Cows-Impact of Feed and Rumen Microbiota**. Faculty of Veterinary Medicine and Animal Science Department of Animal Nutrition and Management Uppsala. Swedish University of Agricultural Sciences Uppsala, Sweden.

Davidson, S., Hopkins, B. A., Diaz, D. E., Bolt, S. M., Brownie, C., Fellner, V., and Whitlow, L. W. (2003). Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. **Journal of Dairy Science**. 86(5): 1681-1689.

Denman, S. E., Tomkins, N. W., and McSweeney, C. S. (2007). Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. **FEMS Microbiology Ecology**. 62(3): 313-322.

Dennis, S. M., Arambel, M. J., Bartley, E. E., and Dayton, A. D. (1983). Effect of energy concentration and source of nitrogen on numbers and types of rumen protozoa. **Journal of Dairy Science**. 66(6): 1248-1254.

Dijkstra, J. (1994). Production and absorption of volatile fatty acids in the rumen. **Livestock Production Science**. 39(1): 61-69.

Evitayani, W. L., Fariani, A., Ichinohe, T., and Fujihara, T. (2004). Study on nutritive value of tropical forages in North Sumatra, Indonesia. **Asian-Australasian Journal of Animal Sciences**. 17(11): 1518-1523.

Fahey, G. C., and Berger, L. L. (1988). Carbohydrate nutrition of ruminants. In: **The Ruminant Animal: Digestive Physiology and Nutrition**. Prentice Hall, Englewood Cliffs, New Jersey. pp. 269-298.

Firkins, J. L., Hristov, A. N., Hall, M. B., Varga, G. A., and St-Pierre, N. R. (2006). Integration of ruminal metabolism in dairy cattle. **Journal of Dairy Science**. 89(E. Suppl.): E31-E51.

Giusti, M. M., Ghanadan, H., and Wrolstad, R. E. (1998). Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins using one- and two-dimensional nuclear magnetic resonance techniques. **Journal of Agricultural and Food Chemistry**. 46(12): 4858-4863.

Goering, H. K., and Van Soest, P. J. (1970). Forage fiber analysis (apparatus, Reagent, Procedures and some Application). **Agriculture Handbook**. No. 397, Agricultural Research Service, United States Department of Agriculture, Washington, D. C.

Goetsch, A. L., Merkel, R. C., and Gipson, T. A. (2011). Factors affecting goat meat production and quality. **Small Ruminant Research**. 101(1-3): 173-181.

González, L. A., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K. S., and Ferret, A. (2012). Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). **Animal Feed Science and Technology**. 172(1-2): 66-79.

Hobson, P. N., and Steward, C. S. (1997). **The rumen microbial ecosytem**. Springer, Netherlands.

Holden, L. A., Muller, L. D., Varga, G. A., and Hillard, P. J. (1994). Ruminal digestion and duodenal nutrient flows in dairy cows consuming grass as pasture, hay, or silage. **Journal of Dairy Science**. 77(10): 3034-3042.

Hook, S. E., Steele, M. A., Northwood, K. S., Wright, A. D. G., and McBride, B. W. (2011). Impact of high-concentrate feeding and low ruminal pH on methanogens and protozoa in the rumen of dairy cows. **Microbial Ecology**. 62(1): 94-105.

Hosoda, K., Eruden, B., Matsuyama, H., and Shioya, S. (2012). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Journal of Animal Science**. 83(6): 453-459.

Hove, L., Topps, J. H., Sibanda, S., and Ndlovu, L. R. (2001). Nutrient intake and utilization by goats fed dried leaves of shrub legumes *Acacia Augustissima, Calliandracalothyrsus* and *Leucaena leucocephala* as supplements to native pasture hay. **Animal Feed Science and Technology**. 91(1-2): 95-106.

Hristov, A. N., Etter, R. P., Ropp, J. K., and Grandeen, K. L. (2004). Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. **Journal of Animal Science**. 82(11): 3219-3229.

Hunt, W. G., and Moore, R. O. (1958). The proteolytic system of a gram negative rod isolated from the bovine rumen. **Applied Microbiology and Biotechnology**. 6(1): 36-39.

Iqbal, M. F., Cheng, Y. F., Zhu, W. Y., and Zeshan, B. (2008). Mitigation of ruminant methane production: Current strategies, constraints and future options. **World Journal of Microbiology and Biotechnology**. 24(12): 2747-2755.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Islam, M., Dahlan, I., Rajion, M. A., and Jelan, Z. A. (2000). Rumen pH and ammonia nitrogen of cattle fed different levels of oil palm (*Elaeis guineensis*) frond based diet and dry matter degradation of fractions of oil palm frond. **Asian Australasian Journal of Animal Sciences**. 13(7): 941-947.

Kanani, J., Lukefahr, S. D., and Stanko, R. L. (2006). Evaluation of tropical forage legumes (*Medicago sativa*, *Dolichos lablab*, *Leucaena leucocephala* and *Desmanthusbicornutus*) for growing goats. **Small Ruminant Research**. 65(1-2): 1-7.

Kaneko, J. J. (1989). **Clinical biochemistry of domestic animal, 4th ed**. Academic Press Inc.

Kariuki, J. N., Tamminga, S., Byachuiri, C. K., Gitau, G. K., and Muia, J. M. K. (2001). Intake and rumen degradation in cattle fed Napier grass (Pennisetum purpureum) supplemented with various levels of Desmodium intortum and Ipomoea batatas vines. **South African Journal of Animal Science**. 31(3): 149-157.

Khaing, K. T., Loh, T. C., Ghizan, S., Jahromi, M. F., Halim, R. A., and Samsudin, A. A. (2016). Profiling of rumen fermentation and microbial population changes in goats fed with Napier grass supplemented with whole corn plant silage. **Asian Journal of Animal Sciences**. 10(1): 1-14.

Kinfemi, A. A., Mohamedand, M. I., and Ayoade, J. A. (2009). Biodegradation of Cowpea shells by *Pleurotus* species for it use as ruminant feed. **World Journal of Agricultural Sciences**. 5(5): 639-645.

Kis, G., Grbesa, D., Kostelic, A., and Karolyi, D. (2005). Estimating grass and grass silage degradation characteristics by in situ and in vitro gas production methods. Ital. **Journal of Animal Science**. 4(3): 142-144.

Klieve, A. V., Hennessy, D., Ouwerkerk, D., Forster, R. J., Mackie, R. I., and Attwood, G. T. (2003). Establishing populations of *Megasphaera elsdenii* YE 34 and *Butyrivibrio fibrisolvens* YE 44 in the rumen of cattle fed high grain diets. **Journal of Applied Microbiology**. 95(3): 621-630.

Koike, S., and Kobayashi, Y. (2001). Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens.* **FEMS Microbiology Letters**. 204(2): 361-366.

Leng, R. A., and Brett, D. J. (1966). Simultaneous measurements of the rates of production of acetic, propionic and butyric acids in the rumen of sheep on different diets and the correlation between production rates and concentrations of these acids in the rumen. **British Journal of Nutrition**. 20(3): 541-552.

Lettat, A. (2011). **Efficacy and mode of action of propionic and/or lactic acid bacteria to prevent latent acidosis in ruminants**. Ph.D. Thesis, Blaise Pascal University, French.

Lewis, D. (1957). Blood-urea concentration in relation to protein utilization in the ruminant. **Journal of Agricultural Science**. 48(4): 438-446.

Lounglawan, P., Lounglawan, W., and Suksombat, W. (2014). Effect of cutting interval and cutting height on yield and chemical composition of King Napier grass (*Pennisetum purpureum* x *Pennisetum americanum*). **APCBEE Procedia**. 8: 27-31.

Mahadevamma, S., Shamala, T. R., and Tharanathan, R. N. (2004). Resistant starch derived from processed legumes: *in vitro* and in vivo fermentation characteristics. **International Journal of Food Sciences and Nutrition**. 55(5): 399-405.

Martin, C., Morgavi, D. P., and Doreau, M. (2010). Methane mitigation in ruminants: From microbe to the farm scale. **Animal**. 4(3): 351-365.

McDonald, P., Greenhalgh, J., and Edwards, R. A. (2002). Silage, Hay, Artificially Dried Forages, Straw and Chaff. In: **Animal Nutrition, McDonald, P. (Ed.). 6th Edn.**, Prentice Hall, London,UK., ISBN-13: 9780582419063. pp: 515-537.

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 and Development**. 28: 9-55.

Moran, J. (2005). Feeding management for small holder dairy farmers in the humid tropics. In **Tropical Dairy Farming**; Landlinks Press: Collingwood, VIC, Australia. 312p.

Morgavi, D. P., Martin, C., Jouany, J. P., and Ranilla, M. J. (2012). Rumen protozoa and methanogenesis: Not a simple cause-effect relationship. **British Journal of Nutrition**. 107(3): 388-397.

Mugeriwa, J. S., Christianson, J. A., and Ochetim, S. (1973). Grazing behavior of exotic dairy cattle in Uganda. **East African Agricultural and Forestry Journal**. 39(1): 1-11.

Newbold, C. J., Lopez, S., Nelson, N., Ouda, J. O., Wallace, R. J., and Moss, A. R. (2005). Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. **British Journal of Nutrition**. 94(1): 27-35.

NRC. (1981). **Nutrient requirements of goats: Angora, dairy, and meat goats in temperate and tropical countries**. Washington, DC: The National Academies Press.

มหาวิทยาลัยเทคโนโลยีสุรนารี

O'Doherty, J. V., and Crosby, T.F. (1998). Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. **Animal Science**. 66(3): 675-683.

Oba, M. (2011). Review: Effects of feeding sugars on productivity of lactating dairy cows. **Canadian Journal of Animal Science**. 91(1): 37-46.

Ørskov, E. R., and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. **The Journal of Agricultural Science**. 92(2): 499-503.

Owens, F. N., Secrist, D. S., Hill, W. J., and Gill, D. R. (1998). Acidosis in cattle: A review. **Journal of Animal Science**. 76(1): 275-286.

Paengkoum, P. (2010a). Protein requirements for maintenance of thai native male cattle fed Pangola hay based diets. **Research Journal of Biological Sciences**. 5(1): 33-35.

Paengkoum, P., and Bunnakit, K. (2009b). Replacement of soybean meal with cassava pulp mixed with urea gelatinizes (caspurea) in concentrate diets of beef cattle. **Journal of Animal and Veterinary Advances**. 8(12): 2594-2601.

Paengkoum, P., Tatsapong, P., Pimpa, O., Traiyakun1, S., and Hare, M. D. (2013c). Nitrogen requirements for maintenance of growing Thai native Buffalo fed with rice straw as roughage. **Buffalo Bulletin**. 32(1): 35-52.

Patra, A. K., and Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesisin the rumen. **Phytochemistry**. 71(11-12): 1198-1222.

Petit, H. V., and Flipot, P.M. (1992). Feed utilization of beef steers fed grass as hay or silage with or without nitrogen supplementation. **Journal of Animal Science**. 70(3): 876-883.

Potter, E. L., Cooley, C. O., Richardson, L. F., Raun, A. P., and Rathmacher, R. P. (1976). Effect monensin on performance of cattle forage. **Journal of Animal Science**. 43(3): 665-669.

Pralomkarn, W., Kochapakdee, S., Saithanoo, S., and Norton, B. W. (1995). Energy and protein utilisation for maintenance and growth of Thai native and AngloNubian x Thai native male weaner goats. **Small Ruminant Research**. 16(1): 13-20.

Prasanpanich, S., Sukpituksakul, P., Tudsri, S., Mikled, C., Thwaites, C. J., and Vajrabukka, C. (2002). Milk production and eating patterns of lactating cows under grazing and indoor conditions in central Thailand. **Tropical Grasslands**. 36(2): 107-115.

Preston, T.R., and Leng, R.A. (1987). **Matching ruminant production systems with available resources in the tropics and sub-tropics**. Penambull book armidale, Australia.

Robbins, R.J. (2003). Phenolic acids in foods: an overview of analytical methodology. **Journal of Agricultural and Food Chemistry**. 51(10): 2866-2887.

Russell, J. (1998). The importance of pH in the regulation. **Journal of Dairy Science**. 81(12): 3222-3230.

Sahlu, T., Goetsch, A. L., Luo, J., Nsahlai, I. V., Moore, J. E., Galyean, M. L., Owens, N., Ferrell, C. L., and Johnson, Z. B. (2004). Nutrient requirements of goats: developed equations, other considerations and future research to improve them. **Small Ruminant Research**. 53(3): 191-219.

SAS. (1990). SAS **User's guide: statistics**. Version 6. 14th ed. SAS Inst., Carry, NC.

Steel, R. G. D., and Torrie, J.N. (1980). **Principles and Procedures of Statistics**. 2nd ed. McGraw-Hill. Book C, New York.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Sun, X.-h., Zhou, T.-t., Wei, C.-h., Lan, W.-q., Zhao, Y., Pan, Y.-j., and Wu, V. C. H. (2018). Antibacterial effect and mechanism of anthocyanin rich Chinese wild blueberry extract on various food borne pathogens, **Food Control**. 94: 155-161.

Sylvester, J. T., Karnati, S. K., Yu, Z., Morrison, M., and Firkins, J. L. (2004). Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. **Journal of Nutrition**. 134(12): 3378-3384.

Torskangerpoll, K., Noerbaek, R., Nodland, E., Oevstedal, D. O., and Andersen, Ø. M. (2005). Anthocyanin content of Tulipa species and cultivars and its impact on tepal colours. **Biochemical Systematics and Ecology**. 33(5): 499-510.

Trach, N. X., Mo, M., and Dan, C. X. (2001). Effects of treatment of rice straw with lime and/or urea on its chemical composition, *in vitro* gas production and *in sacco* degradation characteristics. **Livestock Research for Rural Development**. 13(4): 5-12.

Van Soest, P. J., 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.

Van Soest, P.J. (1965). Symposiumon factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. **Journal of Animal Science**. 24(3): 834-843.

Varga, M. (2014). Chapter 1-rabbit basic science. In **Textbook of Rabbit Medicine**, **2nd ed**. Butterworth Heinemann Publisher: Oxford, UK. pp. 3-108.

Virtanen, A. I. (1933). The AIV method of preserving fresh fodder. **Empire Journal of Experimental Agriculture**. 1: 143-155.

Wanapat, M., Kang, S., Hankla, N., and Phesatcha, K. (2013). Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. **African Journal of Agricultural Research**. 8(17): 1677-1687.

Widiawati, Y., and Thalib, A. (2009). Comparison of fermentation kinetics (*in vitro*) of grass and shrub legume leaves: The pattern of VFA concentration, estimated CH_4 and microbial biomass production. **Indonesian Journal of Agriculture**. 2(1): 21-27.

Wijitphan, S., Lorwilai, P., and Arkaseang, C. (2009). Effect of cutting 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, A. G., and Coleman, G. S. (1992). **The rumen protozoa**. Springer-Verlag New York Inc.: New York, NY, USA.

Williams, B. A. (2000). Cumulative gas-production Techniques for forage evaluation. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed. (Editors), **Forage Evaluation in Ruminant Nutrition**. CAB International. pp. 189-213.

Wolin, M. J. (1960a). A theoretical rumen fermentation balance. **Journal of Dairy Science**. 43(10): 1452-1459.

Wolin, M. J. (1979b). The rumen fermentation: a model for microbial interactions in anaerobic ecosystems. In Alexander M. (Ed.), Springer US New York & London. **Advances in Microbial Ecology**. 3: 49-77.

Woolford, M. K. (1990). The detrimental effects of air on silage. **Journal of Applied Bacteriology**. 68(2): 101-116.

Yu, Z., and Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. **BioTechniques**. 36(5): 808-812.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Zailan, M. Z., Yaakub, H., and Jusoh, S. (2015). **Yield and nutritive value of Napier cultivars at different harvesting ages**. Proceedings of the 35th Malaysian Society of Animal Production (MSAP), Port Dickson, Negeri Sembilan, Malaysia, 1-3 June 2015.

Zhou, Z., Zhou, B., Ren, L., and Meng, Q. (2014). Effect of ensiled mulberry leaves and sun-dried mulberry fruit pomace on finishing steer growth performance, blood biochemical parameters, and carcass characteristics. **PLOS ONE**. 9(1): e85406.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER V

EXPERIMENT III

EFFECT OF ANTHOCYANIN FROM PURPLE NAPIER GRASS SILAGE ON MILK YIELD, MILK COMPOSITION AND BLOOD ANTIOXIDANT ACTIVITY IN LACTATING DAIRY GOATS

5.1 Abstract

The objective of this experiment was to investigate the effect of anthocyanin from Purple Napier grass silage on milk yield, milk composition and blood antioxidant activity in lactating dairy goats. Eighteen female crossbred Saanen lactating goats (approximately 52.34±2.86 kg body weight; mean ± standard deviation (SD)) with healthy and symmetrical udders. Goats were divided into three blocks of six goats based on lactation period in a randomized completed block design (RCBD) and divided into 3 treatments. There were three treatments: (1) control, fed Napier Pakchong-1 grass silage 100%; (2) PNS 50%, fed Napier Pakchong-1 grass silage replaced with Purple Napier grass silage 50%; (3) PNS 100%, fed Purple Napier grass silage 100%.

The results showed that Purple Napier grass silage had crude protein (CP), ether extract (EE), digestible energy (DE), gross energy (GE), anthocyanin composition and

total anthocyanin higher (P<0.05) than Napier Pakchong-1 grass silage. The intake of dry matter intake (DMI), 3.5% FCM, fat (g/d), protein (g/d), lactose (g/d), total solid (g/d) and solid not fat (g/d) in dairy goats fed PNS 100% were higher (P<0.05) than control and PNS 50% treatments. There was no significant different in milk pH by treatment diets. Dairy goats fed 100% of PNS showed that milk composition particularly to such milk lactose was higher (P<0.05) than other treatments, while the somatic cell count (SCC) in milk was lower (P<0.05) than other treatments. These parameters are increased quadratically (P<0.05), while the SCC is decreased quadratically (P<0.05).

The dairy goats fed PNS 100% treatment showed that anthocyanin intake, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione S-transferase (GST) in plasma and milk were higher (P<0.05) than other treatments, while the malondialdehyde (MDA) in plasma and milk was lower (P<0.05) than other treatments. These parameters are increased quadratically (P<0.05), while the MDA is decreased quadratically (P<0.05). The cyanidin-3-glucoside (C3G), pelargonidin-3-glucoside (P3G), peonidin-3-O-glucoside (Peo3G), malvidin-3-O-glucoside (M3G), cyanidin (Cya), pelargonidin (Pel) and total anthocyanin in milk of dairy goats fed PNS 100% were higher (P<0.05) than other treatments and these anthocyanin composition are increased quadratically (P<0.05), whereas the Peo3G, M3G, Cya and total anthocyanin are increased linearly (P<0.05). In addition, it was found that the Del and Mal composition could not be analyzed. The significant difference of the parameters by increasing replacement with the level of Purple Napier grass silage. Based on the evidence in the present study, it can be

concluded that Purple Napier grass silage can be used as a good source of roughage for feeding lactating dairy goats.

Key words: anthocyanin, Purple Napier grass silage, milk composition, antioxidant activity, lactating dairy goats.

5.2 Introduction

Numerous biochemical mechanisms, like the respiratory rupture during phagocytosis, are accountable for the production of ROS. Reactive oxygen species (ROS) result from catabolic pathways in living organisms and thus are principally created in the transportation chain of mitochondrial electrons (Ames et al., 1995). ROS will be sensitive to oxidation with self-biomolecules, as well as lipids are especially vulnerable to certain molecules (Miller et al., 1993).

Aerobic organisms have an efficient antioxidant protection system which depends largely mostly on a dietary supply with antioxidants to counterbalance ROS action (Bendich, 1993). There is an imbalance between oxidants and antioxidants, as well as the organism interactions a status known as oxidative stress, that can affect oxidative destruction when oxidative modifications occur in cell molecules (Celi, 2011a). The equilibrium between pro-oxidants and the antioxidant capacity is impacted by diets, season, calving condition, heat stress, and milk yield. In addition, almost disease and also some physiological phases, such as the peripartum period of dairy cows and dairy goats, have shown to occur in oxidative stress (Castillo et al., 2006; Celi, 2011a; Di Trana et al., 2006).

Plant extracts or natural plant molecules of many plant species provide antioxidant property leading from many compounds, like polyphenols (Celi and

Raadsma, 2010b; Luciano et al., 2011; Makkar et al., 2007). Anthocyanins are pigments commonly present in plants. Anthocyanins can be used as a food colouring additive and may also be used to reduce the risk of many diseases (Wrolstad et al., 2005; Szajdek and Borowska, 2008). Previous study, the Purple corn anthocyanin has been reported to show several functional and biological attributes, such as antioxidant in *in vitro* and *in vivo* experiments with ruminant animals. Purple corn (*Zea mays* L.) stover silage have been reported to show enhancing effects on the activity of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) which is an important antioxidant enzyme in lactating dairy goats (Tian et al., 2019). The use of forages containing bioactive compounds is a natural and viable alternative to encourage a beneficial antioxidant balance, enhance animal performance and ensure certain animal products are safe for human consumption. This strategy is a new direction for sustainable and environmentally responsible agriculture. Consequently, there was little study in providing Purple forage with ruminant animals, so the impact of feeding anthocyanin to ruminant animals remains unclear.

Hence, the aim of this study was to investigate the effect of anthocyanin from Purple Napier grass silage on milk yield, milk composition and blood antioxidant activity in lactating dairy goats.

5.3 Objective

The objective of this experiment was to investigate the effect of anthocyanin from Purple Napier grass silage on milk yield, milk composition and blood antioxidant activity in lactating dairy goats.

5.4 Materials and methods

5.4.1 Animals, treatments, and experimental design

Eighteen female crossbred Saanen lactating goats (approximately 52.34 ±2.86 kg body weight; mean±standard deviation (SD)) with healthy and symmetrical udders. Dairy goats were split into three blocks on the basis of lactation period during the prior lactation. For each block, six animals were randomly allocated treatment according to a randomized completed block design (RCBD). The first 2 weeks of the trial were the adjustment period followed by a 6 week measurement period for a total of 8 weeks. The chemical and anthocyanin composition in Napier Pakchong-1 and Purple Napier grass silage from experiment II were shown in Table 5.1 and 5.2. Dairy goats fed total mixed ration diets (TMR; Table 5.3), split in three treatments:

Control = Napier Pakchong-1 grass silage 100%

PNS 50% = Replaced with Purple Napier grass silage 50%

PNS 100% = Purple Napier grass silage 100%

Animals were housed in dry and clean pens with available access to water. These goats fed TMR diets twice per day at 07:00 and 16:00 h for ad libitum intake and allowed for approximately 10% refusal. According to the National Research Council (NRC, 1981), TMR diets have been formulated to meet the requirements for milk goat with a body weight of 50 kg.

5.4.2 Data collection, sampling and chemical analysis

5.4.2.1 Feed and milk sampling

Feed offered and refused were weighed daily prior to the morning feeding, and then calculated DMI throughout the experiment. The dairy goats

milked once a day at 19:00 h through a portable milking machine (Condor Company, made in Italy) and recorded milk yield. The milk production from dairy goats accumulated throughout that early milking was removed. The milking schedule provided preparation of milking devices, preparation of udder, clean and dry of teats (1.0% sodium hypochlorite solution) and milking. The 36 kPa operating vacuum was used, as well as at 120 cycles/min a pulsator was functioned with such a 50:50 milk: rest ratio. The milk yield was sampled in the experiment's final 2 days from 3rd to 8th weeks (2 days for each collection period). Samples (100 g per kg of recorded milk yield) were completely mixed as well as collected for each milking and the pH value of the milk was instantly determined using a portable pH metre. Afterwards, the milk samples were separated into two portions. One aliquot was stored with such a preservative (bronopol tablet; D&F Control System Inc., San Ramon, CA, USA) at 4°C till the fat, protein, lactose, total solid (TS), solid not fat (SNF) and somatic cell count (SCC) were analysed with a MilkoScan analyzer (MilkoScanTMFT2, FOSS, Hillerod, Denmark) after 10 minutes of incubation at 40°C in water bath. The other aliquot was stored at -20°C until analysed for antioxidant and anthocyanin activity. The milk and fat outputs were calculating 3.5% fat-corrected milk (3.5% FCM) using the following formulas by Hamzaoui et al. (2013):

$$3.5\%\ FCM = Kg\ of\ milk\ yield \times [0.432 + 0.162 \times (fat\ \%)]$$

5.4.2.2 Blood sampling

At the end of the experiment, blood (approximately 3 mL) from the jugular vein was sampled at 0, 2 and 4 hours after feeding by vacuette® tubes (Greiner Bio-One, Greiner Bio-One GmbH Bad Haller Str. 324550 Kremsmünster,

Austria) with K_3-EDTA. After centrifugation at 4000 r/min at 4°C for 15 minutes (Sorvall™ Legend™ XT/XF Centrifuge Series, Thermo Fisher Scientific Pte Ltd., Waltham, USA), the plasma was transferred to a 1.5 mL tube and placed at -20°C for the further analysis.

5.4.3 Laboratory analyses

5.4.3.1 Chemical analysis

For grass silage's nutritive value analysis, the grass silage were freeze-dried, then ground to pass through a 1 mm^2 mesh screen and analyzed for chemical composition. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, ether extract (EE) and ash contents were quantified by AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) estimated by the methods described by Van Soest et al. (1991).

5.4.3.2 Antioxidant activity

Scavenging activity of 2,2-diphenyl-1-picrylhydrazile (DPPH) was analyzed by spectrophotometric methods (Wei and Chiang, 2009; Zarban et al., 2009) utilizing stable free radical DPPH (Sigma-Aldrich, Pcode: 101845869) with minor modification. In a 1.5 mL tube, 50 µL of each plasma and milk sample was mixed with 1 ml of DPPH reagent methanol solution (25 µmol/L). At the room temperature, the mixture was strenuously shaken and incubated for 30 minutes in the dark, afterward centrifuged at 4,000 r/min at 4°C for 10 minutes. The supernatant was instantly relocated to a 96-well plate with 200 µL and a microplate reader was detected at 517 nm. (Epoch, BioTek, Luzern, Switzerland) The activity of DPPH scavenging was calculated using the following equation:

$$\text{DPPH activity for scavenging activity (\%)} = \frac{Ac - As \times 100}{Ac}$$

where A = the absorbance of the control

As = the absorbance of the sample

The Sigma-Aldrich kits (MAK187, 19160 SOD determination kit, CS0410 and MAK085, respectively) were used to measure enzyme activity of total antioxidant capability (TAC), superoxide dismutase (SOD), glutathione S-transferase (GST) and malondialdehyde (MDA) and all samples were tested through the use of a microplate reader.

5.4.3.3 Anthocyanin composition

The grass silage specimen (50 g) was extracted using 1% hydrochloric acid (HCl) dissolved in 95% methanol solution (15:85, v/v), the supernatant has been collected 24 hours after incubation at 50°C (Hosoda et al., 2009). Subsequently, the supernatant was filtered through with a 13 mm 0.45 μm Nylon Syringe Filter (Xiboshi, TNL1345PP, Tianjin Fuji Science & Technology Co., Ltd., China) for the identification of anthocyanin composition by high performance liquid chromatography (HPLC; 1260 Infinity II LC, Agilent Technologies, Santa Clara, CA, USA). The anthocyanin composition in milk has been assessed using a slightly adjusted (Seeram et al., 2006; Tadapaneni et al., 2012).

Milk samples modified to pH 4 with 1% hydrochloric acid and pre-treated with acetone/water liquid-liquid extraction (70:30, v/v) and afterwards centrifuged 10,000 r/min at 4°C for 15 minutes after incubation at room temperature for 4 hours, the supernatant was collected for anthocyanin composition. The analysis of the specimen has been performed with the HPLC and Diode Array Detector

(DAD). Anthocyanin content extraction was achieved on the column C_{18} Symmetry (mobile phase: A, acetonitrile (CH_3CN); B, 10% acetic acid/5% CH_3CN/1% phosphoric acid in deionized water). The time period was 30 minutes, followed by a delay of 5 minutes before the next injection. Another conditions were determined sample temperature at 4°C and injection volume of 20 µL, flow rate of 0.8 mL/min, column temperature of 25°C and DAD wavelength of 520 nm.

5.4.4 Statistical analysis

All statistical calculations were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System 9.1.3 (SAS, 1990) according to a Randomized Completely Block Design (RCBD) and using milk lactation period as block (fixed effect) and orthogonal polynomials was used for trend analysis. Significant differences ($P<0.05$) among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1980). The statistical model for the analysis of data was:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where: Y_{ij} = an observation in treatment i (i = 1-3) and block j (j = 1-6)

μ = the overall mean

τ_i = the effect of treatment i (i = 1-3)

β_j = the fixed effect of block j (j = 1-6)

ε_{ij} = residual

5.4.5 Experimental location

The experiment was conducted at Sukjai Farm, Nakhon Ratchasima, Thailand (14°53'36.7"N, 102°03'21.2"E). All goats were handled in accordance with

the Guide for the care and use of laboratory animals (Institute of Laboratory Animal Resources, Commission on Life Sciences).

5.4.6 Experimental period

The experiment was from September 2019 to November 2019.

5.5 Results

5.5.1 Feed nutrient value and anthocyanin composition

The nutrient value of Napier Pakchong-1 and Purple Napier grass in the experimental treatments were demonstrated in the Table 5.1. For chemical composition, neutral detergent fiber (NDF), hemicellulose and crude fiber (CF) content, respectively, in Napier Pakchong-1 grass silage were higher ($P<0.05$) than Purple Napier grass silage. The crude protein (CP) content, ether extract (EE) content, digestible energy (DE) and gross energy (GE), respectively, in Purple Napier grass silage were higher ($P<0.05$) than Napier Pakchong-1 grass silage. The anthocyanin composition of Napier Pakchong-1 and Purple Napier grass in the experimental treatments were demonstrated in the Table 5.2. All of anthocyanin derivatives and total anthocyanin in Purple Napier grass silage were higher ($P<0.05$) than Napier Pakchong-1 grass silage.

5.5.2 Feed intake and milk yield

The ingredient and nutrient composition of experimental diets in current study has shown in the Table 5.3. The experimental diets were TMR feeding dairy goats including control (Napier Pakchong-1 grass silage 100%), PNS 50% (replaced with 50% of Purple Napier grass silage) and PNS 100% (Purple Napier grass silage 100%), respectively. Feed intake and milk yield has shown in the Table

5.4. The intake of dry matter intake (DMI), 3.5% FCM, fat (g/d), protein (g/d), lactose (g/d), total solid (g/d) and solid not fat (g/d), respectively in dairy goats fed Purple Napier grass silage 100% treatment were higher ($P<0.05$) than other treatments and showed that with increasing of feed intake in the level of Purple Napier grass silage replacing caused increased quadratically ($P<0.05$).

5.5.3 Milk composition

The milk composition including pH, fat, protein, lactose, total solid (TS), solid-not-fat (SNF) and somatic cell count, respectively, of dairy goats in current study has shown in the Table 5.5. There was no significant different in milk pH.

Milk composition parameters including fat, protein, lactose, total solid (TS) and solid not fat (SNF), respectively, of dairy goats in Purple Napier grass silage 100% treatment were higher ($P<0.05$) than other treatments and these parameters are increased quadratically ($P<0.05$), whereas the SCC was lower ($P<0.05$) than other treatments and this parameter is decreased quadratically ($P<0.05$). The significant difference of these parameters by increasing replacement with the level of Purple Napier grass silage.

5.5.4 Antioxidant activity in plasma and milk

The anthocyanin intake and antioxidant activity parameters including DPPH scavenging activity, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione S-transferase enzyme (GST) and malondialdehyde (MDA) in plasma and milk of dairy goats in current study has shown in the Table 5.6. The anthocyanin intake of dairy goats in Purple Napier grass silage 100% treatment was higher ($P<0.05$) than other treatments and this parameter is increased quadratically ($P<0.05$).

Plasma antioxidant activity parameters, the DPPH, TAC, SOD and GST (2, 4 hours after feeding and the mean value of these parameters), respectively, of dairy goats in Purple Napier grass silage 100% treatment were higher ($P<0.05$) than other treatments and these parameters are increased quadratically ($P<0.05$), whereas the plasma MDA (2, 4 hours after feeding and the mean of plasma MDA value) was lower ($P<0.05$) than other treatments and this parameter is decreased quadratically ($P<0.05$).

Milk antioxidant activity parameters, the DPPH, TAC, SOD and GST, respectively, of dairy goats in Purple Napier grass silage 100% treatment were higher ($P<0.05$) than other treatments and these parameters are increased quadratically ($P<0.05$), whereas the milk MDA was lower ($P<0.05$) than other treatments and this parameter is decreased quadratically ($P<0.05$). The significant difference of these parameters by increasing replacement with the level of Purple Napier grass silage.

5.5.5 Anthocyanin composition in milk

The anthocyanin composition in raw milk including cyanidin-3-glucoside (C3G), pelargonidin-3-glucoside (P3G), delphinidin (Del), peonidin-3-O-glucoside (Peo3G), malvidin-3-O-glucoside (M3G), cyanidin (Cya), pelargonidin (Pel), malvidin (Mal) and total anthocyanin, respectively, of dairy goats in current study has shown in the Table 5.7.

The milk C3G, P3G and Pel, respectively, of dairy goats in Purple Napier grass silage 100% treatment were higher ($P<0.05$) than other treatments and these anthocyanin compositions are increased quadratically ($P<0.05$), whereas the milk Peo3G, M3G, Cya and total anthocyanin, respectively, were higher ($P<0.05$) than other treatments and these anthocyanin compositions are increased linearly

(P<0.05). The significant difference of these parameters by increasing replacement with the level of Purple Napier grass silage. In addition, it was found that the Del and Mal composition could not be analyzed.

Table 5.1 Nutrient composition of Napier Pakchong-1 and Purple Napier grass silage.

Item[1]	Napier Pakchong-1	Purple Napier	SEM[2]	P-value
Chemical composition				
DM (%)	23.32	22.11	0.439	0.183
	---------- On dry basis % ----------			
OM (% DM)	85.95	88.83	0.839	0.084
CP (% DM)	8.85[b]	9.26[a]	0.095	0.019
NDF (% DM)	77.52[a]	68.02[b]	1.7144	0.0001
ADF (% DM)	45.04	43.80	0.6583	0.378
Hemicellulose (% DM)	32.47[a]	24.21[b]	1.451	<.0001
EE (% DM)	1.25[b]	1.78[a]	0.110	0.006
CF (% DM)	34.66[a]	32.07[b]	0.650	0.036
ME (kJ/g DM)	2,253.60	2,387.30	143.848	0.669
DE (kJ/g DM)	2,748.67[b]	2,911.46[a]	42.915	0.050
GE (kJ/g DM)	3,404.60[b]	3,740.60[a]	73.710	0.011

[a, b] Means with different superscripts in the same row differ significantly (P< 0.05).

[1] DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; CF = crude fiber; ME = metabolizable energy; DE = digestible energy; GE = gross energy.

[2] SEM = standard error of mean.

Table 5.2 The different of anthocyanin composition (mg/kg DM) of Napier Pakchong-1 and Purple Napier grass silage.

Item[1]	Napier Pakchong-1	Purple Napier	SEM[2]	P-value
C3G	307.64[b]	813.65[a]	84.507	<.0001
P3G	58.19[b]	153.38[a]	15.874	<.0001
Del	50.30[b]	132.40[a]	13.693	<.0001
Peo3G	109.54[b]	289.70[a]	30.163	<.0001
M3G	34.45[b]	90.48[a]	9.419	<.0001
Cya	54.41[b]	143.40[a]	14.908	<.0001
Pel	55.58[b]	147.56[a]	15.411	<.0001
Mal	223.43[b]	590.64[a]	61.656	<.0001
Total (mg/kg DM)	893.53[b]	2,361.22[a]	245.395	<.0001

[a, b] Means with different superscripts in the same row differ significantly (P< 0.05).

[1] C3G = cyanidin-3-glucoside; P3G = pelargonidin-3-glucoside; Del = delphinidin; Peo3G = peonidin-3-O-glucoside; M3G = malvidin-3-O-glucoside; Cya = cyanidin; Pel = pelargonidin; Mal = malvidin.

[2] SEM = standard error of mean.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 5.3 Ingredient and nutrient composition of experimental diets for dairy goats.

Item	Treatment[1]		
	Control	**PNS 50%**	**PNS 100%**
Ingredient (% DM)	---------- On dry basis % ----------		
Napier Pak Chong 1 silage	50.00	25.00	-
Purple Napier silage	-	25.00	50.00
Soybean hull	4.50	4.50	4.50
Soybean residue	30.00	30.00	30.00
Concentrate 21 %CP	15.00	15.00	15.00
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Chemical composition			
DM	36.27	35.97	35.67
	---------- On dry basis % ----------		
OM	90.86	89.96	89.24
CP	17.28	17.39	17.49
NDF	72.16	69.79	67.41
ADF	41.30	40.99	40.68
Hemicellulose	30.86	28.80	26.73
Ash	10.31	9.66	9.02
CF	22.71	22.07	21.42
EE	4.55	4.68	4.81
ME (kJ/g DM)	2,464.30	2,497.76	2,531.22
DE (kJ/g DM)	3,005.25	3,046.05	3,086.85
GE (kJ/g DM)	3,998.61	4,082.64	4,166.67
Anthocyanin (mg/kg DM)	446.92	813.69	1,180.61

[1] Control = Napier Pakchong-1 grass silage 100%; PNS 50% = replaced with 50% of the Purple Napier grass silage; PNS 100% = Purple Napier grass silage 100%.
ME (kJ/g DM) = 0.82 × DE (kJ/g DM)

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 5.4 Effects of experiment diets on DMI, milk yield and efficiency of dairy goats.

Item	Treatment			SEM	P-value	
	Control	PNS 50%	PNS 100%		L	Q
DMI (g/d)	1,130.71[c]	1,162.81[b]	1,208.44[a]	7.793	<.0001	0.004
Milk production						
Milk yield (kg/d)	1.18[c]	1.36[b]	1.56[a]	0.038	0.058	0.035
3.5% FCM (kg/d)	1.19[c]	1.42[b]	1.69[a]	0.050	<.0001	0.020
Fat (g/d)	42.34[c]	51.34[b]	62.94[a]	2.083	<.0001	0.042
Protein (g/d)	39.72[c]	48.05[b]	56.65[a]	1.679	<.0001	0.028
Lactose (g/d)	51.83[c]	63.92[b]	82.95[a]	3.289	<.0001	0.015
Total solid (g/d)	133.88[c]	163.32[b]	202.54[a]	7.146	<.0001	0.033
SNF (g/d)	91.55[c]	111.98[b]	139.60[a]	4.903	<.0001	0.034

[a, b, c] Means followed by a different letter within the same row are significant different ($P<0.05$); DMI = dry matter intake; FCM = fat-corrected milk; SNF = solid not fat; Control = Napier Pakchong-1 grass silage 100%; PNS 50% = replaced with 50% of the Purple Napier grass silage; PNS 100% = Purple Napier grass silage 100%; SEM = standard error of the mean; L = linear; Q = quadratic.

Table 5.5 Effects of diets on milk composition of dairy goats.

Item	Treatment			SEM	P-value	
	Control	PNS 50%	PNS 100%		L	Q
pH	6.57	6.58	6.60	0.012	0.283	0.886
Fat (%)	3.60[c]	3.77[b]	4.02[a]	0.043	<.0001	0.002
Protein (%)	3.37[c]	3.53[b]	3.63[a]	0.028	<.0001	0.008
Lactose (%)	4.40[c]	4.68[b]	5.29[a]	0.098	<.0001	0.018
TS (%)	11.37[c]	11.98[b]	12.94[a]	0.170	<.0001	0.016
SNF (%)	7.77[c]	8.21[b]	8.92[a]	0.124	<.0001	0.031
SCC (cells x 10^6/mL)	1.73[a]	1.27[b]	0.97[c]	0.085	<.0001	<.0001

[a, b, c] Means followed by a different letter within the same row are significant different ($P<0.05$); TS = total solid; SNF = solid not fat; SCC = somatic cell count; Control = Napier Pakchong-1 grass silage 100%; PNS 50% = replaced with 50% of the Purple Napier grass silage; PNS 100% = Purple Napier grass silage 100%; SEM = standard error of the mean; L = linear; Q = quadratic.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 5.6 Effects of diets on antioxidant activity in plasma and milk of dairy goats.

Item	Treatment			SEM	P-value	
	Control	PNS 50%	PNS 100%		L	Q
Anthocyanin intake (mg/day)	505.34[c]	946.17[b]	1,426.69[a]	91.351	<.0001	0.028
Plasma						
DPPH (%)	25.35[b]	27.42[b]	36.55[a]	1.422	<.0001	0.020
TAC (nmole/μL)						
0 h	107.54	107.61	109.23	1.684	0.303	0.576
2 h	112.51[c]	116.19[b]	120.66[a]	0.891	<.0001	0.002
4 h	108.87[c]	111.95[b]	115.69[a]	0.749	<.0001	0.026
Mean	109.64[c]	111.91[b]	115.19[a]	0.613	<.0001	0.003
SOD (inhibition rate, %)						
0 h	67.50	68.13	67.20	1.316	0.828	0.511
2 h	76.28[c]	86.63[b]	91.67[a]	1.716	<.0001	<.0001
4 h	72.64[c]	82.39[b]	86.71[a]	1.624	<.0001	0.017
Mean	72.14[c]	79.05[b]	81.86[a]	1.126	<.0001	0.005
GST (mmol/min/mL)						
0 h	31.84	32.82	30.54	0.959	0.219	0.091
2 h	38.37[c]	51.99[b]	61.17[a]	2.527	<.0001	0.044
4 h	34.73[c]	47.75[b]	56.20[a]	2.389	<.0001	0.029
Mean	34.98[c]	44.19[b]	49.30[a]	1.914	<.0001	0.011
MDA (umol/L)						
0 h	0.76	0.74	0.75	0.017	0.887	0.527
2 h	0.32[a]	0.19[b]	0.06[c]	0.028	<.0001	0.039

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 5.6 (Continue).

Item	Treatment			SEM	P-value	
	Control	PNS 50%	PNS 100%		L	Q
4 h	0.68[a]	0.61[b]	0.38[c]	0.036	<.0001	0.001
Mean	0.59[a]	0.51[b]	0.40[c]	0.021	<.0001	0.002
Milk						
DPPH (%)	20.65[c]	25.72[b]	27.42[a]	0.820	<.0001	0.037
TAC (nmole/µL)	79.94[c]	97.16[b]	106.45[a]	3.618	<.0001	0.025
SOD (inhibition rate, %)	65.95[b]	67.87[b]	73.86[a]	1.701	<.0001	0.011
GST (mmol/min/mL)	33.34[c]	45.71[b]	55.37[a]	2.427	<.0001	0.007
MDA (umol/L)	0.39[a]	0.36[b]	0.34[c]	0.006	<.0001	0.043

[a, b, c] Means followed by a different letter within the same row are significant different ($P<0.05$); DPPH = 2, 2-diphenyl-1-picrylhydrazyl; TAC = total antioxidant capacity; SOD = superoxide dismutase; GST = glutathione S-transferase; MDA = malondialdehyde at 0 and 2 to 4 hours after feeding; Control = Napier Pakchong-1 grass silage 100%; PNS 50% = replaced with 50% of the Purple Napier grass silage; PNS 100% = Purple Napier grass silage 100%; SEM = standard error of the mean; L = linear; Q = quadratic.

Table 5.7 Comparison of anthocyanin composition in goat's milk.

Item[1]	Treatment[2]			SEM[3]	P-value[4]	
	Control	PNS 50%	PNS 100%		L	Q
C3G	1.00[c]	1.85[b]	2.60[a]	0.176	<.0001	0.028
Del	-	-	-	-	-	-
P3G	0.54[c]	1.03[b]	1.54[a]	0.110	<.0001	0.015
Peo3G	0.60[c]	1.05[b]	1.52[a]	0.104	<.0001	0.626
M3G	0.24[c]	0.42[b]	0.62[a]	0.042	<.0001	0.192
Cya	0.57[c]	1.02[b]	1.47[a]	0.101	<.0001	0.917
Pel	0.61[c]	0.90[b]	1.36[a]	0.086	<.0001	0.005
Mal	-	-	-	-	-	-
Total (mg/kg)	3.56[c]	6.27[b]	9.11[a]	0.624	<.0001	0.599

[a, b, c] Means followed by a different letter within the same row are significant different ($P<0.05$); C3G = cyanidin-3-glucoside; P3G = pelargonidin-3-glucoside; Del = delphinidin; Peo3G = peonidin-3-O-glucoside; M3G = malvidin-3-O-glucoside; Cya = cyanidin; Pel = pelargonidin; Mal = malvidin; - = not detected; Control = Napier Pakchong-1 grass silage 100%; PNS 50% = replaced with 50% of the Purple Napier grass silage; PNS 100% = Purple Napier grass silage 100%; SEM = standard error of the mean; L = linear; Q = quadratic.

5.6 Discussion

5.6.1 Feed nutrient value and anthocyanin composition

The chemical composition, structural carbohydrate of Napier Pakchong-1 grass silage were higher than Purple Napier grass, whereas CP content, EE content, energy and anthocyanin composition were higher in Purple Napier grass silage due to the different of chemical composition at the first 21 days of ensilling. The most of the reduction occurred during the first 21 days of fermentation. A rapid synthesis of lactic acid in the lack of oxygen had a positive correlation with nutrients and anthocyanin composition due to a rapid decrease of pH below 4 under anaerobic conditions is improve the conservation of nutrients and anthocyanin content as a good silage quality (Virtanen, 1933).

5.6.2 Feed intake, milk yield and composition

The dairy goats fed Purple Napier grass silage had higher milk composition due to dry matter intake (DMI) and intake of nutrient. The type of feed and dry matter intake affects nutrient composition of milk produced by cattle and feed quality will affect the metabolism in the body of livestock that will affect the availability of energy and nutrients for the synthesis of milk components (Bruhn, 2006). Harvatine and Allen (2005) reported that milk yield and milk protein yield responses were linearly increased with increased DMI response. Fat-corrected milk, milk fat percentage,and milk fat yield responses were affected quadratically by increasing DMI response.

Marginal milk and milk protein yield were linearly increased and marginal milk fat yield was affected quadratically with increasing DMI response. In this experiment, the increasing of milk lactose with the higher level of Purple Napier

grass silage replacement probably anthocyanins affect rumen fermentation particularly propionic acid (C_3) due to propionic acid is precursor in milk lactose synthesis (Rigout et al., 2003). The most widespread anthocyanin is cyanidin 3-glucoside was highest in Purple Napier grass cultivars. The chemical structure of anthocyanin attached with different sugar moieties such as glucose, galactose, rhamnose, xylose and arabinose (Giusti et al., 1998; Torskangerpoll et al., 2005). These sugars may be acylated with ruminal aromatic acids (Robbins, 2003) resulting in a breakdown of anthocyanin. Tian et al. (2019) reported that the TPSS group anthocyanin-rich Purple corn (*Zea mays* L.) stover silage led to an elevation in the content of milk lactose relative to the CSSS (sticky corn stover silage).

It can be indicated that these sugar moieties after anthocyanin breakdown are the precursors for ruminal C_3 synthesis and lactose synthesis by absorption in digestive tract (Passamonti et al., 2003). The genetic influences on nutritional composition of milk and it has a heritability value of 50%. In other words, 50% of high and low nutrient composition of milk is determined by dietary factors and management (Haenlein, 2002). The yield and composition of goat milk very widely and this variation is attributed to breed, parity, stage of lactation, age, geographical location, season, diet, health and management of goats (Pal et al., 1996; Sing and Sengar, 1990).

The decreasing of milk SCC with the higher level of Purple Napier grass silage replacement probably it is obvious that the antibacterial activity was positively correlated with the increasing of anthocyanin intake. Anthocyanins inhibited mechanism by destroying the cell wall of the bacteria and there was cytoplasmic leakage (Lacombe et al., 2013). The effects on the bacterial cell

membrane could be due to antibacterial agents combination with membrane proteins forming hydrogen bonds or through hydrophobic interactions (Apostolidis et al., 2008). This structure of cell membrane, the ions necessary to maintain protein stability were isolated, and the anthocyanin was free to attach or donate electrons at the interface of the membrane to make it an antibacterial agent (Guo et al., 2007; Kwon et al., 2007).

Membrane potential, which is the dominant source of energy for almost every chemical reactions in living cells, is the greatest essential component for the preservation and growth of microbial cells. The respiratory chains of bacterial membranes consist of a primary dehydrogenase, coenzyme Q, cytochrome b, c, o, and a and constituents unique to bacteria (Hellingwerf and Konings, 1985). Hellingwerf and Konings (1985) reported that the Licochalcone and echinatin, retrochalcones isolated from the *Glycyrrhiza inflata* roots. These retrochalcones prevented the intake of oxygen in sensitive Gram-positive bacterial cells (*Micrococcus luteus*). They as well blocked the oxidation of NADH in cell membrane formulations. NADH-cytochrome c reductase has been blocked by licochalcones, whereas cytochrome c oxidase has not been inhibited. NADH-CoQ reductase and NADH-FMN oxidoreductase have not been inhibited. The location of respiratory suppression of licochalcones in the microbial respiratory electron transport chain has been assumed to be between CoQ and cytochrome c (Hellingwerf and Konings, 1985).

Peptidoglycan is a crucial element of the cell wall and preventing its formulation is indeed a main mode of action of traditional antimicrobial drugs and flavonoids. Wu et al. (2008) reported the kinetic studies of D-alanine-D-alanine ligase, produced as a result, in the peptidoglycan catalyst terminal dipeptide UDPMurNAc-

pentapeptide and the study have found quercetin and apigenin inhibit this enzyme (Singh et al., 2013). This antimicrobial activity affected to the bacteria by stopping their cell wall function besides attaching to newly formed peptidoglycan precursors (D-Ala-D-Ala) and establishing a cap causing a loss of cross-linking throughout the polypeptide chain (Gardete and Tomasz, 2014).

5.6.3 Antioxidant activity in plasma and milk

Purple Napier grass silage 100% resulting in a higher DPPH, TAC, SOD and GST enzyme, respectively in plasma and milk, whereas the MDA was lower due to positively correlated with the increasing of anthocyanin intake. The free radical reactions are modulated by two types of antioxidants such as the enzymatic antioxidants and non-enzymatic antioxidants. Anthocyanins can donate electrons under its own native forms to reactive oxygen species (ROS), thus preventing oxidizable biomolecules, along with such polyunsaturated fatty acids (PUFAs), proteins and DNA, which are linked to metal ion-chelating action and free radical scavenging of anthocyanin.

The structural groups are major factors of anthocynidine radical scavenging activity including the structure of ortho-dihydroxy in the B-ring, the 2, 3 double bond in conjugation and the 4-oxofunction in the C-ring, respectively (Pekkarinen et al., 1999). Flavonoids condition, metal ion complexes that use the B-ring 3-or 5-hydroxyl and 4-keto-substituents or hydroxyl groups (Pekkarinen et al., 1999). Interactions with structural function demonstrate that hydroxylation at C3′ and C5′ enhances H-doning efficiency, implying that the B-ring is mainly implicated in electron donation (Kähkönen et al., 2003). Oxygen radical absorbing capacity (ORAC) among aglycones (non-sugar part of anthocyanin, i.e. anthocyanin structure)

with the identical hydroxylation sequence in the A and C rings, enhanced hydroxylation in the B ring gives rise to an increment in antioxidant potential (3′, 4′ di-OH especially in comparison with 3′-OH has greater ORAC capacity) (Wang et al., 1997).

Cyanidine was found to have a higher ORAC absorbing capacity of oxygen radical than malvidin and peonidin, but delphinidine having 3 hydroxyl groups on the C ring had been an exception and had been discovered to get a low potential (Wang et al., 1997). This was considered due to the likely declining effect of the 5′-OH in the existence of 3′, 4′-OH (delphinidine) as compared to the existence of 3′, 4′-OH only (cyanidine) (Wang et al., 1997). Such as in anthocynidins (cynidin-3-glucoside) an electron (supplemented by a hydrogen nucleus) can be donated to free radicals from ˙OH groups bonded to phenolic rings (Ramos et al., 2014; Nijveldt et al., 2001). The electron is steadying and immobilising the free radical. In this procedure, the polyphenol-reducing agent transforms to the aroxyl radical, which is relatively more reliable due to its resonance than the free radical it has decreased. The overall result is the termination of harmful oxidative chain reactions (Nijveldt et al., 2001). It was also noted that anthocyanidins get a greater radical scavenging capacity than anthocyanin, in which the radical scavenging ability has also been revealed to reduce with an increment in the amount of sugar moieties (Wang and Stoner, 2008).

The Nrf2/ARE route might be another method for anthocyanins to enhance the SOD and GST enzyme action in cells (Nguyen et al., 2009; Shih et al., 2007). Nrf2 (nuclear factor E2 related factor 2) primarily regulates the antioxidant response element (ARE) stimulation. Nrf2 is ordinarily bound to Keap1 (Kelch-like erythroid CNC homologue (ECH)-associated protein 1). The reactive oxygen species

(ROS) and reactive nitrogen species (RNS) are naturally found free radicals throughout the body. ROS are by-products of a variety of metabolic and immunological processes, including superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl ($^{\bullet}OH$), ozone (O_3) and singlet oxygen (Jones and DeLong, 2000).

The ROS responds with cysteine bonds on the Keap1 protein, and therefore the Nrf2 detaches with Keap1. Then, the Nrf2 is able to move into the nucleus in which ARE is triggered, enabling ARE to stimulate antioxidant enzymes. The ARE is stimulated, it instructs messenger RNA creation to render antioxidant enzymes code besides peptides and proteins. Nrf2 is susceptible to ROS and other pro-oxidant levels but also to antioxidants, such as phenolic compounds as anthocyanins, the enzymes SOD and GST scavenge free radicals (ROS). SOD situated in the cytosol and mitochondria transform $O_2^{-\bullet}$ to oxygen and H_2O_2 in the co-factors existence of metal ion such as copper (Cu), zinc (Zn) or manganese (Mn) (Gough and Cotter, 2011).

Glutathione (GSH) is one of the greatest essential cellular antioxidant agents because, in combination with both of glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) enzymes, glutathione enzyme is discovered both within the cytoplasm and extracellularly in almost every human tissue. The glutathione enzyme transforms the H_2O_2 to water molecule. The glutathione enzyme has powerful action toward more H_2O_2 and fatty acid hydroperoxides (Arthur, 2000; Cabiscol et al., 2000). The enzyme peroxy redox in catalyze the reduction of H_2O_2, organic hydroperoxides and the peroxynitrite ($ONOO^-$).

Antioxidant enzymes decrease lipid hydroperoxide and H_2O_2 concentrations, therefore they are effective in the prevention of lipid peroxidation and

sustaining cell membrane structure and function, which lead to the highest concentration of DPPH and TAC, as contrasted to the lowest concentration of MDA. Tian et al. (2019) reported that the lactating dairy goats receiving anthocyanin-rich Purple corn stover silage exhibited a higher level of superoxide dismutase (SOD) in plasma and milk relative to the sticky corn stover silage.

The current study could indicate that anthocyanin from Purple Napier Grass Silage could enhance SOD, GST enzyme activity, DPPH and TAC resulting in lower concentration of MDA in the antioxidant system of dairy goats, that also functions to prevent oxidative stress due to MDA by-products derived from DNA and lipid oxidation, respectively, and have been mainly used it to measure oxidant status (Fee et al., 1975).

5.6.4 Anthocyanin composition in milk

The dairy goats fed Purple Napier grass silage 100% had higher milk anthocyanin composition particularly cyanidin-3-glucoside (C3G) had a lot more than other anthocyanin composition due to positive relation with anthocyanin intake. Anthocyanins are plant secondary metabolites of the flavonoid family. Red to blue fruits are significant dietary sources of anthocyanin (up to 1 g/100 g FW), among the most best known being cyanidin-3-O-glucoside (Felgines et al., 2009b). The absorption of anthocyanin mechanism probably glycosylation property. The glycosylation property can be the biggest impact on partitioning coefficients. The compound will diffuse passively across the biological membrane and it might divide internally in different cell phases. Aglycones are primarily hydrophobic and can be diffuse passively through biological membranes. The linkage with sugars improves their water solubility and restriction passive diffusion.

The two possible mechanism role in the transport of flavonoid glycosides are perhaps the transport of preserved glucoside through a sodium-glucose co-transporter (SGLT1 and GLUT2) or the extracellular hydrolysis of glycoside via lactate phlorizin hydrolase at the brush border, accompanied by the passive diffusion of aglycone (Gee et al., 2000; Hollman et al., 1999c; Manach et al., 2005; Williamson et al., 2000). In the first position, anthocyanin glycosides could well be hydrolysed by lactate phlorizin hydrolase mostly to mucosal brush-border membrane (Gee et al., 2000; Williamson et al., 2000). The second potential absorption pathway is probable to appear through the transfer of preserved glycoside of anthocyanin into the enterocyte, probably through a sodium-glucose co-transporter, as indicated for several other flavonoids (Hollman et al., 1995a; Hollman and Katan, 1998b; Mu¨lleder et al., 2002; Williamson et al., 2000; Wolffram et al., 1995).

While inside the cell, the preserved glycoside could then immediately pass the membrane of basolateral into the portal circulation or be hydrolyzed by cytosolic β-glucosidase before the metabolism and transport process of intestinal (Day et al., 1998; Hollman, 2001; Mu¨lleder et al., 2002; Walle et al., 2000; Williamson et al., 2000). For lactate phlorizin hydrolase and cytosolic β-glucosidase, cyanidine and delphinidin glucosides are not substrates (Ne`meth et al., 2003). It remains to be shown that cyanidin glycosides are absorbed and occur as parent glycosides and also glucuronide derivatives in bloodstream, results suggest both the transport of preserved compounds and hydrolysis prior to transport (Mazza et al., 2002; Wu et al., 2002; Felgines et al., 2003a; Galvano et al., 2004; Kay et al., 2005). Felgines et al. (2009b) reported study the ratios of anthocyanin derivatives in different organs in rats fed with anthocyanin-enriched blackberry diet for a 12 days.

The bladder showed the highest concentrations of anthocyanin, accompanied by the prostate. Prostate, testes and heart stored indigenous cyanidin-3-glucoside and a low amount of cyanidin monoglucuronide. Cyanidin-3-glucoside and methylated derivatives were present in adipose tissue (Felgines et al., 2009b). Moreover, Tian et al. (2019) reported that anthocyanin-rich Purple corn stover silage with abundant anthocyanins can transfer anthocyanins to the milk and enhance the amount of antioxidants in lactating dairy goats. As a result of this experiment, it can be shown that the composition of anthocyanin is metabolized and absorbed into the mammary gland and contains in milk of lactating dairy goats.

5.6 Conclusion

The results of this study, it can be concluded that Purple Napier grass silage 100% treatment for feeding dairy goats enhance efficiency in terms of milk composition, antioxidant activity and anthocyanin composition in raw milk. There was no significant difference on milk yield by dietary treatments. The antioxidant activity in plasma, the Purple Napier grass silage 100% treatment enhance DPPH scavenging activity, TAC, SOD and GST enzyme at 2 and 4 hours after feeding. For antioxidant activity in milk, the dairy goats fed Purple Napier grass silage at 100% improve DPPH scavenging activity, TAC, antioxidant enzyme (SOD and GST) and anthocyanin composition in milk. However, it was found that the lowest concentration of MDA in plasma and milk were observed in dairy goats fed Purple Napier grass silage 100% treatment. Based on the evidence in the present study, it can be concluded that Purple Napier grass silage can be used as a source of roughage for feeding lactating dairy goats.

5.8 References

Ames, B. N., Shigenaga, M. K., and Hagen, T. M. (1995). Mitochondrial decay in aging. **Biochimica et Biophysica Acta**. 1271(1): 165-170.

AOAC. (2005). **Association of Official Analytical Chemists**. Official Methods of Analysis. 18th ed., *Association of Official Analytical Chemists*. Washington, DC, USA.

Apostolidis, E., Kwon, Y. I., and Shetty, K. (2008). Inhibition of Listeria monocytogenes by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. **International Journal of Food Microbiology**. 128(2): 317-324.

Arthur, J. R. (2000). The glutathione peroxidases. **Cellular and Molecular Life Sciences**. 57(13-14): 1825-1835.

Bendich, A. (1993). Physiological role of antioxidants in the immune system. **Journal of Dairy Science**. 76(9): 2789-2794.

Bruhn, J. C. (2006). **Dairy goat milk composition**. Colorado: Agriculture Research Service, Departement of Agricultural.

Cabiscol, E., Tamarit, J., and Ros, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. **International Microbiology**. 3(1): 3-8.

Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M. L., and Benedito, J. L. (2006). Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. **Research in Veterinary Science**. 80(2): 133-139.

Celi, P. (2011a). Oxidative stress in ruminants. In **Studies on Veterinary Medicine, Oxidative Stress in Applied Basic Research and Clinical Practice 5**. L.

Mandelker, and P. Vajdovich, ed. Humana Press/Springer Science + Business Media LLC, New York, NY, 191-231.

Celi, P., and Raadsma, H. W. (2010b). The effects of Yerba Mate (*Ilex paraguarensis*) supplementation on the productive performance of lactating dairy cows. **Animal Production Science**. 50(6): 339-344.

Day, A. J., DuPont, M. S., Ridley, S., Rhodes, M., Rhodes, M. J., Morgan, M. R., and Williamson, G. (1998). Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver betaglucosidase activity. **FEBS Letters**. 436(1): 71-75.

Di Trana, A., Celi, P., Claps, S., Fedele, V., and Rubino, R. (2006). The effect of hot season and nutrition on the oxidative status and metabolic profile in dairy goats during mid lactation. **Animal Science Journal**. 82(5): 717-722.

Fee, J., Bergamini, R., and Briggs, R. (1975). Observation on the mechanism of the oxygen dialuric acid induced hemolysis of vitamin E-deficient rat blood cells and the protective roles of catalase and superoxide dismutase. **Archives of Biochemistry and Biophysics**. 169(1): 160-167.

Felgines, C., Talavera, S., Gonthier, M. P., Texier, O., Scalbert, A., Lamaison, J. L., and Remesy, C. (2003a). Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. **Journal of Nutrition**. 133(5): 1296-1301.

Felgines, C., Texier, O., Garcin, P., Besson, C., Lamaison J. L., and Scalbert, A. (2009b). Tissue distribution of anthocyanins in rats fed a blackberry anthocyanin-enriched diet. **Molecular Nutrition and Food Research**. 53(9): 1098-1103.

Galvano, F., La Fauci, L., Lazzarino, G., Fogliano, V., Ritieni, A., Ciappellano, S., Battistini, N. C., Tavazzi, B., and Galvano, G. (2004). Cyanidins: metabolism and biological properties. **Journal of Nutritional Biochemistry**. 15(1): 2-11.

Gardete, S., and Tomasz, A. (2014). Mechanisms of vancomycin resistance in *Staphylococcus aureus*. **Journal of Clinical Investigation**. 124(7): 2836-2840.

Gee, J. M., DuPont, M. S., Day, A. J., Plumb, G. W., Williamson, G. and Johnson, I. T. (2000). Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. **Journal of Nutrition**. 130(11): 2765-2771.

Giusti, M. M., Ghanadan, H., and Wrolstad, R. E. (1998). Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins using one- and two-dimensional nuclear magnetic resonance techniques. **Journal of Agricultural and Food Chemistry**. 46(12): 4858-4863.

Gough, D. R., and Cotter, T. G. (2011). Hydrogen peroxide: a Jekyll and Hyde signaling molecule. **Cell Death and Disease**. 2(10): e213.

Guo, M., Perez, C., Wei, Y., Rapoza, E., Su, G., Bou-Abdallah, F., and Chasteen, N. (2007). Iron-binding properties of plant phenolics and cranberry's bio-effects. **Dalton Transactions**. 43: 4951-4961.

Haenlein, G. F. W. (2002). Composition of goat milk and factors affecting It. In: **Feeding Goats for Improved Milk and Meat Production (G.F.W. Haenlein, ed.)**. Department of Animal and Food Science University of Delaware. USA.

Hamzaoui, S., Salama, A. A. K., Albanell, E., Such, X., and Caja, G. (2013). Physiological responses and lactational performances of late-lactation dairy

goats under heat stress conditions. **Journal of Dairy Science**. 96(10): 6355-6365.

Harvatine, K. J., and Allen, M. S. (2005). The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. **Journal of Dairy Science**. 88(11): 4018-4027.

Hellingwerf, K. J., and Konings, W. N. (1985). The energy flow in bacteria: the main free energy intermediates and their regulatory role. **Advances in Microbial Physiology**. 26: 125-154.

Hollman, P. C. H. (2001) Evidence for health benefits of plant phenols: local or systemic effects?. **Journal of the Science of Food and Agriculture**. 81(9): 842-852.

Hollman, P. C., and Katan, M. B. (1998b). Bioavailability and health effects of dietary flavonols in man. **Archives of Toxicology Supplement**. 20: 237-248.

Hollman, P. C., and Katan, M. B. (1999c). Health effects and bioavailability of dietary flavonols. **Free Radical Research**. 31(Suppl): S75-S80.

Hollman, P. C., de Vries, J. H., van Leeuwen, S. D., Mengelers, M. J., and Katan, M. B. (1995a). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. **American Journal of Clinical Nutrition**. 62(6): 1276-1282.

Hosoda, K., Eruden, B., Matsuyama, H., and Shioya, S. (2009). Silage fermentative quality and characteristics of anthocyanin stability in anthocyanin-rich corn (*Zea mays* L.). ***Asian-Australasian Journal of Animal Sciences***. 22(4): 528-533.

Jones, D. P., and DeLong, M. J. (2000). Detoxification and Protective Functions of Nutrients. In: **Biochemical & Physiological Aspects of Human Nutrition**. (Stipanuk, MH, ed.) St. Louis: W.B. Saunders Company. p. 901-916.

Kähkönen, M. P., and Heinonen, M. (2003). Antioxidant activity of anthocyanins and their aglycons. **Journal of Agricultural and Food Chemistry**. 51(3): 628-633.

Kay, C. D., Mazza, G., and Holub, B. J. (2005). Anthocyanins exist in the circulation primarily as metabolites in adult men. **Journal of Nutrition**. 135(11): 2582-2588.

Kwon, Y. I., Apostolidis, E., Labbe, R. G., and Shetty, K. (2007). Inhibition of *Staphylococcus aureus* by phenolic phytochemicals of selected clonal herbs species of *lamiaceae* family and likely mode of action through proline oxidation. **Food Biotechnology**. 21(1): 71-89.

Lacombe, A., Tadepalli, S., Hwang, C. A., and Wu, V. C. (2013). Phytochemicals in low bush wild blueberry inactivate *Escherichia coli* O157:H7 by damaging its cell membrane. **Food bourne Pathogens & Disease**. 10(11): 944-950.

Luciano, G., Vasta, V., Monahan, F. J., Lòpez-Andréz, P., Biondi, L., Lanza, M., and Priolo, A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet. **Food Chemistry**. 124(3): 1036-1042.

Makkar, H. P. S., Francis, G., and Becker, K. (2007). Bioactivity of phytochemicals in some lesser known plants and their effects and potential applications in livestock and aquaculture production systems. **Animal**. 1(9): 1371-1391.

Manach, C., Williamson, G., Morand, C., Scalbert, A., and Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. **American Journal of Clinical Nutrition**. 81(1 Suppl): S230-S242.

Mazza, G., Kay, C. D., Cottrell, T., and Holub, B. J. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. **Journal of Agricultural and Food Chemistry**. 50(26): 7731-7737.

Miller, J. K., Brzezinska-Slebodzinska, E., and Madsen, F. C. (1993). Oxidative stress, antioxidants, and animal function. **Journal of Dairy Science**. 76(9): 2812-2823.

Mu¨lleder, U., Murkovic, M., and Pfannhauser, W. (2002). Urinary excretion of cyanidin glycosides. **Journal of Biochemical and Biophysical Methods**. 53(1-3): 61-66.

Ne`meth, K., Plumb, G. W., Berrin, J. G., Juge, N., Jacob, R., Naim, H. Y., Williamson, G., Swallow, D. M., and Kroon, P. A. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. **European Journal of Nutrition**. 42(1): 29-42.

Nguyen, T., Nioi, P., and Pickett, C. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. **Journal of Biological Chemistry**. 284(20): 13291-13295.

Nijveldt, R. J., van Nood, E., van Hoorn, D. E., Boelens, P. G., van Norren, K., and van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. **The American Journal of Clinical Nutrition**. 74(4): 418-425.

NRC. (1981). **Nutrient requirements of goats: Angora, dairy, and meat goats in temperate and tropical countries**. Natl. Acad. Press, Washington, DC.

Pal, U. K., Saxena, V. K., Agnihottri, M. K., and Roy, R. (1996). Effect of season, parity and stage of lactation on the composition of Jamunapari goats milk. **International Journal of Animal Science**. 11(1): 245-248.

Passamonti, S., Vrhovsek, U., Vanzo, A., and Mattivi, F. (2003). The stomach as a site for anthocyanins absorption from food. **FEBS Letters**. 544(1-3): 210-213.

Pekkarinen, S. S., Heinonen, I. M., and Hopia, A. I. (1999). Flavonoids quercetin, myricetin, kaempferol and (+)-catechin as antioxidants in methyl linoleate. **Journal of the Science of Food and Agriculture**. 79(4): 499-506.

Ramos, C. G., Sousa, S. A., Grilo, A. M., Feliciano, J. R., and Leitão, J. H. (2014). Retraction for the second RNA chaperone, Hfq2, is also required for survival under stress and full virulence of *Burkholderia cenocepacia* J2315. **Journal of Bacteriology**. 196(22): 3980.

Rigout, S., Hurtaud, C., Lemosquet, S., Bach, A., and Rulquin, H. (2003). Lactational effect of propionic acid and duodenal glucose in cows. **Journal of Dairy Science**. 86(1): 243-253.

SAS. (1990). **SAS User's guide: statistics**. Version 6. 14th ed. Carry, NC: SAS Inst.

Seeram, N. P., Lee, R., Scheuller, H. S., and Heber, D. (2006). Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. **Food Chemistry.** 97(1): 1-11.

Shih, P. H., Yeh, C. T., and Yen, G. C. (2007). Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. **Journal of Agriculture and Food Chemistry**. 55(23): 9427-9435.

Singh, S. N., and Sengar, O. P. S. (1990). Studies on the combining ability of desirable characters of important goat breeds. **Final Technical Report**. RBS College Bichpuri, Agra, India. 1-480.

Singh, S. P., Konwarh, R., Konwar, B. K., and Karak, N. (2013a). Molecular docking studies on analogues of quercetin with D-alanine:D-alanine ligase of *Helicobacter pylori*. **Medicinal Chemistry Research**. 22(5): 2139-2150.

Steel, R. G. D., and Torrie, J. N. (1980). **Principles and Procedures of Statistics**. 2nd ed. McGraw-Hill. Book C, New York.

Szajdek, A., and Borowska, E. J. (2008). Bioactive compounds and health-promoting properties of berry fruits: a review. **Plant Foods for Human Nutrition**. 63(4): 147-156.

Tadapaneni, R. K., Banaszewski, K., Patazca, E., Edirisinghe, I., Cappozzo, J., Jackson, L., and Burton-Freeman, B. (2012). Effect of high-pressure processing and milk on the anthocyanin composition and antioxidant capacity of strawberry-based beverages. **Journal of Agricultural and Food Chemistry**. 60(23): 5795-5802.

Tian, X. Z., Paengkoum, P., Paengkoum, S., Chumpawadee, S., Ban, C., and Thongpea, S. (2019). Short communication: Purple corn (*Zea mays* L.) stover silage with abundant anthocyanins transferring anthocyanin composition to the milk and increasing antioxidant status of lactating dairy goats. **Journal of Dairy Science**. 102(1): 413-418.

Torskangerpoll, K., Noerbaek, R., Nodland, E., Oevstedal, D. O., and Andersen, Ø. M. (2005). Anthocyanin content of Tulipa species and cultivars and its impact on tepal colours. **Biochemical Systematics and Ecology**. 33(5): 499-510.

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

Virtanen, A.I. (1933). The AIV method of preserving fresh fodder. **Empire Journal of Experimental Agriculture**. 1: 143-155.

Walle, T., Otake, Y., Walle, U. K., and Wilson, F. A. (2000). Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. **Journal of Nutrition**. 130(11): 2658-2661.

Wang, H., Cao, G., and Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. **Journal of Agricultural and Food Chemistry**. 45(2): 304-309.

Wang, L. S., and Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. **Cancer Letters**. 269(2): 281-290.

Wei, J. T., and Chiang, B. H. (2009). Bioactive peptide production by hydrolysis of porcine blood proteins in a continuous enzymatic membrane reactor. **Journal of the Science of Food and Agriculture**. 89(3): 372-378.

Williamson, G., Day, A. J., Plumb, G. W., and Couteau, D. (2000). Human metabolic pathways of dietary flavonoids and cinnamates. **Biochemical Society Transaction**. 28(2): 16-22.

Wolffram, S., Weber, T., Grenacher, B., and Scharrer, E. A. (1995). Na(+)-dependent mechanism is involved in mucosal uptake of cinnamic acid across the jejunal brush border in rats. **Journal of Nutrition**. 125(5): 1300-1308.

Wrolstad, R. E., Dursta, R. W., and Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. **Trends in Food Science and Technology**. 16(9): 423-428.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Wu, D., Kong, Y., Han, C., Chen, J., Hu, L., Jiang, H., and Shen, X. (2008). D-Alanine: D-alanine ligase as a new target for the flavonoids quercetin and apigenin. **International Journal of Antimicrobial Agents**. 32(5): 421-426.

Wu, X., Cao, G., and Prior, R. L. (2002). Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. **Journal of Nutrition**. 132(7): 1865-1871.

Zarban, A., Taheri, F., Chahkandi, T., Sharifzadeh, G., and Khorashadizadeh, M. (2009). Antioxidant and radical scavenging activity of human colostrum, transitional and mature milk. **Journal of Clinical Biochemistry and Nutrition**. 45(2): 150-154.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER VI

SUMMARY

6.1 Conclusions

The main objective of this experiment was to study the effect of anthocyanin from Purple Napier grass (*Pennisetum purpureum* "Prince") on rumen fermentation, milk yield, milk composition and blood antioxidant activity in dairy goats.

Experiment 1 was focused on the effect of cultivars, plant spacing and harvesting age of Napier grass on forage yield, morphological characteristics, chemical composition, and anthocyanin composition, Purple Napier grass was greater than Napier Pakchong-1 grass in terms of number of tillers per plant, leafiness (high LSR), high CP content and anthocyanin composition, but Napier Pakchong-1 grass had higher DM yield, plant height and chemical composition such as NDF, ADF, CF, cellulose and lignin content with developed forage maturity. The wider plant spacing of Napier grass had a higher yield of DM, number of tillers per plant, LSR, chemical and anthocyanin composition, while a plant height value reduced with developed maturity. Longer harvesting age had reduced quality, particularly decreased CP content and increased DM yield, NDF and ADF content at advanced maturity.

The Purple Napier grass planted 75×75 cm with harvesting day at 45 days would contain proper number tillers per plant, LSR value, chemical composition for ruminants and highest anthocyanin composition.

Experiment 2 was to investigate the effect of fresh and silage of Napier grass

on *in vitro* gas production, growth performance, rumen fermentation and microbial population in goat's rumen, the fresh Purple Napier grass in goat diets improve efficiency in terms of a high *in vitro* gas production parameters, *in vitro* digestibility, ruminal NH_3-N, ruminal propionic acid (C_3), total VFA, growth performance, nitrogen balance, blood urea nitrogen (BUN) and ruminal *Butyrivibrio fibrisolvens*, respectively. However, it was found that the ruminal acetic acid (C_2) and cellulolytic bacteria population of the goats in fresh Purple Napier grass cultivars treatment were lower than fresh Napier Pakchong-1 treatment, and also had lowest of ruminal methane gas production (CH_4), methanogen and protozoa population, respectively. Moreover, the ruminal pH value and total microbial population were not significantly different by dietary treatments. Based on the information in the current study, it can be concluded that fresh Purple Napier grass can be used as a source of roughage for feeding goats.

Experiment 3 was to investigate the effect of anthocyanin from Purple Napier grass silage on milk yield, milk composition and blood antioxidant activity in lactating dairy goats, the Purple Napier grass silage 100% treatment for feeding dairy goats enhance efficiency in terms of milk composition, blood antioxidant activity and anthocyanin composition in raw milk.

The antioxidant activity in plasma of lactating dairy goats, Purple Napier grass silage 100% enhance DPPH scavenging activity, TAC, SOD and GST enzyme at 2 and 4 hours after feeding. For antioxidant activity in milk of lactating dairy goats, Purple Napier grass silage at 100% improve DPPH scavenging activity, TAC, antioxidant enzyme (SOD and GST) and anthocyanin composition. However, it was found that the lowest concentration of MDA in plasma at 2 and 4 hours after feeding and raw milk

were observed in dairy goats fed Purple Napier grass silage 100% treatment. Based on the evidence in the present study, it can be concluded that Purple Napier grass silage can be used as a source of roughage for feeding lactating dairy goats.

6.2 Implications

Although, the fresh Purple Napier grass was the best choice in experiment 2 but grass silage condition was chosen in experiment 3. Because of the actual situation of goat farming, a farmer was inconvenient to cut the fresh grass for feeding lactating dairy goats in the morning every day. The fresh grass cutting for feeding goats requires farm labor and time, and the grass silage condition has many benefits such as grass silage can be made when weather is not favorable and utilize every part of the fresh grass for ensiling process, grass silage saves storage space, grass silage convenient to carry and reduces risk of fire and grass silage can be stored long time as source of roughage for feeding animal.

From these benefits of the grass silage, there is a possibility of success in the actual situation of goat farming for feeding goats, which is the reason why grass silage condition was chosen in experiment 3. In this study, the metabolism of anthocyanins in ruminants is also unclear, and Purple Napier grass contains not only anthocyanin compositions, but also other natural antioxidants, as well as there is no antioxidant gene expression result to confirm an increasing level of antioxidant enzyme in plasma and milk.

Further studies should be conducted to investigate the bioavailability, metabolism, excretion pathways of degradation and antioxidant gene expression of anthocyanins from Purple Napier grass in ruminants

APPENDICES

มหาวิทยาลัยเทคโนโลยีสุรนารี

APPENDIX A

Rainfall data of the experimental area

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table A1 Rainfall data of the experimental area.

Items	King's 80th birthday Station		Khok Kruat Station	
	2016	**2017**	**2016**	**2017**
Rainfall (mm)				
January	0.0	0.0	0.0	0.0
February	36.0	2.4	29.6	9.7
March	3.7	7.8	3.2	14.9
April	24.1	158.4	10.0	82.3
May	361.8	138.8	268.7	152.9
June	176.0	58.3	178.9	32.1
July	110.5	157.5	107.7	136.3
August	273.2	100.7	161.7	70.7
September	335.3	178.9	178.3	127.8
October	150.0	138.2	192.9	133.4
November	55.5	8.3	66.2	8.3
December	19.5	1.0	10.3	0.0
Total	1,545.6	950.3	1,207.5	768.4
Average	128.8	79.2	100.6	64.0
Day of rainfall (day/year)	84.0	89.0	90.0	88.0

Source: Lower Northeastern Region Hydrological lrrigation Center-RID, Nakhon Ratchasima, Thailand.

มหาวิทยาลัยเทคโนโลยีสุรนารี

APPENDIX B

Anthocyanin extraction

มหาวิทยาลัยเทคโนโลยีสุรนารี

B1 Acetone extraction and chloroform partition of anthocyanins

Materials

1) Powdered plant material

2) Acetone 70% (v/v) aqueous acetone or aqueous acidified acetone: 70% aqueous acetone

3) Chloroform

4) Acidified water: 0.01% (v/v) HC1 in deionized, distilled water

5) Waring Blender with stainless steel container (Waring) or general-purpose homogenizer

6) Whatman no. 1 filter paper

7) Buchner funnel

8) Separatory funnel

9) 500-ml boiling flask

10) Rotary evaporator with vacuum pump or water aspirator, 40^{o}C

Extraction

1) Mix 50 g powdered plant material (accurately weighed and recorded) 1:1(w/v) with acetone using a Waring Blender with stainless steel container or a general-purpose homogenizer.

2) Separate the anthocyanin extract (filtrate) from insoluble plant material by filtering the slurry through a Whatman no. 1 filter paper by vacuum suction using a Buchner funnel.

3) Reextract plant material with 70% (v/v) aqueous acetone until a clear or faintly colored solution is obtained. If plant material has a pH 2-4, use aqueous acidified acetone. Pool filtrates and discard plant material.

4) Transfer filtrate to a separatory funnel, add 2 vol chloroform, and gently mix by turning funnel upside down a few times. Store sample overnight at 4°C or until a clear partition between the two phases is obtained.

5) Transfer the aqueous phase (upper portion) to a 500-ml boiling flask. Remove residual acetone/chloroform in a rotary evaporator at 40°C under vacuum.

6) Make up remaining aqueous extract to a known volume (usually 100 ml) with acidified deionized distilled water. If the sample is to be analyzed within 2 days, store extract at 4°C. For longer periods (up to 1 year or even longer), store at -18°C. Avoid repeated freezing and thawing.

B2 Methanol extraction of anthocyanins

Additional Materials (also see materials of acetone extraction) Acidified methanol: 0.01% (v/v) HCl in methanol.

Extraction

1) Homogenize 50 g powdered plant material (accurately weighed and recorded) in 2 vol (w/v) acidified methanol. Allow it to macerate 1 hours.

2) Filter slurry through a Whatman no. 1 filter paper by vacuum suction using a Buchner funnel.

3) Reextract plant material with acidified methanol until a faint-colored extract is obtained. Pool filtrates and discard plant material.

4) Transfer filtrates to a boiling flask and evaporate methanol in a rotary evaporator at 40°C under vacuum.

5) Make up remaining aqueous extract to a known volume with acidified deionized distilled water, water, methanol, or other appropriate solvent. If the sample

is to be analyzed within 2 days, store extract at 40°C. For longer periods (up to 1 year or even longer), store at -18°C. Avoid repeated freezing and thawing.

B3 Sample preparation

Materials

1) Liquid nitrogen

2) Plant material

3) Waring Blender with stainless steel container (Waring).

4) Teflon or stainless steel container (or equivalent container able to withstand -210°C

5) Freeze-resistant container, high-density polyethylene (HDPE) or equivalent

Preparation

1) Pour liquid nitrogen into a dry stainless steel Waring Blender container and allow to evaporate. If operating a blender, turn on for few seconds. Repeat this procedure until container is well chilled.

2) Freeze 50 g plant material with liquid nitrogen in a separate container of Teflon, stainless steel, or other material that can withstand temperatures of -210°C.

3) Blend frozen plant material using the chilled container.

4) Open blender or mill, add liquid nitrogen, allow to evaporate, and repeat grinding process until a fine powder is obtained.

5) Transfer powdered material to a freeze-resistant container for analysis, or store it immediately at -20°C.

B4 Anthocyanin purification

Materials

1) Methanol

2) Acidified water: 0.01%(v/v) HCl in deionized, distilled water

3) Aqueous anthocyanin extract

4) Ethyl acetate

5) Acidified methanol: 0.01% (v/v) HC1 in methanol

6) C_{18} cartridge (with C_{18} sorbent bonded on silica: C_{18} Sep-Pak Cartidge (360 mg sorbent), Waters Chromotography; ODS-4 Octadecyl Silane (500 mg sorbent), Whatman; or equivalent)

7) 50- to 100-ml boiling flask

8) Rotary evaporator with vacuum pump or water aspirator, 40°C

9) Freeze-resistant container (optional)

Purification

1) Condition a C_{18} cartridge by passing two column volumes methanol through the sorbent bed.

2) Pass three column volumes acidified deionized distilled water through cartridge to remove remaining methanol.

3) Force an aqueous anthocyanin extract through cartridge.

4) Wash cartridge with two column volumes acidified water to remove compounds not adsorbed (e.g., sugars, acids).

5) Wash cartridge with two column volumes ethyl acetate to remove polyphenolic compounds such as phenolic acids and flavonols.

6) Elute anthocyanin pigments with acidified methanol and collect in a 50 to 100-ml boiling flask.

7) Remove methanol in a rotary evaporator at 40°C under vacuum.

8) Redissolve pigments in acidified deionized distilled water or an appropriate HPLC mobile-phase solvent.

9) Store purified anthocyanin extract at 4°C if subsequent analysis will be performed within 24 hours. Store sample for longer periods at -15°C or lower (preferably at -70°C) in a freeze-resistant container to minimize pigment degradation.

มหาวิทยาลัยเทคโนโลยีสุรนารี

APPENDIX C

The primer of reference sequence for real-time PCR

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table C1 Rumen microbial primer sets used for real-time PCR assay.

Target group	Product size (bp)	Sequence (5'-3')	References
Total bacteria	130	F: CGGCAACGAGCGCAACCC	Koike et al., (2001)
		R: CCATTGTAGCACGTGTGTAGCC	
Methanogen	140	F: TTCGGTGGATCDCARAGRGC	Denman et al., (2007)
		R: GBARGTCGWAWCCGTAGAATC	
Protozoa	223	F: CTTGCCCCTCYAATCGTWCT	Sylvester et al., (2004)
		R: GCTTTCGWTGGTAGTGTATT	
Butyrivibrio fibrisolvens	64	F: ACACACCGCCCGTCACA	Klieve et al., (2003)
		R: TCCTTACGGTTGGGTCACAGA	
Fibrobacter succinogenes	446	F: GGTATGGGATGAGCTTGC	Koike et al., (2001)
		R: GCCTGCCCCTGAACTATC	
Ruminococcus flavefaciens	295	F: TCTGGAAACGGATGGTA	Koike et al., (2001)
		R: CCTTTAAGACAGGAGTTTACAA	

BIOGRAPHY

Mr. Narawich Onjai-uea was born on December 22, 1986 in Ratchaburi Province, Thailand. In 2005, he graduated high school level from Benjamarachutit Ratchaburi School. In 2009, he obtained his Bachelor's degree in Animal Sciences and Agricultural Technology from Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi Province. In 2013, he graduated his a Master of Science (Animal Science) with a major in reproductive physiology from the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok. He studied in a field of ruminant nutrition for Ph.D. Program at the School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima Province with the thesis entitled "The effect of anthocyanin from Purple Napier grass (*Pennisetum purpureum* "Prince") on rumen fermentation, milk yield, milk composition and blood antioxidant activity in dairy goats" which supported financial from Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program (Grant no. PHD/0085/2557 to Narawich Onjai-uea and Assoc. Prof. Dr. Pramote Paengkoum).