UTILIZATION OF ANTHOCYANIN RICH NAPIER GRASS SILAGE ON GROWING GOAT DIETS

NGO THI MINH SUONG

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 2017

การใช้หญ้าเนเปียร์สีม่วงหมักเป็นแหล่งแอนโทไซยานินในอาหารแพะระยะกำลังเจริญเติบโต

นางสาวโงว ถิ มิน ซึง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2560

UTILIZATION OF ANTHOCYANIN RICH NAPIER GRASS SILAGE ON GROWING GOAT DIETS

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 Paengkoum

(Assoc. Prof. Dr. Pramote Paengkoum)

Member (Thesis Advisor)

Siwaporn Paenglerun

(Dr. Siwaporn Paengkoum)

Member

(Assoc. Prof. Dr. Nguyen Nhut Xuan Dung)

Member

P. Loonglawan

(Asst. Prof. Dr. Pipat Lounglawan)

Member

(Assoc. Prof. Dr. Amorat Molee)

Member

(Prof. Dr. Santi Maensiri)
Vice Rector for Academic Affairs
and Internationalization

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

นางสาวโงว ถิ มิน ซึง : การใช้หญ้าเนเปียร์สีม่วงหมักเป็นแหล่งแอนโทไซยานินในอาหารแพะระยะกำลังเจริญเติบโต (UTILLIZATION OF ANTHOCYANIN RICH NAPIER GRASS SILAGE ON GROWING GOAT DIETS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. ปราโมทย์ แพงคำ, 139 หน้า.

วัตถุประสงค์ของการศึกษาครั้งนี้คือ (1) เพื่อประเมินผลกระทบของกากน้ำตาลและไอรอนซัลเฟต ($FeSO_4$) ต่อคุณภาพของหญ้าหมัก องค์ประกอบของโภชนะ และกระบวนการหมักในหลอดทดลอง ความสามารถในการย่อยสลายของแอนโทไซยานินของหญ้าเนเปียร์หมักที่มีสารแอนโทไซยานิน (2) เพื่อศึกษาผลของหญ้าเนเปียร์ที่อุดมไปด้วยแอนโทไซยานินที่มีเปอร์เซ็นต์ของกากน้ำตาล และ $FeSO_4$ ที่เหมาะสมต่อสมรรถนะการเจริญเติบโต, จุลินทรีย์ในกระเพาะหมัก และการย่อยได้ของโภชนะของแพะในระยะกำลังเจริญเติบโต และ (3) เพื่อตรวจสอบผลของหญ้าเนปียร์หมักที่อุดมไปด้วยแอนโทไซยานิน ที่ได้รับกากน้ำตาลและ $FeSO_4$ ต่อการต้านอนุมูลอิสระ และกิจกรรมของเอนไซม์ในการต้านอนุมูลอิสระในพลาสมาของแพะในระยะกำลังเจริญเติบโต และแพะที่ติดพยาธิ *Haemonchus contortus* ตามธรรมชาติ ในการศึกษาครั้งนี้มีการทดลองทั้งหมด 3 การทดลอง

การทดลองที่ 1 ศึกษาในหลอดทดลอง พบว่าผลของการเติมกากน้ำตาล และ $FeSO_4$ ในปริมาณที่แตกต่างกันของหญ้าเนเปียร์หมักที่อุดมไปด้วยแอนโทไซยานิน หลังจากหมัก และเก็บรักษา 21 วัน การทดลองใช้การออกแบบการทดลอง แบบแฟคทอเรียลที่สุ่มแบบ (CRD) 3x3 (ปัจจัย A คือกากน้ำตาล (M) และปัจจัย B คือ $FeSO_4$ (Fe)) นอกจากนี้ กากน้ำตาล และ $FeSO_4$ ยังส่งผลต่อค่า pH lactate butyrate ammonia-N DM CP EE NDF ADF สารประกอบฟีโนลิก คอนเดนซ์แทนนิน ค่า DPPH และปริมาณแอนโทไซยานิน มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) การหมักด้วยกากน้ำตาล 4% และไอรอนซัลเฟต 0.03% พบว่ามีค่าแอนโทไซยานินที่ดีที่สุด คุณภาพการหมักและองค์ประกอบสารอาหารเมื่อเทียบกับการหมักในรูปแบบอื่นๆ ในส่วนของวิธีการทดลองแบบประเมินการผลิตแก๊ส (gas production) พบว่าหญ้าเนเปียร์หมักที่มีสารแอนโทไซยานินกับสารเสริม มีปริมาณกรดโพรพิโอนิก กรดไขมันที่ระเหยได้รวม และ *Streptococcus bovis* เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) แต่พบว่าค่า pH อัตราส่วนกรดอะซิติกต่อโพรพิโอนิก (C2/C/3) แบคทีเรียกลุ่มที่ผลิตมีเทนมีค่าลดลง และแอนโทไซยานินในกระเพาะรูเมน มีความเสถียรหลังจากบ่มที่ 24 ชั่วโมง

การทดลองครั้งที่ 2 ใช้ชุดการทดลองแบบการสุ่มแบบสมบูรณ์ (CRD) โดยใช้ทรีตเมนต์ 3 ชนิด ได้แก่ หญ้าเนเปียร์หมัก (T1) หญ้าเนเปียร์หมักที่มีสารแอนโทไซยานินโดยไม่มีสารเสริม (T2) และเสริมกากน้ำตาล 4%+$FeSO_4$ 0.03% (T3) ใช้แพะเนื้อลูกผสมพันธุ์แองโกลนูเบียน 18 ตัว (น้ำหนักเฉลี่ย 14.4±0.6 กิโลกรัม) แบ่งออกเป็น 6 กลุ่ม ใช้แพะกลุ่มละ 6 ตัว แพะแต่ละตัวจะได้รับ

อาหารทดลอง ผลการทดลองพบว่าการเพิ่มขึ้นของโปรตีน ADF ปริมาณการกินได้ของแอนโทไซยานิน สัดส่วนโพรพิโอเนต อัตราส่วน C2/C3 กรดไขมันระเหยได้รวม จำนวนแบคทีเรีย *S. bovis* การย่อยได้ของไนโตรเจน การกักเก็บไนโตรเจน การเพิ่มขึ้นของน้ำหนักตัวเฉลี่ยต่อวัน และน้ำหนักตัวของแพะกลุ่มที่ได้รับอาหารในทรีตเมนต์ที่ 3

การทดลองที่ 3 ใช้อาหารสูตร 3 ชนิดเดียวกันกับการทดลองที่สอง ใช้แพะเนื้อลูกผสมพันธุ์พื้นเมืองและแองโกลนูเบียน จำนวน 18 ตัว (น้ำหนักเฉลี่ย 23.8±2.4 กิโลกรัม) สุ่มเลือกเพื่อที่จะให้อาหารทดลอง ผลการทดลองคล้ายกับการทดลองที่สอง แพะที่ได้รับอาหารในทรีตเมนต์ที่ 3 พบว่าลดกิจกรรมการทำงานของ non-enzymatic (Total Antioxidant Capacity, TAC) เอนไซม์ Superoxide Dismutase (SOD) และการเกิด lipid peroxidation ในพลาสมาของแพะระยะกำลังเจริญเติบโต และแพะที่ติดพยาธิ *H. contortus* ตามธรรมชาติ แต่สามารถส่งเสริมกิจกรรมการทำงานของแคตตาเลส (CAT) กลูตาไธโอน (Glutathione, GSH) และกลูตาไธโอนเอสทรานเฟอเรส (Glutathione -S-transferase, GST) นอกจากนี้การทดลองที่ 3 พบว่ามีค่า เม็ดเลือดแดง และค่าฮีมา-โตคริท (Hematocrit) สูง และมีค่าเม็ดเลือดขาวต่ำ อย่างไรก็ตาม จำนวนไข่พยาธิลดลงในแพะที่ได้รับหญ้าเนเปียร์หมักที่มีสารแอนโทไซยานินกับการเติมกากน้ำตาล และ $FeSO_4$ ร่วมด้วย ผลการทดลองแสดงให้เห็นว่าสามารถใช้หญ้าเนเปียร์หมักที่มีส่วนผสมของแอนโทไซยานินร่วมกับสารเติมเสริมในอาหาร สามารถปรับปรุงสมรรถภาพการผลิตและลดความเครียดของแพะได้

สาขาวิชาเทคโนโลยีการผลิตสัตว์
ปีการศึกษา 2560

ลายมือชื่อนักศึกษา _____
ลายมือชื่ออาจารย์ที่ปรึกษา _____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

NGO THI MINH SUONG : UTILLIZATION OF ANTHOCYANIN RICH NAPIER GRASS SILAGE ON GROWING GOAT DIETS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 139 PP.

ANTHOCYANIN/SILAGE/OXIDATIVE STRESS

The objectives of this study were (1) to evaluate the effect of molasses and $FeSO_4$ on silage fermentation nutrient components *in vitro* ruminal fermentation anthocyanin degradability of anthocyanin-rich Napier grass silage (2) to study the effect of anthocyanin-rich Napier grass silage with the suitable percentage of molasses and $FeSO_4$ on growth performance rumen fermentation microorganism and nutrient digestibility of growing goat and (3) to examine the effect of Anthocyanin rich Napier grass silage treated with molasses and $FeSO_4$ on oxidative status and antioxidant enzyme activities in plasma of the growing goat and naturally *Haemonchus contortus* infected goat. Three experiments were conducted in this study. The first experiment *in vitro* observed the effect of the addition of different levels of molasses and $FeSO_4$ on anthocyanin-rich Napier grass silage (ARNGS) after ensiling twenty-one days. The experiment used a 3 x 3 Factorial design in Completely Randomized Design (factor A is molasses (M) and factor B is $FeSO_4$ (Fe)). Additionally, M and F affected the pH, lactate, butyrate, ammonia-nitrogen, dry matter, crude protein (CP), ether extract, neutral detergent fiber, acid detergent fiber (ADF), total phenolic compound, condensed tannin, DPPH value and anthocyanin content ($P<0.05$). Treatment with 4% M and 0.03% F (additive) showed the best value of anthocyanin content, silage fermentation quality and nutrient composition as compared to other treatments. In the gas production technique, ARNGS with additive showed a significant

increase in gas volume, propionic acid (C3), total volatile fatty acids (TVFAs), and the number of *Streptococcus bovis* but a decrease in pH value, acetic acid, acetate/propionate (C2/C3) ratio, Methanogen bacteria. Anthocyanin was stable in the rumen fluid after 24 hours of incubation. The second experiment used a Completely Randomized Design with three treatments including Napier grass silage (T1) anthocyanin-rich Napier grass silage without additive (T2) and with additive M4%-F0.03% (T3). Eighteen crossbred Thai native x Anglo-Nubian meat goats (average BW 14.42±0.6 kg) were divided into three groups of six goats each to receive the experimental diet. The results showed a significant ($P<0.05$) increase in CP, ADF, anthocyanin intake, C3 proportion, C2/C3 ratio, TVFAs, number of *S. bovis* bacteria, nitrogen digestibility, nitrogen retention, average daily gain, and body weight of the goats fed treatment T3. The third experiment used three similar diets to the second experiment on eighteen crossbred Thai native x Anglo-Nubian goats (average BW 23.78±2.4kg) randomly selected to feed on one of treatment diets. Similar results were found in the second and third experiments: treatment T3 reduced activity of total antioxidant capacity, superoxide dismutase enzyme and lipid peroxidation in the plasma of growing goats and the naturally *H. contortus* infected goats but enhanced the activity of catalase, glutathione, and glutathione-s-transferase antioxidant enzymes. Additionally, the third experiment found a high value of red blood cell and hematocrit and a low value of white blood cell; however, the fecal egg count was reduced in the goats that received the treatment ARNGS with additive.

School of Animal Production Technology Student's Signature _____

Academic Year 2017 Advisor's Signature _____

Co-advisor's Signature _____

ACKNOWLEDGEMENTS

I am deeply grateful to my advisor, Associate Professor Dr. Pramote Paengkoum, School of Animal Production Technology, 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-advisors, 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 Associate Professor Dr. Nguyen Nhut Xuan Dung and Associate Professor Dr. Luu Huu Manh, Associate Professor Do Vo Anh Khoa, and Associate Professor Nguyen Thi Kim Khang, Canho University 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 Thansamay Phorlavin, May Phonvisay, Tian Xingzhou, Nittiya Taethaisong, Ban Chao, Anan Petlum, Bhutharit Vittayaphattananurak Raksasiri, Krung Wilachai, Kanokwan Kamkajon, Narawich Onjai-uea, Sorasak Thongpea, Rayudika Aprilia Patindra Purba and Jiravan Khotsakdee) and I would like to thank Master degree student and PhD student of

School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of technology.

Finally, I would like to express my deepest gratitude to my father, my mother, my brother, my boyfriend and my aunt for their understanding, moral support and permission for me to study in SUT. 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.

Ngo Thi Minh Suong

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
ADG	=	Average daily gain
ADL	=	Acid detergent lignin
ANCY	=	Anthocyanin
AOAC	=	Association of Official Analytical Chemists
BW	=	Body weight
CAT	=	Catalase
CP	=	Crude protein
D	=	Day
DM	=	Dry matter
DMI	=	Dry matter intake
EE	=	Either extract
FAO	=	Food and Agriculture Organization
FEC	=	fecal egg count
FeSO4	=	Iron (II) sulfate
g	=	gram
GC	=	Gas chromatography
GE	=	Gross energy
GLM	=	General Linear Model
GSH	=	Glutathione

LIST OF ABBREVIATIONS (Continued)

GSH	=	Glutathione
GST	=	Glutathione-s-tranferase
GST	=	Glutathione-s-tranferase
H	=	Hour
HCT	=	Hematocrit
HGB	=	Hemoglobin
kcal	=	Kilo calorie
L	=	liters
MCH	=	Mean Corpuscular Hemoglobin
MCHC	=	Mean Corpuscular Hemoglobin Concentration
MCV	=	Mean Corpuscular Volume
MDA	=	Malondialdehyde
nmol	=	nano mole
pg	=	pico gram
RBC	=	Red Blood Cell
RDW	=	Red distribution width
SOD	=	Superoxide dismutase
TAC	=	Total antioxidant capacity
WBC	=	White Blood Cell
mg	=	milligram
mmol	=	millimole
N	=	nitrogen

LIST OF ABBREVIATIONS (Continued)

NDF	=	Neutral detergent fiber
NH_3-N	=	Ammonia nitrogen
NRC	=	National Research Council
OM	=	Organic matter
OMD	=	Organic matter digestibility
SAS	=	Statistical Analysis System
SEM	=	Standard error of mean
VFA	=	Volatile fatty acid
$W^{0.75}$	=	Metabolic weight

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Anthocyanins is glycosides of anthocyanidin which is synthesized via the flavonoid biosynthetic pathway in plants (Dixon et al., 2013). They are a strong natural antioxidant compound appear in the colored plant as a feed source for the animal such as purple corn, purple grass, and purple rice. They play a definite role in the attraction of animals for pollination and seed dispersal, and hence they are of considerable value in the co-evolution of these plant-animal interactions (Kong et al., 2003). Anthocyanin and 3-deoxy anthocyanidins have roles in flowering plants other than as attractants. They can act as antioxidants, phytoalexins or as antibacterial agents and have more result on in vitro fermentation (LeatherWood et al., 2014), increasing the aspartate aminotransferase (AST) activity and superoxide dismutase (SOD) activity in the plasma of lactating dairy cows (Hosada et al., 2012a), improving the plasma activity of SOD-an important antioxidant enzyme in sheep (Hosada et al., 2012b). Digestion and metabolism of anthocyanin were shown clearly by studies on rat and human, in contrast, in ruminant there is very little research on digestion and metabolism of anthocyanin from feed to blood plasma.

Anthocyanin-rich Napier grass is a native cultivate of Napier grass in Thailand, their feature are leaves and stems in purple color from anthocyanin which protect plant cell from UV radiation, oxidative stress from environmental pathogen and herbivores

(Lev-Yadun et al., 2009). Napier grass grows in tropical and sub-tropical regions with a wide range of annual moisture from 750 to 2,500 mm rainfall (Skerman and Riveros, 1990) and has high production and short cutting cycle (Nyambati et al., 2010). Ensiling is the simple method to keep forage in a long time as a feed for small ruminant for centuries. The main key of good fermentation processing is the level of water soluble carbohydrate (WSC) and the number of epiphytic lactic acid bacteria (LAB) attachment in grass but Napier grass is low in these values (Ohmomo et al., 2002; Yahaya et al., 2004). In addition, Anthocyanin-rich feedstuffs were easy to lost anthocyanin in ensiling progress (Song et al., 2012) and anthocyanin are only stable when pH in silage lower than 4 (Hosada et al., 2009). Therefore, to obtain the high quality silage, many additives have been used during the time ensiling (Melkamu et al., 2014) and sugar is a limiting factor in silage fermentation (Seale et al., 1986). Molasses was used in silage to made fermentation quickly by decreasing the pH value and providing the energy source for lactic bacteria (Pipat et al., 2011). Besides, Iron (Fe) is an essential component of several cytochromes and iron-sulfur proteins involved in the electron transport chain and several important biological processes (Ganz and Nemeth, 2006). The level of $FeSO_4$ at 0.03%/DM was reported no effect on the performance and meat characteristics of lamp (Abdelrahim et al., 2012). Addition $FeSO_4$ in silage under fermentation, Fe^{2+} were received electron transfer to Fe^{3+} by lactic bacteria (Scheers et al., 2016), then Fe^{3+} combine with anthocyanidin to form Metallo-anthocyanin compound (Yoshida et al., 2009, Scheers et al., 2016). Thus, using molasses and $FeSO_4$ in silage can improve the fermentation and enhance the anthocyanin value.

In small ruminant, oxidative stress is a negative imbalance between antioxidants and free radicals from high concentrations of a by-product of Reactive oxygen species (ROS) in physiological processes of biological systems (Betteridge, 2000). There are many factors including mastitis, reproductive disorders, parasitic infections, metabolic changes mediated by ROMs (reactive oxygen metabolites, milk production, heat - stress), feed quality, imbalance nutrient, and management which make oxidative stress in the small ruminant (Celi, 2010, Celi, 2011 and Celi et al., 2015). Parasitic diseases seem to be a causative source of oxidative stress, indeed, several studies have reported on the presence of oxidative stress in small ruminant infected with parasites (Esmaeilnejad et al., 2012a, Esmaeilnejad et al., 2012b, Angulo-Valadez et al., 2011, Dey et al., 2010) as well as the antioxidant defense mechanism that exists between parasites and the mammalian host. *Haemonchus Conturtus* (Barber pole worm) is the most popular gastro-intestine nematode in goat. Anemia, low packed cell volume (PCV), diarrhea, dehydration, peripheral, and internal fluid accumulation are common signs of barber pole worm infestation. Infested goats have lower growth rates, markedly reduced reproductive performance, and have higher rates of illness and death. This parasite compete with their host for nutrients and destroy tissues of the host in gastro-intestine as they migrate. This damage cause the metabolic changes and oxidative stress for animal (Andrew et al., 2010).

From these reasons, Anthocyanin rich-Napier grass can be used as a source of anthocyanin for goat and the additive is mixed in silage necessary to improve the silage fermentation and the level of anthocyanin. This study was conducted to evaluate the effect of different level of molasses and $FeSO_4$ in Anthocyanin rich-Napier grass

silage on the anthocyanin value and silage fermentation quality after 21 days ensiling, to exam the effect of the Anthocyanin rich-Napier grass silage with the best percentage of molasses and $FeSO_4$ on growing goat performance and measure antioxidant status in the plasma of the growing goat and the naturally *Haemonchus contortus* infected goat.

1.2 Research objectives

1.2.1 To evaluate the effect of molasses and $FeSO_4$ on silage fermentation, chemical composition, *in vitro* ruminal fermentation, anthocyanin degradability of Anthocyanin-rich Napier Grass silage.

1.2.2 To examine the effect of Anthocyanin-rich Napier Grass silage with the 4 % molasses and 0.03 % $FeSO_4$:

On performance, rumen fermentation, microorganism, and nutrient digestibility of growing goat.

On oxidation status and antioxidant enzyme activities in plasma of growing goat and the naturally *Haemonchus contortus* infected goat.

1.3 Research hypothesis

Molasses and $FeSO_4$ have an effect on silage fermentation, nutrient components, *in vitro* ruminal fermentation, nutrient degradability, anthocyanin stability and degradability of Anthocyanin-rich Napier Grass silage.

Anthocyanin-rich Napier Grass silage with 4 % molasses and 0.03 % $FeSO_4$.

- To improve performance, rumen fermentation, microorganism, nutrient digestibility and meat quality of growing goat.

- To enhance the oxidation status and antioxidant enzyme activities in growing goat plasma.

- To improve the oxidation status and antioxidant enzyme activities in plasma of the naturally *Haemonchus contortus* infected goat.

1.4 Scope and limitation of the study

This study focused only on:

- Anthocyanin-rich Napier Grass (*Pennisetum Purpurreum*) was planted in SUT farm on the rainy season, were used to make silage at 120 days. The crossed (Thai x Anglo Nubian) goats were used in these experiments.

- Effect of molasses and $FeSO_4$ on silage fermentation, nutrient components, in vitro ruminal fermentation, and degradability of Anthocyanin-rich Napier Grass silage

- Effect of Anthocyanin-rich Napier Grass silage with 4% molasses and 0.03% $FeSO_4$ on rumen fermentation, microorganism, nutrient digestibility, oxidative status and antioxidant enzyme activity in growing goat plasma.

- Effect of Anthocyanin-rich Napier Grass silage with 4% molasses and 0.03% FeSO4 on oxidation status and antioxidant enzyme activity in plasma of naturally *Haemonchus contortus* infected growing goat.

1.5 Expected results

According to this research procedures, it could be obtained for expected benefits as following:

- Anthocyanin rich-Napier grass silage composition and Characteristics of anthocyanin in Anthocyanin - rich Napier grass silage.

- To apply the best percentage of molasses and $FeSO_4$ in maintaining the high level of anthocyanin and good fermentation in silage.

- Anthocyanin in Anthocyanin rich-Napier silage improve goat performance, oxidative stress, and antioxidant activity in goat.

- To encourage farmer use Anthocyanin-rich Napier silage for goat as a source of antioxidant.

1.6 References

Abdelrahim, G.M., Khatiwada J. and Gueye A (2012). Effect of Dietary Supplementation of Ferrous Sulfate on Performance and Carcass Characteristics of Finishing Lambs. **Journal of Animal Research and Technology**, 1 (1): 7-12.

Angulo-Valadez, C.E., Reyes-Becerril M.C., Romero G.M.J., Cepeda-Palacios R., López-Aguilar D.R., Zenteno T., Ascencio F. (2011). Antioxidant enzymes in erythrocytes from goats seropositive to the sheep nose bot fly (Oestrus ovis L., Diptera: Oestridae) infection. **Veterinary Parasitology**, 183: 140-145.

Betteridge, D., and John D. (2000). What Is Oxidative Stress? **Journal of Metabolism**. Volume (9): 3-8.

Celi, P. (2011). Oxidative stress in ruminants. In: Mandelker L, Vajdovich P, editors. Studies on Veterinary Medicine. Oxidative Stress in Applied Basic Research and Clinical Practice. **New York: Humana Press**. 191-231.

Celi, P. and Gabai G. (2015). Oxidant/ antioxidant balance in animal nutrition and health:

the role of protein oxidation. **Veterinary Sciences** 2:48-50.

Celi, P., 2010. Biomarkers of oxidative stress in ruminant medicine. **Review article. Immunopharmacology and Immunotoxicology**. 33(2): 233-240.

Dey, S.U.K.D. (2010). Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. **Tropical Animal Health Production**, 42:1663-1668.

Dixon, R.A., Chenggang L. and Jun J.H. (2013). Metabolic engineering of anthocyanins and condensed tannins in plants. **Current Opinion in Biotechnology**, 24:329-335.

Esmaeilnejad, B., Tavassoli M., Asri-Rezaei S., Dalir-Naghadeh B. and Malekinejad H. (2012a). Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with Babesia ovis. Acta Parasitologica, **Veterinary Parasitology**, 57(3), 228-234.

Esmaeilnejad, B., Tavassolia M., Asri-Rezaei S., Dalir-Naghadeh B. (2012b). Evaluation of antioxidant status and oxidative stress in sheep naturally infected with Babesia ovis. **Veterinary Parasitology**, 185:124-130.

Ganz, T., and Nemeth E. (2006). Regulation of iron acquisition and iron distribution in mammals. In: Hansen et al. (2009). Bioaccessibility of iron from soil is increased by silage fermentation, **Journal of Dairy Science**, 92 (6): 2896-2905.

Hosada, 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-Australia Journal of Animal Sciences** 22 (4): 528-533.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2012a). Effect of anthocyanin-

rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Animal Science Journal**, 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 Japanese Society of Grassland Science, **Grassland Science**, 58: 161-169.

Hosoda, K., Miyaji M., Matsuyama H., Haga S., Ishizaki H., 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. **Livestock Science**, 145: 266-270.

Kong, J.M., Chia L.S., Goh N.K., Chia T.F. and Brouillard R. (2008). Analysis and biological activities of anthocyanins. **Phytochemistry**, 69 (9): 1939-1940.

Leatherwood, W.L. (2014). Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on in vitro Fermentation by Rumen Microbial Cultural Research. A thesis submitted to the Graduate Faculty of North Carolina State University. **North Carolina**, 80-88.

Lev-Yadun, S. and Gould K.S. (2009). Role of Anthocyanins in Plant Defence Anthocyanins, **Springer Science Business Media**, 18-22.

Nyambati, E.M., Muyekho F.N., Onginjo E. and Lusweti C.M. (2010). Production, characterization and nutritional quality of Napier grass [*Pennisetum purpureum* (Schum.)] cultivars in Western Kenya. **African Journal of Plant Science**. 4(12): 496-502.

Okaraonye, C.C. and Ikewuchi J.C. (2009). Nutritional and Antinutritional Components

of Pennisetum purpureum (Schumach). **Pakistan Journal of Nutrition**, 8 (1): 32-34.

Pipat, L., Mek K. and Wisitiporn S. (2011). Silage production from cassava peel and cassava pulp as energy source in cattle diets. **Journal of Animal and veterinary Advances**, 10 (8): 1007-1011.

Scheers, N., Lena R., Inga T. and Ann S. (2016). Increased iron bioavailability from lactic-fermented vegetables is likely an effect of promoting the formation of ferric iron (Fe^{3+}). **European Journal of Nutrition**, 55:373-382.

Skerman, P.J., and Riveros F. (1990). Tropical Grasses. FAO, **Rome**, 75-12.

Song, T.H., Han O.K., Tae I.P., Kim D.K., Yoon C., Kim K.J. and Park K.H. (2012). Anthocyanin Stability and Silage Fermentation Quality of Colored Barley. **Journal of the Korean Society of Grassland and Forage Science,** 32(4): 335-342.

Song, T.H., Park T., Han O.K., Yoon C., Kang H.J. and Park K.G. (2013). Effects of Drying Methods on Anthocyanin Contents of Colored Barley (*Hordeum vulgare* L.) cv. Boanchalbori. **Journal of the Korean Society of Grassland and Forage Science**, 33(4): 234-239.

Yoshida, K., Mori M. and Kondo T. (2009). Review. Blue flower color development by anthocyanins: from chemical structure to cell physiology. **Current developments in natural products chemistry**, 26: 857-964.

CHAPTER II

LITERATURE REVIEW

2.1 Oxidative stress in goat

Oxidative stress was defined by an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses which damage the tissue (Betteridge, 2000). Free radicals are chemical species that contain unpaired electrons which were produced by increasing the chemical activity of an atom or molecule (Betteridge, 2000). Several different biochemical processes within the body including aerobic respiration yielding superoxide and hydroxyl radicals (reduction of molecular oxygen); the chemistry by-products as oxidation, activation catecholamines, and arachidonic cascade acid to produce electrons, which can neutralize molecular oxygen to superoxide (Betteridge, 2000). In addition, the response of the cell to external electromagnetic radiation can be produced by free radicals, such as gamma rays can split water to produce hydroxyl radical. The common free radicals are the hydroxyl radical (OH^-), superoxide anion (O^{2-}), transition metals such as iron and copper, nitric oxide (NO^-), and peroxynitrite ($ONOO^-$) (Betteridge, 2000). In small ruminant, oxidative stress is an interesting field of research in veterinary medicine generally, as new as in feed nutrition which the animal receive every day and effect on their healthy state beside the diseases. The factors make oxidative stress in small ruminant including goat and sheep come from the internal and external ways.

2.1.1 Oxidative stress in goat physiology

It was detected differences in models and methods, which quantify oxidative stress mostly by direct or indirect measures the amount of oxidant and antioxidant (Celi, 2010). According to Miller et al. (1993a), oxidants are most of the abundant free radicals in biological systems are the oxygen-centered free radicals and their metabolites, which usually called as “reactive oxygen metabolites” (ROMs), the normal by-products from a cellular metabolism continuously. In low concentrations, they are presented as an essential chemical for several physiological processes, including protein phosphorylation, transcription factors activation, cell differentiation, apoptosis, oocyte maturation, steroidogenesis, cell immunity and cellular defenses against microorganisms (adapt to Celi, 2010). In contrast, when excessed producing, ROMs can harm cellular lipid, proteins, and DNA (Miller et al., 1993b; Sgorlon et al, 2008). Plasma level of ROMs is considered an indicator of free radical production such as protein and lipid oxidation (Celi, 2010). Advanced oxidation protein products (AOPP) are terminal products of proteins exposed to free radicals and arise from the reaction between plasma proteins and chlorinated oxidants mediated by a neutrophil enzyme myeloperoxidase (adapt to Celi, 2010). Lipid, which is polyunsaturated particularly, are prone to oxidation. Georgieva (2005) indicated that lipids were one of the most susceptible substrates to free radicals damage and biomarkers of lipid peroxidation were considered the best indicators of oxidative stress. The method to measure lipid oxidation by using the reaction of Malondialdehyde (MDA) with thiobarbituric acid, which produces red pigment can be detected by spectrophotometry in the form of thiobarbituric acid reactive substances (TBARS) (Janero, 1990). Antioxidants were divided into three major groups including enzymatic antioxidants

such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px); albumin; non-enzymatic low-molecular-weight antioxidants such as glutathione, α-tocopherol, β-carotene, and uric acid (Halliwell & Gutteridge, 1989).

2.1.2 Oxidative stress in goat health

Mastitis is the popular disease of dairy animals because the inflammatory reaction is caused by bacterial infection (Agarwal et al., 2005). Several inflammation damages the mammary secretory epithelium and cytotoxic radicals and pro-inflammatory cytokines are released by the phagocytic cells (Knaapen et al., 1999). Oxidative stress indicator in mastitis is reactive nitrogen intermediates, which were important radicals that play a complex role in the inflammatory process (Goff et al., 1996). Reproductive disorders are another factor made by oxidative stress in goat health. The evidence in human medicine show the effects of oxidative stress in the female reproduction (Agarwal et al., 2005) as well as in dairy ruminant. ROMs affected on physiological functions of the reproductive tract (Celi, 2010), which present in some pathological conditions including retained placenta, udder edema, and mastitis.

Parasitic infections seemed to be a causative source of oxidative stress, indeed, many studies have reported on the presence of oxidative stress in goat infected with parasites (Sengupta and Basu, 2008; Esmaeilnejad et al., 2012a, b; Kucukkurt et al., 2014) as well as the antioxidant defense mechanism that exists between parasites and the host animal (El-Deeb and Younis, 2009; Kucukkurt *et al*., 2014; Dey et al., 2010; Turrens, 2004).

2.1.3 Oxidative stress in goat metabolism and production

Celi (2010) indicated that metabolic changes were mediated by ROMs.

damaged of animal performance by ROMs might be involved altered metabolism of anabolic and catabolic processes (Dröge, 2002). In dairy ruminant, increasing nutrient metabolism during the periparturient period of dairy animal has been made oxidative stress for them (Miller et al., 1993) as well as inducing metabolic stress in pregnancy and lactation (Drackley, 1999).

In the post-partum period, negative energy balance is the direct factor effect on oxidative stress of dairy ruminant as energy demands outstrip energy intake (Ingvartsen and Andersen, 2000). The changing of body condition at calving, milk yield and feed intake of dairy ruminant were affected by oxidative stress (Bernabucci et al., 2005). Therefore, Pedernera et al (2009) reported that medium and high energy balance diet and milk yield categories were the evidence investigate the relationship with oxidative stress.

2.2 Effect of parasite infection on antioxidant status and oxidative stress in goat and sheep

2.2.1 Blood parameter

Red blood cell or erythrocyte is the important cell in the body, which carry oxygen to the cell by hemoglobin, one type of protein contain iron. Red blood cell count (RBC) is the important parameter that shows the health state of the animal. Packed cell volume (PVC) measures the percentage of blood cell into total blood volume including red blood cell and plasma (Angulo-Valadez et al., 2011).

Table 2.1 Red blood cell count (RBC) and packed cell volume (PCV) in the healthy and infected goat and sheep.

Parasite type	Animal	RBC, x10^{12}/L		PCV %		Author
		Healthy	Infected	Healthy	Infected	
Babesia ovis	Goat	6.79[a]	5.58[b]	37[a]	30[b]	Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	8.64[a]	6.84[b]	29[a]	25[b]	Esmaeilnejad et al., 2012b
	Sheep (1-2%)		5.14[b]		22[b]	
	Sheep (2-3%)		3.5[b]		19[b]	
	Sheep (>3%)		2.52[b]		11[b]	
Oestrus ovis	Goat			12.87[a]	8.68[b]	Angulo-Valadez et al., 2011
Sarcoptic mange	Goat	9.55[a]	6.78[b]	28.06[a]	20.27[b]	Dey et al., 2010

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

Hematocrit (HCT) is another method to evaluate the volume of red blood cell by calculating from red blood cell count and mean cell volume while PVC directly measures the value. Hemoglobin (Hb) value show the number of red iron protein in red blood cell. This parameter presents the capacity of red blood cell to bind with oxygen. Table 2.1 and 2.2 show the value of RBC, PCV, Hb, and HCT in the parasite-infected goat lower than the healthy group. Parasites destroyed host cells where they

attached and damage to animal health. In addition, the animal infected in a long time with the parasite that made RBC, Hb and Hematocrit decreased showed a negative effect on small ruminant health.

Table 2.2 Hemoglobin (Hb) concentration and Hematocrit (HCT) (%) in the healthy and infected goat and sheep.

Parasite type	Animal	Hb g/dL		HCT (%)		Author
		Healthy	Infected	Healthy	Infected	
Babesia ovis	Goat	12.33^{a}	10^{b}			Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	9.91^{a}	8.61^{b}			Esmaeilnejad et al., 2012b
	Sheep (1-2%)		7.56^{b}			
	Sheep (2-3%)		6.62^{b}			
	Sheep (>3%)		4.03^{b}			
Haemonchus contortus	Lamp 15d			30.7^{a}	28.5^{b}	Machadoa et al., 2014
	Lamp 45d			30.6^{a}	22^{b}	
	Lamp 75d			37.5^{a}	24.8^{b}	
Sarcoptic mange	Goat	9.55^{a}	6.35^{b}			Dey et al., 2010

a, b Mean in the same row with different superscripts are significantly different ($p<0.05$).

2.2.2 Antioxidant enzyme activity

2.2.2.1 Total antioxidant capacity

From table 2.3, the data shows total antioxidant capacity (TAC) in infected small ruminant with *Babesia ovis* is lower than the healthy animal. This result focus that anemia parasite is very harmful because they decreased antioxidant status of the host animal. It seems that antioxidant mechanisms of erythrocytes that protect them against oxidative damage may be distributed by *Babesia* infection (Esmaeilnejad et al., 2012a, b; Kucukkurt et al., 2014). In table 2.3, *Haemochus contortus* increase FRAP in infected lamp after 75 days infection.

Infection by *Haemochus contortus* caused injury or cell death, due to a decrease in TAC in the serum, confirmed by histological examination of the abomasum by inflammatory infiltrate.

Table 2.3 Total antioxidant capacity (TAC) and ferric reducing ability of plasma (FRAP) level in the healthy and infected goat and sheep.

Parasite type	Animal	TAC (nmol/L)		FRAP (µmol/L)		Author
		Healthy	Infected	Healthy	Infected	
Babesia ovis	Goat	0.47[a]	0.36[b]			Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	0.61[a]	0.57[b]			Esmaeilnejad et al., 2012b
	Sheep (1-2%)		0.49[b]			
	Sheep (2-3%)		0.38[b]			
	Sheep (>3%)		0.21[b]			
Babesia ovis	Goat	3.1[a]	1.56[b]			Kucukkurt et al., 2014
Haemonchus contortus	Lamp 15d			*295.9*	*265.1*	Machado et al., 2014
	Lamp 45d			*298.6*	*262.6*	
	Lamp 75d			283[b]	339.2[a]	

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

2.2.2.2 Lipid peroxidation

According to Bagshani et al. (2011), in the cells of hosts infected with parasites, the value of reactive oxygen species (ROS) were increased, thereby causing cell and tissue damage. ROS induce the oxidation of polyunsaturated fatty acids in biological systems and lead to the formation of lipid peroxidation (LP) products (Dey et al., 2010). Lipid peroxidation used ROS biomarkers, which is MDA detect several from products of LP (Serarslan et al., 2005). In table 2.4, *Babesia* infection increased blood MDA levels in sheep (Esmaeilnejad et al., 2012b), the same results in goat infected with *Babesia bigemina* (Esmaeilnejad et al., 2012a; Kucukkurt

et al., 2014). With Theileriosis and Dicrocoelium dendriticum parasite infected sheep, the value of MDA is very highly significant as similar as the infected goat (Bagshani et al., 2011; Dey et al., 2010; Simsek et al., 2006). We concluded that MDA can be used as an indicator of oxidative stress in the parasite-infected animal.

Table 2.4 Malondialdehyde (MDA) level in the healthy and infected goat and sheep.

Parasite type	Animal	MDA (nmol/g Hb)		Author
		Healthy	Infected	
Babesia ovis	Goat	8.1^{b}	9.33^{a}	Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	6.78^{b}	7.3^{a}	Esmaeilnejad et al., 2012b
	Sheep (1-2%)		8.9^{a}	
	Sheep (2-3%)		10.01^{a}	
	Sheep (>3%)		11.37^{a}	
Babesia ovis	Goat	5.06^{b}	9.59^{a}	Kucukkurt et al., 2014
Theileriosis	Sheep	98.8^{b}	188.43^{a}	Bagshani et al., 2011
Sarcoptic mange	Goat	3.09^{b}	4.1^{a}	Dey et al., 2010
Dicrocoelium Dendriticum	Sheep	2.83^{b}	4.1^{a}	Simsek et al., 2006

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

In addition, the study of Angulo-Valadez et al. (2011), the value of TBARS (one method to measure lipid peroxidation in erythrocytes) is not a significant difference in comparison two goat groups. But the level of lactate dehydrogenase

(LDH) is significant highly in lamp infected with *Haemonchus contortus* (Machado et al., 2014). As we known, LDH is an enzyme in the carbohydrate metabolism, increasing of this level expected that oxidative stress in animal (Machado et al., 2014).

Table 2.5 Lipid peroxidation (TBARS) in erythrocytes and lactate dehydrogenase (LDH) level in plasma of healthy and infected goat and lamp.

Parasite type	Animal	TBARS (nmol/mg protein)		LDH (mg/dL)		Author
		Control	Infected	Control	Infected	
Oestrus ovis	Goat	26.16	24.66			Angulo-Valadez et al., 2011
Haemonchus contortus	Lamp 15d			8.6	17.4	Machado et al., 2014
	Lamp 45d			9^{b}	14.7^{a}	
	Lamp 75d			9.9^{b}	15.9^{a}	

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

2.2.2.3 Superoxide dismutase (SOD)

SOD is an enzyme convert O- to H_2O_2 and plays an important role in the process protect the cell from lipid peroxidation (Esmaeilnejad et al., 2012a & b). From table 2.6, most of parasite-infected animal show decrease of SOD value including erythrocytic SOD activity in *Theileria* infected sheep was significantly lower than the parasitologically free controls (Bagshani et al., 2011); similar findings had been reported in goat and sheep infected *Babesia ovis* (Esmaeilnejad et al., 2012a & b) in goat infected with *Oestrus ovis*; Gastrointestinal parasitism, and Pneumonia (Angulo-Valadez et al., 2011, Pilania et al., 2013). However, decreased SOD

antioxidant enzyme levels indicated that enhancing ROS production stimulate utilization of the antioxidant enzymes in the cell (Pilania et al., 2013).

Table 2.6 Superoxide dismutase (SOD) level in the healthy and infected goat and sheep.

Parasite type	Animal	SOD (U/mg Hb)		Author
		Control	Infected	
Babesia ovis	Goat	8.01[a]	5.86[b]	Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	10.17[a]	8.13[b]	Esmaeilnejad et al., 2012b
	Sheep (1-2%)		7.01[b]	
	Sheep (2-3%)		6.15[b]	
	Sheep (>3%)		5.43[b]	
Oestrus ovis	Goat	3.85[a]	3.09[b]	Angulo-Valadez et al., 2011
Gastrointestinal parasitism	Goat	1.89[b]	9.02[a]	Pilania et al., 2013
Pneumonia	Goat		12.11[a]	
Theileriosis	Sheep	1022.35[a]	794.97[b]	Bagshani et al., 2011

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

2.2.2.4 Catalase

Catalase is an antioxidant enzyme of erythrocytes catalyze H_2O_2 to H_2O and O_2. According to table 2.7, catalase levels were decreased in parasite-infected animal except for the study of Angulo-Valadez et al. (2011), which

show no changes in the CAT of *Oestrus ovis* infected goat as compare to control. Several studies on cattle, dog, and camels reported a significant increase in the activity of CAT in the animals were infected with *Theileria, Babesia*, and *Trypanosoma* (Asri-Rezaei and Dalir-Naghadeh, 2006; Chaudhuri et al., 2008; Saleh et al., 2009). However, Grewal et al. (2005) reported no changes in the CAT activity in *Theileria* infected cattle and in goat while Nazifi et al. (2011) reported a significant decrease in the CAT activity in the *Theileria* infected buffaloes and sheep, respectively. In summary, increase or decrease CAT value depend on the type and health state of the animal, but the general trend shows decreasing in CAT activity when the animal occurs in oxidative stress.

Table 2.7 Catalase (CAT) level in the healthy and infected goat and sheep.

Parasite type	Animal	CAT (katal/g Hb)		Author
		Control	Infected	
abesia ovis	Goat	91.26[a]	69.96[b]	Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	111.26[a]	104.7[b]	Esmaeilnejad et al., 2012b
	Sheep (1-2%)		93.26[b]	
	Sheep (2-3%)		63.26[b]	
	Sheep (>3%)		60.51[b]	
Oestrus ovis	Goat	107.88	108.75	Angulo-Valadez et al., 2011
Gastrointestinal parasitism	Goat	1.83[b]	4.34[a]	Pilania et al., 2013
Pneumonia	Goat		5.61[a]	
Theileriosis	Sheep	298.38[b]	378[a]	Bagshani et al., 2011

a, b mean in the same row with different superscripts are significantly different ($p<0.05$).

2.2.2.5 Glutathione peroxidase (GSH-Px), Glutathione (GSH), and glutathione S-transferase (GST)

GSH enzyme is required for the disposal of H_2O_2 by the reaction catalyzed by GPx (Kucukkurt et al., 2014). GSH enzyme is one of the primary factors that permit lipid peroxidation.

Table 2.8 Glutathione peroxidase (GSH-Px) level in the healthy and infected goat and sheep.

Parasite type	Animal	GSH-Px (IU/mg Hb)		Author
		Control	Infected	
Babesia ovis	Goat	74.25[a]	70.75[b]	Esmaeilnejad et al., 2012a
Babesia ovis	Sheep (<1%)	83.67[a]	80.5[b]	Esmaeilnejad et al., 2012b
	Sheep (1-2%)		75.12[b]	
	Sheep (2-3%)		71.12[b]	
	Sheep (>3%)		68[b]	
Babesia ovis	Goat	30.7[a]	19.53[b]	Kucukkurt et al., 2014
Theileriosis	Sheep	6.3[b]	223.2[a]	Bagshani et al., 2011
Sarcoptic mange	Goat	0.24[a]	0.16[b]	Dey et al., 2010

[a, b] mean in the same row with different superscripts are significantly different ($p<0.05$).

The significant reduction in GSH-Px, GSH, and GST in the infected animal was shown in table 2.8 and 2.9, respectively. The same results in line with the findings

of El-Deeb and Younis (2009), who reported a significant reduction in the levels of glutathione in *Theileriosis annulata* infected buffaloes compared with healthy buffaloes. We can found that trend in *Babesiosis* infected sheep (Bicek et al., 2005) and horse (Deger et al., 2009) and *Trypanosomiasis* in camel (Saleh et al., 2009). Those parasitic diseases can lead to significant reduction in GSH, GST, and GSH-Px levels by increasing the lipid peroxidation in erythrocyte.

Table 2.9 Glutathione (GSH) and glutathione S-transferase (GST) level in the healthy and infected goat and sheep.

Parasite type	Animal	GSH (µmol/g Hb)		GST (U/mg protein)		Author
		Control	Infected	Control	Infected	
Oestrus ovis	Goat			28.86[a]	21.23[b]	Angulo-Valadez et al., 2011
Theileriosis	Sheep	6.3[a]	4.2[b]			Bagshani et al., 2011

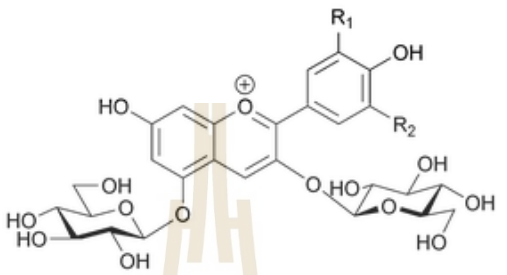
[a, b] Mean in the same row with different superscripts are significantly different ($p<0.05$).

2.3 Anthocyanins

2.3.1 Introduction

Anthocyanins are a group of pigment present in most of the plant flowers, leaves, stem, seed covers, roots, fruits, and vegetables that visible to human eyesight. They are called flavonoids, belongs phenolic compound group and structure from glycoside of polyhydroxy, polymethoxy of 2-phenylbenzopyrylium or flavylium salt (Kong et al., 2003). There are many types of anthocyanins were classified by the number of hydroxyl, sugar group, aromatic acid and the position where they attached to the flavylium cation (figure 2.1). According to Kong et al. (2003), in the plant, there

is six commonly anthocyanins present in plants including cyanidin (Cy), delphinidin (Dp), Pelargonidin (Pg), Peonidin (Pn), Malvidin (Mv), and Petunidin (Pt). Only Cyanidin, Delphinidin, and pelargonidin distribute in most of higher plant (Yoshida et al., 2003).



R_1	R_2	anthocyanin	aglycon
H	H	pelargonin	pelargonidin
OH	H	cyanin	cyanidin
OCH_3	H	peonin	peonidin
OH	OH	delphin	delphinidin
OCH_3	OH	petunin	petunidin
OCH_3	OCH_3	malvin	malvidin

Figure 2.1 Structure of common anthocyanins and their aglycons. (adapt to Yoshida et al., 2009).

2.3.2 Anthocyanin in animal feedstuffs

Anthocyanin is widely present in various plant species used as feedstuffs for the animal (table 1). The most significant function of anthocyanin is their ability to impart color to the plants or plant products in which they occur (Kong et al., 2003). Anthocyanin may be important factors along with other flavonoids in the resistance of plants to insect attack (Harborne, 2001) by cyanidin 3-glucoside which was shown to protect cotton leaves against the tobacco budworm (Hedin et al., 1983). According to Hosada et al (2009, 2012 a, 2012b, 2012c), anthocyanin-rich corn and

purple rice silage are a source of natural antioxidant for ruminants with a high level of anthocyanin as compared to others feedstuffs. Besides, genetic science had opened a new door for the transgenic plant to improve the level of anthocyanin and nutrition (Jonker et al., 2010 and Wang et al., 2006) used as good feedstuffs for the animal. There are limited about the research about anthocyanin content in Anthocyanin-rich Napier grass or silage because this is a local breed of Napier grass in Thailand, they are quite different with the imported Napier grass have purple pigments such as Prince or Princess Napier grass. The local Anthocyanin-rich Napier grass characteristic by short, sharp leaf and strong, hard body with deep red-purple color, and the number of the leaf is less than Prince or Princess Napier grass.

Table 2.10 Anthocyanin in animal feedstuffs.

Feedstuffs	Anthocyanins (%DM)	Authors
Anthocyanin-rich Corn (Zea mays L.) Silage	0.34	Hosoda et al, 2009
Purple rice (Oryza sativa L.) silage	0.36	Hosoda et al, 2012b
Rambler Lc-alfalfa	0.22	Jonker et al, 2010
Rangelander Lc-alfalfa	0.14	Jonker et al, 2010
Beaver Lc-alfalfa	0.23	Jonker et al, 2010
Lc1-transgenic alfalfa from maize	0.114	Wang et al, 2006
Lc2-transgenic alfalfa from maize	0.097	Wang et al, 2006
Lc3-transgenic alfalfa from maize	0.102	Wang et al, 2006
Lc4-transgenic alfalfa from maize	0.136	Wang et al, 2006
Colored barley (4 months)	0.41	Song et al, 2012
Colored barley (6 months)	0.418	Song et al, 2012
Colored barley (12 months)	0.396	Song et al, 2012
Hulled purple colored barley germplasms	0.35	Kim et al, 2007
Unhulled black colored barley germplasms	0.085	Kim et al, 2007
Unhulled blue colored barley germplasms	0.34	Kim et al, 2007
Unhulled purple colored barley germplasms	0.313	Kim et al, 2007
Homnil cultivar rice bran	0.24	Supaporn et al, 2014
Rice berry cultivar rice bran	0.231	Supaporn et al, 2014
Hommali dang cultivar rice bran	0.195	Supaporn et al, 2014

2.3.3 Anthocyanin stability

Table 2.11 Changes in anthocyanin content according to drying method and time in colored barley (Song et al., 2013).

Drying method	Drying time (h)	Anthocyanin content (mg/g)								
		C3G	P2G	Del	M3G	Cya	Pel	Peo	Mal	Total
Sun-dried	0	0.177[a]	0.037[a]	0.116[a]	0.558[a]	0.040	0.026	0.024	0.067	1.043[a]
	4	0.156[b]	0.028[c]	0.096[c]	0.452[c]	0.035	0.024	0.023	0.062	0.877[c]
	8	0.152[b]	0.030[b]	0.093[d]	0.449[c]	0.036	0.025	0.021	0.061	0.866[c]
	24	0.155[b]	0.029[c]	0.092[d]	0.452[c]	0.036	0.026	0.021	0.060	0.871[c]
	32	0.146[c]	0.028[c]	0.092[d]	0.444[d]	0.035	0.024	0.021	0.061	0.847[d]
	Mean	0.152[B]	0.028[B]	0.093[B]	0.448[B]	0.035	0.025	0.022	0.061	0.865[B]
Shade-dried	4	0.179[a]	0.032[ab]	0.117[a]	0.562[a]	0.040	0.027	0.022	0.060	1.039[a]
	8	0.173[a]	0.035[a]	0.109[a]	0.555[a]	0.037	0.026	0.020	0.061	1.016[a]
	24	0.170[a]	0.033[ab]	0.101[b]	0.509[b]	0.037	0.025	0.019	0.060	0.955[b]
	32	0.170[a]	0.031[b]	0.100[b]	0.493[b]	0.037	0.027	0.020	0.062	0.938[b]
	Mean	0.173[A]	0.033[A]	0.106[A]	0.522[A]	0.038	0.026	0.020	0.063	0.981[A]

[a-d] mean in the same column with different superscripts are significantly different ($p<0.05$). C3G:cyanidin-3-glucoside, P3G:pelargonidin-3-glucoside, Del:delphinidin, M3G:malvidin3-O-glucoside, Cya:cyanidin, Pel:pelargonidin, Peo: peonidin, Mal: malvidin.

The stability of anthocyanin may become compromised when they are isolated and are thus extremely prone to degradation (Giusti & Wrolstad, 2001). Several factors contribute to the stability of anthocyanin including storage temperature, pH, concentration, light, chemical structure, oxygen, solvents, proteins, metallic ions, flavonoids, and presence of enzymes (Castaneda-Ovando et al., 2009). Cevallos-Casals et al. (2004) studied the optimal pH for anthocyanin stable around 3, higher this

value, anthocyanin were degraded. Table 2.11 and 2.12 showed anthocyanin decrease quickly in sun-dried method and lost about 50 % after 2 months ensiling, respectively (Song et al., 2013; song et al., 2012). The same results in the research of Hosada et al. (2009) on anthocyanin-rich corn silage (figure 2.2). There are several strong pieces of evidence show that anthocyanin in feedstuff is very sensitive to light, pH, and temperature. In silage making, the time for fermentation and decrease pH is the main factor effect on losing anthocyanin. Improving and protecting anthocyanin in feedstuff is a new field to study in animal nutrition, which can provide the antioxidant for enhancing animal health to adapt to climate change.

Table 2.12 Change in anthocyanin composition after ensiling of colored barley (Song et al., 2012).

Month after ensiling (month)	Anthocyanin content (mg/g)								Total
	C3G	P2G	Del	M3G	Cya	Pel	Peo	Mal	
0	0.194[a]	0.03[a]	0.060[a]	0.543[a]	0.027	0.017	0.018	0.029	0.922[a]
2	0.096[b]	0.028[b]	0.041[b]	0.229[b]	-	-	-	-	0.393[b]
4	0.101[b]	0.028[b]	0.042[b]	0.239[b]	-	-	-	-	0.410[b]
6	0.103[b]	0.029[b]	0.042[b]	0.244[b]	-	-	-	-	0.418[b]
12	0.103[b]	0.028[b]	0.040[b]	0.217[b]	-	-	-	-	0.396[b]

[a,b] Treatments with different letters are different at $p<0.05$. C3G: cyanidin-3-glucoside, P3G : pelargonidin-3-glucoside, Del : delphinidin, M3G : malvidin3-O-glucoside, Cya : cyanidin, Pel : pelargonidin, Peo : peonidin, Mal : malvidin. - : Non detected.

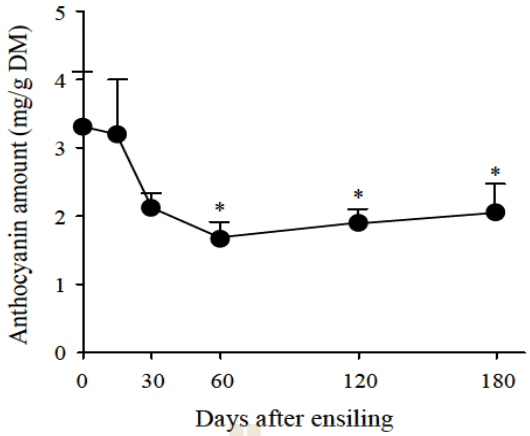


Figure 2.2 Change in anthocyanin content of anthocyanin-rich corn during ensilage. Data are displayed as mean with standard deviations. Anthocyanin (mg)/corn sample (g DM).* Asterisks show statistical difference from the level at day 0 ($p<0.05$). (Hosada et al., 2009).

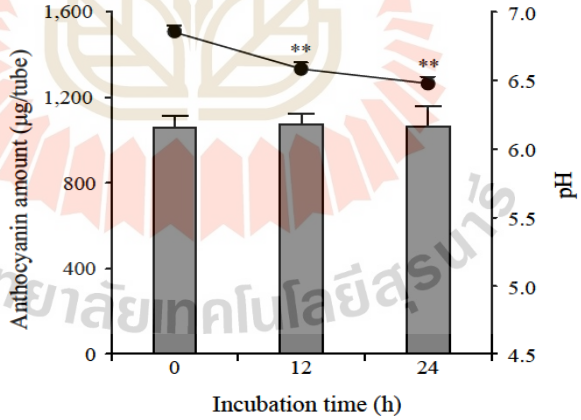


Figure 2.3 Changes in anthocyanin content (solid bar) and pH value (solid circle) in incubation tube during in vitro fermentation with ruminal fluid. Data are displayed as mean with standard deviations. ** Asterisks show statistical difference from the level at 0 h ($p<0.01$) (Hosada et al., 2009).

2.3.4 Digestion and Metabolism of Anthocyanin

In laboratory animal and mono-gastric, there is many researches about anthocyanin digestibility and metabolism. The form of anthocyanin absorbance is mainly glycoside form and present in circulation quickly after 15-120 minutes belong to dose and source of anthocyanin supplement (McGhie et al., 2007). Anthocyanins can be absorbed directly in stomach and gut of digestive system.

In ruminant, there are not many studies about anthocyanin digestibility in the rumen and can be absorbed in the rumen, which is the question up to now, there are only 3 studies were conducted in vitro incubation to measure digestion of anthocyanin in the rumen. Firstly, Hosada et al. (2009) reported that anthocyanin in anthocyanin-rich corn silage was stable under rumen fermentation after 12 and 24 Hours. With the study of Song et al. (2012) conducted the time of incubation up to 48 hours but the results showed that no significant difference could be found in changing the amount of anthocyanin of colored barley silage in rumen fermentation. Two type of anthocyanin sources from two studies indicated that when anthocyanin in feedstuff substrate could not digest by the ruminal microbe, however, in the third study from Leatherwood (2014) presented that anthocyanin extract from purple-fleshed sweet potatoes degraded significantly after 24 hours incubation in rumen fluid. The different about the form of anthocyanin were incubated with rumen fluid suggested that relationship between the structure of anthocyanin in feedstuff and other chemical composition presents an effect on degradation of anthocyanin in rumen fermentation. The hypothesis is anthocyanin pass through the rumen without degrading, it mean they can be absorbed in abomasum and gut.

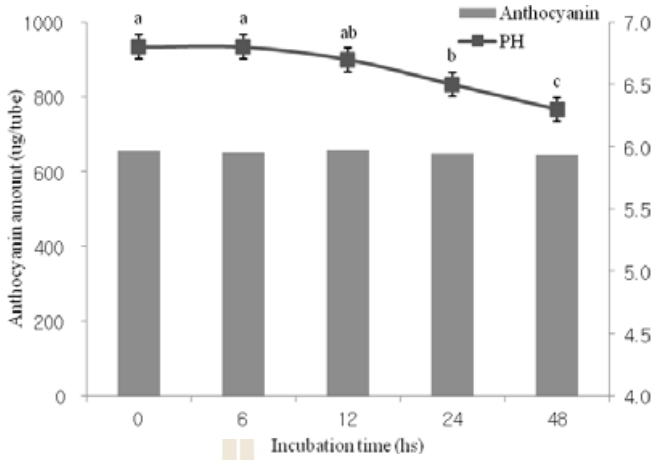


Figure 2.4 Changes in anthocyanin of colored barley content during in vitro ruminal digestion. a,b,c Treatments with different letters are different at $p<0.05$ (Song et al., 2012).

2.3.5 Effect of anthocyanin on rumen fermentation

2.3.5.1 Gas production

In the study of Spanghero et al. (2009) about the effect of seeds and pulp of Californian (USA) and Italian grape pomaces on gas production (table 4). The pulp fraction of the gas production (GP) had a much higher rumen fermentability than seeds at the beginning of the incubation (after 4 h) because of the lower lignified fiber content and the possible presence of rapidly fermentable substrates in pulps. During and at the end of fermentation (after 24 and 48 h) pulps treatments had a higher gas production (from 40 to 50%) than seed treatments. Although anthocyanin content in seed was higher than pulp, a difference of anthocyanin structure can effect on gas production. We can conclude that the structure of anthocyanin effect on fermentation of rumen microbe.

Table 2.13 Effect of seeds and pulp of Californian (USA) and Italian grape pomaces on Gas production.

	Item	Cultivars			P value
		White Italy	Red Italy	Red Califonia	
Grape Seed	Anthocyanin (mg/kg DM)	3	99	85	NS
	Gas volume (ml/0.2 g DM)				
	4 h incubation	5	5	5.5	NS
	24 h incubation	12.1	11.9	12.2	NS
	48 h incubation	17.8	18	19.2	NS
Grape Pulp	Anthocyanin (mg/kg DM)	6	44	34	NS
	Gas volume (ml/0.2 g DM)				
	4 h incubation	7.8^{c}	9.2^{b}	12.2^{a}	<0.01
	24 h incubation	14.8^{c}	16.6^{b}	21.3^{a}	<0.01
	48 h incubation	24.6^{c}	26.3^{b}	32.7^{a}	<0.01

$^{a-d}$ mean in the same column with different superscripts are significantly different ($p<0.05$)

On the other hand, Ray et al. (2003) conducted one study about three anthocyanin regulatory genes of maize (Zea mays; Lc, B-Peru, and C1) were transformed into alfalfa (Medicago sativa) in a strategy designed. From table 5, gas production was different ($P\leq0.001$) among the genotypes (different level of anthocyanin) throughout the 48 h of the incubation. When the level of anthocyanin is higher, the gas volume is increased.

Table 2.14 In vitro ruminal fermentation characteristics of non-transgenic (NT) and *Lc*-transgenic alfalfa incubated in batch culture.

Item	Genotypes						P - value	
	NT	Lc1	Lc2	Lc3	Lc4	SEM	GT	Lc
Anthocyanidins (μg g–1 DM)	0	114.6	96.9	102.2	135	19.22	<0.001	<0.001
Gas production (mL g–1 DM)								
4 H	75.8	76	78.2	81.9	84	0.52	<0.001	<0.001
12H	151.3	148	151.4	155.6	158.7	0.79	<0.001	0.028
24 H	185.9	177.1	183.9	183.8	187.5	0.59	<0.001	0.001
48 H	195.9	191.1	194.5	196.1	198.7	0.9	0.001	0.464

Notes: Lc1, Lc2, Lc3, and Lc4 are genotypes developed from NT as interim germ plasma in a longer-term study undertaken to produce pro-anthocyanidins in alfalfa (Ray et al2003). GT = effect of genotype; Lc = effect of transformation with Lc (NT vs. Lc) (Ray et al., 2003)

2.3.5.2 Volatile fatty acid profile in rumen fluid

In the in vivo study of Hosada et al. (2012b), there were no effects of the control treatment and anthocyanin-rich corn silage treatment on ruminal pH, total and individual VFA concentrations and acetate-propionate ratio (Table 2.15). The acetate and propionate proportions of the total VFA were significant (P<0.01). By contrast, in the in vitro study of Leatherwood (2014), the results were no different on acetate and propionate proportions of the total VFA but the type of purple-fleshed sweet potatoes had effected on butyrate acid proportion. The same difference was found in the second experiment of Leatherwood (2014) about the effect of sample weight on gas production.

Table 2.15 Effect of anthocyanin and sample weight on fatty acid profiles in rumen fluid in vivo and in vitro.

Treatment	pH	Volatile Fatty acid				References
		C_2(%)	C_3(%)	C_4(%)	Total VFA (mmol)	
Control	6.57	63.2	21	11.6	108	Hosoda et al(2012b) (*In vivo*)
AR	6.48	62.3	21.2	12.3	109	
P-value	NS	<0.01	NS	<0.01	NS	
PE	5.9	52.2	36.54[a]	11.9	15.6	Leatherwood (2014) (*In vitro*)
DE	6.1	59.5	30.19[b]	10.5	7.85	
P-value	NS	NS	<0.05	NS	NS	
GP (1g)	4.4	67.2	27.52[a]	5.41	47.9	
GP (5g)	4.4	80.3	14.89[b]	3.98	62.8	
P-value	NS	NS	<0.01	NS	NS	

Notes: AR: anthocyanin rich corn silage, PFSP: purple-fleshed sweetpotatoes, PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

2.3.6 Effect of anthocyanin on ruminal plasma antioxidant enzymes

In three studies of Hosada et al., (2012a, 2012b, and 2012c), sheep and cow were fed different sources of anthocyanin but the results showed increasing of superoxide dismutase (SOD enzyme while total antioxidant capacity (TAC), glutathione, alanine aminotransferase (ALT), and Total antioxidant status of blood plasma were not different.

2.4 Molasses improve anthocyanin degradability in silage ensiling

Anthocyanins is very sensitive to pH, the problem when we make silage is the time for fermentation, which decreases pH and effect directly on the degradation of anthocyanin in silage. However, the quality of silage depends on the water-soluble carbohydrate, which decide the fermentation process quickly or slowly. Napier (*Pennisetum purpureum*) grass is a popular grass used for ruminant in the tropical

country. However, their dry matter and water-soluble carbohydrates are quite low to make a good silage (Booreenok et al., 2012). According to Ohmomo et al. (2002) and Yahaya et al (2004), the lactic acid bacteria attached in Napier grass was low considered poor fermentation quality. Addition of molasses is a simple method for supplying the water-soluble carbohydrate to the substrate by providing energy immediately for lactic acid bacteria increase their copies and produce lactic acid to decrease pH in the short time after ensiling (Van Niekerk et al., 2007). When pH drop as short as possible, the amount of anthocyanin loss were decreased. Molasses can be used for improving anthocyanin stability in Anthocyanin-rich Napier grass silage.

2.5 Addition of $FeSO_4$ in silage

In ruminant, they receive high level of Fe from soil and feedstuff in dietary (Standish et al., 1971). Fe content is high in feedstuffs for ruminant such as alfalfa (300 mg/kg of DM), corn gluten feed (400 mg/kg of DM), distillers dried grains with soluble (DDGS) (600 mg/kg of DM), and soy hulls (600 mg/kg of DM) (Abdelrahim et al., 2012). When Fe excess, it can make oxidative stress (Ganz and Nemeth, 2006). In sheep, supplement of $FeSO_4$ at two levels (75 and 150 mg/kg bodyweight) did not effect on dry matter intake, growth rate, and carcass characteristics of meat sheep. In addition, the information from study of Scheers et al. (2016) show that Fe^{2+} under lactic fermentation will change to Fe^{3+}; this form of ferris will combine with anthocyanin make metallic anthocyanin compound to enhance the color pigment (Wang et al., 2013). On the other hand, addition of Fe^{3+} increased the degradation of fiber in silage (Wang et al., 2018) by increasing the hydrolysis of lignocellulose to sugar, which is fermentable to acid or enzyme. Addition $FeSO_4$ is an additive to help

fiber degradation better as well as maintain the amount of anthocyanin in anthocyanin rich Napier grass.

Table 2.16 Effect of anthocyanin on ruminal plasma antioxidant enzymes.

Animal	Item	Treatment				Authors
		Control	**Purple corn pigment**	**SEM**	**P value**	
Sheep	Plasma TAC, µmol/L	453.8	458.5	6.8	NS	Hosada et al, 2012 c
	Plasma Glutathione, µmol/L	5.1	5.9	0.2	NS	
	Plasma SOD, U/mL	164.2[b]	184.4[a]	4	P<0.05	
	Urinary 8-OHdG, µg/MBS/day	2.8	2.3	0.2	NS	
		Control	Purple rice silage	SEM	P value	
Sheep	Plasma TAC, µmol/L	463.2	470.2	6.4	NS	Hosada et al, 2012a
	Plasma Glutathione, µmol/L	4.2	4	0.1	NS	
	Plasma SOD, U/mL	318.7[b]	360.2[a]	11.7	P<0.01	
	Urinary 8-OHdG, µg/MBS/day	261.9	247.3	34.8	NS	
		Control	Anthocyan in-rich corn silage	SEM	P value	
Cow	AST (IU/L)	97[a]	89.6[b]	0.7	P<0.05	Hosada et al, 2012b
	ALT (IU/L)	36.9	34.3	1.5	NS	
	Total antioxidant status (mmol/L)†	1.06	1.04	0.01	NS	
	SOD (U/mL)	2.4[b]	3.9[a]	0.2	P<0.01	

Notes: TAC = total antioxidant capacity, SOD = superoxide dismutase, 8-OHdG = 8-hydroxy-2′-deoxyguanosine. AST, aspartate aminotransferase; ALT, alanine aminotransferase

2.6 References

Abdelrahim, G.M., Khatiwada J. and Gueye A. (2012). Effect of Dietary Supplementation of Ferrous Sulfate on Performance and Carcass Characteristics of Finishing Lambs. **Journal of Animal Research and Technology,** 1 (1): 7-12.

Agarwal, A., Gupta S., Sharma R. (2005). Role of oxidative stress in female reproduction. **Reproductive Biology and Endocrinology**, 28(3).

Angulo-Valadez, C.E., Reyes-Becerril M.C., Romero G.M.J. , Cepeda-Palacios R., López-Aguilar D.R., Zenteno T., Ascencio F. (2011). Antioxidant enzymes in erythrocytes from goats seropositive to the sheep nose bot fly (*Oestrus ovis* L., Diptera: Oestridae) infection. **Veterinary Parasitology** 183: 140-145.

Asri-Rezaei, S., Dalir-Naghadeh B. (2006). Evaluation of antioxidant status and oxidative stress in cattle naturally infected with Theileria annulata. **Veterinary Parasitology**, 142: 179-186.

Baghshani, H., Razmi G.R., Yaghfouri S. And Dezaki A.A. (2011). Status of some oxidative stress biomarkers in sheep naturally infected with theileriosis. **Research opinion in animal & veterinary sciences**, 1(8): 499-504.

Bernabucci, U., Ronchi B., and Latera N. (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. **Journal of Dairy Science**, 88: 2017-2026.

Betteridge, D. John D. (2000). What Is Oxidative Stress? **Journal of Metabolism**. Volume (9): 3-8.

Bicek, K., Deger Y. and Deger S. (2005). Some biochemical and haematological parameters of in sheep infected with Babesia species. **Yüzüncü Yıl Üniversitesi**

Veteriner Fakültesi Dergisi, 16: 33-35.

Bureenok, S., Yuangklang C., Vasupen K., Schonewille J.T. and Kawamoto Y. (2012). The Effects of Additives in Napier Grass Silages on Chemical Composition, Feed Intake, Nutrient Digestibility and Rumen Fermentation. Asian-Australian **Journal of Animal Science**. 25(9): 1248-1254.

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

Celi, P., (2010). Biomarkers of oxidative stress in ruminant medicine. Review article. **Immunopharmacology and Immunotoxicology**. 33(2): 233-240.

Cevallos-Casals, B.A. and Cisneros-Zevallos L. (2004). Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. **Food Chemistry**, 86:69-77.

Chaudhuri, S., Varshney, J.P. and Patra, R.C. (2008). Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with Babesia gibsoni. **Research in Veterinary Science**, 85: 120-124.

Deger, S., Deger Y., Bicek K., Ozdal N. and Gul A. (2009). Status of lipid peroxidation, antioxidants, and oxidation products of nitric oxide in equine babesiosis: status of antioxidant and oxidant in equine babesiosis. **The Journal of Equine Veterinary Science**, 29 (10): 743-747.

Dey, S.U.K.D. (2010). Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. **Tropical Animal Health Production** (2010) 42:1663-1668.

Drackley, J.K. (1999). Biology of dairy cows during the transition period: the final frontier. **Journal of Dairy Science**, 82: 2259-2273.

Droge, W. (2002). Free radicals in the physiological control of cell function. **Physiological Reviews**, 82: 47-95.

El-Deeb, W.M. and Younis, E.E. (2009). Clinical and biochemical studies on Theileria annulata Egyptian buffaloes (Bubalus bubalis) with particular orientation to oxidative stress and ketosis relationship. **Veterinary Parasitology**, 164: 301-305.

Esmaeilnejad, B., Tavassoli M., Asri-Rezaei S., Dalir-Naghadeh B. and Malekinejad H. (2012a). Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with Babesia ovis. Acta Parasitologica, **Veterinary Parasitology**, 57(3), 228-234.

Esmaeilnejad, B., Tavassolia M., Asri-Rezaei S., Dalir-Naghadeh B. (2012b). Evaluation of antioxidant status and oxidative stress in sheep naturally infected with Babesia ovis. **Veterinary Parasitology** 185 (2012) 124-130.

Ganz, T., and Nemeth E. (2006). Regulation of iron acquisition and iron distribution in mammals. Biochim. Biophys. Acta. 1763:690-699. In: Hansen *et al*(2009). Bioaccessibility of iron from soil is increased by silage fermentation. **Journal of Dairy Science**, 92 (6): 2896-2905.

Georgieva, N.V. (2005). Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems-a review. **Bulgarian Journal of Veterinary Medicine**, 8:.1-11.

Giusti, M., and Wrolstad, R. (2001). Characterization and measurement of anthocyanins

by UV-Visible Spectroscopy. In Current Protocols in Food Analytical Chemistry. **John Wiley & Sons**, Inc.

Goff, W.L., Carl J.W., Wyatt C.R. (1996). Assessment of bovine mononuclear phagocytes and neutrophils for induced L-arginine-dependent nitric oxide production. **Veterinary Immunology and Immunopathology**, 55: 45-62.

Grewal, A., Ahuja C.S., Singha S.P. and Chaudhary, K.C. (2005). Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with Theileria annulata. **Veterinary Research Communications**, 29: 387-394.

Halliwell, B., Gutteridge J.M.C. (1989). Free radicals in biology and medicine. **Oxford: Oxford University Press**, 543.

Harborne, J.B. and Williams, C.A. (2001). Anthocyanins and other flavonoids. **Natural Product Reports** 18, 310-333.

Hedin, P.A., Jenkins, J.N., Collum, D.H., White, W.H., Parrot, W.L. (1983). In P. A. Hedin (Ed.), Plant Resistance to Insects. **American Chemical Society**, Washington, DC, 347.

Ingvartsen K.L. and Andersen J.B. (2000). Integration of metabolism and intake regulation: a review focusing on periparturient animals. **Journal of Dairy Science**, 83: 1573-1597.

Janero, D.R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. **Free Radical Biology and Medicine**, 9: 515-540.

Jonker, A., Gruber M.Y., McCaslin M., Wang Y., Coulman B., McKinnon J.J., Christensen D.A., and Yu P. (2010). Nutrient composition and degradation

profiles of anthocyanidin-accumulating Lc-alfalfa populations. **Canadian Journal of Animal Science**, 401-412.

Kim, M.J., Hyun J.N., Kim J.A., Park, Kim M.J., Kim J.G., Lee S.J., Chun S.C. and Chung I.M. (2007). Relationship between Phenolic Compounds, Anthocyanins Content and Antioxidant Activity in Colored Barley Germplasm, **Journal of Agricultural and Food Chemistry**, 55: 4802-4809.

Knaapen, A.M., Seiler F., Schilderman P.A.E.L. (1999). Neutrophils cause oxidative DNA damage in alveolar epithelial cells. Free Radical, **Biology and Medicine**, 27: 234-240.

Kong, J.M., Lian S.C., Ngoh K.F., Tet F.C. Brouillard R. (2003). Review Analysis and biological activities of anthocyanins. **Phytochemistry** 64: 923-933.

Kucukkurt, I., Cigerci H.I., Ince S., Kozan E., Aytekin E., Eryavuz A., Fidan F.A. (2014). The Effects of Babesiosis on Oxidative Stress and DNA Damage in Anatolian Black Goats Naturally Infected with Babesia ovis. **Iranian Journal of Parasitol**, 9(1): 90-98.

Leatherwood, W.L. (2014). Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on in vitro Fermentation by Rumen Microbial CultuResearchA thesis submitted to the Graduate Faculty of North Carolina State University. **North Carolina.**

Machado, V., Da Silva A.S., Schaferb A.S., Airesb A.R., Toninc A.R., Oliveirad C.P., Hermese C.L., Almeidae T.A., Morescoe R.N., Stefania L.M., Lopesc S.T.A., Mendesf R.E., Schwertzf C.I. and Leal M.L.R. (2014). Relationship between oxidative stress and pathological findings in abomasum of infected lambs by Haemonchus contortus. **Pathology-Research and Practice** 210: 812-817.

McGhie, T.K., Ainge G.D., Barnett L.E., Cooney J.M., and Jensen D.J. (2003). Anthocyanin Glycosides from Berry Fruit Are Absorbed and Excreted Unmetabolized by Both Humans and Rats. **Agricultural of Food Chemistry**, 51 (16): 4539-4548.

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

Miller, N.J., Rice-Evans C., and Davies M.J. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. **Clinical Science**, 84: 407-412.

Nazifi, S., Razavi S.M., Kianiamin P. And Rakhshandehroo E. (2011). Evaluation of erythrocyte antioxidant mechanisms: antioxidant enzymes, lipid peroxidation, and serum trace elements associated with progressive anemia in ovine malignant theileriosis. **Parasitology Research**, 109:275-281.

Ohmomo, S., Tanaka O., Kitamoto H.K and Cai Y. (2002). Silage and microbial performance, old story but new problems. **Japanese Agriculture Research** Q. 36:59-71.

Pedernera, M., Celi P., and Garcia S. C. (2009). Effect of diet, energy balance and milk production on oxidative stress in early lactating dairy cows fed on a predominantly grazed pasturediet. **The Veterinary Journal**, 85: 119-130.

Pilania, P.L., Solanki S., Mohammed N. Asopa S., Joshi A., Sankhala L.N., Mathur M., Thori M.K., Gaur J.S., Meena A. and Kataria N. (2013). Oxidative stress Vis a Vis Gastrointestinal Parasitiem and Pneumonia in Marwari Goat. **Veteinary Research**, 6 (3): 54-57.

Ray, H., Yu M., Auser P., Blahut-Beatty L., McKersie B., Bowley S., Westcott N., Coulman B., Lloyd A. and Gruber M.Y. (2003). Expression of anthocyanins and proanthocyanidins after transformation of alfalfa with maize Lc. **Plant Physiology**. 132: 1448-1463.

Saleh, M.A. (2009). Erythrocytic oxidative damage in crossbred cattle naturally infected with Babesia bigemina. **Research in Veterinary Science**, 86: 43-48.

Scheers, N., Lena R., Inga T. and Ann S. (2016. Increased iron bioavailability from lactic-fermented vegetables is likely an effect of promoting the formation of ferric iron (Fe^{3+}). **European Journal of Nutrition** 55:373-382.

Sengupta, P.P. and Basu A. (2008). Haematological changes and clinical manifestations in the experimental caprine sarcoptic mange. **Journal of Veterinary Parasitolog**y, 22, 65-68.

Sgorlon, S., Stradaioli G., Gabai G., Stefanon B. (2008). Variation of starch and fat in the diet affects metabolic status and oxidative stress in ewes. **Small Ruminant Research**, 74: 123-129.

Simsek, S., Yuce A., and Utuk A.E. (2006). Determination of serum malondialdehyde levels in sheep naturally infected with Dicroelium Dendriticum. **Fırat Üniversitesi Sağlık Bilimleri Dergisi**, 20(3), 217-220.

Song, T. H., Han O. K., Tae Il Park, Kim D. K., Yoon C., Kim K. J. and Park K. H.. (2012). Anthocyanin Stability and Silage Fermentation Quality of Colored Barley. **Journal of the Korean Society of Grassland and Forage Science** 32(4): 335-342.

Song, T.H., Park T., Han O.K., Yoon C., Kang H.J. and Park K.G. (2013). Effects of Drying Methods on Anthocyanin Contents of Colored Barley (Hordeum

vulgare L.) cv. Boanchalbori. **Journal of the Korean Society of Grassland and Forage Science**, 33(4): 234-239.

Spanghero M., Salem A.Z.M. and Robinson P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. **Animal Feed Science and Technology**, 152 (2009) 243-255.

Standish, J.F., Ammerman C.B., Palmer A. Z. and Simpson C. F. (1971). Influence of dietary iron and phosphorus on performance, tissue mineral composition and mineral absorption in steers. **Journal of Animal Science** 33: 171-178.

Supaporn, P. and Theerakulkait C. (2014). Effects of rice cultivars and some factors on anthocyanin extraction from pigmented rice bran on anthocyanin, total phenolic content and DPPH scavenging activity. **Proceedings of 52nd Kasetsart University Annual Conference: Agro-Industry** 2014. 4-7.

Turrens, J.F. (2004). Oxidative stress and antioxidant defences: a target for the treatment of diseases caused by parasitic protozoa. **Molecular Aspects of Medicine, Oxidative Stress in Aging and Disease**, 25: 211-220.

Van Neikerk, W.A., Hassen A., Bechaz F.M. and Coertze R.J. (2007). Fermentative attributes of wilted vs. unwilted Digitaria eriantha silage treated with or without molasses at ensiling. **South African journal of Animal Science** 37:261-268.

Wang, S., Mengdie L.V., Yang J., Zhou Y. and Xu B. (2018). Effects and Mechanism of Metal Ions on Enxymatic Hydrolysis of Wheat Straw after Pretreatment. **Bioresources**, 13(2): 2617-2631.

Wang, Y., Frutos P., M.Y. Gruber, H. Ray, and McAllister T.A. (2006). In vitro ruminal

digestion of anthocyanidin-containing alfalfa transformed with the maize Lc regulatory gene. **Canada Journal of Plant Science**, 1119-1130.

Yahaya, M.S., Goto M., Yimiti W., Smerjai B. and Kawamoto Y. (2004). Additives effects of fermented juice of epiphytic lactic acid bacteria and acetic acid on silo fermentation and ruminal degradability of tropical elephant grass. **journal animal veterinary advances** 3: 115-121.

Yoshida, K., Mori M. and Kondo T. (2009). Review. Blue flower color development by anthocyanins: from chemical structure to cell physiology. **Natural Product Reports www.rsc.org/npr Current developments in natural products chemistry**, 26: 857-964.

CHAPTER III

EFFECT OF MOLLASSES AND $FESO_4$ ON FERMENTATION, ANTHOCYANIN STABILITY, NUTRIENT COMPOSITION, GAS PRODUCTION OF ANTHOCYANIN–RICH NAPIER GRASS SILAGE *IN VITRO*

3.1 Abstract

This study was conducted to evaluate effect of different levels of molasses and $FeSO_4$ on anthocyanin-rich Napier grass silage, the total was nine treatments were conducted in the *in vitro* to evaluate fermentation quality, nutrient composition and anthocyanin stable after ensiling twenty-one days. The experiment used a 3 x 3 Factorial design in Completely Randomized Design (factor A is molasses (M) and factor B is FeSO4 (Fe)). Addition of molasses and $FeSO_4$ affected on lactate, butyrate, ammonia-N, dry matter, Ash, organic matter, acid detergent fiber, pH, lactate, butyrate, ammonia-N, organic matter, crude protein, acid detergent fiber and crude fiber. Addition of molasses at 4% and $FeSO_4$ at 0.03 % improved anthocyanin level, silage quality, and nutrient composition. Gas production study was conducted to measure effects of different anthocyanin levels from three types of Napier grass Silage and Fresh Anthocyanin-rich Napier grass. There were four treatments including Napier grass silage (Negative control: T2), Anthocyanin-rich Napier grass silage (Positive control: T2), Anthocyanin-rich Napier grass silage with 4% molasses and

0.03% $FeSO_4$ (Treatment: T3), and Fresh Anthocyanin Napier grass (Fresh grass: T1). The results showed significant differences in in gas volume, propionic acid (C3), total volatile fatty acids (TVFAs), and the number of *Streptococcus bovis* but a decrease in pH value, acetic acid, acetate/propionate (C2/C3) ratio, Methanogen bacteria among treatments. Anthocyanin was stable in the rumen fluid after 24 hours of incubation. Anthocyanin-rich Napier grass silage added with molasses and $FeSO_4$ can apply for feeding trial.

Keywords: anthocyanin rich-Napier grass, molasses, $FeSO_4$, anthocyanin, silage quality, gas production technique.

3.2 Introduction

Anthocyanin-rich Napier grass is a cultivate of Napier grass with leaves and stems in purple color which pigment was made from anthocyanin to protect plant cell from UV radiation, oxidative stress from environmental pathogen and herbivores (Lev-Yadun et al., 2009). Napier grass is a good source of protein for ruminant but low soluble carbohydrate (Okaraonye et al., 2009). Besides, Napier grass grows in tropical and sub-tropical regions with a wide range of annual moisture from 750 to 2,500 mm rainfall (Skerman and Riveros, 1990) and has high production and short cutting cycle (Nyambati et al., 2010). On the other hand, silage is the best method to keep forage in a long time as a feed for small ruminant. Molasses was used as an additive in silage to made fermentation quickly by decrease pH and provide energy for lactic bacteria (Pipat et al., 2011). Anthocyanin-rich feedstuffs were easy to lost anthocyanin in ensiling progress (Song et al., 2012, Hosada et al., 2009) and stable when pH lower than 4. In addition, Iron (Fe) is an essential component of several

cytochromes and iron-sulfur proteins involved in the electron transport chain and several important biological processes (Parish and Rhine¬hart, 2008; Ganz and Nemeth, 2006). Level of $FeSO_4$ at 0.03%/DM was reported not effect on lamp performance and meat characteristics (Abdelrahim et al., 2012). Addition $FeSO_4$ in silage under fermentation, Fe^{2+} were received electron become Fe^{3+} by lactic bacteria (Scheers et al., 2016), then Fe^{3+} will combine with anthocyanidin become Metallo-anthocyanin compound (Yoshida et al., 2009, Scheers et al., 2016). Using molasses and $FeSO_4$ in silage will help anthocyanin stable better. Hence, this study were conducted to evaluate the effect of the additive on the stable of anthocyanin after 21 day ensiling and in gas production technique.

3.3 Objectives

3.3.1 To collect the suitable percentage of molasses, $FeSO_4$ which make good silage fermentation and high level of anthocyanin stability of anthocyanin-rich Napier grass silage at 21^{st} ensiling.

3.3.2 To evaluate the gas production of anthocyanin-rich Napier grass silage with the suitable percentage of molasses and $FeSO_4$ as compared with fresh anthocyanin-rich Napier grass, Napier grass silage, and anthocyanin-rich Napier grass silage.

3.3.3 To detect the degradation of anthocyanin component in rumen fermentation of anthocyanin-rich Napier grass silage with the suitable percentage of molasses and $FeSO_4$ as compared with fresh anthocyanin-rich Napier grass, Napier grass silage, and anthocyanin-rich Napier grass silage.

3.4 Materials and methods

3.4.1 Experiment 1.1

3.4.1.1 Anthocyanin component in anthocyanin - rich Napier grass silage preparation

Anthocyanin-rich Napier Grass (*Pennisetum Purpureum*) was used in this study. The Thai local cultivar was planted at the Suranaree University of Technology Farm in August 2016 and were harvested at 120 days (the first cutting) and after regrowth (60 days) and cut into 2-3 cm long for making silage. The materials were prepared in a 1 kg silage nylon bag, taken out the air by vacuum pumps, closed and then stored in a dark place at a room temperature (25^{o}C) for 21 days.

3.4.1.2 Chemical analysis

At 21st of ensiling, 500g of samples were dried immediately in an air-forced oven at 70ºC to constant weight to determine the dry matter (DM) content before being ground over a 1 mm screen using a Wiley hammer mill. Ash content was determined by combustion at 550ºC for 3 h in a muffle furnace. Ash-free neutral detergent fiber (NDF) was analyzed by a modified method of (Van Soest et al., 1991) with addition of a heat stable amylase, and ash-free acid detergent fiber (ADF) and acid detergent lignin (ADL) 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. Gross energy measured by 6100 Automatic Compensated Jacket Bomb Calorimeter (Parr Instrument Company, USA). Phenolic compound and condensed tannin were analysis follow the method of Makkar (2003). For the pH and

organic acid analysis, 100 gram of silage were mixed with 900 ml of pure de-ionized water and extracted at 4°C in a shaking incubator for 24 hours. The liquid extract was passed through a syringe filter paper and used for pH and organic acid analysis. The pH and organic acid were determined by pH meter and high-performance liquid chromatography (HPLC), respectively. The HPLC condition for analysis VFAs was shown in table 3.1.

Table 3.1 HPLC conditions for the analysis of organic acid.

Items	Conditions
Column	SBC18
Detector	UV, 240 nm
Flow rate	0.7 ml/min
Solvent	A: KH2PO4 0.1 mM pH 2.4
	B: Acetonitrile
Absorbance	240 nm
Injection volume	20µl

For anthocyanin analysis, 400 grams of sample was lyophilized, ground and extracted using 0.01N HCl-80% Methanol solution and determined by spectrophotometer and HPLC. The HPLC conditions for the analysis of anthocyanin was shown in Table 3.2. DPPH free radical scavenging activity was followed the method of Nile et al (2013).

Table 3.2 HPLC conditions for the analysis of anthocyanin.

Items	Conditions
Column	SBC18
Detector	UV, 520 nm
Flow rate	0.8 ml/min
Solvent	A: 10% acetic acid, 5% acetonitrile, and 1% phosphoric acid B: Acetonitrile
Absorbance	520 nm
Injection volume	20µl

3.4.2 Experiment 1.2

3.4.2.1 Animals

Three matured rumen fistulated crossed (Thai native x Angelo Nubian) breed male goats fed with 2.5% of body weight (% BW) DM/day containing Napier grass and commercial concentrate (16% crude protein) (60:40) were used as donors of rumen fluid.

3.4.2.2 In vitro gas technique

3.4.2.2.1 Reagents preparation

Preparation the reagents were follow the method of Menke and Steingass (1988) as below:

Buffer solution

- 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.

Macro-mineral solution

- 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.

Micro-mineral solution

- 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.

- **0.1% (wt/vol) Resazurin**

- Dissolve 0.1 g of resazurin 100 mL water.

- Store in dark (amber coloured) bottle at $4^{\circ C}$ (infridge).

3.4.2.2.2 Substrate preparation

Substrates were dry at 55°C until dry (~48 h) and ground with mill through 1 mm screen after that weigh 0.5 g of substrate into each syringe.

3.4.2.2.3 Source of rumen fluid for *in vitro* incubations

Rumen fluid were sampled before the morning feeding on the three goats and then placed in warm (39°C) insulated flasks under anaerobic conditions. All sample were pooled in equal proportions and strained through 4 layers of cheesecloth under anaerobic conditions and then use for anthocyanin degradability experiment and gas production technique. Rumen fluid were sampled before the morning feeding on the three goats and then placed in warm (39°C) insulated flasks under anaerobic conditions. All sample were pooled in equal proportions and strained through 4 layers of cheesecloth under anaerobic conditions and then use for anthocyanin degradability experiment and gas production technique.

3.4.2.3 Source of rumen fluid for in vitro incubations of anthocyanin degradability

Anthocyanin degradability were measured by gas technique method. Rumen fluid were obtained from Source of rumen fluid for in vitro incubations. The fluids were passed through 4 layers of cheesecloth in pour fluids in Erlenmeyer flask an equal volume. Culture fluid were prepared by rumen fluid and buffers (ratio 1:2), which were preliminarily purged with CO_2 gas.

3.4.2.4 Sample collection and processing

At pre-determined time points, headspace gas production (GP) were measured at 0, 3, 6, 9, 12, 24, 48, 72, and 96 h post incubation, using in vitro gas production of (Ørskov and McDonald, 1979). Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio *et al*(1999) as:

Gas volume = 0.18 + (3.697 × gas pressure) + (0.0824 × gas pressure2)

The kinetic parameters of GP were calculated using the equation of France *et al*(2000) as:

$$A = b \times (1- e^{-c(t-L)})$$

Where A is the volume of GP at time t; b is the asymptotic GP (mL/g DM); c is the rate of GP (/h), and L (h) is the discrete lag time prior to gas produced.

3.4.2.5 Gas components, VFAs, DNA extraction and anthocyanin

For gas composition analysis by Gas Chromatography Aligent 6780. The gas at 6, 12, and 24H were removed by syringe with cap and detected directly by Gas Chromatography machine every time for getting the sample. Each sample was measured for 12 min. pH and VFAs detected at 6, 12, 24, and 48 H. Sample was taken out and filter with 4 layer cheeses cloth, detect pH, and 16ml fluid will mix 4 ml HCl 20%, 2ml was centrifuge at 10000 rpm in 10 min at 4^{o}C, the supernatant filter by filter syringe 0.45 um and detect by High Performance Liquid Chromatography Aligent 1260 FLD (table 3.2). DNA extraction followed QIAamp PowerFecal DNA kit (Catalog Number: 12830-50, QIAGEN company, USA). The sequence of nine genes and PCR temperature condition as shown in table 3.3.

Anthocyanin in the in vitro incubation were performed in a glass tube containing 1 g of grass silage with 50 ml of culture fluid for 0, 6, 12, 24, and 48 h. The tube were kept at 38^{o}C in a shaking incubator and purged continuously with CO_2 gas. After incubation, the fermentation were stopped by sinking the tube into ice-cold water and the pH of the fluid were measured.

3.5 Statistical Analysis

For experiment 1.1, the data used for the statistical analyses consisted of 3 levels of molasses supplementation, 3 levels of $FeSO_4$ supplementation, 3 replications, and runs making a total of 27 observations. All obtained data were subjected to the general linear models procedures of the Statistical Analysis System Institute (SAS, 1998) according to a 3×3 factorial arrangement in CRD. The statistical model including molasses level, $FeSO_4$ level and interaction effects were: $Yij = \mu+Ai+Bj+ABij+\varepsilon ij$; where Yijk is an observation, μ is the overall mean, A is molasses level effect (i = 1, 2, 3), B is $FeSO_4$ level effect (j = 1, 2, 3), A*B is interaction effect of molasses level and $FeSO_4$ level, and εij the residual effect. Multiple comparisons among treatment mean were performed by Duncan's New Multiple Range Test (DMRT) and orthogonal contrast (Steel and Torrie, 1980) was used for trend analysis after interaction between 2 factors were detected. Significant differences were based on a probability level <0.05. For experiment 1.2, the data obtained from the experiment were statistically subjected to ANOVA and analyzed as a Completely Randomized Design (CRD) design using the PROC GLM (SAS, 1998). Significant differences between treatments were determined using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980) and orthogonal contrast was used for trend analysis. The following statistical model was used: $Yijk = \mu+ \tau i + \varepsilon ij$ .Where Yij = represents of observation from animals; μ= overall mean; τi= Effect of treatment (i = 1-3); εij= Error of the term.

Table 3.3 Gene sequence.

Items	Forward/Reward	primer sequence (5'- 3')	Temperature (°C)	References
Total bacteria	F	CGGCAACGAGCGCAACCC	55	Koike et al(2001)
	R	CCATTGTAGCACGTGTGTAGCC		
Ruminococcus albus	F	CCCTAA AAGCAG TCTTAGTTCG	55	Koike et al(2001)
	R	CCTCCTTGCGGTTAGAACA		
Ruminococcus flavefaciens	F	TCTGGAAACGGATGGTA	60	Koike et al(2001)
	R	CCTTTAAGACAGGAGTTTACAA		
Fibrobacter succinogens	F	GTTCGGAATTACTGGGCGTAAA	55	Lane (1991)
	R	CGCCTGCCCCTGAACTATC		
Methanogen	F	TTCGGTGGATCDCARAGRGC	58	Denman et al., (2006)
	R	GBARGTCGWAWCCGTAGAATC		
Protozoa	F	CTTGCCCCTCYAATCGTWCT	55	Sylwesters etal. (2004)
	R	GCTTTCGWTGGTAGTGTATT		
Butyrivibrio fibrisolvens	F	ACACACCGCCCGTCACA	58	Klieve et al(2003)
	R	TCCTTACGGTTGGGTCACAGA		
Megasphaera elsdenii	F	GACCGAAACTGCGATGCTAGA	59	Klieve et al(2003)
	R	CGCCTCAGCGTCAGTTGTC		
Streptococus bovis	F	GCCA GGCTATTTAGGTGACACTATAG	58	Ghali et al(2004)
	R	GGGT AATACGACTCACTATAGGG		

3.6 Treatment

3.6.1 Experiment 1

Total is nine treatments was shown below:

T1 (control): Anthocyanin-rich Napier grass silage no additive

T2 (M4%): Anthocyanin-rich Napier grass silage with 4% molasses

T3 (M8%): Anthocyanin-rich Napier grass silage with 8% molasses

T4 (F0.015%): Anthocyanin-rich Napier grass silage with 0.015% $FeSO_4$

T5 (F0.03%): Anthocyanin-rich Napier grass silage with 0.03% $FeSO_4$

T6 (M4%F0.015%): Anthocyanin-rich Napier grass silage with 4% molasses and 0.015% $FeSO_4$

T7 (M4%F0.03%): Anthocyanin-rich Napier grass silage with 4% molasses and 0.03% $FeSO_4$

T8 (M8%F0.015%): Anthocyanin-rich Napier grass silage with 8% molasses and 0.015% $FeSO_4$

T9 (M8%F0.03%): Anthocyanin-rich Napier grass silage with 8% molasses and 0.03% $FeSO_4$.

3.6.2 Experiment 2

Total is four treatments:

T1: Fresh Anthocyanin rich Napier grass

T2: Napier Grass silage

T3: Anthocyanin rich Napier grass silage without additive

T4: Anthocyanin rich Napier grass silage with additive (level of molasses and $FeSO_4$ was selected from experiment 1.1).

3.7 Experimental site

The experiment was conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

3.8 Duration

The duration of the present experiment was from October 2016 to March 2016.

3.9 Results and Discussions

3.9.1 Experiment 1.1

3.9.1.1 Chemical composition

The chemical composition of nine treatments was shown in table 3.4. Molasses and $FeSO_4$ effected on dry matter, crude protein, ammonia nitrogen, neutral detergent fiber, acid detergent fiber, ether-extract and gross energy. When increasing the level of molasses and $FeSO_4$, DM value of treatments is higher than control ($P<0.05$) but OM was not affected by the additive. The value of NDF, ADF, EE, and GE in the contrast way with DM and CP. When supplement of molasses, silage fermentation is better with high value of lactate and decreasing of NDF, ADF can explain that addition of molasses to silage and stimulating fermentation, increase the population of lactic acid bacteria, improve quality of silage and avoid of losses dry matter (McDonald et al., 2002). Addition and mixing some additives to forage before ensiling resulted to increase dry matter content of silage due to improving quality of fermentation (Harrison and Blauwiekel, 1994). EE and GE losses were explained that in the second phase of silage fermentation, lactic acid bacteria used water-soluble carbohydrate from substrate to mainly lactic acid, they

made losing of the gross energy and ether extract from silage. This result is different with the study of Sakhawat (2011), he reported that no effect on gross energy loss of clover grass silage (22 % DM in fresh grass) with the additive.

3.9.1.2 Phenolic compound and DPPH

Table 3.5 present the data of phenolic compound and DPPH (% inhibition rate). Additive effected on the percentage of the total phenolic compound, total tannin, non-phenolic compound, condensed tannin, and DPPH value. Increase level of molasses and $FeSO_4$, the level of the total phenolic compound, total tannin and condensed tannin were improved, but DPPH was decreased. Song et al. (2012) reported that antioxidation ability as the content of anthocyanins is high and anthocyanin content has a significant relationship on antioxidant activity. Alonzo-Macías et al. (2013) reported that the antioxidant activity of strawberries was directly correlated with anthocyanin content in the fruit. In this study, antioxidant activity and anthocyanin content have a negative correlation. These results showed that if addition molasses and $FeSO_4$ changing chemical compositions can prevent a decrease in anthocyanin content but the compound of Fe^{3+} and anthocyanin, which affected on the antioxidant capacity or the DPPH value.

3.9.1.3 pH, lactic acid and volatile fatty acids

The data of pH and volatile fatty acids in table 3.6 showed that addition molasses and $FeSO_4$ effect on pH, lactic acid, and volatile fatty acid. According to Catchpoole et al. (1971) quality silage has a pH at 4.2 or below, lactic acid between 3-13%, a butyric acid concentration less than 0.5%, acetic acid from 1-3 %, propionic acid less than 1% and ammonia nitrogen less than 12%. These results presented in table 3.6 show that the silage pH of treatments no added molasses were higher 4.2, the butyric less than 0.5% and lactic acid were from 5 to 8%. Addition of

molasses and $FeSO_4$ increased the number of lactic acids higher than 13% and decreased pH lower than 4.2. However, acetic acid, propionic acid, and butyric acid were followed by the standard. In contrast, ammonia nitrogen in treatments no molasses were higher the standard. We can explain Anthocyanin-rich Napier grass is a cultivar of Napier so they were high in fiber and low in soluble-carbohydrate when ensiling, their pH was still high before ensiling (5.67) and appearance of water in the grass made protein breakdown to increase the number of ammonia nitrogen.

In addition, high level of butyric showed an association with protein degradation. Yunus et al. (2000) indicated that Napier grass was known to have a high moisture content, an effective procedure in silage making. Water soluble carbohydrate concentration in fresh grass give a high probability of lactate type silage and preservation of silage being better (Wilkinson, 1983). Molasses is used in silage making was found to produce more lactate and less acetate and ammonia nitrogen than untreated treatment (McDonald et al., 1991).

Table 3.4 Anthocyanin rich Napier grass silage chemical composition (%DM).

Item	%DM	% DM						
		OM	CP	NH3-N	NDF	ADF	EE	GE(kcal/kg)
T1 (CONTROL)	15.45^{f}	85.97^{ed}	6.64^{e}	0.03^{a}	78.50^{a}	47.04^{a}	4.60^{d}	4107.85
T2 (M4%)	17.00^{de}	86.00^{cd}	7.18^{d}	0.02^{b}	71.06^{bc}	39.88^{cd}	4.34^{f}	4082.99
T3 (M8%)	18.07^{bc}	86.09^{a}	7.34^{cd}	0.01^{c}	67.71^{c}	37.09^{e}	4.24^{g}	4090.81
T4 (F0.015%)	16.26^{e}	86.12^{cde}	6.33^{f}	0.02^{b}	77.87^{a}	44.07^{b}	4.2^{h}	4029.20
T5 (F0.03%)	15.38^{f}	83.45^{f}	6.42^{ef}	0.02^{b}	77.94^{a}	46.81^{b}	4.72^{a}	4094.04
T6 (M4%F0.015%)	17.34^{cd}	86.8a^{bc}	7.18^{d}	0.02^{b}	74.64^{ab}	40.93^{c}	4.48^{i}	4029.92
T7 (M4%F0.03%)	17.61^{cd}	86.87^{ab}	7.70^{b}	0.02^{b}	70.54^{bc}	38.64^{d}	4.47^{b}	4058.45
T8 (M8%F0.015%)	19.46^{a}	85.49^{e}	8.37^{a}	0.02^{b}	62.87^{d}	34.92^{e}	4. 78^{c}	4009.45
T9 (M8%F0.03%)	18.59^{b}	86.05^{ed}	8.53^{bc}	0.02^{b}	63.31^{d}	36.40^{f}	4.54^{e}	4003.46
SEM	0.08	0.08	0.03	0.01	0.48	0.14	0.00	1.35
P VALUE	<0.0001	<0.0001	<0.0001	0.01	<0.0001	<0.0001	<0.0001	0.46
CONTRAST								
CON VS SUPP	**	NS	**	**	**	**	**	NS
CON VS M	**	NS	**	**	**	**	**	NS
CON VS F	**	NS	**	**	**	**	**	NS
CON VS MF	**	NS	**	**	**	**	**	NS
M4% VS M8%	**	*	**	*	**	**	**	NS
F0.015% VS F0.03%	*	*	NS	NS	NS	NS	**	NS

$^{a, b, c, d, e, f, g, h, i}$ mean in the same column with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.05$ and $P<0.01$, respectively). NS mean not significant. M= molasses, F= FeSO4, SUPP= supplement, DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, NH3-N= ammonia nitrogen, GE=gross energy. SEM: standard error of the mean.

3.9.1.4 Anthocyanin component

Anthocyanin content of Anthocyanin-rich Napier grass silage was shown in table 3.7. Addition of molasses and $FeSO_4$ affected on total anthocyanin of treatment with the additive as compared with control. Increasing level of molasses and $FeSO_4$ will enhance anthocyanin but no difference between 2 level of molasses and $FeSO_4$ addition on total anthocyanin. No difference was found in Cyanidin and Pelarnidin. When a combination of molasses and $FeSO_4$ helped pH decrease quickly and maintain anthocyanin in silage. Sugar in molasses blocked the activity of water and enhanced the activity of lactic bacteria by providing an energy source. High level of molasses made pH drop less than 4 but silage was found much wet but kept anthocyanin stable better. $FeSO_4$ addition in silage played a role like salt to absorbed water from cell membrane and inhibit the activity of negative bacteria with lactic bacteria. In addition, under fermentation, Fe^{2+} from $FeSO_4$ received electron become Fe^{3+} by lactic bacteria (Scheers et al., 2016), then Fe^{3+} combined with anthocyanin become Metallo-anthocyanin compound (Yoshida et al., 2009, Scheers et al., 2016). No difference was found between two level of molasses and FeSO4 on total anthocyanin but strong effect on pelargonidin-3-glucoside (P3G), delphinidin (Del), and peonidin-3-o-glucoside (Peo3G). Three type of anthocyanins are sensitive with the changing of pH and they degraded quickly by high temperature (Song et al., 2013) and pH (Hosada et al., 2009; Song et al., 2012).

Table 3.5 Anthocyanin rich Napier grass silage phenolic compound (%DM).

Items	%DM				
	TOTAL PHENOL	**TOTAL TANNIN**	**NON PHENOL**	**CONDENSED TANNIN**	**DPPH (% inhibition rate)**
T1 (CONTROL)	9.13^{g}	2.54^{e}	6.59^{g}	0.10^{e}	87.54^{e}
T2 (M4%)	11.46^{d}	2.75^{a}	8.71^{d}	0.13^{c}	85.59^{bc}
T3 (M8%)	12.12^{c}	2.89^{g}	9.23^{c}	0.16^{a}	82.05^{d}
T4 (F0.015%)	8.70^{f}	2.59^{d}	7.23^{f}	0.11^{d}	87.64^{a}
T5 (F0.03%)	9.01^{h}	2.72^{b}	6.29^{h}	0.11^{d}	87.78^{a}
T6 (M4%F0.015%)	9.82^{e}	2.64^{c}	8.14^{e}	0.10^{e}	85.04^{c}
T7 (M4%F0.03%)	10.78^{i}	2.73^{b}	5.97^{i}	0.14^{b}	85.45^{bc}
T8 (M8%F0.015%)	13.99^{a}	2.59^{d}	11.40^{a}	0.11^{d}	82.02^{b}
T9 (M8%F0.03%)	12.99^{b}	2.45^{d}	10.54^{b}	0.14^{b}	82.74^{d}
SEM	0.005	0.001	0.004	0.002	0.155
P VALUE	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CONTRAST					
CON VS SUPP	**	NS	**	**	**
CON VS M	**	**	**	**	**
CON VS F	**	**	**	**	**
CON VS MF	**	**	**	**	**
M4% VS M8%	**	**	**	**	**
F0.015% VS F0.03%	**	**	**	**	**

$^{a, b, c, d, e, f, g, h, i}$ mean in the same column with different superscripts are significantly different ($p<0.05$).*,** mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant, M= molasses, F= $FeSO_4$, SUPP= supplement, MF=molasses & $FeSO_4$. SEM: standard error of the mean.

Table 3.6 Anthocyanin rich Napier grass fermentation quality.

Items	pH	%DM			
		Lactic acid	Acetic acid	Propionic acid	Butyric acid
T1 (CONTROL)	4.76^{a}	5.51^{c}	2.27^{c}	0.01^{c}	0.39^{c}
T2 (M4%)	3.79^{d}	15.90^{bc}	3.65^{b}	4.43^{bc}	0.29^{c}
T3 (M8%)	3.65^{e}	29.84^{ab}	4.83^{ab}	5.23^{ab}	1.14^{a}
T4 (F0.015%)	4.68^{b}	6.33^{bc}	3.62^{c}	0.01^{c}	0.36^{c}
T5 (F0.03%)	4.51^{c}	8.13^{bc}	2.76^{c}	0.01^{c}	0.29^{c}
T6 (M4%F0.015%)	3.80^{d}	22.36^{ab}	4.98^{b}	4.73^{bc}	0.31^{c}
T7 (M4%F0.03%)	3.83^{d}	35.04^{ab}	4.78^{ab}	5.46^{ab}	0.59^{b}
T8 (M8%F0.015%)	3.70^{e}	37.22^{a}	6.16^{ab}	5.92^{ab}	0.01^{d}
T9 (M8%F0.03%)	3.69^{e}	39.32^{a}	6.87^{a}	6.59^{a}	0.01^{d}
SEM	0.008	0.495	0.250	0.170	0.150
P VALUE	<0.0001	<0.0001	0.02	<0.0001	<0.0001
CONTRAST					
CON VS SUPP	**	**	*	**	NS
CON VS M	**	**	*	**	NS
CON VS F	**	**	*	**	*
CON VS MF	**	**	*	**	**
M4% VS M8%	**	**	NS	*	NS
F0.015% VS F0.03%	*	**	NS	NS	NS

$^{a, b, c, d, e}$ mean in the same column with different superscripts are significantly different ($p<0.05$).*,** mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant, M= molasses, F= $FeSO_4$, SUPP= supplement, MF=molasses & $FeSO_4$.
SEM: standard error of the mean.

Table 3.7 Anthocyanin content (%DM) of anthocyanin rich Napier grass silage**.**

Items	C3G (mg/g)	P3G (mg/g)	Del (mg/g)	Peo3G (mg/g)	M3G (mg/g)	Cya (mg/g)	Pel (mg/g)	Mal (mg/g)	Total (mg/g)
T1 (CONTROL)	0.0144^{b}	0.0210^{f}	0.0489^{f}	0.0473^{c}	0.0405^{d}	0.0743	0.0047	0.0004^{bc}	0.2501^{c}
T2 (M4%)	0.0331^{b}	0.0338^{d}	0.0857^{cd}	0.0983^{cd}	0.0819^{bc}	0.1509	0.005	0.0006^{b}	0.4889^{ab}
T3 (M8%)	0.0841^{a}	0.0398^{bc}	0.0979^{bc}	0.1355^{ab}	0.0792^{bcd}	0.1712	0.0055	0.0005^{bc}	0.6132^{a}
T4 (F0.015%)	0.0154^{b}	0.0236^{ef}	0.0609^{ef}	0.0609^{e}	0.0525^{cd}	0.0373	0.0019	0.0001^{d}	0.2525^{c}
T5 (F0.03%)	0.033^{b}	0.0291^{de}	0.0716^{de}	0.0766^{de}	0.0595^{bc}	0.0712	0.005	0.0001^{d}	0.3459^{bc}
T6 (M4%F0.015%)	0.0334^{b}	0.0344^{cd}	0.0852^{cd}	0.1196^{bc}	0.0797^{ab}	0.1744	0.0066	0.0006^{b}	0.5334^{a}
T7 (M4%F0.03%)	0.0216^{b}	0.0412^{b}	0.0982^{bc}	0.1326^{abc}	0.0945^{a}	0.1867	0.0078	0.0007^{ab}	0.5826^{a}
T8 (M8%F0.015%)	0.0497^{ab}	0.0455^{ab}	0.1129^{b}	0.1301^{abc}	0.1078^{a}	0.1704	0.0112	0.0007^{ab}	0.6277^{a}
T9 (M8%F0.03%)	0.0459^{ab}	0.0495^{a}	0.1363^{a}	0.1605^{a}	0.1025^{ab}	0.1176	0.0067	0.0009^{a}	0.6189^{a}
SEM	0.013	0.005	0.008	0.012	0.009	0.022	0.007	0.032	0.026
P VALUE	0.012	<0.0001	<0.0001	<0.0001	<0.0001	0.125	0.606	0.000	0.001
CONTRAST									
CON VS SUPP	NS	**	**	**	**	NS	NS	NS	**
CON VS M	*	**	**	**	**	*	NS	*	**
CON VS F	NS	**	**	**	**	NS	NS	NS	**
CON VS MF	NS	**	**	**	**	*	NS	**	**
M4% VS M8%	*	**	**	*	NS	NS	NS	NS	NS
F0.015% VS F0.03%	NS	**	**	*	NS	NS	NS	*	NS

[a, b, c, d, e, f] mean in the same column with different superscripts are significantly different ($p<0.05$).*,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant, M= molasses, F= $FeSO_4$, SUPP= supplement, MF=molasses & $FeSO_4$. C3G: cyanidin-3-glucoside, P3G: pelargonidin-3-glucoside, Del : delphinidin, M3G : malvidin-3-O-glucoside, Cya : cyanidin, Pel : pelargonidin, Peo3G : peonidin-3-o-glucoside, Mal : malvidin. SEM: standard error of the mean.

There were many factors affecting the stability of anthocyanin the factors, oxygen, light, high temperature, and high pH were associated with anthocyanin stability during the ensiling period (Francis, 1989; Mazza and Miniati, 1993). In this study, oxygen, light, and high temperature could not affect anthocyanin content during the ensiling period because the silage kept in a nylon bag was stored in a dark place at an ambient temperature. Cevallos-Casals and Cisneros- Zevallos (2004) reported that the pH affected anthocyanin stability in an extract form anthocyanin-rich corn, and anthocyanin was degraded above pH 3. In this study, all silage pH value was from 3.65 to 4.67 and therefore, the decrease of anthocyanin content was attributed to the pH condition of the treatment.

3.9.1.4 Conclusion

Addition 4% molasses and 0.03% $FeSO_4$ is the best treatment because high in total anthocyanin after 21 days ensiling. In addition, pH, lactic acid, dry matter, crude protein, and DPPH is better than others.

3.9.2 Experiment 1.2

3.9.2.1 Gas volume and gas composition

Table 3.8 show the chemical composition of four treatments, the gas production of 4 treatments is presented in Table 3.8, and gas composition was indicated in table 3.9. The results of gas production characteristics demonstrated that soluble fraction of OM (a) and potential degradability (a+b) fractions of DM are not different between two groups: fresh Anthocyanin rich Napier grass in comparing to silage and Napier silage in comparing to Purple Napier silage. The differences was found between Anthocyanin Napier grass silage no additive and additive ($P<0.01$).

The degradation rate of insoluble fraction (c) was not different. Gas volume of 4 treatments was different at 3H, 6H, and 9H from 12H ($P<0.05$) but no difference was found between fresh Anthocyanin rich Napier grass and silage, but up to 48H and 96 H, gas volume is significantly different again. The high gas volume was found in treatment T1 and T4, which are high anthocyanin. This results are in agreement with the study of Spanghero et al. (2009) on gas production of Grape pulp and grape seed with different level of anthocyanin in substrate. Ray et al. (2003) conducted one study about three anthocyanin regulatory genes of maize in in vitro gas production show the similarly trend of results at treatment high anthocyanin.

Methane gas (CH_4) of treatments with additive show amount of CH_4 is significantly smaller than treatment no additive. But no difference was found between fresh grass and silage. Carbonic dioxide is the most compound in gas produced from rumen fermentation. This makes the aerobic condition for microbe population work to digest substrate. CH_4 is produced principally from microbial fermentation of hydrolyzed dietary carbohydrates such as cellulose, hemi-cellulose, pectin and starch in the rumen and emitted primarily by eructation. The primary substrates for ruminal methanogenesis are hydrogen and CO_2. Most of the hydrogen produced during the fermentation of hydrolyzed dietary carbohydrates, much of which is generated during the conversion of hexose to acetate or butyrate, ends up in CH_4 (Broocek, 2015). Significant quantities of CH_4 can also arise from microbial fermentation of amino acids, the end products of which are ammonia, volatile fatty acids, CO_2 and CH4.

Gas volume and gas production characteristics as the good parameters in vitro trial, which can predict fermentation, digestibility, microbial and end-product for the

host animal (Sommart et al., 2000). The results of this trial indicated gas volumes before 96 h (consist of 3, 6, 9, 12, 24, 48, 72, 96, respectively) incubation, in T1 and T4, gas production was highest than T2 and T3. In the fresh grass, sugar does not lose so this is the source of energy for microbe population to use for fermentation. In the silage, the carbohydrate was used by lactic bacteria to reduce pH and inhibit the activity of yeast and other factor effect silage quality. Yunus et al (2000) indicated that Napier grass was known to have a high moisture content, an effective procedure in silage making. Water soluble carbohydrate concentration in fresh grass give a high probability of lactate type silage and preservation of silage being better (Wilkinson, 1983). Molasses is used in silage making was found to produce more lactate and less acetate and ammonia nitrogen than untreated treatment (McDonald et al., 1991). When we add 4% molasses in treatment T4, this energy use for refill the sugar loss in the ensiling process, so the gas volume of this treatment was similar with Fresh Grass. We can found the same result at a value, but at b, c and |a|+|b| value were not effect by treatment, it mean the trend of gas kinetic is similar, they are different on the starting point.

3.9.2.2 pH, Ammonia nitrogen and Volatile fatty acid

The value of C2, C3, C4, pH, TVFAs, and C2/C3 were shown in table 3.11. The contents of the C2, C3, C4 at 6, 12, 24 and 48Hh were significantly different (P>0.05) between 4 treatments. The pH was not different at 6H but at 12h, 24h, and 48h, the low pH in T1 and T4 was found. TVFAs at 6, 12, 24 and 48h were significant differences between 4 treatments and there was an increase in value by the time of incubation. The C2/C3 ratio at 12h, 24h, and 48h were found lowest in T4 and

highest in T2. Leatherwood (2014) reported there has a relationship between the acetate to propionate ratio to methane production. Decreased methane production is common with increased feed intake but it is also common with a decrease in the acetate to propionate ratio (Russell, 1998). In table 3.10, at 24h of incubation, methane production of T4 is the lowest value, it can explain why the C2/C3 ratio of T4 is lower than others.

In addition, VFAs was mainly concluding of acetic acid, propionic acid, and butyric acid, they are an important intermediate metabolite when the host utilized energy, which was mainly energy storage, growth, reproduction and lactation and other basic activities for ruminants (Hosada et al., 2009). In in vivo study of Hosada et al. (2012b), there were no effects of the control treatment and anthocyanin-rich cornsilage treatment on ruminal pH, total and individual VFA concentrations and acetate–propionate ratio. By contrast, in vitro study of Leatherwood (2014), the results were no different on acetate and propionate proportions of the total VFA but the type of purple-fleshed sweet potatoes had effected on butyrate acid proportion. The same difference was found in the second experiment of Leatherwood (2014) about the effect of sample weight on gas production.

The pH value is of crucial importance indicator, which was markedly affected ammonia concentration, acetate: propionate ratio deamination and ruminal methane production (Lana et al., 1998). The pH at 24H of T1 was significantly different with silage group, the similar result was found in compare T3 and T4. The pH depends on the concentration of VFAs, by the time of incubation, the concentration of VFAs was higher, so pH will drop following the time of incubation and TVFAs.

Table 3.8 Chemical composition of four treatments.

Items	T1	T2	T3	T4
%Dry Matter (DM)	15.43	16.18	15.32	16.99
		%DM		
ASH	16.19	16.56	13.99	12.95
Organic Matter (OM)	83.81	83.44	86.01	87.05
Crude Protein (CP)	7.62	7.22	7.38	7.85
Neutral Detergent Fiber (NDF)	79.49	78.22	79.71	72.11
Acid Detergent Fiber (ADF)	49.01	46.72	47.73	41.28
Hemicellulose	35.5	31.5	31.98	30.83
Crude Fiber (CF)	47.04	48.14	47.31	38.95
Ether Extract (EE)	2.19	4.2	4.6	5.48
Anthocyanin	0.12	0.01	0.03	0.06
Phenolic Compound	9.86	3.72	9.12	9.72
Total Tannin	2.62	2.05	2.54	2.73
Non Phenolic Compound	7.23	1.68	6.59	6.99
Condensed Tannin	0.09	0.09	0.1	0.11
Gross Energy (GE) (kcal/kg DM)	4188.45	4141.39	4191.83	4105.05

Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.9 Gas kinetic of four treatments.

Items	T1	T2	T3	T4	SEM	Treatment	P value		
							Fresh vs silage	Napier vs Purple	Purple vs additive
a	6.40[a]	5.07[b]	5.90[b]	7.96[a]	0.402	0.018	NS	NS	**
b	103.17	100.57	102.16	98.05	0.936	0.509	NS	NS	NS
c	0.05	0.05	0.05	0.05	0.003	0.687	NS	NS	NS
a+b	109.05	105.65	107.06	106.01	0.951	0.568	NS	NS	NS
Gas volume (ml)									
3h	22.5 [a]	10.00[c]	13.67[b]	22.50[a]	0.323	<0.0001	**	**	**
6h	34.67[a]	17.67[c]	23.33[b]	36.00[a]	0.771	0.001	**	**	**
9h	45.00[a]	27.67[b]	34.67[b]	47.83[a]	1.313	0.014	*	**	**
12h	55.33[ab]	37.33[c]	45.67[bc]	58.67[a]	1.757	0.044	NS	**	*
24h	80.00[a]	61.33[b]	70.00[ab]	80.00[a]	2.062	0.046	NS	*	NS
48h	103.00[a]	87.33[c]	91.67[bc]	97.67[ab]	1.104	0.026	**	*	NS
72h	106.67[a]	90.00[c]	97.00[b]	103.33[ab]	0.949	0.009	**	**	NS
96h	108.00[a]	91.33[c]	99.67[b]	108.17[a]	1.03638	0.009	**	*	*

[a, b, c, d] mean in the same row with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.10 Gas component of four treatments.

items	Time	T1	T2	T3	T4	SEM	Treatment	P value		
								Fresh vs silage	Napier vs Purple	Purple vs additive
CH_4	6h	25.35^{b}	27.76^{a}	23.92^{c}	24.96^{b}	0.0604	<0.0001	NS	**	**
	12h	25.69^{b}	27.11^{a}	27.3^{a}	25.69^{b}	0.1403	0.0211	*	NS	*
	24h	25.56^{b}	26.29^{a}	26.75^{a}	25.02^{b}	0.0848	0.0033	NS	NS	**
CO_2	6h	51.74^{c}	49.79^{d}	57.53^{a}	55.63^{b}	0.1129	<0.0001	**	**	**
	12h	53.65	55.68	52.1	54.5	0.4387	0.1567	NS	NS	NS
	24h	54.46^{a}	53.19^{b}	52.7^{b}	55.6^{a}	0.1820	0.0105	NS	NS	**
O_2	6h	2.81^{a}	0.34b	0.48^{b}	0.48^{b}	0.0474	<0.0001	**	NS	NS
	12h	0.92^{a}	0.11^{d}	0.30^{c}	0.47^{b}	0.0174	<0.0001	**	**	*
	24h	0.43	0.48	0.39	0.49	0.0374	0.6389	NS	NS	NS
N_2	6h	1.19^{b}	1.45^{a}	0.21^{c}	0.44^{c}	0.0332	<0.0001	**	**	NS
	12h	0.60	0.39	0.33	0.32	0.0584	0.3239	NS	NS	NS
	24h	0.49	0.27	0.32	0.32	0.0712	0.7306	NS	NS	NS
CO	6h	18.91^{b}	20.66^{a}	17.86^{d}	18.5^{c}	0.0443	<0.0001	NS	**	**
	12h	19.15^{ab}	16.72^{b}	19.96^{a}	19.02^{ab}	0.4035	0.1633	NS	NS	NS
	24h	19.05^{b}	19.77^{a}	19.84^{a}	18.58^{c}	0.0636	0.0027	NS	*	**

$^{a, b, c, d}$ mean in the same row with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

NH3-N concentration was found highly significant differences in the treatments T2 at 24h after incubation as compared to T3 and T4 ($P<0.01$). The value of NH_3-N in treatment T1 is the lowest among four treatments with $P<0.05$ at 6h, 12h, and 24h. The availability of NH_3-N is an important determinant of microbial protein production as the majority of rumen bacteria use NH_3 as a nitrogen source (Phesatcha et al., 2016). It is essential to know what concentration of NH_3-N will support maximal microbial growth in order to make judgments regarding utilization of non-protein N. The NH3-N concentration of all treatments ranged from 11.22 to 18.52 mg/dL (Table 3.10).

However, Satter and Slyter (1974) suggested NH_3-N concentrations from 3 to 5 mg/dL as optimal to produce ruminal microorganism growth, which was relatively less than those observed in this study. It appears that, once NH_3 starts to accumulate, the growth of bacteria utilizing NH_3 is not enhanced by increasing NH_3 concentration (Satter and Slyter, 1974).

3.9.2.3 Microbe population

The microbe population was shown in table 3.11, 3.12, and 3.13. No different was found between treatments for total bacteria, *Ruminococcus albus, Fibrobacter succinogens, Butyrivibrio fibrisolvens, Megasphaera elsdenii, Ruminococcus flavefaciens* and protozoa at 24h. But the group of methanogen bacteria was significantly different between T2 with T3 and T4 at 24h. The number of *Streptococcus bovis* copy, which type of bacteria produce lactic acid was significantly different between fresh grass and silage, because in silage the water soluble carbohydrate was used by lactic acid bacteria during fermentation time, so the number of this bacteria in silage were lower. There is an interesting observed about correlation

of anthocyanin amount in substrate on *Streptococcus bovis* population. When the level of anthocyanin was increased, the number of copies was be higher. This evidence suggest that anthocyanin effect on this group of *Streptococcus bovis.* In contrast, the amount of Methanogen bacteria in T2 is the highest. These results demonstrated that anthocyanin effect slightly on methane production of rumen fermentation, the treatment T2 with low anthocyanin so CH_4 production is higher than others.

3.9.2.4 Anthocyanin degradation

The degradation of anthocyanin of 4 treatments after incubation showed not significant on changing amount of anthocyanin from 0h, 3h, 6h, 9h, 12h, and 24h. We can conclude anthocyanin cannot be absorbed in the rumen, this result is similar with the study of Song et al (2012), Hosada et al. (2009), and Leatherwood (2010). Hosada et al. (2009) reported that anthocyanin in anthocyanin-rich corn silage was not decomposed by ruminal fluid, which suggests that the anthocyanin-rich corn is appropriate to provide a functional substance for ruminants. Song et al. (2012) reported anthocyanin content in colored barley was not affected in the ruminal fluid. Leatherwood (2014) presented that anthocyanin extract from purple-fleshed sweet potatoes degraded significantly after 24 hours incubation in rumen fluid. He explained the difference about the form of anthocyanin were incubated with rumen fluid related with the degradation of anthocyanin in rumen fermentation. In this study, anthocyanin in Anthocyanin rich Napier grass was not degraded in rumen fermentation.

Table 3.11 Volatile fatty acid, pH, and NH3-N of four treatments.

Items	Time	T1	T2	T3	T4	P value				
						SEM	Treatment	Fresh vs silage	Napier vs Purple	Purple vs additive
Acetate %	6h	55.93^{a}	54.36^{b}	52.84^{c}	52.30^{c}	0.1633	0.0024	**	**	NS
	12h	55.14^{c}	56.84^{b}	57.70^{a}	51.54^{d}	0.1145	<0.0001	NS	**	**
	24h	55.58^{c}	58.37^{a}	56.05^{b}	52.32^{d}	0.0438	<0.0001	NS	**	**
Propionate %	6h	26.01^{b}	34.60^{a}	36.00^{a}	34.87^{c}	0.2198	<0.0001	**	NS	NS
	12h	34.38^{b}	31.41^{d}	32.75^{c}	38.37^{a}	0.0666	<0.0001	NS	**	**
	24h	34.16^{b}	30.81^{d}	32.46^{c}	37.60^{a}	0.0418	<0.0001	**	**	**
Butyrate %	6h	18.06^{a}	11.04^{c}	11.16^{c}	12.82^{b}	0.0434	<0.0001	**	*	**
	12h	10.48^{b}	11.75^{a}	9.54^{c}	10.09^{bc}	0.0511	0.0083	NS	**	NS
	24h	10.26^{c}	10.82^{b}	11.49^{a}	10.08^{c}	0.0106	0.0010	*	NS	**
C2/C3	6h	2.15^{a}	1.57^{b}	1.47^{b}	1.50^{b}	0.0014	0.0002	**	NS	NS
	12h	1.60^{c}	1.81^{a}	1.76^{b}	1.34^{a}	0.0001	<0.0001	*	**	**
	24h	1.63^{c}	1.89^{a}	1.73^{b}	1.39^{d}	0.0000	<0.0001	**	**	**
Total VFAs mmole/L	6h	137.16^{a}	120.46^{b}	120.56^{b}	111.99^{c}	2.1356	0.0005	**	NS	*
	12h	125.75b^{c}	132.63^{b}	122.97^{c}	178.16^{a}	3.7977	<0.0001	**	**	**
	24h	140.88^{c}	148.64^{b}	150.05^{b}	189.48^{a}	1.92725	<0.0001	**	**	**

$^{a, b, c}$ mean in the same row with different superscripts are significantly different ($p<0.05$). *, ** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.11 Volatile fatty acid, pH, and NH3-N of four treatments (continue).

Items	Time	T1	T2	T3	T4	P value				
						SEM	Treatment	Fresh vs silage	Napier vs Purple	Purple vs additive
pH	6h	6.84	6.87	6.86	6.85	0.0067	0.5613	NS	NS	NS
	12h	6.74^{b}	6.79^{a}	6.74^{b}	6.71^{b}	0.0042	0.0064	NS	**	NS
	24h	6.55^{bc}	6.62^{ab}	6.68^{b}	6.54^{c}	0.0104	0.0194	*	NS	**
NH_3-N mg/DL	6h	11.22	14.20	14.93	15.86	0.1339	0.6100	NS	NS	NS
	12h	12.62^{c}	17.73^{a}	15.04^{b}	15.97^{b}	0.1620	0.0487	*	NS	NS
	24h	15.16^{c}	18.52^{a}	15.67^{c}	16.01^{b}	0.1340	0.0345	*	**	NS

[a, b, c] mean in the same row with different superscripts are significantly different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.12 Microbe population in vitro gas production 1.

Items	T1	T2	T3	T4	P value				
					SEM	Treatment	Fresh vs silage	Napier vs Purple	Purple vs additive
Total bacteria (lg10 copies/ml)									
6h	9.26	9.42	9.29	9.38	0.01184	0.047	NS	NS	NS
12h	9.74[a]	9.51[b]	9.35[b]	9.51[b]	0.01332	0.0182	**	NS	NS
24h	9.47	9.44	9.45	9.31	0.02616	0.5839	NS	NS	NS
Ruminococcus albus (lg10 copies/ml)									
6h	7.65[a]	7.65[a]	7.33[b]	7.75[a]	0.0686	0.0002	NS	NS	*
12h	7.32[b]	7.68[ab]	7.75[ab]	7.87[a]	0.07877	<0.0001	*	NS	NS
24h	7.32[c]	7.87[b]	7.88[b]	7.94[a]	0.26777	0.0054	NS	NS	NS
Methanogen (lg10 copies/ml)									
6h	5.57[bc]	5.83[ab]	5.88[a]	5.42[c]	0.02881	0.0016	NS	NS	**
12h	6.03[a]	6.03[a]	5.65[b]	5.72[b]	0.00938	<0.0001	**	**	NS
24h	5.39[c]	5.78[a]	5.57[b]	5.57[b]	0.00708	<0.0001	**	**	NS

[a, b, c] mean in the same row with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.13 Microbe population in vitro gas production 2.

Items	T1	T2	T3	T4	SEM	P value			
						Treatment	Fresh vs silage	Napier vs Purple	Purple vs additive
Butyrivibrio fibrisolvens (lg10 copies/ml)									
6h	6.72[b]	6.99[a]	6.96[a]	6.94[a]	0.049	0.000	*	NS	NS
12h	7.03[a]	6.98[b]	7.00[ab]	7.00[ab]	0.007	<0.0001	*	NS	NS
24h	6.99[ab]	7.01[a]	6.89[b]	7.02[a]	0.021	<0.0001	NS	NS	*
Megasphaera elsdenii (utilization lactic acid) (lg10 copies/ml)									
6h	3.69	3.73	3.71	3.72	0.010	0.085	NS	NS	NS
12h	3.97	3.78	3.73	3.71	0.063	0.099	NS	NS	NS
24h	3.67	3.71	3.68	3.94	0.051	0.052	NS	NS	NS
Streptococus bovis (producing lactic (lg10 copies/ml)									
6h	5.60[a]	4.94[b]	4.53[c]	4.66[c]	0.050	<0.0001	**	**	NS
12h	5.56[a]	4.64[b]	5.20[a]	5.22[a]	0.097	0.065	*	**	NS
24h	5.44[a]	4.72[b]	4.38[b]	5.14[a]	0.067	0.003	*	NS	**

[a, b, c] mean in the same row with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

Table 3.14 Microbe population in vitro gas production 3.

Items	T1	T2	T3	T4	P value				
					SEM	Treatment	Fresh vs silage	Napier vs Purple	Purple vs additive
Protozoa (lg10 copies/ml)									
6h	6.04^{b}	6.05^{b}	6.35^{a}	6.13^{ab}	0.041	0.029	NS	NS	NS
12h	6.05	6.05	6.19	6.21	0.034	0.052	NS	NS	NS
24h	6.33	6.32	6.31	6.00	0.078	0.552	NS	NS	NS
Fibrobacter succinogens (lg10 copies/ml)									
6h	7.91	8.35	8.37	7.70	0.141	0.079	NS	NS	NS
12h	8.40^{b}	8.61^{a}	8.28^{c}	8.41^{b}	0.012	<0.0001	NS	**	**
24h	7.78	7.93	7.69	7.51	0.088	0.592	NS	NS	NS
Ruminococcus flavefaciens (lg10 copies/ml)									
6h	4.71^{b}	4.93^{b}	5.22^{b}	6.60^{a}	0.055	0.007	*	**	*
12h	6.62	6.34	5.97	6.86	0.082	0.208	NS	NS	NS
24h	6.40	6.31	6.08	6.69	0.076	0.133	NS	NS	NS

$^{a, b, c}$ mean in the same row with different superscripts are significantly different ($p<0.05$). *,** Mean significant different ($P<0.01$), *mean significant different ($p<0.05$), NS mean not significant. SEM: standard error of the mean. Fresh Anthocyanin rich Napier grass (T1), Napier grass silage (T2), anthocyanin rich Napier grass silage (T3) without additive and Anthocyanin rich Napier grass silage with additive (T4).

3.9.2.4 Anthocyanin degradation

The degradation of anthocyanin of 4 treatments after incubation showed not significant on changing amount of anthocyanin from 0H, 3H, 6H, 9H, 12H, and 24H. We can conclude anthocyanin cannot be absorbed in the rumen, this result is similar with the study of Song et al (2012), Hosada et al. (2009), and Leatherwood (2014). Hosada et al. (2009) reported that anthocyanin in anthocyanin-rich corn silage was not decomposed by ruminal fluid, which suggests that the anthocyanin-rich corn is appropriate to provide a functional substance for ruminants. Song et al. (2012) reported anthocyanin content in colored barley was not affected in the ruminal fluid. Leatherwood (2014) presented that anthocyanin extract from purple-fleshed sweet potatoes degraded significantly after 24 hours incubation in rumen fluid. He explained the difference about the form of anthocyanin were incubated with rumen fluid related with the degradation of anthocyanin in rumen fermentation. In this study, anthocyanin in Anthocyanin rich Napier grass was not degraded in rumen fermentation.

3.9.2.5 Conclusions

Anthocyanin in anthocyanin rich Napier grass effected on increasing gas volume, total volatile fatty acid, the number of Streptococcus bovis bacteria. Anthocyanin reduced CH_4 production by decreasing the number of Methanogen bacteria, and effected on propionic acid proportion and acetate propionate ratio. Anthocyanin is stable under fermentation up to 24 hours of incubation. The useful of anthocyanin in treatment with additive (4% molasses and 0.03% $FeSO_4$) is similarly to treatment fresh grass. In rainy season, we can feeding animal with fresh Anthocyanin rich Napier fresh grass, in hot season, we can make anthocyanin rich Napier grass silage to feeding animal because the effect on rumen fermentation is

similar. It is called to conduct the study on animal to compare the effect of anthocyanin in silage.

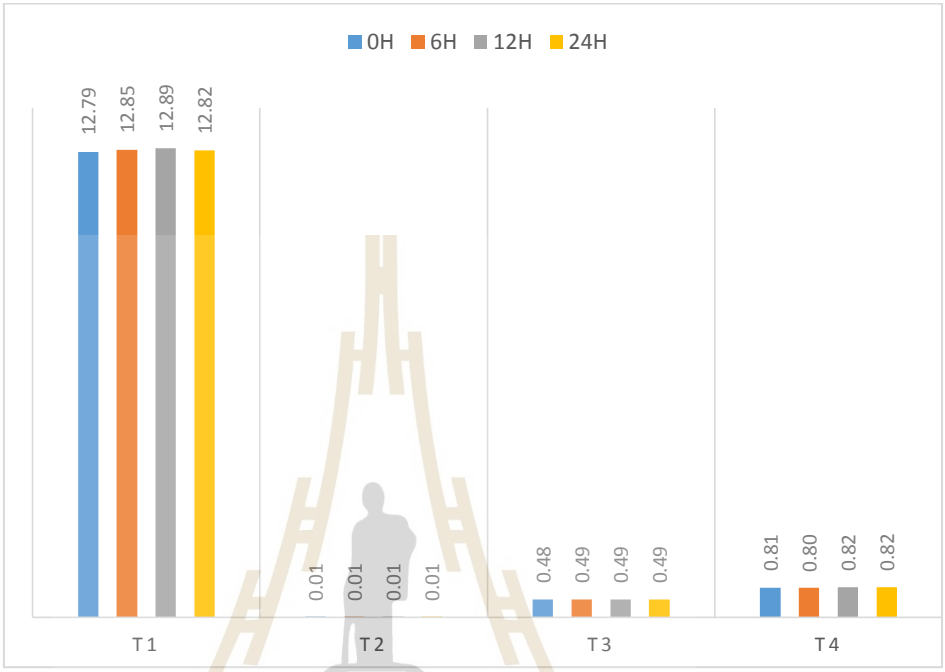


Figure 3.1 Anthocyanin content (mg/g DM) incubated with goat rumen fluid at 0, 6, 12, and 24 hours.

3.10 Conclusion

Addition molasses and $FeSO_4$ enhance the stable of anthocyanin, DM, CP and improve digestibility of NDF, ADF. 4% molasses and 0.03% $FeSO_4$ is the best level to add in silage. The result about gas production demonstrate anthocyanin effect on propionic acid production, development of Methanogen bacteria and enhance the group of *Streptococcus bovis* bacteria. Anthocyanin cannot be absorbed in the rumen

in vitro incubation. The anthocyanin rich Napier grass silage should be applied on feeding trial to evaluate the effect of this silage on animal performance.

3.11 References

Abdelrahim, G.M., Khatiwada J. and Gueye A. (2012). Effect of Dietary Supplementation of Ferrous Sulfate on Performance and Carcass Characteristics of Finishing Lambs. **Journal of Animal Research and Technology**, 1 (1): 7-12.

Alonzo-Macías, M., Cardador-Martínez A., Mounir S., Montejano-Gaitán G., and Allaf K. (2013). Comparative Study of the Effects of Drying Methods on Antioxidant Activity of Dried Strawberry (Fragaria Var. Camarosa) **Journal of Food Research**, 2(2): 92-107.

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

Brouček, J. (2015). Methane yeld from cttle, sheep, and goats housing with emphasis on emission factors: a review. **Slovak Journal of Animal Science**, 48 (3): 122-139.

Catchpoole, V.R. and Henzell E.F. (1971). Silage and silage making from tropical herbage speicies. **Herbage abstracts**. 41:213-219.

Cevallos-Casals, B.A. and Cisneros-Zevallos L. (2004). Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. **Food Chemistry**, 86:69-77.

Denman, S.E. and McSweeney C.S. (2006). Development of a real-time PCR assay for

monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. **FEMS Microbiology Ecology**, 58: 572-582.

Detmann, E., Paulino M.F., Mantovani H.C., Valadares Filho S.D.C., and Sampaio C.B. (2009). Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis-Menten kinetics. **Livestock Science**, 126: 136-146.

Francis,,F. (1989). Food colourants: Anthocyanins. **Critical Reviews in Food Science and Nutrition,** 28:273-314.

Ganz, T, and Nemeth E. (2006). Regulation of iron acquisition and iron distribution in mammals. Biochimica et Biophysica Acta . 1763:690-699. In: Hansen et al. (2009). Bioaccessibility of iron from soil is increased by silage fermentation, **Journal of Dairy Science**, 92 (6): 2896-2905.

Ghali, M.B., Scott P.T., and Al Jassim R.A.M. (2004). Characterization of Streptococcus bovis from the rumen of the dromedary camel and Rusa deer. **Letters in Applied Microbiology**, 39: 341-346.

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.**

Hosada, 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-Australia Journal of Animal Sciences** 22 (4): 528-533.

Hosoda, K., Miyaji M., Matsuyama H., Haga S., Ishizaki H., 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. **Livestock Science,** 145: 266-270.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2012a). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Animal Science Journal**, 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 Japanese Society of Grassland Science, **Grassland Science**, 58: 161-169.

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 Microbiol. Lett., 204: 361-366.

Lane, D.J. (1991). 16S/23S rRNA Sequencing. In: Nucleic Acid Techniques in Bacterial Systematic, Stackebrandt, E. and M. Goodfellow (Eds.). **John Wiley and Sons,** New York, USA, 115-175.

Lana, R.P., Russell J.B., and an Amburgh M.E. (1998). The role of pH in regulating ruminal methane and ammonia production. **Journal of animal Sciences**. 76: 2190-2196.

Leatherwood, W.L. (2014). Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on in vitro Fermentation by Rumen Microbial Cultural Research. A thesis submitted to the Graduate Faculty of North Carolina State University. **North Carolina**, 80-88.

Lev-Yadun, S. and Gould K.S. (2009). Role of Anthocyanins in Plant Defence Anthocyanins, **Springer Science+Business Media**, LLC 2009.

Makkar, H.P.S. (2003). Quantification of Tannins in Tree and Shrubs Foliage. IAEA. Kluwer. Academic Publisher Dordrect, **The Netherlands**.

Mauricio, R.M., Mould F.L., Dhanoa M.S., Owen E., Channa K.S., and Theodorou M.K. (1999). A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. **Animal Feed Science and Technology**, 79(4): 321-330.

Mazza, G. and E. Miniati. (1993). Anthocyanins in fruits, vegetables, and grains. CRC Press, **Boca Raton**, FL.

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**., 515-537.

McDonald, P., Henderson A.R., and Heron S.J.E. (1991). The Biochemistry of silage. 2nd Ed. **Cambrian Printers Ltd. Aberystwyh**. 184-236.

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

Nile, S.H., Kim S.H., Ko E.Y., Park S.W. (2013). Polyphenolic contents and antioxidant properties of different grape (V. vinifera,V. labrusca, and V. hybrid)

Cultivars. **BioMed Research International**, 1-5.

Nyambati, E.M., Muyekho F.N., Onginjo E. and Lusweti C.M. (2010). Production, characterization and nutritional quality of Napier grass [Pennisetum purpureum (Schum.)] cultivars in Western Kenya. **African Journal of Plant Science**, 4(12): 496-502

Okaraonye, C.C., and Ikewuchi J.C. (2009). Nutritional and Antinutritional Components ofPennisetum purpureum (Schumach). **Pakistan Journal of Nutritio**n, 8 (1): 32-34.

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

Parish, J. and Rhinehart J. (2008). Mineral and Vitamin Nutrition for Beef Cattle. It is available at http://www.thebeefsite.com/articles/1549/mineral-and-vitamin-nutrition-for-beef-cattle Accessed on June 7, 2012.

Phesatcha, K. and Wanapat M. (2016). Improvement of Nutritive Value and In vitro Ruminal Fermentation of Leucaena Silage by Molasses and Urea Supplementation. **Asian-Australasian Journal of Animal Science**, 29(8): 1136-1144.

Pipat, L., Mek K. and Wisitiporn S. (2011). Silage production from cassava peel and cassava pulp as energy source in cattle diets. **Journal of Animal and veterinary Advances**, 10 (8): 1007-1011.

Ray, H., Yu M., Auser P., Blahut-Beatty L., McKersie B., Bowley S., Westcott N., Coulman B., Lloyd A. and Gruber M.Y. (2003). Expression of anthocyanins

and proanthocyanidins after transformation of alfalfa with maize Lc. **Plant Physiology**, 132: 1448-1463.

Russell, J. (1998). The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production in vitro. **Journal of Dairy Science**, 81: 3222-3230.

Sakhawat, I. (2011). The effect of silage quality on gross energy losses. Degree Project 360, Department of Animal Nutrition and Management, Swedish University of Agricultural Science, **Sweden**.

SAS, (1998). User's Guide: Statistic, Version5.Edited 1996. SAS.Inst Cary,NC.; U.S.A.

Satter, L.D. and Slyter L.L. (1974). Effect of ammonia concentration on ruminal microbial protein production in vitro. **British Journal of Nutrion**, 32:199-208.

Scheers, N., Lena R., Inga T. and Ann S. (2016). Increased iron bioavailability from lactic-fermented vegetables is likely an effect of promoting the formation of ferric iron (Fe^{3+}). **European Journal of Nutrition**, 55:373-382.

Skerman, P.J., and Riveros F. (1990). Tropical Grasses. FAO, Rome, 75-12.

Sommart, K., Wanapat M., Rowlinson P., Parker D.S., Climee P., Panishying S. (2000). The use of cassava chips as an energy source for lactating dairy cows fed with rice straw. **Asian-Australian Journal of Animal Science**, 13: 1094-1101.

Song, T.H., Han O.K., Tae I.P., Kim D.K., Yoon C., Kim K.J. and Park K.H. (2012). Anthocyanin Stability and Silage Fermentation Quality of Colored Barley **Journal of the Korean Society of Grassland and Forage Science** 32(4): 335-342.

Song, T.H., Park T., Han O.K., Yoon C., Kang H.J. and Park K.G. (2013). Effects of Drying Methods on Anthocyanin Contents of Colored Barley (Hordeum vulgare L.) cv. Boanchalbori. **Journal of the Korean Society of Grassland and Forage Science**, 33(4): 234-239.

Spanghero, M., Salem A.Z.M., and Robinson P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. **Animal Feed Science and Technology** 152: 243-255.

Sylvester, J.T., Karnati S.K.R., 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: 3378-3384.

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.

Wilkinson, I.M. (1983). Silage make from topical and temperate crops. **World Animal Review**, 45:36-40.

Yoshida, K., Mori M. and Kondo T. (2009). Review. Blue flower color development by anthocyanins: from chemical structure to cell physiology. Natural Product Reports. **Current developments in natural products chemistry**, 26: 857-964.

Yunus, M., Ohba N., Shimojo M., Furuse M., and Masuda Y. (2000). Effects of adding urea and molasses on Napiergrass silage quality. **Asian-Australasian Journal of Animal Sciences**, 13:1542-1547.

CHAPTER IV

EFFECT OF ANTHOCYANIN RICH NAPIER GRASS SILAGE ON NUTRIENT DIGESTIBILITY RUMEN FERMENTATION AND GROWTH PERFORMANCE IN GROWING GOATS

4.1 Abstract

The present research aims to investigate the effect of anthocyanin rich Napier grass silage on digestibility, rumen fermentation, and growth performance in meat growing goats. Eighteen crossbred Thai native x Anglo-Nubian meat goats (average BW 14.42±0.6kg) were randomly assigned to feed on one of treatment diets according to completely randomized design. The goats were divided into three groups of six goats, each to receive Napier grass silage (negative control: T1), Anthocyanin rich Napier grass silage without additive (positive control: T2), and Anthocyanin rich Napier grass silage with 4% molasses and 0.03% $FeSO_4$ (treatment: T3). All animals were offered *ad-libitum* daily throughout 90 days with concentrate 16% crude protein (1.5% body weight). The result shows a significant ($p<0.05$) increase in crude protein intake (DMI) and nutrient intakes for goats fed treatment diets; the goats fed with treatment had significant higher digestibility, ammonia nitrogen (NH_3-N) concentration for 4 h post feeding in the rumen, nitrogen (N) balance, blood urea nitrogen (BUN), and average daily gain (ADG), body weight gain, total volatile fatty

acid (VFA) as well as body weight; while pH value, and bacteria population there were not significantly difference among diet treatments. The results of this study indicated that using anthocyanin rich Napier improved activity of non-enzymatic (total antioxidant capacity: TAC), effect on superoxide dismutase (SOD) antioxidant enzyme, and reduced lipid oxidation in plasma of growing goats. Anthocyanin rich Napier grass silage can be used as animal feed, that indicate to help the goat limit the harmful of oxidative stress from environment.

Key Words: Napier grass, anthocyanin. Nutrient utilization, Goat.

4.2 Introduction

Anthocyanin-rich Napier grass is a cultivate of Napier grass with leaves and stems in purple color which pigment was made from anthocyanin to protect plant cell from UV radiation, oxidative stress from environmental pathogen and herbivores (Lev-Yadun et al., 2009). Anthocyanin is glycosides of anthocyanidin which is synthesized via the flavonoid biosynthetic pathway in plants (Dixon et al., 2013). They are a strong natural antioxidant compound appear in the color plant as a feed source for the animal such as purple corn, purple grass, purple rice. Silage is the best method to keep forage in a long time as a feed for small ruminant. Molasses was used as the additive in silage to made fermentation quickly by decrease pH and provide energy for lactic bacteria (Pipat et al., 2011). Anthocyanin-rich feedstuffs were easy to lost anthocyanin in ensiling progress (Song et al., 2012, Hosada et al., 2009) and stable when pH lower than 4. In addition, Iron (Fe) is an essential component of several cytochromes and iron-sulfur proteins involved in the electron transport chain and several important biological processes (Parish and Rhinehart, 2008; Ganz and Nemeth,

2006). Level of FeSO4 at 0.03%/DM was reported not effect on lamp performance and meat characteristics (Abdelrahim et al., 2012). The aim of this study to evaluate the effect of anthocyanin-rich Napier grass silage with additives on performance, rumen fermentation, microorganism, nutrient digestibility and antioxidant enzyme activity on male growing goat and compare with Napier grass silage, anthocyanin-rich Napier grass silage.

4.3 Objectives

This study was designed to investigate the effect of anthocyanin-rich Napier grass silage with additives on performance, rumen fermentation, microorganism, nutrient digestibility and antioxidant enzyme activity on male growing goat and compare with Napier grass silage, anthocyanin-rich Napier grass silage without additive.

4.4 Materials and methods

4.4.1 Animals, treatments, and experimental design

Eighteen meat goats crossbred Thai native x Anglo Nubian an average body weight (BW) of 14.42±2.11 kg were allocated in Completed randomized design (CRD). The goats were housed in individual pens and allowed 2 weeks to adapt to the experimental conditions. Animals were received dietary treatments as followed:

Negative control (T1): Napier grass silage

Positive Control (T2): anthocyanin rich Napier grass silage

Treatment (T3): anthocyanin rich Napier grass silage with 4% molasses and 0.0 % $FeSO_4$.

Silage was offered twice daily ad libitum; approximately at 07:00 and 17:00 h. concentrate was fed 1.5% BW. Diets were allowed to have 5% left over. Feed ingredients of the experimental diets are shown in Table 5.1. Water was available at all times. Animals were individually housed and intensively cared according to procedures of goat farm at Suranaree University of Technology.

4.4.2 Data collection, sampling and chemical analysis

4.4.2.1 Feed sampling

Feed was sampled daily during the collection period and was composition prior to analyses. During the last seven days of 30th, 60th and 90th days of fermentation. Feed samples were collected every day and divided into two parts, the first part be analyzed for DM, while the second part was kept and pooled at the end of each period for chemical analysis. Samples were dried 60°C and ground (1mm screen) and then analyzed for DM, ash, EE and CP content (AOAC, 1995), NDF, ADF (Van Soest et al., 1991), GE used adiabatic bomb calorimeter (Parr Instrument Company). Intakes and digestibility of GE, OM, CP, NDF, and ADF were calculated based on DMI (g/d), their concentrations (g/kg DM) in the diet components and fecal samples. Digestible energy (DE) content of the pasture was calculated as the difference between gross energy intake and the fecal energy. Digestible energy (DE) content of the pasture was calculated as the difference between gross energy intake and the fecal energy.

Table 4.1 Chemical composition of the experimental diets and concentrate (% DM).

Items	Concentrate	T1	T2	T3
%Dry Matter (DM)	89.75	19.83	15.09	17.98
		%DM		
Ash	9.67	11.16	11.76	12.54
Organic Matter	90.33	88.84	88.24	87.46
Crude Protein	15.92	6.68	8.39	8.97
Ether Extract	3.05	4.75	4.81	4.41
Acid Detergent Fiber	17.25	41.81	41.17	33.08
Neutral Detergent Fiber	54.78	72.50	71.66	62.53
Hemicellulose	37.53	30.69	30.49	29.45
Acid Detergent Lignin	2.82	6.74	6.27	5.79
Crude Fiber	12.83	36.17	35.24	26.92
Cellulose	14.44	35.07	34.90	27.29
Gross Energy (kcal/kg DM)	3900.45	3849.01	3898.13	3818.23
Anthocyanin (mg/kg DM)	-	14.79	280.15	596.61
Lactic acid (g/100 g DM)	-	5.42	5.58	35.84

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive.

4.4.2.2 Fecal and Urine samplings

Fecal samples were total collected and weighed during the last 7 days of day 30th, 60th and 90th. The fecal samples were collected about 5% of total fresh weight and divided into two parts, the first part being analyzed for DM, the second part kept for chemical analysis at the end of each period.

Urine samples were collected the same time with fecal sampling, recording total collection and urine samples were collected 10% of total of the day acidified with

50% H_2SO_4 then mixed together of each goat after that were stored at -20°C until analyzed NH_3-N by the method of (Bremner and Keeney, 1965).

4.4.2.3 Blood sampling

Blood sample (about 10 ml) was collected from a jugular vein (at the same time as rumen fluid sampling) into tubes containing 12 mg of EDTA, and plasma was separated by centrifugation at 500×g for 10 min and stored at -20°C until analysis of blood urea-N (BUN) according to the method of Crocker (1967) and analysis antioxidant enzymes.

The total antioxidant capacity (TAC), representing the plasma's non-enzymatic antioxidative systems, was assayed using a commercial kit (Total Antioxidant Capacity Assay Kit MAK187; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as nmole of copper reducing power per µl of plasma.

The plasma activity of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion, was measured as an endogenous enzyme using a commercial kit (19160 SOD determination Kit; Sigma-Aldrich, Missouri,USA) following the manufacturer's instructions. Values were expressed as units per mL of plasma.

The malondialdehyde (MDA) concentration in the plasma sample was measured as an index of lipid peroxidation by thiobarbituric acid reaction using a commercial kit (Lipid Peroxidation (MDA) Assay Kit MAK085; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as nmole per µl of plasma.

The Catalase (CAT) concentration in the plasma sample was measured as an index of antioxidant enzyme by decompostion of hydrogen peroxide using a

commercial kit (Lipid Peroxidation (MDA) Assay Kit MAK085; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as unit per ml of plasma.

The plasma concentration of Glutathione reductase, an enzymatic antioxidant catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), was analyzed by a commercial kit (Glutathione Reductase Assay Kit GRSA; Sigma-Aldrich, Missouri, USA) according to the manufacturer's instructions. The assay was performed with extracts prepared immediately from the fresh plasma samples. Values were expressed in unit per mL of plasma.

The plasma concentration of Glutathione-S-Transferase (GST), a group of enzymes detoxicate xenobiotics in the mammals, was analyzed by a commercial kit (Glutathione-S-Transferase (GST) Assay Kit CS0410; Sigma-Aldrich, Missouri, USA) according to the manufacturer's instructions. The assay was performed with extracts prepared immediately from the fresh plasma samples. Values were expressed in umole per ml per min of plasma.

Table 4.2 Gene sequences.

Items	Forward/Reward	Primer sequence (5'- 3')	Temperature (°C)	References
Total bacteria	F	CGGCAACGAGCGCAACCC	55	Koike et al(2001)
	R	CCATTGTAGCACGTGTGTAGCC		
Ruminococcus albus	F	CCCTAA AAGCAG TCTTAGTTCG	55	Koike et al(2001)
	R	CCTCCTTGCGGTTAGAACA		
Ruminococcus flavefaciens	F	TCTGGAAACGGATGGTA	60	Koike et al(2001)
	R	CCTTTAAGACAGGAGTTTACAA		
Fibrobacter succinogens	F	GTTCGGAATTACTGGGCGTAAA	55	Lane (1991)
	R	CGCCTGCCCCTGAACTATC		
Methanogen	F	TTCGGTGGATCDCARAGRGC	58	Denman et al., (2006)
	R	GBARGTCGWAWCCGTAGAATC		
Protozoa	F	CTTGCCCCTCYAATCGTWCT	55	Sylwesters etal. (2004)
	R	GCTTTCGWTGGTAGTGTATT		
Butyrivibrio fibrisolvens	F	ACACACCGCCCGTCACA	58	Klieve et al(2003)
	R	TCCTTACGGTTGGGTCACAGA		
Megasphaera elsdenii	F	GACCGAAACTGCGATGCTAGA	59	Klieve et al(2003)
	R	CGCCTCAGCGTCAGTTGTC		
Streptococcus bovis	F	TTCCTAGAGATAGGAAGTTTCTTCGG	58	Klieve et al(2003)
	R	ATGATGGCAACTAACAATAGGGGT		

4.4.2.4 Rumen fluid sampling

Rumen fluid samples were collected by stomach tube at 0, 2 and 4 h-post morning feeding in the day 30th, 60th and 90th. Approximately 300 ml of rumen fluid was taken using stomach tube at each time at the end of each period. Rumen fluid was immediately measured for pH using a portable pH meter. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into three portions; first portion was used for NH3-N analysis where 20 ml of 50% HCl solution was added to 80 ml of rumen fluid. The mixture was centrifuged at 16,000g for 15 minutes and supernatant was stored at -20°C prior to NH3-N measurement (Bremner and Keeney, 1965) and VFA analysis (HPLC; model RF-10AXmugiL; Shimadzu; Japan) according to Zinn and Owens (1986). Second portion was collected for microbial analyzed DNA technique.

Table 4.3 HPLC conditions for the analysis of organic acid.

Items	Conditions
Column	SBC18
Detector	UV, 240 nm
Flow rate	0.7 ml/min
Solvent	A: KH2PO4 0.1 mM pH 2.4 B: Acetonitrile
Absorbance	240 nm
Injection volume	20µl

The pH and volatile fatty acids were determined by pH meter and high-performance liquid chromatography (HPLC), respectively. The HPLC condition for analysis VFAs was shown in table 4.3. For anthocyanin analysis, 300 grams of silage sample was lyophilized, ground and extracted using 0.01N HCl -80% Methanol solution and determined by spectrophotometer and HPLC. The HPLC conditions for the analysis of anthocyanin was shown in Table 4.4.

Table 4.4 HPLC conditions for the analysis of anthocyanin.

Items	Conditions
Column	SBC18
Detector	UV, 520 nm
Flow rate	0.8 ml/min
Solvent	A: 10% acetic acid, 5% acetonitrile, and 1% phosphoric acid B: Acetonitrile
Absorbance	520 nm
Injection volume	20µl

4.4.3 Data Statistical Analysis

All data obtained from the experiment were statistically subjected to ANOVA and analyzed as a Complete Randomized Design (CRD) design using the PROC GLM (SAS, 1998). Significant differences between treatments were determined using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980) and orthogonal contrast was used for trend analysis. The following statistical model was used:

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

Where Y_{ij} = represents of observation from animals

μ = overall mean

τ_i = Effect of treatment (i = 1-3)

ε_{ij} = Error of the term (j=1-6)

4.5 Results and discussions

4.5.1 Feed intake

Feed intake data are presented in table 4.5. Feed intake were no significantly different ($p>0.05$) among Napier grass silage, Anthocyanin rich Napier grass

silage without additive, and Anthocyanin rich Napier grass silage with additive (362.87, 334.07 and 354.92 g DM/d respectively). When compared by percentage of body weight and gram per kilo gram body weight 0.75 (g/kg $BW^{0.75}$) found no significant ($p>0.05$). The anthocyanin content in silage do not effect on the feed intake of goat.

Table 4.5 Effect of treatments on feed intake, nutrient intake of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Feed intake					
g DM/d	362.87	334.07	354.92	0.75	0.36
%BW	2.37	2.12	2.22	0.05	0.30
g/kg$BW^{0.75}$	46.87	42.12	44.32	0.05	0.28
Nutrient intake					
OMI g DM/d	325.31	299.01	316.01	0.71	0.36
CPI g DM/d	40.85^{b}	41.61^{b}	48.25^{a}	0.27	0.01
EEI g DM/d	13.88	12.52	13.01	0.15	0.23
NDFI g DM/d	228.09	205.22	206.85	0.59	0.16
ADFI g DM/d	103.22^{a}	89.11^{b}	86.59^{b}	0.40	0.02
ANI mg DM/d	1.65^{c}	65.73^{b}	104.62^{a}	0.41	0.001

[a, b, c] mean in the same row with different superscript differ ($p<0.05$). T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive.DM= dry matter, BW= bodyweight, OMI= Organic matter intake, CPI= crude protein intake, EEI= ether extract intake, NDFI= neutral detergent fiber intake, ADFI= acid detergent fiber intake, ANI: anthocyanin intake, and SEM = Standard error of the mean.

Crude protein intake per day was apparent ($p<0.05$) highest level in goats fed anthocyanin rich Napier grass silage with additive (48.25 g DM/d), Anthocyanin rich Napier grass no additive (41.61 g DM/d), and Napier grass silage (40.85 g DM/d). Organic matter intake, ether extract intake, and neutral detergent fiber intake were not different among treatment. Acid detergent fiber intake in treatment T1 is highest value (103.22 g DM/d), significant different with treatment T2 and T3 (89.11 and 86.59 g DM/d, respectively) with P=0.02. Anthocyanin intake in treatment T3 is significant

higher treatment T2 and T1 (124.76, 62.15, and 1.64 mg DM/d, respectively P=0.01). In treatment T3 and T2, the CP is higher than T1 (8.97, 8.39 in compare to 6.68%, table 4.1), the goats received similarly concentrate intake, so the amount of CP were different from the silage composition. For anthocyanin and ADF were explain in the same way. Three treatments were different mainly about anthocyanin content to evaluate the effect of anthocyanin pigment on feed intake and nutrient intake. In the study of Bureenok et al. (2012), DM intake of silage was significantly higher when molasses was used as a silage additive as compare to the use of cassava meal resulted in the lowest DM intake of Napier grass silage. Hosada et al. (2012a) reported that anthocyanin corn silage was not effect on the feed intake of animal. In this study, the different about nutrient intake from the chemical composition of the silage.

Table 4.6 Effect of treatments on apparent digestibility of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Apparent Digestibility, % of intake					
DDM	71.99^{c}	80.67^{b}	83.61^{a}	0.78	0.020
DOM	73.72^{c}	82.48^{b}	85.32^{a}	0.74	0.010
DCP	75.16^{c}	81.51^{b}	84.74^{a}	0.90	0.040
DEE	45.04^{c}	61.22^{b}	68.87^{a}	1.07	0.010
DNDF	67.60^{b}	78.99^{ab}	82.33^{a}	0.80	0.010
DADF	52.22^{b}	68.79^{ab}	72.24^{a}	0.99	0.010
MCP(g/day)	31.29	32.04	35.04	0.62	0.260

$^{a, b, c}$ Mean in the same row with different superscript differ ($p<0.05$); T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive, MCP = Microbial crude protein (kg/d) = 0.13*kg DOMI; SEM = Standard error of the mean.

4.5.2 Nutrient digestibility

Nutrient digestibility was shown in table 4.6. Digestibility of DM, OM, EE, CP, NDF, and ADF was different among treatments ($p<0.05$). DM, OM, EE, CP,

NDF, and ADF digestibility of anthocyanin rich Napier grass silage with additive was highest and significant different when compared with other treatments. No significant difference was observed in the digestibility of gross energy and microbial crude protein. According to Bereenok et al. (2012), addition of molasses to Napier grass versus no additive resulted in a 1.4 times higher intake of silage, the silages prepared with molasses are advantageous to stimulate dry matter intake. The increase in DM and OM digestibility for silages ensiled with molasses are in line with the observed lower NDF and ADF content of this silage. Thus, it has been discussed that the aerobic stability of the silage with molasses was increased thereby potentially preventing the growth of spoilage organisms (Danner et al., 2003). On the other hand, addition of Fe^{3+} increased the degradation of fiber in silage (Wang et al., 2018) by increasing the hydrolysis of lignocellulose to sugar, which is fermentable to acid or enzyme. Inclusion, Anthocyanin rich Napier grass silage with additive (4% molasses and 0.03% FeSO4) is the good feed for goat by increasing the digestibility of the silage.

4.5.3 Nitrogen balance

The effect of dietary treatments on nitrogen (N) utilization is presented in table 4.7. These present results expressed nitrogen retention as the cumulative nitrogen balances. N intake, N absorbed, and N balance, were higher significant different ($p<0.01$) level in goats fed treatment T3 have additive compared with both of negative control T1 and positive control T2, while N in urine and N in feces were not significant different level among treatments ($p>0.05$). Moreover, N absorption (% of N intake) and N balance (% of N intake) of treatment T3 significant different when compared with negative control (T1) and positive control (T2). Therefore, animals fed with Anthocyanin rich Napier grass Silage with 4% molasses and 0.03% $FeSO_4$ was

improved retention of nitrogen. This results in agreement with Yokota et al. (1992) that molasses improve the nutrient digestibility so nitrogen absorbed were increase.

4.5.4 Average daily gain and body weight change

Effect of treatments on daily gain and body weight change of growing meat goats was shown in table 4.8. Treatment 3 was increased average daily gain (ADG) apparent significantly ($p<0.05$) when compared to negative control and positive control, 46.63, 38.49 and 34.13 g/d, respectively. The data about weight change is similar with daily gain because nitrogen intake and nitrogen absorb of treatment 3 is higher than treatment 2 and 1, so the body weight is heavier. According to Mohammad et al. (2015), the Napier silage showed the lower BW gain compared to the animals fed other treatments, he indicated that Napier silage is a low-quality roughage. Besides, Khaing et al. (2016) reported that Napier grass silage is low in voluntary intake and that it can only provide the required amount of energy and protein for maintenance. Addition molasses and $FeSO_4$ was improved the body weight of goat.

Table 4.7 Effect of treatments on nitrogen digestibility of growing meat goats.

Items	T1	T2	T3	SEM	P Value
N intake g/d	6.54[b]	6.65[b]	7.72[a]	0.25	0.014
Fecal N excreted g/d	1.59	1.23	1.19	0.19	0.140
Urine N excreted g/d	0.43	0.38	0.32	0.12	0.546
N digestibility (g/d)	4.94[b]	5.42[b]	6.53[a]	0.25	0.003
N retention g/d)	4.52[b]	5.05[ab]	6.21[a]	0.25	0.002
%N digestibility	75.16[c]	81.51[b]	84.74[a]	0.90	0.040
%N retention	68.66[b]	75.81[ab]	80.42[a]	0.74	0.009

[a, b, c] Mean in the same row with different superscript differ ($p<0.05$); N=nitrogen. T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

Table 4.8 Effect of treatments on daily gain and body weight change of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Body weight					
Initial weight, kg	14.38	14.42	14.45	0.45	0.99
Final weight, kg	17.25[b]	17.65[b]	18.37[a]	0.44	0.04
Weigh change, kg	2.87[c]	3.23[b]	3.92[a]	0.26	0.04
ADG, g/d	34.13[b]	38.49[b]	46.63[a]	1.06	0.04

[a, b, c] Mean in the same row with different superscript differ ($p<0.05$), ADG= average daily gain. T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

4.5.5 Blood urea nitrogen

Blood BUN was shown in table 4.9. No significant different can find among 3 treatments. Treatment 3 tend to higher than treatment 2 and 1 because Nitrogen absorb in treatment 3 is better than others. The mean BUN in this study was range from 16.26-21.89 mg%. It was similar with the optimal level in normal goats which has been reported by Lloyd (1982), the range of BUN start from 11.2 to 27.7 mg%. Preston et al. (1965) reported that the concentration of BUN is correlated to the level of ammonia production in the rumen. Furthermore, Wanapat et al. (2008) suggested that concentrations of blood urea N are highly correlated to the concentration of N_{H3}-N production in the rumen.

4.5.6 Rumen fermentation characteristics

Nitrogen-N and pH was shown in table 4.9. Volatile fatty acid and total volatile fatty acid were shown in table 4.10. No significant different were observed in the pH and NH_3-N in rumen fluid. Acetic acid production was significant among 3 treatments at H0 and H2 ($P<0.05$) but not significant at 4H. Mean of acetic acid show that treatment 2 is highest (59.35% total VFAs), next to treatment 1 (57.43% TVFAs)

and treatment 3 (52.9% TVFAs). In contrast, propionic acid treatment T3 is significant higher than others with P<0.05. Butyric acid production different at H2 and H4 but mean is not different. C2/C3 ratio shows that at H4 no different but significant in mean. Mean of total VFAs production is highest in treatment 3 (243.18 mmole/l), treatment 2 (211.06 mmole/l) and treatment 1(194.76 mmole/l) with P<0.05.

Table 4.9 Effect of treatments on blood urine nitrogen (BUN), nitrogen and pH in rumen fluid of growing meat goats.

Items	T1	T2	T3	SEM	P-value
BUN mg/DL					
0h	16.26	16.95	17.82	0.46	0.47
2h	20.01	20.79	21.89	0.52	0.51
4h	18.67	19.37	20.72	0.51	0.42
Mean	18.31	19.04	20.15	0.49	0.45
NH3-N mg/L					
0h	17.89	18.17	19.29	0.58	0.50
2h	19.55	18.95	20.12	0.47	0.90
4h	15.06	14.73	15.48	0.50	0.88
Mean	17.5	17.28	18.3	0.48	0.74
pH					
0h	7.21	7.21	7.14	0.10	0.38
2h	6.71	6.73	6.66	0.11	0.55
4h	6.80	6.78	6.67	0.11	0.21
Mean	6.90	6.90	6.82	0.10	0.29

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. h: hour, NH3-N: ammonia nitrogen, BUN: blood urine nitrogen. SEM = Standard error of the mean

Muia et al. (2000) reported that the values of pH were in the range of 6.3-7.0 and Erdman (1988) stated that the pH value were 6.0-7.0 in ruminant fluid, where the activity of rumen microbial population was optimal for growth and absorption VFA (Dijkstra et al., 1993). In this study, the pH of three treatments at 2 and 4 hours post feeding were from 6.6 to 6.9, which was suitable for the activity of microbe in digestion.

Detmann et al. (2009) reported the concentration of NH_3-N has been used as a qualitative reference to detect the microbial activity in fibrous carbohydrates in the rumen. According to Leng (1990), the level of NH_3- N within the range of 10-20 mg dL-1 is required to optimize digestion of fibrous feed for rumen microorganisms. In the present study, the average ruminal fluid NH_3- N concentrations of goats in all diets from 15.06 to 20.12 mg dL-1, it indicates the level of NH_3-N obtained in all dietary treatments were sufficient for optimum rumen fermentation and microbial growth. In treatment T3 tend to higher than T1 and T2.

According to Firkins et al. (2006) 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 the rumen is affected by numerous factors such as substrate composition and availability of specific types of rumen microbes to degrade the received diets (Dijkstra, 1994). High fiber diet has resulted in greater proportions of acetate, whereas diet with high starch content has resulted in greater proportions of propionate. All of the goats resumed the same concentrate and different about silage, treatment T3 with additive was low in acetate but high in propionate proportion as compare to T1 (Napier grass silage) and T2 (anthocyanin rich Napier grass silage without additive). This study was in agreement with Kariuki et al. (2001) who reported that a high proportion of acetate and low proportion of propionate were observed in Napier grass diet.

Firkins et al. (2006) reported that high degradation of fiber in the diets will produce a high proportion of acetic acid and low amount of propionic acid in the rumen. On the other hand, when the content of starch and sugar level was increased in the diet, the proportion of acetic acid decreased; resulting in the increased of propionic acid concentration (Leek, 1993). Kariuki et al. (2001) and Widiawati (2009) have

reported that if the level of propionic acid is increased, the energy of the ruminant ration were more efficiently used.

Table 4.10 Effect of treatments on volatile fatty acid in rumen fluid of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Acetate (%)					
0h	59.86[a]	56.23[a]	47.77[b]	0.76	0.01
2h	54.13[b]	60.96[a]	53.26[b]	0.60	0.01
4h	58.3	60.86	57.66	0.63	0.44
Mean	57.43[b]	59.35[a]	52.90[c]	0.59	0.02
Propionate (%)					
0h	28.70[b]	32.00[b]	37.77[a]	0.72	0.03
2h	35.68[b]	26.69[c]	37.33[a]	0.66	0.01
4h	28.49[b]	23.19[c]	29.80[a]	0.63	0.03
Mean	30.96[b]	27.30[c]	34.97[a]	0.57	0.01
Butyrate (%)					
0h	11.44	11.77	14.46	0.51	0.14
2h	10.19[b]	12.35[a]	9.41[b]	0.42	0.03
4h	13.21[b]	15.95[a]	12.54[b]	0.44	0.02
Mean	11.61	13.35	12.14	0.37	0.14
C2:C3					
0h	2.35[a]	2.07[a]	1.48[b]	0.23	0.04
2h	1.60[b]	2.39[a]	1.51[b]	0.20	0.01
4h	2.14	2.74	2.06	0.22	0.07
Mean	2.03[b]	2.40[a]	1.68[c]	0.18	0.01
Total VFAs (mmol/L)					
0h	177.88[c]	178.10[b]	222.39[a]	1.68	0.03
2h	203.35[c]	241.28[b]	265.73[a]	1.60	0.01
4h	203.06[c]	213.79[b]	241.41[a]	1.34	0.01
Mean	194.76[c]	211.06[b]	243.18[a]	1.42	0.01

[a, b, c] Mean in the same row with different superscript differ ($p<0.05$), T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. h: hour, VFAs: total volatile fatty acids. SEM = Standard error of the mean

Addition molasses and $FeSO_4$ enhance anthocyanin content and increase the lactic acid in silage, which stimulate the group of bacteria, which produce propionate from lactate in rumen such as *Mesasphaera elsdenii* (Kung, 2010). Propionate is an important precursor for gluconeogenesis and increased propionate concentrations in the rumen has resulted in increased of glucose synthesis, providing more energy to the animals, increasing production response. According to Weinberg et al (2004), lactic acid bacteria from silage was used as probiotic which improve the host animal's intestinal microbial balance. In the rumen butyric acid also is primarily used as a source of energy for the host animal (McDonald et al., 2002) but in this study the mean of butyrate proportion was not significant different. The different was found after feeding 2 and 4 hours because the animal transfer their status from low energy-before feeding to high energy- after feeding, so the amount of butyrate change to the diet but the mean is similarly. It mean three treatments did not effect on butyrate proportion.

Leedle et al. (1995) stated that the proportion of butyric acid has significantly increased when the diet is changed from a low energy diet with a high energy diet. In contrast, addition additive in treatment T3 increase the total volatile fatty acids and decrease the ratio of acetate to propionate. It can be explain by the number of total bacteria in treatment T3 tend to higher than treatment T1 and T2 (table 4.10). Treatment T3 provide a large of total volatile fatty acid, especially propionate, so the resulted in increasing the bodyweight of this treatment was better than others.

4.5.7 Rumen micro-organism population

Table 4.11, 4.12, and 4.13 illustrated the result on ruminal microorganism population affected by 3 treatments. No significant different on total bacteria, *Ruminococcus albus*, Methanogen, *Butyrivibrio fibrisolvens*, *Megasphaera elsdenii*, Protozoa, *Fibrobacter succinogens*, *Ruminococcus flavefaciens* bacteria but

significant different on number of *Streptococus bovis* copies/ml among 3 treatments. Treatment 2 is highest (4.636 log10 copies/ml), treatment 1 (4.363 log10 copies/ml) and treatment 3 (4.353 log10 copies/ml) with P<0.05.

Table 4.11 Effect of treatments on total bacteria, *Ruminococcus albus*, Methanogen bacteria in rumen fluid of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Total bacteria (lg10 copies/ml)					
0h	9.38	9.54	9.58	0.19	0.51
2h	9.27	9.40	9.38	0.15	0.43
4h	9.40	9.45	9.56	0.19	0.67
Mean	9.35	9.46	9.51	0.17	0.53
Ruminococcus albus (lg10 copies/ml)					
0h	5.84	5.94	5.88	0.35	0.99
2h	5.68	5.63	5.84	0.37	0.95
4h	5.57	5.92	5.76	0.35	0.85
Mean	5.70	5.83	5.83	0.35	0.97
Methanogen (lg10 copies/ml)					
0h	6.59	6.49	6.48	0.24	0.91
2h	6.61	6.64	6.41	0.22	0.59
4h	6.50	6.26	6.31	0.20	0.47
Mean	6.57	6.46	6.40	0.22	0.77

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

In addition, diet is one of the main factors influencing the rumen microbial populations and specifically the milieu of substrate derived from microbial fermentation of ingested feed (Carberry et al., 2012). Fiber degradability mainly depends on the accessibility of rumen microbes which may be influenced by the morphological structure and chemical composition of the fibrous tissue (Ngwe et al., 2012). The predominant rumen cellulolytic bacteria are *Ruminococcus albus*,

Fibrobacter succinogens, and *Ruminococcus flavefaciens* (Hobson and Stewart, 1997) and they possess a greater ability to degrade fiber than other cellulolytic bacteria species (Carberry et al., 2012). According to Lettat (2011), the effect of diets on the growth of cellulolytic bacteria varies due to changes of pH, the capacity of the different species to adapt to dietary changes and the interactions among different bacterial species.

Besides, Carberry et al (2012) reported that the populations of protozoa have a negative relationship with the level of propionate, whereas, positive relationship appears when butyric acid is present, treatment T3 was high in propionate proportion and the number of protozoa was not different as compare to others but the number of Methanogen bacteria showed a trend to lower than treatment T1 and T2. This is in agreement with Leatherwood (2014) reported that high concentration of propionate will effect on methane expression. The high production of propionic acid in T3 diets that reduces the uses of hydrogen availability by rumen methanogen and inhibits the activity of their population (Martin et al., 2010; Hook et al., 2011). Similarly, animals fed with high fiber in diet 1 resulted in more production of acetic acid together with high production of hydrogen and leading to an increase number of *methanogenic archaea* (Adeyosoye et al., 2010).

The relationship of anthocyanin content and the number of protozoa and methanogen bacteria was not clear, from this study, the results show that a lightly effect of treatment high in anthocyanin content on propionate producing and methanogen group. *Butyrivibrio fibrisolvens* is the group of bacteria, which are responsible for ruminal biohydrogenation (Shokryazdan et al., 2016) but no significant about the number of this bacteria among treatments.

Table 4.12 Effect of treatments on *Butyrivibrio fibrisolvens, Megasphaera elsdenii, Streptococus bovis* bacteria in rumen fluid of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Butyrivibrio fibrisolvens (lg10 copies/ml)					
0h	6.99	7.02	7.04	0.08	0.13
2h	6.86	6.95	6.80	0.11	0.10
4h	6.90	6.88	6.96	0.10	0.29
Mean	6.92	6.95	6.94	0.30	0.52
Megasphaera elsdenii (utilization lactic acid) (lg10 copies/ml)					
0h	4.23	4.26	4.39	0.14	0.25
2h	4.28	4.42	4.41	0.17	0.50
4h	4.38	4.47	4.47	0.16	0.73
Mean	4.30	4.39	4.43	0.26	0.52
Streptococus bovis (producing lactic (lg10 copies/ml)					
0h	4.45	4.54	4.61	0.16	0.49
2h	4.28^{b}	4.27^{b}	4.64^{a}	0.13	0.01
4h	4.32^{b}	4.27^{b}	4.65^{a}	0.11	0.01
Mean	4.35	4.36	4.64	0.13	0.02

[a, b, c] Mean in the same row with different superscript differ ($p<0.05$). T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

When ruminants are fed high concentrate diets, *Streptococcus bovis* proliferates rapidly to produce lactic acid, easily causing rumen acidosis (Chen et al., 2016). *Megasphaera elsdenii* is an anaerobic gram-negative bacterium that consumed 60 to 80% of the lactic acid metabolism in the rumen (Counotte et al., 1981), it is an ideal candidate for aiding in the mitigation of lactic acidosis and use as probiotic for animal. The relationship between two groups effect on the host animal healthy. In this study, treatment T3 was low in *Streptococcus bovis* population but high in *Megasphaera elsdenii* population. In contrast, treatment T1 was high in in *Streptococcus bovis* population but high in *Megasphaera elsdenii* population. We can explain the substrate for lactic acid bacteria utilize in treatment T3 is lower than treatment 1 and 2 because the carbohydrate source was used by lactic bacteria in silage to make lactic acid. Thus, lactic acid in silage treatment 3 is higher treatment 1 and 2 (table 4.1). This result indicated the

relationship of anthocyanin and lactic acid in silage on changing the number of *Megasphaera elsdenii* and *Streptococcus bovis* population in rumen fluid. High anthocyanin content followed to high lactic acid in silage, high lactic acid in silage increased the number of lactic acid utilization bacteria (*Megasphaera elsdenii*) and decreased the number of lactic acid producing bacteria (*Streptococcus bovis*).

Table 4.13 Effect of treatments on Protozoa, *Fibrobacter succinogens*, *Ruminococcus flavefaciens* bacteria in rumen fluid of growing meat goats.

Items	T1	T2	T3	SEM	P-value
Protozoa (lg10 copies/ml)					
0h	6.33	6.26	6.26	0.19	0.90
2h	6.15	6.31	6.26	0.18	0.63
4h	6.18	6.37	6.15	0.19	0.45
Mean	6.22	6.31	6.22	0.19	0.83
Fibrobacter succinogens (lg10 copies/ml)					
0h	6.44	6.19	6.15	0.25	0.63
2h	5.75	5.80	6.04	0.27	0.69
4h	5.80	5.99	6.24	0.27	0.49
Mean	5.99	5.99	6.15	0.26	0.88
Ruminococcus flavefaciens (lg10 copies/ml)					
0h	6.31	6.31	6.07	0.38	0.86
2h	6.43	6.31	6.30	0.16	0.42
4h	6.47	6.05	6.16	0.23	0.22
Mean	6.40	6.22	6.18	0.26	0.63

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

4.5.8 Antioxidant enzymes activity

Antioxidant enzymes activity in plasma was shown in table 4.14. The total antioxidant capacity (TAC), representing the plasma's non-enzymatic antioxidative systems, the plasma activity of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion and the malondialdehyde (MDA) concentration in the plasma sample were significant different among three treatments.

The goat from treatment T3 fed highest amount of anthocyanin so increased TAC and decreased SOD and MDA in plasma with P<0.05. But CAT, GST, GSH and cholesterol were not significant. Anthocyanin effect on antioxidant enzyme activities include SOD, TAC and MDA but tend to effect on CAT, GR, GSH and cholesterol. The study of Hosada et al. (2012a, 2012b, 2012c) show the improving in the value of TAC and SOD when supplement the feedstuff contain anthocyanin pigment.

Table 4.14 Effect of treatments on Antioxidant enzymes activity in plasma of growing meat goats.

Items	T1	T2	T3	SEM	P-value
TAC nmole/µl	0.25[b]	0.32[b]	0.36[a]	0.06	0.00
SOD unit/ml	40.89[a]	34.34[b]	31.97[b]	0.72	0.03
MDA nmole/µl	0.44[a]	0.40[ab]	0.37[b]	0.05	0.05
CAT unit/ml	244.0	305.9	365.6	2.96	0.10
GR umole/ml/min	192.4	239.3	284.0	2.57	0.10
GSH unit/ml	2.0	2.4	2.9	0.26	0.11
Cholesterol mg%	68.1	62.4	61.2	1.07	0.58

[a, b] Mean in the same row with different superscript differ (p<0.05). T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. TAC= total antioxidant capacity, SOD= superoxide dismutase. MAD= Malondialdehyde, CAT= catalase, GR= glutathione reductase, GST= glutathione S-transferase. SEM = Standard error of the mean

4.6 Conclusions

Anthocyanin rich Napier grass silage with 4 % molasses and 0.03% $FeSO_4$ can be used as animal feed, that was indicator by apparently increased of CP intake, nutrient intakes, nutrients digestibility, N utilization, and ADG as well as body weight gain of growing goats. In addition, Anthocyanin rich Napier grass silage with additive enhance the number of *Streptococcus bovis* and tend to reduce Methanogen bacteria

group in rumen fluid, resulted om methane emission. Using Anthocyanin rich Napier silage improved activity of antioxidant enzymes and help the animal limit the harmful of oxidative stress from environment.

4.7 References

Abdelrahim, G.M., Khatiwada J. and Gueye A. (2012). Effect of Dietary Supplementation of Ferrous Sulfate on Performance and Carcass Characteristics of Finishing Lambs. **Journal of Animal Research and Technology**, 1 (1): 7-12.

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**, 4: 388-391.

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

Bremner, J.M., and Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. **Analytica Chimica Acta**. 32: 485-495.

Bremner, J.M., and Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. **Analytica Chimica Acta**. 32: 485-495.

Bureenok, S., Yuangklang C., Vasupen K., Thomas Schonewille J., and Kawamoto K. (2012). The Effects of Additives in Napier Grass Silages on Chemical Composition, Feed Intake, Nutrient Digestibility and Rumen Fermentation. **Asian-Australian Journal of Animal Sciences**, 25(9): 1248-1254.

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 Environ. **Microbiology**, 78: 4949-4958.

Chen, L., Luo Y., Wang H., Liu S., Shen Y., and Wang M. (2016). Effects of Glucose and Starch on Lactate Production by Newly-Isolated Streptococcus bovis S1 from Saanen Goats. **Applied and Environmental Microbiology**, 8:123-134.

Counotte, G.H.M., Prins R.A., Janssen R.H.A.M., and De Bie M.J.A. (1981). Role of Megasphaera elsdenii in the fermentation of DL-[2-'3C] lactate in the rumen of dairy cattle. **Applied and Environmental Microbiology**, 42:649-655.

Crocker, C.L. (1967). Rapid determination of urea-nitrogen in serum or plasma without deproteinization. **American Journal of Medical Technology**. 33: 361-365.

Dahlan, I., Islam M., Rajion M.A. (2000). Nutrient intake and digestibility of fresh, ensiled and pelleted oil palm (Elaeis guineensis) frond by goats. Asian-**Australian Journal of Animal Science**, 13:1407-1413.

Danner, H., Holzer M., Mayrhuber E., and Braun R. (2003). Acetic acid increases stability of silage under aerobic conditions. **Applied and Environmental Microbiology**. 69:562-567.

Detmann, E.M.F., Mantovani H.C., Valadares Filho S.D.C., and Sampaio C.B. (2009). Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis-Menten kinetics. **Livestock Science**, 126: 136-146.

Dijkstra, J., Boer H., van Bruchem J., Bruining M., and Tamminga S. (1993). Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. **British Journal of Nutrition**, 69: 385-396.

Erdman, R.A. (1988). Dietary buffering requirements of the lactating dairy cow: A review. **Journal of Dairy Science**, 71: 3246-3266.

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 Sciences**, 89: E31-E51.

Ganz, T, and Nemeth E. (2006). Regulation of iron acquisition and iron distribution in mammals. Biochimica et Biophysica Acta . 1763:690-699. In: Hansen et al. (2009). Bioaccessibility of iron from soil is increased by silage fermentation, **Journal of Dairy Science**, 92 (6): 2896-2905.

Hobson, P.N., and Steward C.S. (1997). The Rumen Microbial Ecosytem. **Springer**, Netherlands.

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. **Microbiology Ecology**, 62: 94-105.

Hosada, 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-Australia Journal of Animal Sciences** 22 (4): 528-533.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2012a). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Animal Science Journal**, 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 Japanese Society of Grassland Science, **Grassland Science**, 58: 161-169.

Hosoda, K., Miyaji M., Matsuyama H., Haga S., Ishizaki H., Nonaka K. (2012c). Effect of

Kariuki, J.N., Tamminga S., Gachuiri 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 batatus vines. **South African Journal**, 12: 25-34.

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.

Kung, J.L. (2010). Aerobic stability of silage. Proceedings of the California Alfalfa and Forage Symposium and Corn/Cereal Silage Conference, December 1-2, 2010, **Visalia**, CA: 234-331.

Leatherwood, W.L. (2014). Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on in vitro Fermentation by Rumen Microbial Cultural Research. A thesis submitted to the Graduate Faculty of North Carolina State University. **North Carolina**, 80-88.

Leedle, J.A., Coe M.L., and Frey R.A., (1995). Evaluation of health and ruminal variables during adaptation to grain-based diets in beef cattle. American **Journal of Veterinary Research**, 56: 885-892.

Leek, B.F. (1993). Digestion in the Ruminant Stomach. In: Dukes' Physiology of Domestic Animals, Swenson, M.J. and W.O. Reece (Eds.). 11th Edn. Cornell University Press, Ithaca, New York, **USA**, 387-416.

Leng, R.A. (1990). Factors affecting the utilization of 'poor-quality' forages by ruminants particularly under tropical conditions. **Nutrition Research Review**, 3: 277-303.

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**.

Lev-Yadun, S. and Gould K.S. (2009). Role of Anthocyanins in Plant Defence Anthocyanins, **Springer Science+Business Media**, LLC 2009.

Lloyd, S. (1982). Blood characteristics and the nutrition of ruminants. **British Veterinary Journal**. 138:70-85.

Martin, C., Morgavi D.P., and Doreau M. (2010). Methane mitigation in ruminants: From microbe to the farm scale. **Animal**, 4: 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**., 515-537.

Mohammad, M.R., Abdullah R.B., Wan W.E., Khadijah T.N., and Akashi R. (2015). Feed intake and growth performance of goats fed with Napier grass and oil palm frond supplemented with soya waste. **Journal of Applied Animal Research**, 43(3): 256-260.

Muia, J.M.K., Tamminga S., Mbugua P.N., and Kariuki J.N., (2000). The nutritive value of Napier grass (Pennisetum purpureum) and its potential for milk production with or without supplementation: A review. **Tropical Science**, 40: 109-131.

Ngwe, T., Nukui Y., Oyaizu S., Takamoto G., and Koike S. (2012). Bean husks as a supplemental fiber for ruminants: Potential use for activation of fibrolytic rumen bacteria to improve main forage digestion. **Animal Science Journal**, 83: 43-49.

Nyambati, E.M., Muyekho F.N., Onginjo E. and Lusweti C.M. (2010). Production, characterization and nutritional quality of Napier grass [Pennisetum purpureum (Schum.)] cultivars in Western Kenya. **African Journal of Plant Science**, 4(12): 496-502

Okaraonye, C.C., and Ikewuchi J.C. (2009). Nutritional and Antinutritional Components of Pennisetum purpureum (Schumach). **Pakistan Journal of Nutritio**n, 8 (1): 32-34.

Parish, J. and Rhinehart J. (2008). Mineral and Vitamin Nutrition for Beef Cattle. It is available at http://www.thebeefsite.com/articles/1549/mineral-and-vitamin-nutrition-for-beef-cattle Accessed on June 7, 2012.

Pipat, L., Mek K. and Wisitiporn S. (2011). Silage production from cassava peel and cassava pulp as energy source in cattle diets. **Journal of Animal and veterinary Advances**, 10 (8): 1007-1011.

Preston, R.L., Schnakanberg D.D., and Pander, W.H. (1965). Protein utilization in ruminants. 1. Blood urea nitrogen as affected by protein intake. **Journal of Nutrition**. 86: 281-288.

Shokryzadan, P., Rajion M.A., Goh Y.M., Ishak I., Ramlee M.F., Faseleh Jahromi M., and Ebrahimi M. (2016). Mangosteen peel can reduce methane production and rumen biohydrogenation in vitro. **South African Journal of Animal Science**, 46(4): 419-431.

Skerman, P.J., and Riveros F. (1990). Tropical Grasses. FAO, Rome, 75-12.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedure of Statistics. **McGraw-Hill Publishing Co**., New York.

supplementation of purple pigment from anthocyanin-rich corn (*Zea mays* L.) on blood antioxidant activity and oxidation resistance in sheep. **Livestock Science,** 145: 266-270.

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

Wanapat, M., Cherdthong A., Pakdee P., and Wanapat S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratesStapf.) powder supplementation. **Journal of Animal Science**. 86: 3497-3503.

Wang, Y., Frutos P., Gruber M.Y., Ray H., and McAllister T.A. (2006). In vitro ruminal digestion of anthocyanidin-containing alfalfa transformed with the maize Lc regulatory gene. **Canada Journal of Plant Sience**, 1119-1130.

Weinberg, Z.G., Chen Y., and Gamburg M. (2004). The passage of lactic acid bacteria from silage into rumen fluid, in vitro studies. **Journal of Dairy Science**, 87: 3386-3397.

Widiawati, Y. and Thalib A. (2009). Comparison of fermentation kinetics (in vitro) of grass and shrub legume leaves: The pattern of VFA concentration, estimated CH4 and microbial biomass production. **Indonesian Journal of Agriculture**, 2: 21-27.

Yokota, H., Okajima T. and Ohshima M. (1991). Effect of environmental temperature and addition of molasses on the quality of Napier grass (Pennisetum purpureum) silage. **Asian-Australian Journal of Animal Sciences**, 4:377-382.

Zinn, A.R. and Owen, F.N. (1986). A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. **Canadian Journal of Animal Science.** 66: 157-163.

CHAPTER V

EFFECT OF ANTHOCYANIN RICH NAPIER GRASS SILAGE WITH ADDITIVES ON OXIDATION STATE AND ANTIOXIDANT ENZYME ACTIVITY IN PLASMA OF THE NATURALLY *HAEMONCHUS CONTORTUS* INFECTED GOAT

5.1 Abstract

The present research aims to evaluate the effect of anthocyanin rich Napier grass silage on oxidative state and antioxidant enzyme activity in the growing goats. Eighteen crossbred Thai native x Anglo-Nubian meat goats (average BW 23.78 ±2.4kg) were randomly assigned to feed on one of treatment diets according to completely randomized design. The goats were divided into three groups of six goats each to receive Napier grass silage (negative control), Anthocyanin rich Napier grass silage without additive (positive control), and Anthocyanin rich Napier grass silage with 4% molasses and 0.03% $FeSO_4$ (treatment). All animals were offered ad-libitum daily throughout 90 days with concentrate 16% crude protein (1.5% body weight). The result shows a significant ($P<0.05$) increase in ADF, CP, anthocyanin, total phenol, and condensed tannin intake; improving red blood cell (RBC), white blood cell (WBC), and hematocrit (HCT) parameter in goat blood; enhancing activity of catalase (CAT), glutathione reductase (GR) and glutathione-s-tranferase (GST) antioxidant

enzyme and reducing lipid oxidation (MDA), superoxide dismutase (SOD), and antioxidant state (TAC) in goat plasma. Fecal egg count (FEC) was reduced after 4 week feeding and body weight was increased better. With the *Haemonchus conturtus* injected goat, using Anthocyanin rich Napier silage improved activity of antioxidant enzymes and help the goat inhibit oxidative stress from parasite injection.

Keywords: Anthocyanin rich Napier grass, *Haemonchus conturtus*, oxidative stress, goat

5.2 Introduction

Oxidative stress is negative imbalance between antioxidants and free radicals from high concentrations of by-product of Reactive oxygen species (ROS) in physiological processes of biological systems (Betteridge, 2000). Oxidative stress is a normal occurrence during the immune system's inflammatory response to infection or injury. Inflammation plays a vital role in the body's response to pathogens and tissue damage. Some factors such as mastitis, reproductive disorders, parasitic infections, metabolic changes mediated by ROMs (reactive oxygen metabolites, milk production, heat-stress), feed quality, imbalance nutrient and management which make oxidative stress in ruminant (Celi, 2010, Celi, 2011 and Celi et al., 2015). Parasitic diseases seem to be a causative source of oxidative stress, indeed, several studies have reported on the presence of oxidative stress in small ruminant infected with parasites (Esmaeilnejad et al., 2012a, Esmaeilnejad et al., 2012b, Angulo-Valadez et al., 2011, Dey et al., 2010) as well as the antioxidant defense mechanism that exists between parasites and the mammalian host. Anthocyanin rich Napier grass is a cultivate of Napier grass with leaves and stems in purple color which pigment was made from anthocyanin to protect plant cell from UV radiation, oxidative stress from

environmental pathogen and herbivores (Lev-Yadun et al., 2009). The results of the previous study indicated that using anthocyanin rich Napier improved activity of nonenzymatic (total antioxidant capacity: TAC), effect on superoxide dismutase (SOD) antioxidant enzyme, and reduced lipid oxidation in plasma of growing goats. Anthocyanin rich Napier grass silage can be used as animal feed, that indicate to help the goat limit the harmful of oxidative stress from environment. Hence, this study were conducted to evaluate effect on anthocyanin rich Napier silage with additive on antioxidant status in plasma of natural Haemonchus contortus infected growing goat.

5.3 Objectives

To compare the feeding time and the effect of Anthocyanin rich Napier grass silage with additives on oxidative status and antioxidant enzyme activity of growing goat plasma under naturally *Haemonchus contortus* infection condition and compare with Napier grass silage, anthocyanin-rich Napier grass silage without additive

5.4 Materials and methods

5.4.1 Treatments

Three treatments follow:

Negative control (T1): Napier grass silage

Positive control (T2): anthocyanin rich Napier grass silage without additive

Treatment (T3): anthocyanin rich Napier grass silage with 4%molasses and 0.03% $FeSO_4$.

5.4.2 Experimental design

Eighteen growing goats crossbred Thai native x Anglo nubain an average body weight (BW) of 23.78±2.4kg were allocated in Completed randomized design (CRD). These goats were natural infected with *Haemonchus conturtus* and selected from SUT farm.

5.4.3 Management, sampling and measurements

Before going to experiment, the goats were test the fecal egg count and blood parameters including: HCT (Hematocrit), HGB (Hemoglobin), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume), RBC (Red Blood Cell), RDW (Red Distribution Width), and WBC (White blood cell). EFC was followed the method of McMaster (Raynaud, 1970). EDTA blood samples were collected before treatment and every two weeks from the vein of animal with sampling the feces and recording the body weight. Plasma was recovered and stored at -20°C until analysed antioxidant enzymes. Eight Haematological parameters were estimated using an Auto Haematology analyser BC- 2800Vet® (Shenzen Mindray Bio-Medical Electronics Co., Ltd, Hamburg, Germany). Plasma samples were also used to estimate antioxidant enzymes.

The total antioxidant capacity (TAC), representing the plasma's non-enzymatic antioxidative systems, was assayed using a commercial kit (Total Antioxidant Capacity Assay Kit MAK187; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as nmole of copper reducing power per µl of plasma.

The plasma activity of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion, was measured as an endogenous enzyme using a commercial kit (19160 SOD determination Kit; Sigma-Aldrich, Missouri, USA) following the manufacturer's instructions. Values were expressed as units per mL of plasma.

The malondialdehyde (MDA) concentration in the plasma sample was measured as an index of lipid peroxidation by thiobarbituric acid reaction using a commercial kit (Lipid Peroxidation (MDA) Assay Kit MAK085; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as nmole per µl of plasma.

The Catalase (CAT) concentration in the plasma sample was measured as an index of antioxidant enzyme by decompostion of hydrogen peroxide using a commercial kit (Lipid Peroxidation (MDA) Assay Kit MAK085; Sigma-Aldrich, Missouri, USA) with fresh plasma samples according to the manufacturer's instructions. Values were expressed as unit per ml of plasma.

The plasma concentration of Glutathione reductase, an enzymatic antioxidant catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), was analyzed by a commercial kit (Glutathione Reductase Assay Kit GRSA; Sigma-Aldrich, Missouri, USA) according to the manufacturer's instructions. The assay was performed with extracts prepared immediately from the fresh plasma samples. Values were expressed in unit per mL of plasma.

The plasma concentration of Glutathione-S-Transferase (GST), a group of enzymes detoxicate xenobiotics in the mammals, was analyzed by a commercial kit (Glutathione-S-Transferase (GST) Assay Kit CS0410; Sigma-Aldrich, Missouri, USA)

according to the manufacturer's instructions. The assay was performed with extracts prepared immediately from the fresh plasma samples. Values were expressed in umole per ml per min of plasma.

Table 5.1 Ingredients and chemical composition of the experimental diets (% DM).

Items	T1	T2	T3
% Dry Matter (DM)	18.01	15.21	17.49
		%DM	
ASH	13.86	12.88	12.75
Organic Matter (OM)	86.14	87.13	87.26
Crude Protein (CP)	6.95	7.89	8.41
Neutral Detergent Fiber (NDF)	75.36	75.69	67.32
Acid Detergent Fiber (ADF)	44.27	44.45	37.18
Hemicellulose	31.1	31.24	30.14
Crude Fiber (CF)	42.16	41.28	32.94
Ether Extract (EE)	4.48	4.71	4.95
Anthocyanin	0.01	0.03	0.06
Phenolic Compound	4.14	9.05	10
Total Tannin	2.28	2.52	2.81
Non Phenolic Compound	1.87	6.54	7.19
Condensed Tannin	0.1	0.1	0.11
Gross Energy (kcal/kg DM)	3952.15	4015.05	4008.64

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. SEM = Standard error of the mean

Animals were individually housed and intensively cared according to procedures of goat farm at Suranaree University of Technology. Silage was offered twice daily *ad libitum*; approximately at 7 am and 5 pm. concentrate was fed 1.5%

BW. Diets were allowed to have 5% left over. Feed ingredients of the experimental diets are shown in Table 5.1. Fresh water was available at all times.

5.4.4 Statistical analysis

The data of this experiment were subjected to SAS Ver. 9.1 program using the general linear model procedure and statistically significant differences among mean were determined using Duncan's multiple range test at 5% probability (SAS, 1998). Blood measurements were analyzed as repeated measures using the autoregressive repeated covariance structure in the MIXED procedure. The model fixed effects included treatment, sampling time and the treatment* sampling time interaction, with animal as the random effect. Values of $P < 0.05$ and $P < 0.01$ were considered to indicate statistical significance and to show tendency, respectively.

5.5 Results

5.5.1 Feed intake

Feed intake was shown in table 5.2. DM, OM, EE, NDF and total tannin intake were not different but ADF, CP, anthocyanin, total phenol, and condensed tannin were significant different ($P<0.05$). The animal in treatment received the highest amount of phenolic compound and anthocyanin because the chemical composition of 3 treatments are differences about CP, ADF, anthocyanin, total phenolic compound, and condensed tannin while they received the similarly amount of dry matter intake. These results are agreement with the results from the previous experiment, which reported that the group of goat fed anthocyanin rich Napier grass, received more crude protein and have the digestibility of nutrient better.

Table 5.2 Effect of treatments on feed intake of parasite infected goat.

Items	T1	T2	T3	SEM	P-value
DMI g/d	615.08	603.26	601.32	67.71	0.93
OMI g/d	551.17	539.82	535.65	60.63	0.90
ADFI g/d	179.13[a]	162.51[b]	145.30[c]	20.04	0.03
NDFI g/d	389.63	371.70	349.77	42.80	0.30
EEI g/d	23.82	22.72	21.93	2.64	0.48
CPI g/d	70.44[b]	77.64[b]	85.36[a]	8.09	0.02
ANI mg/d	2.97[c]	101.34[b]	171.47[a]	11.96	0.00
TPI g/d	17.82[c]	29.91[b]	35.34[a]	2.75	0.00
TTI g/d	12.26	13.18	13.76	1.39	0.20
CTI g/d	0.53[b]	0.55[b]	0.64[a]	0.06	0.01

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. DMI= dry matter intake, OMI= organic matter intake, ADFI= acid detergent fiber intake, NDFI= neutral detergent fiber intake, EEI= ether extract intake, CPI= crude protein intake, ANI= anthocyanin intake, TPI= total phenol intake, TTI= total tannin intake, CTI= condensed tannin intake. SEM = Standard error of the mean.

5.5.2 Blood parameters

Blood parameters were shown in table 5.3. WBC, RBC and HCT were significant different ($P<0.05$). HGB, MCV, MCHC, MCH, and RDW were not different. Anthocyanin rich Napier grass silage improved the blood parameters than other treatment. When the goat infected with *Haemonchus conturtus*, they lost amount of blood, their haematological alterations and histopathological changes (Mir et al., 2007; Williamson et al., 2003). In addition, trace literatures were conducted regarding gastrointestinal nematodes effect on the oxidative stress parameters and antioxidant defenses in the sheep (Dede et al., 2002) in spite of the exceeded exposure of sheep to *Haemonchus contortus* parasite now and later which considered amongst the main parasitic infestations in Egypt. Under parasite condition, hematocrit and red blood cell was decreased, which made anemia in the host animal (Esmaeilnejad et al., 2012a; Machado et al., 2014; Dey et al., 2010). The WBC improved better after 4 week feeding silage, this results are in un-agreement with Rahman and Collins (1990) who stated that infection with *Haemonchus contortus* did not lead to significant changes in total white cell counts with marked eosinophilia and Al-Salahy et al (2017), who used garlic juice on sheep infected with *Haemonchus conturtus* . From these findings, our result assumed that anthocyanin can enhance the response to haemonchosis probably is an immunological regulator against any excessive harmful parasitic effect on the host. On the other way, nutrition is the important in process against parasite, the goat change the living condition from grazing to feeding by human, who provide them nutrient requirement daily.

Table 5.3 Effect of treatments on blood parameters of parasite infected goats.

Items	RBC 106 cell/µL	WBC 103cell/µL	HCT %	HGB g/dL
Treatment effect				
T1	14.95[a]	15.87[a]	19.98[a]	6.54
T2	15.53[a]	17.95[a]	22.13[a]	7.01
T3	11.29[b]	12.38[b]	16.89[b]	7.2
SEM	0.65	0.91	0.47	0.62
P value	0.01	0.02	0.04	0.6
Week effect				
0w	11.62[c]	17.95[a]	16.98[a]	6.05b
2w	13.79[b]	12.38[b]	19.93[b]	7.26b
4w	16.36[a]	15.87[a]	22.08[b]	7.43a
SEM	0.65	0.91	0.47	0.62
P value	0.01	0.03	0.02	0.06
Treatment *Week effect				
0w* T1	17.15[a]	15.18[c]	11.21[d]	7.45b
0w* T2	13.36[ab]	19.47[a]	20.58[abc]	6.01b
0w* T3	16.08[ab]	19.21[a]	19.16[bc]	7.56b
2w* T1	15.89[ab]	16.92[bc]	16.67[c]	7.61b
2w* T2	16.89[a]	18.21[b]	20.35[abc]	6.38b
2w* T3	11.08[c]	11.99[cd]	22.77[ab]	7.60b
4w* T1	16.04[ab]	17.02[b]	22.78[ab]	7.23b
4w* T2	14.61[b]	14.36[cd]	19.00[bc]	7.76a
4w* T3	14.21[b]	16.22[bc]	24.46[a]	7.63a
SEM	0.65	0.91	0.47	0.62
P value	0.02	0.01	0.02	0.98
Treatment effect				
T1	13.56	20.93[b]	4.11	35.85[b]
T2	14.34	22.88[a]	3.94	32.56[b]
T3	15.3	20.38[b]	4.83	45.89[a]
SEM	0.41	0.97	0.28	0.66
P value	0.07	0.23	0.32	0.13

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. HCT=hematocrit, HGB= hemoglobin, MCH= Mean Corpuscular Hemoglobin, MCHC= mean corpuscular hemoglobin concentration, MCV= Mean Corpuscular Volume, RBC= red blood cell, RDW= red distribution width, WBC= white blood cell. SEM = Standard error of the mean

Table 5.3 Effect of treatments on blood parameters of parasite infected goats (continue).

Items	MCV IL	RDW %	MCH pg	MCHC g/dL
Week effect				
0w	15.08^{a}	20.08^{c}	4.67	40.34^{a}
2w	14.56^{b}	20.39^{b}	4.48	37.69^{b}
4w	13.55^{b}	23.72^{a}	3.72	36.26^{b}
SEM	0.41	0.97	0.28	0.66
P value	0.04	0.03	0.1	0.61
Treatment *Week effect				
0w* T1	14.27^{b}	25.52^{a}	3.626^{b}	30.52^{c}
0w* T2	14.58^{b}	20.77^{bc}	3.758^{b}	33.82^{c}
0w* T3	14.16^{b}	22.37^{b}	4.441^{ab}	33.34^{c}
2w* T1	14.36^{b}	22.48^{b}	3.855^{b}	33.83^{c}
2w* T2	16.47^{a}	19.93^{d}	5.833^{a}	58.15^{a}
2w* T3	15.08^{a}	18.73^{d}	4.807^{ab}	45.67^{b}
4w* T1	12.03^{c}	23.17^{b}	3.691^{b}	44.43^{b}
4w* T2	14.19^{b}	19.55^{d}	5.434^{a}	29.05^{d}
4w* T3	14.45^{b}	20.07^{c}	5.205^{a}	34.06^{c}
SEM	0.41	0.97	0.28	0.66
P value	0.26	0.75	0.25	0.03

T1: Napier grass silage, T2: Anthocyanin rich Napier grass silage without additive, T3: Anthocyanin rich Napier grass silage with additive. HCT=hematocrit, HGB= hemoglobin, MCH= Mean Corpuscular Hemoglobin, MCHC= mean corpuscular hemoglobin concentration, MCV= Mean Corpuscular Volume, RBC= red blood cell, RDW= red distribution width, WBC= white blood cell. SEM = Standard error of the mean.

Besides, condensed tannin effected on digestive parasite (Hoste et al., 2012) by limit the action of them and increased the by-pass protein for host animal (Whitley et al., 2009). When the action of parasite was limited, and the supplement nutrient was provide enough for the host animal, the number of RBC, HCT, and WBC were improved. Anthocyanin rich Napier grass silage contain anthocyanin and tannin with high protein is the good feed for goat to against *Haemonchus conturtus* parasite. Feeding anthocyanin rich Napier grass silage after 2 weeks will show the effect on the goat blood parameters.

5.5.3 Antioxidant enzymes

Antioxidant enzyme activity were shown in table 5.3. TAC, SOD, MDA, CAT, GST, GR, FEC, and body weight were significant different. From the previous experiment, the animal received diet with anthocyanin rich Napier grass silage have the body weight increasing better than others because they took the higher amount of crude protein, digestibility percentage and nitrogen absorbed, so the body weight of treatment is higher than control group. After 4 weeks, the control and treatment were improved the animal body weight, difference was found between week but not significantly different between treatments. With fecal egg count, condensed tannin in anthocyanin rich Napier grass silage and Napier grass silage showed the slightly effect on number of parasite egg after 4 weeks but no interaction between week and treatment. There are many studies suggested that condensed tannin decreased fecal

Table 5.4 Effect of treatments on antioxidant enzymes of parasite infected goat plasma.

Items	TAC nmole/ul	SOD U/ml	MDA nmole/ul	CAT unit/ml
Treatment effect				
T1	0.30^{a}	298.59^{a}	0.41^{a}	334.32^{a}
T2	0.33^{a}	335.00^{a}	0.45^{a}	375.09^{a}
T3	0.23^{b}	232.55^{b}	0.34^{b}	269.57^{b}
SEM	0.06	17.86	0.02	18.04
P value	0.04	0.03	0.03	0.03
Week effect				
0w	0.33^{a}	329.47^{a}	0.44^{a}	279.66^{b}
2w	0.29^{ab}	293.07^{ab}	0.40^{ab}	331.66^{ab}
4w	0.24^{b}	243.60^{b}	0.35^{b}	367.67^{a}
SEM	0.06	17.86	0.02	18.04
P value	0.03	0.03	0.02	0.04
Treatment *Week effect				
0w* T1	0.28^{bc}	283.60^{bc}	0.39^{bc}	314.02^{b}
0w* T2	0.37^{a}	373.01^{a}	0.48^{a}	323.46^{b}
0w* T3	0.35^{ab}	348.39^{ab}	0.46^{b}	387.80^{a}
2w* T2	0.35^{ab}	348.83^{ab}	0.46^{b}	260.99^{d}
2w* T3	0.22^{abc}	224.91^{c}	0.33^{c}	263.65^{c}
2w* T1	0.32^{abc}	223.90^{c}	0.24^{d}	384.08^{a}
4w* T1	0.32^{ab}	323.29^{ab}	0.44^{bc}	304.90^{b}
4w* T2	0.26^{d}	266.58^{bc}	0.38^{bc}	354.53^{ab}
4w* T3	0.30^{abc}	305.91^{ab}	0.42^{bc}	343.51^{ab}
SEM	0.06	17.86	0.02	18.04
P value	0.01	0.01	0.01	0.06

TAC= total antioxidant capacity, SOD= superoxide dismutase. MAD= Malondialdehyde, CAT= catalase, GSH= glutathione, GST= glutathione S-transferase, FEC=fecal egg count, BW=bodyweight. SEM = Standard error of the mean

Table 5.4 Effect of treatments on antioxidant enzymes of parasite infected goat plasma (continue).

Items	GST umole/ml/min	GR unit/ml	BW kg	FEC egg/g
Treatment effect				
T1	177.92[a]	1.82[a]	25.2	124123[b]
T2	197.91[a]	2.02[a]	24.78	160630[a]
T3	144.97[b]	1.49[b]	25.12	165910[a]
SEM	9.28	0.09	0.48	6469
P value	0.03	0.03	0.75	0.04
Week effect				
0w	149.14[b]	1.53[b]	24.00[b]	172995[a]
2w	175.40[ab]	1.80[a]	25.00a[b]	145896[b]
4w	196.26[a]	1.99[a]	26.00[a]	131772[b]
SEM	9.28	0.09	0.48	6469
P value	0.03	0.04	0.01	0.01
Treatment *Week effect				
0w* T1	119.66	1.23[cd]	25.00[ab]	157920
0w* T2	171.07[b]	1.74[b]	24.00[b]	171553
0w* T3	202.99[a]	2.07[a]	25.00[ab]	152417
2w* T2	90.38[d]	0.95[d]	24.00[b]	182833
2w* T3	141.81[c]	1.47[c]	25.00[ab]	166366
2w* T1	102.71[d]	2.06[a]	26.00[a]	148532
4w* T1	166.41[c]	1.69[b]	25.00[ab]	88864
4w* T2	185.96[b]	1.90[ab]	24.00[b]	164600
4w* T3	181.40[b]	1.86[ab]	25.00[ab]	118904
SEM	9.28	0.09	0.48	6469
P value	0.04	0.01	0.8	0.06

TAC= total antioxidant capacity, SOD= superoxide dismutase. MAD= Malondialdehyde, CAT= catalase, GR= glutathione reductase, GST= glutathione S-transferase, FEC=fecal egg count, BW=bodyweight. SEM = Standard error of the mean

Fecal egg counts (FECs) is a reduction in larval development for Spanish weather goats and Angora does grazing Sericea lespedeza (Min et al., 2003 and 2004). After 4 week feeding treatment, total antioxidant capacity, superoxide dismutase, and lipid peroxidation (MDA) were lower than beginning time, there are significantly different from week, treatment, interaction between week and treatment. Condensed tannin from three diets effect direct on the parasite, under feeding program is better than grazing system, there are 2 factors make the goat is more healthy by the time, but anthocyanin in treatment is highest as compared to others and the value of TAC, SOD, and MDA in treatment was show the lowest result. From this finding, Anthocyanin decreased the value of TAC, SOD, and MDA and improve the oxidative stress of the animal. In addition, Al-Salahy et al. (2017) reported the garlic juice effected to decrease MDA but increase SOD after 8 weeks feeding the sheep infected with *Haemonchus conturtus* in Egypt. Anthocyanin effect strongly on SOD, TAC and MDA (Hosada et al., 2012 a, 2012b). However, decreased SOD antioxidant enzyme levels still indicated that enhancing ROS production stimulate utilization of the antioxidant enzymes in the cell (Pilania et al., 2013).

5.5.3 Antioxidant enzymes

Antioxidant enzyme activity were shown in table 5.3. TAC, SOD, MDA, CAT, GST, GR, FEC, and body weight were significant different. From the previous experiment, the animal received diet with anthocyanin rich Napier grass silage have the body weight increasing better than others because they took the higher amount of crude protein, digestibility percentage and nitrogen absorbed, so the body weight of treatment is higher than control group. After 4 weeks, the control and treatment were improved the animal body weight, difference was found between week but not significantly

different between treatments. With fecal egg count, condensed tannin in anthocyanin rich Napier grass silage and Napier grass silage showed the slightly effect on number of parasite egg after 4 weeks but no interaction between week and treatment. There are many studies suggested that condensed tannin decreased fecal

Fecal egg counts (FECs) is a reduction in larval development for Spanish weather goats and Angora does grazing Sericea lespedeza (Min et al., 2003 and 2004). After 4 week feeding treatment, total antioxidant capacity, superoxide dismutase, and lipid peroxidation (MDA) were lower than beginning time, there are significantly different from week, treatment, interaction between week and treatment. Condensed tannin from three diets effect direct on the parasite, under feeding program is better than grazing system, there are 2 factors make the goat is more healthy by the time, but anthocyanin in treatment is highest as compared to others and the value of TAC, SOD, and MDA in treatment was show the lowest result. From this finding, Anthocyanin decreased the value of TAC, SOD, and MDA and improve the oxidative stress of the animal. In addition, Al-Salahy et al. (2017) reported the garlic juice effected to decrease MDA but increase SOD after 8 weeks feeding the sheep infected with *Haemonchus conturtus* in Egypt. Anthocyanin effect strongly on SOD, TAC and MDA (Hosada et al., 2012 a, 2012b). However, decreased SOD antioxidant enzyme levels still indicated that enhancing ROS production stimulate utilization of the antioxidant enzymes in the cell (Pilania et al., 2013).

The value of catalase (CAT), Glutathione reductase (GR), and glutathione S-transferase (GST) of diet contain anthocyanin were increased but diet with Napier grass silage was change a little bit in the amount of CAT, GR, and GST. The different were significantly from week, treatment, and interaction between week and treatment. This

results is the same trend with the study of Al-Salahy et al. (2017) but in his research, the GST was reduced. In summary, anthocyanin effect on the antioxidant status of animal, especially with the animal under strong oxidative stress-parasite infection, decreasing TAC, SOD, and MDA; and increasing action of CAT, GR, and GST.

5.5 Conclusion

Using Anthocyanin rich Napier silage improved activity of antioxidant enzymes and help the animal limit the harmful of oxidative stress from environment. With *Haemonchus conturtus* injected goat, this silage improve activity of antioxidant enzymes and enhance number of red blood cell, hematocrit and reduce white blood cell while increase the body weight by increasing of CP intake, nutrient intakes, nutrients digestibility, N utilization, and ADG as well as body weight gain of growing goats, decreasing TAC, SOD, and MDA; and increasing action of CAT, GR, and GST. The Anthocyanin rich Napier grass silage showed the effect on the animal after feeding two weeks.

5.6 References

Al-Salahy, M.B. Kamel H.H., Mostafa A.M., Arafaa I.M., and Abdel-Salam M. (2017). Evaluation The Antioxidants Effect of Garlic Juice on Experimental Sheep Haemonchosis **Egyptian Journal of Chemistry and Environmental Health**, 3 (2):75 -92.

Angulo-Valadez, C.E., Reyes-Becerril M.C., Romero G.M.J., Cepeda-Palacios R., López-Aguilar D.R., Zenteno T., Ascencio F. (2011). Antioxidant enzymes in erythrocytes from goats seropositive to the sheep nose bot fly (*Oestrus ovis* L., Diptera: Oestridae) infection. **Veterinary Parasitology** 183: 140– 145.

Betteridge, D. John D. (2000). What Is Oxidative Stress? **Journal of Metabolism. Volume** (9): 3-8.

Celi, P. (2011). Oxidative stress in ruminants. In: Mandelker L, Vajdovich P, editors. Studies on Veterinary Medicine. Oxidative Stress in Applied Basic Research and Clinical Practice. **New York: Humana Press.** 191-231.

Celi, P. and Gabai G. (2015). Oxidant/ antioxidant balance in animal nutrition and health: the role of protein oxidation. Front. **Veterinary Sciences** 2:48-50.

Celi, P., (2010). Biomarkers of oxidative stress in ruminant medicine. Review article. **Immunopharmacology and Immunotoxicology**. 33(2): 233-240.

Dede, S., Deger, Y., Alkan, M. and Cemek, M. (2002). Oxidation products of nitric oxide and the concentration of antioxidant vitamins in parasitized goats. Acta Veterinaria Brunensis, 71: 1-5.

Dey, S. U. K. D. (2010). Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. **Tropical Animal Health and Production**, 42: 1663-1668.

Esmaeilnejad, B., Tavassoli M., Asri-Rezaei S., Dalir-Naghadeh B. and Malekinejad H. (2012a). Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with Babesia ovis. **Acta Parasitologica**, 57(3): 228-234.

Esmaeilnejad, B., Tavassolia M., Asri-Rezaei S., Dalir-Naghadeh B. (2012b). Evaluation of antioxidant status and oxidative stress in sheep naturally infected with Babesia ovis. **Veterinary Parasitology**, 185: 124-130.

Hosada, 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-Australia Journal of Animal Sciences**, 22 (4): 528- 533.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2012a). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. **Animal Science Journal**, 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 Japanese Society of Grassland Science, **Grassland Science**, 58: 161-169.

Hosoda, K., Miyaji M., Matsuyama H., Haga S., Ishizaki H., 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. **Livestock Science**, 145: 266-270.

Hoste, H., Martinez-Ortiz-de-Montellano C., Manolaraki F., Brunet S., Ojeda-Robertos N., Fourquaux I., Torres-Acosta J.F.J., Sandoval-Castro C.A. (2012). Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infection. **Veterinary Parasitology**, 186: 18-27.

Leatherwood, W.L. (2014). Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on in vitro Fermentation by Rumen Microbial CultuResearchA thesis submitted to the Graduate Faculty of North Carolina State University. **North Carolina.**

Lev-Yadun, S. and Gould K.S. (2009). Role of Anthocyanins in Plant Defence Anthocyanins, **Springer Science+Business Media**, LLC.

Machado, V., Da Silva A.S., Schaferb A.S., Airesb A.R., Toninc A.R., Oliveirad C.P., Hermese C.L., Almeidae T.A., Morescoe R.N., Stefania L.M., Lopesc S.T.A., Mendesf R.E., Schwertzf C.I. and Leal M.L.R. (2014). Relationship between

oxidative stress and pathological findings in abomasum of infected lambs by Haemonchus contortus. **Pathology-Research and Practice**, 210: 812-817.

Min, B.R., and Hart S.P. (2003). Tannins for suppression of internal parasites. **Journal of Animal Science**, 81(2): E102-E109.

Min, B.R., Pomroy W.E., Hart S.P., and Sahlu T. (2004). The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing weather goats. **Small Rumininant Research**, 51:279-283.

Mir, A.R., Chishti, M.Z., Zarger, M.A., Tak, H. and Ganie, S.A. (2007). Clinicopathological changes in sheep experimentally infected with Haemonchus contortus. **World Journal of Agricultural Sciences**, 3(5), 562-566.

Pilania, P.L., Solanki S., Mohammed N. Asopa S., Joshi A., Sankhala L.N., Mathur M., Thori M.K., Gaur J.S., Meena A. and Kataria N. (2013). Oxidative stress Vis a Vis Gastrointestinal Parasitiem and Pneumonia in Marwari Goat. **Veteinary Research**, 6 (3): 54-57.

SAS, (1998). User's Guide: Statistic, Version5.Edited 1996. SAS.Inst Cary,NC., U.S.A.

Whitleya, N.C., Miller J.E., Burkec J.M., Cazac D., O'Briena, D.J., Dykes L., Muire J. P. (2009). Effect of high tannin grain sorghum on gastrointestinal parasite fecal egg counts in goats. **Small Ruminant Research**, 87: 105-107.

Williamson, A.L., Brindley, P.J., nox, D.P., Hotez, P.J. and Loukas A. (2003). A digestive proteases of blood feeding nematodes. **Trends Parasitology**, 19(9): 417-423.

CHAPTER VI

OVERALL CONCLUTION AND IMPLICATION

6.1 Conclusions

Addition molasses and $FeSO_4$ enhanced the stable of anthocyanin, DM, CP and improved digestibility of NDF, ADF. The suitable level of molasses is 4% and FeSO4 is 0.03%. The data of gas production demonstrated anthocyanin increases propionic acid proportion, decreased the number of Methanogen and *Streptococcus bovis* bacteria. Anthocyanin cannot be absorbed in the rumen by *in vitro* incubation. Anthocyanin rich Napier grass silage can be used as animal feed, that was indicator by apparently increased of CP intake, nutrient intakes, nutrients digestibility, N utilization, and ADG as well as body weight gain of growing goats. In feeding trial, Anthocyanin rich Napier grass silage with additive reduce the number of *Streptococcus bovis* and tend to reduce Methanogen bacteria group in rumen fluid, which was resulted on methane emission. Using Anthocyanin rich Napier silage improved activity of antioxidant enzymes and help the animal limit the harmful of oxidative stress from environment by enhancing the activity of Superoxide dismutase (SOD) enzyme, reducing the oxidative stress by decreasing total antioxidant capacity (TAC) and lipid peroxidation (MDA) in the plasma of goats.

With the naturally *Haemonchus conturtus* injected goats, Anthocyanin rich Napier grass silage improved number of red blood cell (RBC), hematocrit (HCT) and reduce white blood cell (WBC) while increase the body weight by increasing of CP

intake. The most important, this silage effected to reduce TAC, SOD, MDA, and FEC (fecal egg count), it mean the oxidative stress was improved. Thus, the increasing in action of CAT (catalase), GR (glutathione reductase), and GST (glutathione-s-transferase) is the evidence for effect of anthocyanin in this silage. Anthocyanin can used for animal to improve the performance and oxidative stress status. The farmer will save cost when feeding their animal by Anthocyanin rich grass silage with additive.

6.2 Implications

From these results, it is called to conduct more and more studies about anthocyanin pigment from anthocyanin rich feedstuff on the animal, how and where the anthocyanin can be absorbed in the digestive system and their presence is in circulation, urine, feces, and muscle. On the other hand, the study should be done about the effect of anthocyanin on antioxidant enzyme with different type of parasites, which infect the animal to record clearly the role of Anthocyanin in the completion to against oxidative stress.

BIOGRAPHY

Miss Ngo Thi Minh Suong was born on the 3rd of January 1986 in Dong Thap province, Vietnam. She graduated Bachelor of Animal Science from College of Agriculture & Applied Biology, Cantho University of Vietnam in 2008. After graduation, she was studied master degree at College of Agriculture & Applied Biology, Cantho University of Vietnam In 2008, she obtained the scholarship from Suranaree University of technology for Asean countries to presence a Doctor degree at school of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in July 2015, under the supervision of Associate Professor Dr. Pramote Paengkoum. She conducted the research in the topic of Utilization Anthocyanin rich Napier grass silage in growing goat diets from October 2016 to May 2018.