UTILIZATION OF ANTHOCYANIN FROM PURPLE NEEM FOLIAGE
ON ANTIOXIDANT ACTIVITY AND MEAT QUALITY
IN GROWING GOATS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the

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การใช้ประโยชน์ของแอนโทไซยานินจากใบรวมก้านสะเดาสีม่วงต่อการต้านอนุมูลอิสระ และคุณภาพเนื้อในแพะกำลังเจริญเติบโต
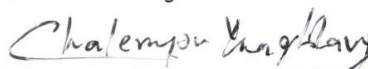
นางสาวนิตยา แท้ไธสง

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

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อนุมูลอิสระ และคุณภาพเนื้อในแพะกำลังเจริญเติบโต (UTILIZATION OF ANTHOCYANIN
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คำสำคัญ: ใบรวมก้านสะเดาสีม่วง/การต้านอนุมูลอิสระ/คุณภาพเนื้อ/แพะกำลังเจริญเติบโต

วิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อ ศึกษาผลของการใช้ประโยชน์ของแอนโทไซยานินจากใบรวม
ก้านสะเดาสีม่วงต่อการต้านอนุมูลอิสระ และคุณภาพเนื้อในแพะกำลังเจริญเติบโต การศึกษาครั้งนี้
ประกอบด้วย 3 การทดลอง

การทดลองที่ 1 เป็นการศึกษาผลของการใช้แอนโธไซยานินจากใบรวมก้านสะเดาม่วงต่อการ
หมักในกระเพาะหมักและการเจริญเติบโตของแพะที่กำลังเติบโต แพะเพศผู้จำนวน 25 ตัว สายพันธุ์
ลูกผสมบอร์ น้ำหนักตัวประมาณ 20±2 กก. โดยได้จัดทำแผนการทดลองแบบสุ่มสมบูรณ์ แบ่ง
ออกเป็น 5 ทรีทเมนต์ ได้แก่ T1 = กลุ่มควบคุม T2 = 3% ใบรวมก้านสะเดา T3 = 6% ใบรวมก้าน
สะเดา T4 = 3% ใบรวมก้านสะเดาม่วง T5 = 6% ใบรวมก้านสะเดาม่วง ผลการทดลองพบว่า แพะที่
ได้รับอาหารที่มี 6% ใบรวมก้านสะเดาม่วง เพิ่มการกินได้ การย่อยได้ของโภชนะ การเจริญเติบโต
และเพิ่มระดับของแอมโมเนียไนโตรเจน ยูเรียไนโตรเจนในเลือด กรดอะซิติก, กรดโพรพิโอนิก, กรด
บิวทิริก, ปริมาณกรดไขมันระเหยได้ทั้งหมด (VFA) เพิ่มจำนวนประชากรแบคทีเรีย, *Butyrivibrio
fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*,
Streptococcus bovis เพิ่มระดับของสารต้านอนุมูลอิสระทั้งหมด total antioxidant (TAC),
superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-
picrylhydrazyl (DPPH), and catalase (CAT) ในพลาสมา และลดจำนวนประชากรโปรโตซัว
methanogen ในกระเพาะรูเมน และ malondialdehyde (MDA) ในพลาสมา

การทดลองที่ 2 การศึกษาผลของแอนโทไซยานินจากใบรวมก้านสะเดาม่วงต่อการต้านอนุมูล
อิสระในพลาสมาและคุณภาพเนื้อในแพะกำลังเจริญเติบโต แพะเพศผู้จำนวน 25 ตัว สายพันธุ์ลูกผสมบอร์
น้ำหนักตัวประมาณ 20±2 กก. โดยได้จัดทำแผนการทดลองแบบแฟกทอเรียล 2×2+1 ในการทดลอง
ใช้เวลาในการทำการทดลองทั้งหมด 60 วัน โดยแบ่งออกเป็น 5 ทรีทเมนต์ ได้แก่ T1 = กลุ่มควบคุม
T2 = 3% ใบรวมก้านสะเดาม่วง+3% น้ำมันทานตะวัน T3 = 6% ใบรวมก้านสะเดาม่วง+3% น้ำมัน
ทานตะวัน T4 = 3% ใบรวมก้านสะเดาม่วง+6% น้ำมันทานตะวัน T5 = 6% ใบรวมก้านสะเดาม่วง+
6% น้ำมันทานตะวัน ผลการทดลองพบว่า แพะที่ได้รับอาหารที่มี 6% ใบรวมก้านสะเดาม่วง+6% น้ำมัน
ทานตะวัน เพิ่มปริมาณของการต้านอนุมูลอิสระทั้งหมด (total antioxidant) ซุปเปอร์ออกไซด์
ดิสมูเทส (SOD), กลูตาไธโอน เปอร์ออกซิเดส (GPX), DPPH, และ คะตาเลส (CAT) เพิ่มการ

แสดงออกของยีนต้านอนุมูลอิสระ GADPH, GPX, CAT, SOD ในกล้ามเนื้อส่วน *longissimus dorsi* และลดระดับของมาลอนไดอัลดีไฮด์ (MDA) ในพลาสมา แพะที่ได้รับอาหารที่มี 6% ใบรวมก้านสะเดาม่วง+6% น้ำมันทานตะวัน ลดค่าการสูญเสียน้ำ ลดการสูญเสียน้ำขณะการปรุงอาหาร ลดค่าแรงตัดเฉือนของเนื้อแพะ และเพิ่มกรดไขมันไม่อิ่มตัวเชิงเดี่ยว (MUFA) กรดไขมันไม่อิ่มตัวเชิงซ้อน (PUFA)

การทดลองที่ 3 การศึกษาผลของแอนโทไซยานินจากใบรวมก้านสะเดาม่วงต่อการแสดงออกของยีนต้านอนุมูลอิสระในกล้ามเนื้อ และคุณภาพเนื้อในแพะกำลังเจริญเติบโต แพะเพศผู้จำนวน 25 ตัว สายพันธุ์ลูกผสมบอร์ น้ำหนักตัวประมาณ 20±2 กก. โดยได้จัดทำแผนการทดลองแบบสุ่มสมบูรณ์ ในการทดลองใช้เวลาในการทำการทดลองทั้งหมด 60 วัน โดยแบ่งออกเป็น 5 ทรีทเมนต์ ได้แก่ T1 = กลุ่มควบคุม T2 = 6% ใบรวมก้านสะเดาม่วง T3 = 6% ใบรวมก้านสะเดาม่วง+โพลีเอทิลีนไกลคอล T4 = 6% ใบรวมก้านสะเดาม่วง+สารสีจากสะเดาม่วง T5 = 6% ใบรวมก้านสะเดาม่วง+สารสีจากสะเดาม่วง+โพลีเอทิลีนไกลคอล ผลการทดลองพบว่า แพะที่ได้รับอาหารที่มี 6% ใบรวมก้านสะเดาม่วง+สารสีจากสะเดาม่วง+โพลีเอทิลีนไกลคอล สามารถเพิ่มปริมาณการต้านอนุมูลอิสระทั้งหมด (TAC), SOD, GPX, DPPH และ CAT เพิ่มการแสดงออกยีนการต้านอนุมูลอิสระ GADPH, GPX, CAT, SOD ในกล้ามเนื้อส่วน *longissimus dorsi* และลด MDA ในพลาสมา นอกจากนี้แพะที่ได้รับอาหารที่มี 6% ใบรวมก้านสะเดาม่วง+สารสีจากสะเดาม่วง+โพลีเอทิลีนไกลคอล ลดค่าการสูญเสียน้ำ ลดการสูญเสียน้ำขณะการปรุงอาหาร ลดค่าแรงตัดเฉือนของเนื้อแพะ และเพิ่มกรดไขมันไม่อิ่มตัวเชิงเดี่ยว (MUFA) กรดไขมันไม่อิ่มตัวเชิงซ้อน (PUFA) ในเนื้อแพะ

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์

ปีการศึกษา 2565

ลายมือชื่อนักศึกษา

ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

NITTAYA TAETHAISONG : UTILIZATION OF ANTHOCYANIN FROM PURPLE NEEM
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Keyword: PURPLE NEEM FOLIAGE/ANTIOXIDANT ACTIVITY/MEAT QUALITY/GROWING
         GOATS

The objectives of this thesis were to investigate the effect of utilization of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats. This report was divided into 3 experiments.

Experiement 1 investigated the effect of utilization of anthocyanin from Purple Neem foliage on rumen fermentation and growth performance in growing goats. Twenty-five Boer crossbreed male goats (about 20±2 kg body weight; mean standard deviation (SD)) were assigned to a completely randomized design. There were five treatments: (1) control, (2) 3% Normal Neem foliage in concentrate, (3) 6% Normal Neem foliage in concentrate, (4) 3% Purple Neem foliage in concentrate, and (5) 6% Purple Neem foliage in concentrate. The results show that feed goats at 6% Purple Neem foliage in concentrate had higher nutrient intake, nutrient digestion, final weight, weigh change and ADG, ammonia nitrogen, BUN, acetic acid, propionic acid, butyric acid, total VFA, total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, *Streptococcus bovis*, levels of Total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) antioxidant activity in plasma and reduced protozoa methanogen and malondialdehyde (MDA) in plasma.

Experiement 2 investigated the effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma and meat quality in growing goats. Twenty-five Boer crossbreed male goats (approximately 20±2 kg body weight; mean±standard deviation (SD)) were assigned to 2×2+1 Factorial in a completely randomized design. All goats received a 60-d routine feeding consisting of five treatments: (1) control, (2) 3% Purple Neem foliage (PNF)+3% sunflower oil (SFO) in concentrate, (3) 3% Purple Neem foliage (PNF)+6% sunflower oil (SFO) in concentrate, (4) 6% Purple Neem foliage (PNF)+3% sunflower oil (SFO) in concentrate, and (5) 6% Purple Neem foliage

(PNF)+6% sunflower oil (SFO) in concentrate. The results demonstrate that goats fed 6% PNF+6% SFO had increased plasma levels of TAC, SOD, GPX, DPPH, and CAT, as well as strong antioxidant gene expression of GADPH, GPX, CAT, and SOD in the *longissimus dorsi* and decreased MDA in the plasma. Goats fed 6%PNF+6%SFO had reduced drip loss, cooking loss, shear force, and increased monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in goat meat.

Experiement 3 investigated the effect of anthocyanin from Purple Neem foliage on antioxidant genes expression in muscle and meat quality in growing goats. Twenty-five Boer crossbreed male goats (approximately 20±2 kg body weight; mean ±standard deviation (SD)) were assigned to a completely randomized design. All goats received a 60-d routine feeding consisting of five treatments: (1) control, (2) 6% Purple neem foliage (PNF) in concentrate, (3) 6% Purple neem foliage (PNF)+ Polyethylene glycol PEG in concentrate, (4) 6% Purple neem foliage (PNF)+Purple Neem pigment (PNP) in concentrate, and (5) 6% Purple neem foliage (PNF)+Purple Neem pigment (PNP)+Polyethylene glycol (PEG) in concentrate. The results show that feed goats fed a concentrate containing 6%PNF+PNP+PEG had higher TAC, SOD, GPX, DPPH, and CAT in plasma and antioxidant gene expression in *longissimus dorsi* of GADPH, GPX, CAT, SOD, and reduced MDA in plasma after feeding. Moreover, goats fed 6%PNF+PNP+PEG had reduced drip loss, cooking loss, shear force, and increased MUFA and PUFA in goat meat.

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Nittaya Taethaisong

CONTENTS

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF ABBREVIATIONS

a*	=	Redness
ADF	=	Acid detergent fiber
ADFI	=	Acid detergent fiber intake
ANOVA	=	Analysis of Variance
ADG	=	Average daily gain
AOAC	=	Association of Official Analytical Chemists
b*	=	Yellowness
BUN	=	Blood urea nitrogen
BW	=	Body weight
C_2	=	Acetic acid
C_3	=	Propionic acid
C_4	=	Butyric acid
CAT	=	Catalase
CP	=	Crude protein
CPI	=	Crude protein intake
DCP	=	Protein digestibility
DDM	=	Dry matter digestibility
DHA	=	Docosahesaenoic acid
DM	=	Dry matter
DMI	=	Dry matter intake
DOM	=	Digestibility of organic matter
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl
EE	=	Either extract
EEI	=	Either extract intake
EPA	=	Eicosapentaenoic acid
FR	=	Free radical
GADPH	=	Glyceraldehyde-3-phosphate dehydrogenase
GPX	=	Glutathione peroxidase

LIST OF ABBREVIATIONS (Continued)

L*	=	Lighhtness
LD	=	Longissimus dorsi
MDA	=	Malondialdehyde
MUFA	=	Monounsaturated fatty acid
NDF	=	Neutral detergent fiber
NDFI	=	Neutral detergent fiber intake
NH_3-N	=	Ammonia nitrogen
NRC	=	National Research Council
OM	=	Organic matter
OMD	=	Organic matter digestibility
OMI	=	Organic matter intake
PEG	=	Polyethylene glycol
PNF	=	Purple Neem foliage
PNP	=	Purple Neem pigment
PUFA	=	Polyunsaturated fatty acid
SAS	=	Statistical Analysis System
SD	=	Standard deviation
SEM	=	Standard error of mean
SFA	=	Saturated fatty acid
SFO	=	Sunflower oil
SOD	=	Superoxide dismutase
TAC	=	Total antioxidant
VFA	=	Volatile fatty acid

CHAPTER I

INTRODUCTION

1.1 Introduction

Goats are small ruminants adapted well to the environment. They are resistant to dry weather and heat. The production of goats are rapidly expanding which the number of goats production in Thailand is approximately 1,263 (Department of Livestock Development, 2017). Most of goat will have a lot of production in Thailand is located. Because consumers are Muslims but nowadays, consumption of meat and milk of goat is not limited to Muslims. The production of goat meat is not enough to demand because it has been exported to neighboring countries such as Malaysia, and Vietnam.

Presently, new antioxidants are quite popular in nutritional sciences because they are safe for consumers. Anthocyanin is one of substance belonging to the group of phenolic compounds found in the kingdom of plants. In plants, anthocyanin occur in at least 27 families, 73 genera, and various species (Bridle and Timberlake, 1997). have antioxidant properties (Castañedaovando et al., 2009; Canuto et al., 2016) Therefore, it plays an important role in the protection of free radicals and the ability to recognized free radicals that cause damage to cells. A number of reported studies of flavonoid compounds, including anthocyanin, can reduce stress in ruminants (Suman et al., 2015). Anthocyanin belongs to the group of flavonoids and are widely found in flowers, fruits and vegetables. Anthocyanin molecules are often linked to sugar, glucose, galactose, rhamnose and arabinose are the most common monosaccharides such as rutinose, sophorose, sambubiose (Clifford, 2000). The mechanism hypothesizes that anthocyanin acts as a potent antioxidant by giving hydrogen atoms to free radical reactions, which in turn destroys free chain reactions of free radicals (Rice-Evans, Miller, and Paganga, 1996). Because the benefits of anthocyanin are able to help fight free radicals and can be used as ruminant food, it Will be beneficial to animal health and beneficial to consumers as well.

Goat meat is found to have nutrients that are highly nutritious and beneficial to the body. Goat meat is a food with higher digestible protein than beef, pigs and chickens (Winai, 1999). Goat meat is an alternative health production. Goat meat is very low fat (3% goat, 7.4% chicken, 18.8% beef), less calories and lower cholesterol than chicken, pork, lamb, but goat meat have high protein, low cholesterol and has more calcium, potassium, and vitamins than beef. Goat milk is also still easily digested and has less than 7% lactose (Weerachai, 2013). Which is a good choice for people with digestive system. Goat production in Thailand can be an alternative to consumers who choose goat meat, goat milk for good health.

Anthocyanin is a group of phenolic compounds that are widely found in the plant kingdom (Bridle and Timberlake, 1997). In plants, both flowers and fruits of most plants, found in purple plants, such as grapes, purple corn, berries, strawberries and mulberry etc. Which is a plant that is easily found in Asia which they contains a substance that dissolves in water well (Bridle and Timberlake, 1997). It has an antioxidant effect, inhibits lipolytic oxidation, helps to slow the degeneration of cells, reduces the risk of heart disease and stroke in the brain by inhibiting blood clotting (Konczak and Zhang, 2004). May be another option for consumers who are interested in health to turn to consume more goat meat, causing the body to receive nutrients such as protein. And more useful substances that resulting in the body to create better immunity, have better health.

Purple neem leaves can be used as a source of feed ingredients. Purple Neem leaves is a native herbal vegetable that is full of nutritional value, rich in protein nutrients minerals and vitamins that are essential to the body and also found in purple neem leaves contain anthocyanin, which has the effect of antioxidants helps to slow down the degeneration of various cells in the body that can cause health problems such as a weakened immune system and cancer (Postharvest Technology Innovation Center Chiang Mai University, 2009). Therefore, the use of purple neem leaves as ingredients is suitable for use as feed for ruminant, and can also help reduce the cost of expensive raw materials. Thailand is a tropical country with high temperatures and different climates from other countries and has a long dry season. Therefore, the feed ingredients used may not be enough to the demand for ruminant feed. Recently, farmers and researchers in Thailand are looking for new

ways to improve ruminant health from the perspective of animal nutrition in terms of antioxidant activity and increasing meat quality. Therefore, the purpose of this study is to investigate the utilization of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats.

1.2   Research objectives

1.2.1 To examine the effect of utilization anthocyanin from Purple Neem foliage on rumen fermentation and growth performance in growing goats.

1.2.2 To study effect of utilization anthocyanin from Purple Neem foliage on antioxidant activity, antioxidant gene expression and meat quality in growing goats.

1.3   Research hypothesis

1.3.1 Anthocyanin from Purple Neem foliage improves rumen fermentation and growth performance in growing goats.

1.3.2 Supplementation of anthocyanin from Purple Neem foliage increases antioxidant capacity in growing goats.

1.3.3 Supplementation of anthocyanin from Purple Neem foliage increases meat quality in growing goats.

1.4   Scope and limitation of the research

1.4.1 Anthocyanin from Purple Neem foliage planted in Suranaree University of Technology (SUT) farm.

1.4.2   Boer crossbreed male goats used in this study

1.4.3 Evaluation of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats.

1.5   Expected benefit

1.5.1 Utilization of anthocyanin-rich from purple Neem leaves has to improves meat quality of goats, antioxidant status and antioxidant gene expression in muscle of meat goat.

1.5.2 Nutritional strategy of anthocyanin-rich from purple Neem leaves as a source of excellence feed ingredient for ruminant providing a suitable feed for small holder farming.

1.6   References

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CHAPTER II

LITERATURE REVIEW

2.1 Neem leaves

2.1.1 Nutritional composition of neem foliage

Siamese neem tree (*Azadirachta indica* A. Juss var. siamensis Valeton) is one of two varieties of neem (*Azadirachta indica* A. Juss) of the family Meliaceae and is found throughout Southeast Asia including Laos, Mynmar, Cambodia and Thailand. Phenotype's characteristic of Siamese neem tree has reportedly included less branching, longer and thicker leaflets, larger and denser inflorescence and larger fruit. Both *Azadirachta indica* varieties are used for the treatment of some pathological conditions related to oxidative disorders, such as inflammation (Sithisarn et al., 2005). *Azadirachta indica* has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterolwere first polyphenolic flavonoids purified from fresh leaves of neem and were known to have antifungal and antibacterial activities (Mohammad et al., 2016).

In Thailand, the young leaves and inflorescences of these plants are commonly eaten as a vegetable. After the fresh young leaves and flowers are soaked in boiled water for a few minutes, they are eaten with sweet source, cooked fish and rice. In each meal, some 300–500 g of the material is consumed. Several parts of the Siamese neem tree are used for various medicinal proposes (Sithisarn et al., 2005). The leaves are alternate and the leaflets contain 8-19 leaves that may appear in March-April. The leaves are bitter in taste. Neem leaves contain 0.13 per cent essential oil (Subapriya et a., 2005). It is a fast-growing tree with 20–23m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the months of June – August (Mohammad et al., 2016).

The neem leaves are reported to contain high level of polyphenolic compounds. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals (Ghimeray et al., 2009).

Table 2.1 Chemical composition of neem leaves.

	Content (on dry matter basis)
Crude protein*	14.01 - 18.82
Crude fiber*	11.20 - 23.80
Fat*	2.31 - 6.93
Ash*	7.73 - 8.52
Moisture (g/100g)	59.49
Amino acids (mg/100g)	
●Glutamic acid	73.3
●Tyrosine	31.5
●Aspartic acid	15.5
●Alanine	6.4
●Proline	4
●Glutamine	1
Minerals (mg/100g)	
●Calcium	3.4
●Iron	510
●Phosphorus	0.13 - 0.24
●Thiamine	80
●Niacin	17.1
●Vitamin C	0.04
●Carotene	1.4

Adapted from: Subapriya et al., 2005.

2.1.2 Antioxidants found in neem leaves

The neem leaves are a large ever green tree which belongs to the Meliaceae family is had high level of polyphenolic compounds. The high antioxidant activities of plant phenolic compounds are attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals have a significant role in preventing many diseases (Nahak et al., 2011). Neem leaf has been documented to decrease the extent of lipid peroxidation. Antioxidant activity free radical or reactive oxygen species are one of the main culprits in the genesis of various diseases. However, neutralization of free radical activity is one of the important steps in the disease's prevention. Antioxidants stabilize/deactivate free radicals, often before they attack targets in biological cells. and also play role in the activation of ant oxidation enzyme that plays role in the control of damage caused by free radicals/reactive oxygen species. Plants have been reported to have antioxidant activity. Plants fruits, seeds, oil, leaves, bark, and roots show an important role in diseases prevention due to the rich source of antioxidant (Mohumed et al., 2016).

- Neem role as free radical scavenging properties due to rich source of antioxidant.
- Neem modulates the activity of various tumor suppressor genes transcription factors (NF- kB).
- Neem also plays role as anti-inflammatory via regulation of pro inflammatory enzyme activity.

Table 2.2 Summary effect of neem on antioxidant activity.

Reference	Anthocyanin source	Dosage	Effects
Sithisarn et al., 2005	Neem leaves	-	- Inhibition of lipid peroxidation. - Exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 26.5, 27.9 and 30.6 g/mL, respectively. - The results suggest that extracts from leaf, flower and stem bark of the Siamese neem tree has strong antioxidant potential.
Subapriya et al., 2005	Neem Leaves	-	- Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. - Neem leaf has been documented to decrease the extent of lipid peroxidation. - Reduce peroxidation, by enhancing the activities of glutathione dependent antioxidants as well as superoxide dismutase (SOD) and catalase (CAT).

Table 2.2 Summary effect of neem on antioxidant activity (Cont).

Reference	Anthocyanin source	Dosage	Effects
Ghimeray et al., 2009	Azadirachta indica A. Juss. (neem)	23.85 to 237.00 µg/mg in the leaf extracts	-Inhibition of peroxidation, decrease in the absorbance of DPPH radical caused by phenolic compound. -Inhibition percentage of lipid peroxidation can react with lipids, amino acid, polysaccharides and nucleotides in the body and the prevention of such harmful reactions is highly advantageous in terms of both human health and the shelf-life of foodstuffs.
Schumacher et al., 2010	Neem leaves	-	- Inhibition of NF-kB activity. - anti-inflammatory potential via the nuclear factor-kB (NF-kB) signaling pathway, linked to cancer, inflammation. - neem leaf extract may exert its chemopreventive effects by down-regulation of lipid peroxidation, simultaneously increasing the level of glutathione (GSH) and GSH-dependent enzymes.
Nahak et al., 2011	flower and seed oil of neem	-	- The Neem oil has the highest amount of total phenol content (132 µg/mL) which is responsible for highest percentage of inhibition of DPPH radical. - Neem flower revealed highest percentage of antioxidant activity. - The in vitro antioxidant study of Neem flower and oil demonstrate that the oil has more ability to scavenge DPPH. - High antioxidant activity in seed oil is attributed to total phenol content.

Table 2.2 Summary effect of neem on antioxidant activity (Cont).

Reference	Anthocyanin source	Dosage	Effects
Kiranmai et al., 2011	Neem tree	100 g of plant	- The results showed that the root bark extract exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 27.3 µg/mL. - Azadirachta indica had antioxidant activity against the DPPH radical and also had significant total antioxidant capacity.
Mohammad et al., 2016	Neem		- modulation of various molecular pathways including NF-kB - inhibition of growth of numerous microbes such as viruses, bacteria, and pathogenic fungi, prevention of microbial growth.
		200 mg/kg	-showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats

2.2   Anthocyanin

Anthocyanin is classified as a phenolic compound. Polyphenol is a pigment found in plants both in flowers and in fruits. Therefore, if it is in an acidic state with a pH lower than 3 (high acidity), the anthocyanin will be red. In a relatively neutral condition or with a pH value of about 7-8, anthocyanin is purple. And when the condition is bass or has a pH value greater than 11 (a high base), anthocyanin will turn blue. Water soluble, anthocyanin is a pigment that has received a lot of attention from researchers. Because it is an antioxidant and from being an antioxidant, anthocyanin plays a role in preventing the occurrence of various chronic diseases, helping to slow down the degeneration of cells. Such as cardiovascular disease   helps reduce the growth of cancer cells and slows the formation of tumors. In the past, anthocyanin extracts have been used to treat many health problems including urinary tract problems, kidney stones, liver disease, high blood pressure, dysentery, colds, influenza and diarrhea.

2.2.1   The structure of anthocyanin

Anthocyanin is mainly present in nature in the form of heteroses where a hydroxyl group attached to the first carbon is substituted by an alcoholic, a phenolic, or a specific sugar moiety. Structurally, AC are derivatives of 2-phenylbenzopyrylium (flavylium cation) and consist of an aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s) (Saija et al., 2014). Depending on the numerous existing structural characteristics (number and position of hydroxyl and methoxy groups as substituents, the nature and the number of bonded sugars to their structure, the aliphatic, or aromatic carboxylates bonded to the sugar in the molecule and the position of these bonds), the literature reports more than 500 different anthocyanin. Among them, the six anthocyanidins most commonly found in fruits and vegetables are pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin (Pg, Cy, Dp, Pt, Pn, Mv) (Saija et al., 2014). Their distribution in plants usually consumed in the western diet is roughly as follows: Cy 50%, Dp 12%, Pg 12%, Pn 12 %, Pt 7%, and Mv 7%. 3-monoglycosides, diglycosidic, 3,5-diglycosides, and 3-diglycoside-5-monoglycosides are the more known glycosidic variations among these pigments, glucose being the most common conjugated sugar (Pati et al. 2009). The

frequency of 3-glucoside derivatives is 2.5-fold than that of 3,5-diglucosides, and the most common AC in the western diet is Cy-3-O-glucoside (C3G) (Saija et al., 2014).

Type 1 is anthocyanidin (Aglycone). The basic structure of anthocyanin is composed of 6 carbon atoms, 3 carbon atoms (C-6-C-3-C-6) Connect. the anthocyanin is common ground. Currently there are 6 types of Pelargonidin, cyanidin, Delphinidin, Peonidin, Petunidin and Malvidin which is different from position 3′ or 5′ that contains hydroxyl or Methoxy group.

R_1	R_2	Anthocyanin
H	H	Pelargonidin-3-glucoside
OH	H	Cyanidin-3-glucoside
OH	OH	Delphinidin-3-glucoside
OCH_3	H	Peonidin-3-glucoside
OCH_3	OH	Petunidin-3-glucoside
OCH_3	OCH_3	Malvidin-3-glucoside
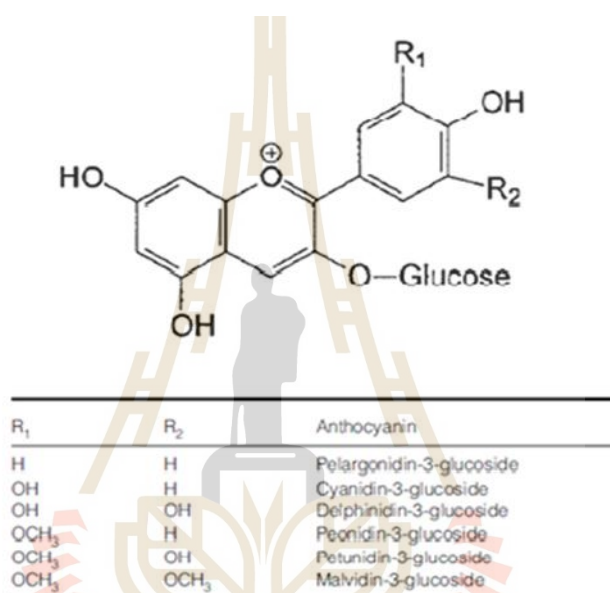
Figure 2.1 Structure of anthocyanin. Adapted from: Michael et al., 2000.

Type 2 is sugar, in which sugar is bonded to carbon at position 3 or position 3 and 5 by bonding sugar such as glucose (Glucose), sugar, galactose, rutinose sugar. Rhamnose sugar, etc.

Type 3 is acid, which may or may not contain this part. If anthocyanins are acidic, they are called Nonacylated anthocyanin, but if there is no acid, it is called acylated anthocyanin. The acid will cause the esters. Aries (Esterification) and sugar captured with Carbon, position 3 and/or position 5, acid-induced ester bonding with sugar such as Coumaric acid, Ferulic acid, Carpal acid Ichigo (Caffeic acid), etc. The formation of the acylation in the structure of anthocyanin will make the anthocyanin more stable.

2.2.2 Anthocyanin color

Anthocyanin is a color substance that is commonly found in flowers, some fruits, leaves or stems of some plants that have colors ranging from red to dark blue. In acidic conditions, the pH value is lower than 3 (high acidity) will cause anthocyanin to be red. In a relatively neutral condition or with a pH value of about 7-8, anthocyanin is purple. And when the condition is bass or has a pH greater than 11 (a high base), anthocyanin will turn blue.
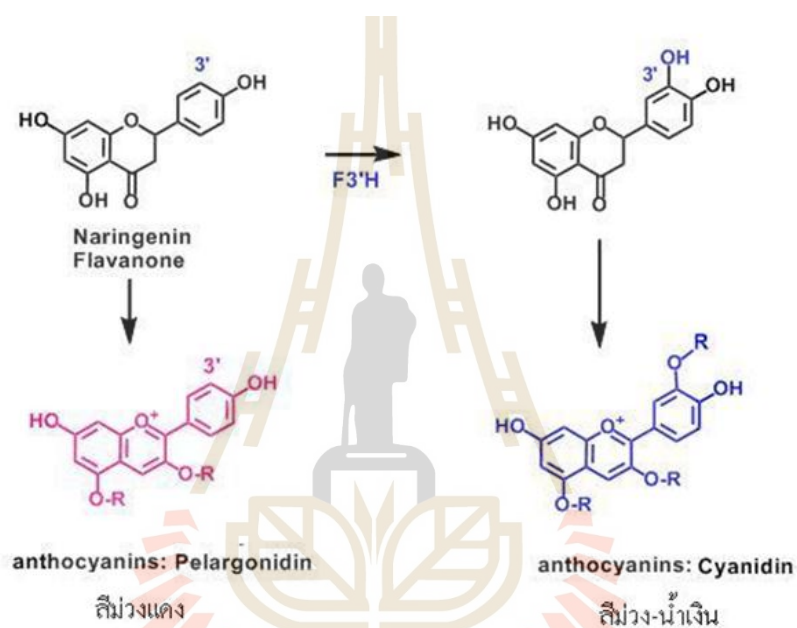
Figure 2.2 Anthocyanin color. Adapted from: Michael et al., 2000.

2.2.3   The structure of anthocyanin at different pH
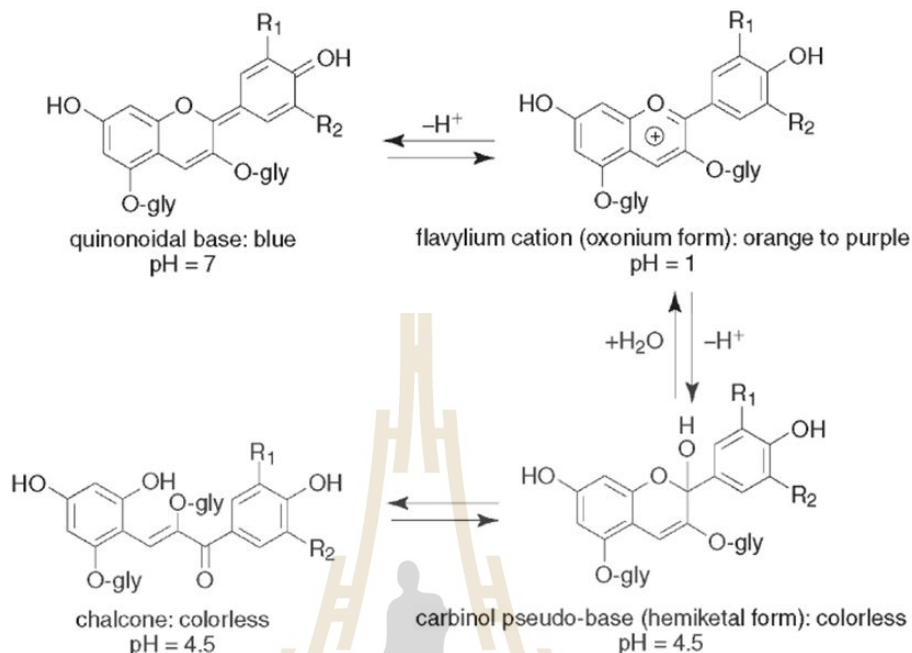
Figure 2.3 The structure of anthocyanin at different pH. Adapted from: Arasa, 2054.

2.2.4   The health benefits of substance Anthocyanin

Anthocyanin has many health benefits, classified as functional food because this substance has antioxidant activity, reduce the risk of birth coronary artery disease and cancer (Lazze et al., 2004). Which when consumers consume products containing anthocyanin, they can help reduce the occurrence of such diseases and can be used as an alternative to consumers who are interested in health in order to provide a better health, free from disease.

2.2.5   Source of anthocyanin

Anthocyanin, which can be found both in vegetables and fruits, in which the amount of anthocyanin in each plant has different amounts of anthocyanin. Depend on the pigment of each plant.

Table 2.3 Anthocyanin content from different sources.

Sources	Anthocyanin content (mg/100 g)
Blackberry	1150
Blueberry	825-4200
Boysenberry	1609
Cherry	20-4500
Chokeberry	5060-10000
Cranberry	600-2000
Cowberry	1000
Currant (black)	1300-4000
Elderberry	2000-10000
Grape (red)	300-7500
Loganberry	774
Orange, blood (juice)	2000
Plum	20-250
Raspberry (black)	1700-4277
Raspberry (red)	100-600
Raspberry (red) single strength juice	4-1101
Sloe	1600
Strawberry	150-350
Cabbage (red)	250
Eggplant	7500
Onion	up to 250
Rhubarb	up to 20000
Wines (red)	240-350
Wines (Port)	140-1100
Purple corn	1642
Purple corn leaves	10× more than in kernels

Adapted from: Michael et al., 2000.

2.2.6 Bioavailability and metabolism of anthocyanin

In experiments in vitro, the effect on health is important to consider the absorption of anthocyanin in the body levels of anthocyanin detected in blood and urine After the digestion of urine, the presence of anthocyanin is low the amount reported in vitro studies may have little relevance to the physical condition due to levels of anthocyanin that are not yet exposed to tissue (except GIT luminal sidetissues) may be limited due to low blood concentration (Kroon et al. 2004). Another important issue is the form of substances present in tissues, such as flavonoids, in which some flavonoids have the same biological activity or potential higher than the substrate (Setchell et al. 2002). Therefore, the biological evaluation of anthocyanin is therefore important to understand the absorption and metabolism of anthocyanin.

The absorption process of anthocyanin is different from common flavonoids because it has been found that only anthocyanin is not harmful in urine and blood (Felgines et al. 2002). However, improved analytical methods have revealed that anthocyanin is methylated, sulfated and glucuronidated. Which is currently believed to be absorbed Metabolic processes and excretion of anthocyanin, some processes, are similar to the flavonoids.

2.2.7 Absorption, metabolism and excretion of anthocyanin

The potential for treatment and prevention of anthocyanin is directly proportional to the absorption and metabolism of the organism's body. Several studies in animal models show that the absorption of anthocyanin may begin in the stomach and appear rapidly in the bloodstream after eating a few minutes (6 to 20 minutes). And achieve maximum blood levels after 15 to 60 minutes (Matsumoto et al., 2006). There are many factors related to the absorption of anthocyanin in the intestines, including the nature of food, chemical structure, type, level of processing and food preparation, interaction with other macro and micro phytonutrients, as well as pathophysiological, nutritional and genetic factors of individual (Riaz et al., 2016).

The absorption and metabolism of anthocyanin is based on the same mechanism. Related to general hydrophilic glyavides glycosides and may occur through the transport of glucosides intact through sodium glucose co-transporter or hydrolysis extracellular of glycosides (Gee et al., 2000). Anthocyanin is not absorbed

by passive spread. Because it is larger and has water-like properties Therefore, the absorption of anthocyanin is generally possible through the movement mechanism, otherwise large molecules are hydrolyzed to β-glycoside before absorption (Riaz et al., 2016). Anthocyanin glycosides are hydrolyzed may convene a serosal fluid before transportation or into the systemic circulation as aglycone (Hollman, 2001). Another possible mechanism for the absorption of anthocyanin is to transport together with sodium and glucose, according to reports (Williamson et al., 2000).

In research published earlier, there is a great variety of paths to the absorption of anthocyanin. Current evidence suggests that both are involved in the absorption of anthocyanin however, the mechanism behind the absorption of anthocyanin and the factors affecting its pharmacokinetics must continue to be explored. After absorption through the digestive tract, the enzyme will change anthocyanin into methylates, sulphates and glucuronids in the liver and kidneys (Kroon et al., 2004). These conjugated forms may be passed through the bile to the jejunum and reused by the intestinal circulatory system/colon. Urine is an important excretion path of anthocyanin and aglycones that are not harmful. There are many reports showing that most of the flavonoids are excreted by the urinary tract. However, their simple part may return into the jejunum through the bile and be absorbed through the colon and achieve circulation. entero-hepatic Again or being excreted with feces lungs are important excretory organs for many bioactive flavonoids, including cursein (Walle, 2004). However, there is no evidence to suggest that the excretion of anthocyanin through respiration.

2.2.8 Metabolism and digestion of anthocyanin in ruminants

There are no reports of metabolism and digestion of anthocyanin in ruminants. However, Hosoda and the faculty (2009) revealed that the incubation of corn that is rich in anthocyanin and rumen fluids does not cause the degradation of all anthocyanin. Similarly, music and faculty (2012) Found that the anthocyanin in barley has a higher stability in the rumen fluid than with a glass tube technique. However, Leatherwood (2013) Indicates that anthocyanin, purple sweet potato extract, are sensitive to rumen fluids. Therefore, these literatures are the basis for us

that anthocyanin in plants are not decomposed in the rumen. In contrast, the pigment or powder of anthocyanin or plant extracts can be degraded in the ruminal fluid.
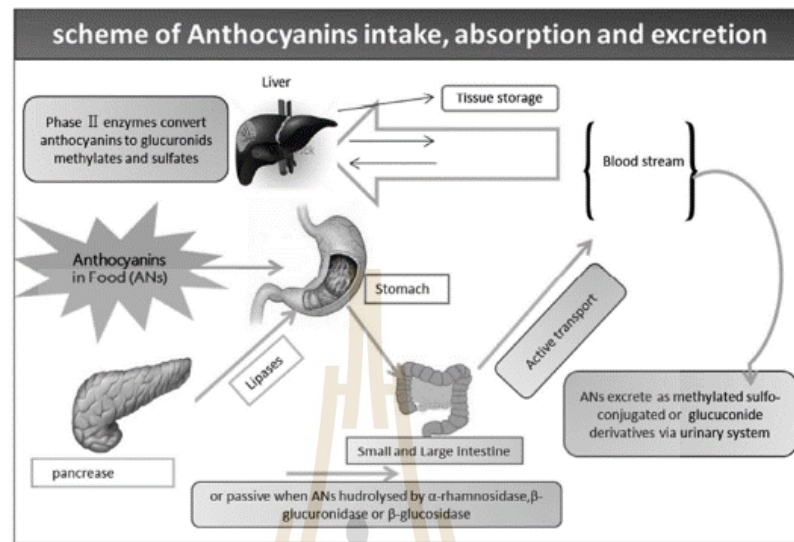
Figure 2.4 An overview of anthocyanin (ANs) intake, absorption, and excretion. Adapted from: (Gee et al., 2000).

Anthocyanin are flavonoids, so the path to possible absorption of anthocyanin in plants in ruminants (Fig. 2.5): (1) anthocyanin can pass through the rumen to the small intestine. A small part of it is absorbed into the blood with blood sent to the liver; (2) another small part of the decomposition and absorption in the colon, microorganisms to reach the liver; (3) part of anthocyanin can create a cycle in the liver with bile salts secreted in the small intestine; (4) anthocyanin can be absorbed into the tissues and substances that are excreted from the kidneys with urine; and (5) non-absorbent parts are drained by feces (Gonthier et al., 2003).
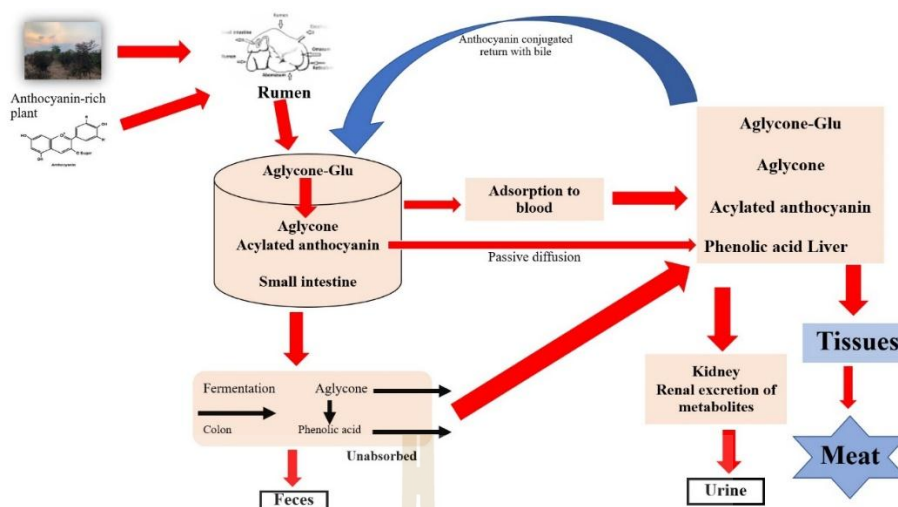
Figure 2.5 Possible pathways of anthocyanin absorption, metabolism, distribution, and excretion in ruminant. Adapted from: He and Giustia, 2010.

2.2.9 Antioxidants of anthocyanin

Physiological stress is a result of the reactive oxygen species (ROS) that leads to changes in free radical balance and damage to cells, Inflammation and may result in degeneration such as cancer, wrinkles and metabolic (Allen and Tresini, 2000). Anthocyanin has a stronger antioxidant effect α-tocopherol, Trolox and catechin (Kahkonen and Heinonen, 2003). However, other factors may have an influence on anthocyanin in reducing or reducing the activity of antioxidants in the body and must be considered (Pojer et al., 2013). The test of the ability to absorb free radicals of oxygen (ORAC) has been used to evaluate the antioxidant potential of anthocyanin and glycolic derivatives. And found to have antioxidant activity similar to vitamin E (Tsuda et al., 1996). And other studies have found that anthocyanin help prevent DNA damage and act as free radicals in a manner that depends on the size of the drug significantly (P<0.05) and found that inhibition of xanthine oxidase activity.

2.3 Anthocyanin's function in antioxidants

Anthocyanin is an antioxidant or antioxidant. Which can be used as an alternative to free radicals due to the anthocyanin compounds that can be found in

the purple plant which anthocyanin acts to prevent the oxidation reaction in the chain reaction of the integrative and proactive step thus able to inhibit or prevent free radicals that damage cells (Radical scavenging antioxidants).

2.3.1 Free radicals

Free radicals are produced by the body, producing free radicals from the metabolic processes to generate energy. Is a byproduct that even if we don't want but it is inevitable stress from the environment or from the animal's own body Causing more free radicals Which increases the density in raising the animals to cause stress Affecting free radicals and lipid oxidation reactions in the cell membrane in the body more (Manoli et al., 2004). Causing a lot of damage to cells in addition, stress also increases the formation and secretion of cortisal hormones, which inhibit the body's immune response. Causing slower growth (Tankson et al., 2001) and affect the work of the body, therefore, not enough antioxidants to inhibit the free radicals that result in animal stress and free radicals play a role in inflammation and tissue damage affecting degeneration or the aging of cells causes various diseases.

2.3.2 Antioxidant

Antioxidant is an amino acid enzyme that is present in the body that is created for use in antioxidants, or if the body produces more free radicals than the antioxidants. Will be able to inhibit free radicals, may need to use supplements, supplements, vitamins and minerals in order to help inhibit free radicals that occur. Helps protect the body from free Radical caused by uncontrolled oxidation which can damage cells and weaken the immune system.
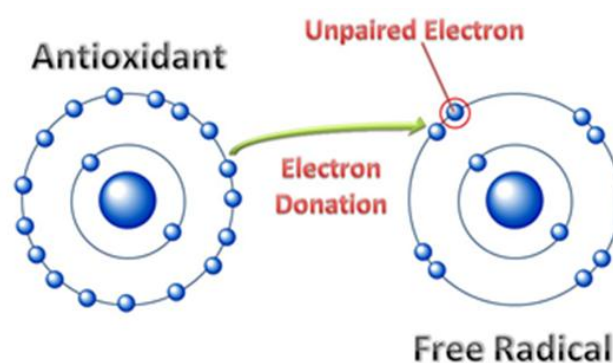
Figure 2.6 Antioxidant. Adapted from: http://syromonoed.com/antioxidant-protocol.

2.3.3   Anti-oxidants outside of animals consist of three main groups

The first group, enzymatic antioxidants, is represented by superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). They are the main intracellular antioxidant defense system and the first defense system against ROM such as peroxides (ROO-) and hydroperoxides (ROOH). SOD enzymes are Mn, Zn, and Cu dependent and GSH-Px is Se dependent.

The second group consists of protein antioxidants in the intracellular fluids such as the sulfhydryl groups of albumin, cysteine and homocysteine.

The third group consists of low molecular weight chain-breaking antioxidants such as the water-soluble vitamin C, glutathione and the lipid-soluble vitamin E.
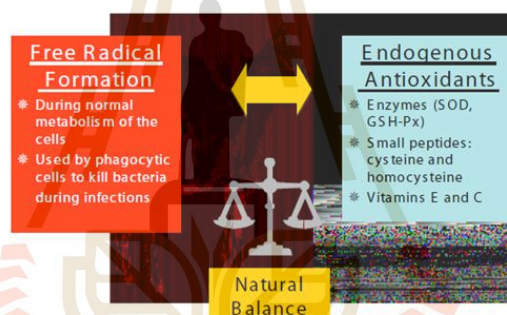
Figure 2.7   Natural   antioxidant balance in the body. Adapted from; Vazquez-Anon et al., 2007.

2.3.4   The relationship between anthocyanin and free radicals

In condition of Superoxide ($O_2\bullet-$) is generated during normal metabolism by the activity of the NADPH oxidase. Dietary imbalances and high metabolic load also stimulate the activity of the NADPH oxidase leading to oxidative stress if not removed by the antioxidant system show in figure 2.8. In normal conditions, $O_2\bullet-$ is converted in hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD); H_2O_2 is then converted in water (H_2O) by the activity of glutathione peroxidase (GSH-Px) and catalase (CAT). Reduction of peroxides is accompanied by oxidation of reduced glutathione (GSH) which can be regenerated from glutathione disulfide

(GSSG) by reducing equivalents from NADPH, which is generated by the pentose monophosphate shunt. The resulting destruction of GSH increases consumption of reducing equivalents, diverting glucose from important physiological pathways and competing with NADPH dependent metabolic pathways such as energy metabolism, immunological functions, antioxidation capacity and calcium homeostasis (Celi et al., 2014). Accordingly, there are two main ways to alleviate OS status: (1) the neutralization of FRs by antioxidants; (2) the reduction of oxidized molecules by enzymatic system. The possible antioxidant mechanism for anthocyanins as follows: (1) the phenolic hydroxyl group possesses the function of directly scavenging oxygen FRs through its own structure. (2) improve the body's antioxidant-related enzyme activity to achieve the purpose of improving the body's antioxidant capacity (Sakano et al., 2005; Soobrattee et al., 2005). (3) may provide a solution to boost ruminants' health and productive performance (Leanet al., 2013).
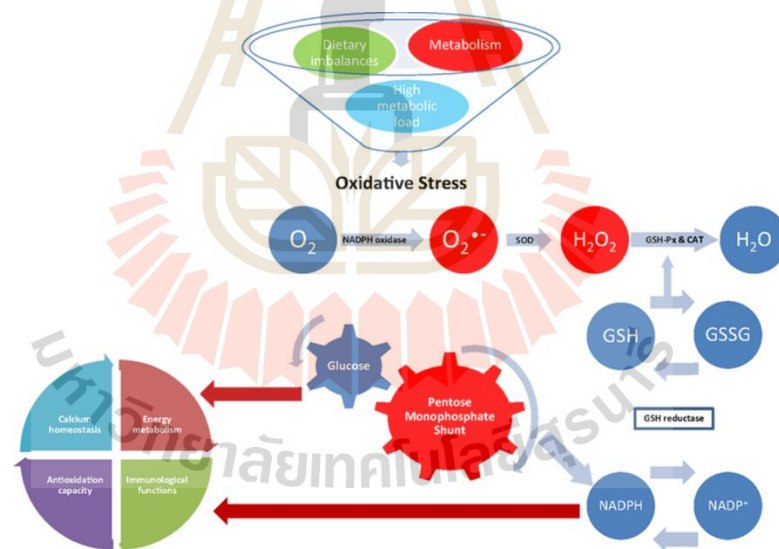
Figure 2.8   The relationship between anthocyanin and free radicals during normal condition (modified from Celi et al., 2014).

2.3.5 The mechanism of anthocyanin affected on gene expression

The ability of anthocyanin and proanthocyanidin to limit oxidative stress and inflammation (Figure 2.9) has been studied found in grape seeds and red rice grains and anthocyanin (cyanidin-3-glucoside and peonidin-3-glucoside) found in

black rice grains scavenge superoxide (O_2-), hydroxyl radicals and showed a decreased production of NO, as well as a lower concentration of reactive oxygen species (ROS), due to the black rice anthocyanin, cyaniding-glucoside and peonidin-3-glucoside (Kruger et al., 2014). In vivoeffects included increased antioxidant capacity from a higher total radical scavenging capacity and from an adaptive increase in activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Chiang et al., 2006).
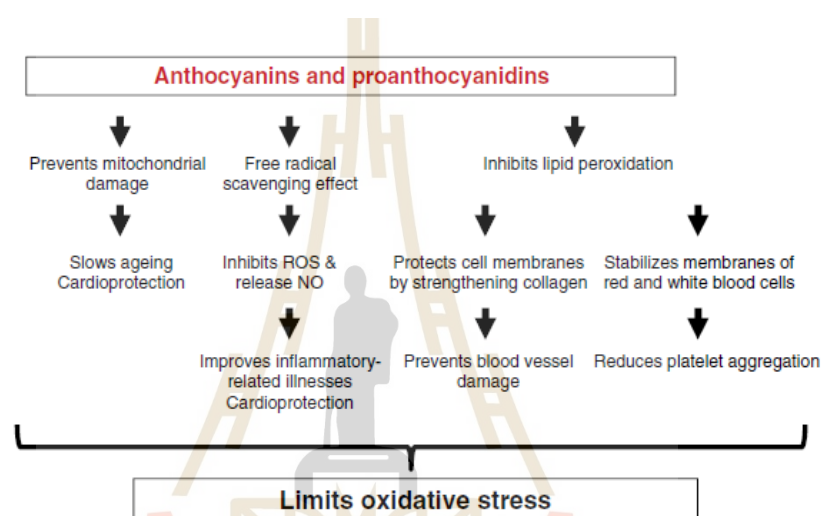
Figure 2.9 Anthocyanin and proanthocyanidin can limit oxidative stress as a result of its antioxidant and free radical scavenging effect. Abbreviations: ROS, reactive oxygen species; NO, nitric oxide (modified from Kruger et al., 2014).

2.3.5.1 Effects of Anthocyanin on ruminant meat quality

The agricultural wastes and by-products such as seeds, stalks and leaves can reduce the economic and environmental livestock costs. Additionally, optimal inclusion of by products and wastes of *Hibiscus sabdariffa* L. By-Products, Neem leaves in balanced ruminant diets should not have negative effects on animal productive behavior but rather improve the meat and milk quality.
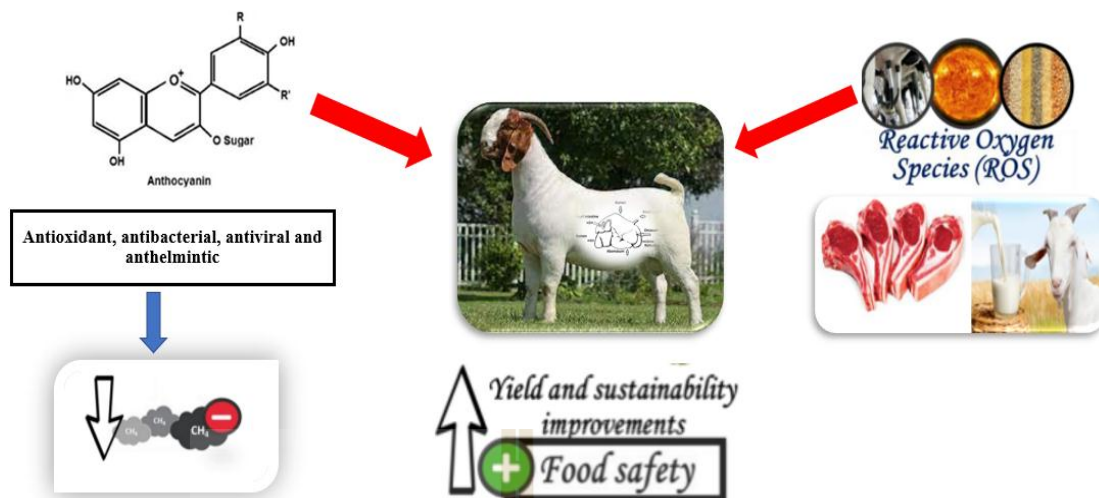
Figure 2.10 Overall economic, environmental, and productive effects of anthocyanin and by-products in ruminant diets (modified from Lazalde-Cruz et al., 2021).

2.3.6 Utilization of anthocyanin in feed on malondialdehyde production, α-tocopherol and total antioxidant status

From the table 2.4 Utilization of anthocyanin in feed on malondialdehyde production, α-tocopherol and total antioxidant status. According to a study of Gladine et al., 2007, it was found that the use of anthocyanin 5% in concentrate is likely to help reduce malondialdehyde production (MDA) and increased α-tocopherol concentration Which is consistent with the study of Gobert et al., 2009 found that use anthocyanin 10% in concentrate the results have the same direction. However, according to a study of Hosada et al., 2012 use anthocyanin from corn 0.5% it was found that the use of anthocyanin in feed can help increase antioxidant activity. Which results in the use of anthocyanin in the diet as an alternative because the diet containing anthocyanin can inhibit MDA and α-tocopherol concentration in the blood caused by the animal being squeezed and found that anthocyanin can reduce the occurrence of MDA. MDA is a non-enzyme that helps in the fight against free radicals and can help reduce lipid peroxidation which can result in the reduction of free radicals caused by stress.

Table 2.4 Utilization of anthocyanin in feed on malondialdehyde production, **α**-tocopherol and total antioxidant status.

Reference	Treatment	MDA (**μ**g/mL)	**α**-Tocopherol (**μ**g/mL)	TAS (mmol/L)
Gladine et al., 2007	Control	0.4	7.21	1.74
	Anthocyanin 5%	0.34	7.26	1.74
Gobert et al., 2009	Control	0.112	3.05	1.89
	Anthocyanin 10%	0.111	3.19	1.89
Hosada et al., 2012	Control	0.16	-	4.63
	Corn 0.5%	0.16	-	4.7

Note: MDA: malondialdehyde production, TAS: total antioxidant status.

2.3.7 Utilization of anthocyanin on Activity of antioxidant enzyme

From the table 2.5 Utilization of anthocyanin in feed from the study of Mario et al., 2003 use anthocyanin from grape 10% found that the use of anthocyanin in feed tends to can help increase superoxide dismutase (SOD), glutathione peroxidase (GPx) and tri-glutathione reductase (GR), which is consistent with the study of Han et al., 2007 use purple potato flakes, Hosada et al., 2011 use anthocyanin from corn 10% and Hosada et al., 2012 use anthocyanin from corn 0.5% was found to result in the same direction. From the table of utilization of anthocyanin in food, which is likely to be able to help increase the antioxidant enzymes such as SOD, GPx such enzymes will reduce the reaction of oxygen species and peroxides produced in organs to help maintain the balance of free radicals and may result in reducing the occurrence of oxidative stress.

2.3.8 Utilization of anthocyanin in feed on fatty acid composition

From the table 2.6 Utilization of anthocyanin in feed from studies of Vecera et al., 2003 found that the use of anthocyanin in foods tends to help increase Saturated fatty acids and both unsaturated fatty acids are Monounsaturated fatty acid (MUFA), Polyunsaturated fatty acid (PUFA), which is consistent with Gladine et al., 2007 found that the results in the same direction. However, according to a study of Gobert et al., 2009, found that the use of anthocyanin in foods can reduce the formation of saturated fatty acids and can help to increase the fatty acids. Both

saturated types are MUFA and PUFA. Which from the use of anthocyanin in foods that will help to increase unsaturated fatty acids is considered good for the animals that will accumulate unsaturated fatty acids which are fatty acids that useful when consumers consume goat meat, allowing consumers to receive fatty acids that are beneficial to the body, help prevent coronary heart disease.

Table 2.5 Utilization of anthocyanin on activity of antioxidant enzyme.

Reference	Treatment	SOD (U/mL)	Glutathione (μmol/L)	Catalase (U/mg)	GR (mU/mg)	GPX (mU/mg)
Mario et al., 2003	Control	166	-	14.74	96	147.68
	Grape 10%	176	-	14.74	85	184.87
Han et al., 2007	Control	774.7	5.12	123.1	75.5	661
	Purple potato flakes	798.4	5	106	76.7	562
Hosada et al., 2011	Control	164.2	5.1	-	-	-
	Corn 10%	184	5.9	-	-	-
Hosada et al., 2012	Control	318.7	4.2	-	-	-
	Corn 0.5%	360.2	4	-	-	-

Note: SOD: superoxide dismutase, GR: glutathione reductase, GPx: glutathione peroxidase.

Table 2.6 Utilization of anthocyanin in feed on fatty acid composition.

Reference	Treatment	SFA (%)	MUFA (%)	PUFA (%)
Gladine et al., 2007	Control	25.3	12.3	58.8
	Anthocyanin	26.3	13.5	57.2
Gobert et al., 2009	Control	30.8	12.9	48.8
	Anthocyanin	23.5	15.1	59.2
Wang et al., 2010	Control	65.66	29.17	51.4
	Anthocyanin	63.35	32.06	45.9
Vecera et al., 2003	Control	31.1	31.1	30.9
	Anthocyanin	43.1	44.1	11.1

Note: SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

2.4   Rumen bio-hydrogenation of fatty acids

The process of biodiversity of unsaturated fatty acids occurs by rumen microbes and ruminant fats are more saturated than single-stomach animals (Jenkins et al., 2008). The bacteria in the rumen decompose polyunsaturated fatty acids (PUFAs) in food by complex processes of reaction isomerization of enzymes and reducing the amount of chemicals that lead to intermediates such as conjugated linoleic acid (CLA) and other fatty acids using stearic acid (18: 0) as the final product, as shown in Figure 11. In which various media were found to flow out of the rumen and be absorbed for a longer period of time, resulting in shorter tissue fatty acids (Wallace et al., 2007; Shingfield et al., 2010). The competition between the rate of bio-hydrogenation process and the ratio of the concentration ratio to the rumen flow pattern (Aldai et al., 2013). Linoleic acid (LA); c9, c12-18: 2) and Linolenic acid (LNA); c9, c12, c15-18: 3 were found that while seeds Some oils are rich in unsaturated fatty acids (MUFA; oleic acid c9-18: 1) Linoleic acid is converted to CLA in most of the rumen, in the form of rumeric acid (RA); c9, t11-18: 2) which is converted into Vuccenic acid (VA); t11-18: 1) and finally changed to stearic acid (18: 0) as shown in the figure 4. Linoleic acid is burned the same way (Fig. 5) because there are three double bonds that are reduced and the process has a more complex structure (Jenkins et al., 2008). The process of metabolism of linolenic acid in the product of intermediate sets (Fig. 11) (Chilliard et al., 2007). Oleic acid can be changed to form t-18: 1, the rate of change of isomers at different locations of mono-fatty acids that will eventually be converted to stearic acid (Jenkins et al., 2008).

Consumption of saturated fatty acids in meat and ruminant dairy products is associated with an increased rate of coronary heart disease (Jenkins et al., 2008). The World Health Organization recommends reducing the intake of 12:0, 14:0, 16:0 and trans-fatty acids (FAO/WHO, 2010) depending on the type of food that ruminants receive. Which different foods will cause the fatty acids that accumulate in the meat and milk of the animals are different, such as fatty acids 14:0, 16:0 and t10-18:1 that accumulate in cow's concentrated food (Leheska et al., 2008; Aldai et al., 2011) found that these fatty acids are associated with an increase in coronary heart disease (FAO/WHO., 2010).
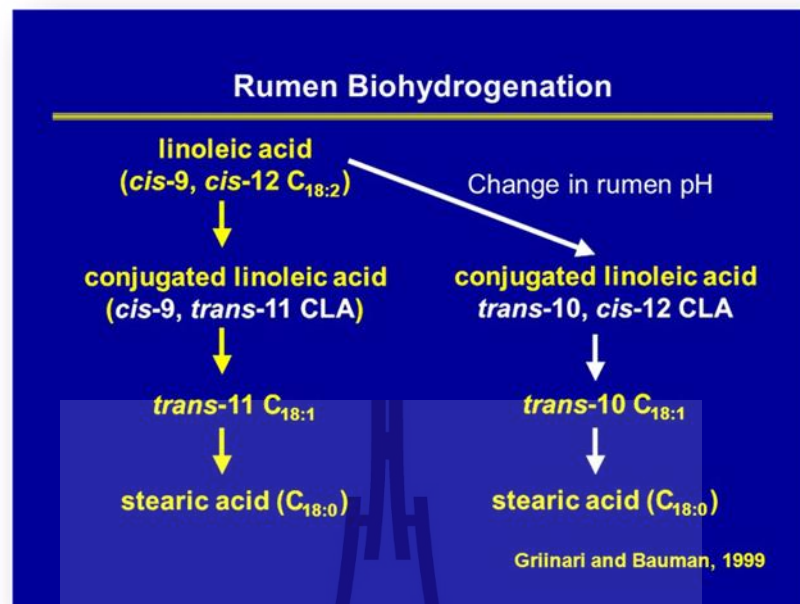
Figure 2.11 Bio-hydrogenation process of linoleic acid and linoleic acid in the rumen, Source; Modified from Griinari and Bauman, 1999.

Rumeric acid in the bio-hydrogenation process in rumen is an intermediary that can prevent cancer and arteriosclerosis, improve immunity. Regulate blood flow rate, protein circulation rate and promotes tissue accumulation (Jenkins et al., 2008; Dilzer and Park., 2012). Vacinic acid, which is interesting because they can transform from rheumatic acid through endogenous desaturation to accumulate in meat (Jenkins et al., 2008; or-Rashid et al., 2009).

Controlling the occurrence of bio-hydrogification processes is very interesting in improving the nutritional value of ruminant products. If the goal is to raise the level of rumeric acid in meat or milk, then it will try to stop the final stages of the bio-hydrogenation production process without affecting the changes in linoleic acid and linolenic acid to rumeric acid and vaccenic acid. Reducing rumeric to vaccenic acid does not need to be inhibited, because vaccenic acid will still produce rumeric acid. In animal tissues through Δ9-desaturase (Grinarii et al., 2000). In fact, 90% of rumeric acid in meat and milk originated externally, which is synthesized from vaccenic acid (Pierova et al., 2002). Therefore, the best strategy for Increasing the amount of

rumeric acid in meat or milk will increase the production of vaccenic acid in the rumen and absorption in the duodenum (Vasta and Luciano., 2011).

Bio-hydrogenation is affected by factors such as pH, quantity and type of substrate (Martin and Jenkins., 2002). Consumption of fish and algae oil can inhibit the formation of bio-hydrogenation and increase the flow of vaccenic acid in the rumen (Boeckaert et al., 2007; Or-Rashid et al., 2009). Other nutritional strategies to increase beneficial fatty acids in ruminant products by coping with the digestion of fats in the rumen and the bio-hydrogenation process in grazing animals will cause linoleic acid. High-lenic, vegetable oil-containing or oil-based vegetable oils or to prevent fat (Lourenco et al., 2010). Different fatty acids affect different microbes on the microbes in the lumen and the number of fatty acids that are excreted from the rumen (Lourenco et al., 2010) and large amounts of coarse food will go Reducing the negative effects of unsaturated fatty acids on the bio-hydrogenation process (Lourenco et al., 2010).

The bio-hydrogenation process of linoleic acid and linoleic acid is complete when the final product is stearic acid (C18:0). It has been found that there are 3 chemical processes. In which the medium contains 2 groups of bacteria, group A bacteria, group B bacteria. The enzyme process of biodiversity of linoleic acid (C-9, C-12 C18:2) in the first step of the biodiesel production process starting from the group. A Bacteria with C-12 t-11 isomers changed into C-9 t-11CLA, Step 2. Group A Bacteria will reduce the double-bonded C-9 into a single bond, which will be in the form of trans-vaccenic acid (t-11 C18:1) and Step 3 uses group B. Bacteria will change the t-11 bond to the form of trans-vaccenic acid (t-11 C18:1). Then, vaccenic acid is transformed into stearic acid (C18:0), which is the final product of the bio-hydrogenation production process of linoleic acid and linoleic acid.

2.4.1 Rumen fermentation

The rumen fats are first decomposed and reduced after being converted to saturated fatty acids. In which the formation of bio-hydrogenation (BH) of linoleic acid (cis-9, cis-12; 18:2) can be produced in 3 reactions. First, the reaction isomerization Which leads to the synthesis of cis-9, trans-11CLA) followed by a reduction of the number of continuous bonds twice The first is to change to oleic acid (trans-11 C18:1) and then to be transformed into stearic acid (C18:0; Harfoot and

Hazelwood) 1988) but under changing environment conditions The environment and the formation of bio-hydrogenation processes can follow other pathways that create specialized intermediates such as trans-10, cis-12 CLA and trans-10 18:1 (Bauman and Griinari.2001; figure 6). These intermediates can leave the stomach and the amount that flows through to the small intestine and for absorption depends on the rate of flow through the capacity of the gastric bacteria and the concentration and food that is in the form of unsaturated fatty acids. Enters the stomach (Lock et al. 2006). Once absorbed, it is circulated to the mammary gland, which leads to a reduction in the synthesis of milk fat by interfering with the expression of genes that contain lipoprotein enzymes and important molecules. In conclusion, MFD production has two conditions. One is the presence of PUFA in the rumen and the other, the changing stomach environment that causes incomplete BH (Bauman and Griinari 1998). Changes in the stomach environment are caused by the antibacterial activity of unsaturated fatty acids (PUFA), which can penetrate the cell membranes of certain bacteria and cause damage to cells by cell arrangement of new cell membranes (Jenkins, 2002). Since the MFD-induced fatty acids during PUFA's bio-hydrogenation process can predict that the amount of its precursors (linoleic and linolenic acid) may determine the amount of linolenic acid production media Igg and Linolenic Acid (Jenkins and Lock, 2008).

2.4.2 Effect of anthocyanin on fatty acid profile of meat goats

Effect of anthocyanin on the fatty acid profile of meat goats, as shown in Table 7 Study of Gomez et al., 2018 use grape 5% in concentrate found that anthocyanin effects tend to increase useful fatty acids, including polyunsaturated fatty acids the same result with study of Andrea et al., 2019 and Roberta et al., 2018 use anthocyanin 3.2 mg/d which gives results in the same direction. However, study of Kafantaris et al., 2018 use grape 9% in concentrate found that anthocyanin effects tend to increase useful fatty acids, including monounsaturated fatty acids and saturated fatty acids which the same result with study of Roberta et al., 2018 found that the results were in the same direction. The effect of anthocyanin on the fatty acid profile will help the bio-hydrogenation of microbial process that convert unsaturated fatty acids into more saturated fatty acids, which will help improve the quality of goat meat because of the acid fats that are beneficial to both the animals

themselves and are beneficial to consumers as well fatty acids will help prevent cancer, arteriosclerosis, improve immunity and control blood flow.

Table 2.7 Effect of anthocyanin on fatty acid profile of meat goats.

Reference	Treatment	C18:0	C18:1 cis-9	C18:1 tran-11	SFA	MUFA	PUFA
Gomez et al., 2018	Control	11.5	-	1.75	36.1	37.6	26.3
	Grape 5%	11.5	-	2.10	36.6	34.9	28.4
Kafantaris et al., 2018	Control	13.08	29.85	1.67	39.06	34.93	18.73
	Grape 9%	11.93	32.33	1.39	40.49	37.50	16.39
Roberta et al., 2018	Control	15.60	27.61	1.08	49.22	34.35	16.47
	Anthocyanin 3.2 mg/day	14.31	36.01	1.64	39.51	42.22	18.26
Andrea et al., 2019	Control	18.64	25.79	1.74	50.99	36.45	10.94
	Anthocyanin	16.33	26.20	1.55	49.74	33.96	15.21

Note; C18:0: steric acid, C18:1 cis-9: oleic acid, C18:1 tran-11: vaccenic acid, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid and PUFA: polyunsaturated fatty acid.

2.4.3 Effect of anthocyanin on carcass characteristic, ultimate pH and color of goats

The effect of anthocyanin on carcass characteristic, ultimate pH and color of meat goats, as shown in table 2.8 Overall, anthocyanin has a tendency to improve the quality of Carcass characteristic because anthocyanin, when supplemented in food, has the effect of reducing pathogenic microorganism and increase probiotic bacteria. Which will help increase Polyunsaturated fatty acids help improve production (Ioannis et al., 2018). In addition, the effect of anthocyanin reduces the occurrence of PUFA bio-hydrogenation in the ramen, which will increase by-pass in the production of useful fatty acids, such as CLA, which has a positive effect on animal health and allows consumers to consume goats to receive healthy fatty acids.

Table 2.8 Effect of anthocyanin on carcass characteristic, ultimate pH and color of goats.

Reference	Treatment	Carcass weight (kg)	Carcass yield (%)	Ultimate pH	Color		
					Lightness (L*)	Redness (a*)	Yellowness (b*)
Ngamba et al., 2013	Control	9.4	49.7	5.4	4.1	12.5	10.1
	Anthocyanin 200 g/day	7.0	43.4	6.6	39.8	8.2	4.4
Gomez et al., 2018	Control	6.23	53.1	5.74	49.3	2.82	13.4
	Grape 5%	6.09	52.7	5.68	49.8	3.22	9.54
Nallely et al., 2018	Control	-	-	5.05	37.02	20.78	6.96
	Anthocyanin 2%	-	-	5.64	36.74	19.63	6.64
Andrea et al., 2019	Control	18.3	55.83	5.55	41.89	18.15	12.55
	Anthocyanin	19.6	55.91	5.55	40.20	17.94	12.64

2.4.4 Effect of anthocyanin on cooking loss and shear force of goats

The effect of anthocyanin on cooking loss and shear force shown in the table 2.9 the study from Karami et al., 2011 use anthocyanin 0.5% in concentrate found that have a tendency to reduce cooking loss and shear force the same result with Zhao et al., 2018 found use anthocyanin at 5% and 10% in concentrate and study from Monika et al., 2018 found that use anthocyanin at 20 g/d and 40 g/d can reduce cooking loss and use anthocyanin at 20 g/d can increase shear force. Demonstrate that anthocyanin can improve meat quality because can reduce cooking loss and after cooking the meat loses little water.

Table 2.9 Effect of anthocyanin on cooking loss and sheer force of goats.

Reference	Treatment	Cooking loss (%)	Shear force (%)
Karami et al., 2011	Control	29.3	4.51
	0.5%	29.0	4.11
Monika et al., 2018	Control	30.91[a]	58.1[b]
	20g/d	27.21[ab]	71.04[a]
	40 g/d	23.28[b]	46.16[c]
Zhao et al., 2018	Control	22.9	43.8[a]
	5%	19.3	36.4[b]
	10%	19.4	33.2[b]

2.4.5 Effect of anthocyanin on growth performance of goats

The effect of anthocyanin on goat performance shown in the table 2.10 from the study of Gomez et al., 2018 use grape 5% found that the effect of anthocyanin has not negative affect on growth performance of goats. The same result with the study of (Roberta et al., 2018) found that the results were in the same direction. However, study of (Nallely et al., 2018) use anthocyanin 200g/d found that the effect of anthocyanin tends to result in the improvement of goat growth the same result with the study of (Ngamba et al., 2013) found that the results are in the same direction the effect of anthocyanin, which has a tendency to improve performance of goats. Because anthocyanin is able to bind to proteins, when the animal is fed with the right amount of anthocyanin, it is beneficial to animals, which causes digestion of anthocyanin bind to the protein in the rumen, therefore decreasing the flow through the stomach more resulting in digestion and absorption in the small intestine, resulting in animals receiving more protein or amino acids, allowing the body of the animal to be able to create resistance and prevent disease.

Table 2.10 Effect of anthocyanin on growth performance of goats.

Reference	Treatment	Initial body weight (kg)	Final body weight (kg)	ADG (g/d)
Gomez et al., 2018	Control	41.9	118	295
	Grape 5%	45.3	116	283
Nallely et al., 2018	Control	23.95	35.64	292
	Anthocyanin 200 g/day	23.48	35.53	301
Roberta et al., 2018	Control	69.8	179	90
	Anthocyanin 3.2 mg/day	68.9	186	100

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CHAPTER III

Experiment I

Effect of utilization anthocyanin from Purple Neem foliage on rumen fermentation and growth performance in growing goats.

3.1 Abstract

The purpose of this study was to investigate the effect of utilization of anthocyanin from Purple Neem foliage on rumen parameters and growth performance in growing goats. In total, 25 Boer crossbreed male goats (about 20 ± 2 kg body weight; mean standard deviation (SD)) were assigned to a completely randomized design. There were five treatments: (1) control, (2) 3% Normal Neem foliage in concentrate, (3) 6% Normal Neem foliage in concentrate, (4) 3% Purple Neem foliage in concentrate and (5) 6% Purple Neem foliage in concentrate. The results show that the goats that were fed at 6% Purple Neem foliage in concentrate had a higher ($p<0.05$) feed intake gDM/d, %BW, $g/kgBW^{0.75}$, nutrient intake, nutrient digestion, final weight, weigh change and ADG than did the goats that were fed 3% purple neem foliage in concentrate, 3% normal neem foliage in concentrate, 6% normal neem foliage in concentrate and control treatment. The feeding of 6% Purple Neem foliage in concentrate had higher ($p<0.05$) N intake, N Urine, N digestion, N digestion (%), N retention and N retention (%). Goats receiving 6% Purple Neem foliage in concentrate had no negative effect ($p<0.05$) on pH but had a higher ($p<0.05$) level of ammonia nitrogen, BUN, acetic acid, propionic acid, butyric acid, total VFA at 2 and 4 h after feeding compared to the other treatments. of utilization anthocyanin from 6% Purple Neem foliage show higher ($p<0.05$) of total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, *Streptococcus bovis* at 2 and 4 h after feeding respectively. Goats feed 6% Purple Neem foliage displayed higher ($p<0.05$) levels of Total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) antioxidant activity in plasma at 2 and 4 h

after feeding. Goats feed 6% Purple Neem foliage had lower (p<0.05) on protozoa methanogen and malondialdehyde (MDA) at 2 and 4 h after feeding. It can be concluded that Purple Neem foliage can be used as a good source of anthocyanin for feeding growing goats.

Key word: anthocyanin, Purple Neem foliage, Normal Neem foliage, rumen fermentation growth performance, antioxidant activity, growing goats.

3.2 Introduction

Thailand is a tropical country with a distinct environment than other countries and locations, with high temperatures that can cause oxidative stress in animals, causing the animal body to destroy cells (Sies, 1997). Roughage must be fed to ruminants during the dry season since it is not accessible during that time. Purple Neem foliage, which is high in anthocyanin, may be fed to animals to minimize environmental effect and production costs. Furthermore, its phytochemicals, like polyphenols and vitamins, may increase the quality of meat and milk as well as their shelf-life stability. As a result, they may eliminate free radicals to reduce oxidative stress in ruminants. (Jomova and Valko, 2011). Overall, anthocyanin from purple neem foliage has the potential to influence ruminal digestibility and fermentation, as well as animal production behavior, hence minimizing the consequences of oxidative stress in ruminants. In order to offer a preliminary grasp of the potential of anthocyanin from purple neem foliage as a high grain and concentrate proportion on ruminant diets. Therefore, in this study we investigated its chemical composition, anthocyanin composition of Purple Neem foliage.

Purple Neem foliage (*Azadirachta indica* A. Juss var. siamensis Valeton) is a popular crop in Thailand and is one of the world's most significant cereal crops, particularly in Asian nations. Because of its potential biological and pharmacological uses, neem shoots and leaves are regarded one of the most essential features for improving grain quality. Purple Neem is a native fodder that contains anthocyanins. Anthocyanin is a collection of flavonoid compounds that are natural colorants that belong to the bioflavonoids category. It is classified into two categories of chemical compounds: anthocyanins (ANS) and anthocyanidins (ACN) (Cisowska et al., 2011).

Anthocyanins are water-soluble plant pigments found in the flesh, skin, and roots of a variety of brightly colored fruits and vegetables, including berries, plums, and berries (Sun et al., 2019). In human, animal, and in vitro research, anthocyanins have been linked to reactive oxygen and nitrogen species (ROS and RNS). (Speer et al., 2020).

Feeding Purple Neem foliage to animals can minimize both economic and environmental expenses. Because of the presence of bioactive substances such as phytochemicals and vitamins. Natural antioxidants may be found in Purple Neem foliage (*Azadirachta indica* A. Juss var. siamensis Valeton). Anthocyanins and phenolic compounds are abundant in Purple Neem foliage. It has an antioxidant role in that their structure may donate electrons to free radicals with unpaired electrons and decrease agents in the electron transfer reaction route (Prommachart et al., 2021). The information of anthocyanin in Purple Neem foliage can be exhibit potent antioxidant activity and reduce oxidative stress in ruminants, thus the purple neem foliage is balance ruminant diets do not have a negative effect on animal productivity and also improve rumen fermentation, rumen digestibility in ruminants, and improve plasma antioxidant of ruminants. In this study were to examine the effect of utilization anthocyanin from purple neem foliage on rumen parameter and growth performance in growing goats.

3.3 Objective

To examine the effect of utilization anthocyanin from Purple Neem foliage on rumen parameter and growth performance in growing goats.

3.4 Materials and methods

3.4.1 Preparation of plant harvested

Samples of forage species such as purple neem foliage planted on a Suranaree University of Technology (SUT) farm in Nakhon Ratchasima, Thailand were prepared by cutting tips of about 30 cm from the youngest leaves from several trees. The harvested samples were immediately brought back to the laboratory, where all leaves of the forages were collected and dried at 65°C for 48 h, processed

into a powder, and kept in sealed plastic bags until the extraction time. After grinding through a 1.0 mm sieve, the samples were stored at 4°C in an airtight container in darkness until analysis.

3.4.2 Preparation for plant extract

Five grams of crude or residue content was extracted with 20 mL of solvents on the basis of their polarity. Methanol was used in this section. The extraction process was run with Soxhlet apparatus for 3–4 h. The extract was filtered and kept. The remaining solid residue on the filter paper was reextracted three times with the fresh solvent, Soxhlet, and then filtered. All the filtrates were pooled and combined based on the solvent, followed by evaporation using a Rotary evaporator. The extracts were finally filtered through a 0.45 μm PVDF syringe filter, and the volume was increased to 10 mL using the same solvent, then stored at −20°C until the analysis of the anthocyanin content

3.4.3 Determination of Anthocyanin Content by HPLC: Isolate Anthocyanin Content

The HPLC-grade solvents, including acetonitrile, methanol, and chloroform used in the extraction process, were purchased from Labscan (Bangkok, Thailand) and Duksan (Gyunggido, Korea) for Acetonitrile. Standard chemicals with anthocyanin content (total anthocyanin, cyanidin, delphinidine, petunidine, pelagonodine, peonidinea and malvidine) were purchased from Sigma Chemical Co. (Sigma, Saint Louis, Missouri, USA). All water used in all the preparations was of the double distillated water grade (Millipore, Illkirch-Graffenstaden, France). A standard stock solution (1 mg/mL) was created by diluting 1 mg of selecting standard with 0.5 mL of HPLC-grade methanol, followed by sonication for 15 min in ice and vortex. The standard stock solution was then adjusted to 1 mL with the mobile phase solution (1:9, HPLC-grade acetonitrile:1% acetic acid). HPLC analyses were performed with HPLC Agilent Technologies 1260 Infinity, Santa Clara, CA, USA with four solvent delivery system quaternary pumps (61311B), including a diode array detector (DAD 61315D) with a 10 mm flow cell, an automatic sample injection valve equipped with a 100 loop and Agilent Open LAB CDS 1.8.1 system manager as the data processor. The separation was achieved using a reversed-phase Zorbax SB-C18 column (3.5 μm

particle size, i.d. 4.6 mm×250 mm). The method for chromatographic analysis followed Van Soest et el. (1991) with modifications.

3.4.4 Experimental design, animal diets and managements

The experiment was conducted to recommendation by Animal Care and use committee of Suranaree University of Technology, Suranaree University of Technology, Nakhon Ratchasima province, Thailand. The treatments were completely randomized design. Twenty-five Boer crossbreed male goats have 5 treatments that were consisted (1) control, (2) 3% Normal Neem foliage in concentrate, (3) 6% Normal Neem foliage in concentrate, (4) 3% Purple Neem foliage in concentrate and (5) 6% Purple Neem foliage in concentrate. During the 60 days of experimental period goats will be fed with 1.5% of BW DM/day containing Pangola (*Digitaria eriantha*) hay and (16% crude protein) at the ratio of 60:40. All goats were be received feeds and supplemented with their respective treatment. Goats were feed in the morning and afternoon at approximately (07.00 and 16.00). During the adjusting period (14 days before starting each experimental period), all animal were be fed with the same feed. Moreover, nutrient composition and anthocyanin composition in two types of Neem foliage was shown in Table 3.1 and Table 3.1. All goats were kept in the individual feeding pens during the 60 days of experimental period. Mineral blocks and clean water were available ad libitum offered for all animals.

3.4.5 Sample collection

The experimental periods were used 60 day a period which 14 days for adjusted the animal and digestive tract. Samples of offered and refused diets were collected every day, while urine and feces samples will be collected by total collection from each individual goat during the last 7 days of experimental period. Samples of offered and refused diets and feces will be oven dried (60°C) and ground through a 1.0 mm sieve prior to analysis for DM, ash, ether extract (EE) and crude protein (CP) (AOAC, 2005) and NDF and ADF (Van Soest et al., 1991). Total urine excretion were collected and acidified using 20% H2SO4 solution prior to analyses for ammonia nitrogen concentration using Kjeldahl method according to AOAC (2005).

Table 3.1 Feed ingredients and chemical composition of experimental diets.

Items	Pangola hay	Control	3% Normal Neem foliage	6% Normal Neem foliage	3% Purple Neem foliage	6% Purple Neem foliage
Ingredients						
soybean meal	-	18.00	19.00	17.00	19.00	17.00
Rice bran	-	27.00	24.00	20.80	23.00	22.00
cassava chip	-	27.00	30.00	32.30	35.00	33.10
corn	-	26.40	22.40	22.30	18.40	20.30
salt	-	0.40	0.40	0.40	0.40	0.40
limestone	-	0.20	0.20	0.20	0.20	0.20
premix	-	1.00	1.00	1.00	1.00	1.00
Purple Neem	-	0.00	3.00	6.00	3.00	6.00
Chemical composition (% DM)						
Dry matter	86.72	74.06	75.32	74.57	75.37	74.84
OM	93.52	93.83	93.05	93.81	93.90	93.58
Ash	6.48	6.17	6.95	6.19	6.10	6.42
Crude protein	5.91	16.71	16.01	16.29	16.27	16.62
Ether extract	1.66	4.27	4.59	5.57	4.57	5.08
Non-fibrous car-bohydrate	5.55	28.21	31.12	38.13	38.55	34.49
Neutral detergent fiber	80.40	44.64	41.33	33.82	34.51	37.39
Acid detergent fiber	49.56	30.70	33.22	30.87	32.60	32.15
%TDN	55.00	88.69	84.77	87.18	87.37	86.91
Metabolizable energy, Mcal/ kg DM	1.99	3.21	3.06	3.15	3.16	3.14

Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g iron; 40 g zinc; 40 g manganese; 0.1 g cobalt; 10 g copper; 0.1 g selenium; 0.5 g iodine; Calculated as: NFC =100−(%NDF+%CP+%EE+%ash); Estimated by the equation TDN = (%)DCP+ DNFC)+DEE×2.25+(DNDF); Estimated by the equation ME (Mcal/kg DM) = (TDN×0.04409×0.82).

Table 3.2 Chemical composition of Purple Neem foliage.

Ingredient		
	Purple Neem foliage	Normal Neem foliage
Chemical composition (% DM)		
Dry matter	57.37	51.10
Crude protein	9.24	9.20
Ash	7.45	6.20
Ether extract	1.80	1.70
Neutral detergent fiber	56.51	51.00
Acid detergent fiber	44.85	37.50

NDF: neutral detergent fiber, ADF: acid detergent fiber.

Table 3.3 Anthocyanin composition (mg/g DM) in Purple Neem foliage.

Athocyanin composition	Purple Neem foliage (mg/g DM)
Total Anthocyanin	138.77
Cyanidin	39.96
Delphinidine	26.12
Petunidine	32.60
Pelagonodine	10.93
Peonidine	9.49
Malvidine	19.67

Ruminal fluid will be sampled on last day of experiment using a stomach tube connected with vacuum pump at 0, 2 and 4 h-post feeding. Rumen fluid samples were immediately measured for pH using a portable pH Meter, and then filtrated through 4 layers of cheesecloth under anaerobic conditions. Ruminal fluid sample were then divided into three portions; the first portion were used for community DNA extraction for estimation of microbial populations. The second portion of ruminal fluid sample were used for volatile fatty acid and the third portion of ruminal fluid sample were used for NH_3-N measurement using Kjeldahl method according to AOAC (2005).

With the same frequency of ruminal fluid sampling, blood samples were taken from jugular vein and immediately placed on ice and brought back to the laboratory and refrigerated at -20°C for 1 h. Blood plasma were then separated by centrifuge at 3,500×g for 20 min, then plasma were collected and stored at -20°C for further analysis of blood urea nitrogen (BUN) according to Crocker (1967).

3.4.6 Analysis of antioxidant activity enzymes in plasma

The TAC in the plasma was determined using a commercially available kit (Catalog Number MAK187-1KT, Sigma-Aldrich, Product of USA), according to manufacturers' protocol. In the kit, either the concentration of the combination of both small molecule and protein antioxidants, or the concentration of only small molecule antioxidants can be determined with a colorimetric probe, giving a broad absorbance peak at 570 nm.

The activity of SOD enzyme was measured according to a commercial assay kit (PCode 101869510, 19160-1KT-F, Sigma-Aldrich, Product of Japan). The Kit-WST allows very convenient SOD assaying by utilizing a water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. Absorbance was measured at 440 nm using a microplate reader (Epoch, BioTek, Luzern, Switzerland). Calculation the SOD activity (inhibition rate %) was done using the following equation.

$$\text{SOD activity (inhibition rate\%)} = \frac{[(A_{blank\ 1} - A_{blank\ 3}) - (A_{sample} - A_{blank\ 2})]}{(A_{blank\ 1} - A_{blank\ 3})} \times 100.$$

Glutathione Peroxidase (GPX) enzyme activity was determined by an indirect method, using the Sigma Glutathione Peroxidase Cellular Activity Assay kit (Catalog number: CGP1-1KT, Sigma-Aldrich, Product of Israel). The absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPX activity, since GPX is the rate limiting factor of the coupled reactions.

The catalase (CAT) enzyme activity was determined by a colorimetric assay, using a commercial kit (Pcode 1002565186, CAT100-1KT, Sigma-Aldrich,

Product of Israel). This assay method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase that absorbs at 520 nm.

The DPPH scavenging activity in the plasma was assayed spectrophotometrically according to using a stable free radical DPPH (Sigma-Aldrich, Pcode: 101845869) with a slight modification. Briefly, 50 μL of each sample was added to 1 mL of methanolic solution of DPPH reagent (25 μmol/L) in a 1.5 mL tube. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min, and then centrifuged at 4000 rpm for 10 min at 4°C. 200 μL of the supernatant was transferred to a 96-well plate immediately and the absorbance was detected at 517 nm via a microplate reader (Epoch, BioTek, Luzern, Switzerland). DPPH scavenging activity was calculated by the following formula:

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

where Ac is the absorbance of the control, and as is the absorbance of the sample.

The malondialdehyde (MDA) enzyme activity was determined by a colorimetric assay, using a commercial kit (Sigma-Aldrich, catalog number MAK085). All samples and standards should be run in duplicate. Use ultrapure water for the preparation of all standards and samples. Sample Preparation use plasma samples (20 μL) should be gently mixed with 500 μL of 42 mM sulfuric acid in a microcentrifuge tube. Add 125 μL of Phosphotungstic acid Solution and mix by vortexing. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000×g for 3 min. In a separate tube, add 2 μL of BHT (100x) to 100 μL of water. Resuspend the pellet on ice with the water/BHT solution. Adjust the volume to 200 μL with water. In this kit, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present. Malondialdehyde was calculated by the following formula:

Concentration of MDA for samples without 1-butanol concentration step:

$$(Sa/Sv) \times D = C$$

Sa, Amount of MDA in unknown sample (nmole) from standard curve; Sv, Sample volume (mL) or amount (mg) added into the wells; C, Concentration of MDA in sample; D, Sample dilution factor (if applicable)

Sample Calculation:

Amount of MDA (Sa) = 5.84 nmole; Sample volume (Sv) = 0.020 mL; Concentration of MDA in sample; (5.84 nmole/0.020 mL) × 1 = 292 nmole/mL

3.4.7 Microbial population analyses

Rumen fluid mixture were used for quantitative analysis of microbial population. Ruminal fluid sample will be extracted for community DNA using RBB+C method (Yu and Morrison, 2004). Population of rumen microbes which were studied in this experiment including of bacteria involved in Total bacteria, methanogen, protozoa. *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Streptococcus bovis* and *Ruminococcus albus* were identified.

3.4.8 Chemical composition

Analysis chemical composition of Purple Neem foliage were analysis dry matter (DM), crude protein (CP), ether extract (EE), ash according to feed proximate analysis of AOAC (2005); organic matter (OM) calculates from the difference between ash and dry matter; neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicelluloses following by (Van Soest et al., 1991).

3.4.9 Statistical analyses

All results were analyzed as a completely randomized design using the general linear model procedure of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC, USA). Differences between treatments means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). The relative mRNA abundance was calculated averaged abundance of the gene in data was considered as the calibrator, and the data was analyzed by ANOVA procedure. The level of significance difference at ($P <$ 0.05) were accepted as representing statistically significant differences. The statistical model for the analysis of data was:

$$X_{ij} = \mu + B_j + \varepsilon_{ij}$$

where: X_{ij}, the observed value of treatment j, replication I, μ = the overall mean, B_j, Influence of treatment at j = μ_j - μ and ε_{ij}, tolerance of the experimental unit at ij

3.4.10 Location of the study

These experiments were conducted at Suranaree University of Technology (SUT) Farm, the Center for Scientific and Technologyical Equipment Building 10, Suranaree University of Technology, Nakhon Ratchasima, Thailand, which provided the planting site and chemical analysis facilities for this study.

3.4.11 Experimental period

The experiments were carried out from November 2019 to October 2020.

3.5 Results

3.5.1 Feed intake, nutrient intake, and growth performance

In all experimental periods, there was a significant difference (P<0.05) in feed intake (gDM/d, %BW, and g/kgBW$^{0.75}$) and nutrient intake for 5 treatments (Table 3.4). Total dry matter consumption %BW, and g/kgBW$^{0.75}$, OM, CP, EE, NDF and ADF was higher (P<0.05) in the goats feed the 6% Purple Neem foliage diet than in the control diet respectively.

According to Table 3.5, the apparent digestibility of DM, OM, CP, EE in goats fed the 6% Purple Neem foliage diet was significantly higher (P<0.05) than those in the other treatment.

Table 3.6 shows that there was a significant difference (P<0.05) in cultivar × levels on final weight, weight change, and average daily increase (ADG). The goats fed 6% Purple Neem foliage had the highest (P<0.05) final weight, weight change, and ADG than those fed the other treatments.

Table 3.4 Effect of utilization anthocyanin from Purple Neem foliage on feed intake of growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Feed intake							
gDM/d	642.00[e]	755.20[d]	809.60[c]	853.00[b]	949.00[a]	20.94	0.01
%BW	3.03[e]	3.18[d]	3.24[c]	3.34[b]	3.49[a]	0.03	0.01
g/kgBW$^{0.75}$	50.82[d]	60.17[c]	62.54[c]	66.54[b]	72.94[a]	1.53	0.01
Nutrient intake ġ DM/d							
OMI	603.48[e]	702.34[d]	761.02[c]	801.82[b]	892.06[a]	19.83	0.01
CPI	109.14[e]	120.83[d]	129.54[c]	136.48[b]	161.33[a]	3.59	0.01
EEI	25.68[e]	37.76[d]	42.65[c]	47.45[b]	48.58[a]	1.70	0.01
NDFI	288.90[d]	309.63[b]	275.26[e]	298.55[c]	351.13[a]	5.34	0.01
ADFI	199.02[d]	249.22[c]	250.98[c]	281.49[b]	303.68[a]	7.24	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), ġ DM/d: daily intake of dry matter, %BW: edible quantity per body weight per day, g/kgBW$^{0.75}$: daily consumption per daily metric weight, SEM: standard error of the mean.

Table 3.5 Effect of utilization anthocyanin from Purple Neem foliage on nutrient digestion in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Apparent Digestibility, % of intake							
DDM	51.61[e]	53.54[d]	55.62[c]	63.54[b]	65.62[a]	1.14	0.01
DOM	52.57[e]	54.54[d]	54.42[c]	64.18[b]	66.52[a]	1.12	0.01
DCP	50.56[e]	55.48[d]	63.49[c]	68.02[b]	73.20[a]	1.68	0.01
DEE	50.39[e]	53.14[d]	55.59[c]	57.21[b]	60.51[a]	0.71	0.01
DNDF	75.35[a]	73.81[b]	64.94[c]	63.01[d]	55.07[e]	1.53	0.01
DADF	68.68[a]	67.45[b]	62.06[c]	53.99[d]	52.21[e]	1.39	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e = in the same row, there is a statistically significant difference (P<0.05(, DDM: digestibility of dry matter, DOM: digestibility of organic matter, DCP: protein digestibility, DEE: fat digestibility, DEE: digestibility fatty digestibility, DNDF: the digestibility of the crude fiber that

cannot be dissolved in neutral solution, DADF: the digestibility of the fiber that cannot be digested in an acidic solution, SEM: standard error of the mean.

Table 3.6 Effect of utilization anthocyanin from Purple Neem foliage on performance in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Body weight							
Initial weight, kg	20.14	20.40	20.40	20.80	20.89	0.14	0.43
Final weight, kg	30.03[e]	31.10[d]	31.60[c]	32.45[b]	33.05[a]	0.22	0.01
Weigh change, kg	9.89[e]	10.70[d]	11.12[c]	11.65[b]	12.16[a]	0.23	0.01
ADG, g/d	155.53[e]	173.57[d]	183.27[c]	192.67[b]	207.50[a]	3.77	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e: in the same row, there is a statistically significant difference (P 0.05), SEM: standard error of the mean.

3.5.2 Nitrogen utilization

As a consequence, in Table 3.7, there was a significant different (P< 0.05) in the interaction between cultivars' levels of nitrogen utilization parameters. Feed the goats 6% Purple Neem foliage, which had the highest levels (P<0.05) of N intake (g/d), N excretion from urine (g/d), N digestion, N digestion (%), N retention, and N retention (%) respectively among all the treatment.

Table 3.7 Effect of utilization anthocyanin from Purple Neem foliage on nitrogen utilization in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
N intake (g/day)	7.25[e]	8.65[c]	8.51[d]	9.40[b]	10.59[a]	0.22	0.01
N Faces (g/day)	3.75[a]	2.14[b]	1.82[c]	1.77[c]	1.62[d]	0.16	0.01
N Urine (g/day)	0.16[d]	0.11[e]	0.17[c]	0.21[b]	0.23[a]	0.01	0.01
N digestion (g/day)	3.50[e]	6.51[d]	6.69[c]	7.63[b]	8.97[a]	0.37	0.01
N digestion (%)	50.56[e]	55.48[d]	63.49[c]	68.02[b]	73.20[a]	1.68	0.01
N retention (g/day)	3.34[e]	6.40[d]	6.53[c]	7.42[b]	8.74[a]	0.36	0.01
N retention (%)	46.13[e]	73.93[d]	76.71[c]	78.92[b]	82.52[a]	2.67	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e = in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean

3.5.3 Rumen fermentation parameters

At 0 h, treatments showed no effect (P<0.05) on pH, ammonia nitrogen (NH_3-N), blood urea nitrogen (BUN), acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), acetic acid: propionic (C_2/C_3), and total VFA (TVFA). There was a highest (P<0.05) in NH_3-N, BUN, C_2, C_3, C_4 and TVFA levels after feeding (2 to 4 h) in the 6% Purple Neem foliage treatment compared to the other groups.

Table 3.8 Effect of utilization anthocyanin from Purple Neem foliage on pH, ammonia nitrogen and blood urea nitrogen in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
			pH				
0-H	6.94	6.95	6.96	6.97	6.99	0.01	0.62
2-H	6.75	6.77	6.78	6.79	6.80	0.01	0.11
4-H	6.82	6.87	6.83	6.86	6.85	0.01	0.06
Mean	6.84	6.86	6.85	6.88	6.88	0.01	0.25
			Ammonia nitrogen mg/dl				
0-H	12.38	12.4	12.14	12.36	12.11	0.05	0.10
2-H	12.41[e]	13.42[d]	14.12[c]	15.25[b]	16.60[a]	0.30	0.01
4-H	13.50[e]	14.40[d]	15.14[c]	16.40[b]	17.20[a]	0.27	0.01
Mean	12.76[e]	13.41[d]	13.80[c]	14.67[b]	15.30[a]	0.19	0.01
			BUN mg/dl				
0-H	10.11	10.14	10.15	10.16	10.18	0.01	0.14
2-H	11.44[e]	12.46[d]	13.60[c]	14.28[b]	15.08[a]	0.27	0.01
4-H	15.58[d]	16.32[c]	16.34[c]	17.36[b]	17.50[a]	0.16	0.01
Mean	12.38[e]	12.97[d]	13.36[c]	13.93[b]	14.25[a]	0.14	0.01

NNF: normal neem foliage, PNF: purple neem foliage, BUN: blood urea nitrogen, CT: condensed tannin, ANT: anthocyanin, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 3.9 Effect of utilization anthocyanin from Purple Neem foliage on Volatile fatty acid in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Acetic acid (%molar)							
0-H	56.02	52.06	56.03	56.08	56.04	0.01	0.23
2-H	57.51[e]	59.14[d]	59.43[c]	60.29[b]	61.45[a]	0.27	0.01
4-H	59.55[e]	60.77[d]	61.25[c]	62.66[b]	63.88[a]	0.31	0.01
Mean	57.69[e]	58.66[d]	58.90[c]	59.68[b]	60.46[a]	0.19	0.01
Propionic acid (%molar)							
0-H	29.7	29.71	29.72	29.73	29.74	0.01	0.55
2-H	31.03[e]	33.04[d]	35.32[c]	36.49[b]	37.38[a]	0.48	0.01
4-H	32.44[e]	33.33[d]	36.12[c]	37.18[b]	39.08[a]	0.50	0.01
Mean	31.06[e]	32.02[d]	33.72[c]	34.47[b]	35.40[a]	0.32	0.01
Butyric acid (%molar)							
0-H	14.21	14.24	14.19	14.2	14.22	0.01	0.09
2-H	15.04[e]	17.67[d]	18.82[c]	19.83[b]	20.64[a]	0.40	0.01
4-H	17.19[e]	18.94[d]	20.32[c]	21.66[b]	22.59[a]	0.39	0.01
Mean	15.48[e]	16.95[d]	17.78[c]	18.57[b]	19.15[a]	0.26	0.01
Acetic acid : Propionic							
0-H	1.89	1.75	1.89	1.89	1.88	0.01	0.20
2-H	1.85[a]	1.79[b]	1.68[c]	1.65[d]	1.64[e]	0.07	0.01
4-H	1.84[a]	1.82[b]	1.70[c]	1.69[d]	1.63[e]	0.08	0.01
Mean	1.86[a]	1.79[b]	1.75[c]	1.74[d]	1.72[e]	0.05	0.01
Total VFA (mmol/L)							
0-H	54.63	54.64	54.65	54.69	54.73	0.01	0.12
2-H	56.41[e]	58.42[d]	63.19[c]	67.59[b]	75.82[a]	1.42	0.01
4-H	54.46[e]	61.36[d]	67.83[c]	74.43[b]	82.20[a]	1.98	0.01
Mean	55.17[e]	58.14[d]	61.89[c]	65.57[b]	70.92[a]	1.13	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

3.5.4 Microbial population

Table 3.10 demonstrate the effect of dietary treatments had no effect on the microbial population (P<0.05). At 0 h, total bacteria, Protozoa, Methanogen, *Butyrivibrio- fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Streptococcus bovis* at 0 h. Total bacteria, Protozoa, Methanogen, *Butyrivibrio-fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Streptococcus bovis* showed a significant different (P<0.05) change

after feeding (2 to 4 h). When compared to other treatments, goats receiving 6% Purple Neem foliage at 2 and 4 h after feeding had the highest (P<0.05) levels of Total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Streptococcus bovis*. When compared to other treatments, goats receiving 6% Purple Neem foliage had lower levels of Protozoa and Methanogen at 2 and 4 h after feeding (P<0.05).

Table 3.10 Effect of utilization anthocyanin from Purple Neem foliage on rumen microbial population in growing goats.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Total bacteria (lg10 copies/ml)							
0-H	5.55	5.54	5.56	5.62	5.51	0.05	0.98
2-H	5.36[e]	6.34[d]	7.46[c]	8.58[b]	10.59[a]	0.38	0.01
4-H	5.38[e]	6.41[d]	7.58[c]	8.55[b]	9.59[a]	0.32	0.01
Mean	5.43[e]	6.10[d]	6.87[c]	7.58[b]	8.56[a]	0.23	0.01
Butyrivibrio fibrisolven (lg10 copies/ml)							
0-H	7.58	7.57	7.55	7.56	7.59	0.03	1.00
2-H	4.92[e]	6.66[d]	7.59[c]	8.15[b]	8.75[a]	0.28	0.01
4-H	5.15[e]	6.43[d]	7.56[c]	8.07[b]	8.64[a]	0.27	0.01
Mean	5.88[d]	6.89[c]	7.57[b]	7.93[ab]	8.33[a]	0.18	0.01
Fibrobacter succinogenes (lg10 copies/ml)							
0-H	3.54	3.58	3.59	3.58	3.67	0.06	0.97
2-H	5.03[d]	5.14[d]	6.04[c]	7.09[b]	9.25[a]	0.33	0.01
4-H	4.83[e]	5.03[d]	5.99[c]	7.03[b]	9.02[a]	0.32	0.01
Mean	4.47[e]	4.58[d]	5.21[c]	5.90[b]	7.31[a]	0.22	0.01
Ruminococcus albus (lg10 copies/ml)							
0-H	3.55	3.63	3.60	3.66	3.70	0.05	0.93
2-H	5.01[e]	6.09[d]	6.94[c]	7.95[b]	9.06[a]	0.30	0.01
4-H	4.95[e]	6.05[d]	6.74[c]	7.55[b]	8.78[a]	0.28	0.01
Mean	4.50[e]	5.26[d]	5.76[c]	6.39[b]	7.18[a]	0.20	0.01
Ruminococcus flavefacises (lg10 copies/ml)							
0-H	3.47	3.57	3.6	3.61	3.63	0.05	0.90
2-H	4.50[e]	6.03[d]	7.03[c]	8.11[b]	9.47[a]	0.33	0.01
4-H	4.95[e]	5.91[d]	6.99[c]	7.99[b]	9.36[a]	0.33	0.01
Mean	4.31[e]	5.17[d]	5.87[c]	6.57[b]	7.49[a]	0.22	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 3.10 Effect of utilization anthocyanin from Purple Neem foliage on rumen microbial population in growing goats (Con).

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Streptococcus bovis (lg10 copies/ml)							
0-H	3.30	3.49	3.60	3.63	3.70	0.05	0.13
2-H	4.04[d]	5.03[c]	5.18[c]	6.53[b]	9.03[a]	0.36	0.01
4-H	3.91[d]	4.73[c]	5.03[c]	6.31[b]	8.95[a]	0.37	0.01
Mean	3.75[d]	4.42[c]	4.60[c]	5.49[b]	7.23[a]	0.25	0.01
Protozoa (lg10 copies/ml)							
0-H	4.59	4.50	4.70	4.59	4.39	0.05	0.28
2-H	8.61[a]	7.16[b]	6.70[b]	5.51[c]	3.97[d]	0.36	0.01
4-H	8.55[a]	7.12[b]	6.19[c]	5.15[d]	3.18[e]	0.39	0.01
Mean	7.25[a]	6.26[b]	5.86[b]	5.08[c]	3.85[d]	0.25	0.01
Methanogen (lg10 copies/ml)							
0-H	1.70	1.55	1.58	1.54	1.53	0.06	0.93
2-H	8.24a	6.15[b]	5.30[c]	2.91[d]	1.99[e]	0.47	0.01
4-H	7.74[a]	6.12[b]	4.78[c]	2.59[d]	1.97[e]	0.45	0.01
Mean	5.89[a]	4.61[b]	3.89[c]	2.34[d]	1.83[e]	0.31	0.01

NNF: normal neem foliage, PNF: purple neem foliage, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

3.5.5 Antioxidant activity in plasma

Table 3.11 demonstrates showed at 0 h, there was no effect (P<0.05) of dietary treatments on total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) antioxidant activity in plasma. However, there was a significant difference (P<0.05) in the antioxidant activity in plasma of TAC, SOD, GPX, MDA, DPPH, and CAT in the goats receiving the dietary treatments after feeding at 2 and 4 h. Total antioxidant, superoxide dismutase, glutathione peroxidase, 2, 2-diphenyl-1-picrylhydrazyl, and catalase levels were significantly higher (P<0.05) in goats receiving 6% Purple Neem foliage than in the other treatment. In terms of

antioxidant activity in plasma, goats receiving 6% Purple Neem foliage had reduce (P <0.05) levels of malondialdehyde after feeding at 2 and 4 h than the other groups.

Table 3.11 Effect of utilization anthocyanin from Purple Neem foliage on antioxidant activity in plasma of growing goat.

Items	Control	3%NNF	6%NNF	3%PNF	6%PNF	SEM	p-value
Total antioxidant (nmol/ul)							
0H	0.85	0.83	0.81	0.85	0.86	0.01	0.81
2H	0.87^c	0.71^d	1.05^b	1.08^b	1.27^a	0.04	0.01
4H	0.81^b	0.84^b	0.86^b	0.88^b	0.99^a	0.02	0.01
Mean	0.84^c	0.79^d	0.91^b	0.93^b	1.04^a	0.02	0.01
SOD (inhibition rate %)							
0H	86.50	87.72	88.27	88.70	89.30	1.38	0.98
2H	91.95^d	92.19^d	93.25^c	94.06^b	95.40^a	0.26	0.01
4H	89.74^d	90.29^c	90.49^c	90.90^b	91.42^a	0.12	0.01
Mean	89.40	90.06	90.67	91.22	92.04	0.48	0.48
GPX (Units/ml)							
0H	68.45	68.2	68.53	68.57	68.49	0.05	0.19
2H	83.35^d	84.02^c	84.11^b	84.16^b	84.28^a	0.03	0.01
4H	75.94^d	76.03^c	76.11^b	76.18^b	76.28^a	0.03	0.01
Mean	75.91^b	76.08^b	76.25^a	76.30^a	76.35^a	0.03	0.01

NNF: normal neem foliage, PNF: purple neem foliage, superoxide dismutase (SOD) glutathione peroxidase (GPX), Malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT). a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

3.6 Discussion

3.6.1 Feed intake, nutrient intake and growth performance

In the current study, we found that although Purple Neem foliage displayed a high level of anthocyanin, anthocyanins are phenolic compounds that contribute a characteristic bitter flavor to plants but not has effect to palatability for goats (Hosoda et al., 2012). Our current study revealed that Goats consumed 3%, 6% normal neem foliage and 3%, 6% Purple Neem foliage had high levels of feed intake (g DM/d, percent BW, and g/kgBW$^{0.75}$), nutrient intake than control group. However, in the current report, goats receiving 6% Purple Neem foliage diet had higher of feed intake (g DM/d, percent BW, and g/kgBW$^{0.75}$), nutrient intake, when compare which goats fed 3%, 6% normal neem foliage and 3% Purple neem foliage. Because Purple Neem foliage have been successfully used as a protein source for ruminant without any negative effect on palatability (Acosta-Estrada et al., 2014). Purple Neem foliage containing anthocyanin content has to the relatively high quality of the basal diet can indicating that the feeding of the anthocyanins did not lead to lower palatability for goats. Although anthocyanins had the capacity to affect palatability, they also had the ability to boost antioxidant activity without reducing DMI. According to Cheeke (1999) feed intake of the animal is directly related to the disappearance rate of DM from reticulo-rumen by the process of digestion. Our study found that was consistent with (Hosoda et al., 2012), report that Effect of anthocyanin-rich corn silage can improve feed intake and nutrient intake indicating that anthocyanins did not lead to poor palatability for goats.

Previous studies have reported that feeding Neem fodder have been successfully used as protein source for ruminant (Paengkoum, 2010). Utilization of anthocyanin from Purple Neem foliage improve apparent digestibility of DM, OM, CP, EE in present study. This is may be because the sugar structure in anthocyanin might be involved in digestion and metabolism (Ribnicky et al., 2008). The feeding experiment demonstrated that goats consumed 3%, 6% Normal Neem foliage and 3%, 6% Purple Neem foliage had high apparent digestibility in all nutrient compare with control group. However, the feeding experiment demonstrated that goats consumed 6% Purple Neem foliage diet had higher apparent digestibility in all nutrient when compare with other treatment. In addition, it is well known that the

feed nutritional value usually dependents on its digestive characteristics and nutrient composition, especially for the CP and fiber fractions. From our results was consistent with (Sanon, 2007) goats fed leaves and pods of Pterocarpus lucens or A. senegal displayed an improved total DM intake and digestibility. Additionally, anthocyanins might be bound to the nutrients to be digested, inhibiting the digestive enzymes and exerting an anti-microbial effect (Acosta-Estrada et al., 2014).

Growth performance of goats, such as the final weight, weight change and ADG were relatively high in goats fed 6% Purple Neem foliage diet. The significant different in final weight, weight change and ADG of goats in the treatment of 6% Purple Neem foliage could be due to relatively good quality of the basal diet. Because of the comparatively high intake DM of Purple Neem foliage, total intake per day of CP content in Purple Neem foliage was relatively high in final weight, weigh change, and ADG of goats consumed 6% Purple Neem foliage diet. Although anthocyanins had the capacity to affect palatability, they also had the ability to boost antioxidant activity without reducing DMI. Our results consistent with Sanon, (2007), report that supplementation with mixtures and sole multipurpose trees rather than with conventional supplements like wheat bran improves performance of animals in terms of live weight gain. From this study can conclude that the feeding experiment demonstrated growth performance of goats throughout the experimental period, indicating that the Purple Neem foliage diet had nutritional composition and high anthocyanin composition is more effective in improving growth performance

3.6.2 Nitrogen utilization

Nitrogen utilization in the present experiment, goats receiving 3%, 6%Normal Neem foliage and 3%, 6% Purple Neem foliage has highly significant different compare with control group. Goats receiving 6% Purple Neem foliage diet increased their level of nitrogen intake, N Urine, N digestion, N digestion (%), N retention and N retention (%) than other treatment groups. This might be the high CP content and anthocyanin in Purple Neem foliage have a potential to improve the ruminal fermentation and microbial protein production. As a result, it appears that anthocyanins do not affect digestibility when the nutrient balance is unchanged. In addition, anthocyanins can bind to dietary proteins and reduce rumen fermentation,

thereby increasing nitrogen utilization (Correddu et al., 2020), who demonstrated that ewes receiving polyphenol-rich plants exhibited increased nitrogen utilization and CP digestibility (Nudda et al., 2020), As expected, we found that feeding purple corn pigment CP could increase CP digestibility, which may be related to the high anthocyanin content in purple corn pigment, (Nudda et al., 2020), Consistent with our resalt.

3.6.3 Rumen fermentation parameters

The ruminal pH, ammonia nitrogen and blood urea nitrogen post feeding were not significant different among treatment. The ruminal pH is decrease after feeding at 2 and 4 h. The decrease ruminal pH is relative to the accumulation of VFA (Owens et al., 1998). In this study, it was found that the pH value was in the appropriate range. Our study results are consistent with (Bryszak et al., 2019) report that higher level of black currant diet had no negative effects on fermentation in the rumen. The rumen pH had average values ranged from 6.75 to 7.00, which is optimal for microbial digestion in rumen (Cherdthong et al., 2014). This result indicated the possibility that the microbial digestion of feed could decrease pH value in the rumen.

The NH_3-N has significant different all among treatments. In addition, ruminal concentration of NH_3-N is generally affected by CP content in the diet (Hristov et al., 2004). In the current study, goats receiving 6% Purple Neem foliage diet has high NH_3-N at 2 and 4 hours after morning feeding. Because high level CP of anthocyanin in Purple Neem foliage did not effect on ruminal pH value but have high level of NH_3-N concentration. This result was according with (Hosoda et al., 2012), who demonstrated that lactating dairy cows receiving higher level CP of anthocyanin-rich corn silages did not effect on the ruminal fluid pH value, but showed a higher level of NH_3-N concentration compared with the control silage group. The ruminal concentration of ammonia is generally affected by CP content in a diet (Hristov et al., 2004). Furthermore, urea is synthesized in the liver from ammonia absorbed from the digestive organs, and so a blood urea nitrogen concentration in the blood is positively associated with ammonia concentration in ruminal fluid (Fee et al., 1975). In this study, the average ruminal NH_3-N value for goats in the different treatment groups was 12.11-17.20 mg/dL and was within the range of optimal rumen NH_3–N concentration for microbial growth is reported

differently. An average range value the optimal NH_3–N concentration of 15 to 30 mg/dL recommended by Wanapat and Pimpa, (1999). It can be indicated that the 6% Purple Neem foliage was likely affected by the high level of CP, that is a supplemental source of nitrogen to increase NH_3-N.

The BUN concentration was no different among experimental treatment at 0 h and there was significant different. Goats fed 6% Purple Neem foliage diet has high BUN than other treatment at 2 and 4 h after morning feeding. In this study, the BUN concentration was 10.11-17.50 mg/dL. These results show that the BUN concentration for all treatment was within or above the optimal level of 10-20 mg/dL (Kaneko et al., 1997). The increasing of ruminal ammonia and BUN concentration in goats fed 6% Purple Neem foliage were probably caused by higher CP content of Purple Neem foliage by higher concentration of ruminal ammonia. It can be indicated that Purple Neem foliage has effect to change the protein content. Our study was consisted with Bryszak et al. (2019). Whoreport that high level of black currant diet had no negative effects on fermentation in the rumen.

In the present study, there was no significant different in volatile fatty acid among five groups treatment at 0 h and there was significant different on acetic acid, propionic acid, butyric acid, acetic acid: propionic acid ratio and total VFA at 2 and 4 h after feeding. In this study, goats receiving 6% Purple Neem foliage has higher acetic acid, propionic acid, butyric acid and total VFA in rumen fluid at 2 and 4 h post feeding than compare with other treatment. The volatile fatty acid are the main sources of energy for metabolism in ruminants. This study consists with (Bryszak et al., 2019), report that high level of black currant diet increased concentration of propionate, butyrate, increase VFA concentration. Anthocyanins could increase the formation of volatile fatty acid by regulating intestinal flora (Tian et al., 2019). volatile fatty acid, produced by fermentation of organic matter in the rumen, can have a major effect on production and product composition in ruminants (Cherdthong et al., 2014). The relative proportions in which VFA are produced, are influenced by a number of factors, including substrate composition, substrate availability and rate of depolymerization, and microbial species present (Cherdthong et al., 2014).

Anthocyanins might be able to affect carbohydrate metabolism to provide more energy for ruminant by increasing the proportion of propionic acid (Tian et al., 2019). The acetic acid concentration was higher goats fed 6% Purple Neem foliage when compare with Normal Neem foliage at 2 and 4 h post feeding. This likely due to the high digestibility of NDF and ADF content. Our previous study showed that anthocyanins might be able to affect carbohydrate metabolism to provide more energy for ruminants by increase acetic acid production, Furthermore, demonstrated that rumen digesta transfer from buffalo to cattle was fluctuated in acetic acid concentration while propionic acid and butyric acid were similar indicating an active role of rumen microbes and on-going fiber fermentation by cellulolytic bacteria (Wanapat and Pimpa, 1999). The formation of acetic acid is always accompanied by H_2 and CO_2 production. In the current study, goats fed a 6% Purple Neem foliage diet had significantly higher levels of propionic acid, butyric acid, and total VFA at 2 and 4 h after feeding. Because of the high fiber content of the Purple Neem foliage diet treatment, it will be difficult to digest in the rumen. According to the study, this might be because, while anthocyanin-rich purple corn silage had a higher level of anthocyanin, it did not appear to be broken down in the rumen (Hosoda et al., 2009). Furthermore, the type of VFA generated in the rumen is determined by the substrate fermented, the rumen environment, and the microbial population (Mensor et al., 2001).

3.6.4 Microbial population

Anthocyanins have been reported to modulate microorganisms in the gastrointestinal tract. Moreover, the fermentation substrate, rumen environment, and microbial population are the important factors affecting the type of microbial population in the rumen (Mensor et al., 2001). Hence, this difference may be caused by fermentation products, anthocyanin sources, and animal physiological stages. In this study has significant difference on microbial population in growing goats at 2 and 4 h after feeding among the treatment. Our study showed that goats fed a 6% Purple Neem foliage diet increase total bacteria, *butyrivibrio fibrisolven*, *fibrobacter succinogenes*, *ruminococcus albus*, *ruminococcus flavefacises* and *streptococcus bovis* respectively. It was likely that the microorganisms could improve anthocyanin bioavailability in the gastrointestinal tract, enhancing the gut antioxidant capacity

(Cardona et al., 2013). In addition, anthocyanins have strong antioxidant activity, which can protect the body from peroxidation damage, thus improving rumen microflora. As a consequence, anthocyanins can reduce heat stress and change the rumen microflora, thus improving rumen health could promote the proliferation of healthy anaerobic bacteria. Therefore, we found that this study was consistent with the study of (Bryszak et al., 2019), who demonstrated that feeding of anthocyanin could increase various bacteria abundances. showed that anthocyanin-rich berry seed residues could increase ruminal fluid total bacteria in a batch culture system. Moreover, (Sun et al., 2019) report that dairy cows receiving anthocyanin-rich plants had increased relative abundance of ruminal fluid.

Anthocyanins as gut modifiers could promote the proliferation of healthy anaerobic bacteria and inhibit pathogenic bacteria (Igwe et al., 2019). In addition, anthocyanin have antibacterial activity and inhibit the growth of a variety of pathogens due to intracellular interactions (Cisowska et al., 2011). In the current study, has significant difference was observe for protozoa and methanogen at 2 and 4 after feeding among the treatment. Show that goats fed 6% Purple Neem foliage diet decreased protozoa and methanogen when compare with other treatment suggesting that high anthocyanin content inhibited microorganisms in the rumen. This result was according with (Gutierrez-Banuelos et al., 2011), who demonstrate that phenolic-rich plants had antimicrobial effects in feedlot cattle and (Colombo et al., 2021), report that anthocyanin from purple corn affecting rumen microflora by regulating the relative abundance of rumen microbes and improving rumen microbial diversity and (Bryszak et al., 2019), report that Phyto factors including Strawberry (SB), black currant (BC), and raspberry seed (RB) residues can reduce the number of protozoa and methane activity influencing the rumen fermentation.

3.6.5 Antioxidant activity in plasma

metabolites of plants that are powerful natural antioxidants and free radical (FR) scavengers, have a variety of major physiological activities for consumers, and have broad development and application potential (Tian et al., 2021). Previous research has demonstrated that anthocyanins are absorbed into the bloodstream in their entirety (McGhie et al., 2003). As a result, anthocyanins can boost the activity of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase

(GSH-Px), and catalase (CAT) to further limit malondialdehyde formation (MDA). In our study, there was a significant difference in total antioxidant, SOD, GPX, DPPH, and According to the literature, anthocyanins are a source of secondary CAT levels at 2 and 4 h after feeding, indicating that goats given a 6% Purple Neem foliage diet increased total antioxidant, SOD, GPX, DPPH, and CAT levels in plasma.

Goats fed a 6% Purple Neem foliage diet dramatically increased plasma SOD activity, possibly indicating that anthocyanins were absorbed into the blood, supplying electrons to O_2, and therefore alleviating the OS condition. It is worth noting that the activity of total antioxidant, GPX, and CAT in plasma increased in goats fed a 6% Purple Neem foliage diet, maybe because they improved SOD activity in plasma, alleviating the stress on the antioxidant defense system. Furthermore, anthocyanin-rich foods may increase messenger RNA production, influencing SOD activity in the blood (Tian et al., 2021). The current findings are consistent with the findings of (Hosoda et al., 2012), who observed a considerable increase in SOD in the plasma of lactating dairy cows given anthocyanin-rich purple corn silage (Hosoda et al., 2009). Furthermore, our findings are consistent with the findings (Tian et al., 2019), who found that anthocyanin-rich purple corn stover silage can boost plasma antioxidant capacity in nursing dairy goats.

Because anthocyanins have considerable antioxidant activity, studies have demonstrated that anthocyanins are absorbed intact into blood goats given a 6% Purple Neem foliage diet dramatically raise plasma DPPH. DPPH is a kind of FR that is reduced in an aqueous solution containing an antioxidant (Mensor et al., 2001). As a result, anthocyanins in plasma can donate electrons to DPPH, boosting the degree of DPPH scavenging activity. Anthocyanin-rich grape juices, as effectively highlighted by Toaldo et al. (2016), can boost blood antioxidant capacity in humans.

In this study, MDA concentrations in plasma were considerably lowered after goats given a 6% Purple Neem foliage diet compared to other treatments. Because FRs' high reactivity has an effect on antioxidant enzymes, lipid peroxidation occurs (Toaldo et al., 2016). Antioxidant enzymes reduce lipid hydroperoxide and H_2O_2 concentrations, making them efficient in preventing lipid peroxidation and maintaining cell membrane integrity and function, resulting in the lowest MDA concentration. Previous research (Fee et al., 1975) found that it serves to minimize

oxidative stress caused by MDA byproducts formed from DNA and lipid oxidation and has mostly been utilized to monitor oxidation state.

3.7 Conclusion

According to the results of this study, goats feeding a 6% Purple Neem foliage diet enhance efficiency in terms of DMI, nutrients apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, and antioxidant activity in plasma of growing goats. Additionally, high total bacteria, *butyrivibrio fibrisolven*, *fibrobacter succinogenes*, *ruminococcus albus*, *ruminococcus flavefacises* and *streptococcus bovis* respectively were discovered, as well as low protozoa and methane production. Improve DPPH scavenging and SOD activity, as well as TAC, CAT, GPX, and reduce MDA in growing goat plasma. Based on the findings of the current study, it is possible to conclude that Purple Neem foliage can be utilized as a source of anthocyanin for ruminant feeding.

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CHAPTER IV

Experiment II

Effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma and meat quality in growing goats.

4.1 Abstract

The objective of this experiment was to investigate the effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma and meat quality in growing goats. Twenty-five Boer crossbreed male goats (approximately 20±2 kg body weight; mean±standard deviation (SD)) were assigned to 2×2+1 Factorial in completely randomized design. All goats received a 60-d routine feeding consisting of five treatments: (1) control, (2) 3% Purple Neem foliage (PNF)+3% sunflower oil (SFO) in concentrate, (3) 3% Purple Neem foliage (PNF)+6% sunflower oil (SFO) in concentrate, (4) 6% Purple Neem foliage (PNF)+3% sunflower oil (SFO) in concentrate and (5) 6% Purple Neem foliage (PNF)+6% sunflower oil (SFO) in concentrate. The results show that feed goats at 6%PNF+6%SFO in concentrate had higher (P<0.05) of feed intake gDM/d, %BW, g/kgBW$^{0.75}$, nutrient intake, nutrient digestion, final weight, weigh change, ADG, N intake, N Urine, N digestion, N digestion (%), N retention and N retention (%). Compare to the feed goat at 3%PNF+ 3%SFO in concentrate, 3%PNF+6%SFO in concentrate, 6%PNF+3%SFO in concentrate and control treatment. Ammonia nitrogen, BUN, acetic acid, propionic acid, ratio of acetic acid to propionic acid, butyric acid and total VFA at 2 and 4 h after feeding had higher (P<0.05) in the feed goat at 6%PNF+6%SFO in concentrate. Individual microbial population with 6%PNF+6%SFO higher (P<0.05) of total bacteria, *butyrivibrio fibrisolven*, *fibrobacter succinogenes*, *ruminococcus albus*, *ruminococcus flavefacises*, *streptococcus bovis* and lower protozoa and methanogen at 2 and 4 h after feeding. Antioxidant in plasma indices varied, with 6%PNF+6%SFO having higher Total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) antioxidant activity in plasma

and antioxidant gene expression in *longissimus dorsi* of GADPH, GPX, CAT, SOD and lower malondialdehyde (MDA) in plasma at 2 and 4 h after feeding. Goat meat from the 6%PNF+6%SFO had lower (P<0.05) of drip loss, cooking loss, shear force and was higher monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) compared to goat meat feed from the other treatment. The feeding of anthocyanin from Purple Neem foliage appears to be an attractive alternative for other native anthocyanin source in the nutrition of meat goats. The current results indicate that the feeding of 6% Purple Neem foliage+6% sunflower oil alleviates oxidative stress and promotes the production of meat

4.2 Introduction

Goat meat is a high-quality protein source with an excellent balance of key amino acids and a high biological value (Das et al., 2020). Meat is also a good source of minerals including selenium, iron, magnesium, salt, and vitamins (A, B12, folic acid). Better weights, higher growth performance, and meat tenderness are all advantages of meles for raising meat goats. Goat meat has the potential to replace beef meat, yet it is now in short supply due to exports to neighboring nations such as Malaysia, Vietnam, and China.

The natural active anthocyanin has polyphenol compounds are responsible for the total antioxidant potential of many fruits and other purple materials (Tian et al., 2021b). They are important components of plant-based foods because of their contribution to the taste, odor, aroma, and color of plant-based foods, such as fruits, vegetables, cereal, whole grains, coffee, and tea (Zhao et al., 2022). Anthocyanins are a source of secondary metabolites of plants that are effective natural antioxidant and free radical (FR) scavengers, have various kinds of significant physiological functions for consumers, and have broad prospects for development and application (Tian et al., 2022). Therefore, anthocyanins can improve the activities of antioxidant enzymes. Indeed, anthocyanins have been reported to show strong antioxidant potential to protect against lipid oxidation in meat goats (Tian et al., 2020).

Feeding purple neem foliage to growing goats not only reduces environmental problems caused by residue accumulation and lowers animal production's carbon footprint, but it also improves meat shelf-life stability and quality due to the

presence of bioactive compounds such as anthocyanins and phenolic compounds as natural antioxidants and their role in minimizing oxidative degradation of meat and meat production. Natural antioxidants derived from purple neem foliage are regarded as two key dietary components engaged in human health promotion and display antioxidant activity. As a result, the inclusion of components containing purple neem leaf, which has antioxidant activity, might be an opportunity to improve the quality and storage durability of meat products while also encouraging good behaviors (Das et al., 2020). Anthocyanin's antioxidant action also boosted the activity of desaturase enzymes, which are responsible for converting monounsaturated fatty acids (MUFA) to polyunsaturated fatty acids (PUFA) or inserting new unsaturated links into previously existing unsaturated bonds (PUFA) (Prommachart et al., 2021).

Despite the fact that anthocyanin from purple neem foliage has antioxidant properties and improves in meat production. There has been no research conducted to explore the effect of dietary anthocyanin consumption from purple neem foliage on antioxidant gene expression in muscle and the fatty acid profile of developing goats. Therefore, the objective of this study was to evaluate the effect of anthocyanin from purple neem foliage on antioxidant activity and meat quality in growing goats.

4.3 Objective

To study effect of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats.

4.4 Material and methods

4.4.1 Experimental design, animal diets and managements

The experiment was conducted to recommending by Animal Care and use committee of Suranaree University of Technology, Suranaree University of Technology, Nakhon Ratchasima province, Thailand.

The treatments were 2×2+1 Factorial in completely randomized design. This study has 5 treatments that were consisted T1, control; T2, 3% Purple Neem foliage+3% sunflower oil in concentrate; T3, 6% Purple Neem foliage (PNF)+3%

sunflower oil (SFO) in concentrate in concentrate; T4, 3% Purple Neem foliage+6% sunflower oil in concentrate in concentrate and T5, 6% Purple Neem foliage+6% sunflower oil in concentrate.

Twenty-five Boer crossbreed male goats were randomly assigned according to 2×2+1 Factorial in completely randomized design to receive 5 dietary treatments. All goats were kept in the individual feeding pens during the 60 days of experimental period. Mineral blocks and clean water were available ad libitum offered for all animals.

During the 28 days of experimental period goats will be fed with 1.5 % of BW DM/day containing Pangola (*Digitaria eriantha*) hay and (16 % crude protein) at the ratio of 60:40. All goats were received feeds and supplemented with their respective treatment diet prepared in accordance with the NRC requirements. Goats were feed in the morning and afternoon at approximately (07.00 and 16.00). During the adjusting period (14 days before starting each experimental period), all animal were fed with the same feed. Moreover, nutrient composition of experimental diets was shown in Table 4.1.

4.4.2 Sample collection

The experimental periods were used 28 day a period which 14 days for adjusted the animal and digestive tract. Samples of offered and refused diets were collected every day, while urine and feces samples were collected by total collection from each individual goat during the last 7 days of experimental period. Samples of offered and refused diets and feces will be oven dried (60°C) and ground through a 1.0 mm sieve prior to analysis for DM, ash, ether extract (EE) and crude protein (CP) (AOAC, 2005) and NDF and ADF (Van Soest et al., 1991). Total urine excretion were collected and acidified using 20% H_2SO_4 solution prior to analyses for ammonia nitrogen concentration using Kjeldahl method according to AOAC (2005).

Table 4.1 Feed ingredients and chemical composition of experimental diets.

Items	Pangola hay	Control	3%PNF+ 3% SFO	6%PNF + 3% SFO	3%PNF+ 6%SFO	6%PNF+ 6%SFO
Ingredients (% air-dry basis)						
Soybean meal	-	18.00	16.00	14.00	20.00	14.00
Rice bran	-	32.00	24.00	25.80	20.00	22.00
Cassava chip	-	28.00	27.00	24.30	31.00	30.10
Corn	-	20.40	25.40	25.30	18.40	20.30
Salt	-	0.40	0.40	0.40	0.40	0.40
Limestone	-	0.20	0.20	0.20	0.20	0.20
Premix	-	1.00	1.00	1.00	1.00	1.00
Sunflower oil	-	0.00	3.00	3.00	6.00	6.00
Purple Neem	-	0.00	3.00	6.00	3.00	6.00
Chemical composition (% DM)						
Dry matter	86.72	88.66	88.87	89.02	88.25	88.78
OM	93.52	93.83	93.05	93.81	93.90	93.58
Ash	6.48	6.17	6.95	6.19	6.10	6.42
Crude protein	5.91	16.71	16.01	16.29	16.27	16.62
Ether extract	1.66	3.78	4.44	4.48	6.44	6.74
Non-fibrous carbohydrate	5.55	53.51	46.21	39.22	38.68	32.83
Neutral detergent fiber	80.40	19.83	26.39	33.82	34.51	37.39
Acid detergent fiber	49.56	10.71	13.33	15.92	17.74	17.20
TDN, %	55.00	80.76	84.80	85.84	90.93	91.92
Metabolizable energy, Mcal / kg DM	1.99	2.92	3.07	3.10	3.29	3.32

Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g iron; 40 g zinc; 40 g manganese; 0.1 g cobalt; 10 g copper; 0.1 g selenium; 0.5 g iodine; Calculated as: NFC =100−(% NDF+% CP+% EE+% ash); Estimated by the equation TDN = (%) DCP+DNFC)+DEE×2.25+(DNDF); Estimated by the equation ME (Mcal/kg DM) = (TDN×0.04409×0.82), PNF: purple neem foliage, SFO: sunflower oil.

Table 4.2 Fatty acid composition of experimental diets.

Fatty Acids, g/100 g of Total Fatty Acids	Control	3%PNF+3%SFO	6%PNF+3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Palmitic C16:0	20.16[e]	21.15[d]	21.25[c]	22.15[b]	23.17[a]	0.21	0.01	0.01	0.01	0.01
Palmitoleic C16:1	1.25[e]	1.35[d]	1.55[c]	1.65[b]	1.85[a]	0.04	0.01	0.01	0.01	1.00
Stearic C18:0	3.15[e]	3.55[d]	4.25[c]	4.55[b]	5.15[a]	0.15	0.01	0.01	0.01	0.01
Oleic C18:1	28.69[d]	30.50[c]	30.85[c]	33.50[b]	35.50[a]	0.49	0.01	0.01	0.01	0.01
Linoleic C18:2 n-6	28.50[e]	30.15[d]	33.25[c]	35.35[b]	38.55[a]	0.73	0.01	0.01	0.01	0.46
α-Linolenic C18:3 n-3	2.56[b]	2.58[b]	3.70[a]	3.59[a]	3.77[a]	0.12	0.01	0.01	0.01	0.001
Total saturated fatty acids	23.31[e]	24.70[d]	25.50[c]	26.70[b]	28.32[a]	0.35	0.01	0.01	0.01	0.01
Total monounsaturated fatty acids	29.94[e]	31.85[d]	32.40[c]	35.15[b]	37.35[a]	0.54	0.01	0.01	0.01	0.01
Total polyunsaturated fatty acids	31.06[e]	32.73[d]	36.95[c]	38.94[b]	42.32[a]	0.84	0.01	0.01	0.01	0.02
n-6: n-3 fatty acid ratio	11.23[ab]	11.79[a]	9.02[c]	9.90[c]	10.23[bc]	0.26	0.04	0.41	0.01	0.01

PNF = Purple Neem foliage, SFO=Sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05)

Ruminal fluid will be sampled on last day of experiment using a stomach tube connected with vacuum pump at 0, 2 and 4 h post feeding. Rumen fluid samples were immediately measured for pH using a portable pH Meter, and then filtrated through 4 layers of cheesecloth under anaerobic conditions. Ruminal fluid sample were then divided into three portions; the first portion were used for community DNA extraction for estimation of microbial populations. The second portion of ruminal fluid sample were used for volatile fatty acid and the third portion of ruminal fluid sample were used for NH_3-N measurement using Kjeldahl method according to AOAC (2005).

With the same frequency of ruminal fluid sampling, blood samples were taken from jugular vein and immediately placed on ice and brought back to the laboratory and refrigerated at -20°C for 1 h. Blood plasma were then separated by centrifuge at 3,500×g for 20 min, then plasma were collected and stored at -20°C for further analysis of blood urea nitrogen (BUN) according to Crocker (1967).

4.4.3 Analysis of antioxidant activity enzymes in plasma

The TAC in the plasma was determined using a commercially available kit (Catalog Number MAK187-1KT, Sigma-Aldrich, Product of USA), according to manufacturers' protocol with a colorimetric probe, giving a broad absorbance peak at 570 nm, which is proportional to the total antioxidant capacity. The kit gives antioxidant capacity in Trolox (a water-soluble vitamin E analog, serves as an antioxidant standard) equivalents (ranging from 4-20 nmole/well).

The activity of SOD enzyme was measured according to a commercial assay kit (PCode 101869510, 19160-1KT-F, Sigma-Aldrich, Product of Japan). The Kit-WST allows very convenient SOD assaying by utilizing a water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. Absorbance was measured at 440 nm using a microplate reader (Epoch, BioTek, Luzern, Switzerland). Calculation the SOD activity (inhibition rate %) was done using the following equation.

SOD activity (inhibition rate %) = [(Ablank 1–Ablank 3)–(Asample–Ablank 2)]/(Ablank 1–Ablank 3)×100.

Glutathione Peroxidase (GPX) enzyme activity was determined by an indirect method, using the Sigma Glutathione Peroxidase Cellular Activity Assay kit (Catalog number: CGP1-1KT, Sigma-Aldrich, Product of Israel). The kit is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced (NADPH). The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPX activity, since GPX is the rate limiting factor of the coupled reactions.

The catalase (CAT) enzyme activity was determined by a colorimetric assay, using a commercial kit (Pcode 1002565186, CAT100-1KT, Sigma-Aldrich, Product of Israel). This assay method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase. The colorimetric method uses a substituted phenol (3,5-dichloro-2-hydroxybenzenesulfonic acid), which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to give a red quinoneimine dye (N-(4-antipyryl)-3-chloro-5-sulfonatep-benzoquinone-monoimine) that absorbs at 520 nm.

The DPPH scavenging activity in the plasma was assayed spectrophotometrically according to Wei and Chiang (2009), using a stable free radical DPPH (Sigma-Aldrich, Pcode: 101845869) with a slight modification. Briefly, 50 µL of each sample was added to 1 mL of methanolic solution of DPPH reagent (25 µmol/L) in a 1.5 mL tube. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min, and then centrifuged at 4000 r/min for 10 min at 4°C. 200 µL of the supernatant was transferred to a 96-well plate immediately and the absorbance was detected at 517 nm via a microplate reader (Epoch, BioTek, Luzern, Switzerland). DPPH scavenging activity was calculated by the following formula:

DPPH scavenging activity = (Ac-As) × 100/Ac

where Ac is the absorbance of the control, and as is the absorbance of the sample

The malondialdehyde (MDA) enzyme activity was determined by a colorimetric assay, using a commercial kit (Sigma-Aldrich, catalog number MAK085). All samples and standards should be run in duplicate. Use ultrapure water for the preparation of all standards and samples. Sample Preparation use plasma samples (20 µL) should be gently mixed with 500 µL of 42 mM sulfuric acid in a microcentrifuge tube. Add 125 µL of Phosphotungstic acid Solution and mix by vertexing. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000 × g for 3 minutes. In a separate tube, add 2 µL of BHT (100x) to 100 µL of water. Resuspend the pellet on ice with the water/BHT solution. Adjust the volume to 200 µL with water. In this kit, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present. Each sample was added MDA-TBA adduct, add 600 µL of the TBA solution into each vial containing standard and sample. Incubate at 95 C for 60 minutes. Cool to room temperature in an ice bath for 10 minutes, and then Pipette 200 µL from each reaction mixture, into a 96 well plate for analysis. Malondialdehyde was calculated by the following formula: Concentration of MDA for samples without 1-butanol concentration step:

$$(S_a/S_v) \times D = C$$

S_a, Amount of MDA in unknown sample (nmole) from standard curve; S_v, Sample volume (mL) or amount (mg) added into the wells; C, Concentration of MDA in sample; D, Sample dilution factor (if applicable)

Sample Calculation:
Amount of MDA (S_a) = 5.84 nmole; Sample volume (S_v) = 0.020 mL; Concentration of MDA in sample; (5.84 nmole/0.020 mL) × 1 = 292 nmole/mL

4.4.4 Microbial population analyses

Rumen fluid mixture were used for quantitative analysis of microbial population. Ruminal fluid sample will be extracted for community DNA was determined using a commercially available kit method (QIAGEN, Inc., Hilden, Germany). Population of rumen microbes which were studied in this experiment including of bacteria involved in Total bacteria, methanogen, protozoa. *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Streptococcus bovis* and *Ruminococcus albus*.

4.4.5 Slaughter procedures

At the end of the experiment three goats from each group were selected ran-domly for slaughter after overnight fasting. After obtaining the slaughter live weight these goats were slaughtered. After bleeding the head, skin, feet, lung and trachea, liver, heat, spleen, gastrointestinal tract and testicles were removed and hot carcass was obtained. The hot carcass was weighed, and weights of the skin and limbs, liver, kidneys and heart were recorded and expressed as a percentage of slaughter weight. Approximately 30 g samples were taken from: the *longissimus dorsi* (LD) of the carcass. These samples were immediately frozen in liquid nitrogen and stored at −80℃ for antioxidant indicator analyses and RNA extraction and were used to analyses parameters of meat quality.

4.4.6 Analysis gene expression of *longissimus dorsi* tissue

Total RNA was extracted from *longissimus dorsi* tissue using TRIzol Reagent method according to Labrecque et al. (2009), with a minor modification. Briefly, the sample was transferred into 2 mL tube, and added 1 mL TRIzol Reagent (Thermo Fisher Scientific, USA) and 0.1 g of the 3 mm dia. Zirconia/silica (Cat. No. 11079105z, BioSpec Products) and run in a homogenizer. The total RNA was dissolved with DEPC water (Ambion by Life Technologies, made in USA) and was DNase digested the RNase-Free DNase. After that, purity of the extracted RNA was analyzed by assaying the 260/280 absorbance ratio, using a Nano value spectrophotometer (GE Healthcare Bio-Sciences, Pittsburgh, United States). Integrity of the extracted RNA was assessed by verifying the presence of 18S and 28S RNA bands, using an Image Quant LAS 500 imager (GE Healthcare Bio-Sciences, Pittsburgh, United States) electrophoresis on a 2% agarose gel. Complementary DNA (cDNA)

synthesis was performed as described by ImProm-IITM Reverse Transcription System (Promega Corporation, Madison, USA).

All samples were assayed using quantitative real-time Polymerase Chain Reaction (real-time PCR) amplification by a Roche LightCycler® 480 real-time PCR System (Roche Diagnostics GmbH, Penzberg, Germany). Five target genes (TG) were: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase. Nucleotide sequences obtained from the National Center for Biotechnology Information (NCBI) and the primers set for all genes were designed using GenScript real-time PCR (TaqMan) Primer Design tool and were synthesized by the Bio Basic Inc. Company (Table 4.2). The real-time PCR amplifications were performed in a 10 µL reaction volume, which consisted of 5 µL of 2× Roche 04707516001 LightCycler® 480 SYBR Green I Master (Roche Diagnostics GmbH, Mannheim, Germany), 2 µL of 10×diluted cDNA, and 1 µL of forward (500 nM) and reverse (500 nM) primers, respectively. After that, the plates (LightCycler®480multiwell plate 96, white; Roche Diagnostics GmbH, Mannheim, Germany) were centrifuged at 4°C, 1500 rpm for 3 min (Universal 320, Hettich Zentrifugen, Germany). The program uses for the amplification of genes consisted of denaturing cycle of 10 min at 95°C, followed by 40 cycles (95°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s) and a dissociation step consisting of 95°C for 15 s, 60°C for 15 s and 95°C for 15 s (annealing temperature optimized depending on primers), and cooling at 40°C for 30 s. At the end of PCR, melt-curve analyses were performed for all genes. Amplifications were performed in triplicate for each gene.

4.4.7 Meat quality analysis

pH measurement

The pH value of meat was measured using a portable pH-meter (testo-PH2, Germany) at 1 and 24 h post-mortem, the pH meter was recalibrated after every six samples.

Color measurement

The color of meat dorsi was assessed on the cut surface using a Minolta Chromameter (Minolta CR-400, Tokyo, Japan) with D65 illuminant and two-degree standard observer. The results were expressed in terms of L* (lightness), a* (redness) and b* (yellowness) in the CIELAB color space model (CIE, 1976).

Drip losses

The drip losses of the meat in this study by according to the protocol followed by (Honikel et al., 1998). Meat pieces (2.5 cm thick) were weighed, placed in a container equipped with a plastic bag and lid, suspended in 24h at 4°C, and then the sample was reweighed. The drip loss was calculated as the percentage ratio of the initial weight using the following formula: Drip loss = $(W1 - W2)/W1 \times 100\%$.

Cooking losses

Each sample of meat was weighed, placed in a vacuum sealed plastic bag and cooked for 1 h in a water bath maintained at 80°C (Ramirez et al., 2004). After cooking, the samples were removed from the water bath, and then cooled under running water for 30 min the sample was then taken from the bag, blotted dry and weighed. The cooking losses were calculated as a percentage of the initial weight, according to the following formula: Cooking loss = $(M1 - M2)/W1 \times 100\%$.

Shear force

Shear force was measured using the method of Warner Bratzler shear force at 24 h post-mortem. The sample of longissimus dorsi, cooked as described above, was cut into rectangular cross-section strips (3×1.5×1.5 cm). All pieces were sheared using an Instron 5543 equipped with a Warner–Bratzler shear device and crosshead speed set at 200 mm/ min (Wheeler et al., 1997). The sheer force of each sample was measured three times and recorded in Newtons (N).

Proximate Composition

To calculate the proximate composition of GM, the separable fat and connective tissue were removed manually and thoroughly ground and homogenized with a homogenizer Ultra-Turrax (IKA Werke, GMBH & Co. KG, Staufen, Germany). The moisture, crude fat, crude protein and crude ash contents of the goat meat were determined according to (AOAC,2000).

Fatty acid

Extracted for fatty acids using the method as described by (Wheeler et al., 1997). Extracted fatty acids will be converted to fatty acid methyl esters by the procedure as described by (Garcés and Mancha, 1993), and methyl esters will be separated by using a gas chromatograph (GC).

4.4.8 Statistical analyses

All results were analyzed as a factorial completely randomized design (CRD) design using the general linear model procedure of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC, USA). Differences between treatments means will be determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Data obtains from meat quality were subjected to general linear model procedure of SAS. The relative mRNA abundance was calculated averaged abundance of the gene in data was considered as the calibrator, and the data was analyzed by ANOVA procedure. Data for physicochemical properties were analyzed using the PROC MIXED procedure of SAS. The level of significance difference at (p<0.05) will be accepted as representing statistically significant differences. The statistical model for the analysis of data was:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$$

where: where: Y_{ijk}, all dependent variables, μ = the overall mean, A_i, the effect of cultivar of Normal Neem foliage and Purple Neem foliage, B_j, the effect of level of Normal Neem foliage and Purple Neem foliage, AB_{ij} = the interaction of cultivar× level of Neem foliage, and ε_{ijk}, the residual effect

4.4.9 Experimental places

The Suranaree University of Technology part of Goat and Sheep farm and the center of Scientific and Technology Equipment Building 3 10 11 and 14, where were used for field experiment and laboratory, respectively.

4.5 Results

4.5.1 Feed intake, nutrient intake, nutrient digestion and growth performance

Table 4.1 shows the ingredient and nutrient composition of the experimental diets fed to growing goats in this study, which included control, 3%PNF +3%SFO, 6%PNF+3%SFO, 6%PNF+3%SFO, 6%PNF+3%SFO, and 6%PNF+6%SFO.

Feed intake and nutrient intake are shown in Table 4.3, with a significant difference (P<0.05) between feed intake (gDM/d, %BW, g/kgBW$^{0.75}$) and nutrient

intake (OM, CP, EE, NDF, and ADF). As a result, there was a significant difference on g DM/d and g/kgBW$^{0.75}$ and gDM/d, %BW, and g/kgBW$^{0.75}$ in growing goats fed 6%PNF+ 6%SFO diet was higher (P<0.05) than other treatments, indicating that with increasing feed intake. Nutrient intake (OM, CP, EE) there was significantly different among treatments due to (P<0.05), with goats feeding the 6%PNF+6%SFO treatment doing the highest (P<0.05).

According to Table 4.4, the apparent digestibility of DM, OM, CP, EE differed significantly among treatments (P<0.05). Feeding the goats at 6%PNF+6%SFO the best apparent digestibility (P<0.05) when compared to other treatments.

Table 4.5 shows the growth performance. There was a significant difference (P <0.05) in the final weight, weight change, and average daily gain (ADG) when compared to other treatments, goats receiving 6%PNF+ 6%SFO had the highest final weight, weight change, and average daily gain (ADG).

4.5.2 Nitrogen utilization

As shown in Table 4.6, there were significant differences in nitrogen utilization (P<0.05). Among all treatments, 6%PNF+ 6%SFO had the highest levels of N intake (g/d), N excretion from urine (g/d), N digestion (g/d), N digestion (%), N retention (g/d), and N retention (%).

4.5.3 Rumen fermentation parameter

At 0 h, the treatments had no effect on pH, ammonia nitrogen (NH$_3$-N), blood urea nitrogen (BUN), acetic acid (C$_2$), propionic acid (C$_3$), butyric acid (C$_4$), acetic acid: propionic (C$_2$/C$_3$), and total VFA (TVFA) (P<0.05) (Table 4.7 and 4.8). There were significant differences (P<0.05) in pH, NH$_3$-N, BUN, C$_2$, C$_3$, C$_4$, C$_2$/C$_3$, and TVFA among treatments after 2 and 4 h of feeding. In terms of ammonia nitrogen, blood urea nitrogen, acetic acid, propionic acid, butyric acid, and total VFA feeding, 6%PNF+ 6%SFO has the highest value when compared to the other groups.

Table 4.3 Effect of anthocyanin from Purple Neem foliage on feed intake of growing goat.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatment	Cultivars (C)	Levels (L)	C*L
Feed intake										
gDM/d	855.60[e]	971.56[d]	1055.11[c]	1260.09[b]	1480.19[a]	45.79	0.01	0.01	0.01	0.01
%BW	3.05[e]	3.13[d]	3.27[c]	3.39[b]	3.46[a]	0.05	0.01	0.01	0.21	0.64
g/kgBW$^{0.75}$	37.31[e]	48.21[d]	50.68[c]	61.96[b]	71.09[a]	2.38	0.01	0.01	0.01	0.01
Nutrient intake g DM/d										
OMI	804.27[e]	903.55[d]	991.80[c]	1184.49[b]	1391.38[a]	43.31	0.01	0.01	0.01	0.02
CPI	145.45[e]	155.45[d]	168.82[c]	201.62[b]	251.63[a]	7.95	0.01	0.01	0.01	0.01
EEI	34.22[e]	38.86[d]	42.20[c]	75.61[b]	103.61[a]	5.46	0.01	0.01	0.01	0.01
NDFI	171.12[e]	252.61[d]	358.74[c]	441.03[b]	547.67[a]	27.28	0.01	0.01	0.01	0.96
ADFI	94.12[e]	126.30[d]	168.82[c]	226.82[b]	251.63[a]	12.11	0.01	0.01	0.01	0.01

PNF= Purple Neem foliage, SFO=Sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), g DM /d: daily intake of dry matter% BW: edible quantity per body weight per day g/kgBW$^{0.75}$: daily consumption per daily metric weight, SEM: standard error of the mean.

Table 4.4 Effect of anthocyanin from Purple Neem foliage on nutrient digestion in growing goats.

Items	Control	3%PNF+3%SFO	6%PNF+3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Apparent Digestibility, % of intake										
DDM	46.80[e]	51.61[d]	54.30[c]	55.44[b]	57.48[a]	0.76	0.01	0.01	0.01	0.04
DOM	50.57[e]	52.27[d]	56.65[c]	58.58[b]	60.47[a]	0.77	0.01	0.01	0.01	0.01
DCP	50.70[e]	55.64[d]	60.70[c]	70.53[b]	75.60[a]	1.88	0.01	0.01	0.01	0.01
DEE	43.38[e]	45.49[d]	45.54[c]	61.50[b]	75.26[a]	2.45	0.01	0.01	0.01	0.01
DNDF	74.50[a]	71.07[b]	65.48[c]	62.53[d]	60.61[e]	1.06	0.01	0.01	0.01	0.04
DADF	68.39[a]	65.44[b]	62.68[c]	60.26[d]	55.48[e]	0.90	0.01	0.01	0.01	0.03

PNF = Purple Neem foliage, SFO=Sunflower oil, a, b, c, d, e = in the same row, there is a statistically significant difference (P<0.05), DDM: digestibility of dry matter, DOM: digestibility of organic matter, DCP: protein digestibility, DEE: fat digestibility, DEE: digestibility fatty digestibility, DNDF: the digestibility of the crude fiber that cannot be dissolved in neutral solution, DADF: the digestibility of the fiber that cannot be digested in an acidic solution, SEM: standard error of the mean.

Table 4.5 Effect of anthocyanin from Purple Neem foliage on performance in growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value Con vs Treatment	Cultivars (C)	Levels (L)	C*L
							Body weight			
Initial weight, kg	20.75	20.66	20.65	20.78	20.61	0.04	0.5	0.67	0.38	0.43
Final weight, kg	32.63[e]	34.10[d]	35.60[c]	37.45[b]	38.11[a]	0.42	0.01	0.01	0.01	0.002
Weigh change, kg	11.88[e]	13.44[d]	14.95[c]	16.67[b]	17.50[a]	0.43	0.01	0.01	0.01	0.06
ADG, g/d	197.97[e]	223.97[d]	249.10[c]	277.80[b]	291.67[a]	7.09	0.01	0.01	0.01	0.06

PNF = Purple Neem foliage, SFO=Sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.6 Effect of anthocyanin from Purple Neem foliage on nitrogen utilization in growing goats.

Items	Control	3%PNF+3%SFO	6%PNF+3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	Con vs Treatments	Cultivars (C)	Levels (L)	C*L
							p-value			
N intake, g/d	11.24[e]	12.78[d]	13.68[c]	15.41[b]	17.56[a]	0.45	0.01	0.01	0.01	0.01
N Feces, g/d	4.71[a]	3.56[b]	3.25[c]	2.47[d]	1.66[e]	0.21	0.01	0.01	0.01	0.01
N Urine, g/d	0.13[e]	0.15[d]	0.17[c]	0.19[b]	0.20[a]	0.01	0.01	0.01	0.01	1.00
N digestion, g/d	4.08[e]	6.43[d]	8.46[c]	11.24[b]	14.43[a]	0.74	0.01	0.01	0.01	0.01
N digestion (%)	50.70[e]	55.64[d]	60.70[c]	70.53[b]	75.60[a]	1.88	0.01	0.01	0.01	0.01
N retention, g/d	4.74[e]	6.28[d]	8.49[c]	11.45[b]	14.22[a]	0.70	0.01	0.01	0.01	0.07
N retention (%)	33.72[e]	47.72[d]	61.40[c]	71.05[b]	81.19[a]	3.43	0.01	0.01	0.01	0.01

PNF = Purple Neem foliage, SFO=Sunflower oil, a, b, c, d, e = in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean

Table 4.7 Effect of anthocyanin from Purple Neem foliage on pH, ammonia nitrogen and blood urea nitrogen in growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
pH										
0-h	6.95	6.96	6.93	6.98	6.99	0.01	0.34	0.02	0.53	0.16
2-h	6.75	6.76	6.77	6.79	6.80	0.01	0.07	0.06	0.63	0.73
4-h	6.81	6.83	6.84	6.85	6.87	0.01	0.03	0.04	0.27	0.71
Mean	6.84	6.85	6.85	6.88	6.88	0.01	0.12	0.04	0.78	0.78
Ammonia nitrogen mg/dL										
0-h	12.43	12.65	12.52	12.55	11.63	0.06	0.31	0.97	0.85	0.45
2-h	12.24[e]	13.34[d]	14.49[c]	15.17[b]	16.34[a]	0.29	0.01	0.01	0.01	0.03
4-h	13.24[e]	14.34[d]	15.49[c]	16.17[b]	17.34[a]	0.29	0.01	0.01	0.01	0.03
Mean	12.36[e]	13.10[d]	13.86[c]	14.32[b]	15.10[a]	0.19	0.01	0.01	0.01	0.03
BUN Mg%										
0-h	11.23	11.22	11.24	11.26	11.27	0.01	0.20	0.008	0.22	0.68
2-h	12.35[e]	13.42[d]	14.35[c]	15.46[b]	16.45[a]	0.30	0.01	0.01	0.01	0.75
4-h	13.48[d]	14.20[c]	15.28[b]	16.14[a]	16.60[a]	0.24	0.01	0.01	0.01	0.01
Mean	12.35[e]	12.95[d]	13.62[c]	14.29[b]	14.77[a]	0.18	0.01	0.01	0.01	0.06

PNF= Purple Neem foliage, SFO=Sunflower oil, BUN: blood urea nitrogen, CT: condensed tannin, ANT: anthocyanin, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.8 Effect of anthocyanin from Purple Neem foliage on Volatile fatty acid in growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF+ 6%SFO	6%PNF+ 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Acetic acid (%molar)										
0-h	58.64	58.65	58.66	58.67	58.68	0.02	0.63	0.59	0.82	0.96
2-h	59.74[a]	61.07[c]	62.29[b]	63.95[a]	63.99[a]	0.34	0.01	0.01	0.01	0.01
4-h	60.12[e]	62.08[d]	63.37[c]	64.32[b]	65.36[a]	0.37	0.01	0.01	0.01	0.02
Mean	59.50[e]	60.60[d]	61.44[c]	62.31[b]	62.68[a]	0.24	0.01	0.01	0.01	0.01
Propionic acid (%molar)										
0-h	23.64	23.67	23.68	23.69	23.71	0.01	0.20	0.37	0.52	0.81
2-h	24.80[e]	25.15[d]	26.48[c]	27.71[b]	28.99[a]	0.32	0.01	0.01	0.01	0.24
4-h	25.43[e]	26.07[d]	27.45[c]	28.73[b]	29.80[a]	0.33	0.01	0.01	0.01	0.01
Mean	24.63[e]	24.96[d]	25.87[c]	26.71[b]	27.50[a]	0.22	0.01	0.01	0.01	0.01
Butyric acid (%molar)										
0-h	17.71	17.73	17.74	17.75	17.76	0.01	0.17	0.39	0.69	1.00
2-h	18.78[d]	19.21[d]	20.46[c]	21.65[b]	22.99[a]	0.32	0.01	0.01	0.01	0.70
4-h	19.87[e]	20.19[d]	21.36[c]	22.77[b]	24.06[a]	0.32	0.01	0.01	0.01	0.14
Mean	18.79[e]	19.04[d]	19.85[c]	20.72[b]	21.60[a]	0.21	0.01	0.01	0.01	0.39
Acetic acid : Propionic										
0-h	2.48	2.47	2.47	2.48	2.47	0.01	0.08	0.10	0.28	0.92
2-h	2.41[b]	2.43[a]	2.35[c]	2.31[d]	2.21[e]	0.16	0.01	0.01	0.01	0.01
4-h	2.36[b]	2.38[a]	2.31[c]	2.24[d]	2.19[e]	0.27	0.01	0.01	0.01	0.01
Mean	2.42[b]	2.43[a]	2.38[c]	2.34[d]	2.29[e]	0.09	0.01	0.01	0.01	0.60
Total VFA (mmol/L)										
0-h	86.15	86.19	86.21	86.22	86.28	0.04	0.50	0.64	0.70	0.81
2-h	89.50[e]	92.19[d]	95.38[c]	97.67[b]	100.17[a]	0.78	0.01	0.01	0.01	0.01
4-h	92.29[e]	96.07[d]	98.56[c]	101.13[b]	104.04[a]	0.83	0.01	0.01	0.01	0.01
Mean	89.31[e]	91.48[d]	93.38[c]	95.01[b]	96.83[a]	0.54	0.01	0.01	0.01	0.51

a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

4.5.4 Microbial population

As shown in Table 4.9. There were significant differences (P<0.05) in the microbial population after feeding at 2 and 4 h. After feeding at 2 and 4 h, goats receiving 6%PNF+6%SFO treatment had higher (P<0.05) levels of Total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* than other treatments. When goats were fed 6%PNF+6%SFO (Table 4.8). The levels of protozoa and methanogen were lower (P<0.05) after 2 and 4 h of feeding when compared to other treatments.

4.5.5 Antioxidant activity in plasma

Table 4.10 shows the antioxidant activity in plasma. Total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) levels were significantly different (P<0.05). As a result, goats fed 6%PNF+6%SFO had significantly increased (P<0.05) levels of total antioxidant, superoxide dismutase, glutathione peroxidase, malondialdehyde, 2, 2-diphenyl-1-picrylhydrazyl, and catalase after 2 and 4 h of feeding. Malondialdehyde levels were lower (P<0.05) in goats fed 6%PNF+6%SFO at 2 and 4 h than in other treatments.

4.5.6 Antioxidant gene expression in *longissimus dorsi*

The Antioxidant gene expression in meat goats (*Longissimus dorsi*) (Table 4.11). There was significant difference (P<0.05) on glyceraldehyde-3-phosphate dehydrogenase (GADPH), glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD). After feeding at 2 and 4 h, goats fed the 6%PNF+6%SFO diet had significantly higher (P<0.05) levels of glyceraldehyde-3-phosphate dehydrogenase, glutathione peroxidase, catalase, and superoxide dismutase than those fed the 3%PNF+6%SFO, 3%PNF+3%SFO, 6%PNF+3%SFO and control.

Table 4.9 Effect of anthocyanin from Purple Neem foliage on rumen microbial population in growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF+ 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Total bacteria (lg10 copies/mL)										
0-h	4.52	4.63	4.70	4.58	4.57	0.06	0.54	0.52	0.82	0.80
2-h	4.07^{c}	6.18^{b}	7.03^{b}	7.08^{b}	8.18^{a}	0.29	0.01	0.01	0.01	0.59
4-h	4.03^{c}	6.08^{b}	6.71^{b}	6.91^{ab}	8.03^{a}	0.29	0.01	0.01	0.01	0.39
Mean	4.21^{c}	5.63^{b}	6.15^{ab}	6.19^{ab}	6.93^{a}	0.20	0.01	0.01	0.01	0.61
Butyrivibrio fibrisolven (lg10 copies/mL)										
0-h	5.40	5.41	5.43	5.44	5.58	0.05	0.67	0.51	0.56	0.63
2-h	6.15^{d}	7.26^{c}	8.29^{b}	9.08^{b}	10.31^{a}	0.31	0.01	0.01	0.01	0.65
4-h	6.11^{d}	7.22^{c}	8.09^{bc}	8.95^{b}	10.21^{a}	0.30	0.01	0.01	0.01	0.40
Mean	5.89^{e}	6.63^{d}	7.27^{c}	7.82^{b}	8.70^{a}	0.20	0.01	0.01	0.01	0.28
Fibrobacter succinogenes (lg10 copies/mL)										
0-h	3.59	3.54	3.53	3.57	3.68	0.06	0.96	0.52	0.70	0.68
2-h	4.06^{d}	4.50^{d}	7.32^{c}	8.19^{b}	10.30^{a}	0.48	0.01	0.01	0.01	0.05
4-h	3.54^{d}	4.05^{d}	7.23^{c}	8.11^{b}	10.07^{a}	0.51	0.01	0.01	0.01	0.01
Mean	3.73^{c}	4.03^{c}	6.03^{b}	6.62^{b}	8.02^{a}	0.33	0.01	0.01	0.01	0.06
Ruminococcus albus (lg10 copies/mL)										
0-h	3.59	3.45	3.57	3.61	3.79	0.06	0.92	0.17	0.28	0.81
2-h	4.46^{d}	5.25^{d}	6.26^{c}	8.25^{b}	10.10^{a}	0.43	0.01	0.01	0.01	0.04
4-h	4.26^{d}	5.08^{d}	6.15^{c}	7.14^{b}	9.82^{a}	0.40	0.01	0.01	0.01	0.01
Mean	4.10^{d}	4.59^{d}	5.33^{c}	6.33^{b}	7.90^{a}	0.29	0.01	0.01	0.01	0.03

PNF = Purple Neem foliage, SFO=Sunflower oil, a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.
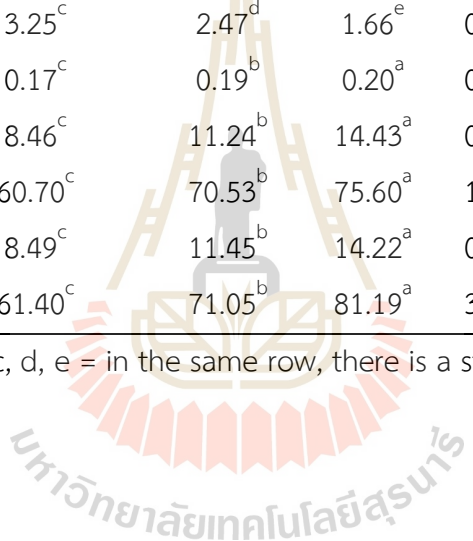
Table 4.9 Effect of anthocyanin from Purple Neem foliage on rumen microbial population in growing goats (Con).

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF+ 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Ruminococcus flavefacises (lg10 copies/mL)										
0-h	3.57	3.58	3.59	3.60	3.99	0.07	0.50	0.19	0.22	0.24
2-h	4.34^e	6.31^d	8.30^c	10.34^b	12.15^a	0.58	0.01	0.01	0.01	0.71
4-h	4.14^e	6.11^d	8.15^c	10.26^b	12.10^a	0.59	0.01	0.01	0.01	0.71
Mean	4.02^e	5.33^d	6.68^c	8.07^b	9.41^a	0.40	0.01	0.01	0.01	1.00
Streptococcus bovis (lg10 copies/mL)										
0-h	4.55	4.56	4.57	4.59	4.61	0.06	0.86	0.82	0.93	0.97
2-h	5.05^e	6.08^d	7.15^c	9.33^b	11.34^a	0.47	0.01	0.01	0.01	0.05
4-h	3.92^e	5.09^d	7.09^c	9.06^b	11.12^a	0.54	0.01	0.01	0.01	0.88
Mean	4.51^e	5.24^d	6.27^c	7.66^b	9.06^a	0.34	0.01	0.01	0.01	0.36
Protozoa (lg10 copies/mL)										
0-h	7.58	7.55	7.21	7.24	7.28	0.06	0.10	0.38	0.28	0.17
2-h	9.07^a	6.29^b	5.24^c	4.31^d	3.59^e	0.40	0.01	0.01	0.01	0.42
4-h	8.03^a	6.14^b	5.03^c	4.29^c	3.26^d	0.35	0.01	0.01	0.01	0.87
Mean	8.23^a	6.66^b	5.83^c	5.28^d	4.71^e	0.26	0.01	0.01	0.01	0.48
Methanogen (lg10 copies/mL)										
0-h	8.33	8.55	8.57	8.50	8.22	0.06	0.46	0.16	0.36	0.29
2-h	9.38^a	8.14^b	6.94^c	5.26^d	5.34^d	0.34	0.01	0.01	0.02	0.01
4-h	9.34^a	8.06^b	6.93^c	3.99^d	3.29^d	0.48	0.01	0.01	0.01	0.31
Mean	9.02^a	8.25^b	7.48^c	5.92^d	5.62^d	0.28	0.01	0.01	0.01	0.22

PNF = Purple Neem foliage, SFO = Sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.10 Effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma of growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF+ 6%SFO	6%PNF+ 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Total antioxidant (nmol/uL)										
0-h	1.72	1.64	1.64	1.65	1.72	0.04	0.47	0.79	0.73	0.73
2-h	2.07^d	2.28^c	2.46^b	2.56^b	2.81^a	0.05	0.01	0.01	0.01	0.31
4-h	1.79^e	2.05^d	2.17^c	2.32^b	2.50^a	0.05	0.01	0.01	0.01	0.17
Mean	1.86^e	1.99^d	2.09^c	2.18^b	2.34^a	0.04	0.01	0.01	0.01	0.42
SOD (inhibition rate %)										
0-h	88.91	88.83	89.04	88.66	89.61	0.16	0.75	0.57	0.11	0.30
2-h	89.17^e	90.45^d	92.62^c	94.69^b	96.67^a	0.57	0.01	0.01	0.01	0.75
4-h	90.40^d	90.87^c	91.19^c	91.86^b	92.44^a	0.15	0.01	0.01	0.01	0.20
Mean	89.49^d	90.05^d	90.95^c	91.74^b	92.91^a	0.26	0.01	0.01	0.01	0.50
GPX (Units/mL)										
0-h	67.60	67.41	67.71	67.87	67.81	0.10	0.71	0.24	0.62	0.44
2-h	72.83^d	74.15^c	76.35^b	76.69^b	78.14^a	0.40	0.01	0.01	0.01	0.13
4-h	67.37^d	69.93^c	70.39^c	73.01^b	75.28^a	0.59	0.01	0.01	0.01	0.08
mean	69.27^e	70.50^d	71.48^c	72.52^b	73.74^a	0.33	0.01	0.01	0.01	0.55

PNF = Purple Neem foliage, SFO = Sunflower oil, superoxide dismutase (SOD) glutathione peroxidase (GPX), Malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT). a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.10 Effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma of growing goats (Con).

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF+ 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
DPPH scavenging activity (%)										
0-h	35.43	35.48	35.74	35.49	38.31	0.42	0.40	0.15	0.09	0.15
2-h	43.37^e	47.79^d	52.92^c	62.31^b	74.00^a	2.27	0.01	0.01	0.01	0.00
4-h	38.99^d	52.14^c	56.11^{bc}	57.89^b	63.08^a	1.73	0.01	0.01	0.01	0.61
Mean	39.26^e	45.14^d	48.26^c	51.90^b	58.46^a	1.35	0.01	0.01	0.01	0.04
CAT (nmol/min/mL)										
0-h	9.61	9.80	9.79	9.73	10.20	0.10	0.31	0.48	0.34	0.31
2-h	14.79^d	14.87^c	14.93^{bc}	14.97^b	15.08^a	0.02	0.01	0.01	0.01	0.10
4-h	13.81^d	13.89^c	13.96^c	14.04^b	14.11^a	0.02	0.01	0.01	0.01	0.73
Mean	12.74^b	12.85^b	12.89^{ab}	12.91^{ab}	13.13^a	0.04	0.02	0.07	0.11	0.26
MDA (μg/mL)										
0-h	28.04	27.75	27.76	27.63	27.18	0.23	0.45	0.52	0.69	0.68
2-h	36.07^a	30.50^b	25.13^c	23.27^c	19.99^d	1.20	0.01	0.01	0.01	0.19
4-h	32.14^a	30.13^b	28.42^b	24.65^c	17.63^d	1.06	0.01	0.01	0.01	0.01
Mean	32.08^a	29.46^b	27.10^c	25.18^d	21.60^e	0.75	0.01	0.01	0.01	0.16

PNF = Purple Neem foliage, SFO=Sunflower oil, superoxide dismutase (SOD) glutathione peroxidase (GPX), Malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT). a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.11 Effect of anthocyanin from Purple Neem foliage on antioxidant gene expression in *longissimus dorsi* tissue of growing goats.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
GADPH (µmol/min per mg protein)										
	2.54[d]	3.76[c]	5.97[b]	6.09[b]	6.96[a]	0.34	0.01	0.01	0.01	0.01
GPX (nmol/min per mg protein)										
	3.04[e]	4.74[d]	6.77[c]	7.74[b]	8.87[a]	0.43	0.01	0.01	0.01	0.02
CAT (µmol/min per mg protein)										
	2.76[e]	3.41[d]	4.81[c]	5.68[b]	6.97[a]	0.32	0.01	0.01	0.01	0.76
SOD (µmol/min per mg protein)										
	2.74[e]	3.76[d]	4.67[c]	5.76[b]	6.94[a]	0.31	0.01	0.01	0.01	0.42

PNF = Purple Neem foliage, SFO=Sunflower oil, superoxide dismutase (SOD) glutathione peroxidase (GPX), catalase (CAT), glyceraldehyde-3-phosphate dehydrogenase (GADPH), a, b, c, d, e: in the same row, there is a statistically significant difference ($P<0.05$), SEM: standard error of the mean.
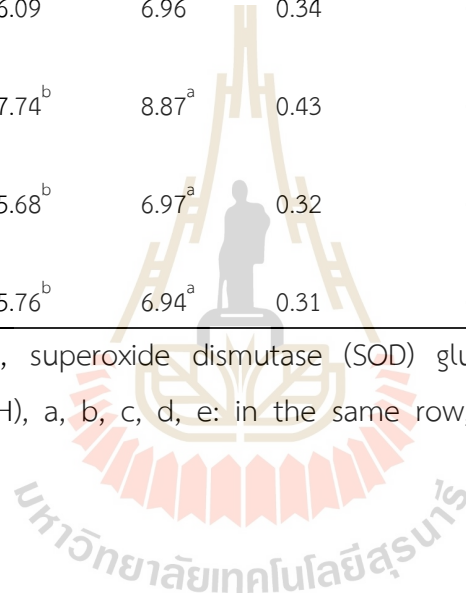
4.5.7 Carcass and Meat Characteristics

As the result on carcass weight, skin, White offal, red offal, pH, meat color of L*, a*, and b* had no effect (P>0.05). However, goat fed 6%PNF+6%SFO had higher slaughter weight (P<0.05) and lower carcass (%) shown in (Table 4.12). Drip loss, cooking loss, and sheer force were significantly lower (P<0.05) in the 6%PNF+6%SFO group shown in Table 4.13 The effect of anthocyanin from Purple Neem foliage was reported to did not affect (P>0.05) on proximate composition of goat meat (Table 4.14).

4.5.8 Fatty acid profile

As the result shown in Table 4.15. There were has significant different (P<0.05) on saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Goats fed 6%PNF+6%SFO lower were saturated fatty acid (P<0.05). However, goats fed 6%PNF+6%SFO were significantly higher (P<0.05) monounsaturated fatty acid and polyunsaturated fatty acid than those in the other treatments.
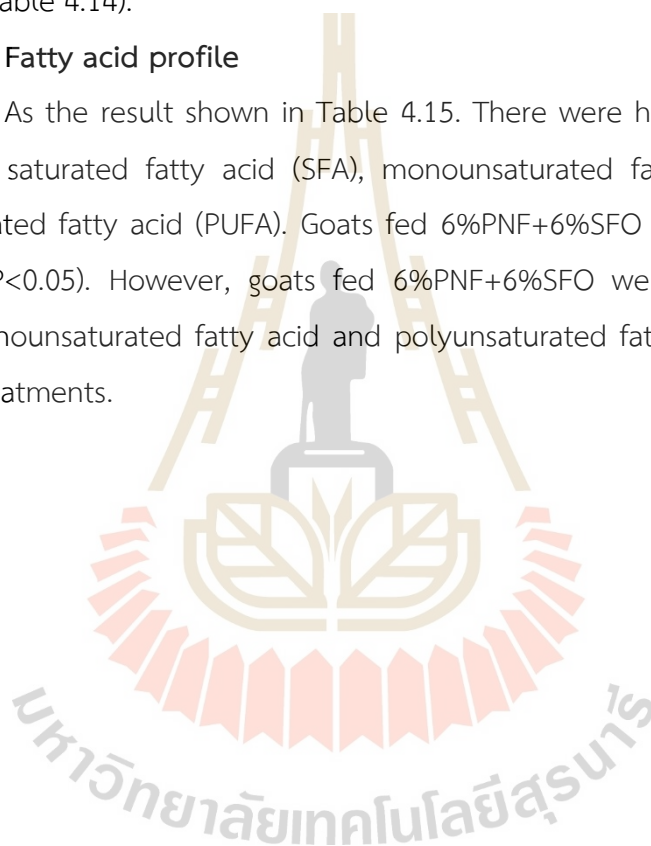
Table 4.12 Effect of anthocyanin from Purple Neem foliage on carcass characteristics of growing goats.

Items	Control	3%PNF +3%SFO	6%PNF +3%SFO	3%PNF+ 6%SFO	6%PNF+ 6%SFO	SEM	Con vs Treatments	Cultivars (C)	Levels (L)	C*L
								p-value		
Slaughter weight (SW), kg	32.63[e]	34.10[d]	35.60[c]	37.45[b]	38.11[a]	0.42	0.01	0.01	0.01	0.02
Carcass weight (kg)	16.61	16.62	16.64	16.66	16.67	0.03	0.66	0.67	0.86	0.95
Carcass (%)	50.90[a]	48.74[b]	46.75[b]	44.49[c]	43.74[d]	0.55	0.01	0.01	0.01	0.04
Skin g/100g SW	13.50	13.52	13.56	13.58	13.60	0.04	0.59	0.64	0.78	0.93
White offal, g/100 g SW	8.50	8.52	8.54	8.58	8.59	0.04	0.58	0.58	0.86	0.98
Red offal, g/100g SW	0.56	0.57	0.58	0.58	0.59	0.04	0.02	0.15	0.31	0.91

PNF : purple neem foliage, SFO: sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.
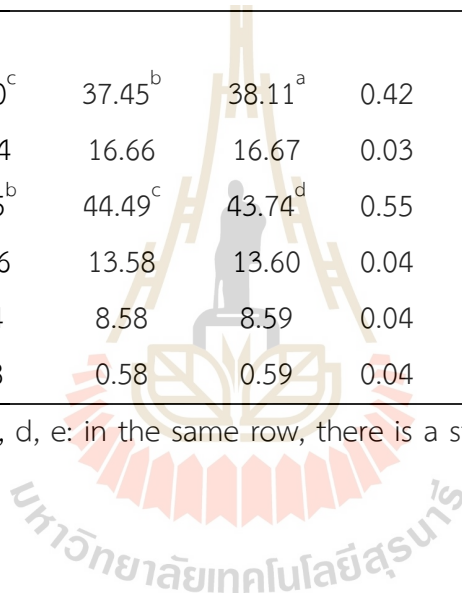
Table 4.13 Effect of anthocyanin from Purple Neem foliage on meat quality characteristics of growing goats.

Items	Control	3%PNF+3%SFO	6%PNF+3%SFO	3%PNF + 6%SFO	6%PNF+ 6%SFO	SEM	Con vs Treatments	Cultivars (C)	Levels (L)	C*L
									p-value	
pH value at										
1- h	7.33	7.34	7.35	7.36	7.37	0.01	0.03	0.04	0.25	0.85
24 - h	6.71	6.73	6.74	6.75	6.76	0.01	0.04	0.07	0.48	1.00
Mean	7.02[b]	7.04[ab]	7.05[ab]	7.06[ab]	7.07[a]	0.01	0.01	0.02	0.25	0.91
Meat Color										
L*	34.68	34.63	34.60	34.61	34.65	0.04	0.65	0.90	0.94	0.77
a*	1.87	1.86	1.85	1.84	1.83	0.01	0.05	0.06	0.55	0.93
b*	5.34	5.35	5.37	5.38	5.39	0.01	0.28	0.35	0.58	0.87
Drip loss	4.85[a]	4.51[a]	4.42[b]	3.41[c]	2.66[d]	0.18	0.01	0.01	0.02	0.06
Cooking loss (%)	34.99[a]	35.72[a]	35.44[a]	23.64[b]	20.98[c]	1.41	0.02	0.01	0.24	0.34
Shear force (N)	12.70[a]	9.93[b]	8.08[c]	5.88[d]	3.40[e]	0.66	0.01	0.01	0.01	0.23

PNF : purple neem foliage, SFO: sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 4.14 Effect of anthocyanin from Purple Neem foliage on chemical composition of goat meat.

Items	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Moisture	75.60	75.63	75.64	75.65	75.69	0.01	0.05	0.10	0.36	0.50
Dry matter	24.40	24.37	24.36	24.35	24.31	0.01	0.05	0.10	0.36	0.50
CP	20.42	20.43	20.45	20.47	20.48	0.01	0.08	0.04	0.50	0.76
Ash	5.16	5.17	5.18	5.19	5.20	0.01	0.04	0.05	0.36	0.85
EE	11.10	11.11	11.13	11.16	11.17	0.01	0.23	0.13	0.53	0.77

PNF : purple neem foliage, SFO: sunflower oil, CP: Crude protein, EE: Ether extract, SEM: standard error of the mean.

Table 4.15 Effect of anthocyanin from Purple Neem foliage on fatty acid (FA) composition (% of all fatty acids analyzed) of the lipids in meat of growing goats.

Fatty Acids, g/100 g of Total Fatty Acids	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Saturated FA (SFA)										
C14:0	3.94[a]	3.41[b]	2.88[c]	2.36[d]	1.96[e]	0.15	0.01	0.27	0.02	0.62
C15:0	0.57[a]	0.50[b]	0.45[b]	0.40[c]	0.30[d]	0.02	0.01	0.02	0.03	0.14
C16:0	23.71[a]	22.72[b]	21.68[c]	20.68[d]	20.13[e]	0.27	0.01	0.01	0.01	0.04
C17:0	1.96[a]	1.86[b]	1.76[c]	1.56[d]	1.50[e]	0.04	0.01	0.01	0.01	0.20
C18:0	20.72[a]	19.90[b]	18.71[c]	16.71[d]	15.68[e]	0.39	0.01	0.01	0.01	0.42
C20:0	0.21[a]	0.20[b]	0.19[c]	0.16[d]	0.15[e]	0.04	0.01	0.01	0.01	0.45
C22:0	2.17[a]	2.07[b]	1.85[c]	1.66[d]	1.56[e]	0.05	0.01	0.01	0.01	0.02
Other SFA	0.87[a]	0.65[b]	0.55[c]	0.47[d]	0.40[e]	0.03	0.01	0.01	0.01	0.24
Total SFA	54.15[a]	51.31[b]	48.07[c]	44.00[d]	41.68[e]	0.57	0.01	0.01	0.01	0.16
Monounsaturated FA (MUFA)										
C16:1 cis-9	1.57[e]	1.75[d]	1.85[c]	1.95[b]	2.10[a]	0.04	0.01	0.01	0.03	0.40
C17:1 cis-10	1.26[e]	1.57[d]	1.85[c]	1.95[b]	2.17[a]	0.06	0.01	0.01	0.01	0.03
C18:1 cis-9	16.04[e]	17.80[b]	19.13[c]	22.73[b]	24.63[a]	0.66	0.01	0.01	0.01	0.31
C20:1 cis-11	0.27[e]	1.05[d]	1.55[c]	1.75[b]	1.95[a]	0.12	0.01	0.01	0.01	0.01
Other MUFA	0.56[e]	0.75[d]	0.85[c]	1.05[b]	1.15[a]	0.04	0.01	0.01	0.01	0.94
Total MUFA	19.70[e]	22.92[d]	25.23[c]	29.43[b]	32.00[a]	0.91	0.01	0.01	0.01	0.69

PNF : purple neem foliage, SFO: sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean, C14:0: myristic, C15:0: pentadecanoic, C16:0: palmitic, C17:0: heptadecanoic, C18:0: stearic, C20:0: arachidic, C22:0: behenic.
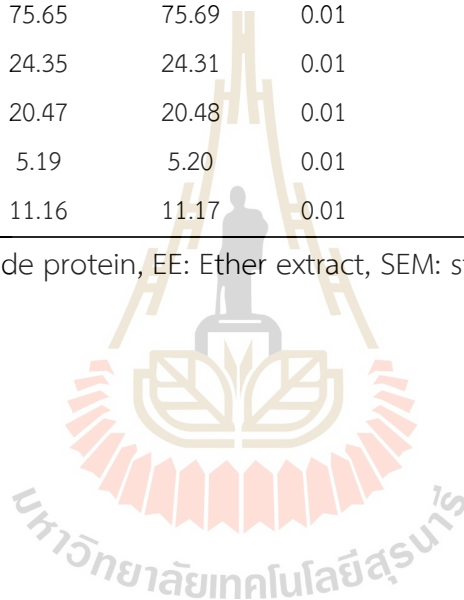
Table 4.15 Effect of anthocyanin from Purple Neem foliage on fatty acid (FA) composition (% of all fatty acids analyzed) of the lipids in meat of growing goats (Con).

Fatty Acids, g/100 g of Total Fatty Acids	Control	3%PNF+ 3%SFO	6%PNF+ 3%SFO	3%PNF + 6%SFO	6%PNF + 6%SFO	SEM	p-value			
							Con vs Treatments	Cultivars (C)	Levels (L)	C*L
Polyunsaturated FA (PUFA)										
C18:2 n-6	6.56[e]	8.58[d]	10.70[c]	11.59[b]	12.58[a]	0.45	0.01	0.01	0.01	0.04
C18:3 n-3	0.75[e]	1.55[d]	1.65[c]	1.77[b]	1.95[a]	0.08	0.01	0.01	0.01	0.01
C20:3 n-6	0.87[e]	1.25[d]	1.47[c]	1.65[b]	1.87[a]	0.07	0.01	0.01	0.01	1.00
C20:4 n-6	3.73[e]	5.68[d]	7.73[c]	8.73[b]	10.78[a]	0.50	0.01	0.01	0.01	0.97
C20:5 n-3 (EPA)	0.56[e]	1.15[d]	1.27[c]	1.35[b]	1.45[a]	0.06	0.01	0.01	0.01	0.49
C22:6 n-3 (DHA)	0.27[e]	0.35[d]	0.55[c]	0.75[b]	0.87[a]	0.05	0.01	0.01	0.01	0.01
Other PUFA	0.35[e]	0.45[d]	0.55[c]	0.65[b]	0.75[a]	0.03	0.01	0.01	0.01	0.95
Total PUFA	13.09[e]	19.01[d]	23.92[c]	26.49[b]	30.25[a]	1.23	0.01	0.01	0.01	0.10
Total PUFA n-3	1.58[e]	3.05[d]	3.47[c]	3.87[b]	4.27[a]	0.19	0.01	0.01	0.01	0.86
Total PUFA n-6	11.17[e]	15.52[d]	19.90[c]	21.96[b]	25.23[a]	1.01	0.01	0.01	0.01	0.05
PUFA/SFA ratio	0.24[e]	0.37[d]	0.50[c]	0.60[b]	0.73[a]	0.05	0.01	0.01	0.01	0.01
MUFA/SFA ratio	0.36[e]	0.45[d]	0.52[c]	0.67[b]	0.77[a]	0.05	0.01	0.01	0.01	0.09
n-6/n-3 ratio	7.06[a]	5.09[d]	5.73[c]	5.68[c]	5.91[b]	0.13	0.01	0.01	0.01	0.01

PNF : purple neem foliage, SFO: sunflower oil, a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

4.6 Discussion

4.6.1 Feed intake, nutrient intake, nutrient digestion and growth performance

In the current study, we discovered that anthocyanin from Purple Neem foliage has a significant different on feed intake (g DM/d), % BW, g/kgBW$^{0.75}$, and nutrient intake. Although goats fed anthocyanin-rich 6%PNF+6%SFO had higher level of g DM/d, % BW, g/kgBW$^{0.75}$ and nutrient intake than goats fed 3%PNF+3%SFO, 3% PNF+6%SFO, 6%PNF+3%SFO, and control. Goats fed 6%PNF+6%SFO had high feed intake and nutritional intake, demonstrating that giving anthocyanins did not result in poorer palatability for goats, and sunflower oil will not have a detrimental effect on feed intake since oil contains vitamin E, which can promote palatability for goats. Further intake is expected to be inversely related to the fiber content of the forage, since the slower digesting portion gets larger in ratio to the volume of the digestive system (Van Soest, 1965). Our findings, which are similar to those of (Antunovic et al., 2022), show that red maize high in anthocyanins can boost lamb productivity. As a result, polyphenol-enriched feeds, such as anthocyanins, are increasingly being utilized as supplements added into solid feed after weaning, potentially leading to decreased oxidative stress and enhanced immunological response, health status, and meat quality in lambs (Correddu et al., 2020).

The nutrient digestion in this experiment differed significantly across treatments. In this study, we discovered that anthocyanin from 6%PNF + 6%SFO has a high nutritional digestion of DM, OM, CP, EE, NDF, and ADF when compared to other treatments. The effect of anthocyanin from Purple Neem foliage has high nutrient digestion because Purple Neem foliage has high crude protein and Neem, despite its bitter taste, has been introduced and investigated in the composition of livestock diet as Neem Seed Cake, due to the presence of essential amino acids, crude protein, fiber, Sulphur and Nitrogen (Esonu et al., 2006), as a replacement for groundnut or soya beans due to its high protein component without any adverse effects. According to (Paengkoum, 2010), Neem leaves may substitute up to 50% of soya bean meal in ruminant diets with no detrimental impacts on feed intake, dry matter and fiber digestibility, or body weight increase. As a result, the higher intake of Purple Neem foliage may be explained by its enhanced rumen degradability and a

rise in the outflow of Purple Neem foliage cell wall to the abomasum (Trach et al., 2001). In this trial, the effect of anthocyanin from Purple Neem foliage on final weight, weight change, and ADG was significant different all treatments. The significant difference in final weight, weight change, and ADG of goats in the treatment given 6%PNF + 6%SFO might be attributed to the comparatively high quality of the basal diet. Because goat given Purple Neem foliage may boost feed intake and nutrient digestion, this will allow the animal to obtain more nutrients and improve animal performance. Moreover, because sunflower oil is a source of energy, this will allow the animal to get nutrients and sustain performance. (Harikrishnan et al., 2003), also found that the Neem plant possesses antibacterial, antifungal, antiviral, and pesticidal properties, as well as the ability to improve general development and health performance while having no adverse impact on important organs. According to (Paengkoum, 2010), the effect of Neem foliage showed protein locally produced shrubs can replace imported feedstuffs concentrate as a protein supplement for goat production.

4.6.2 Nitrogen utilization

In this investigation, the results of nitrogen consumption differed significantly among treatments. When compared to other treatments, goat fed 6%PNF+6%SFO had greater nitrogen utilization of N intake, N Urine, N digestion, N digestion (%), N retention, and N retention (%). This might be due to the high CP level in Purple Neem foliage, which has the ability to boost ruminal fermentation and so increase microbial protein synthesis (Anantasook et al., 2016). The increased CP intake may make N accessible for rumen microorganisms to use as an energy source to manufacture their cells. However, the addition of PEREM may offer additional nutrients to the rumen microbes while also supporting the animal host (Cherdthong et al., 2019). The anthocyanin may aid in the coordinated release of nitrogen and carbohydrates from purple field corn stover, which is responsible for increased microbial efficiency (Cherdthong et al., 2019). All treatments demonstrated good N consumption, indicating that the nutrients in each treatment properly met the goats' protein maintenance requirements. Our findings are congruent with the findings of (Nudda et al., 2017), who found that feeding sheep polyphenol-rich plants

improved nitrogen utilization and CP digestibility. Because anthocyanin may bind to dietary proteins, it can reduce rumen fermentation and improve nitrogen uptake.

4.6.3 Rumen fermentation parameter

In this study, the rumen fermentation parameters of pH, ammonia nitrogen, and BUN found that anthocyanin from Purple Neem foliage had no significant difference on pH, ammonia nitrogen, and BUN at 0 h, but had a significant difference on pH, ammonia nitrogen, and BUN at 2 and 4 h after feeding. However, our study discovered that goats fed a diet containing 6%PNF + 6%SFO had significantly higher ammonia nitrogen and BUN levels at 2 and 4 h after feeding, but had no detrimental influence on pH levels at 2 and 4 h. In the current study, there was a significant difference in pH at 2 and 4 h after feeding, and goats fed 6%PNF+ 6%SFO can reduce pH from our results, which were similar to (Mattos et al., 2017) report that animals that received the highest dietary lipid content (60 g/kg), rumen pH decreased quadratically with an increase in the lipid content. According to (Russell and Wilson, 1996) and (Mertens, 1987), pH values greater than 6.2 have little effect on ruminal fermentation. The rumen pH varied from 6.75 to 7.00 on average, which is optimum for microbial digestion in the rumen (Cherdthong et al., 2019).

Furthermore, the ruminal fluid concentration of NH_3-N is often altered by the CP content of the diet (Hristov et al., 2004). In the current study, goats fed 6%PNF+6%SFO had higher NH_3-N levels at 2 and 4 h compared to other treatments. The NH_3-N content in this research ranged from 12.43 to 17.34 mg/dL. When (Onetti et al., 2001) evaluated the impact of dietary fat type and concentration on ruminal fermentation in dairy cattle, they found comparable results to ours. The cows were fed corn silage-based diets, and the results revealed that adding 40 g/kg fat resulted in a higher (P<0.001) ammonia content than adding 20 g/kg fat. These findings reveal that the ruminal NH_3-N concentration was sufficient to support microbial development (5 mg/dL) (NRC, 1981) and was within or above the optimum level of 12-20 mg/dL at 2 and 4 h post feeding (Islam et al., 2000). Furthermore, Purple Neem foliage contains CP, and the goats are likely to obtain increased protein as a result. According to (Hosoda et al., 2012), nursing dairy cows consuming greater levels of CP of anthocyanin-rich corn silages had a higher level of NH_3-N concentration as compared to the control silage group.

The results demonstrate that the effect of anthocyanin from Purple Neem foliage on BUN is significantly different at 2 and 4 h after feeding in this research. However, we discovered that goats given 6%PNF+6%SFO had greater BUN when compared to other treatments. This was most likely due to the higher CP content of Purple Neem foliage, which was followed by a higher concentration of ammonia nitrogen. The concentration of BUN in plasma also indicated the animal feed balance. In this investigation, the BUN in plasma ranged from 12.22 to 16.60 mg/dL. These findings reveal that the BUN in plasma was within or above the recommended limit of 10-20 mg/dL for all treatments (Kaneko et al., 1997).

The volatile fatty acid concentrations in this investigation differed significantly between 2 and 4 h after feeding in terms of acetic acid, propionic acid, butyric acid, acetic acid: propionic acid, and total VFA. VFAs are the primary sources of energy for ruminant metabolism. Anthocyanins may promote VFA generation by altering gut flora (Purba et al., 2020). The acetic acid concentration was higher in the goats fed 6%PNF+6%SFO. This is likely due to higher content and digestibility of NDF and ADF in goats fed 6%PNF+6%SFO treatment. However, the rumen VFA concentration is affected by feed intake, feeding frequency, and diet composition. The concentration of acetic acid in this experiment was determined to be (58.64-65.36 %molar), which was within the usual range. Similarly, to the current work, (Mensor et al., 2001), found that the rumen molar concentration of acetate was altered by dietary fat content. The development of AA is always accompanied by the generation of H_2 and CO_2, but the formation of PA necessitates the formation of H_2 as PA (Liu et al., 2016).

Propionic acid levels were increased in goats fed 6%PNF + 6%SFO at 2 and 4 h after feeding because Purple Neem foliage contains a high anthocyanin content, and anthocyanin may affect carbohydrate metabolism to provide greater energy for ruminants. Similarly, (Tian et al., 2019) claim that anthocyanins may be able to influence carbohydrate metabolism in order to supply more energy to ruminants by raising the fraction of PA. The synthesis of anthocyanin-rich PSS revealed a higher concentration of anthocyanin, which did not appear to be broken down in the rumen (Hosoda et al., 2009). Furthermore, the kind of VFA generated in the rumen is determined by the substrate fermented, the rumen environment, and

the microbial community (McGhie et al., 2003). Enhanced rumen propionic acid levels are associated with increased insulin secretion, fat deposition, and protein synthesis, while blocking lipolysis and protein breakdown (Zhao et al., 2018). According to the findings of this investigation, the propionic acid concentration in all treatments was within the optimal range of 18-20%. (Leng and Brett, 1966).

In this investigation, the butyric acid levels differed significantly among treatments at 2 and 4 h after feeding. Our findings reveal that goats given 6%PNF+ 6% SFO had greater butyric acid levels than other treatments. This study, similar (Tian et al., 2019) report that the addition of purple corn pigment or anthocyanin rich to the dairy goats increased the levels of butyric acid. It was demonstrated that flavonoid-rich plants might alter the kind of rumen fermentation in goats. This might be due to the fact that the hydroxyl groups in anthocyanins are the major groups responsible for inhibitory action and, as a result, may destroy bacterial cell membranes (Mattos et al., 2017). This might be because, despite the fact that anthocyanins are present, they do not appear to be broken down in the rumen (Hosoda et al., 2009).

The acetic acid: propionic acid ration shows a significant difference between treatments at 2 and 4 h after feeding. However, we discovered that goats given 6%PNF+6%SFO had a lower ratio of acetic acid: propionic acid compared to other treatments because Purple Neem foliage has lower NDF and ADF digestibility, which leads to an lower in the ratio of acetic acid: propionic acid. As a result, cell wall fiber fermentation boosted the AA:PA ratio in the Normal Corn treatment (Tian et al., 2019). Our findings are congruent with those of (Hosoda et al., 2012), They observed that lactating dairy cows fed anthocyanin-rich maize silage had a difference in ruminal fluid VFA production from those fed control silage.

Total VFA levels in this research demonstrate a significant difference between treatments at 2 and 4 h after feeding. Our findings show that goats given 6%PNF+6% SFO had greater total VFA than other treatments. It was demonstrated that flavonoid-rich plants might alter the kind of rumen fermentation in goats (Purba et al., 2020). As a result, feeding Purple Neem foliage altered the manner of VFA fermentation. VFAs are the primary sources of energy for ruminant metabolism. Anthocyanins may promote VFA generation by altering gut flora (Li et al., 2019). Our

findings are consistent with those of (Hosoda et al., 2009), who found that feeding purple corn pigment or anthocyanin-rich purple corn stover silage to dairy goats raised overall VFA levels. Furthermore, the kind of VFA generated in the rumen is determined by the substrate fermented, the rumen environment, and the microbial community (Mensor et al., 2001). This might be due to the fact that the hydroxyl groups in anthocyanins are the major groups responsible for inhibitory action and, as a result, may destroy bacterial cell membranes (Mattos et al., 2017).

4.6.4 Microbial population

The microbial community in the rumen in this experiment shows a significant difference at 2 and 4 h after feeding. In the current study, we discovered that goats fed a diet containing 6%PNF+6%SFO had a greater microbial population of total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, *Streptococcus bovis*, and reduce protozoa and methanogen compare with other treatment. It is worth noting that, while both additions impacted the general structure of the bacterial population in distinct ways, they had a comparable ruminal environment and fermentation in terms of fermentation acids. The effect of anthocyanin from Purple Neem foliage can improve ruminal cellulolytic activities by favoring the growth of cellulolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* because Purple Neem foliage appears to have anthocyanin content and the function of anthocyanin has been reported to modulate microorganisms in the gastrointestinal tract. Furthermore, the fermentation substrate, rumen environment, and microbial population are critical variables influencing the kind of rumen microbial population (Mattos et al., 2017). Our findings, which are consistent with those of (Ogunade et al., 2020), show that either PROB or SYNB enhanced the relative abundance of cellulolytic bacteria such as *Ruminococcaceae*, and that supplementation with mangosteen peel powder considerably boosted the cellulolytic bacteria population (Wanapat and Pimpa, 1999). Anthocyanin from Purple Neem foliage has been shown to suppress protozoal populations, increase bacteria and fungi populations, produce propionate, the partitioning factor, increase the yield and efficiency of microbial protein synthesis, and decrease methanogenesis, all of which improve ruminant performance. *Butyrivibrio fibrisolven*

is the group of proteolytic bacteria in this study that has higher in goats fed 6%PNF+ 6%SFO at 2 and 4 h after feeding because previous research has shown that plant extracts rich in flavonoids increase the degradation of cell wall constituents as well as the yield and efficiency of microbial protein synthesis (Sirohi et al., 2009). Indicated that anthocyanin is also known to influence both the composition and number of ruminal bacterial species through specific inhibition or selective enhancement of the growth of individual species. Thus, anthocyanins can influence rumen fermentation characteristics because they have substantial regulatory effects on ruminal bacteria, hence promoting gastrointestinal tract health (Prommachart et al., 2021). Furthermore, anthocyanins have potent antioxidant properties that can protect the organism from peroxidation damage, hence boosting rumen microbiota. As a result, anthocyanins can reduce heat stress and alter rumen microbiota, so boosting rumen health (Cardona et al., 2013). In the current study, goats fed a diet of 6%PNF + 6%SFO had lower protozoa and methanogen levels than other treatments because anthocyanin can reduce the number of ruminal ciliate protozoa and increase the flow of microbial protein from the rumen, increasing feed utilization efficiency and decreasing methanogenesis. Our findings were similar with the findings of (Wanapat and Pimpa, 1999), who found that mangosteen peel supplementation significantly reduced rumen protozoa generation while increasing the populations of the major cellulolytic bacteria and decreasing methanogen levels. Anthocyanins, as gut modifiers, may encourage the growth of beneficial anaerobic bacteria while inhibiting pathogenic bacteria (Igwe et al., 2019).

4.6.5 Antioxidant activity in plasma

Many fruits and other purple materials have a high antioxidant capacity due to the presence of naturally active polyphenol chemicals. Anthocyanins are a source of secondary plant metabolites that are powerful natural antioxidants and free radical (FR) scavengers, have a variety of important physiological roles for consumers, and have vast research and application potential (Zhao et al., 2022). As a result, anthocyanin can boost the activity of antioxidant enzymes such superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), which can further suppress FR in milk. In the current study, anthocyanin from Purple Neem had a significant difference on antioxidant activity in plasma of total antioxidant, SOD,

GPX, DPPH, CAT, and MDA at 2 and 4 h after feeding all treatments. However, this study found that goats fed 6%PNF+6%SFO had higher total antioxidant, SOD, GPX, DPPH, and CAT levels, possibly because Purple Neem foliage provided a high concentration of exogenous natural antioxidant, resulting in higher antioxidant activity and lower MDA levels in plasma when compared to other treatments. According to (Tian et al., 2022), the additional anthocyanin groups had greater levels of SOD, GSH-Px, and CAT than the no additional anthocyanin groups. According to (Dijkstra et al., 2005), the lack of substantial increases in antioxidant enzyme activity (SOD, GPx, SOD) might be explained by small ruminant absorption of anthocyanin. Furthermore, anthocyanins have high antioxidant activity DPPH is a kind of FR that is reduced in an aqueous solution containing an antioxidant (Mensor et al., 2001). Thus, anthocyanins in plasma can give electrons to DPPH, raising the degree of DPPH scavenging action (Toaldo et al., 2016). Previous research has found that goats given 6%PNF+6%SFO have higher DPPH levels in their plasma. Anthocyanin-rich foods can boost human blood antioxidant capacity. Because of their unique features, anthocyanins have been shown to inhibit lipid oxidation (Tian et al., 2020). The following mechanisms have been proposed for anthocyanins' inhibition of lipid metabolism: (1) anthocyanins can inhibit cholesterol synthesis by decreasing the gene expression of 3-hydroxy-3-methylglutaryl coenzyme A; (2) anthocyanins can reduce blood apo B–and apo C-III; and (3) anthocyanins can inhibit cholesteryl ester transfer protein (Liu et al., 2016). MDA levels in plasma were found to be lower in goats given 6%PNF+6%SFO in this investigation. According to (Chen et al., 2018), mice given black soybean seed coat extract (BSSCE), a rich source of anthocyanins, had a substantial drop in MDA concentrations as well as an increase in antioxidant enzyme (SOD, GPx, and catalase) activities. (Hosoda et al., 2012) found that feeding anthocyanin-rich corn silage to dairy cows reduced plasma MDA concentrations.

4.6.6 Antioxidant gene expression in *longissimus dorsi*

This study on antioxidant gene expression in *longissimus dorsi* found that anthocyanin from Purple Neem foliage had a significant different on GADPH, GPX, CAT, and SOD in meat goats. Because anthocyanins are natural antioxidants found in many plants. The anthocyanin is a kind of water-soluble phenolic chemical (Tian et al., 2019). A previous study found that adding anthocyanin-containing feed

to ruminant feed influenced meat quality (Colombo et al., 2021). (Love and Pearson, 1971) demonstrate that feeding purple maize anthocyanins to tiny ruminants can considerably improve antioxidant activity. Furthermore, anthocyanins have the potential to influence gene and protein expression, hence avoiding lipid oxidation and enhancing volatile components (Vasta et al., 2019). This is due to the fact that anthocyanins utilized as FR terminators contribute hydrogen to the FRs, halting oxidation events and improving meat compounds (Ladikos and Lougovois, 1990). Thus, feeding anthocyanin derived from 6%PNF+6%SFO leads in increased levels of GADPH, GPX, CAT, and SOD in *longissimus dorsi*. It may be stated that no muscle injury has occurred as a result of their actions. SOD, GPx, and catalase are all antioxidant enzymes that assist maintain a healthy cellular antioxidant state, which might explain why tiny ruminants absorb more anthocyanin.

4.6.7 Carcass and Meat Characteristics

This study found that anthocyanin from Purple Neem foliage had no influence on carcass parameters such as carcass weight, skin, white offal, and red offal regardless of treatment. However, goats fed 6%PNF+6%SFO had higher slaughter weight and lower carcass (%). Because anthocyanin's function might increase the rate of meat production. Our findings are consistent with the findings of (Duan et al., 2019), who found that melatonin treatment had no effect on carcass characteristics in these cashmere goats.

The pH value is connected to pre-slaughtering, which can also affect meat texture and color (Ponnampalam et al., 2017). The influence of anthocyanin from Purple Neem foliage on pH at 1 and 24 h was not observed to be significant, however our pH readings were 5.71-5.84. According to the findings of our study, the pH value is within the normal range and has no effect on meat quality, such as color or shelf life. Previous research has indicated that pH values of meat from cashmere goats in the current study (range 5.83 to 6.54) are indicative of high carcass quality (Liu et al., 2016) and (Zhao et al., 2018) demonstrate that the influence of dietary grape pomace had no effect on pH value among different groups. The pH, on the other hand, is determined by the species of animal, as well as the age of slaughter and storage duration.

Meat color has been recognized as the most important feature when customers evaluate meat quality since color is associated with freshness. Meat color in this study shows that anthocyanin from Purple Neem foliage has a tendency to reduce meat color (a*) tends to brown the meat, implying that the oxymyoglobin to metmyoglobin conversion stage and the lipid peroxidation interaction were engaged in meat discoloration (Tian et al., 2021a). Our findings are consistent with those of (Zhao et al., 2018), who found that dietary grape pomace had no effect on meat color coordinates (L*, a*, and b*) because anthocyanin, a dietary antioxidant, can be better incorporated into cellular membranes containing oxidation susceptible phospholipids than the superficial contact made by antioxidants added postmortem. The color of meat is determined by a variety of individual elements and their interactions; however, chevon has been observed to be lighter in color and more redness than lamb, owing to the decreased intramuscular fat content of goat carcasses (Babiker et al., 1990).

In the current experiment, the effect of anthocyanin from Purple Neem foliage on drip loss and cooking loss was considerable across all treatments. However, drip loss and cooking loss were decreased in goats given 6%PNF+6%SFO compared to other treatments. Cooking loss, according to (Trout, 1988), is more reliant on the final pH and the cooking condition. Similar to (Babiker et al., 1990), our investigation indicated that goat muscles had lower cooking loss than lamb muscles. According to the current study, goats fed 6%PNF+6% SFO had decreased drip loss and cooking loss. Juiciness of meat is strongly connected to intramuscular lipids and moisture content of the meat, and lean meat contains around 75% water (Karami et al., 2011). It concludes that anthocyanin is a natural antioxidant that may be transferred to the muscle where, in conjunction with the native defense system, it can counteract the action of pro-oxidants and protect meat water loss, quality, and nutritious value of goat meat.

Shear force value of muscle can be influenced by many factors, including nutrition, stress prior to and at slaughter, age and breed of animal, type of muscle, and cooking operation. It has been reported that the change in collagen fiber arrangement in connective tissue, as well as the decrease in soluble collagen content during animal growth, are important factors influencing shear force value

(Sekhon and Bawa, 1996). Tenderness of goat meat is frequently acceptable to consumers (Webb et al., 2005). Shear force was shown to be significantly varied across all treatments in this investigation. However, according to the current study, goat fed 6%PNF+6%SFO has lower shear force compared to other treatments because anthocyanin is a natural phenolic compound with health-improving properties, such as their potential as a viable alternative to antibiotics and synthetic growth promoters in the production of sustainable animal feed can act as an antioxidant and has been shown to be instrumental in preventing the oxidation of milk and meat (Lazalde-Cruz et al., 2021). Nonetheless, dietary anthocyanin bioactive compounds obtained from Purple Neem foliage may result in a decrease in shear force by enhancing juiciness and meat softness by reducing cooking loss and drip loss. Similar to the findings of (Chuntang, 2020), treatment with wine grape pomace reduced shear force in the lamb's *longissimus dorsi* muscle. The chemical composition of the goat meat in this investigation was within the range of previously published values (Manso et al., 2011) and, as predicted, was unaffected by dietary treatments. Our findings are consistent with those of (Morán et al., 2012), who found no influence on the protein, fat, and moisture content of suckling lamb meat. Nieto (2013) discovered no alterations in muscular lamb chemical composition when thyme and rosemary were added to sheep diets.

4.6.8 Fatty acid profile

Plant polyphenols can change the composition of fatty acids by reducing oxidation processes in unsaturated fatty acids (Cao et al., 2012). Plant polyphenolic flavonoids have been shown to protect UFA against oxidants and to stimulate antioxidant response element (ARE) mediated gene expression (Chen et al., 2000). In the current study, goats fed a diet of 6%PNF+6%SFO lowered the percentage of saturated fatty acids in goat meat because anthocyanin inhibited the process of biohydrogenation in the rumen. (Purba et al., 2020) discovered that include flavonoid-rich plants in the diet of dairy goats might reduce milk individual SFA and total SFA concentrations while increasing the UFA profile. In contrast, the content of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) was higher in goats fed a diet containing 6%PNF+6%SFO. Consistent with our findings, (Bryszak et al., 2019) proposed that a phenolic-rich plant extract increased

MUFA and PUFA concentrations in the longissimus dorsi of crossbred young kids. Previous research (Ahmed et al., 2016) demonstrated that fermented herbs alter the makeup of fatty acids by decreasing SFA levels and boosting PUFA concentrations. Characterizing the individual and combined impacts of particular phytochemicals in Purple Neem foliage with anthocyanin concentration will have biohydrogenation products that might assist increase the quantities of human-health-promoting PUFAs in ruminant meat. (Purba et al., 2020) showed that PUFA chevon is more than meat that rapidly oxidizes but can be very important in human nutrition. Furthermore, polyphenols might modulate rumen PUFA biohydrogenation to increase lipid fraction by lowering rumen skatole production, increasing the beneficial fatty acid content, and raising the product's oxidation stability (Correddu et al., 2020).

4.7 Conclusion

This study, to investigate effect of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats. The current study results indicated that goats fed 6%PNF + 6%SFO had the potential to improve DMI, nutrients apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, antioxidant activity in plasma, antioxidant gene expression in *longissimus dorsi*, carcass, meat characteristics and fatty acid profile in goat meat. In addition, it was found that high total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises* and *Streptococcus bovis* respectively and low protozoa and methane production. Whereas it did improve the DPPH scavenging activity and SOD activity, TAC, CAT, GPX and reduce MDA in the plasma of growing goats. That antioxidants in the feed of goats improve antioxidant gene expression in *longissimus dorsi* leading to improve meat quality and fatty acid profile in goat meat.

4.8 References

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CHAPTER V

Experiment III

Effect of anthocyanin from Purple Neem foliage on antioxidant gene expression in meat and meat quality in growing goats

5.1 Abstract

The objective of this experiment was to investigate the effect of anthocyanin from Purple Neem foliage on antioxidant gene expression in *longissimus dorsi* and meat quality in growing goats. Twenty-five Boer crossbreed male goats (approximately 20±2 kg body weight; mean±standard deviation (SD)) were assigned to a completely randomized design. All goats received a 60-d routine feeding consisting of five treatments: (1) control, (2) 6%Purple neem foliage (PNF) in concentrate, (3) 6%Purple neem foliage (PNF)+Polyethylene glycol PEG in concentrate, (4) 6%Purple neem foliage (PNF)+Purple Neem pigment (PNP) in concentrate and (5) 6%Purple neem foliage (PNF)+Purple Neem pigment (PNP)+Polyethylene glycol (PEG) in concentrate.

The results show that feed goats fed a concentrate containing 6%PNF+PNP+PEG had higher (P<0.05) feed intake gDM/d, %BW, g/kgBW$^{0.75}$, nutrient intake, nutrient digestion, final weight, weigh change, ADG, N intake, N Urine, N digestion, N digestion (%), N retention, and N retention (%). In comparison to the feed goat at 6%PNF in concentrate, 6% PNF+PEG in concentrate, 6% PNF+PNP in concentrate, and the control treatment.

Ammonia nitrogen, BUN, acetic acid, propionic acid, acetic acid to propionic acid ratio, butyric acid, and total VFA levels were greater (P<0.05) in the fed goat at 6%PNF+PNP+PEG in concentrate at 2 and 4 h after feeding. At 2 and 4 h after feeding, individual microbial populations with 6%PNF+PNP+PEG in concentrate showed greater (P<0.05) total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, *Streptococcus bovis*, and lower protozoa and methanogen. Antioxidant indices in plasma goats fed 6%PNF +PNP+PEG showed higher Total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase

(CAT) antioxidant activity in plasma and antioxidant gene expression in *longissimus dorsi* of GADPH, GPX, CAT, SOD, and reduce malondialdehyde (MDA) in plasma at 2 and 4 h after feeding. When compared to goat meat feed from the other treatment, goat meat from the 6%PNF+PNP+PEG had reduced (P<0.05) drip loss, cooking loss, shear force, and greater monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Feeding anthocyanin from Purple Neem foliage looks to be an appealing option to other local anthocyanin sources in meat goat diet. According to the present findings, feeding 6% Purple Neem foliage+Purple Neem pigment+ Polyethylene glycol alleviates oxidative stress and stimulates meat production, implying that the meat is better for customers.

5.2 Introduction

Anthocyanins are water-soluble plant pigments found in the flesh, skin, and roots of many different colored fruits and vegetables, including berries, plums, and berries (Speer et al., 2020). Temperature, pH, light, oxygen, and enzymes are all known to destroy anthocyanin molecules (Serra et al., 2021). Clinical investigations have recently revealed that anthocyanin intake is connected with a lower risk of chronic diseases such as cancer, obesity, diabetes, and cardiovascular disease.

Growing interest in supplementing animal diets with plant antioxidants to increase the nutritional content of meat for the health advantages of consumers (Serra et al., 2021). In Africa and Asia, goat meat is widely consumed (Ali et al., 2019). Although interest in goat meat differs per community, goat meat has carved itself a place as a popular choice for ethnic cuisine consumers (Ivanović et al., 2020). Due to rising customer demand, Thailand has increased its production of goat meat in recent years. (Wasiksiri et al., 2010) Production and consumption of goat meat has also spread to other niche markets in the Middle East and Asia. Feed additives are becoming more popular due to their purported antioxidant activity and positive impacts on farm animal performance and animal product quality.

Purple neem foliage high in anthocyanins is increasingly being employed as a feed ingredient in ruminant diets to attenuate oxidative stress damage, demonstrate antioxidant activity, and enhance meat quality. The oxidative processes that occur

during the conversion of muscle to meat, meat processing, or storage are one of the primary reasons of meat degradation. However, feeding purple neem foliage rich in anthocyanin improves meat quality and shelf-life stability (Lazalde-Cruz et al., 2021). In this study were to study effect of anthocyanin from purple neem foliage on antioxidant activity and meat quality in growing goats.

5.3 Objective

To study effect of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats.

5.4 Material and methods

5.4.1 Experimental design, animal diets and managements

The experiment was conducted to recommending by Animal Care and use committee of Suranaree University of Technology, Suranaree University of Technology, Nakhon Ratchasima province, Thailand.

The treatments were completely randomized design. This study has 5 treatments that were consisted T1, control; T2, 6% Purple neem foliage (PNF) in concentrate; T3, 6% Purple neem foliage (PNF)+Polyethylene glycol (PEG); T4, 6% Purple neem foliage (PNF)+Purple Neem pigment (PNP) and T5, 6% Purple neem foliage (PNF)+Purple Neem pigment (PNP)+Polyethylene glycol (PEG).

Twenty-five Boer crossbreed male goats were randomly assigned according to completely randomized design to receive 5 dietary treatments. All goats were kept in the individual feeding pens during the 60 days of experimental period. Mineral blocks and clean water were available ad libitum offered for all animals.

During the 28 days of experimental period goats were fed with 1.5 % of BW DM/day containing Pangola (*Digitaria eriantha*) hay and (16 % crude protein) at the ratio of 60:40. All goats were received feeds and supplemented with their respective treatment. Goats were feed in the morning and afternoon at approximately (07.00 and 16.00). During the adjusting period (14 days before starting each experimental period), all animal were fed with the same feed. Moreover, nutrient composition experimental diets were shown in Table 5.1.

Table 5.1 Feed ingredients and chemical composition of experimental diets.

Items	Pangola hay	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG
				Diets		
Ingredients (% air-dry basis)						
Soybean meal	-	18.00	15.00	17.00	16.00	16.00
Rice bran	-	32.00	25.00	21.00	22.00	22.00
Cassava chip	-	28.00	29.10	33.10	33.10	32.10
Corn	-	20.40	23.30	20.30	20.30	20.30
Salt	-	0.40	0.40	0.40	0.40	0.40
Limestone	-	0.20	0.20	0.20	0.20	0.20
Premix	-	1.00	1.00	1.00	1.00	1.00
Purple Neem	-	0.00	6.00	6.00	6.00	6.00
Purple Neem pigment (PNP)	-	0.00	0.00	0.00	1.00	1.00
Polyethylene glycol (PEG)	-	0.00	0.00	1.00	0.00	1.00
Chemical composition (% DM)						
Dry matter	86.72	80.42	80.26	81.25	83.04	80.99
OM	93.52	93.81	93.48	93.09	93.96	93.94
Ash	6.48	6.19	6.52	6.91	6.04	6.06
Crude protein	5.91	16.17	16.27	16.35	16.72	16.74
Ether extract	1.66	5.96	4.63	5.73	4.38	4.43
Non-fibrous carbohydrate	5.55	3.29	7.06	5.49	11.64	12.11
Neutral detergent fiber	80.40	68.39	65.52	65.52	61.22	60.66
Acid detergent fiber	49.56	44.86	34.93	32.57	37.73	35.93
%TDN	55.00	87.76	86.76	85.96	86.46	85.40
Metabolizable energy, Mcal/kg DM	1.99	3.17	3.14	3.11	3.13	3.09

Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g iron; 40 g zinc; 40 g manganese; 0.1 g cobalt; 10 g copper; 0.1 g selenium; 0.5 g iodine; Calculated as: NFC = 100−(%NDF+%CP+%EE+% ash); Estimated by the equation TDN = (%) DCP+DNFC)+DEE×2.25+(DNDF); Estimated by the equation ME (Mcal/kg DM) = (TDN×0.04409×0.82), PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol.

Table 5.2 Fatty acid composition of experimental diets.

Fatty Acids, g/100 g of Total Fatty Acids	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP +PEG	SEM	p-value
Palmitic C16:0	10.16[e]	11.15[d]	11.25[c]	12.15[b]	13.17[a]	0.21	0.01
Palmitoleic C16:1	1.13[e]	1.24[d]	1.36[c]	1.47[b]	1.49[a]	0.03	0.01
Stearic C18:0	2.15[c]	2.55[b]	2.56[b]	2.66[a]	2.69[a]	0.04	0.01
Oleic C18:1	25.69[d]	26.50[c]	26.85[b]	27.50[a]	27.65[a]	0.15	0.01
Linoleic C18:2 n-6	26.50[d]	27.15[c]	27.25[c]	28.35[b]	28.55[a]	0.16	0.01
α-Linolenic C18:3 n-3	1.56	1.58	1.59	1.70	1.77	0.05	0.63
Total saturated fatty acids	12.31[e]	13.70[d]	13.81[c]	14.81[b]	15.86[a]	0.24	0.01
Total monounsaturated fatty acids	26.82[d]	27.74[c]	28.21[b]	28.97[a]	29.14[a]	0.18	0.01
Total polyunsaturated fatty acids	28.06[e]	28.73[d]	28.84[c]	30.05[b]	30.32[a]	0.84	0.01
n-6: n-3 fatty acid ratio	17.42	17.64	17.61	16.97	16.13	0.5	0.89

PNF : Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05),

5.4.2 Sample collection

The experimental periods were used 28 day a period which 14 days for adjusted the animal and digestive tract. Samples of offered and refused diets were collected every day, while urine and feces samples were collected by total collection from each individual goat during the last 7 days of experimental period. Samples of offered and refused diets and feces were oven dried (60 °C) and ground through a 1.0 mm sieve prior to analysis for DM, ash, ether extract (EE) and crude protein (CP) (AOAC, 2005) and NDF and ADF (Van Soest et al., 1991). Total urine excretion were collected and acidified using 20% H_2SO_4 solution prior to analyses for ammonia nitrogen concentration using kjeldahl method according to AOAC (2005).

Ruminal fluid will be sampled on last day of experiment using a stomach tube connected with vacuum pump at 0, 2 and 4 h-post feeding. Rumen fluid samples were immediately measured for pH using a portable pH Meter, and then filtrated through 4 layers of cheesecloth under anaerobic conditions. Ruminal fluid sample were then divided into three portions; the first portion were used for community DNA extraction for estimation of microbial populations with the same procedures as described in the Experiment-1, the second portion of ruminal fluid sample were used for volatile fatty acid and VFA analysis by the methods as described in the Experiment-1 and the third portion of ruminal fluid sample were used for NH_3-N measurement using Kjeldahl method according to AOAC (2005).

With the same frequency of ruminal fluid sampling, blood samples were be taken from jugular vein and immediately placed on ice and brought back to the laboratory and refrigerated at -20°C for 1 h. Blood plasma were then separated by centrifuge at 3,500×g for 20 min, then plasma were collected and stored at -20°C for further analysis of blood urea nitrogen (BUN) according to Crocker (1967).

5.4.3 Slaughter procedures

At the end of the experiment three goats from each group were selected ran-domly for slaughter after overnight fasting. After obtaining the slaughter live weight these goats were slaughtered. After bleeding the head, skin, feet, lung and trachea, liver, heat, spleen, gastrointestinal tract and testicles were removed and hot carcass was obtained. The hot carcass was weighed, and weights of the skin and

limbs, liver, kidneys and heart were recorded and expressed as a percentage of slaughter weight. Approximately 30 g samples were taken from: the longissimus dorsi (SD) of the carcass. These samples were immediately frozen in liquid nitrogen and stored at −80°C for antioxidant indicator analyses and RNA extraction and were used to analyses parameters of meat quality.

5.4.4 Analysis of antioxidant activity enzymes in plasma

The TAC in the plasma was determined using a commercially available kit (Catalog Number MAK187-1KT, Sigma-Aldrich, Product of USA), according to manufacturers' protocol with a colorimetric probe, giving a broad absorbance peak at 570 nm, which is proportional to the total antioxidant capacity.

The activity of SOD enzyme was measured according to a commercial assay kit (PCode 101869510, 19160-1KT-F, Sigma-Aldrich, Product of Japan). The Kit-WST allows very convenient SOD assaying by utilizing a water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. Absorbance was measured at 440 nm using a microplate reader (Epoch, BioTek, Luzern, Switzerland). Calculation the SOD activity (inhibition rate %) was done using the following equation.

$$SOD \text{ activity (inhibition rate \%)} = \{[(Ablank\ 1 - Ablank\ 3) - (Asample - Ablank\ 2)]/ (Ablank\ 1 - Ablank\ 3)\} \times 100.$$

Glutathione Peroxidase (GPX) enzyme activity was determined by an indirect method, using the Sigma Glutathione Peroxidase Cellular Activity Assay kit (Catalog number: CGP1-1KT, Sigma-Aldrich, Product of Israel). The absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPX activity, since GPX is the rate limiting factor of the coupled reactions. The GPX positive control was purchased glutathione peroxidase from erythrocytes (Catalog number: G6137-100UN, Sigma-Aldrich, Product of USA).

The catalase (CAT) enzyme activity was determined by a colorimetric assay, using a commercial kit (Pcode 1002565186, CAT100-1KT, Sigma-Aldrich, Product of Israel). This assay method is based on the measurement of the hydrogen

peroxide substrate remaining after the action of catalase. The colorimetric method uses a substituted phenol (3,5-dichloro-2-hydroxybenzenesulfonic acid), which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to give a red quinoneimine dye (N-(4-antipyryl)-3-chloro-5-sulfonatep-benzoquinone-monoimine) that absorbs at 520 nm.

The DPPH scavenging activity in the plasma was assayed spectrophotometrically according to Wei and Chiang (2009), using a stable free radical DPPH (Sigma-Aldrich, Pcode: 101845869) with a slight modification. Briefly, 50 µL of each sample was added to 1 mL of methanolic solution of DPPH reagent (25 µmol/L) in a 1.5 mL tube. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min, and then centrifuged at 4000 r/min for 10 min at 4°C. 200 µL of the supernatant was transferred to a 96-well plate immediately and the absorbance was detected at 517 nm via a microplate reader (Epoch, BioTek, Luzern, Switzerland). DPPH scavenging activity was calculated by the following formula:

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

where Ac is the absorbance of the control, and as is the absorbance of the sample.

The malondialdehyde (MDA) enzyme activity was determined by a colorimetric assay, using a commercial kit (Sigma-Aldrich, catalog number MAK085). All samples and standards should be run in duplicate. Use ultrapure water for the preparation of all standards and samples. Sample Preparation use plasma samples (20 µL) should be gently mixed with 500 µL of 42 mM sulfuric acid in a microcentrifuge tube. Add 125 µL of Phosphotungstic acid Solution and mix by vortexing. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000×g for 3 minutes. In a separate tube, add 2 µL of BHT (100x) to 100 µL of water. Resuspend the pellet on ice with the water/BHT solution. Adjust the volume to 200 µL with water.In this kit, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present. For Plasma samples, mix with 300 µL of 1-

butanol and 100 µL of 5 M NaCl with each reaction mixture. Vortex and then centrifuge for 3 minutes at 16,000 × g at room temperature. Transfer the 1-butanol layer (the top layer) to a new centrifuge tube and remove the 1-butanol. The 1-butanol can be removed either by freeze-drying, or heating on a hot block at 55 C. Resuspend the remaining material in 200 µL of ultrapure water. Mix well and add 200 µL into a 96 well plate and the absorbance was detected at absorbance at 532 nm (A532). Malondialdehyde was calculated by the following formula: Concentration of MDA for samples without 1-butanol concentration step:

$$(Sa/Sv) \times D = C$$

Sa, Amount of MDA in unknown sample (nmole) from standard curve; Sv, Sample volume (mL) or amount (mg) added into the wells; C, Concentration of MDA in sample; D, Sample dilution factor (if applicable)

Sample Calculation:

Amount of MDA (Sa) = 5.84 nmole; Sample volume (Sv) = 0.020 mL; Concentration of MDA in sample; (5.84 nmole/0.020 mL) × 1 = 292 nmole/mL

5.4.5 Analysis gene expression of *longissimus dorsi* tissue

Total RNA was extracted from *longissimus dorsi* tissue using TRIzol Reagent method according to (Li et al., 2009; Milne et al., 2009), with a minor modification. Briefly, the sample was transferred into 2 mL tube, and added 1 mL TRIzol Reagent (Thermo Fisher Scientific, USA) and 0.1 g of the 3 mm dia. Zirconia/silica (Cat. No. 11079105z, BioSpec Products) and run in a homogenizer. The total RNA was dissolved with DEPC water (Ambion by Life Technologies, made in USA) and was DNase digested the RNase-Free DNase. After that, purity of the extracted RNA was analyzed by assaying the 260/280 absorbance ratio, using a Nano value spectrophotometer (GE Healthcare Bio-Sciences, Pittsburgh, United States). Integrity of the extracted RNA was assessed by verifying the presence of 18S and 28S RNA bands, using an Image Quant LAS 500 imager (GE Healthcare Bio-Sciences, Pittsburgh, United States) electrophoresis on a 2% agarose gel. Complementary DNA

(cDNA) synthesis was performed as described by ImProm-IITM Reverse Transcription System (Promega Corporation, Madison, USA).

All samples were assayed using quantitative real-time Polymerase Chain Reaction (real-time PCR) amplification by a Roche LightCycler® 480 real-time PCR System (Roche Diagnostics GmbH, Penzberg, Germany). Five target genes (TG) were: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase. Nucleotide sequences obtained from the National Center for Biotechnology Information (NCBI) and the primers set for all genes were designed using GenScript real-time PCR (TaqMan) Primer Design tool and were synthesized by the Bio Basic Inc. Company (Table 6.1). The real-time PCR amplifications were performed in a 10 µL reaction volume, which consisted of 5 µL of 2× Roche 04707516001 LightCycler® 480 SYBR Green I Master (Roche Diagnostics GmbH, Mannheim, Germany), 2 µL of 10×diluted cDNA, and 1 µL of forward (500 nM) and reverse (500 nM) primers, respectively. After that, the plates (LightCycler®480multiwell plate 96, white; Roche Diagnostics GmbH, Mannheim, Germany) were centrifuged at 4℃, 1500 rpm for 3 min (Universal 320, Hettich Zentrifugen, Germany). The program uses for the amplification of genes consisted of denaturing cycle of 10 min at 95℃, followed by 40 cycles (95℃ for 15 s, annealing at 60℃ for 30 s and extension at 72℃ for 30 s) and a dissociation step consisting of 95℃ for 15 s, 60℃ for 15 s and 95℃ for 15 s (annealing temperature optimized depending on primers), and cooling at 40℃ for 30 s. At the end of PCR, melt-curve analyses were performed for all genes. Amplifications were performed in triplicate for each gene.

5.4.6 Meat quality analysis

pH measurement

The pH value of the *longissimus dorsi* was measured using a portable pH-meter (testo-pH2, Germany) at 1 and 24 h post-mortem, the pH meter was recalibrated after every six samples.

Color measurement

The color of the *longissimus dorsi* was assessed on the cut surface using a Minolta Chromameter (Minolta CR-400, Tokyo, Japan) with D65 illuminant and two-degree standard observer. The results were expressed in terms of L* (lightness), a* (redness) and b* (yellowness) in the CIELAB color space model (CIE, 1976).

Drip losses

Drip losses of the meat in this study according to the protocol followed by (Honikel et al., 1998). Meat pieces (2.5 cm thick) were weighed, placed in a container equipped with a plastic bag and lid, suspended in 24 h at 4℃, and then the sample was reweighed. The drip loss was calculated as the percentage ratio of the initial weight using the following formula: Drip loss = (W1–W2)/W1×100%

Cooking losses

Each sample of the *longissimus dorsi* was weighed, placed in a vacuum sealed plastic bag and cooked for 1 h in a water bath maintained at 80℃ (Ramírez et al., 2004). After cooking, the samples were removed from the water bath, and then cooled under running water for 30 min the sample was then taken from the bag, blotted dry and weighed. The cooking losses were calculated as a percentage of the initial weight, according to the following formula: Cooking loss = (M1–M2)/W1 100%

Shear force

Shear force was measured using the method of Warner Bratzler shear force at 24 hr post-mortem. The sample of *longissimus dorsi*, cooked as described above, was cut into rectangular cross-section strips (3×1.5×1.5 cm). All pieces were sheared using an Instron 5543 equipped with a Warner–Bratzler shear device and crosshead speed set at 200 mm/ min (Lorenzen et al., 2010). The sheer force of each sample was measured three times and recorded in Newtons (N).

5.4.7   Statistical analyses

All results were analyzed as a completely randomized design (CRD) design using the general linear model procedure of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC, USA). Differences between treatments means will be determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Data obtain from meat quality were subjected to general linear model procedure of SAS. The relative mRNA abundance was calculated averaged abundance of the gene in data was considered as the calibrator, and the data was analyzed by ANOVA procedure. Data for physicochemical properties were analyzed using the PROC MIXED procedure of SAS. The level of significance difference at ($P<0.05$) will be accepted as representing statistically significant differences. The statistical model for the analysis of data was:

$$X_{ij} = \mu + B_j + \varepsilon_{ij}$$

where: where: where: X_{ij}, the observed value of treatment j, replication I, μ = the overall mean, B_j, Influence of treatment at j = $\mu_j - \mu$ and ε_{ij}, tolerance of the experimental unit at ij

5.4.8 Experimental places

The Suranaree University of Technology part of Goat and Sheep farm and the center of Scientific and Technology Equipment Building 3 10 11 and 14, where were used for field experiment and laboratory, respectively.

5.5 Results

5.5.1 Feed intake, nutrient intake, nutrient digestion and growth performance

Table 5.3 shows that the use of Purple Neem foliage has a significant difference (P<0.05) on feed intake. Goats fed 6%PNF+PNP PEG had significantly higher (P<0.05) gDM/d, %BW, and g/kgBW$^{0.75}$ values than other treatments. As a result, there was a significant difference in the interaction between cultivars' nutritional consumption levels (Table 5.2). When compared to other treatments, goats receiving 6%PNF+PNP+PEG had significantly higher (P 0.05) OM, CP and EE respectively.

As shown in Table 5.4, the apparent Digestibility differs significantly (P<0.05). Nutrient digestion of DM, OM, CP and EE was highest (P<0.05) in goats fed 6%PNF+PNP+PEG compared to 6%PNF+PNP, 6%PNF, 6%PNF+PEG, and control group.

The result is shown in Table 5.5. There were significant differences in the interaction of cultivars×levels of final weight, weight change, and average daily growth (ADG). The goats fed 6%PNF+PNP+PEG had a significantly higher (P<0.05) final weight, weight change, and average daily gain than the other treatments.

Table 5.3 Effect of anthocyanin from Purple Neem foliage on feed intake of growing goat.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
			Feed intake				
gDM/d	746.59[e]	851.70[d]	1056.92[c]	1279.17[b]	1355.55[a]	48.14	0.01
%BW	3.05[e]	3.15[d]	3.26[c]	3.35[b]	3.44[a]	0.03	0.01
g/kgBW$^{0.75}$	51.40[d]	62.30[c]	62.39[c]	69.94[b]	71.72[a]	1.47	0.01
			Nutrient intake g DM/d				
OMI	701.80[e]	792.08[d]	982.94[c]	1202.41[b]	1274.22[a]	45.57	0.01
CPI	119.45[e]	136.27[d]	169.11[c]	217.46[b]	230.44[a]	8.91	0.01
EEI	44.79[d]	42.58[e]	63.41[a]	51.17[c]	54.22[b]	1.52	0.01
NDFI	507.68[e]	562.12[d]	697.57[c]	780.29[b]	826.89[a]	25.14	0.01
ADFI	335.97[c]	298.10[d]	348.79[b]	486.08[a]	488.00[a]	16.34	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), g DM/d: daily intake of dry matter% BW: edible quantity per body weight per day g/kgBW$^{0.75}$: daily consumption per daily metric weight, SEM: standard error of the mean.

Table 5.4 Effect of anthocyanin from Purple Neem foliage on nutrient digestion in growing goats.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
			Apparent Digestibility, % of intake				
DDM	50.90^e	53.92^d	57.48^c	60.68^b	63.07^a	0.91	0.01
DOM	51.52^e	54.40^d	58.00^c	61.40^b	64.57^a	0.96	0.01
DCP	56.51^e	65.15^d	76.86^c	82.70^b	86.47^a	2.29	0.01
DEE	44.63^e	56.40^d	58.61^c	63.05^b	65.80^a	1.51	0.01
DNDF	84.29^a	74.59^b	67.58^c	54.46^d	46.85^e	2.77	0.01
DADF	75.65^a	63.45^b	56.17^c	52.41^d	42.94^e	2.26	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a b c d = in the same row, there is a statistically significant difference (P<0.05), DDM: digestibility of dry matter, DOM: digestibility of organic matter, DCP: protein digestibility, DEE: fat digestibility, DEE: digestibility fatty digestibility, DNDF: the digestibility of the crude fiber that cannot be dissolved in neutral solution, DADF: the digestibility of the fiber that cannot be digested in an acidic solution, SEM: standard error of the mean.

Table 5.5 Effect of anthocyanin from Purple Neem foliage on performance in growing goats.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
			Body weight				
Initial weight, kg	20.14	20.40	20.40	20.80	20.89	0.14	0.43
Final weight, kg	31.03[e]	32.10[d]	33.60[c]	34.45[b]	35.25[a]	0.32	0.01
Weigh change, kg	10.89[e]	11.70[d]	13.20[c]	13.65[b]	14.36[a]	0.29	0.01
ADG, g/d	170.63[e]	192.03[d]	231.07[c]	223.13[b]	236.23[a]	4.77	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a b c d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.
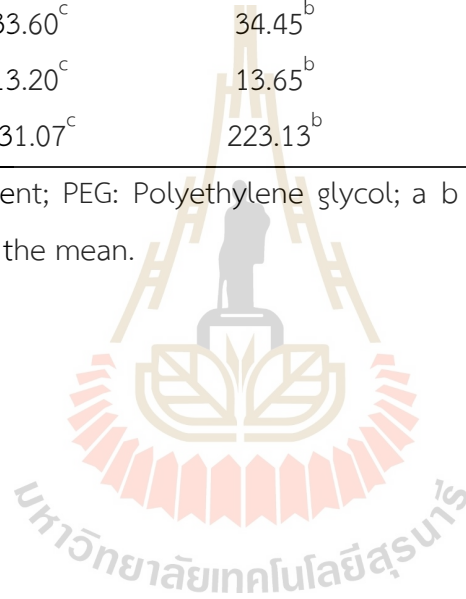
5.5.2   Nitrogen utilization

As a result, in Table 5.6, there was a significant difference (P<0.05) in nitrogen utilization characteristics. Goats fed 6%PNF+PNP+PEG had a significant difference in N intake, N urine, N digestion, N digestion (%), N retention, and N retention (%) (P<0.05). However, N feces were significantly lower (P<0.05) in goats fed 6%PNF+PNP+PEG compared to other treatments.

5.5.3   Rumen fermentation parameter

Not significant differences (P>0.05) were observed in the pH among treatment (Table 5.7). Ammonia nitrogen (NH$_3$-N) and blood urea nitrogen (BUN) levels differed significantly (P<0.05). Goats fed 6%PNF+PNP+PEG showed a higher (P <0.05) level of ammonia nitrogen and blood urea nitrogen after feeding at 2 and 4 hours than those fed the other treatment.

There was significant different (P<0.05) in the acetic acid, propionic acid, butyric acid, Acetic acid: Propionic and total VFA after feeding at 2 and 4 h for five treatments in all experimental (Table 5.8). After feeding at 2 and 4 h, goats receiving 6%PNF+PNP+PEG had the highest (P<0.05) levels of acetic acid, propionic acid, butyric acid and total VFA.

5.5.4   Microbial population

Table 5.9 shows the effects of Purple Neem foliage on microbial populations. Total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* all showed significant differences (P<0.05). After 2 and 4 h of feeding, goats fed 6%PNF+ PNP+PEG had the highest (P<0.05) levels of Total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* than other treatments. When compared to the other groups, goats given 6%PNF+PNP+PEG had significantly reduced (P<0.05) levels of protozoa and methanogen after 2 and 4 h of feeding.

Table 5.6 Effect of anthocyanin from Purple Neem foliage on nitrogen utilization in growing goats.

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP +PEG	SEM	p-value
N intake	8.57[e]	10.60[d]	12.52[c]	13.29[b]	14.75[a]	0.44	0.01
N Faces	6.59[a]	4.54[b]	3.55[c]	2.69[d]	1.90[e]	0.33	0.01
N Urine	0.13[e]	0.18[d]	0.19[c]	0.25[b]	0.29[a]	0.01	0.01
N digestion	3.83[e]	4.45[d]	5.47[c]	7.66[b]	8.38[a]	0.37	0.01
N digestion (%)	56.51[e]	65.15[d]	76.86[c]	82.70[b]	86.47[a]	2.29	0.01
N retention	2.75[e]	3.89[d]	4.54[c]	5.81[b]	6.33[a]	0.26	0.01
N retention (%)	45.03[e]	54.66[d]	67.42[c]	73.64[b]	84.45[a]	2.84	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a b c d = in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean

Table 5.7 Effect of anthocyanin from Purple Neem foliage on pH, ammonia nitrogen and blood urea nitrogen in growing goats.

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
pH							
0-H	6.92	6.93	6.95	6.96	6.97	0.01	0.34
2-H	6.75	6.77	6.76	6.78	6.79	0.01	0.16
4-H	6.80	6.81	6.83	6.84	6.85	0.01	0.10
Mean	6.82	6.84	6.85	6.86	6.87	0.01	0.11
Ammonia nitrogen mg/dl							
0-H	13.51	13.37	13.54	13.49	13.35	0.05	0.73
2-H	14.49^d	15.50^c	15.57^c	16.61^b	17.43^a	0.21	0.01
4-H	15.57^d	16.49^c	16.57^c	17.49^b	18.61^a	0.22	0.01
Mean	14.52^d	15.12^c	15.23^c	15.86^b	16.46^a	0.14	0.01
BUN mg/dl							
0-H	10.25	10.26	10.20	10.27	10.23	0.01	0.27
2-H	12.50^d	13.50^c	13.65^c	14.23^b	15.29^a	0.19	0.01
4-H	13.67^e	14.37^d	15.55^c	16.38^b	17.36^a	0.27	0.01
Mean	12.22^e	13.17^d	14.23^c	14.95^b	15.98^a	0.27	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; BUN: blood urea nitrogen, a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.8 Effect of anthocyanin from Purple Neem foliage on Volatile fatty acid in growing goats.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
Acetic acid (%molar)							
0-H	55.59	55.78	55.92	56.11	56.25	0.10	0.21
2-H	56.00[e]	58.07[d]	59.13[c]	60.68[b]	61.94[a]	0.43	0.01
4-H	57.77[e]	59.33[d]	60.59[c]	62.28[b]	63.48[a]	0.42	0.01
Mean	56.45[e]	57.73[d]	58.61[c]	59.63[b]	60.56[a]	0.30	0.01
Propionic acid (%molar)							
0-H	30.30	30.48	30.60	30.72	30.84	0.08	0.20
2-H	31.52[e]	33.51[d]	35.18[c]	37.24[b]	39.26[a]	0.56	0.01
4-H	32.34[e]	34.28[d]	36.34[c]	38.59[b]	41.04[a]	0.63	0.01
Mean	31.39[e]	32.76[d]	34.04[c]	35.52[b]	37.05[a]	0.41	0.01
Butyric acid (%molar)							
0-H	14.44	14.46	14.55	14.58	14.64	0.03	0.08
2-H	15.52[e]	17.23[d]	19.48[c]	20.69[b]	21.60[a]	0.46	0.01
4-H	16.69[e]	18.59[d]	20.47[c]	21.73[b]	23.08[a]	0.46	0.01
Mean	15.55[e]	16.76[d]	18.17[c]	19.00[b]	19.77[a]	0.31	0.01
Acetic acid : Propionic							
0-H	1.83	1.83	1.83	1.83	1.82	0.01	0.10
2-H	1.78[a]	1.73[b]	1.68[c]	1.63[d]	1.58[e]	0.06	0.01
4-H	1.79[a]	1.73[b]	1.67[c]	1.61[d]	1.55[e]	0.06	0.01
Mean	1.80[a]	1.76[b]	1.73[c]	1.69[d]	1.65[e]	0.04	0.01
Total VFA (mmol/L)							
0-H	54.89	54.92	54.95	54.97	54.99	0.09	1.00
2-H	57.17[e]	60.51[d]	63.34[c]	67.73[b]	71.56[a]	1.06	0.01
4-H	63.77[e]	69.81[d]	74.55[c]	78.96[b]	87.34[a]	1.65	0.01
Mean	58.61[e]	61.75[d]	64.28[c]	67.22[b]	71.30[a]	0.90	0.01

a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.9 Effect of anthocyanin from Purple Neem foliage on rumen microbial population in growing goats.

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
Total bacteria (lg10 copies/ml)							
0-H	4.58	4.72	4.60	4.55	4.78	0.06	0.73
2-H	5.09[e]	6.97[d]	8.30[c]	10.00[b]	12.10[a]	0.51	0.01
4-H	5.02[e]	6.34[d]	8.05[c]	10.03[b]	10.94[a]	0.46	0.01
Mean	4.89[e]	5.96[d]	6.98[c]	8.28[b]	9.27[a]	0.33	0.01
Butyrivibrio fibrisolven (lg10 copies/ml)							
0-H	5.59	5.65	5.71	5.77	5.94	0.06	0.33
2-H	6.11[e]	7.02[d]	8.07[c]	9.11[b]	11.10[a]	0.37	0.01
4-H	6.07[e]	6.99[d]	7.99[c]	9.03[b]	10.80[a]	0.35	0.01
Mean	5.92[e]	6.55[d]	7.26[c]	7.97[b]	9.28[a]	0.25	0.01
Fibrobacter succinogenes (lg10 copies/ml)							
0-H	4.55	4.68	4.71	4.73	4.79	0.06	0.75
2-H	5.07[e]	7.27[d]	8.99[c]	10.14[b]	11.09[a]	0.44	0.01
4-H	5.02[e]	7.16[d]	8.15[c]	10.08[b]	11.00[a]	0.44	0.01
Mean	4.88[e]	6.37[d]	7.29[c]	8.32[b]	8.96[a]	0.30	0.01
Ruminococcus albus (lg10 copies/ml)							
0-H	6.60	6.62	6.70	6.71	6.97	0.05	0.19
2-H	7.11[e]	8.12[d]	11.05[c]	13.07[b]	14.95[a]	0.61	0.01
4-H	7.10[e]	8.08[d]	11.00[c]	12.97[b]	14.04[a]	0.56	0.01
Mean	6.94[e]	7.61[d]	9.58[c]	10.92[b]	11.99[a]	0.40	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.9 Effect of anthocyanin from Purple Neem foliage on rumen microbial population in growing goats (Con).

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
Ruminococcus flavefacises (lg10 copies/ml)							
0-H	5.54	5.63	5.66	5.70	5.95	0.07	0.45
2-H	6.03[e]	8.06[d]	10.15[c]	12.02[b]	14.05[a]	0.59	0.01
4-H	5.36[e]	7.19[d]	9.23[c]	11.26[b]	13.18[a]	0.58	0.01
Mean	5.64[e]	6.96[d]	8.34[c]	9.66[b]	11.06[a]	0.40	0.01
Streptococcus bovis (lg10 copies/ml)							
0-H	5.59	5.63	5.65	5.71	5.98	0.06	0.30
2-H	6.95[e]	9.10[d]	11.03[c]	13.08[b]	15.10[a]	0.59	0.01
4-H	6.10[e]	8.14[d]	10.07[c]	12.19[b]	13.11[a]	0.54	0.01
Mean	6.21[e]	7.62[d]	8.92[c]	10.33[b]	11.40[a]	0.38	0.01
Protozoa (lg10 copies/ml)							
0-H	4.72	4.59	4.58	4.55	4.38	0.06	0.52
2-H	12.16[a]	11.09[b]	7.27c	5.06[d]	3.48[e]	0.69	0.01
4-H	12.03[a]	10.38[b]	7.14[c]	4.26[d]	3.34[e]	0.69	0.01
Mean	9.64[a]	8.69[b]	6.33[c]	4.62[d]	3.73[e]	0.47	0.01
Methanogen (lg10 copies/ml)							
0-H	5.73	5.58	5.53	5.43	5.12	0.08	0.12
2-H	11.06[a]	9.33[b]	8.31[c]	7.18[d]	6.25[e]	0.35	0.01
4-H	11.03[a]	8.88[b]	8.37[b]	7.05[c]	6.18[d]	0.35	0.01
Mean	9.28[a]	7.93[b]	7.40[c]	6.56[d]	5.85[e]	0.25	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

5.5.5 Antioxidant activity in plasma

The antioxidant activity in plasma has been shown in Table 5.10. There was significant different ($P<0.05$) on total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) levels after feeding at 2 and 4 h. Goats fed the 6%PNF+PNP+PEG had higher ($P<0.05$) levels of total antioxidant, superoxide dismutase, glutathione peroxidase, 2, 2-diphenyl-1-picrylhydrazyl and catalase in plasma compare with those fed the 6%PNF+PNP, 6%PNF, 6%PNF+PEG and control treatments. Furthermore, the addition of 6%PNF+PNP+PEG in goats reduce ($P<0.05$) malondialdehyde in plasma when compared with the 6%PNF+PNP, 6%PNF, 6%PNF+ PEG and control treatments.

5.5.6 Antioxidant gene expression in *Longissimus dorsi* of growing goat

As shown in Table 5.11. Goats receiving 6%PNF+PNP+PEG displayed a significant different ($P<0.05$) level of Antioxidant gene expression in the (*Longissimus dorsi*) compare to 6%PNF+PNP, 6%PNF, 6%PNF+PEG and control treatments. Goat receiving 6%PNF+PNP+PEG treatment shown the highest ($P<0.05$) levels of the mRNA abundance of glyceraldehyde-3-phosphate dehydrogenase (GADPH), glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) in meat tissue (*Longissimus dorsi*) of goats than of goats fed 6%PNF+PNP, 6%PNF, 6%PNF+PEG and control treatments.

5.5.7 Meat quality characteristics

There was no difference ($P>0.05$) in carcass weight, skin, white offal and red offal among treatments. In addition, goats fed 6%PNF + PNP + PEG in diet had significant higher ($P<0.05$) on slaughter weight and lower %carcass shown in (Table 5.12).

Meat quality characteristics no significant different ($P>0.05$) was observed for pH value at 1, 24 h and meat color between five treatments (Table 5.13). However, the utilization anthocyanin from Purple Neem foliage had significant different ($P<0.05$) on drip loss, cooking loss and sheer force respectively. In addition, fed 6%PNF+PNP+PEG in diet had significant lower ($P<0.05$) on drip loss, cooking loss and sheer force respectively than other those treatments. chemical composition of goat meat no significant different ($P>0.05$) shown in (Table 5.14).

Table 5.10 Effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma of growing goat.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
Total antioxidant (nmol/ul)							
0-H	1.44	1.57	1.64	1.68	1.70	0.04	0.19
2-H	2.23^e	2.51^d	2.78^c	2.89^b	3.37^a	0.08	0.01
4-H	2.06^e	2.22^d	2.60^c	2.82^b	3.06^a	0.08	0.01
Mean	1.91^e	2.10^d	2.34^c	2.46^b	2.71^a	0.06	0.01
SOD (inhibition rate %)							
0-H	87.20	87.09	87.29	87.40	87.92	0.10	0.06
2-H	90.17^d	91.10^c	92.26^b	92.65^b	93.52^a	0.25	0.01
4-H	88.54^e	89.23^d	89.87^c	90.43^b	91.16^a	0.19	0.01
Mean	88.63^e	89.14^d	89.81^c	90.16^b	91.87^a	0.16	0.01
GPX (Units/ml)							
0-H	68.47	68.35	68.08	68.18	68.31	0.07	0.49
2-H	75.04^e	75.33^d	75.82^c	76.02^b	76.21^a	0.09	0.01
4-H	68.28^d	70.22^c	71.33^c	73.25^b	75.60^a	0.54	0.01
Mean	70.59^e	71.30^d	71.75^c	72.48^b	73.37^a	0.20	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol, superoxide dismutase (SOD) glutathione peroxidase (GPX), Malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT). a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.10 Effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma of growing goat (Con).

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
DPPH scavenging activity (%)							
0-H	22.05	21.62	22.59	21.43	21.23	0.17	0.07
2-H	23.97^e	28.28^d	32.42^c	37.55^b	45.81^a	1.59	0.01
4-H	23.41^e	27.08^d	30.43^c	35.29^b	38.90^a	1.15	0.01
Mean	23.14^e	25.66^d	28.48^c	31.42^b	35.31^a	0.88	0.01
CAT (nmol/min/ml)							
0-H	8.90	8.96	8.80	8.60	8.73	0.06	0.32
2-H	14.88^e	15.04^d	15.10^c	15.16^b	15.24^a	0.03	0.01
4-H	13.66^d	13.91^c	14.01^b	14.07^b	14.22^a	0.04	0.01
Mean	12.48^b	12.61^{ab}	12.64^a	12.64^a	12.73^a	0.02	0.01
MDA (µg/mL)							
0-H	28.55	28.38	28.33	27.82	27.54	0.24	0.68
2-H	42.86^a	37.29^b	35.21^c	33.85^c	29.95^d	0.97	0.01
4-H	40.18^a	36.58^b	34.53^c	32.76^d	28.41^e	0.81	0.01
Mean	37.20^a	33.80^b	32.52^c	31.65^c	28.58^d	0.59	0.01

superoxide dismutase (SOD) glutathione peroxidase (GPX), Malondialdehyde (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT). a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.11 Effect of anthocyanin from Purple Neem foliage on antioxidant gene expression in *longissimus dorsi* of growing goat.

Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
GADPH (μmol/min per mg protein)						
8.20[e]	9.35[d]	10.37[c]	11.73[b]	12.73[a]	0.34	0.01
GPX (nmol/min per mg protein)						
2.97[e]	4.54[d]	5.57[c]	6.30[b]	8.78[a]	0.40	0.01
CAT (μmol/min per mg protein)						
5.55[e]	6.55[d]	7.06[c]	8.66[b]	9.58[a]	0.30	0.01
SOD (μmol/min per mg protein)						
3.94[e]	5.54[d]	6.35[c]	7.56[b]	8.69[a]	0.34	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; superoxide dismutase (SOD) glutathione peroxidase (GPX), catalase (CAT), glyceraldehyde-3-phosphate dehydrogenase (GADPH), a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

5.5.8 Fatty acid profile

As the result shown in Table 5.15. There were has significant different (P<0.05) on saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The saturated fatty acid content was also has reduced with goats fed 6%PNF group, 6%PNF+PEG, 6%PNF+PNP+PEG and 6%PNF+ PNP+PEG respectively. However, goats fed 6%PNF+PNP+PEG were significantly higher (P<0.05) monounsaturated fatty acid and polyunsaturated fatty acid than those in the other treatments.

5.6 Discussion

5.6.1 Feed intake, nutrient intake, nutrient digestion and growth performance

The effect of anthocyanin from Purple Neem foliage on feed intake of gDM/d, % BW, g/kgBW$^{0.75}$ and nutrient intake of OMI, CPI, EEI, NDFI, and ADFI was shown to be significant in this experiment. When compared to the other treatments, goats given the 6%PNF+PNP+PEG diet had greater gDM/d, %BW, g/kgBW$^{0.75}$ and nutrient consumption. Because anthocyanin is a plant secondary metabolite, Phenolic substances have been characterized as secondary metabolites that can increase feed intake and animal performance due to improved utilization of dietary protein (Bohluli et al., 2009). PEG was used in this investigation since it can assist boost the CP digestibility of by-products (Abarghuei et al., 2010). However, as previously reported by (Ma et al., 2015), the greater DMI seen in lambs that received polyherbal combination was probably connected with higher digestibility, comparable to the results obtained in our study. This may counteract their detrimental effect on palatability, and therefore feed intake, and increase anthocyanin feed digestion (Robbins et al., 1987); (Austin et al., 1989). These findings imply that phenols can boost productive performance at low dosages, but at large concentrations, they can impair growth rate, most likely owing to toxic consequences.

Table 5.12 Effect of anthocyanin from Purple Neem foliage on carcass characteristics of growing goat.

Items	Control	6%PNF	6% PNF+PEG	6% PNF+PNP	6%PNF +PNP +PEG	SEM	p-value
Slaughter weight (SW), kg	31.03[e]	32.10[d]	33.60[c]	34.45[b]	35.25[a]	0.32	0.01
Carcass weight (kg)	15.63	15.62	15.61	15.64	15.68	0.03	0.97
Carcass (%)	50.36[a]	48.66[b]	46.47[c]	45.40[d]	44.49[e]	0.45	0.01
Skin g/100g SW	12.54	12.55	12.56	12.57	12.59	0.01	0.69
White offal, g/100 g SW	5.52	5.52	5.54	5.56	5.56	0.02	0.97
Red offal, g/100g SW	0.56	0.56	0.57	0.58	0.59	0.04	0.22

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d, e: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean
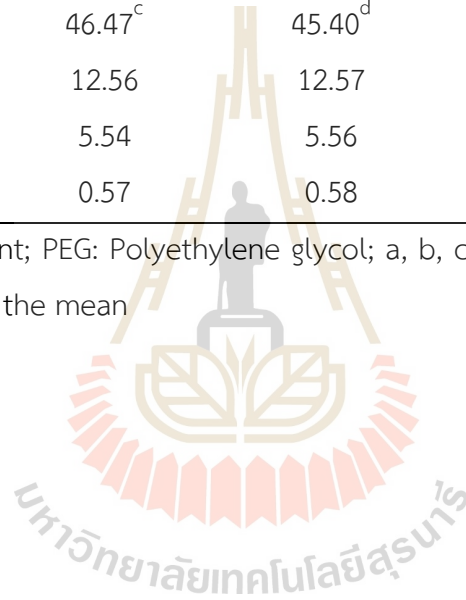
Table 5.13 Effect of anthocyanin from Purple Neem foliage on meat quality characteristics of growing goat.

Items	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
pH value at							
1-H	7.23	7.24	7.25	7.26	7.27	0.01	0.39
24 -H	6.68	6.69	6.72	6.76	6.77	0.01	0.12
Mean	6.96	6.97	6.99	7.01	7.02	0.01	0.08
Meat Color							
L*	42.31	42.32	42.33	42.35	42.39	0.20	1.00
a*	3.57	3.56	3.55	3.53	3.50	0.05	1.00
b*	6.33	6.34	6.36	6.37	6.39	0.01	0.06
Drip loss	5.66^a	4.72^b	3.55^c	2.56^d	2.34^d	0.35	0.01
Cooking loss (%)	18.54^a	16.58^b	15.44^{bc}	13.82^{cd}	13.14^d	0.43	0.01
Shear force (N)	13.54^a	11.54^b	9.08^c	7.03^d	4.43^e	0.68	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

Table 5.14 Effect of anthocyanin from Purple Neem foliage on chemical composition of goat meat.

Items	Control	6%PNF	6%PNF+PEG	6% NF+PNP	6%PNF PNP+PEG	SEM	p-value
Moisture	74.60	74.63	74.64	74.65	74.69	0.01	0.11
Dry matter	25.40	25.37	25.36	25.35	25.31	0.01	0.11
CP	21.42	21.43	21.45	21.47	21.48	0.01	0.11
Ash	6.16	6.17	6.18	6.19	6.20	0.01	0.07
EE	10.10	10.11	10.13	10.16	10.17	0.01	0.38

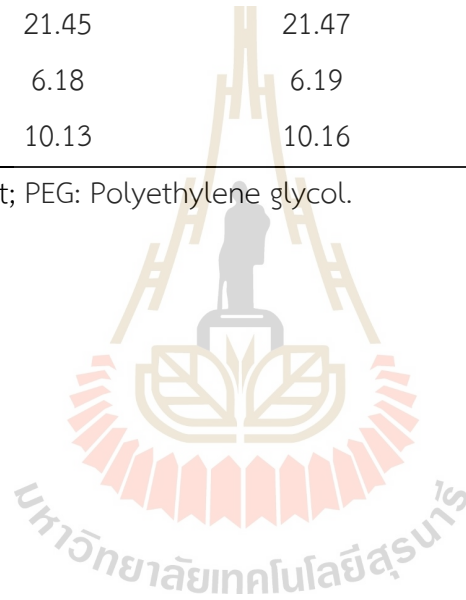PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol.

Table 5.15 Effect of anthocyanin from Purple Neem foliage on fatty acid (FA) composition (% of all fatty acids analyzed) of the lipids in meat of growing goats.

Fatty Acids, g/100 g of Total Fatty Acids	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
Saturated FA (SFA)							
C14:0	2.67[a]	2.63[b]	2.58[c]	2.54[d]	2.51[e]	0.01	0.01
C15:0	0.66[a]	0.62[b]	0.57[c]	0.52[d]	0.47[e]	0.01	0.01
C16:0	20.91[a]	19.74[b]	17.68[c]	16.71[d]	15.68[e]	0.40	0.01
C17:0	1.10[a]	0.87[b]	0.76[c]	0.68[d]	0.48[e]	0.04	0.01
C18:0	19.92[a]	17.71[b]	15.68[c]	14.68[d]	13.75[e]	0.46	0.01
C20:0	0.24[a]	0.21[b]	0.19[c]	0.17[d]	0.15[e]	0.01	0.01
C22:0	1.17[a]	0.99[b]	0.85[c]	0.66[d]	0.45[e]	0.05	0.01
Other SFA	0.69[a]	0.64[b]	0.55[c]	0.47[d]	0.42[e]	0.02	0.01
Total SFA	47.36[a]	43.41[b]	38.86[c]	36.43[d]	33.91[e]	0.54	0.01
Monounsaturated FA (MUFA)							
C16:1 cis-9	1.67[d]	1.81[c]	1.89[b]	1.92[b]	2.10[a]	0.03	0.01
C17:1 cis-10	1.36[e]	1.47[d]	1.56[c]	1.75[b]	1.95[a]	0.04	0.01
C18:1 cis-9	18.04[e]	20.20[d]	21.69[c]	23.43[b]	24.93[a]	0.53	0.01
C20:1 cis-11	1.27[e]	1.41[d]	1.65[c]	1.80[b]	1.90[a]	0.05	0.01
Other MUFA	0.36[c]	0.48[b]	0.49[b]	0.51[a]	0.52[a]	0.01	0.01
Total MUFA	22.71[e]	25.37[d]	27.28[c]	29.41[b]	31.39[a]	0.65	0.01

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean, C14:0: myristic, C15:0: pentadecanoic, C16:0: palmitic, C17:0: heptadecanoic, C18:0: stearic, C20:0: arachidic, C22:0: behenic.

Table 5.15 Effect of anthocyanin from Purple Neem foliage on fatty acid (FA) composition (% of all fatty acids analyzed) of the lipids in meat of growing goats (Con).

Fatty Acids, g/100 g of Total Fatty Acids	Control	6%PNF	6%PNF+PEG	6%PNF+PNP	6%PNF+PNP+PEG	SEM	p-value
Polyunsaturated FA (PUFA)							
C18:2 n-6	3.56[d]	5.58[c]	5.70[c]	7.59[b]	8.58[a]	0.36	0.01
C18:3 n-3	0.95[e]	1.25[d]	1.35[c]	1.57[b]	1.75[a]	0.06	0.01
C20:3 n-6	0.98[e]	1.15[d]	1.25[c]	1.45[b]	1.55[a]	0.04	0.01
C20:4 n-6	2.73[e]	3.27[d]	3.85[c]	4.25[b]	4.76[a]	0.15	0.01
C20:5 n-3 (EPA)	0.84[e]	1.02[d]	1.17[c]	1.30[b]	1.42[a]	0.04	0.01
C22:6 n-3 (DHA)	0.15[e]	0.24[d]	0.26[c]	0.38[b]	0.49[a]	0.02	0.01
Other PUFA	0.25[e]	0.35[d]	0.39[c]	0.47[b]	0.58[a]	0.02	0.01
Total PUFA	9.46[e]	12.86[d]	13.97[c]	17.01[b]	19.13[a]	0.69	0.01
Total PUFA n-3	1.94[e]	2.50[d]	2.78[c]	3.25[b]	3.65[a]	0.12	0.01
Total PUFA n-6	7.27[e]	10.00[d]	10.80[c]	13.29[b]	14.89[a]	0.55	0.01
PUFA/SFA ratio	0.20[e]	0.30[d]	0.36[c]	0.47[b]	0.56[a]	0.04	0.01
MUFA/SFA ratio	0.48[e]	0.58[d]	0.70[c]	0.81[b]	0.93[a]	0.05	0.01
n-6/n-3 ratio	3.74[b]	4.00[a]	3.90[ab]	4.09[a]	4.08[a]	0.04	0.04

PNF: Purple Neem foliage; PNP: Purple Neem pigment; PEG: Polyethylene glycol; a, b, c, d: in the same row, there is a statistically significant difference (P<0.05), SEM: standard error of the mean.

This study demonstrated that anthocyanin from Purple Neem foliage might increase apparent nutritional digestibility. This might be because anthocyanin's sugar structure is important in digestion and metabolism, and anthocyanins can attach to feed proteins (Ribnicky et al., 2014). Furthermore, this study found that include PEG in the diet can assist boost CP digestibility. Previous research indicates that PEG has the ability to increase the CP digestibility of by-products (Abarghuei et al., 2010). The current study discovered that goats fed a diet of 6%PNF+PNP+PEG had a higher significant difference in nutrient digestion compared to other treatments because Purple Neem foliage was greater on NDF and ADF digestibility, which could be related to the greater susceptibility of hemicellulolytic enzymes and Purple Neem foliage has anthocyanin content and anthocyanin can increase cp digestibility from this animal can get more nutrient and amino acid will improve animal health and performance. Furthermore, anthocyanins can attach to feed proteins, enhancing nitrogen consumption and thereby improving rumen health (Correddu et al., 2020). Our findings were consistent with those of (Nudda et al., 2017), who found that sheep given polyphenol-rich plants had higher and CP digestibility, and with the findings of (Sedighi-Vesagh et al., 2015), who found that PBP and PBP+PEG supplementation can improve nutrient digestibility.

This experiment investigated the influence of anthocyanin from Purple Neem foliage on growth performance. This study found that goats fed a diet containing 6% PNF+PNP+PEG had a higher significant difference in final weight, weight change, and ADG when compared to the other treatments. The effect of Purple Neem foliage containing anthocyanin can boost growth performance since our results reveal that goats fed a diet containing 6%PNF+PNP+PEG had greater feed intake, nutrient intake, and nutrient digestion, which may be related with increased growth performance. In addition, PEG can increase the palatability of anthocyanin in the diet, which has no detrimental effects on DMI and can improve animal performance (Makkar, 2003). (Ma et al., 2015) report that the higher DMI observed in lambs that consumed high anthocyanin was potentially associated with higher digestibility and growth performance in lambs supplemented with 0.25 mg of resveratrol, similar to the results observed in our study; and (Lobo et al., 2010) when the effects of supplementing lambs were evaluated using leaf extracts from Ilex Paraguariensis

compounds, DWG, DMI, and FBW increased. These findings imply that anthocyanin from Purple Neem foliage might increase productive performance at low dosages, but at high doses, it can decrease growth rate, most likely owing to toxic effects.

5.6.2 Nitrogen utilization

The investigation shown that anthocyanin extracted from Purple Neem foliage can boost nitrogen consumption. When compared to the other treatments, goats given 6% PNF+PNP+PEG had a higher significant difference in N intake, N Urine, N digestion, N digestion (%), N retention, and N retention (%). In similar research, (Correddu et al., 2020) discovered that anthocyanins may bind to feed proteins, enhancing nitrogen consumption, and (Nudda et al., 2017) discovered that sheep fed polyphenol-rich plants had higher nitrogen utilization. The advantage of goats in managing with anthocyanin may be due to their rumen microbial population's stronger capacity to breakdown anthocyanin (McArthur et al., 1995). As a result, anthocyanin groups demonstrated greater levels of nitrogen consumption, demonstrating that anthocyanins can increase growth performance and can be employed as novel functional feed additives for ruminants.

5.6.3 Rumen fermentation parameter

The ruminal fermentation effect of anthocyanin from Purple Neem foliage was regulated, with some favorable benefits on growth performance. All therapies had no effect on ruminal pH. In this investigation, the average pH ranged from 6.92 to 6.85. The rumen pH varied from 6.75 to 7.00 on average, which is optimum for microbial digestion in the rumen (Cherdthong et al., 2019). However, two hours after eating, the ruminal pH fell somewhat. The drop in ruminal pH is connected to the buildup of VFA, which is induced by an increase in propionate and acetate (Al Ibrahim et al., 2010). To avoid acid buildup, the ruminant animal will remove them from the rumen wall (González et al., 2012). This study indicates that microbial decomposition of grain may cause a fall in pH in the rumen.

At 2 and 4 h after feeding, the ruminal ammonia nitrogen (NH_3-N) contents in goats fed various diets differed significantly between treatments. According to this study, goats fed a meal containing 6% PNF+PNP+PEG had greater levels of NH_3-N at 2 and 4 h after feeding. The average NH_3-N content ranged from 13.35 to 18.61 mg/dl. It might be because goats given 6% PNF+PNP+PEG had a high

intake of CP, resulting in a high amount of CP accessible from Purple Neem foliage for microbial breakdown to NH_3-N in the rumen. Ruminal NH_3-N is the primary byproduct of protein breakdown utilized by rumen bacteria. These findings show that the ruminal NH_3-N content for all treatments was sufficient to maintain microbial growth at the NRC (1988) recommended level of 5 mg/dL, and was within or above the optimal level of 12-20 mg/dL at 2 and 4 h after feeding (Leng, 1990), which is considered non-limiting for microbial growth in tropical conditions. This shows that ruminal ammonia was not a limiting factor in any of the diets' fermentation. Furthermore, the presence of CP in the feed may facilitate NH_3-N rumen accessibility. Protein might be degraded to NH_3-N by microbial extracellular enzymes and deamination, according to (Hristov et al., 2004). Furthermore, the high concentration of NH_3-N in the Purple Neem foliage was most likely influenced by the high quantity of CP, which is a nitrogen supplement.

At 2 and 4 h after feeding, blood urea nitrogen (BUN) content differs significantly from anthocyanin from Purple Neem foliage. The current study found that goats fed a diet containing 6%PNF+PNP+PEG had increased BUN levels at 2 and 4 h after feeding. The increase in ruminal ammonia nitrogen and BUN concentrations in goats given 6%PNF+PNP+PEG was most likely caused by the increased CP content of the 6%PNF+PNP+PEG, which was followed by an increase in ruminal ammonia concentration. The concentration of BUN also represented the CP/energy content balance of the animal diet. The BUN levels in this research ranged from 10.20 to 17.36 mg/dL. These findings reveal that the BUN concentration was within or above the optimum limit of 10-20 mg/dL at 2 and 4 h after feeding for all regimens (Kaneko et al., 1997).

At 0 h, the concentration of volatile fatty acids (VFA) was not significantly changed. However, the effect of anthocyanin from Purple Neem foliage on VFA at 2 and 4 h after feeding all treatments was substantial. The current study found that goats fed a diet containing 6% PNF+PNP+PEG had greater levels of acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), and total VFA at 2 and 4 h post feeding. VFA are the primary byproducts of rumen microbial fermentation (Dijkstra, 1994). They are necessary for the overall metabolism of ruminant animals since they supply up to 75% of ruminant metabolic energy demands (Bergman, 1990). Thus,

higher VFA production in the rumen frequently leads in enhanced metabolic state and animal output (Kolver and Veth, 2002). Acetic acid levels were greater in goats given 6%PNF+PNP+PEG compared to other treatments. This rising acetic concentration trend might be attributed to the stimulating influence of Purple Neem leaves on the proliferation or activities of fiber-degrading microorganisms in the rumen on the activities of cellulolytic bacteria, the major product of which is acetate. Our findings are consistent with (Wiedmeier et al., 1987). VFAs are the primary sources of energy for ruminant metabolism. Anthocyanins might promote VFA synthesis by modulating intestinal flora, and flavonoid-rich plants could affect the type of rumen fermentation in goats. (Li et al., 2019). When compared to other treatments, goats given 6%PNF+PNP+PEG had a greater concentration of propionic acid. The increase could be attributed to the fact that Purple Neem foliage contains soluble energy sources such as starch, which may supply C_3 for rumen microorganisms to synthesize. As a result, an alternative electron sink for a metabolic route to dispose of the reduced power must occur, as well as the relative abundance of *B. fibrisolvens*. Increased ruminal propionate concentrations, which are glucogenic precursors for net glucose production, generally translate to better energy status in ruminants (Acosta-Estrada et al., 2014), which is consistent with our findings. Glucose is the principal energy source for ruminants, and its increasing concentration is frequently indicative of the animals' improved energy and nutritional state (Grummer, 1993). Furthermore, (Anantasook et al., 2016) suggested that the anticipated transfer of hydrogen from the CH_4 route might generate C_3 synthesis. Butyric acid levels were greater in goats fed 6%PNF+PNP+PEG at 2 and 4 h after feeding compared to other treatments in this investigation. The rise in ruminal proportion of C_2 and C_4 after feed 6%PNF+PNP+PEG therapy indicates that the source of anthocyanin influenced the ruminal VFA profile. The C_4 is largely employed as an energy source for the host (Kumar et al., 2019). In this study, substantial levels of C_4 were reported when goats were fed 6%PNF+PNP+PEG diets in high quantities. (Tian et al., 2018) found that feeding purple corn pigment or anthocyanin-rich the findings of this investigation reveal that the C_4 in rumen was within or above the optimum level for all treatments at 2 and 4 h after feeding (Leng and Brett, 1966). At 2 and 4 h after feeding, the ratio of C_2:C_3 was lower in goats fed 6%PNF+PNP+PEG

than in other treatments. The C_2:C_3 ratio in this research was within the usual range for all treatments (Leng, 1990). (Tian et al., 2018) reveal that fermentation of cell wall fiber enhanced the AA:PA ratio in the normal corn treatment, which is consistent with our findings. Furthermore, changes in the VFA profile and the C_2 to C_3 ratio might be another factor influencing CH_4 decrease (Patra et al., 2010). The improved quality of anthocyanin source gave more nutrients to rumen microbial for development, resulting in increased microbial and rumen fermentation to end products evidenced to C_3 and total VFA in goats fed 6%PNF+ PNP+PEG. Furthermore, the fermentation substrate, rumen environment, and microbial population all have an impact on the kind of VFA found in the rumen (Messana et al., 2013). (Suong et al., 2022) show that feeding anthocyanin-rich black cane instead of Napier grass enhanced overall VFA concentrations, which is consistent with our findings.

5.6.4 Microbial population

The microbial population with improvement effect of anthocyanin from Purple Neem foliage, in the current study, effect of anthocyanin from Purple Neem foliage has significant different on microbial population at 2 and 4 h after feeding. Because anthocyanins have strong antioxidant activity, which can protect the body from peroxidation damage, and thus improve rumen microflora, goats fed a 6%PNF+ PNP+PEG treatment had higher levels of total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* at 2 and 4 h after feeding compared. Furthermore, (Zhang et al., 2016) proposed that the lack of this impact might be due to the purple sweet potato anthocyanins, which include anthocyanin monomers, being unable to alter the whole bacterial population. As a result, anthocyanins can lower heat stress and alter rumen microbiota, ultimately enhancing rumen health.

The current study found that adding 6%PNF+PNP+PEG enhanced the relative abundance of ruminal *Butyrivibrio fibrisolven* at 2 and 4 h after feeding, which is associated to rumen biohydrogenation activities. (Guerreiro et al., 2021), dietary supplementation with red cabbage extract may have the ability to increase ruminal biohydrogenation processes, as seen in our investigation. The increased population of these bacteria in the rumen corresponded to a rise in the symbiotic and short-chain fatty acid (SCFA) ratio in blood, which might further stimulate meat

and milk fat production (Kawas et al., 1991) *Butyrivibrio fibrisolven* is an essential proteolytic bacteria because ruminal protein degradation is a mixture of numerous microbial activities such as protein hydrolysis, peptide breakdown, amino acid deamination, and fermentation of amino acid carbon skeletons (Brock et al., 1982).

The rumen cellulolytic bacteria *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* were required in this investigation to break down the hemicellulose and cellulose fraction of goats fed a diet containing 6%PNF+PNP+PEG. Purple Neem foliage contains anthocyanin, and anthocyanin has been shown to influence microbes in the gastrointestinal system. Furthermore, the fermentation substrate, rumen environment, and microbial population are critical variables influencing the kind of rumen microbial population (Messana et al., 2013). (Collings and Yokoyama, 1980) showed that hemicellulose was digested in the same way by *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefacises*.

The low rumen protozoa and methanogen detected in goats fed 6%PNF+PNP+PEG treatment at 2 and 4 h after feeding compared to other treatments because anthocyanin has an inhibitory effect on pathogens bacteria such as rumen methanogen and protozoa due to anthocyanin's high antimicrobial activity. Woodward et al., 2004 conducted a similar trial with cows fed *Lotus corniculatus* and discovered that methane production was reduced; (McAllister and Newbold, 2008) reported that extracts from plants such as rhubarb and garlic could also reduce methane emissions, which is consistent with our study. According to (Wanapat and Pimpa, 1999), the effects of mangosteen peel supplementation on the population of ruminal microorganisms are that supplementation reduced rumen protozoa production significantly, while the numbers of the predominant cellulolytic bacteria increased and methanogen numbers tended to decrease. Anthocyanin plants have been demonstrated to inhibit protozoal populations, increase total bacteria, propionate production, the partitioning factor, the yield and efficiency of microbial protein synthesis, and decrease methanogenesis, all of which enhance ruminant performance.

5.6.5 Antioxidant activity in plasma

Plasma antioxidant activity is an indication of the animal's antioxidant state. The phenolic compounds were absorbed by the digestive tract's colon; a wide range of phenolic substances in plasma can boost antioxidant activity (Ishida et al., 2015). As a result, in this study, feeding anthocyanin from Purple Neem foliage showed significant differences at 2 and 4 h after feeding. However, goats fed a diet containing 6%PNF+PNP+PEG had greater total antioxidant (TAC), SOD, GPX, DPPH, and CAT levels at 2 and 4 h after feeding and decrease MDA in plasma when compared to other treatments. Ruminants can make polymeric proanthocyanidins accessible by rumen microorganisms hydrolyzing proanthocyanidin polymers into bioavailable and bioactive monomers. (Gladine et al., 2007). Previous research (Hosoda et al., 2012c) found that the pigments in the examined pigment-rich forages (anthocyanin-rich whole crop corn and purple whole crop rice), which would enhance ruminant oxidative status indicators, are found in the fibrous sections of the plants such as the stem and leaf. Our findings are comparable with those of (Hosoda et al., 2012a), who found that anthocyanin in purple corn (Zea mays L.) increased SOD plasma enzyme activity in lactating dairy cows. (Gladine et al., 2007) found that extracts, particularly grape extracts, effectively increased TAS in plasma. Similarly, (Safari et al., 2018) found that include pomegranate by-products in dairy cow diets enhanced overall antioxidant status in plasma. where SOD serves as the first line of defense against ROS and GPx serves as the second (Wang et al., 2018). Previous results (Szerlauth et al., 2019) verified the higher concentration of enzymatic GPx, CAT, and DPPH scavenging activities shown in this study, indicating that natural antioxidants from plants may be beneficial. Secondary metabolites may include reactive hydrogen atoms that function as oxidative stress (OS) reductants. Furthermore, higher DPPH scavenging activity and GPx were followed by enhanced SOD, CAT, and TAC activity in this study, indicating that the influence of plant secondary metabolites on antioxidant activity is dependent on the molecular weight or threshold utilized in the diets. According to (Tian et al., 2018), feeding purple-corn stover silage containing 8.75 g of nontannic polyphenols to dairy goats was associated with increased SOD antioxidant activity, GPx activity, and TAC. The current investigation found that goats fed a diet containing 6% PNF+PNP+PEG had lower

levels of MDA in their plasma. Similarly, giving natural flavonoid compounds to mice boosted plasma superoxide dismutase (SOD) activity while decreasing plasma MDA (Nurrofingah et al., 2020). (Safari et al., 2018) revealed that phenolic acid from pomegranate by-products in dairy cow diets had a significant effect on blood antioxidant capacity while lowering blood MDA levels. Taken together, a dietary 6 percent PNF+PNP+PEG diet should improve meat goat growth performance by increasing antioxidant activity (and hence lowering OS) and promoting nutrient digestion by increasing fermentable organic matter (OM).

5.6.6 Antioxidant gene expression in *longissimus dorsi*

Because anthocyanin was engaged in helping avoid production loss owing to oxidative stress, the current study found that anthocyanin from Purple Neem foliage might promote antioxidant gene expression in *Longissimus dorsi* (LD). Anthocyanin was a powerful natural antioxidant that inhibited oxidative stress and inflammation in ruminants by controlling peroxidation reactions and scavenging free radicals (FR) (Tian et al., 2019). Based on the findings of this study, it was proposed that including 6%PNF+PNP+PEG in the diet might increase LD antioxidant status (GADPH, GPX, CAT and SOD). According to (Hosoda et al., 2012b), anthocyanin-rich maize feeding increased SOD activity in the plasma of lactating dairy cows. According to (Hosoda et al., 2012d), providing anthocyanin-rich diets has been shown to ameliorate oxidative stress indicators. Similarly, the inclusion of rosemary extract, widely regarded as one of the spices with the highest antioxidant activity, was found to be effective in avoiding lipid oxidation in dairy products (Gad and Sayd, 2015). In short, anthocyanin was the most important element influencing antioxidant activity since it had a positive link with it (Seo et al., 2011). Our research showed that providing 6%PNF+PNP+PEG in goat diet gave several benefits. When antioxidants in diets are combined, they may be absorbed and boost ruminant production and quality. As a result, anthocyanin from Purple Neem leaf has the potential to transmit anthocyanin composition to organs, so influencing the antioxidant status of growing goats.

5.6.7 Carcass and Meat Characteristics

This study found that anthocyanin from Purple Neem foliage had no significant influence on carcass features of developing goats across all treatments, indicating that the function of anthocyanin might boost meat production rate. Our

findings are comparable with those of (Suong et al., 2022), who found that feeding anthocyanin-rich black cane silage had no effect on carcass characteristics. Similarly, (Duan et al., 2019) show that melatonin therapy had no influence on carcass features in these cashmere goats. The varying outcome between research, however, is most likely due to a wide range of parameters, including the composition of the anthocyanin-based diet utilized in the study, the age of slaughter, and the bioavailability of flavonoids or anthocyanins in ruminants. Our research shows that anthocyanin from Purple Neem foliage, as a source of anthocyanin, can help improve the production of developing goats during the last fattening stage without harming the quality of the meat or the animals' health.

The pH values varied from 5.60 to 5.77 and were unaffected by the treatments. However, the pH levels in this investigation were typical for regular grade beef (Modzelewska-Kapituła et al., 2018). (Oliveira et al., 2010) discovered no variations in pH of Nellore heifers given different diets including or not containing essential oils (clove and/or rosemary essential oil) and/or active principle mix (eugenol, thymol and vanillin). (Rivaroli et al., 2016) investigated the effect of essential oils on meat quality in crossbred young bulls and found that the diet had no effect on meat pH, which is consistent with our findings.

Meat color is determined by several elements, including haeminic pigments (myoglobin), pH, and the chemical condition of the pigments. The purple color of deep muscle is reduced (or deoxy) myoglobin. The findings of color determinations given in this investigation revealed no significant differences between treatments. Furthermore, because consumers associate color with freshness, meat color has been identified as the most essential criterion when assessing meat quality. However, anthocyanin from Purple Neem foliage was found to diminish meat redness in this investigation (a*). These findings were comparable with those of (Prommachart et al., 2020), who found that LD samples derived from calves given high levels of anthocyanin-extracted residue (AER) had lower a* values. Similarly, (Jaturasitha et al., 2016) found that feeding purple rice to fatten pigs resulted in lower a* values than feeding white rice. Previous research found that lowered meat color a tends to brown the meat, implying that the oxymyoglobin to metmyoglobin conversion stage and the lipid peroxidation interaction play a role in meat

discoloration (Tian et al., 2022a). Anthocyanin from Purple Neem foliage is likely to improve meat color in this study because anthocyanins including cyanidin-3-glucoside and malvidin-3-O-glucoside stabilized muscle membranes, resulting in improved meat color (Kil et al., 2006).

The effect of anthocyanin from Purple Neem foliage on drip loss and cooking loss was considerable in the current experiment across all treatments. However, drip loss and cooking loss were decreased in goats fed a diet containing 6%PNF+PNP+PEG. According to earlier research, the juiciness of meat is strongly connected to the intramuscular lipids and moisture content of the flesh, and lean meat contains roughly 75% water (Trout, 1988). (Karami et al., 2011) found that dietary antioxidants minimize drip loss and cooking loss in the LD muscle, which is consistent with our findings. Cooking loss is more reliant on final pH, sarcomere length, and cooking circumstances, according to (Trout, 1988). Normal cooking alters the composition of animal fat and increases the calorie density of meat (Webb et al., 2005).

Tenderness is possibly the most significant eating quality attribute that influences consumer acceptance (Kannan et al., 2002). In the current study, the dietary influence of anthocyanin from Purple Neem foliage on meat softness reported as shear force was significantly different. However, this study discovered that goats fed a diet containing 6% PNF + PNP + PEG can minimize shear force when compared to other treatments. Tenderness of goat meat is frequently acceptable to consumers (Webb et al., 2005). Our results are consistent with those of (Karami et al., 2011), who found that dietary antioxidants can lower shear force in LD muscle. The lowest Warner–Bratzler shear force (WBSF) values were seen in the LD muscles of heifers given a diet supplemented with all bioactive compounds examined (Oliveira et al., 2010). Shear force readings are affected by factors such as how the animals were treated previous to slaughter, postmortem procedures, the muscle sampled, and the manner of sample preparation (Webb et al., 2005). As a result, goats fed a diet containing 6%PNF+PNP+PEG suggest that it can be used to feed ruminants to enhance tenderness. The results of this study suggest that anthocyanin from Purple Neem foliage has the capacity to increase antioxidant defense systems in goats and reduce the formation of reactive oxygen species (ROS).

In the current investigation, no significant differences in nutritional content of longissimus dorsi muscle in growing goats were observed across the groups. This suggests that anthocyanins had no influence on the nutritional components of goats. The cause might be due to anthocyanins' limited bioavailability in animals. Furthermore, pH, temperature, oxygen, light intensity, and enzymes all have an impact on anthocyanin stability (Enaru et al., 2021). These results indicate that the effects of Purple neem foliage on the composition of goat meat may be dose-dependent, botanical origin-dependent, and experimental phase-dependent. According to (Orzuna-Orzuna et al., 2021), the effect of polyherbal mixture (HM) had no influence on the nutritional content of meat.

5.6.8 Fatty acid profile

Anthocyanins are natural antioxidants that are widely present in plants, the fatty acid profiles of goat meat in this study effect of anthocyanin from Purple Neem foliage have significant differences on saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) all among treatments. Previous research has showed that feeding anthocyanin-rich diet to ruminants impacted meat quality. The anthocyanin is a kind of water-soluble phenolic chemical (Tian et al., 2018). The current study discovered that goats fed a diet containing 6%PNF+PNP+PEG can lower SFA compared to other treatments. Our findings, which are similar to those of (Tian et al., 2022b), reveal that including purple maize pigment in the baseline diet of developing goats resulted in a decrease in SFA content, perhaps due to anthocyanins shifting the relative abundance of rumen microbiota in goats. (Vasta et al., 2019) proposed that reducing SFAs increased PUFAs in ruminant products. Furthermore, (Bryszak et al., 2019) shown that anthocyanin-rich plants might change the biohydrogenation pathway, resulting in higher ruminal fluid trans C18:1 in dairy cows. Consistent with our results, (Resconi et al., 2018) discovered that lambs fed phenolic-rich grape pomace had lower C16:0 and C17:0 levels in meat and higher C18:2n-6 content. In the current study, goats fed a diet containing 6%PNF+PNP+PEG increased PUFA levels in goat meat compared to other treatments because anthocyanins may protect PUFAs by modulating lipid mechanisms and ruminal biohydrogenation, thereby improving PUFA levels in ruminant meat, and PUFAs are thought to be important compounds for consumers'

nutrition and health. Indeed, anthocyanins can give H-atoms to peroxyl radicals, limiting PUFA oxidation via chain radical termination (Narayan et al., 1999). Similar to our findings, (Colombo et al., 2021) discovered that including anthocyanin-rich purple corn might improve antioxidant status and unsaturated fatty acids (UFA) in ruminants. Anthocyanin supplementation in sheep feed might reduce oxidative stress (OS) and assist prevent PUFA loss, resulting in considerably higher PUFA concentrations (Colombo et al., 2021). As a result, anthocyanins can donate electrons to FRs with unpaired electrons and decrease agents in the electron-transfer reaction pathway in ruminant flesh, adding unsaturated bonds to previously existing PUFAs (Prommachart et al., 2021). (Flores et al., 2020) proposed that feeding grape pomace silage in a lamb diet might improve overall PUFA content while maintaining meat lipid and protein stability. Furthermore, anthocyanin has been shown to suppress blood lipid mechanism parameters and modify meat-related gene expression in goats (Tian et al., 2021). As a result, we discovered that goats given anthocyanin-rich Purple Neem foliage had higher levels of C18:1, C18:2n-6, and total PUFA. This could be because anthocyanins have hydroxyl groups in the aromatic ring, which can provide extra hydrogen or electron donors and eliminate excess FR in the body, thereby maintaining the PUFA concentration. Overall, dietary supplementation with anthocyanin from Purple Neem foliage may increase antioxidant activity and PUFA concentrations in muscle, thereby improving meat quality in goats, and an intriguing perspective is the possibility of enriching meat goat with substances that have health benefits for its consumers.

5.7 Conclusion

The purpose of this study is to investigate anthocyanin from Purple Neem foliage affects antioxidant activity, antioxidant gene expression in *longissimus dorsi*, and meat quality in growing goats. The current study found that feeding goats a diet containing 6%PNF+PNP+PEG had the potential to improve DMI, nutrient apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, antioxidant activity in plasma, antioxidant gene expression in *longissimus dorsi*, carcass and meat characteristics, and fatty acid profile in goat meat. Furthermore, high total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*,

Ruminococcus albus, *Ruminococcus flavefacises*, and *Streptococcus bovis* were discovered, as well as low protozoa and methane production. Whereas it did improve DPPH scavenging activity and SOD activity, TAC, CAT, GPX, and lower MDA in developing goat plasma. Antioxidants in goat feed promote antioxidant gene expression in *longissimus dorsi*, improving meat quality and fatty acid profile in goat meat.

5.8 References

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CHAPTER VI

OVERALL CONCLUSION AND IMPLICATION

6.1 Conclusion

The main objective of this experiment was to study the effect utilization of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats.

Experiment 1 was to investigate the effect of utilization of anthocyanin from Purple Neem foliage on rumen parameters and growth performance in growing goats. The results revealed that goats feeding a 6% Purple Neem foliage diet enhance efficiency in terms of DMI, nutrients apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, and antioxidant activity in plasma of growing goats. Additionally, high total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises* and *Streptococcus bovis* respectively were discovered, as well as low protozoa and methane production. Improve DPPH scavenging and SOD activity, as well as TAC, CAT, GPX, and reduce MDA in growing goat plasma. Based on the findings of the current study, it is possible to conclude that Purple Neem foliage can be utilized as a source of anthocyanin for ruminant feeding.

Experiment 2 was to investigate the effect of anthocyanin from Purple Neem foliage on antioxidant activity in plasma and meat quality in growing goats. The results indicated that goats fed a 6%PNF+6%SFO diet had the potential to improve DMI, nutrients apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, antioxidant activity in plasma, antioxidant gene expression in meat, carcass and meat characteristics and fatty acid profile in goat meat. In addition, it was found that high total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises* and *Streptococcus bovis* respectively and low protozoa and methane production. Whereas it did improve the DPPH scavenging activity and SOD activity, TAC, CAT, GPX

and reduce MDA in the plasma of growing goats. Based on the information in the current study, it can be concluded that feeding Purple Neem foliage to goats improves antioxidant gene expression in meat, resulting in improved meat quality and fatty acid profile in goat meat.

Experiment 3 was to investigate the effect of anthocyanin from Purple Neem foliage on antioxidant gene expression in longissimus dorsi and meat quality in growing goats. The results showed that feeding goats a diet containing 6%PNF+PNP +PEG had the potential to improve DMI, nutrient apparent digestibility, nitrogen utilization and rumen fermentation parameters, microbial population, antioxidant activity in plasma, antioxidant gene expression in meat, carcass and meat characteristics, and fatty acid profile in goat meat. Furthermore, high total bacteria, *Butyrivibrio fibrisolven*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacises*, and *Streptococcus bovis* were discovered, as well as low protozoa and methane production. Whereas it did improve DPPH scavenging activity and SOD activity, TAC, CAT, GPX, and lower MDA in developing goat plasma. Based on the findings in this study, it is possible to infer that Purple Neem foliage may be utilized as an antioxidant source in goat feed to boost antioxidant gene expression in meat, enhancing meat quality and fatty acid profile in goat meat.

6.2 Implication

In current world, many people are more concerned about functional foods. To produce nutritious feeds from ruminants. Despite the fact that Purple Neem foliage was the best choice in experiments 2 and 3 to boost animal production, meat quality, fatty acid profile, and antioxidant gene expression in muscle has greater interested. These advantages stem from the use of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats. As a result, a better knowledge of Purple Neem foliage and its role in the rumen might be beneficial for strategy implementation. The novelty of our study is that we investigate the usage of Purple Neem foliage as a ruminant feed addition. Therefore, anthocyanins metabolism in ruminants is unclear, and Purple Neem foliage contains not just anthocyanin chemicals but also other natural antioxidants such as phenolic compound.

Further studies should be conducted to investigate the bioavailability, metabolism, milk yield, milk composition, and antioxidant gene expression in the mammary gland of anthocyanin from Purple Neem foliage in dairy goats by LED light and beneficial microorganisms could promoted yield production.

APPENDICES
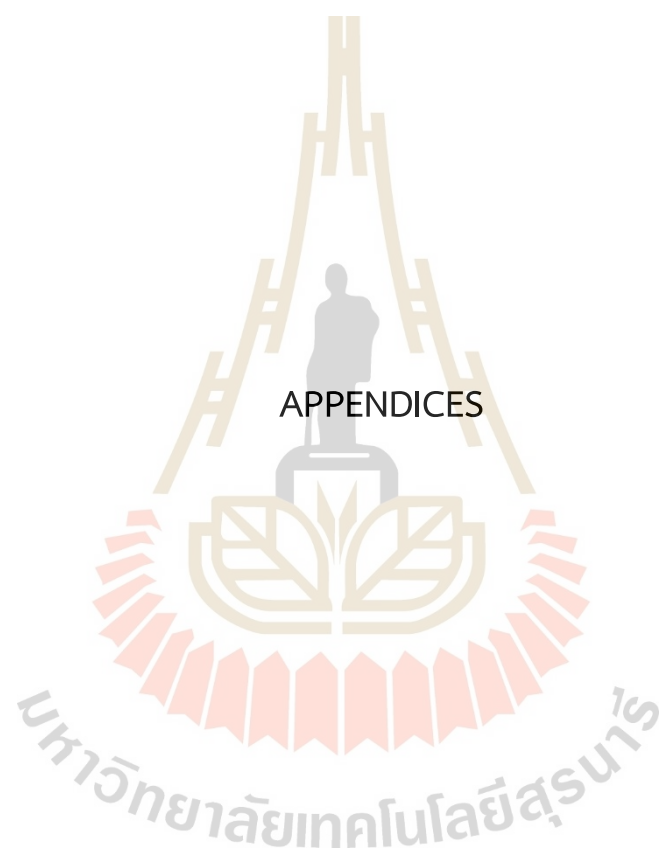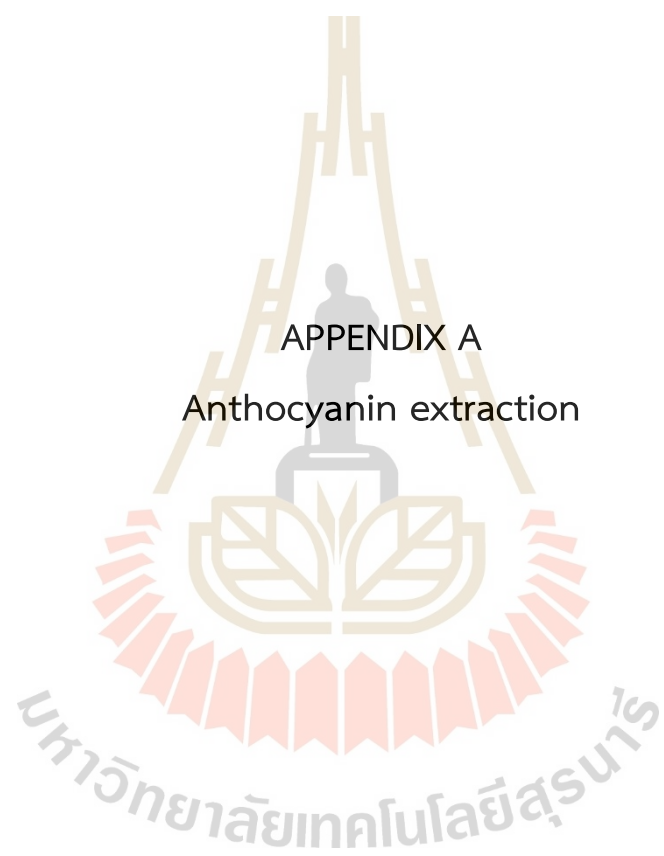
APPENDIX A

Anthocyanin extraction

A1   Methanol extraction of anthocyanin

Extraction

1)   Using a warring Blender with a stainless-steel container or a general-purpose homogenizer, combine 50 g powdered plant material (accurately weighed and documented) 1: 1 (w/v) with ethanol.

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

3)   Extract plant material with 50% ethanol until a clear solution is obtained. Filtrate and discard plant material.

4)   Transfer the filtrate to a separating funnel and gently mix it by flipping the funnel over down several times. Keep the sample at 4°C overnight or until a distinct partition between the two phases is obtained.

5)   Fill a 500-ml boiling flask halfway with the aqueous phase (top section). Remove any remaining ethanol in a rotary evaporator set to 50°C and under vacuum.

6)   With acidified deionized distilled water, dilute the residual aqueous extract to a predetermined volume (typically 100 ml). If the sample will be examined within two days, keep the extract at 4°C. Store at -18°C for extended durations (up to a year or even longer). Avoid repeated freezing and thawing cycles.

A2   Anthocyanin purification

Isolate anthocyanin content

1.   Activate a C18 SPE cartridge through successive applications of 5 ml acidified methanol and 5 ml acidified water.

2.   Using a syringe, apply an anthocyanin containing sample (typically 1 ml) to an activated C, SPE cartridge. Wash twice with 5 ml water each time to remove sugars and acids.

3.   Elute the anthocyanin with two 5-ml aliquots of acidified methanol and collect elute in a flask that can be put on a rotary evaporator.

4.   Remove the acidified methanol from the sample on a rotary evaporator until a small drop of liquid is still present.

Sample preparation

1. Isolate anthocyanin fraction (steps 1 to 4).

2. Dissolve the sample in 10 ml 2 N HCl and transfer to a 20-ml screw-top test tube. Flush the tube with nitrogen gas and seal with Teflon-lined cap.

3. Place tube in a boiling water bath and allow to hydrolyze for 30 min.

4. Cool sample in an ice bath.

5. Apply sample to an activated CIS SPE cartridge and rinse twice with 5 ml water each time. Elute anthocyanin twice with 5 ml acidified methanol each time and collect in a flask appropriate for a rotary evaporator.

6. Remove acidified methanol from the sample on the rotary evaporator until a small drop of liquid is still present.

7. Dissolve the sample in 2 to 5 ml 4% phosphoric acid, filter through 0.45-pm filter and place in a 20-ml screw-top test tube. Flush the tube with nitrogen, seal with a Teflon-lined cap, and store on ice in the dark until injection.

8. Set the flow rate of the HPLC system to 1 ml/min across a 5-pm x 250-mm x 4.6-mm Prodigy ODs-3 column (or equivalent) at ambient temperature. Set the detector at 520 nm. Inject 50 μl sample into the HPLC system.

APPENDIX B

The primer of reference sequence for real-time PCR

Table B1 Rumen microbial primer sets used for real-time PCR assay.

No	Items	product size (bp)	F/R	sequence	References
1	Total bacteria	130	F	CGGCAACGAGCGCAACCC	Denman and McSweeney
			R	CCATTGTAGCACGTGTGTAGCC	(2006)
2	Methanogen	140	F	TTCGGTGGATCDCARAGRGC	Denman et al. (2007)
			R	GBARGTCGWAWCCGTAGAATC	
3	Protozoa	223	F	CTTGCCCCTCYAATCGTWCT	Sylvester et al. (2004)
			R	GCTTTCGWTGGTAGTGTATT	
4	*Butyrivibrio fibrisolvens*	64	F	ACACACCGCCCGTCACA	Klieve et al. (2003)
			R	TCCTTACGGTTGGGTCACAGA	
5	*Fibrobacter succinogenes*	446	F	GGTATGGGATGAGCTTGC	Khafipour et al. (2009)
			R	GCCTGCCCCTGAACTATC	
6	*Ruminococcus flavefaciens*	295	F	TCTGGAAACGGATGGTA	Khafipour et al. (2009)
			R	CCTTTAAGACAGGAGTTTACAA	
7	*Ruminococcus albus*	176	F	CCCTAAAAGCAGTCTTAGTTCG	Khafipour et al. (2009)
			R	CCTCCTTGCGGTTAGAACA	
8	*Streptococcus bovis*	82	F	TTCCTAGAGATAGGAAGTTTCTTCGG	Muyzer et al. (1993)
			R	ATGATGGCAACTAACAATAGGGGT	

Table B2 Antioxidant primer sets used for real-time PCR assay.

Target gene	Primer sequence (5'-3')	Accession number	Product size(bp)
GADPH	F: TACTGGCAAAGTGGACATCG	NM_001190390.1	136
	R: GATGACGAGCTTCCCGTTCT		
SOD	F: CCGTCAGCCTTACACCAAGT	NM_001280703.1	112
	R: GTCAGCTCTTGGGAACGGAA		
CAT	F: TGGGACCCAACTATCTCCAG	JF728302.1	216
	R: AAGTGGGTCCTGTGTTCCAG		
GPX	F: ACATTGAAACCCTGCTGTCC	XM_004016396.3	178
	R: TCATGAGGAGCTGTGGTCTG		
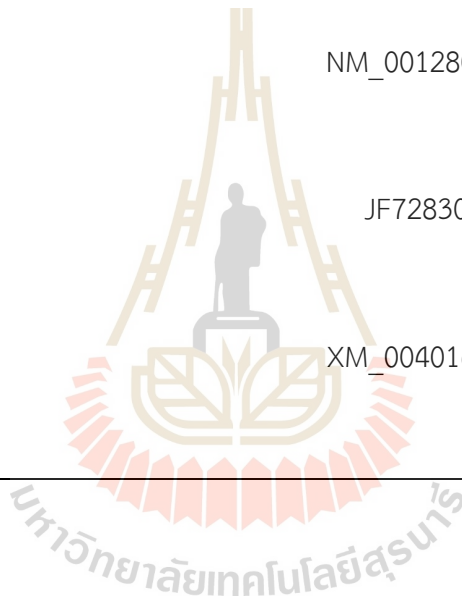
BIOGRAPHY

Miss Nittaya Taethaisong was born on 4 April 1994 in Buriram, Thailand. In 2012, she graduated high school level from Phutthaisong School, Buriram. In 2016, she obtained her Bacherlor's degree in Agricultural Technology from the Program of Animal Production Technology, Suranaree University of Technology. In 2019, she graduated her a Master of Animal Production Technology from the Institute of Agricultural Technology, Suranaree University of Technology. During study in master's degree, she has gotten to scholarship by SUT-OROG scholarship under her Advisor's Assoc. prof. Dr. Pramote Paengkoum. She studied in a field of Animal Production Technology for ph.D. Program at the School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima from November 2020 to April 2022 with the thesis entitled "Utilization of anthocyanin from Purple Neem foliage on antioxidant activity and meat quality in growing goats" which supported financial by SUT-OROG scholarship.