UTILIZATION OF MANGOSTEEN (*Garcinia mangostana* L.) PEEL POWDER IN MEAT GOAT DIETS

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การใช้ประโยชน์ของเปลือกมังคุดผง (*Garcinia mangostana* L.) ในอาหารแพะเนื้อ

นายบัน เชาว์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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UTILIZATION OF MANGOSTEEN (*Garcinia mangostana* L.)

PEEL POWDER IN MEAT GOAT DIETS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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บัน เชาว์ : การใช้ประโยชน์ของเปลือกมังคุดผง (*Garcinia mangostana* L). ในอาหารแพะเนื้อ (UTILIZATION OF MANGOSTEEN (*Garcinia mangostana* L.) PEEL POWDER IN MEAT GOAT DIETS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 75 หน้า.

การวิจัยในครั้งนี้มีวัตถุประสงค์เพื่อ (1) การศึกษาในหลอดทดลองถึงผลของเปลือกมังคุดต่อการหมักในรูเมน และแก๊สมีเทนในแพะเนื้อ (2) การตรวจสอบผลของเปลือกมังคุดต่อการย่อยได้ การหมักในรูเมนและการต้านอนุมูลอิสระในพลาสมาในแพะเนื้อ

การทดลองที่ 1 ในการทดลองแบ่งออกเป็น 3 ทรีตเม้นท์ซึ่งมีระดับของเปลือกมังคุดที่แตกต่างกัน (0 5 และ 10% ของสิ่งแห้ง ตามลำดับ) ตามแผนการทดลองเป็นแบบสุ่มสมบูรณ์ (CRD) สำหรับที่ 0 3 6 9 12 24 และ 48 ชั่วโมง ในการวัดปริมาณการเกิดแก๊สในหลอดทดลอง จากผลการทดลองพบว่า เปลือกมังคุดมีผลต่อการเกิดแก๊สอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) นอกจากนี้พบว่าเปลือกมังคุดไปลดการย่อยได้ของอินทรีย์วัตถุ พลังงาน และผลต่อศักยภาพการย่อยได้เมื่อเปรียบเทียบกับกลุ่มควบคุม และยังพบว่าในกลุ่มที่ใช้เปลือกมังคุดในระดับต่ำจะไปช่วยลดการเกิดแก๊สในส่วนที่ละลายน้ำได้ (a), การผลิตแก๊สจากส่วนไม่ละลายในน้ำ (b), อัตราการเกิดแก๊ส (c) และค่าการผลิตแก๊สรวม (a+b), กรดอะซิติก (AA), กรดบิวทีริก(BA), กรดไขมันระเหยได้ทั้งหมด (TVFA) และมีเทน ที่ระดับ 5% ของเปลือกมังคุดสามารถลดมีเทน และการย่อยได้ของอินทรียวัตถุในรูเมน และยังช่วยเพิ่มโภชนะที่ไหลผ่านจากรูเมน

การทดลองที่ 2 ใช้แพะลูกผสมพื้นเมืองพันธุ์แองโกนูเบียน ทั้งหมด 12 ตัว (น้ำหนัก 23.1± 1.49 กิโลกรัม) แบ่งออกเป็นสองกลุ่ม (1) กลุ่มควบคุม : อาหารพื้นฐาน และ (2) กลุ่มทดลอง : อาหารปกติ +4.125% เปลือกมังคุด ผลการทดลองพบว่า กลุ่มที่เสริมด้วยเปลือกมังคุดไม่ได้มีผลต่อการกินได้ของสิ่งแห้ง, การกินได้ของอินทรียวัตถุ และการย่อยได้ของโภชนะ ในขณะที่น้ำหนักตัวเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) นอกจากนี้พบว่ากลุ่มที่ใช้เปลือกมังคุดมีผลช่วยลดการขับออกของไนโตรเจนในปัสสาวะ มีแนวโน้มในการลดเปอร์เซ็นต์การขับออกของไนโตรเจนทั้งหมด แต่พบว่าเพิ่มการเปอร์เซ็นต์การกักเก็บไนโตรเจน การใช้เปลือกมังคุดพบว่า ช่วยลดความเข้มข้นของแอมโมเนียไนโตรเจน, กรดอะซิติก และกรดไขมันระเหยได้ทั้งหมด ลดมีเทน, กรดอะซิติก และอัตราส่วนของกรดอะซิติกต่อกรดโพรพิโอนิก และเพิ่มกรดโพรพิโอนิก การเสริมเปลือกมังคุดมีแนวโน้มที่จะเพิ่มโปรตีนทั้งหมดในเลือด (TP) และ อะบลูมิน(ALB) นอกจากนี้เปลือกมังคุดเพิ่ม 2,2-diphenyl-1-picrylhydrazyl scavenging activity ซึ่งเพิ่มกิจกรรมของเอนไซม์กลูต้าไทโอน โดยรวมการใช้เปลือกมังคุดสามารถปรับปรุงความสามารถในการต้านการเกิดอนุมูลอิสระใน

พลาสมา และมีศักยภาพในการปรับปรุงการใช้ประโยชน์ของไนโตรเจนในร่างกาย และการช่วยส่งเสริมทำงานของภูมิคุ้มกันในร่างกาย

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BAN CHAO : UTILIZATION OF MANGOSTEEN (*Garcinia mangostana* L.) PEEL POWDER IN MEAT GOAT DIETS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 75 PP.

MANGOSTEEN PEEL POWDER/CONDENDSED TANNIN/RUMEN FERMENTATION/ANTIOXIDANT ACTIVITIES/MEAT GOATS

The purpose of this study was to estimate: (I) *in vitro* study the effect of mangosteen peel powder on rumen fermentation and methane production in meat goats. (II) To investigate the effect of mangosteen peel powder on nutrient digestibility rumen fermentation and plasma antioxidant activity in meat goats.

In experiment 1, the experimental treatments were separated into three treatments with different levels (0 5% and 10% of DM, respectively) of mangosteen peel powder (MPP) according to the completely randomized design (CRD) for 0, 3, 6, 9, 12, 24 and 48 hours gas production *in vitro*. The results showed that MPP had significant effect on gas production ($P<0.05$). Moreover, MPP also significantly decreased ($P<0.05$) the organic matter digestibility (OMD), metabolizable energy (ME), and effective degradability (ED) relative to control. In addition, lower level of MPP decreased ($P<0.05$) gas production from the immediate soluble fraction (a), gas production from the insoluble fraction (b), gas production rate constant (c), and potential extent of gas production (a+b). In addition, acetic acid (AA), butyric acid (BA) total volatile fatty acid (TVFA), and methane (CH_4) production were decreased by MPP. Taken together, the 5% MPP decreased CH_4 production and OMD in the rumen, and also enhanced the amount of nutrient of escape rumen.

In experiment 2, total twelve Anglo-Nubian crossed with Thai native male goat (bodyweight 23.1 ± 1.49kg) were randomly assigned to two groups: (1) control group: basal diet, and (2) treatment: basal diet + 4.125% MPP. The results showed that the feeding of MPP had no effect (P>0.05) on dry matter intake (DMI), organic matter intake (OMI), and nutrient digestibility. Whereas, it significantly increased (P=0.005) BW, %. In addition, urine nitrogen (N) excretion was decreased (P=0.024) by MPP, the percentage of N excretion tended to decrease (P=0.096), but the percentage of N retention tended to increase (P=0.096). The concentration of ammonium nitrogen (NH_3-N), AA, and TVFA were reduced by MPP; MPP was decreased (P<0.05) methane production, percentage of AA and ratio of acetic acid to propionic acid (AA/PA), increased (P<0.001) percentage of PA. Supplementation of MPP indicated a trend to increase serum total protein (TP) (P=0.082) and albumin (ALB) (P=0.077). Moreover, MPP increased (P=0.017) the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity; it also increased (P=0.044) the glutathione peroxidase (GPx) activity. Collectively, MPP could improve plasma antioxidant capacity, and it had the potential to improve N utilization and immunity function.

School of Animal Technology and Innovation Student's Signature

Academic Year 2019 Advisor's Signature

Co-advisor's Signature

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LIST OF ABBREVIATIONS

a	=	Gas production from the immediately soluble fraction
AA	=	Acetic acid
AA/PA	=	Ratio of acetic acid to propionic acid
ADF	=	Acid detergent fiber
ALB	=	Albumin
a+b	=	Potential extent of gas production
b	=	Gas production from the insoluble fraction
BA	=	Butyric acid
BUN	=	Blood urea nitrogen
c	=	Gas production rate constant
CH_4	=	Methane
CP	=	Crude protein
CRD	=	Complete randomized design
CT	=	Condensed tannin
DM	=	Dry matter
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
ED	=	Effective degradability
EE	=	Ether extract
GE	=	Gross energy
GLB	=	Globulin
GPx	=	Glutathione peroxidase

LIST OF ABBREVIATIONS (Continued)

HM	=	High level of mangosteen peel powder
H_2SO_4	=	Sulfuric acid
LM	=	Lower level of mangosteen peel powder
ME	=	Metabolizable energy
MPP	=	Mangosteen peel powder
N	=	Nitrogen
NDF	=	Neutral detergent fiber
NH_3-N	=	Ammonia nitrogen
NRC	=	National Research Council
NS	=	No significant effect
OM	=	Organic matter
OMD	=	Organic matter digestibility
PA	=	Propionic acid
SEM	=	Standard error of the mean
SOD	=	Superoxide dismutase
SUT	=	Suranaree University of Technology
TAC	=	Total antioxidant capacity
TP	=	Total protein
TPs	=	Total phenolics
TVFA	=	Total volatile fatty acid
VFA	=	Volatile fatty acid
↑	=	Significant increase

LIST OF ABBREVIATIONS (Continued)

↓	=	Significant decrease
-	=	Not determine

CHAPTER I

INTRODUCTION

1.1 Introduction

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants, which can occur when antioxidant utilization is increased, buffering is impaired, or immune function is impaired (Celi et al., 2014). Due to heat stress can trigger OS (Akbarian et al., 2016), this has become an important factor limiting animal production in tropical countries (Habeeb, 2018).

The protective effect of antioxidant nutrients on free radical damage in ruminants has become an important part of the research on production of ruminants (Castillo et al., 2003). Condensed tannin (CT) as a natural and safe addition of antioxidant was reported by many studies (Tian et al., 2012; Beninger et al., 2003; Zhang et al., 2010). In addition, CT has been reported that have the potential to regulate rumen fermentation protective proteins escape rumen and reduce methane production in ruminants (Min et al., 2003). Finding of new CT source has become urgent (Singh and Kumar, 2019).

Mangosteen (*Garcinia mangostana* L.) is a tropical evergreen tree whose peel is rich in secondary plant metabolites, but it is wasteful because the peel cannot be eaten directly. Moreover, mangosteen peel rich crude protein can be used for protein source for ruminants (Paengkoum et al., 2015). Therefore, the purpose of this study was to estimate effect of mangosteen peel on growth performance rumen fermentation

nutrient digestibility and plasma antioxidant capacity in meat goats.

1.2 Research objectives

1.2.1 To study effect of mangosteen peel powder on rumen fermentation and methane production *in vitro*.

1.2.2 To investigate mangosteen peel powder on nutrient digestibility rumen fermentation and plasma antioxidant capacity in meat goats.

1.3 Research hypothesis

1.3.1 Mangosteen peel powder could reduce methane production in rumen.

1.3.2 Mangosteen peel powder could improve nitrogen utilization in meat goats.

1.3.3 Mangosteen peel powder could increase plasma antioxidant capacity in meat goats.

1.4 Expected results

1.4.1 Mangosteen peel powder reduce methane production in rumen.

1.4.2 Mangosteen peel powder decrease nitrogen excretion and increase nitrogen retention.

1.4.3 Mangosteen peel powder increase plasma antioxidant enzyme activity in meat goats.

1.5 References

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CHAPTER II

LITERATURE REVIEWS

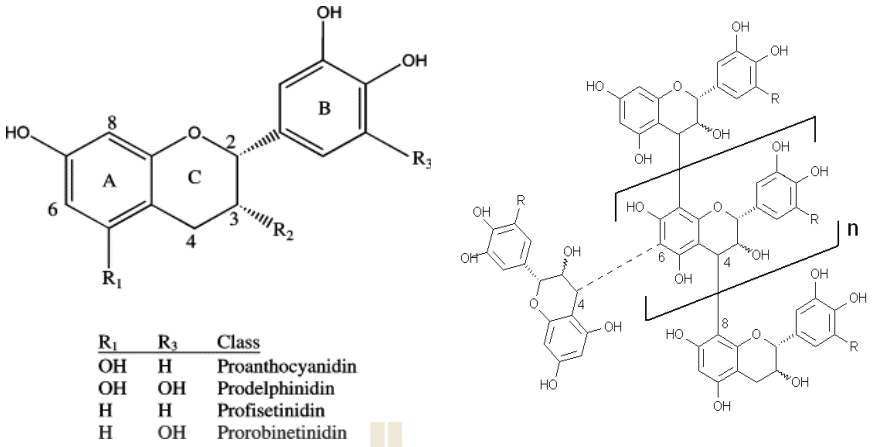
2.1 Condensed tannin and structure

Condensed tannin (CT), a group of flavonoid compounds, widely distributed in plant kingdom (Table 1). As the Figure 1 showed that CT consists of a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon bonds between flavanol subunits (Schofield et al., 2001).

Table 1 CT content in different plant.

Source	CT levels (g/kg DM)	References
Lotus corniculatus	47	Ramirez et al., 2005
Lotus pedunculatus	77	Ramirez et al., 2005
Hedysarum coronarium	84	Ramirez et al., 2005
	177	Foiklang et al., 2016
	158	Suchitra et al., 2008
Mangosteen peel	168	Ngamsaeng et al., 2006
	172	Wanapat et al., 2014
	153	Polyorach et al., 2015
Soap berry tree	121	Poungchompu et al.,2010
Sericea lespedeza	177	Puchala et al., 2005

Abbreviations: CT = condensed tannin; DM = dry matter.



R_1	R_3	Class
OH	H	Proanthocyanidin
OH	OH	Prodelphinidin
H	H	Profisetinidin
H	OH	Prorobinetinidin

Figure 1 CT basial units and structure.

source: Schofield et al., 2001.

2.2 Application of CT in ruminants

2.2.1 Effect of CT on feed intake and nutrient digestibility

For many years, CT have been known as antinutritional factor through reduce voluntary feed intake in ruminants. The bitterness of the CT might be to blame (Okunade et al., 2014). The reason by salivary proteins covalently bind to the oral mucosal cells and form a layer surrounding the soft structure of the mouth (Ma et al., 2014), when CT combine with saliva proteins to make them hard to absorb, increasing friction with the mouth and giving them a bitter taste (Baxter et al., 1997). In general, when CT in feed contain less than 5% did not affect feed intake, whereas dietary CT contain high than 5% could reduce voluntary feed intake (Frutos et al., 2004).

Recently, Poornachandra et al. (2019) who supplement CT-rich Tamarindus indica in diet of cattle did not affects feed intake and nutrient digestibility. Besides, Zhang et al. (2019) compared two source CT on nitrogen utilization in cow, the result shown the different CT source is different affect. In contrast, Caetano et al. (2019) found that

supplement 31.2 g/kg CT from grape marc could increase feed intake. Peng et al. (2016) suggesting that diet contain 49.3 g/kg CT from purple prairie clover could decrease feed intake but increase dry matter digestibility. Kronberg et al. (2018) and Adejoro et al. (2019) reported shown that diet contain CT could decreased nutrient digestibility (shown in Table 2). These studies suggesting that CT from different sources has different effects on different ruminants.

2.2.2 Effect of CT on antioxidant capacity in ruminant

CT not only inhibit the growth of microorganisms, but also could resist microbial degradation (Henis et al., 1964). As show in Table 3. CT is a natural and safe additive to regulate rumen fermentation and reduce methane production. Min et al. (2019a) supplement peanut skin in diet (CT contain 49 g/kg) of goat could increase VFA concentration decrease NH_3-N. Subsequent studies have shown that peanut skin in goat (CT contain 10.5 g/kg) could decrease VFA concentration and reduce methane production (Min et al., 2019b). Conversely, some reports show that tannin does not affect VFA concentration in the rumen (Koenig et al. 2018; Adejoro et al. 2019; Sharifi et al. 2019; Caetano et al., 2019). Moreover, CT could inhibit methane production to varying degrees (Min et al., 2019b; Poornachandra et al., 2019; Caetano et al., 2019; Adejoro et al., 2019; Wang et al., 2018). On the other hand, CT could reduce protein degradation in the rumen, the addition of CT to the ruminant diet could increase the nitrogen utilization efficiency.

Table 2 Effect of CT on feed intake nutrient digestibility and nitrogen utilization.

References	Plant source	Animals	CT g/kg	Feed intake	Nutrient digestibility					N utilization		
					DM	OM	CP	NDF	ADF	Absorbed	Excretion	Retention
Poornachandra, 2019	Tamarindus indica	Cattle	7.1	NS	NS	NS	NS	NS	NS	-	-	-
Zhang et al., 2019	bayberry	Cow	30	-	-	-	-	-	-	NS	NS	↑
	valonia	Cow	30	-	-	-	-	-	-	↓	NS	↓
Caetano et al., 2019	Grape marc	Cattle	31.2	↑	-	-	-	-	-	-	-	-
Koenig et al., 2018	Acacia mearnsii	Cattle	25	NS	-	-	-	-	-	-	-	-
Kronberg et al., 2018	lespedeza pellets	sheep	36.4	-	↓	-	↓	↓	↓	↓	↓	-
Adejoro et al., 2019	Acacia	sheep	40	NS	↓	↓	↓	↓	↓	-	NS	-
Peng et al., 2016	purple prairie clover	lambs	49.3	↓	↑	↑	NS	NS	NS	-	-	-
Wang et al., 2018	Hazel	sheep	14.95	-	-	↓	-	↓	↓	↓	↑	↓

"↑" = significant increase; "↓" = significant decrease; NS = no significant effect; "-" not determine. CT = condensed tannin; N = nitrogen; DM=dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Table 3 Effect of CT on rumen fermentation and methane production.

References	Plant source	Animals	CT additive levels g/kg	VFA				NH_3-N	pH	CH_4	yield
				AA	PA	BA	TVFA				
Min et al., 2019a	peanut skin	Goat	49	↑	↑	↑	↑	↓	NS	-	
Min et al., 2019b	peanut skin	Steers	10.5	NS	NS	↓	↓	-	NS	↓	
Poornachandra et al., 2019	Tamarindus indica	Cattle	7.1	↑	NS	NS	NS	↓	-	↓	
Caetano et al., 2019	Grape marc	Cattle	31.2	-	-	-	-	-	-	↓	
Koenig et al., 2018	Acacia mearnsii	Cattle	25	NS	NS	NS	NS	↓	↓	-	
Adejoro et al., 2019	Acacia	Sheep	40	NS	NS	NS	NS	NS	NS	↓	
Sharifi et al., 2019	pomegranate pee	Lambs	33.5	NS	NS	NS	NS	↓	NS	-	
Lima et al., 2019	mimosa tenuiflora	Sheep	23	-	-	-	-	↑	↓	NS	
Jayanegara et al., 2019	chestnut	Cattle	40	-	-	-	↓	↓	NS	-	
Peng et al., 2016	purple prairie clover	Lambs	49.3	NS	↓	NS	↓	↓	NS	-	
Wang et al., 2018	Hazel	Sheep	14.95	-	-	-	-	-	-	↓	

"↑" = significant increase; "↓" = significant decrease; NS = no significant effect; "-" not determine. CT = condensed tannin; TVFA = total volatile fatty acid;

AA = acetic acid; PA = propionic acid; BA = butyric acid; NH_3-N = ammonia nitrogen; CH_4 = methane.

Table 4 Effect of CT on blood biochemical indicators and antioxidant activity in ruminant.

References	Plant source	Animals	CT additive levels g/kg	Biochemical indicators					Antioxidant activity		
				GLU	BUN	TP	GLB	ALB	TAC	SOD	GPx
Zhang et al., 2019	Bayberry	Dairy cow	30	NS	↓	NS	NS	NS	-	-	-
	Valonia	Dairy cow	30	NS	NS	NS	NS	NS	-	-	-
Trana et al., 2015	Sulla	Goat	25.9	NS	NS	-	-	-	↑	NS	↑
Liu et al., 2013	Chestnut	Dairy cow	10	-	-	-	-	-	↑	↑	↑
Peng et al., 2016	Purple prairie clover	Lambs	49.3	NS	↓	NS	NS	NS	↑	NS	NS
Wang et al., 2019	Hazel	Sheep	14.95	-	-	-	-	-	↑	↑	↑
Liu et al., 2016	Chestnu	Lams	10	NS	-	NS	-	-	-	-	-

"↑" = significant increase; "↓" = significant decrease; NS = no significant effect; "-" = not determine. CT = condensed tannin; GLU = glucose; BUN = blood urea nitrogen; TP = total protein; ALB = albumin; GLB = globulin; GPx = glutathione peroxidase; SOD = superoxide dismutase; TAC = total antioxidant capacity.

2.2.3 Effect of CT on antioxidant capacity in ruminant

GLU and BUN are important indicators for evaluated energy and protein metabolism of ruminants, TP, GLB, and ALB are evaluating immunologic function parameter. A study by Liu et al. (2013) found that chestnut CT could increase TAC SOD GPx capacity in plasma of cow. Similarly, Wang et al. (2019) who used hazel CT in sheep diet, the TAC SOD and GPx capacity were significantly increased. Moreover, Zhang et al. (2019) suggesting that valonia CT was no effect on blood biochemical indicators, bayberry CT was decrease BUN concentration in cow (showed in Table 4). These might prove that CT was no negative affect on animal immunologic function.

2.3 Mechanism of CT

2.3.1 Protect protein in the rumen

As well know, CT was used as an additive to protect protein escaping rumen. CT can combine with protein through hydrogen bonds and hydrophobic bonds to produce stable complex at pH range 3.5-7.0 in the rumen (Getachew et al., 2000; Min et al., 2005), then subsequently release and digest and absorbed of protein in the acidic conditions of the abomasum and small intestine (Min et al., 2005; Martin and Martin 1983). Kumar and Singh (1984) who think there were four main ways that CT bind to protein:

(1) Phenolic rings interact with hydrophobic groups of proteins or amino acids.

(2) The hydroxyl group of the CT polymer forms a hydrogen bond with the oxygen in the protein peptide bond amide.

(3) Phenolic ions form ionic bonds with cations in proteins.

(4) The oxidation process forms irreversible covalent bonds with protein nucleophilic groups.

On the other hand, CT also can though inhibit proteolytic bacteria growth and decrease the number of proteolytic bacteria to reduce protein degradation in rumen (Min et al., 2003). In short, CT protein precipitation is the result of complex interaction of multiple factors (Lorenz et al., 2013).

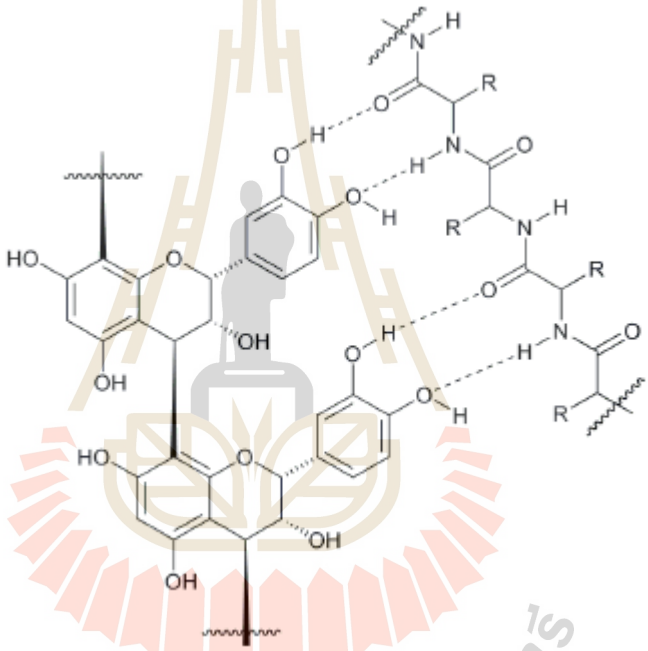


Figure 2 Mechanism of condensed tannin reduce protein degradation.
source: Naumann et al., 2017.

2.3.2 Mechanism of CT on rumen fermentation

CT affects rumen fermentation mainly through the following four aspects:

(1) CT combining with nutrients in the medium, limits the use of substrates by microorganisms to inhibit their growth (Scalbert, 1991).

(2) CT could effect on microbial membranes by inhibiting oxidative phosphorylation or by affecting membrane integrity, may react with sulfhydryl groups of enzymes and form covalent linkages with them and contribute to increase the efficiency of binding to proteins through non-covalent linkages through oxidative polymerization (Scalbert, 1991).

(3) CT is generally considered to inhibit the growth of rumen microorganisms (Patra and Saxena, 2009). CT reduce the biohydrogenation of rumen by inhibiting the growth of microorganism Butyrivibrio fibrisolvens (Vasta et al., 2009). CT has a greater effect on gram positive bacteria than on gram negative bacteria (Ikigai et al., 1993), this due to CT inhibit their growth and protein enzymes (Smith and Mackie, 2004).

(4) Biological systems, including microorganisms, are highly dependent on the metal ion state of the environment (Brown, 1963). CT could cause the loss of metal ions through the formation of chelation with metal ions (Naumann et al., 2017).

The effect of CT on rumen fermentation is not a single factor but the result of the interaction of various factors.

2.3.3 Mechanism of CT on antioxidant capacity

The antioxidant properties of CT are reflected in the reduction of harmful free radicals by providing electrons to free radicals to form stable structures (Koleckar et al., 2008). In addition, two adjacent phenolic hydroxyl groups can form chelates with metal ions in the form of stable oxygen anions (Hemingway, 2012) shown in Figure 4.

The CT antioxidant capacity was determined by its structure, especially the number of hydroxyl groups and their positions (Rice-Evans et al., 1996).

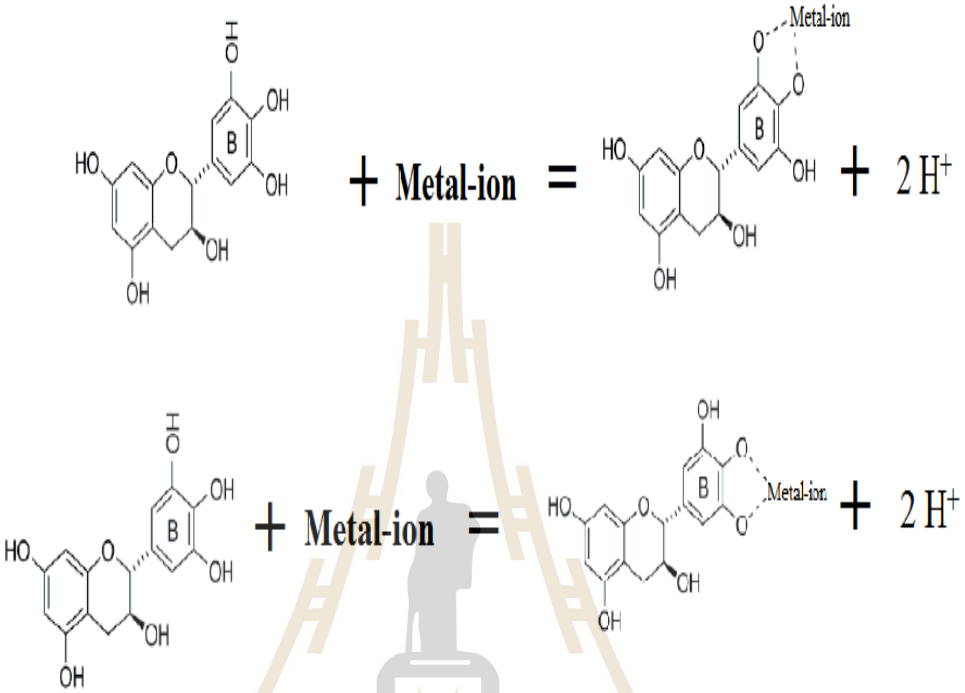


Figure 3 Mechanism of condensed tannin structure on antioxidant.
source: adapted from Naumann et al., 2017.

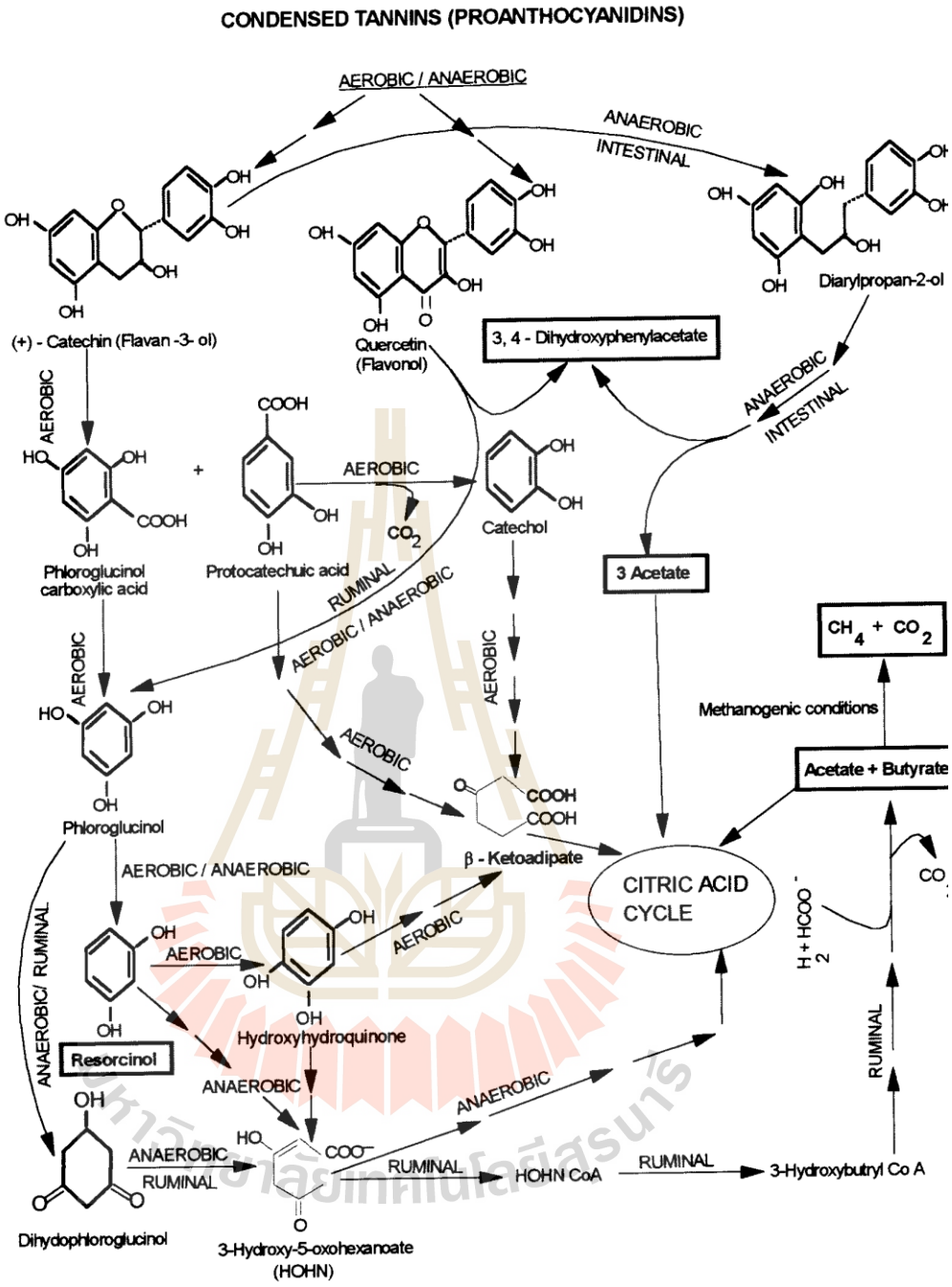


Figure 4 Pathway biodegradation of condensed tannin.

Source: Bhat et al., 1998.

2.4 Pathway biodegradation of condensed tannin

The aerobic breakdown of flavonoid compounds from concentrated tannins occurs in two pathways (Bhat et al., 1998; figure 2). The first degradation mode was to decompose the heterocyclic ring of flavonoid 3-alcohol catechin into resorcinol carboxylic acid and protocatechuic acid (Barz and Hosel, 1975). Phloroglucinol carboxylic acid, by decarboxylation and scission of the aromatic rings by various oxygenase, finally forms β-ketoadipate, an aliphatic acid, through intermediates like phloroglucinol, resorcinol, hydroxy hydroquinone and maleic acetate. Protocatechuic acid is also converted to β- ketoadipate through β-carboxy cis, cis muconate and catechol pathways (Bhat et al., 1998). Quercetin, a flavanol, is broken into phloroglucinol and 3, 4-dihydroxyphenyl acetate through the second pathway of flavonoid degradation. The former ends up as β-ketoadipate, while the latter is not degraded further (Fewson, 1981; Gibson and Subramanian, 1984; William, 1986).

2.5 Mangosteen peel

Mangosteen, a slow-growing tropical evergreen tree with leathery, glabrous leaves. The tree can attain 6-25 m in height and is mainly found in India, Myanmar, Sri Lanka, and Thailand (Jung et al., 2006). Its peel rich condensed tannin (15.3-17.7% DM) and crude protein (15.3-21.5% DM) (Polyorach et al. 2015; Foiklang et al. 2016; Suchitra and Wanapat, 2008). Paengkoum et al. (2015) fund that condensed tannin from mangosteen peel molecular weight defined as Mw, was 2,081 Da and Mn was 1,133. The mangosteen peel have been used as folk medicine for thousands of years. The thick mangosteen rind has been and is used for treating catarrh, cystitis, diarrhea, dysentery, eczema, fever, intestinal ailments, pruritis and other skin ailments (Akao et al., 2008). Wanapat et al. (2014) who suggested that mangosteen peel powder can be

used as good sources of protein to supplement ruminant feeding. Widjastuti et al. (2018) who suggest that addition of mangosteen peel meal (MPM) in the ration until 7.5% gave the best protein efficiency ratio of Sentul chicken. In ruminant, a study by Polyorach et al. (2016) suggested supplementation of mangosteen peel at 300 g/head/day with yeast fermented cassava chip protein as a protein source in the concentrate mixture revealed an enhancement of rumen fermentation and methane reduction in lactating dairy cows.

Table 5 composition of condensed tannin in mangosteen peel (Khundamri et al., 2018).

Compound	**Content (g/kg)**
Catechin	1.8-4.46
Epicatechin	6.48-16.69
Catechin mercapto	31.74-59.50
Catechin gallate mercapto	2.05-3.97
Epigallocatechin gallate	3.19-9.93

2.6 References

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CHAPTER III

EFFECT OF MANGOSTEEN PEEL POWDER ON RUMEN FERMENTATION *in vitro*

3.1 Abstract

The purpose of this study was to estimate the effect of mangosteen peel powder (MPP) on rumen fermentation and methane production *in vitro*. Supplement of different levels (0 5% and 10% of DM, respectively) of mangosteen peel powder according to the completely randomized design (CRD) for 0 3 6 9 12 24 and 48 hours gas production *in vitro*. The results showed that MPP had significant effect on gas production ($P<0.05$). Moreover, MPP also significantly decreased ($P<0.05$) the organic matter digestibility (OMD) metabolizable energy (ME) and effective degradability (ED) relative to control. In addition, lower level of MPP decreased ($P<0.05$) gas production from the immediate soluble fraction (a) gas production from the insoluble fraction (b) gas production rate constant (c) and potential extent of gas production (a+b). In addition, acetic acid (AA) butyric acid (BA) total volatile fatty acid (TVFA) and methane (CH_4) production were decreased by MPP. Taken together, the 5% MPP decreased CH_4 production and OMD in the rumen, and also enhanced the amount of nutrient of escape rumen.

Keywords: mangosteen peel powder, condensed tannin, rumen fermentation, methane

3.2 Introduction

For many years, global warming was become an important factor that restricts economic development. Methane is a major greenhouse gas (GHG), which is 25 times stronger than carbon dioxide (Kerr, 2010) and contributes 20% of GHG (IPCC, 2001). Livestock contribute 33% methane emission of total anthropogenic Methane (calculate from Scheehle et al., 2006) thereinto 81% produced by ruminant (Tapio et al., 2017), in addition, the methane production will lead to loss the dietary energy (Foiklang et al., 2016; Johnson and Johnson, 1995), reducing methane emission from ruminant become urgent affairs. There is research shown plant secondary metabolites (such as condensed tannin, anthocyanins saponin etc.) have the potential to reduce methane in ruminant (Sliwinski et al., 2002; Szumacher-Strabel and Cieslak, 2010). Waghorn et al. (2002) and Woodard et al. (2001) they found that feeding with forage containing condensed tannin could reduce methane production.

Mangosteen (*Garcinia mangostana* L.), a tropical fruit widely distributed in southeast Asian countries such as Thailand, Malaysia and Vietnam. Its peel contains condensed tannin 15.3-17.7% and crude saponin 10-11.9% of dry matter (Polyorach et al., 2015; Ngamsaeng et al., 2006; Wanapat et al., 2014; Foiklang et al., 2016), as a traditional medicine for treat skin infections and other medical conditions (Obolskiy et al., 2009; Mahabusarakam et al., (1987). To note, mangosteen peel have been shown to have the potential to improve rumen fermentation as an additive (Wanapat et al., 2014). A study by Pilajun and Wanapat (2011) showed that additive mangosteen peel could improve rumen fermentation by positively affecting the ruminal on microbial population in swamp buffalo. Moreover, the supplement of mangosteen peel powder

(MPP) significantly increase propionic acid (Wanapat et al., 2014), which can improve feed energy utilization (Feng et al., 1996).

Therefore, mangosteen peel may through affect rumen fermentation and thus affecting health of body. The purpose of this study was to evaluate mangosteen peel powder on rumen fermentation in meat goat.

3.3 Materials and methods

3.3.1 Experimental design and sampling methods

This experiment was adopted one factor completely randomized design (CRD) were: (1) control (without MPP); (2) low level of MPP (5%, LM); and high level of MPP (10%, HM). Total four health male meat goats (Anglo-Nubian cross with Thai native) with similar body weight (18.1±1.49 kg; mean ± standard deviation) were selected from Suranaree University of Technology (SUT) farm used as source of rumen fluid. Prepare artificial saliva with reference Menke et al. (1979), the rumen fluid was collected via mouth by vacuum pump into a sealed glass bottle, then transported to laboratory, immediately filter with four layers of gauze saturate placed in 39℃ water bath with continuously inject carbon dioxide and mix with buffer solution in a ratio of 10:20. The fermentation substrate was ratio of concentrate to roughage is 40:60, the nutrient requirement according to National Research Council (NRC, 1981) show in Table 6. Approximately 200mg of substrate sample were weighed accurately into 100ml glass syringe (Kabuskiki Kaisha, Japan), then mixed with 30ml rumen culture fluid in each syringe for 3 6 9 12 24 and 48 hours to estimate gas production (GP), the GP was calculated according to the formula by Orskov and McDonald (1979):

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

where y is the gas produce value at time t, a is the immediately soluble fraction (ml), b is the insoluble fraction (mL), c is the rate constant for the insoluble fraction b (%/h), t expresses incubation time (h), and a+b represents the potential extent of gas production (mL).

The fermentation was stopped by dip the syringe in ice-cold water, immediately measured pH by pH meter (Sartorius AR company, Gottingen, Germany), then centrifugation at 3000 r/min for 15 min at 4℃ (Sorvall™ XT Centrifuge Series, Thermo Credit, LLC, USA), supernatant was removed for analysis. Ammonia nitrogen (NH_3-N) was to determine reference AOAC (1990) by premium Kjeldahl steam distillation systems (VAPODEST® 200-450, Gerhardt company, Germany)

3.3.2 Chemical analysis

All ingredients of feedstuff were oven dried at 60℃ for 48h (Modell 100-800, Memmert Co. KG, Germany), then passed 1-mm sieve. According to the Association of Official Analytical Chemists (AOAC 1990), the dry matter (DM) was determined after dried-oven at 105℃ for 4h, and the ash was determined after incineration (at 550℃ for 4h, Carbolite AAF1100 Ashing Furnaces, Germany), the crude protein (CP) was analyzed used method of Kjeldahl apparatus (Distillation unit, Kjeltec™ 8100, Foss Co., Ltd, Hillerød, Denmark). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed refer methods of AOAC (1990) and Van Soest et al. (1991). Gross energy (GE) was determined by calorimeter (Parr 6200, Moline, IL, USA). Ether extract (EE) was measured using Soxtec system (Soxtec™ 2050, Foss Co., Ltd, Hillerød, Denmark). The total phenolics (TPs) and condensed tannin (CT) were analyzed by microplate reader (Epoch, BioTek Instruments, Inc.

Winooski, Vermont, USA), according to Folin-Ciocalteu method and method of Porter et al. (1986), respectively. The rumen fluid was centrifugal on 13,000rpm for 10min at 4℃, the supernatants for analyzed ammonium nitrogen (NH3-N) and volatile fatty acid (VFA). The 5ml supernatant rumen fluid was used fort analyzed NH_3-N through premium Kjeldahl steam distillation systems (VAPODEST® 200-450, Gerhardt company, Germany) according to method of Bremner et al. (1965) with a minor modification (steam power 80%, NaOH addition 80ml, water addition 50ml, reaction time 5s, distillation time 4min, sample suction 30s). Removing 1ml supernatant rumen fluid filtered through Nylon Syringe filter (13mm 0.45um with PP prefilter, P/N TNL1345, Xiboshi, China) for analyzed VFA by gas chromatography (GC, CP-3800, Varian Medical Systems Company, California, USA) with a flame ionization detector (FID, H2 flow 30ml/min, air flow 300ml/min) and an Agilent JandW GC Columns (DB-WAX, 30m×0.320mm×0.15μm, California, USA). The detector and injector temperature are 250℃，the columns temperature programed were initial held 80℃ for 5min, then increased to 170℃ at 10℃/min, finally increased to 250℃ at 30℃/min held for 5min. The total VFA was calculated reference to the formula: TVFA= AA+PA+BA. The methane (CH_4) production was calculated according to method of Foiklang et al. (2016), OMD was calculated according to Karabulut et al. (2007), ME, and ED were calculated according to Tian et al. (2018).

$$CH_4 \text{ production} = 0.45*(\%AA)-0.275*(\%PA) + 0.4*(\%BA) \quad (1)$$

$$OMD\ (\%) = 14.88+0.889GP\ (24h) + 0.45CP + 0.0651*XA\ (\text{ash content}) \quad (2)$$

$$ME(MJ/kg) = -0.20 + 0.1410*OMD \quad (3)$$

$$ED(\%) = a+bc/(k+c) \quad (4)$$

Where, k is ruminal outflow rate and the value sets as 0.031 h.

3.4 Statistical analysis

All data were analysis performed by one-way ANOVA using SPSS16.0 software. Differences among treatments were examined using Duncan's multiple comparison method to test the significance of the differences between treatment. The differences were significant at $P < 0.05$.

3.5 Results

3.5.1 Gas production

As Figure 5 shown, the gas production in each group were increase with the prolongation of time, total gas production with supplement MPP was significant lower ($P<0.05$) than control group at all time point, the additive levels do not affect ($P>0.05$) gas production. At 0~12h, the slope of the control group was higher than MPP group, the gas production rate was fast. After incubation 24h, the curves tend to be flat in each of group, the gas production trend to maximum.

3.5.2 Effect of different levels of MPP on rumen fermentation characteristic and Methane production

Supplement MPP were no effect ($P>0.05$) on pH, NH_3-N, PA content, and BA %Molar. All parameter (except for GP) in HM group was no effect ($P>0.05$) compared to control group. The LM group was decreased ($P<0.05$) GP, AA, TVFA, CH_4, AA %Molar, and ratio of AA to PA, increased ($P<0.001$) PA %Molar compared to other groups shown in Table 8.

Table 6 Substrate for gas production *in vitro*.

Ingredient	Control	LM	HM
Rice straw	600.00	600.00	600.00
Soybean meal	170.00	170.00	180.00
Corn	100.00	110.00	20.00
Cassava chip	80.00	30.00	20.00
Rice bran	30.00	20.00	60.00
Limestone	5.00	5.00	5.00
Premix	10.00	10.00	10.00
Salt	5.00	5.00	5.00
MPP	0.00	50.00	100.00
Total	1000.00	1000.00	1000.00
Nutrient composition			
DM	90.26	90.41	90.67
OM	87.58	87.81	87.38
CP	10.98	10.89	11.01
GE Mj/kg of DM	17.02	17.71	18.43
ADF	39.29	40.22	42.41
NDF	60.73	60.56	64.15
TPs	-	1.25	2.51
CT	-	0.75	1.51

LM = lower level of mangosteen peel powder; HM = high level of mangosteen peel powder, DM = dry matter, OM = organic matter, CP = crude protein, GE = gross energy, ADF = acid detergent fiber, NDF = neutral detergent fiber, TPs = total phenolics, CT = condensed tannin, "-" = not detected.

Vitamin premix: vitamin A 12000000 IU, vitamin D3 2400000, vitamin E 750mg, vitamin B1 980mg, vitamin B2 960mg, vitamin B6 654mg, vitamin B12 1658µg, vitamin B9 133mg, calcium pantothenate 2940mg, nicotinamide 8910mg, K 637 mg, vitamin B4 446 g, Na 289.4 g, and citrate 850.5mg.

Mineral premix (per kg): NaCI 825g, Mg 2g, Mn 830mg, Fe 4.3g, Zn 810mg, Co 20mg, I 50mg, Se 10mg, Cu 220mg, P 15g, Ca 50g, other 500mg.

Table 7 Nutrient composition of MPP, g/kg of DM.

GE, Mj/kg	DM	OM	CP	EE	ADF	NDF	TPs	CT
30.55	921.65	957.327	39.82	110.96	618.64	590.06	250.90	150.50

MPP = mangosteen peel powder, GE = gross energy, DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, ADF = acid detergent fiber, NDF = neutral detergent fiber, TPs = total phenolics, CT = condensed tannin

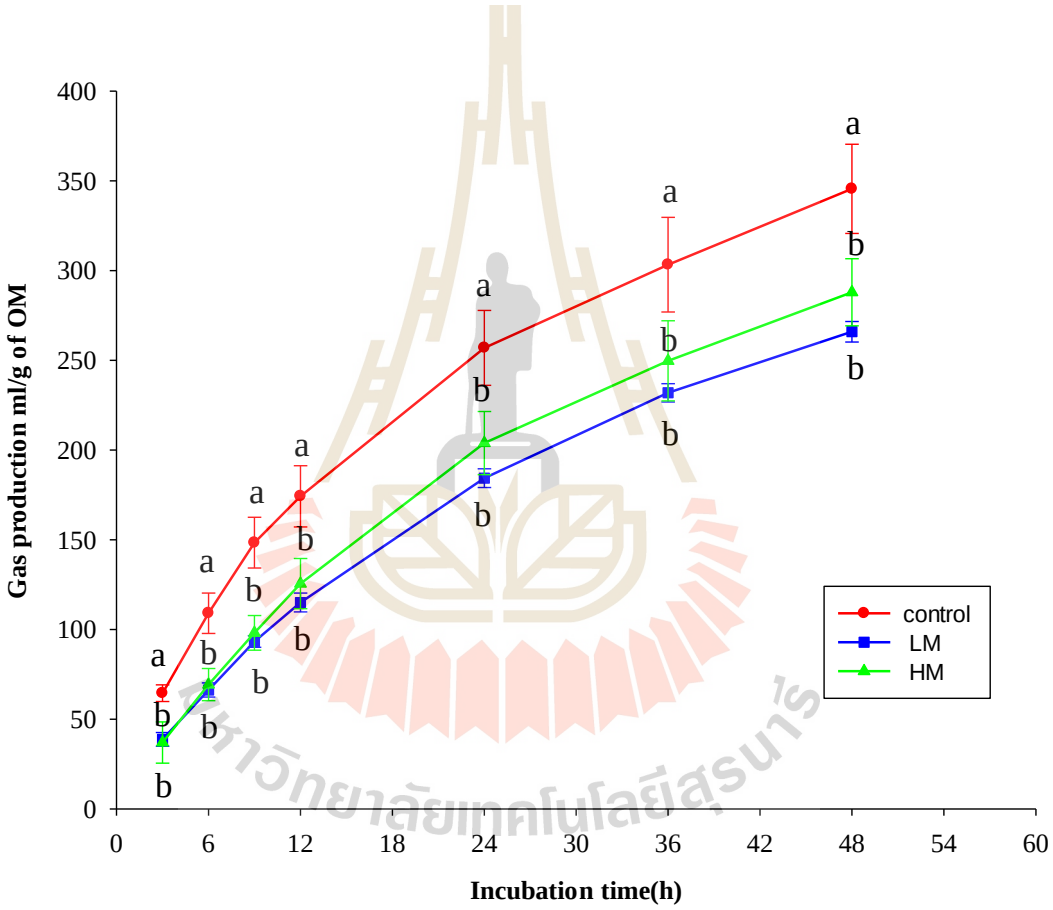

Figure 5 Gas production with or without supplement MPP.

Table 8 Effect of different levels of MPP on ruminal fermentation and methane production *in vitro*.

Item	control	LM	HM	SEM	P-value
GP ml/g of DM	248.20[a]	177.96[b]	196.69[b]	6.93	<0.001
pH	5.97	5.97	6.03	0.02	0.117
NH_3-N, mg/dL	24.17	17.26	16.81	3.17	0.224
AA, mmol/L	43.65[a]	33.49[b]	41.77[a]	1.59	0.001
PA, mmol/L	17.76	16.26	17.90	0.52	0.077
BA, mmol/L	8.52[a]	7.12[b]	9.01[ab]	0.49	0.039
TVFA, mmol/L	69.93[a]	56.86[b]	68.68[a]	2.46	0.003
CH_4	25.97[a]	23.66[b]	25.35[a]	0.30	<0.001
VFA, % Molar					
AA	62.93[a]	58.92[b]	60.78[a]	0.55	0.002
PA	25.40[a]	28.58[b]	26.21[a]	0.42	<0.001
BA	12.20	12.51	13.01	0.38	0.330
AA/PA	2.46[a]	2.07[b]	2.33[a]	0.05	<0.001

Values with different letter superscripts mean significant difference (P<0.05).

LM = lower level of mangosteen peel powder; HM = high level of mangosteen peel powder; SEM = standard error of the mean, GP = gas production, DM = dry matter, NH_3-N = ammonia nitrogen, AA = acetic acid, PA = propionic acid, BA = butyric acid, TVFA = total volatile fatty acid, CH_4 (mol/100mol VFA) = methane production, AA/PA = ratio of acetic acid to propionic acid. CH4 production (mol/100mol of TVFA) = 0.45*(%AA)-0.275*(%PA) + 0.4*(%BA).

3.5.3 Effect of MPP on gas production kinetics

MPP was trend to decrease (P=0.082) on gas production from the insoluble fraction (b). However, the OMD, ME, ED, gas production from the immediately soluble fraction (a), and gas production rate constant (c) were significant decrease (P<0.05) with supplement MPP, to note, for potential extent of gas production (a+b) the LM group was lower than control, HM was no significant difference (P>0.05) with control (Table 9).

Table 9 MPP on gas production kinetics in incubation 24h.

Item	Control	LM	HM	SEM	P-value
OMD (%)	80.55^{a}	69.52^{b}	72.46^{b}	1.09	<0.001
ME (MJ/kg)	11.16^{a}	9.60^{b}	10.02^{b}	0.15	<0.001
ED (%)	39.35^{a}	29.90^{b}	32.49^{b}	0.94	0.001
a (ml)	4.13^{a}	1.56^{b}	1.56^{b}	0.31	0.001
b (ml)	60.25	55.04	58.88	1.40	0.082
c (% h)	0.06^{a}	0.04^{b}	0.04^{b}	0.004	0.001
a+b (ml)	64.39^{a}	56.60^{b}	60.45^{ab}	1.37	0.013

Values with different letter superscripts mean significant difference (P<0.05).

LM = lower level of mangosteen peel powder; HM = high level of mangosteen peel powder; SEM = standard error of the mean, OMD = organic matter digestibility, ME = metabolizable energy, ED = effective degradability, a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, c = gas production rate constant; a+b = potential extent of gas production.

3.6 Discussion

3.6.1 Gas production

Gas production technique does not require sophisticated equipment and the large numbers of samples can be incubated and analyzed at the same time (Aung et al., 2015). The close association between rumen fermentation and gas production has long been recognized, which helps to measure nutrient utilization in feed, and there have been numerous studies in describing animal digestion (Marston, 1948; Sallam et al., 2007; Elghandour et al., 2016), Menke and Steingass (1988) proved that ruminal DM degradation was positive correlated to gas production value.

In current study, the gas production in each of group were experienced a rapid increase and then gentle within 48h (Figure 5), the result similarly with Rufino-moya et al. (2019). Investigate its reason, on the one hand, the fermentation substrate was degradation by microbes lead to substrate decreased with the incubation time increased, on the other hand, due to limitation of syringe system the fermentation product cannot be expelled with the fermentation product (such as NH_3-N, VFA) accumulate might effect fermentation microbes activity and end-product (Sommart et al., 2000). Moreover, MPP rich contain condensed tannin, which can react with other sources of protein such as enzymes secreted by rumen bacteria inhibit rumen carbohydrate fermentation (Min et al., 2003).

3.6.2 Effect of different levels of MPP on rumen fermentation characteristic and methane production

NH_3-N is an important parameter for nutrient in supporting efficient rumen fermentation (Wanapat and Pimpa, 1999), pH is mainly indictor to estimate rumen environment. In this case, was no observed MPP effect (P=0.224) on NH_3-N,

this indicates that MPP has no significant effect on the degradation of dietary protein. This result was similarly with Shokryzadan et al. (2016) who used MPP supplementation accounted for 25% and 50% of the substrate *in vitro* rumen fermentation, Polyorach et al. (2015) who also got similar result in dairy cows. The methane production was reduced by LM the might result of reduce methanogens and/or protozoa (Ngamsaeng et al., 2006; Foiklang et al., 2016). Carbohydrate degradation was effected with addition of LM, thus LM was decreased AA, BA, TVFA content, the transformation of VFA from AA fermentation to PA fermentation might related to the transfer of hydrogen from methane pathway (Anantasook et al., 2012), it also could explain that AA %Molar ($P=0.002$), and ratio of AA to PA ($P<0.001$) were decrease, the PA %Molar was increased ($P<0.001$). However, the HM was no difference with control, the reason might due to NDF and/or crude protein in HM group was higher than control group (Table 8), the exact reasons remain unclear and further observation is required. The GP was decrease 28.30% and 20.75% ($P<0.001$) by addition MPP, the reason might due to condensed tannin limit carbohydrate degradation (Min et al., 2003).

3.6.3 Effect of MPP on rumen gas production kinetics

In this case, the OMD was decreased ($P<0.001$) 13.69% and 10.04% that conform to the gas production result (table 9), thus MPP might has the potential to prevent rumen bloating (McMahon et al., 1999). ME represents the energy available for tissues, which approximately equal 81% of digestibility energy (Savage and Nolan, 2009), in the present study, the ME was decreased with additive MPP, which might due to the reduced rumen degradability, the complex formation of condensed tannins with dietary proteins and carbohydrates, and the decreased proteolysis, cellulose

hydrolysis and general fermentation activity of rumen microorganisms (Muhammed et al., 1994, Schropfer and Meyer, 2016), it might also be the reason for decrease of , gas production from the immediately soluble fraction (a), gas production from the insoluble fraction (b), gas production rate constant (c), the similarly result was observed in report of El-Waziry et al. (2005). For potential extent of gas production (a+b), the HM was no effect compare to control, the reason might due to the DNF in HM group was higher than control, but the specific reasons need to be further studied.

3.6.4 Conclusions and suggestion

In conclusions, we conclusion that 5% MPP could reduce methane production, did not adversely to pH and NH_3-N, in addition, decrease of GP and OMD was observed. However, MPP as additive need to research in feeding trail, because the fermentation products in the syringe system cannot be escape in time and the palatability which is an important index for feed quality cannot considered, failed to simulate the real physiology of the animal.

3.7 References

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CHAPTER IV

UTILIZATION OF MANGOSTEEN (*Garcinia mangostana* L.) PEEL POWDER ON GROWTH PERFORMANCE RUMEN FERMENTATION NITROGEN UTILIZATION AND PLASMA ANTIOXIDANT CAPACITY IN MEAT GOATS

4.1 Abstract

The purpose of present work was to estimate the supplementation of mangosteen peel powder (MPP) on growth performance, rumen fermentation and plasma antioxidant capacity in meat goat. Total twelve Anglo-Nubian crossed with Thai native male goat (bodyweight 23.1 ± 1.49kg) were randomly assign to two groups: (1) control group: basal diet, and (2) treatment: basal diet + 4.125% MPP. The results showed that the feeding of MPP had no effect (P>0.05) on dry matter intake (DMI), organic matter intake (OMI), and nutrient digestibility. Whereas, it significantly increased (P=0.005) BW, %. In addition, urine nitrogen (N) excretion was decreased (P=0.024) by MPP, the percentage of N excretion tended to decrease (P=0.096), but the percentage of N retention tended to increase (P=0.096). The concentration of ammonium nitrogen, acetic acid, and total volatile fatty acids were reduced by MPP; MPP decreased (P<0.05) methane production, acetic acid and ratio of acetic acid to propionic acid (AA/PA), increased (P<0.001) propionic acid. Supplementation of MPP indicated a trend to increase serum total protein (TP) (P=0.082) and albumin (ALB) (P=0.077). Moreover, MPP increased (P=0.017) the 2,2-diphenyl-1-picrylhydrazyl

scavenging activity, it also increased (P=0.044) the glutathione peroxidase activity. Collectively, MPP could improve plasma antioxidant capacity, and it had the potential to improve N utilization and serum immunity function.

Keywords: mangosteen peel powder, condensed tannin, nitrogen balance, antioxidant capacity, meat goat

4.2 Introduction

Oxidative stress (OS) and free radical (FR) are negative effect that occurs when the accumulation of reactive oxygen species (ROS) in an animal exceeds its antioxidant and neutralizing capacity (Samadieh et al., 2016; Halliwell, 2006), FR can trigger the production of ROS (Thatoi et al., 2014). OS might lead to decreased animal productivity and decreased immune function (Yuan et al., 2007). In tropical countries, heat is a major contributor to the increase in ROS, which can lead to OS (Kumar et al., 2011). OS was usually treated by improving antioxidant enzyme activity, which could reduce the activity of ROS and prevent its conversion to harmful forms (Varjovi et al., 2015). The major antioxidant enzymes in mammals are superoxide dismutases (SOD), catalase (CAT), and gluthathione peroxidases (GPX) (Yuan et al., 2007; Varjovi et al., 2015). In brief, the superoxide radical (O_2^-) is initially reduced by SOD to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Varjovi et al., 2015), and then reduced by GPx and CAT to water and alcohol (Halliwell and Gutteridge, 1986; Simko, 2007).

Plant secondary metabolites are a natural and safe additive, they have been proved to have the functions of antioxidant, antibacterial, protective protein and reducing rumen methane production and other function (Gasmi et al., 2019; Wina et al., 2017; Jafari et al., 2019; Jadhav et al., 2017). Plant secondary metabolites such as

condensed tannin can provide electrons to free radicals, allowing them to form stable structures that reduce harmful radicals (Koleckar et al. 2008). A study by Mancini et al. (2019) shown that supplement quebracho and chestnut tannin mix could increase plasma CAT and GPx concentration in rabbits, Peng et al. (2016) found that TAC activity in lamb was increased by the addition of purple prairie clover condensed tannin. Moreover, the ability of condensed tannin on DPPH scavenging has also been widely reported (Wettasinghe and Shahidi, 2000; Myint et al., 2017; Jo et al., 2015). Nevertheless, condensed tannin from different source have different antioxidant ability (Valenti et al., 2008).

Mangosteen (*Garcinia mangostana* L.) is also known as the queen of fruit, which a tropical fruit widely distributed in Thailand (Wanapat et al., 2014; Pedraza-Chaverri et al., 2008). Mangosteen peel contain-rich secondary metabolites such as condensed tannin, saponin, and anthocyanins (Fu et al., 2007; Mai and Tan, 2013). It has been used as a traditional medicine to treat wound infections for years (Mahabusarakam et al., 1987; Obolskiy et al., 2009). However, there was poorly reports showed the mangosteen peel as the feed additive for ruminants in in vivo feeding trial (Paengkoum et al., 2015). There was research in dairy cow show that mangosteen peel was decreased the ratio of acetate to butyrate, greatly increased milk protein, and increased profits (Polyorach et al., 2015). Moreover, Norrapoke et al. (2012) found that mangosteen peel could reduce protozoa content without effect rumen fermentation in lactating dairy crossbreds.

Nevertheless, to the best of our knowledge, there has been no found that mangosteen peel on antioxidant activity in ruminants. In order to fill that gap, we hypothesis that mangosteen peel powder could increase plasma antioxidant activity

without negative effect on growth performance and rumen fermentation in meat goat. Therefore, the current study was to estimate the mangosteen peel powder on growth performance, rumen fermentation and plasma antioxidant activity in meat goats.

4.3 Materials and methods

4.3.1 Experimental design and treatments

The complete randomized design (CRD) was used in this study. Total two treatments were: (1) control group, basal diet, and (2) treatment, basal diet+4.125% mangosteen peel powder, the mangosteen peel powder was the commercial product (Wallawit-herb company, Bangkok, Thailand). The experimental period was 26 days (7 days for acclimatization, 14 days for adapt experimental diet, and 5 days for collected sample).

4.3.2 Animal management and diets

All experimental animals were fed according to Suranaree University of Technology (SUT) animal welfare rules. Total twelve health Anglo-Nubian cross with Thai native male goats (body weight: 23.10 ±1.49kg; mean ± standard deviation) were selected from SUT`s farm (Nakhon Ratchasima, Thailand 30000). All goats were separated into cleanly single pens, free to drink cleanly water. The dietary nutrition (Table 10) was reference to 25 kg body weight of meat goat according to National Research Council (NRC, 1981), the diet was feeding twice per day with equal amounts at 9:00 and 17:00, respectively.

Table 10 Dietary ingredient and nutrient composition.

Ingredients	**Treatment**	
	Control	**MPP**
Pangola hay	670.00	670.00

Soybean meal	148.50	148.50
Corn	41.25	41.25
Cassava chip	82.50	41.25
Rice bran	41.25	41.25
Salt	4.50	4.50
Limestone	4.00	4.00
Vitamin premix	4.00	4.00
Mineral premix	4.00	4.00
MPP	0.00	41.25
Total	1000.00	1000.00
DM	909.26	910.82
Base on DM, g/kg		
OM	914.92	916.15
GE, Mj/kg	24.67	25.26
CP	102.46	101.90
EE	32.50	36.34
ADF	311.43	319.11
NDF	460.65	462.44
TPs	ND	10.35
CT	ND	6.21

Note: MPP=mangosteen peel powder; DM=dry matter; OM= organic matter; GE=gross energy; CP=crude protein; EE= ether extract; ADF = acid detergent fiber; NDF=neutral detergent fiber; TPs= total phenolics; CT=condensed tannin. Vitamin premix: vitamin A 12000000 IU, vitamin D3 2400000, vitamin E 750mg, vitamin B1 980mg, vitamin B2 960mg, vitamin B6 654mg, vitamin B12 1658μg, vitamin B9 133mg, calcium pantothenate 2940mg, nicotinamide 8910mg, K 637 mg, vitamin B4 446 g, Na 289.4 g, and citrate 850.5mg. Mineral premix (per kg): NaCI 825g, Mg 2g, Mn 830mg, Fe 4.3g, Zn 810mg, Co 20mg, I 50mg, Se 10mg, Cu 220mg, P 15g, Ca 50g, other 500mg.

4.3.3 Scanning electron microscopy (SEM)

Recording the daily feeding and residual feed for calculate dry matter intake (DMI). On the 22d to 26d of each period, the feces approximately 100g was

collected after mixed from each day, then dried in oven at 60℃ for 72h and pass through 1-mm sieve stored in -20℃. The urine (approximately 30ml) was used 20% sulfuric acid (H_2SO_4) at a ratio of 4:1 to acidize, stored in -20℃ after mixed from each day.

On the end day of each period, the blood was collected at 0h and 4h after feeding through jugular vein by FUSHINO® Pro-Coagulation tube (4ml) and VACUETTE® K3-EDTA tube (4ml) respectively. The blood in FUSHINO® Pro-Coagulation tube was send to SUT hospital for analysis glucose (GLU) blood urea nitrogen (BUN), total protein (TP), albumin (ALB). Another the blood sample in VACUETTE® tube, the plasma was collected after centrifugation at 3,000rpm for 10min at 4℃ (Eppendorf 5810R refrigerated centrifuge, Hamburg, Germany), then the plasma was transported into clean 1.5ml tube store in -80℃ until analysis in the future.

The rumen fluid was collected on the 26d at 0h and 4h after feeding, through PVC tube by a vacuum pump (Electrolux Lite company, Thailand), then filtered through four layers of gauze, immediate measured pH by pH basic benchtop pH meters (Sartorius AR company, Gottingen, Germany), approximate 20ml rumen fluid mixed with 5ml hydrochloric acid (6N HCl) stored in -20℃ for future analysis.

4.3.4 Chemical analysis

Ingredient nutrient composition

All ingredients of feedstuff were oven dried at 60℃ for 48h (Modell 100-800, Memmert Co. KG, Germany), then passed 1-mm sieve for analysis. According to the Association of Official Analytical Chemists (AOAC 1990), the dry matter (DM) was determined after dried-oven at 105℃ for 4h, and the ash was determined after incineration at 550℃ for 4h (Carbolite AAF1100 Ashing Furnaces, Germany), the

crude protein (CP) was analyzed used method of Kjeldahl apparatus (Distillation unit, KjeltecTM 8100, Foss Co., Ltd, Hillerød, Denmark). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed refer methods of AOAC (1990) and Van Soest et al. (1991). Gross energy (GE) was determined by calorimeter (Parr 6200, Moline, IL, USA). Ether extract (EE) was measured using Soxtec system (SoxtecTM 2050, Foss Co., Ltd, Hillerød, Denmark). The total phenolics (TPs) and condensed tannin (CT) were analyzed by microplate reader (Epoch, BioTek Instruments, Inc. Winooski, Vermont, USA), according to Folin-Ciocalteu method and method of Porter et al. (1986), respectively.

Rumen fermentation parameter

The rumen fluid was centrifugal on 13,000rpm for 10min at 4℃, the supernatants for analyzed ammonium nitrogen (NH_3-N) and volatile fatty acid (VFA). The 5ml supernatant rumen fluid was used for analyzed NH_3-N through premium Kjeldahl steam distillation systems (Distillation unit, KjeltecTM 8100, Foss Co., Ltd, Hillerød, Denmark) according to method of Bremner et al. (1965); removing 1ml supernatant rumen fluid filtered through Nylon Syringe filter (13mm 0.45um with PP prefilter, P/N TNL1345, Xiboshi, China) for analyzed VFA by gas chromatography (GC, CP-3800, Varian Medical Systems Company, California, USA) with a flame ionization detector (FID, H2 flow 30ml/min, air flow 300ml/min) and an Agilent JandW GC Columns (DB-WAX, 30m×0.320mm×0.15μm, California, USA).

Serum biochemical and antioxidative activity in plasma

The ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity in plasma was analyzed refer Tian et al. (2019) with a minor modified, in brief, 100μl sample mixed with 1ml DPPH reagent (50μmol/L, PCode: 101845869, Sigma-Aldrich,

Germany) into a 1.5ml centrifuge tube and vortex for 15s then store at room temperature without light for 35min, then centrifuge at 13,000 rpm for 10min at 4℃, then removed the 200µl suppurate into 96-well plate, then determined at 517nm by microplate reader, the DPPH scavenging ability was calculated according to formula: DPPH scavenging % = (Ac-As)*100/Ac, where, Ac is the absorbance of the control group, and AS is the absorbance of the sample. The activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), and total antioxidant capacity (TAC) were analyzed by microplate reader at 340nm, 450nm, and 570nm, respectively, according to direction of assay kit (Catalog number CGP1, 19160, and MAK 187, respectively. Sigma-Aldrich, USA).

4.4 Statistical analysis

All data were performer t-test for independent samples using SPSS 16.0. The significant difference at $P<0.05$, the tendency to significant difference at $0.05<P<0.1$.

4.5 Results

4.5.4 4.5.1 Effect of MPP on feed intake and nutrient digestibility in meat goat

As shown in Table 11. The MPP was no effect ($P>0.05$) on DMI and OMI. The ADG and FUR did not effect were observed by addition of MPP. Moreover, no difference ($P>0.05$) on the digestion of DM, OM, GE, CP, ADF and NDF were observed. However, the BW (%) was increase ($P=0.005$) with addition of MPP.

Table 11 Effect of MPP on nutrient digestibility in meat goats.

Item	Control	MPP	SEM	P-value
DMI, g/d	626.04	652.16	21.27	0.405
BW, %	2.71	2.82	0.02	0.005
OMI, g/d	573.28	598.28	19.55	0.387
ADG, g/d	47.87	50.45	5.04	0.724
FUR, %	7.35	7.41	0.79	0.962
Apparent digestibility, %				
DM	59.98	60.10	2.38	0.972
OM	61.18	60.74	2.32	0.897
GE	55.77	55.08	2.68	0.859
CP	60.93	57.28	2.35	0.299
ADF	47.58	47.44	3.86	0.981
NDF	46.93	43.73	4.23	0.604

The value represents the average of 6 replicates (n=6).

Abbreviations: MPP, mangosteen peel powder; SEM, standard error of the mean. DMI, dry matter intake; BW, body weight; OMI, organic matter intake;

ADG, average daily gain; FUR, feed utilization rate; DM, dry matter; OM, organic matter; GE, gross energy; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber.

4.5.2 Effect of MPP on nitrogen balance in meat goats

Addition of MPP was no effect (P>0.05) no N intake and absorbed; N excretion (g/d) in feces and total excretion were no effect (P>0.05) compared to

control (Table 12). MPP had decrease (P=0.024) on N in urine, and the total N excretion (%), tendency to decreased (P=0.096). Besides, MPP was found to tend to increase (P=0.096) N retention (%).

Table 12 Effect of MPP on nitrogen balance in meat goats.

Item	Control	MPP	SEM	P-value
N intake, g/d	9.91	10.04	0.30	0.771
N excretion, g/d				
Urine	3.34	2.30	0.28	0.024
Feces	3.87	4.29	0.27	0.310
Total	7.21	6.59	0.28	0.146
N excretion, %	72.86	65.82	2.70	0.096
N absorbed, g/d	6.04	5.75	0.30	0.516
N absorbed, %	60.93	57.28	2.35	0.299
N retention, g/d	2.70	3.45	0.31	0.117
N retention, %	27.14	34.18	2.70	0.096

The value represents the average of 6 replicates (n=6).

Abbreviations: MPP, mangosteen peel powder; SEM, standard error of the mean; N, nitrogen.

4.5.5 4.5.3 Rumen fermentation parameter

Inclusion of MPP was no significantly affect (P>0.05) on pH, concentration of PA and BA (Table 13). However, MPP was significant decreased (P<0.05) on NH_3-N, concentration of AA, TVFA and CH_4. Moreover, in VFA (%

Molar), the MPP was increased (P<0.001) on C_3 and C_4, decreased (P<0.001) AA and AA/PA compared to control group.

Table 13 Effect of MPP on rumen fermentation parameter in meat goats.

Item	Control	MPP	SEM	P-value
pH	6.92	7.07	0.065	0.114
NH_3-N, mg/dL	12.62	9.24	0.594	0.002
AA, mmol/L	43.54	33.75	2.470	0.019
PA, mmol/L	12.61	12.03	0.524	0.447
BA, mmol/L	7.72	7.44	0.366	0.602
TVFA, mmol/L	63.87	53.22	3.240	0.042
CH_4, mol/100mol TVFA	30.01	27.87	0.30	<0.001
% Molar				
AA	67.99	63.34	0.76	0.002
PA	19.84	22.67	0.38	<0.001
BA	12.17	14.00	0.43	0.013
AA/PA	3.44	2.80	0.09	0.01

The value represents the average of 6 replicates (n=6).

Abbreviations: MPP, mangosteen peel powder; SEM, standard error of the mean; NH_3-N, ammonia nitrogen; TVFA, total volatile fatty acid, TVFA=AA+PA+BA; AA, acetic acid; PA, propionic acid; BA, butyric acid; AA/PA, ratio of acetic acid to propionic acid.

4.5.6 Blood biochemical parameter and antioxidant activity in plasma of meat goats

No effect (P>0.05) of MPP on GLU, BUN, GLB and A/G was observed (Table 14), in addition, MPP was tendency to increase, TP (P=0.077) and ALB (P=0.071) content.

Table 14 Effect of MPP on serum biochemical parameter in meat goats.

Item	Control	MPP	SEM	P-value
GLU, mg/dL	53.63	55.79	2.003	0.462
BUN, mg/dL	16.27	16.47	0.660	0.830
TP, mg/dL	6.80	7.26	0.171	0.082
GLB, mg/dL	2.59	2.64	0.086	0.708
ALB, mg/dL	4.23	4.67	0.155	0.077
A/G	1.1650	1.785	0.087	0.298

The value represents the average of 6 replicates (n=6).

Note: MPP=mangosteen peel powder; SEM=standard error of the mean; GLU=glucose; BUN=blood urea nitrogen; GLU=glucose; TP=total protein; ALB=albumin; GLB=globulin, GLB=GLB=TP-ALB.

No significant effect (P>0.05) observed that MPP effect on SOD and TAC, supplementation of MPP was significantly increased (P<0.05) the DPPH scavenging activity and GPx activity show in Table 15.

Table 15 Effect of MPP on plasma antioxidant activity in meat goats.

Item	Control	MPP	SEM	P-value
DPPH, scavenging activity, %	10.73	14.53	0.939	0.017
SOD, (inhibition rate %)	56.91	54.89	3.535	0.695
TAC, nmol/µl	44.15	43.70	1.908	0.327
GPx, mmol/min/ml	0.3008	0.3197	0.00584	0.044

The value represents the average of 6 replicates (n=6).

Abbreviations: MPP, mangosteen peel powder; SEM, standard error of the mean; GLU, glucose; BUN, blood urea nitrogen; GLU, glucose; TP, total protein; ALB, albumin; GLB, globulin, GLB= TP-ALB; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity.

4.6 Discussion

4.6.1 Effect of MPP on DMI and nutrient digestibility of meat goats

Feed intake is one of most important limiting factors in ruminant (Chamberlain and Wilkinson, 1996), which is a better indicator of nutritive value of feed than apparent digestibility (Okunade et al., 2014). The astringency of condensed tannin due to interaction of salivary proteins with condensed tannins (Jerónimo et al., 2016), which is the main factor affecting feed intake (Cooper et al., 1985; Landau et al. 2000), Frutos et al. (2004) who review that feeding high contain (generally >5% DM) condensed tannin could significantly reduce DMI, while low contain (generally <5% DM) condensed tannin not effect it, when the additive levels less than 3% DM was no effect or even a slight increase DMI (Zhang et al., 2019). In the current study, the MPP

no effect (P>0.05) on DMI, OMI, ADG and FUR were observed, the result was similarly to Animut et al. (2008). Consistent with Zhang et al. (2019), in this case, the BW (%) was increased (P=0.005) by additive MPP, the reason might due to the low additive level (MPP contain 0.621% CT), which might also be the reason for the MPP group did not affect nutrient digestibility. Similarly, Ngamsaeng et al. (2006) who got result that feeding 50-150 g/head/d MPP in cattle was no effect nutrient digestibility.

4.6.2 Effect of MPP on N utilization of meat goat

CT can interact with forage protein through hydrophobic and hydrogen bonding (Min et al., 2003), based on this property, CT is frequently as an additive to protect protein escape rumen (Haslam, 1989). As the previous studies, Gunun et al. (2016) found that supplement 1.6% of DM intake Mao seed meal (contain 9.6% CT) was decrease N excretion in urine and total excretion, increase N retention, consistent to current study. Conflict with the current experimental results, Dentinho et al. (2014) addition of different levels of Cistus ladanifer L. tannins (1.40, 13.6, 22.8 g/kg) was no effect N utilization in Merino rams. This might relate to additive levels of CT. Besides, the efficiency of CT combine with protein was influence by many factors, such as structure (Xiao et al., 2010), molecular weight (Paengkoum et al., 2015), and binding sites (Waghorn, 2008; Min et al., 2003).

4.6.3 Effect of MPP on rumen fermentation parameter of meat goats

NH_3-N, which is the end-product of dietary protein (or nonprotein) degradation and are mainly nitrogen source for synthesis bacterial protein by ruminal bacteria (Liu et al., 2019; Cotta and Russell, 1982) and an important index for nitrogen metabolism in the rumen (Allison, 1969). In current study, the MPP was decreased (P<0.001) on NH_3-N concentration in the rumen; the reason probably due to MPP rich

condensed tannin, which could protect dietary protein through CT combine with protein to be stable complexes (Naumann et al., 2017), and/or inhibit proteolytic bacteria (Min et al. 2003; Mcsweeney et al. 2001), the result might could explained urine N content was decreased in MPP group.

VFA is the main energy source for ruminants (Bergman, 1990). It is produced by enzymes after carbohydrate fermentation by rumen microorganisms (Wang et al., 2012), Messana et al. (2013) who report VFA concentration was effect by DMI, dietary composition and feeding frequency. CT could affect VFA by combining CT with specific enzymes to affect microorganisms (Barry and Manley, 1984). In present experiment, concentration of TVFA and AA were decreased by supplement MPP, no effect on pH (Table 13), results consistent with Chanthakhoun et al. (2011). In contrast, Ngamsaeng et al. (2006) supplement feed with 50-150 g/d MPP in cattle was no effect rumen VFA, by reason of the sensitivity of microbial population to CT varies from animal to animal (Toral et al., 2011). Besides, no difference on PA and BA was observed, agreement with Soltan et al. (2012) who supplement Acacia saligna in diet for sheep had no effect on VFA. Also noteworthy is that MPP increased the molar ratio of PA and BA, decrease the molar ratio of AA and ratio of AA to PA. PA is most important energy for live weight gain, AA concentration is positively correlated with milk fat content (Chamberlain and Wilkinson, 1996), lower ratio of AA to PA could an increase energy for nutrient utilization (Moran, 2005). This might indicate that MPP has a positive effect on the improvement of meat animals. Moreover, we observed that methane production was decrease by addition of MPP, this might due to condensed tannin has potential to mitigating methane production by methanogenesis and/or protozoa (Bhatta et al., 2009), on the other, a reason of reduce CH_4 production might

due to reduce the acetic acid content, which provide substrate for methanogens to synthesis methane (Patel et al., 1976).

4.6.4 Effect of MPP on serum biochemical parameter of meat goats

Blood biochemical indictors are important parameters to predictions health status (Olafadehan et al., 2014), and dietary nutrient absorbed and metabolism in animals (Nkrumah et al., 2007). The GLU, BUN levels are important indictors of energy and nitrogen metabolism in ruminants (Graugnard et al., 2012; Abdel-Salam et al., 2014). In current study, we observed that MPP was no effect GLU and BUN content (Table 14) and both in normal range: GLU 50-80mg/dl (Sanhouri et al., 1990) and BUN 6-27mg/dl (Abarghuei et al. 2014). Blood serum protein (such as TP, ALB, GLB and ratio of ALB to GLB) are indicators of the immune function in ruminants. Oni et al. (2012) who found that cassava leaves (approximately contain 1% CT) could increase TP, ALB and GLB content in serum of goats, Zhang et al. (2019) supplement 3% of two different CT (bayberry and Acacia mangium) the result shown that both of two CT had no effect TP, ALB, GLB and ratio of ALB to GLB. In present work, MPP has trend increase TP and ALB content. This might suggest that MPP have the potential to improve the goat's immunity, but more data are needed in the future.

4.6.5 Effect of MPP on antioxidant activity in plasma

Concentrated tannin is a natural antioxidant with stronger antioxidant capacity than vitamin C, vitamin E, and β-carotene (Castelluccio et al., 1995), they antioxidant capacity determined by its structure, especially the number of hydroxyl groups and their positions (Rice-evans et al., 1995). DPPH method was widely used to evaluate the properties constituents for free radicals scavenging (Pyrzynska and Pekal, 2013), SOD play role of the first guard of for defense against ROS (Thatoi et al., 2014),

and the GPx is the next player (Varjovi et al., 2015). Many studies have proved that plant extracts rich in condensed tannins have strong DPPH scavenging ability (Gangwar et al., 2014; Szerlauth et al., 2019), Jaisupa et al. (2018) found that rich-condensed tannin MPP extract has significant DPPH scavenging capability. Di-trana et al. (2015) who feed with Sulla coronarium (contain 1.99% CT) was significant increase TAC and GPx capacity no effect SOD. Besides, Dey and De (2014) conclusion that feeding 1.5% condensed tannin from F. bengalensis leaves can positive effect antioxidant status in crossbred cows. In the current study, we observed MPP could increase plasma DPPH and GPx scavenging capacity, this clarification that condensed tannin could provide the paired electron for DPPH, in addition, the chelation of condensed tannins to metal ions is an important factor to improve their antioxidant properties (Makni et al., 2013). We found no effect of MPP on TAC and SOD. Similarly, Liu et al. (2011) found that 5 g/kg of condensed tannin from chestnut wood was no effect on TAC, SOD capacity in when fed 21 days, significant increase GPx capacity.

4.7 Conclusion

In conclusion, the MPP could improve the antioxidant capacity of plasma, reduce the CH4 production, tendency to positive effect on N utilization and serum immune function, but attention should be paid to its negative effect on TVFA.

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CHAPTER V

OVERALL CONCLUSION AND IMPLICATION

5.1 Conclusion

This study provided the mangosteen peel powder as condensed tannin source that can reduce methane production, improve nitrogen utilization and enhance antioxidant capacity in meat goats.

The first experiment was to study the effect of mangosteen peel powder on gas production, rumen fermentation and methane production *in vitro*. The result shown that (1) mangosteen peel powder could be reduce gas production; (2) mangosteen peel powder reduce organic matter degradation, acetic acid and total volatile fatty acids, not effect on pH and ammonium nitrogen; (3) mangosteen peel powder could reduce methane production.

The second experiment was to investigate the effect of mangosteen peel powder on growth performance, nutrient digestibility, rumen fermentation, plasma antioxidant capacity in meat goats. The result shown that (1) mangosteen peel powder could improve nitrogen utilization without negative effect growth performance and nutrient digestibility; (2) mangosteen peel powder could decrease ammonium nitrogen concentrate in rumen; (3) mangosteen peel powder could enhance antioxidant capacity in meat goats.

Collectively, mangosteen peel powder could be used as additive source. Because mangosteen peel powder can improve nitrogen utilization and enhance antioxidant capacity without negative effect growth performance.

5.2 Implication

The plant secondary metabolite of mangosteen peel is not contain condensed tannin only. It is containing such as anthocyanin and saponin, the mechanism of metabolic in the rumen still not clear. In the future studies are encouraged to estimate the degradation and interaction of secondary metabolite components of mangosteen peel in the rumen.

BIOGRAPHY

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