ผลของกากชาเขียวในอาหารผสมรวม ต่อผลผลิตน้ำนม การย่อยได้ของโภชนะ และฤทธิ์การต้านออกซิเดชัน ในโคนมพันธุ์ผสมที่กำลังให้นม

นางสาว ฐิติมนต์ ธีรภัคสิรินนท์

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คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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EFFECTS OF GREEN TEA WASTE IN TOTAL MIXED RATION ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND ANTIOXIDANT ACTIVITY IN CROSS-BRED LACTATING COWS

Miss Thitimon Theeraphaksirinont

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By	Miss Thitimon Theeraphaksirinont
Field of Study	Animal Nutrition
Thesis Advisor	Professor Somchai Chanpongsang, M.S.
Thesis Co-Advisor	Professor Narongsak Chaiyabutr, Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkron University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Veterinary Science
(Professor Mongkol Techakumpu, Doctorate de 3e cycle)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Uttra Jamikorn, Ph.D.)

..... Thesis Advisor
(Professor Somchai Chanpongsang, M.S.)

..... Thesis Co-Advisor
(Professor Narongsak Chaiyabutr, Ph.D.)

..... Examiner
(Assistant Professor Chackrit Nuengjamnong, D.Tech.Sc.)

.....External Examiner
(Associate Professor Pornsri Chairatanayuth, Ph.D.)

ฐิติมนต์ ธีรภัคสิรินนท์ : ผลของกากชาเขียวในอาหารผสมรวมต่อผลผลิตน้ำนม การย่อยได้ของโภชนะ และฤทธิ์การต้านออกซิเดชันในโคนมพันธุ์ผสมที่กำลังให้นม (EFFECTS OF GREEN TEA WASTE IN TOTAL MIXED RATION ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND ANTIOXIDANT ACTIVITY IN CROSS-BRED LACTATING COWS.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.นสพ. สมชาย จันทร์ผ่องแสง, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.นสพ.ดร. ณรงค์ศักดิ์ ชัยบุตร, 40 หน้า.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมกากชาเขียวในอาหารที่มีผลต่อผลผลิตน้ำนม การย่อยได้ของโภชนะ และฤทธิ์การต้านออกซิเดชันในโคนมพันธุ์ผสมที่กำลังให้นม โดยใช้สัตว์ทดลอง 6 ตัว แบ่งโดยการสุ่มเป็น 3 กลุ่ม กลุ่มละ 2 ตัว วางแผนการทดลองแบบจัตุรัสลาติน (3 × 3 ลาตินสแควร์) ใช้เวลาในการทดลองแต่ละช่วง 25 วัน เป็นระยะปรับตัว 10 วัน ระยะทดลอง 15 วัน อาหารที่ใช้คือ อาหารผสมรวม (TMR) ที่เตรียมมาจากอาหารหยาบหมักและอาหารข้นในอัตราส่วน 39:61 (น้ำหนักแห้ง) สัตว์ทดลองทั้ง 3 กลุ่ม จะได้รับอาหารเหมือนกันต่างกันที่ระดับของกากชาเขียว โดยแต่ละกลุ่มจะมีการเสริมกากชาเขียวในอาหารผสมรวมที่ระดับ 0 , 5 และ 10% ตามลำดับ

จากการทดลองพบว่า การเสริมกากชาเขียวไม่มีผลต่อปริมาณการกินได้ ปริมาณการกินได้ต่อเปอร์เซ็นต์น้ำหนักตัว ปริมาณน้ำนม ปริมาณน้ำนมต่อปริมาณการกินได้ แต่พบความเข้มข้นของโปรตีนในน้ำนมเพิ่มสูงขึ้นในกลุ่มที่เสริมกากชาเขียว 10%GTW ในสูตรอาหาร ($P<0.05$) ส่วนประกอบน้ำนม (ของแข็งทั้งหมด ไขมัน ของแข็งที่ไม่ใช่ไขมัน และ แลคโตส) การย่อยได้ของโภชนะ ปริมาณอะลานโทอินในน้ำนมที่ใช้เป็นดัชนีชี้วัดการเจริญของจุลินทรีย์ในกระเพาะรูเมน ปริมาณกรดไขมันระเหยได้ ไม่พบความแตกต่างทางสถิติ ($P>0.05$) เช่นเดียวกับระดับความเข้มข้นของ TBARS และวิตามินอีในพลาสมา ไม่พบความแตกต่างทางสถิติ ($P>0.05$) เมื่อเสริมกากชาเขียวในอาหารผสมรวม

จากผลการทดลองสรุปได้ว่าการใช้กากชาเขียวเป็นแหล่งโปรตีนที่ระดับ 5% และ 10% ของวัตถุแห้งในสูตรอาหารผสมรวมในโคพันธุ์ผสมช่วงการให้นมระยะกลาง ไม่เกิดผลเสียต่อปริมาณน้ำนม ผลผลิตน้ำนม การย่อยได้ของโภชนะ และการให้ผลผลิตของโคนมพันธุ์ผสมในช่วงของการให้นมระยะกลาง การเสริมกากชาเขียวในอาหารเลี้ยงโคนมสามารถเพิ่มผลกำไรให้แก่เกษตรกรผู้เลี้ยงโคนมได้

ภาควิชา	สัตวบาล	ลายมือชื่อนิสิต ฐิติมนต์ ธีรภัคสิรินนท์
สาขาวิชา	อาหารสัตว์	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก
ปีการศึกษา	2552	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม

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THITIMON THEERAPHAKSIRINONT: EFFECTS OF GREEN TEA WASTE IN TOTAL MIXED RATION ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND ANTIOXIDANT ACTIVITY IN CROSS-BRED LACTATING COWS. THESIS ADVISOR: PROF. SOMCHAI CHANPONGSANG, M.S., THESIS CO-ADVISOR: PROF. NARONGSAK CHAIYABUTR, Ph.D., 40 pp.

The objective of the present experiment was to study the effect of dietary green tea waste supplementation on milk production, nutrient digestibility and antioxidant activity in cross-bred lactating cows. Six cross-bred lactating cows were used in the experiment. They were randomly divided into three groups of two cows each. A Latin Square (3×3) design was used in this study. Each period lasted for 25 days; the adjusting and collecting periods were 10 and 15 days, respectively. Cows were fed ad libitum with total mixed ration (TMR). TMR was the mixture of silage and concentrate at the ratio of 39:61 (DM basis). The compositions of a basal diet (control) and two treatment diets were the same except for the difference of the green tea waste concentration. Dietary green tea waste was varied by using green tea waste. The concentrations of green tea waste in control diet and two treatment diets were 0%, 5% and 10% (DM basis), respectively.

No effects of GTW supplementation on feed intake, DMI/%BW, MY and MY/DMI. Milk protein percentage was increased ($P<0.05$) for cows fed with 10% GTW when compared to the control group. The percentages of milk composition: total solid, fat, protein, solid not fat and lactose were not significantly affected. No significant differences ($P>0.05$) were observed in nutrient digestibility, milk allantoin concentration and VFA concentrations. The oxidative stress index, TBARS concentration and ascorbic acid concentrations were not different in this study.

It can be concluded from this study that GTW can be used as a source of protein, at both level 5 and 10% (DM basis), in the diet fed to cross-bred multiparous cows during mid-lactation period without any deleterious effect on milk yield, nutrient digestibility and performance. Replacement of conventional ingredients with GTW in cow's diet showed a better net income than using conventional feed.

Department:	Animal Husbandry	Student's Signature:	Thitimon Theeraphaksirinont
Field of Study:	Animal Nutrition	Advisor's Signature:	Somchai Chanpongsang
Academic Year:	2009	Co-Advisor's Signature:	Narongsak Chaiyabutr

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ADF	Acid detergent fiber
BW	Body weight
C	Catechin
CG	Catechin gallate
CP	Crude protein
CT	Condensed tannin
d	Day
DMD	Dry matter digestibility
DM	Dry matter
DMI	Dry matter intake
EC	Epicatechin
ECG	Epigallocatechin
EE	Ether extract
EGCG	Epigallocatechin gallate
EVAP	Evaporative cooling system
FM	Fresh matter
GC	Gallocatechin
GCG	Gallocatechingallate
GT	Green tea
GTW	Green Tea Waste
h	Hour
HT	Hydrolysable tannin
H_2O_2	Hydrogen peroxides
Kg	Kilogram
m	meter
MDA	Plasma malonaldehyde
MPS	Microbial protein synthesis
MY	Milk yield

NEVAP	Non evaporative cooling system
NDF	Neutral detergent fiber
O_2^-	Superoxide anion
$ONOO^-$	Peroxynitrite
OH^-	Hydroxyl radical
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Respiration rate
RT	Rectal temperature
RH	Relative humidity
SNF	Solid not fat
SOD	Superoxide dismutase
TBA	Thiobarbituric acid reactive substance
TMR	Total mixed ration
THI	Temperature humidity index
VFA	Volatile fatty acid
WI	Water intake

CHAPTER I

INTRODUCTION AND AIMS

It is known that feed is the major factor affecting to dairy production and milk production cost. Feed cost is shown to be 70 percentage of milk production cost. Major cost of feed originates from the cost of concentrate. Therefore, seeking new materials that can be used to replace the conventional ingredients, might declined milk production cost.

Green tea is made from *Camellia sinensis* (family Theaceae) which is a native plant found in Asian countries. The growing area of tea in Thailand is about 97,355 rai, which is the fourteenth of the world, and 13,961 ton of fresh production or 6,600 ton of dry production. Last decade, green tea has become the focus of attention for a wide range of positive health benefits. In the beverage industry, green tea is used to produce soft drink, which is very popular. Green tea composes of varieties of flavonoids, including the catechins which is found predominantly. Wide range of biological activities along with their effects on the promotion of health and prevention of disease in humans have been studied for many years (Pietta and Simonetti, 1999; Peterson et al., 2005). Low grade green tea has been used as an ingredient in broilers (Kaneko et al., 2001; Cao, 2005), pigs (Suzuki, 2002; Ko et al., 2008) and calves (Ishihara et al., 2001) feeds. The positive effects of green tea on animal performance have been described. The supplementation of green tea in broiler diets had positive effects on growth performance and lean meat production (Kaneko et al., 2001) and positive effects on an increase in lactic acid bacteria and aerobic bacteria counts in ruminants was noted (Bureenok et al., 2007). However, utilizing green tea in the livestock industry would increase feed cost.

By product from green tea beverage is the green tea waste (GTW). Although a small part of GTW is converted into raw compost material, most of them are

generally dumped into landfills. There is an increase in demand for efficient used of local by-product instead of commercial feedstuff due to economic and environmental concerns. Kondo et al. (2006, 2007) reported that 10% fresh matter of green tea waste added in silages for ruminant and goat diets with 5% of green tea by-product showed high nutritive values. GTW may be considered as a valuable protein source consisting 22-35% of crude protein (Kondo et al., 2004a,b). In addition, the addition of 5 to 10% ensiled GTW to replace soybean meal and alfalfa hay in a total mixed ration (Kondo et al., 2004a) or timothy hay based diets (Eruden et al., 2003) as a DM basis had no negative impact on the lactating performance in late lactating dairy cows, so that GTW may be used as an animal feed supplement for dairy cattle. Yang et al. (2003) reported that the thiobarbituric acid and reactive substance (TBA) value of broiler meat decreased significantly when broilers were fed diets containing 0.5 to 2.0% green tea by-product compared to those fed a diet containing antibiotics. Ko et al. (2008) reported that the TBA value of meat was not affected by dietary supplementation with 0.5% green tea by-product and green tea probiotics to finishing pigs ($p>0.05$), but pigs fed diets containing 0.5% green tea probiotic supplementation had a lower meat TBA value compared to that of 0.5% green tea by-product treatment ($p<0.05$). Nishida et al. (2006) reported that administration of green tea waste silage to Holstein steers increased the concentrations of plasma high density lipoprotein cholesterol.

Therefore, the objectives of this experiment were to investigate the effects of GTW supplementation on milk production, nutrient digestibility and antioxidant activity in cross-bred lactating cows.

CHAPTER II

BACKGROUND AND INFORMATIONS

1 Tea

1.1 Origin of tea

Tea plant is believed to have originated in the western Yunnan region of China on the border between Myanmar and China. Around the seventh century, tea cultivation spread from China to Japan and then subsequently spread to other Asian countries and later to Europe. Tea plant has long been cultivated, and consumed widely in Asia, particularly in China and Japan (McCaleb et al., 1999)

In Thailand, Chiang Rai province is known as the biggest tea growing area. Chaing Rai is the mountainous province that touches border with Myanmar (Shan state) and Laos. One of the village of northern Thailand; Mae Salong is also well known place for its tea plantation. Mae salong is the famous Chinese Thai village which perched on the border, a mere mountain pass away from Myanmar. Beside the tea plant *Camellia sinensis*, other mixed varieties are also developed in this area. From the tea plantation in Chiang Rai, different kind of tea products are available in the maket in the form of Green tea, Oolong tea and Jasmine tea.

1.2 Botanical characteristics of tea

Tea plant is classified in the Theaceae family and the Camellia species (*Camellia sinensis*, (L) O. Kuntze) (Hara et al., 1995). *Camellia sinensis* consist mainly of two variaties, *Camellia sinensis* variety sinensis and *Camellia*

sinensis variety assamica. In nature, tea tree is a perennial plant. It can grow to a height of 30 m. The plant is heavily branched with young leaves, pruned and maintained at the height of 2 to 3 m. Leaves and buds are the useful parts of the tea plant. However, it can differ in colour, shapes and size depending on types, species and environmental conditions. For the production, leaves are preferentailly picked as young shoots where older leaves are considered to be of inferior quality (Brown, 1999). Only the apical bud and the first few leaves are picked for tea processing. In tropical countries, tea leaves are harvested all year around while in temperate countries tea leaves harvesting in seasonal.

1.3 Types of tea

There are three types of tea, all coming from the same plant, but differing in the way processing; namely, Green tea, Oolong tea and Black tea. Green tea is unfermented tea, Oolong tea is partially fermented tea and black tea is fully fermented tea (Murray, 1995)

1.4 The chemical constituents in tea

Green tea is made without enzymatic oxidation of polyphenols, as polyphenol oxidase is inactivated by heat during the early stages of green tea processing (Hara et al., 1995). Thus, the polyphenols present in green tea should be the same as those found in fresh tea leaves. Tea leaves comprise of polyphenols

(flavonoids), alkaloids (caffeine, thiophylline and thiobromine) and to the lesser extent of minerals (calcium, iron and maganese) (McCaleb et al., 1999). The chemical compounds present in fresh tea leaves are shown in table 1

1.4.1 Polyphenols

The polyphenol substance in tea leaves is approximately 20-35% dry weight. The chemical compounds in fresh tea leaves (table 1) shown it is unusually rich in the flavonol group of polyphenols, known as catechins which may constitute up to 30% of the dry weight of the water-extractable material. The catechins are abundantly distributed in green tea. Flavonoids mostly occurred as a glycosylated forms. But in catechins groups, they are present as free forms rather than glycosylated forms which is one of the distinct points from other flavonoids.

Flavonoids present in green tea are catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), catechin gallate (CG), gallocatechin gallate (GCG), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). The tea catechins is a term commonly used to refer to both catechins and catechin gallates. In a more specific sense, catechins include EC, C, EGC and GC while catechin gallates include ECG,CG, EGCG and GCG (Hara et al., 1995). Figure 1 shows the structures of the catechins and catechin gallates observed in tea

Table 1 The chemical compounds in fresh tea leaves

Components	Dry weight (%)
Soluble in water	
Flavonols	
(-) – EGCG	9 – 14
(-) – EGC	4 – 7
(-) – ECG	2 – 4
(-) – EC	1 – 3
(+) – GC	1 – 2
(+) – C	0.5 – 1
minor catechin	0.4 – 1
Flavonol glucosides	3 – 4
Proanthocyanidins	2 – 3
Caffeine	3 – 4
Amino acids	2 – 4
Carbohydrates	3 – 5
Organic acids	0.5 – 2
Saponins	0.04 – 0.07
Pigments	0.5 – 0.8
Vitamins	0.6 – 1.0
Soluble minerals	2 – 4
Insoluble or Slightly soluble in water	
Cellulose	6 – 8
Lignin	4 – 6
Polysaccharides	4 – 10
Lipids	2 – 4
Insoluble pigments	0.5
Insoluble minerals	1.5 -3.0
Volatiles	0.01 – 0.02

Source : Zong-mao (2002)

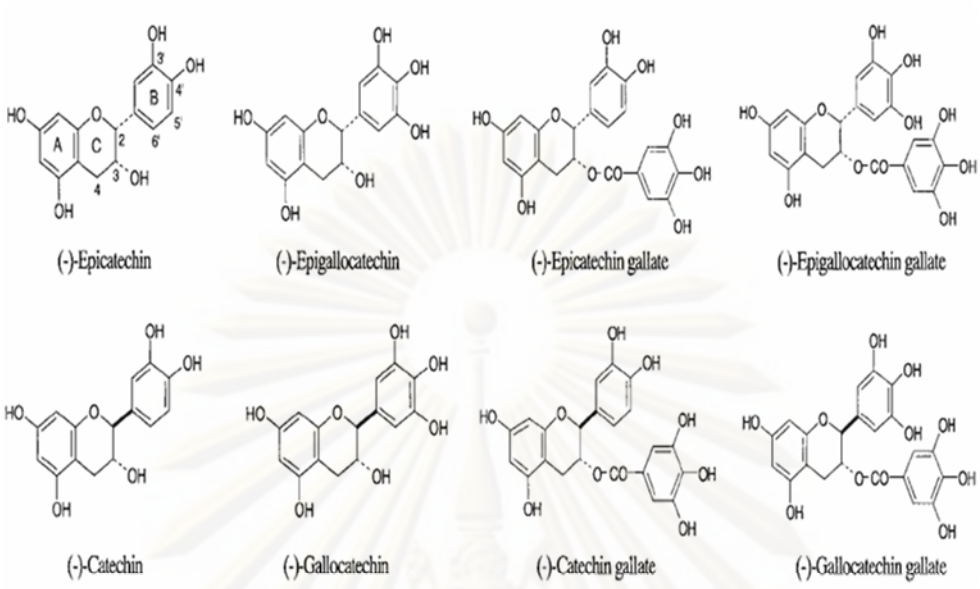

Figure 1. Structures of catechins in green tea (adapted from Unno et al., 2000)
(-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-catechin (C), (-)-catechin gallate (CG), (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG).

Flavonoids are absorbed by the gastrointestinal tracts of humans and animals, and are excreted either unchanged or as their metabolites in the urine and feces (Tarao, 1999). Colonic bacteria spilt the heterocyclic ring and degrade flavonoids to phenyl acids which may be absorbed, conjugated, and excreted or metabolized further by bacteria (Pietta and Simonnetti, 1999)

1.4.2 Tannin

Tannin as water soluble phenolic compounds having molecular weights between 500 to 3000 dalton and containing a large number of phenolic hydroxylic groups (one to two hundred molecular weight) to enable it to form effective cross-links with protein and other molecules. Tannin are usually subdivided into two groups; hydrolysable tannin (HT) and condensed tannin (CT).

The examination of toxicity of tannin by Laudau et al. (2000) found that CT extracted from quabracho over 500 g/day (50 %DM), it is possible that negative effect on feed intake was small after the ration was changed in the short term (within 5 days) in heifers. Aerts et al. (1999) reported that high dietary CT concentration in forage legume (6.0-12.0 %DM) can depress voluntary feed intake, digestibility and animal productivity in sheep. CT 2.0-4.0 %DM had no effect on voluntary feed intake it had beneficial effects on protein metabolism in ruminant (Aerts et al., 1999)

1.4.3 Other compound

Caffeine is present at an average level of 3%. The amino acid theanine (5-N-ethylglutamine) is also unique to tea.

2. Effect of GTW on lactating cow

By-product from green tea beverage is the green tea waste (GTW). The nutrient composition on DM basis of GTW contained 34.8 % protein, 7.1 % fat, 31 % NDF, 24.1 % ADF, 11.4 % total phenolic and 1.7 % CT (Kondo et al., 2004a). However, the nutrient in GTW varies by origin, type, environmental condition of plantation, harvesting and manufacturing processing.

2.1 feed intake

Kondo et al. (2004a) studied influence GTW on palatability in cattle. DM intake of dairy cows fed TMR including GTW at rates of 0, 2.5 and 5.0% of DM was measured. The DM intake of cows fed TMR with GTW was slightly but not significantly increased with the increment of GTW. Eruden et al. (2006) studied effect of main roughage on palatability of total mixed rations with GTW silage in lactating dairy cows. In experiment 1, the main roughage in TMR (basal diet) was timothy hay, and the GTW silage addition rate were 1.25, 2.5, 5, 10 or 20% of TMR on a dry matter basis. In experiment 2, the main roughage in TMR (basal diet) was maize silage, and the GTW silage proportion were 2.5, 5, 10 or 20% of TMR on a dry matter

basis. Feed intake and preference index were measured. Results indicated that the upper limit of green tea waste silage mixing rate is expected to be 8 and 15% in TMR with timothy hay and maize silage as a main roughage, respectively. It could be concluded that GTW had the effect on the palatability of feed. The more pronounced depends on the amount of GTW used and the type of basal diet in the trial.

2.2 milk production

Green tea by-products can be supplied up to 5% on a dry matter (DM) basis of total diet to lactating Holstein cows that produce approximately 30 kg of milk per day without any detrimental effect on their performance, and can also be substituted for a part of soybean meal and alfalfa hay in a total mixed ratio (Kondo et al. 2004a; Eruden et al. 2005). It was reported that decaffeinated black tea by-products could be offered up to approximately 7% on a DM basis of the total diet to lactating crossbred cows with 7.5 kg of milk yield in India (Konwar et al. 1991).

2.3 Antioxidant

As the result of radical chain reaction, reactive oxygen species (ROS) are evolved as by products. The ROS comprises those of oxygen-centered radicals such as superoxide anion (O_2^-), hydrogen peroxides (H_2O_2), hydroxyl radical (OH^-) and peroxynitrite ($ONOO^-$). Free radicals, ROS and reactive nitrogen species (RNA) are chemical species that have one or more unpaired electrons in an outer orbit. They are unstable, highly reactive molecules and capable of reacting with each other (Fenton reaction) or with other molecules to equilibrate its charge and to form more or less reactive molecules. In biological system, free radicals are continuously produced in the body as a result of the normal metabolic processes from mitochondria, phagocytes, inflammation and enzyme action. External environmental stimuli such as toxic substances, microbial attacks, ozone, UV radiation, cigarette smoke, or intensive exercise are another sources of free radicals formation (Dufresne et al., 2001). An antioxidant can be broadly defined as any compound

that delay or prevents oxidation of a substrate (Gordon, 1990). On the other hand, antioxidants are molecules that interact with the free radicals thereby neutralizing them, which results in protecting normal tissue and DNA from potential damage. For instances, enzymatic antioxidant are produced by certain enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase whereas non-enzymatic antioxidants are antioxidant vitamins (vitamin C and vitamin E), some trace elements like zinc, copper and selenium and phytochemicals like carotene and flavonoids.

The catechins are abundantly distributed in green tea. Many epidemiological studied shown that green tea has antibacterial, anticarcinogenic and radical-scavenging activities which can be attributed to the high content of flavonoids; catechins present in it. Many biological activities of green tea are mostly related to the structural activity of catechins. The presence or absence of galloyl group in orthodihidroxy or trihydroxy group of B ring in the basic catechin structure is still a controversial issue as regard the biological activities (see figure 1). Hydroxyl groups on the B ring donate hydrogen and an electron to hydroxyl, peroxyl and peroxylnitrite radicals, stabilizing them and giving rise to relatively stable flavonoid radical. Moreover the radical-scavengering activities of polyphenols greatly depends on their molecular weight. One of the major component of green tea polyphenol; EGCG has a high molecular weight (458.4) and altogether 8 numbers of phenolic hydroxyl group present in contributed to the strongest radical scavenging activity than other catechin derivatives (Hatano et al., 1989). Similar effect was reported with additional remarks on stronger radical scavenging activity of green tea polyphenols than ascorbic acid and alpha tocopherol (Yoshida et al., 1989). Hara (1997) reported that catechin could inhibit the activity of α-amylase in small intestine. As a result it inhibits the activity of bacteria but not lactic acid bacteria. He et al. (2006) found that polyphenol in tea at 0.05 mg/ml could inhibit the activity of α-amylase,

pepsin and trypsin. So polyphenol has the role on protein precipitation. By binding protein with non-covalent legand this activity would increase the stability of protein. GTW had the effect on radical scavenging activity. Nishida et al. (2006) fed GTW at 20% (DM basis) in cows diet found that GTW increased plasma vitamin E compared to control group.

Vitamin C is required in some animal species but domestic animal such as ruminant, swine, horse and poultry can synthesize ascorbic acid. The majority of vitamin C exists as ascorbic acid and ascorbic acid is reversibly oxidized to dehydroascorbic acid in the bodies of animals (McDowell, 1989). Thus both forms are biologically active. Liver of adult cattle synthesizes ascorbic acid from glucose-1-phosphate which is sufficient to cover its vitamin C requirement (Toutain et al., 1997). However, cattle are tend to have vitamin C deficiency when the synthesis of ascorbic acid is impaired because of dietary vitamin C is easily degraded in the rumen (Haiying et al., 2003). Plasma ascorbic acid concentration was also reported to decrease in cattle under unfavorable environmental conditions (heat stress) (Haiying et al., 2003: Tanaka et al., 2007). Temperature of great importance to animal production, especially in Thailand, where there are high ambient temperatures throughout the year. The temperature humidity index (THI) is commonly used to indicate the degree of heat stress on dairy cattle. There has been categorized the severity level of livestock welfare by using THI to four levels. THI $\leq$ 74 generally does not cause significant problems for healthy animals. Under alert conditions (THI = 75 – 78), producers can expect some decrease in the rate of weight gain. Under danger conditions (THI = 79 – 83), animals show noticeable decreases in weight gain and, when handled, transported or overcrowded, may be severely affected. Under emergency conditions (THI $\geq$ 84) without management intervention, animal mortality can occur, especially when such conditions are prolonged (Hahn and Mader, 1997). During heat stress is to decrease metabolic heat production by increase the respiratory rate, panting, reduced activity, reduced

feed intake and adversely affects productivity. Dairy cattle produce large amounts of heat from both ruminal fermentation and metabolic processes. In order to maintain body temperature within thermal range, cows must exchange this heat with the environment. The environmental modification is necessary to maintain productivity by the lactating dairy cow. Padilla et al. (2006) reported that heat stress had the effect on antioxidant activity by reducing the concentration plasma ascorbic acid. In summer dairy cows had significant ($p<0.05$) lower level of plasma ascorbic acid than dairy cows in autumn.

Thiobarbituric acid reactive substance (TBARS) value, expressed as MDA concentration, is a good indicator reflecting the degree of oxidation (Guo et al., 2001). Supplementation of green tea may indirectly decrease TBARS value as affecting by antioxidant activity of catechin in green tea. Castiilo et al. (2006) studied in postparturient dairy cows, MDA found to be increased at high level after parturition and reduced during peak stage of lactation. Tanaka et al. (2007) studied in dairy cows in the winter (average of maximum monthly temperature/day is 14.9 °C) compared to high temperature in hot climate (32.6 °C) found that RT increased from 100.58 to 102.2 °F that effect on vitamin C decreased from 35.4 μmol in winter to 22.9 μmol in hot climate, in the other hand, TBAR increased from 56.3 nm in winter to 68.8 nm in hot climate.

For the temperature in Nakhon Pathom, The monthly average maximum day temperature varied from 34ºC and 93.5% THI in January to 22.5ºC and 45.3% THI in April of 2007. High temperature and THI can effect production in dairy cows such as low milk yield and decreased fertility. Our hypothesis is GTW can be used to decrease the effect of heat stress in crossbred lactating cow by reducing the oxidation activity by using plasma ascorbic level as an indicator of stress condition.

CHAPTER III

MATERIALS AND METHODS

1. Experimental design and animals

1.1 Animals

Six crossbred Friesian lactating cows, days in milk for d 75 to 90, were used in the experiment. A replicated (3 × 3) Latin square design was used in this study. Six cows were assigned randomly into three groups for rotationally receiving three different treatments (control, 5% and 10% GTW) at different period of times (period I, II and III). Three consecutive periods were assigned to each treatment. Each period was 10 d of adjusting period and 15 d of the collecting period. Cows were kept individually in tie stall housing with solid floor and open sides. Animal care procedures were approved by the Animal Care Committee guidelines of Faculty of Veterinary Science, Chulalongkorn University.

1.2 Feed and Feeding

Fresh GTW was obtained from a local beverage company in Nakornphathom, Thailand, then dried by air for 48 h (humidity <13 percentage). After dried, the materials were packed into bags, and tying with string after removing air. All bags were kept at ambient temperature.

Diets were formulated to meet NRC requirements (National Research Council, 2001). All animals received feed in the form of Total Mixed Ration (TMR). The forage to concentrate ratio was 39:61 (DM basis). Ingredient compositions of feed were shown in Table 2. Dietary was varied by GTW. The amount of GTW in the control diet and two treatment diets were 0%, 5% and 10% (DM basis), respectively. Food and water were available *ad libitum*. Chemical analysis of GTW in the treatment diets are presented in Table 3

Table 2 Ingredient compositions of the TMR fed to the cow during the experimental period.

Ingredient composition	Dry matter basis (%)		
	control	5%GTW	10%GTW
Corn silage	38.9	39.1	39.2
Cassava	26.4	26.4	26.4
Green tea waste	-	5.0	10.0
Soybean meal	19.4	17.8	16.5
Soybean hull	11.4	7.8	4.0
Full fat soybean	1.2	1.2	1.2
Dicalcium phosphate	1.2	1.2	1.2
Potassium chloride	0.2	0.2	0.2
Limestone	0.9	0.9	0.9
Salt	0.2	0.2	0.2
Premix*	0.2	0.2	0.2

Premix* 1 kg : Vitamin A 2,400,000 IU, Vitamin D_3 500,000 IU, Vitamin E 500 IU, vitamin B_{12} mg, Mn (Manganese) 8 g, Zn (Zinc) 8 g, Fe (Iron) 10 g, Cu (Copper) 2 g, Mg (Magnesium) 26.4 g, Co (Cobolt) 400 mg, I (Iodine) 400 mg, Se (Selenium) 40 mg

Table 3 Chemical analysis of GTW in the treatment diets (DM basis).

Item (%)	Amount
Crude protein (CP)	25.2
Ether Extract (EE)	1.2
Acid detergent fiber (ADF)	26.1
Neutral detergent fiber (NDF)	42.8

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Experimental diets were formulated iso-nitrogenous and iso-energenous TMR. The amount of feed intake was recorded daily. Cows were milked twice a day and milk yields were recorded at 06:00 and 16:00 h. Body weight of individual animal was measured at the start and end of each period. From the d 13 to d 15 in collection periods, rectal temperature (RT) and respiration rate (RR) were measured every 4 h, between 00:00 to 20:00 h for three consecutive days. RT was recorded with digital electronic thermometer. RR was measured by observing the movement of flank for 1 minute, three times, and an average of RR was calculated. An ambient temperature and relative humidity were recorded for calculating the temperature humidity index (THI), for three consecutive days between 00:00 to 20:00 h by thermometer throughout the experimental period. THI was calculated according to NOAA, (1976) (Equation 1):

Equation 1

$$THI = [1.8(temp) + 32] - [0.55 - 0.0055(rh)][1.8(temp) - 26]$$

Water consumption of each cow was measured once daily at 0600 h using individual water meter for three consecutive days. On d 11 through d 20 of each period, cows were dose with 5 g Cr_2O_3 at each feeding via gelatin capsule for a total of 10 g marker/d. This chromic oxide was used as a digestible marker.

2. Sample collection and chemical analysis

2.1 Feed

Samples of GTW were collected for analysis. Total catechin of GTW samples were analyzed by the methods of ISO (2005), and tannin were analyzed by the methods of Burn (1971).

Offered feed and orts were measured daily during the experiment period. Orts were removed in the morning before next feeding. The amount of offered feed and orts were weighed daily. Feed samples of TMR were collected every other day during the collecting periods for the determination of DM content. The DM content was used to calculate DMI.

Samples of feed were collected and immediately frozen at -20 ^{O}C for further analysis. Samples were composited, dried by a forced air oven (Binder ED53) at 60 ^{O}C for 48 h, and ground with a Wiley mill to pass through a 1-mm screen (cyclotec 1093 sample mill). NDF and ADF were analyzed according by the method of Van Soest et al. (1991), and CP were analyzed using Kjeldahl N according to the Association of Official Analytical Chemists (AOAC, 1990).

2.2 Milk

The total milk yield from individual cows were recorded and collected during milking both at 0600 a.m. and 1600 p.m. in each collection period. Two 60 ml of milk in plastic bottles were collected for 3 days (23th to 25th d) in each period and kept at -20 ^{O}C for analysis.

Milk lactose, fat and protein concentrations were analyzed using Milko scan 133B (N. FOSS ELECTRIC. DENMARK). Milk samples were used to determine the urea and purine derivatives (PD) in milk. Milk fat was removed by centrifugation at 3000 rpm for 15 min at 4°C. Protein in fat free-milk samples were removed with 10% TCA for determination of milk urea concentration and with 5% uranyl acetate for determination of milk allantoin concentration. The milk allantoin analysis was carried out by a colorimetric method according to Young and Conway (1942). Urea concentration in milk was determined by the diacetylmonoxime method (Coulombe and Favreau, 1963). The microbial protein synthesis (MPS) or microbial nitrogen flow was analyzed by indirect method which determined the concentration of allantoin in

milk and predict microbial nitrogen flow by using allantoin output in milk (Timmermans et al., 2000) (Equation 2):

Equation 2

MPS (g/d) = 119 + (11.6X) – (3.3MY)

Where; x = allantoin excretion in milk (mmol/d)

MY = milk yield (kg/d)

2.3 Feces

Chromic oxide (Cr_2O_3) was used as an external marker to estimate digestibility of dry matter (DDM), NDF and ADF. Five grams of Cr_2O_3 in gelatin capsules was dosed twice daily at 0600 and 1600 h (10 g/d) during d 11^{th} to 20^{th} for 10 d (Bargo et al., 2002). Dosing began 7 d prior to the start of fecal collection and continued throughout the fecal sampling. The fecal grab sample from rectum was collected every 4 h from d 7^{th} to d 10^{th} post dosing of the marker. Fecal collections were started at 0000, 0400, 0800, 1200, 1600 and 2000 h. The fecal samples were kept at -20 ^{O}C for chromium analysis by the method from Williams et al. (1962).

Frozen fecal samples were dried by a forced air oven at 60 ^{O}C for 72 h, and ground with a Wiley mill to pass through a 1-mm screen (cyclotec 1093 sample mill). One-gram sample of feces were analyzed for the concentration of chromic oxide using spectrophotometer. The digestibility of NDF and ADF can be determined by application of the formula in equation 3 and DDM was calculated as according to equation 4 and 5.

Equation 3

$$\text{Digestibility of a nutrient (\%)} = 100 - 100\left(\frac{\text{\% marker in feed}}{\text{\% marker in feces}} \times \frac{\text{\% nutrient in feces}}{\text{\% nutrient in feed}}\right)$$

Equation 4

$$\text{Faeces dry matter output (kg/day)} = \frac{\text{Marker dose (g/day)}}{\text{Marker concentration in faces (g / kg DM)}}$$

Equation 5

$$\text{Dry matter digestibility} = \left(\frac{\text{DM intake} - \text{DM excreted}}{\text{DM intake}} \right) \times 100$$

2.4 Ruminal fluid

At the end of experimental period, rumen fluid sample of each animal was collected through the stomach tube using vacuum pump after 2.5 h of morning feeding for determination volatile fatty acids (VFA). The pH was measured immediately after sampling using glass electrode (digital pH measurement device). A 60 ml aliquot of the filtered ruminal fluid was preserved by adding 3 ml of *6 N* hydrochloric acid and kept at -20 ^{O}C. Ruminal fluid was analyzed by the method modified from Erwin (1961).

Briefly, frozen ruminal fluid were thawed at room temperature, this were centrifuged at 9,000 rpm for 8 min and the supernatant aliquots were removed. The volume of 0.4 ml working internal standard solution (isocaproic acid, formic acid and 25% metaphosphoric acid) was mixed with 0.7 ml of the supernatant or standard solution. The aliquots were analyzed for the concentration of VFA using a gas chromatograph equipped with a hydrogen flame ionization detector. The column used for analysis (GL Sciences Inc) was treated with 1% (wt/wt) H_3PO_4 (length 2.1 m, ID 4 mm, OD 7 mm) and packed with 10% FFAP (80 – 100 mesh).

2.5 Blood

Blood samples from the external jugular vein were collected from each cow at the start and end of collecting period, by venipuncture with a G21 needle into

heparinized tube. The blood sample was kept on ice, centrifuged post-sampling at 2500 rpm for 20 min, and the plasma was pipette off and frozen at -20 °C until analysis.

A breakdown product of lipid peroxidation, TBARS concentration in lactating cow plasma, was determined by methods according to Ohkawa et al. (1979). The stock solution contained equal volumes of trichloroacetic acid 15% (w/v) in 0.25 normal hydrochloric acid and 2-thiobarbituric acid 0.37% (w/v) in 0.25 normal hydrochloric acid. One ml of fresh plasma was mixed with 2 ml of the stock reagent in screw-capped tube and heated for 15 min in boiling water. After cooling in ice water, 3 ml of an extracting solution contained pyridine and 1-buthanol (1:15) was added and mixed well. The precipitate was removed by centrifugation at 1900 g for 10 min and the absorbance of the supernatant was measured at 532 nm against the extracting solution without plasma extracts. A standard curve was prepared with a known concentration of malondialdehyde and all the above reagents without plasma

The total ascorbate concentration in lactating cow plasma was measured according to the method described in Omaye et al. (1979). The ascorbic acid was oxidized by copper ions to from dehydroascorbic acid and diketogulonic acid, which react with 2,4-dinitrophenylhydrazine to from the derivative bis-2,4-dinitrophenylhydrazine. One ml of fresh plasma was mixed with 1.0 ml of ice-cold 10% TCA solution. After centrifugation, 1.0 ml of the supernatant was recovered and mixed with 0.2 ml of 2,4-dinitrophenylhydrazine/thiourea/copper solutions. The mixed solution was incubated for 4 h at 38 °C, then 1.5 ml of ice-cold 65% sulfuric acid was added. The absorbance of the solution was determined at 520 nm. A standard curve was made using several concentrations of sodium ascorbate.

3. Statistical Analysis

All data were reported as the mean value ± SD. These data were analyzed as a replicated 3 × 3 Latin-square using the general linear model. Each cows represented an experimental unit. The model included square, cows (square), period, replicate, and treatment. The error was residual error mean square. The mean differences between treatments were tested by Least significant different using the commercially computer program (SAS, 2002). Differences were considered significant when $P < 0.05$.

CHAPTER IV

RESULTS

Effects of green tea waste supplementation on feed intake and water intake in cross-bred lactating cows

Chemical analysis of the experimental diet is show in Table 4. The values of body weight (BW), dry matter intake (DMI), dry matter intake per %body weight (DMI/%BW), water intake (WI) and water intake per dry matter intake (WI/DMI) in control group and two treatment groups are show in Table 5. Cows fed with 10% green tea waste supplementation in diet had slightly lower, but not significant ($p>0.05$), DMI and DMI%BW when compared with control diets and 5% GTW diets. It was found that WI/DMI in GTW diet consumed slightly higher (6.82 and 5.96 litres) than cows in the control group (5.34 litres) ($p>0.05$)

Table 4 Chemical analysis of the experimental diets (dry matter basis)

Nutrients (%)	Dietary		
	control	5%GTW	10%GTW
CP	16.4	16.8	17.0
ADF	28.4	29.1	26.9
NDF	47.0	46.8	43.5
Fat	1.4	1.1	0.9
Catechin*	-	0.35	0.69
Condensed tannin*	-	0.76	1.51
NE_L(Mcal/kg)**	1.61	1.62	1.62

*calculated from the amount of GTW addition

**calculated from NRC 1988

Table 5 Dry matter intake and water intake of cows fed TMR at different level of green tea waste inclusion (mean ± SD)

	Dietary		
	Control	5%GTW	10%GTW
BW (kg)	569 ± 98.5	562 ± 94.7	563 ± 102.6
DMI (kg DM/d)	17.5 ± 2.5	17.0 ± 2.3	15.4 ± 3.8
DMI / %BW	2.68 ± 0.3	2.60 ± 0.2	2.58 ± 0.3
WI (l/d)	91.8 ± 5.7	104.6 ± 34.8	91.8 ± 15.6
WI / DMI (l/kg)	5.34 ± 0.7	6.82 ± 2.1	5.96 ± 1.1

Effects of green tea waste supplementation on milk yield and compositions

The values of total milk yield (MY), MY /DMI, percentage of milk compositions and total yield of milk compositions are shown in Table 6. No difference in MY/DMI was found between control and 10% GTW diet. Compare to 5% GTW MY/DMI was improved, but not significant ($p>0.05$) when 10% of GTW diet was fed to the animals. There were no differences in TS, Fat, SNF, protein and lactose compositions between control group and GTW diet group. 10% GTW diet significantly improved ($p<0.05$) milk protein percentage when compared to control diet. However, no difference in milk composition yield was found.

Table 6 MY, MY/DMI, milk compositions and milk composition yield of cows fed TMR at different level of GTW supplementation (mean ± SD)

Parameters	Dietary		
	Control	5%GTW	10%GTW
MY (kg/d)	16.5 ± 2.0	16.5 ± 3.6	16.0 ± 3.0
MY/DMI	0.96 ± 0.11	1.06 ± 0.09	0.96 ± 0.05
4% FCM (kg/d)	14.4 ± 3.1	13.8 ± 2.8	13.5 ± 1.7
Milk compositions (%)			
TS	11.95 ± 1.9	11.63 ± 1.9	11.77 ± 2.3
Fat	3.16 ± 1.0	2.97 ± 1.1	3.12 ± 1.3
SNF	8.79 ± 1.1	8.65 ± 0.8	8.63 ± 1.0
Protein	3.20 ± 0.9[b]	3.37 ± 0.7[b]	3.71 ± 0.9[a]
Lactose	4.76 ± 0.5	4.57 ± 0.5	4.25 ± 0.3
Milk composition yield (kg/d)			
TS	1.91 ± 0.3	1.82 ± 0.2	1.76 ± 0.2
Fat	0.51 ± 0.2	0.46 ± 0.1	0.45 ± 0.1
SNF	1.40 ± 0.2	1.36 ± 0.2	1.31 ± 0.2
Protein	0.50 ± 0.1	0.53 ± 0.1	0.55 ± 0.1
Lactose	0.76 ± 0.1	0.72 ± 0.1	0.64 ± 0.1

[a,b] means in the row with different superscripts are significantly different ($P<0.05$)

Milk urea nitrogen and milk allantoin concentration

The effect of GTW supplementation on milk urea nitrogen, milk allantoin concentration and microbial protein synthesis (MPS), which reflected the ruminal microbial activity are shown in Table 7. During the experimental period, there were no significant differences in milk urea nitrogen, milk allantoin concentration and MPS. Miik urea nitrogen tended to be lower in animals receiving GTW replacement when compared with control group.

Table 7 The effects of green tea waste supplementation on milk urea nitrogen and milk allantoin concentration (means±SD)

	Dietary		
	Control	5%GTW	10%GTW
Urea nitrogen (mg%)	27.33 ± 6.1	26.40 ± 1.7	23.58 ± 5.5
Allantoin concentration (mg/l)	82.75 ± 14.5	87.3 ± 11.0	83.6 ± 13.6
MPS (g/d)	164.77 ± 19.1	170.09 ± 16.1	164.83 ± 20.1

Effects of green tea waste supplementation on digestibility of nutrients

The means value of the digestibility of DM, ADF, NDF and protein. The digestibility of DM, ADF, NDF and protein were slightly higher in control group as compared to treatment group but no significant difference were found (P>0.05).

Table 8 The effects of green tea waste supplementation on digestibility of nutrients (mean ± SD).

	Dietary		
	Control	5%GTW	10%GTW
Nutrients digestibility (%)			
DM	59.11 ± 3.1	54.38 ± 9.3	53.66 ± 17.0
ADF	45.05 ± 4.7	38.07 ± 11.7	39.88 ± 14.2
NDF	48.95 ± 4.9	41.19 ± 13.5	43.64 ± 14.5
Protein	61.95 ± 4.4	59.73 ± 10.2	56.43 ± 15.4

Volatile fatty acid concentrations

The ruminal volatile fatty acid (VFA) compositions, the total concentration of VFA and pH in the experimental period are presented in Table 9. There were no

differences on pH, concentrations for acetic, propionic, butyric and total volatile fatty acid, among animals fed different diets. However, pH were slightly lower in GTW diets but no significant difference was found (P>0.05).

Table 9 The effect of green tea waste supplementation on pH and volatile fatty acid of rumen fluid (mean ± SD)

	Dietary		
	Control	5%GTW	10%GTW
pH	7.0 ± 0.63	6.6 ± 0.18	6.7 ± 0.06
VFA conposition (mmol/l)			
Acetic acid (C_2)	102 ± 18.1	136 ± 39.1	118 ± 38.1
Propionic acid (C_3)	37.9 ± 7.9	38.4 ± 15.1	33.5 ± 10.2
Butyric acid (C_4)	31.6 ± 7.4	38.7 ± 12.0	37.1 ± 13.7
Total VFA	174 ± 33.0	216 ± 63.6	191 ± 61.0

Effects of GTW on the thiobarbituric acid reactive substance (TBARS) value and vitamin C concentration

The effects of GTW on the plasma TBARS value of plasma and vitamin C concentration are shown in Table 10. Plasma TBAR value was slightly increased in GTW treatment, but no difference was observed between treatment. Plasma vitamin C concentrations was not significant differences among treatments. However, plasma vitamin C concentration tended to decrease in GTW treatments.

Table 10 The effect of green tea waste supplementation on TBARS and vitamin C concentration

	Dietary		
	Control	5%GTW	10%GTW
TBARS concentration (mmol/ml)	2.56 ± 1.0	2.86 ± 1.1	3.15 ± 0.6
VitaminC concentration (µmol/l)	30.37 ± 6.1	28.62 ± 4.4	27.17 ± 8.9

Temperature, relative humidity (RH), temperature humidity index (THI) and physiological change

Average temperature, RH and THI during the experimental periods are presented in Table 11. Average temperature on 06:00-12:00 a.m. and 12:00-06:00 p.m. are 28.9 and 29.2°C respectively. During the day, if ambient temperature was higher than 24 °C, the level which has been suggested to be the critical temperature for dairy cattle. However, when average THI is calculated both time had similar THI (80.8 vs 81.1). While in the relative humidity was high in the morning, getting lower when the ambient temperature increased. On the other hand, THI started to increase when the ambient temperature increased.

Average RR (breaths/min) and RT (°C) between treatments are shown in Table 12. No significant differences were observed.

Table 11 Average ambient temperature, relative humidity and temperature humidity index during the experimental periods (mean ± SD)

Item	Time	
	06.00-12.00 a.m.	12.00-06.00 p.m.
Average Temperature (°C)	28.9 ± 3.1	29.2 ±4.1
Average relative humidity	79.3 ± 9.5	78.8 ±16
Average temperature humidity index	80.8 ± 3.9	81.1 ± 4.7

Table 12 Average rectal temperature (°F) and respiration rate (breath/min) of experimental study (mean ± SD)

Average	Dietary		
	Control	5%GTW	10%GTW
Rectal temperature (°F)	101.8 ± 0.81	102.1 ± 0.89	101.8 ± 0.94
Respiration rate (breaths/min)	59 ± 8.72	68 ± 9.0	65.5 ± 0.94

Effects of GTW on profitability

The estimated costs of all experimental diets, total income and net profit are shown in table 13. Data was shown that GTW supplementation could reduce feed cost as a result more profit was achieved.

Table 13 The estimated costs of feed and total income (baht/day/head)

	Control	5%GTW*	10%GTW*
Cost of total feed	124.78	111.01	91.78
Total income from milk[a]	231.0	231.0	224.0
Net profit[a]	106.22	119.99	132.22

[a] Calculation based on milk yield price.

*GTW price was calculated from transportation and labour cost (2.13 bht/kg fed basis)

Milk price is 14 bht/kg

Calculated of the net profit = Total income from milk - Cost of total feed

CHAPTER V

DISCUSSION

Dry matter intake, water intake,

No negative effects of GTW, as protein replacement in TMR at both level, fed to cross-bred dairy cows, on BW, DMI and MY were observed in this experiment. This result was similar to the study of Kondo et al. (2004a,b). However, there was tendency of slightly decreased on DMI when 10% of GTW replacement diet was fed. This may be caused by the higher content of tannin in GTW used in this experiment, which resulted on the palatability of the GTW diets (Xu et al., 2008).

Milk production and compositions

No negative effects of GTW in TMR fed to cross-bred dairy cows on MY were observed in this experiment. This is consistent with the study of Kondo et al. (2004a) which replaced soybean meal and alfalfa hay with 5% GTW in the diet. MY/DMI was improved when level of GTW was increased from 5% to 10%. There were no significant differences in milk fat compositions from cows when GTW was added to the diet at 5% of DM basis. These results were similar to the study of Kondo et al. (2004a) who found that supplementation with GTW at 5% (DM basis) did not influence milk composition. However, milk fat tended to be lower when the level of GTW was increased to 10% (DM basis). This incidence may partly due to the lower level of NDF content and partly to the higher level of tannin in 10% GTW diet. Tannins are known to be the major compound found in GTW. Kondo et al. (2004a) found that GTW contained 1.67% (DM basis) of tannin. Tannin has the negative effect on fiber digestibility by inhibiting the activity of fiber digestive enzyme (Scalbert, 1991, cited in Reed, 1995). This combination effect could reduce milk fat

composition by reducing the amount of ruminal acetate. Acetate is the major precursor substances for the synthesis of milk fat (Kronfeld, 1969).

No difference in milk compositions except protein content were observed. Milk protein content tended to be higher when cows fed with 5% GTW diet. Greater difference in protein content was found in cows fed 10% GTW. O'Connell and Fox. (2001) concluded that phenolic compounds, in this study is tannin, can interact with protein and protect protein from degradation by rumen microorganisms. Once this complex protein passed through abomasums, then it will be broken down by digestive enzyme and providing amino acids to the animals. Kondo et al. (2004b) reported that goats fed with whole-crop oat silage with and without the addition of green tea waste at a rate of 0, 50 and 200 g/kg fresh matter, nitrogen intake per day in green tea waste treatments was higher than that in control ($P<0.05$) but the excretions of nitrogen into feces and urine were similar among treatments. It is likely that replacement GTW in cow's diet could provide better amino acid for milk protein production in cross-bred dairy cows.

Milk urea nitrogen, milk allantoin and MPS

A major source of dietary protein for ruminants or a subsistence diet is known to be the excess bacteria produced in the rumen. Microbial protein synthesis in the rumen comprises 60 to 85% of the CP requirements for maintenance. Rumen microbial protein provides a quality, similar in AA profile to milk protein and is highly digestible. Milk urea nitrogen and allantoin are used to be as the good indicators of microbial protein synthesis (Giesecke et al., 1994; Timmermans et al., 2000;). Xu et al. (2008) studied influence of replacing brewers' grains with green tea ground on ruminal fermentation of wethers. It was founded that increasing of wet green tea grounds level in TMR silage from 0 to 10% (DM basis) slightly decreased plasma urea nitrogen but no difference was found. Loor et al. (2002) reported that urea nitrogen in plasma reflects the content of dietary CP content, excess ruminal ammonia enters the blood and it is converted to urea in the liver. However, Reed

(1995) suggested that low plasma urea might be ascribed to tannins, which lower the rate of protein degradation and deamination in the rumen and therefore lower ruminal ammonia nitrogen. In this study the amount of tannin in GTW diets was higher than control. This may partly affect the amount of milk urea nitrogen. Purine derivatives have been used to measure microbial protein synthesis in the rumen. In this study, there were no significant differences on milk allantoin concentrations at am and pm collection and also average value between groups. From these results it was shown that feeding GTW at 5% and 10% in cows diet in this study had no effect on microbial protein synthesis

Nutrient digestibility

Apparent digestibility of DM, ADF, NDF and protein were unaffected by increasing GTW dietary. However DMD and CPD of dairy cattle fed GTW diets were slightly lower than control diet. The results from this study were similar to the report of Xu et al. (2008) who reported that DM, ADF,NDF and protein were slightly lower when brewer grain was replaced by wet green tea ground ($p>0.05$). Furthermore, higher fecal N output with lower CP digestibility was observed in goats fed the diet supplemented with black tea by-product silage compared to the control diet (63.9 vs 67.5). It was inferred that tannins in black tea by-product silage bound tightly to dietary proteins and/or endogenous proteins that were fractionated as ADIN and protected the degradation in digestive tract. On the other hand, black tea by-product might protect excess protein degradation of a diet and reduce N loss to urea from much NH_3 production in rumen by tannins, as reported by Ben Salem et al. (2005) using *Acacia cyanophylla* leaves.

Volatile fatty acid

Ruminal pH was not affected by diet treatment ($p>0.05$). In this study, level of pH is in the optimum pH (pH>6.2) because concentrate:roughage ratio in fed.

VFA are the result of fermentative organic matter by microbes present in the rumen. Acetic, propionic and butyric acid are the predominant from of VFA in the rumen. VFA constitute a large proportion of the energy available to the cow. Declining intake reduces the quantity of VFA occur in the rumen because of the reduction of fermentable carbohydrate for bacteria. In the present experiment, the molar percent of acetic acid (C_2), propionic acid (C_3), and butyric acid (C_4) were not affected by addition of GTW in TMR diet. There are in agreement with the finding of the present study Kondo et al. (2004a) who found that GTW supplementation had no effect on the ruminal VFA concentration. Xu et al. (2008) reported that the molar percentage of C2, and C2:C3 ratio in the rumen of wethers fed TMR silage were not different when wet green tea ground replacement in feed was increased from 0% to 15% (DM basis). From this result, it was shown that GTW at both level had no effect on volatile fatty acid.

Rectal temperarture and respiration rate

During experimental period THI was moderately higher than the recommended suitable THI (<74). It we use this THI figure as a standard figure it can be concluded that all experimental animal were in stress condition. THI is widely used in hot areas all over the world to assess the impact of heat stress on dairy cow. RT of animal in control, 5% GTW and 10%GTW were 0.2, 0.3 and 0.2 °C higher than normal RT (38.6 °C). Also RR of experimental animals was slightly higher than animals in thermal zone.

Vitamin C and TBARS

The antioxidative component of ascorbic acid concentration in plasma of animals fed experimental diets were not difference. The increase of TBAR concentration in dairy cows plasma seemed to be derived from oxidative stress under hot conditions. It has been reported that oxidative stress increased TBARS.

No significant difference in plasma TBARS concentration of all experimental animals in this study was found. It could be concluded that GTW had no effect on radical scavenging activity in this study which were shown by no differences in both plasma ascorbic acids and TBAR.

Profit

Total net income was found to be increased when 5% GTW and 10% GTW was supplemented. An increment of profit was range from 0.83 to 1.63 baht/kg of milk, depending on the milk composition. The result from this study showing that there had been the possibility for utilize GTW as a source of protein in the diet fed to crossbred dairy cattle in Thailand.

Conclusion

It can be concluded from this study that GTW can be used as a source of protein, at both level 5 and 10% (DM basis), in the diet fed to cross-bred multiparous cows during mid-lactation period without any deleterious effect on milk yield, nutrient digestibility and performance. Replacement of conventional ingredients with GTW in cow's diet showed a better net income than using conventional feed.

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BIOGRAPHY

Miss Thitimon Theeraphaksirinont was born on June 26, 1983 in Nakornpathom, Thailand. She graduated from the Faculty of Animal Science and Agriculture Technology, Silpakorn University. She received the Bachelor degree of Animal Science and Agriculture Technology in 2005. She admitted with the degree of Master of Science, Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University in 2006.