ผลของสมุนไพรรางจืดต่อการย่อยได้ของสารอาหาร เอนไซม์ต้านออกซิเดชั่นและการทำงานของตับ
ในไก่กระทงที่ได้รับอาหารปนเปื้อนสารพิษจากเชื้อรา

นายฉัตรชัย ดอนโคตรจันทร์

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF *THUNBERGIA LAURIFOLIA LINN.* ON NUTRIENT DIGESTIBILITY, ANTIOXIDANT ENZYME ACTIVITY AND LIVER FUNCTION OF BROILER FED MYCOTOXIN-CONTAMINATED FEED

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ฉัตรชัย ดอนโคตรจันทร์: ผลของสมุนไพรรางจืดต่อการย่อยได้ของสารอาหาร เอนไซม์ต้านออกซิเดชั่นและการทำงานของตับในไก่กระทงที่ได้รับอาหารปนเปื้อนสารพิษจากเชื้อรา (EFFECTS OF *THUNBERGIA LAURIFOLIA LINN.* ON NUTRIENT DIGESTIBILITY, ANTIOXIDANT ENZYME ACTIVITY AND LIVER FUNCTION OF BROILER FED MYCOTOXIN-CONTAMINATED FEED) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.น.สพ. ดร. กฤษ อังคนาพร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. สพ.ญ.ดร. จุฑามาส เบ็ญจนิรัตน์, 100 หน้า.

การทดลองนี้มีวัตถุประสงค์เพื่อศึกษาผลของสมุนไพรรางจืดต่อการย่อยได้ของสารอาหาร, เอนไซม์ต้านออกซิเดชั่นและการทำงานของตับในไก่กระทงที่ได้รับอาหารปนเปื้อนสารพิษจากเชื้อรา การทดลองเบื้องต้นเป็นการศึกษาผลของสมุนไพรรางจืดต่อประสิทธิภาพการเจริญเติบโต ค่าน้ำตาลในเลือดและ อัตราส่วนของเม็ดเลือดขาวเฮทเทอโรฟิล: ลิมโฟซัยท์ ในไก่กระทง แบ่งไก่กระทง 150 ตัว เป็น 3 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐาน (กลุ่มควบคุม) กลุ่มที่ 2 และ 3 ได้รับอาหารพื้นฐานที่ผสมผงสมุนไพรรางจืดในระดับ 2 และ 4 เปอร์เซ็นต์ ตามลำดับ บันทึกน้ำหนักอาหารที่กิน ประสิทธิภาพการใช้อาหาร ค่าน้ำตาลในเลือด และอัตราส่วนของเม็ดเลือดขาวเฮทเทอโรฟิล: ลิมโฟซัยท์ ในวันที่ 21 และ 42 ของการทดลอง ผลการทดลองพบว่าการผสมผงสมุนไพรรางจืดในระดับ 2 เปอร์เซ็นต์มีความเหมาะสมมากที่สุดที่จะใช้ในการทดลอง สำหรับการทดลอง ไก่กระทง 450 ตัว ถูกแบ่งเป็น 6 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐานเป็นกลุ่มควบคุม กลุ่มที่ 2 ได้รับอาหารพื้นฐานผสมสารดูดซับสารพิษจากเชื้อราจากยีสต์ (1% กลูโคแมนแนน) กลุ่มที่ 3 ได้รับอาหารปนเปื้อนสารพิษจากเชื้อราในข้าวโพด กลุ่มที่ 4 ได้รับอาหารเหมือนกลุ่มที่ 3 ผสม 1% กลูโคแมนแนน กลุ่มที่ 5 ได้รับอาหารเหมือนกลุ่มที่ 3 ผสมผงสมุนไพรรางจืด 2% กลุ่มที่ 6 ได้รับอาหารเหมือนกลุ่มที่ 3 ผสมผงสมุนไพรรางจืด 2% และ1%กลูโคแมนแนน บันทึกน้ำหนักอาหารที่กิน อัตราการตาย การย่อยได้ของสารอาหาร เอ็นไซม์ต้านออกซิเดชั่น การทำงานของเอนไซม์ในซีรัมที่บ่งชี้ถึงการทำงานของตับในวันที่ 21 และ 42 ของการทดลอง ผลการทดลองพบว่าในวันที่ 42 ไก่กลุ่มที่ 5 และ 6 มีการย่อยได้ของสารอาหารมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในวันที่ 21 พบว่าไก่กลุ่มที่ 4 มีการทำงานของเอนไซม์มอลเตสมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) อย่างไรก็ตาม ไก่กลุ่มที่ 3 มีการทำงานของเอนไซม์มอลเตสน้อยกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ทั้งวันที่ 21 และ 42 ไก่กลุ่มที่ 4,5 และ6 มีการทำงานของเอนไซม์กลูตาไทโอน เปอร์ออกซิเดสมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) จากการทดลองนี้สามารถสรุปได้ว่าการเสริมสมุนไพรรางจืดช่วยลดระดับน้ำตาลในเลือดของไก่กระทงปกติ สารพิษจากเชื้อราที่ปนเปื้อนในอาหารจะลดสมรรถภาพการเจริญเติบโตของไก่กระทงในวันที่ 21 ถึง 42 การเสริมผงสมุนไพรรางจืดหรือผงสมุนไพรรางจืดร่วมกับ 1% กลูโคแมนแนน จะช่วยลดผลกระทบของสารพิษจากเชื้อราโดยเพิ่มการย่อยได้ของสารอาหารและการทำงานของเอนไซม์ต้านออกซิเดชั่น กลูตาไทโอน เปอร์ออกซิเดส ระดับของสารพิษจากเชื้อราในการทดลองนี้มีผลเล็กน้อยต่อการทำงานของเอนไซม์ในซีรัมและการเปลี่ยนแปลงทางจุลพยาธิวิทยาของตับ

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CHUTCHAI DONKOTJAN: EFFECTS OF *THUNBERGIA LAURIFOLIA LINN.* ON NUTRIENT DIGESTIBILITY, ANTIOXIDANT ENZYME ACTIVITY AND LIVER FUNCTION OF BROILER FED MYCOTOXIN-CONTAMINATED FEED. THESIS ADVISOR: ASSOC. PROF. KRIS ANGKANAPORN, D.V.M., Ph.D., THESIS CO-ADVISOR: CHUTAMAS BENJANIRUT, D.V.M., Ph.D. 100 pp.

The objectives of this experiment were to study the effects of *Thunbergia laurifolia Linn.* (TL) on nutrient digestibility, antioxidant enzyme activity and liver function of broiler fed mycotoxin-contaminated feed. In a preliminary study, the effects of TL on growth performance, blood glucose and heterophil:lymphocyte (H:L) ratio were examined. One hundred and fifty, one day old, male, Arbor Acre broilers were divided into 3 treatment groups: T1, basal diet (control group), T2 and T3, basal diet supplemented with 2 and 4 % TL, respectively. Body weight gain, feed intake, feed conversion ratio (FCR), blood glucose and H:L ratio were measured at day 21 and 42. The results showed that 2% TL was appropriate to be used in the experiment. In the experiment, 450 one day old, male, Arbor Acre broilers were divided into 6 treatment groups: T1, control corn-soybean diet; T2, control diet supplemented with 1% yeast glucomannan (GCM); T3, diet with mixed mycotoxins (aflatoxin, fumonisins and tricothecene) contaminated corn; T4, diet as in T3 supplemented with 1% GCM; T5, diet as T3 supplemented with 2% TL; T6, diet as T3 supplemented with 2% TL and 1% GCM. Body weight gain, feed intake, mortality rate, nutrient digestibility, antioxidant enzyme activity, serum enzyme activity in relation to liver function were measured at day 21 and 42. The results showed that broilers in T5 and T6 groups had significantly ($p<0.05$) higher nutrient digestibility than other groups at day 42. Broilers in T4 groups had significantly ($p<0.05$) higher maltase activity than other groups. However, broilers in T3 groups had significantly lower ($p<0.05$) maltase activity than other groups on day 21. Broilers in T4, T5 and T6 groups had significantly higher ($p<0.05$) glutathione peroxidase activity than other groups on day 21 and 42. In conclusion, TL reduced blood glucose concentrations in normal broilers. Mixed mycotoxins contamination in feed reduced growth performance of broilers during day 21-42 of age. TL and TL plus GCM helped to ameliorate the adverse effect of mixed mycotoxin with improving nutrient digestibility and increasing antioxidant enzyme (glutathione peroxidase) activity. The level of mixed mycotoxins in this study slightly affected serum enzyme activity and histopathological changes.

Department Veterinary Physiology
Field of study Animal Physiology
Academic year 2008

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LIST OF ABBREVIATIONS

ADG	average daily gain
AIA	acid insoluble ash
ANOVA	Analysis of variance
BHT	butyrate hydroxyl toluene
BW	body weight
DFI	daily feed intake
FCR	feed conversion ratio
g	gram
ID	ileal digestibility
kcal	kilocalorie
kg	kilogram
MDA	molandialdehye
ME	metabolizable energy
mg	milligram
mg%	milligram percent
ml	milliliter
mM	millimolar
NADPH	nicotinamide adenine dinucleotide phosphate
nm	nanometer
nmol	nanomole
ppb	part per billion
ppm	part per million
rpm	round per minute
SD	standard deviation
SGOT	serum glutamate oxaloacetate transaminate
SGPT	serum glutamate pyruvate transaminate
TBA	thiobarbituric acid
TL	*Thunbergia laurifolia Linn.*
U	unit
WG	weight gain
µl	microliter
µg	microgram

CHAPTER I
INTRODUCTION

Mycotoxin is one of the important problems in broiler industry. It causes primary immuno-suppression that makes poor performance and increases the susceptibility of broiler to diseases (Lim and Vithia, 2007). In addition, many types of mycotoxin were found simultaneously in broiler feed and they adversely affected feed utilization and growth of broiler (Guo et al., 2004). Moreover, potential transfer of mycotoxins from broiler to man via the food chain is feasible (Maurice et al., 1983).

The important mycotoxins in broiler feed are aflatoxin, fumonisins, and tricothecene (Surai and Mezes, 2005). They are produced from *Aspergillus spp*. and *Fumonisins spp*. These fungi can grow well in high humidity and temperature environment such as in the tropical area (Quezada et al., 2000)

Broiler products are popular for consumers all over the world (Smith, 1992). Therefore, the concern about the safety and hygiene of these products is significant. Not only antibiotic contamination but also mycotoxin problem has been regarded in most integrated broiler company (Zhang et al., 1991).

In Thailand, mycotoxin problem affects the broiler industry as same as Enterobactericiae (*Sallmonella spp*.) and *Clostridium perfringens*, most food borne bacteria. Thailand is located in the tropical area, therefore, many fungal growth and mycotoxin productions in animal feeds are inevitable (จักรกริศน์, 1997).

Nowadays, several parts of broiler industry are trying to solve mycotoxin problem. There are many adsorbents being used to prevent mycotoxins toxicity in broilers such as activated charcoal, hydrated sodium calcium aluminosilicate (HSCAS), clay materials, bentonite, including yeast and products from yeast (Huwig et al., 2001). However, their effects are still controversial and many alternative additives are further investigated.

Herbs have been used as food and for medicinal purposes for centuries. The World Health Organization (WHO) estimated that eighty percentages of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of these therapies involve the use of plant extracts or their active components (Ciftci et al., 2005). Those plants and their components are perceived as natural and safe by consumers. Such compounds are already established as flavorings in human and animal feeds.

Interestingly, Samapundo et al. (2007) reported that phenolic compounds available in most herbs can be used to protect corn from fungal invasion and mycotoxins contamination during harvest and storage. In addition, Atroshi et al. (2002) found that antioxidants and natural constituents can be used against mycotoxin toxicity.

Thunbergia laurifolia Linn. (TL) is the herb of interest due to its wide range of medicinal properties and application reported for treating illnesses or diseases. Many researchers indicated that TL elicited an antidote for ethanol (กรุงไกร, 1973; ชัชวดีและคณะ, 1979; Chanawirat, 2000), insecticide and toxicant (สกุลรัตน์และธานี, 1999; สะเทิม, 2003). Moreover, it was found that there was a significant effect of TL on anti-inflammation in mice (สุพรและคณะ, 1998). Furthermore, it has been reported that TL has hepatoprotective effect in mice (Pramyothin et al., 2005) and can be used as antioxidant agent (ศิริพลและสันติ, 2003). In addition, TL has been used to reduce blood glucose in diabetic rat (สุริยันต์, 1998). Interestingly, Sasisakulporn (1981) reported that TL has pesticide adsorbent property that can be adapted to mycotoxins adsorbent and detoxification in this research.

However, researches involving the effects of TL on mycotoxin adsorbent and detoxification have not been yet investigated. It has been examined only in rats and mice and there is only one research in broilers that involved anti-insecticide (สะเทิม, 2003). It is interesting that TL can be used as mycotoxins adsorbent and to support liver in the process of detoxification. It is hypothesized that TL affects growth performance, and intestinal function in broiler fed mixed mycotoxins contaminated-feed. Moreover, with the antitoxic and antioxidant effects, TL may also help to improve liver function. Therefore, the objectives of this experiment are to examine the effects of *Thunbergia laurifolia Linn.* on growth performance, jejunal disaccharidase activity, ileal digestibility of nutrients, various antioxidant enzyme activities and malondialdehyde concentrations and liver function in broilers fed on mixed mycotoxin-contaminated feed.

CHAPTER II
LITERATURE REVIEW

2.1 *Thunbergia laurifolia Linn.*

Thunbergia laurifolia Linn. is a medicinal plant. The therapeutic use of this herbal remedy with its wide range of applications has been well documented in South East Asia (Sasisakulporn, 1981). In addition, it can grow well in different parts of Thailand, particularly; north and north-eastern. Thai people have used it in different ways.

2.1.1 Nomenclature

"Rang Jued' means taste reduction. In other words, it means no concentration in Thai language (Sasisakulporn, 1981). The name "Rang Jued" might come from the original word "Jang Jued" which Chantaburi people call the herb because the word "Jang" means less concentration which refers to detoxification.

Thunbergia laurifolia Linn. is in the family Acanthacace. This medicinal plant has many common name such as Rang Jeud (รางจืด), Rang Yen (รางเย็น), Duwow (ดุเหว่า), Tid Pud (ทิดพุด), Kob Cha Nang (ขอบคะนาง), Ya Keaw (ยาเขียว), Kam Lung Chang Peork (กำลังช้างเผือก) and Kai (คาย) (Chanawirat, 2000).

2.1.2 Systematic classification (Taxonomy) (Chanawirat, 2000)

Thunbergia laurifolia Linn. is classified by taxonomy method as following:

Classification	Name
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Acanthaceae
Genus	Thunbergia
Species	*Thunbergia laurifolia Linn.*

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Figure 2.1 *Thunbergia laurifoilia Linn.* (Tree) (Forest and Kim sterr, 2009).

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Figure 2.2 *Thunbergia laurifoilia Linn*. (Flower) (สีรี, 2009).

Figure 2.3 *Thunbergia laurifoilia Linn*. (Leaf) (สีรี, 2009).

2.1.3 Morphology

Putiyanan et al. (2009) described that *Thunbergia laurifoilia Linn*. is medium-size, evergreen chamber that have round green stems basal diameter 3 cm: back finely striate, tan with clearly visible node and internodes. Leaves are simple, opposite venations, glabrous surface, long-ovate shape, crenate, leaf blades are dark green, 8 – 10 cm long, 4 -5 cm wide, petioles are 2.5 cm long. Flowers are cymes, 3 -4 florets per cymes, pedicels light green and light yellow, funnel form, 1 cm long, 5 petals split into 5 lobes, light purple or indigo blue; calyx cream; corellas: narrow base cream. Fully-blossom flowers are 3 inches in diameter with white tube inside, green-red spot, light yellowish with violet bract, 4 stamens. Fruits are sharp-end pods, 1 cm long and dehiscence: mature fruits split into 2 pieces.

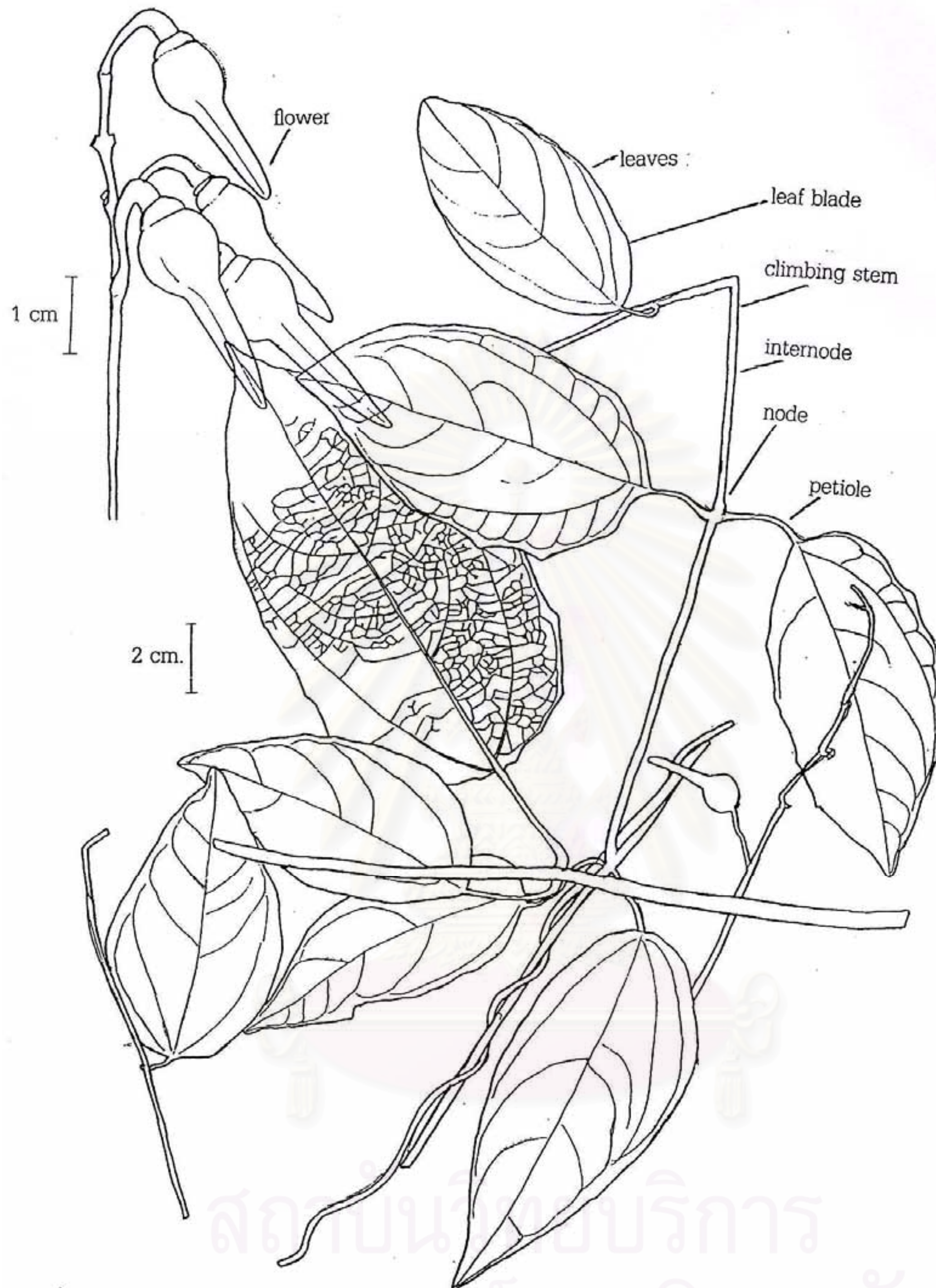

Figure 2.4 Morphological description of *Thunbergia laurifoilia Linn*. (Putiyanan et al., 2009).

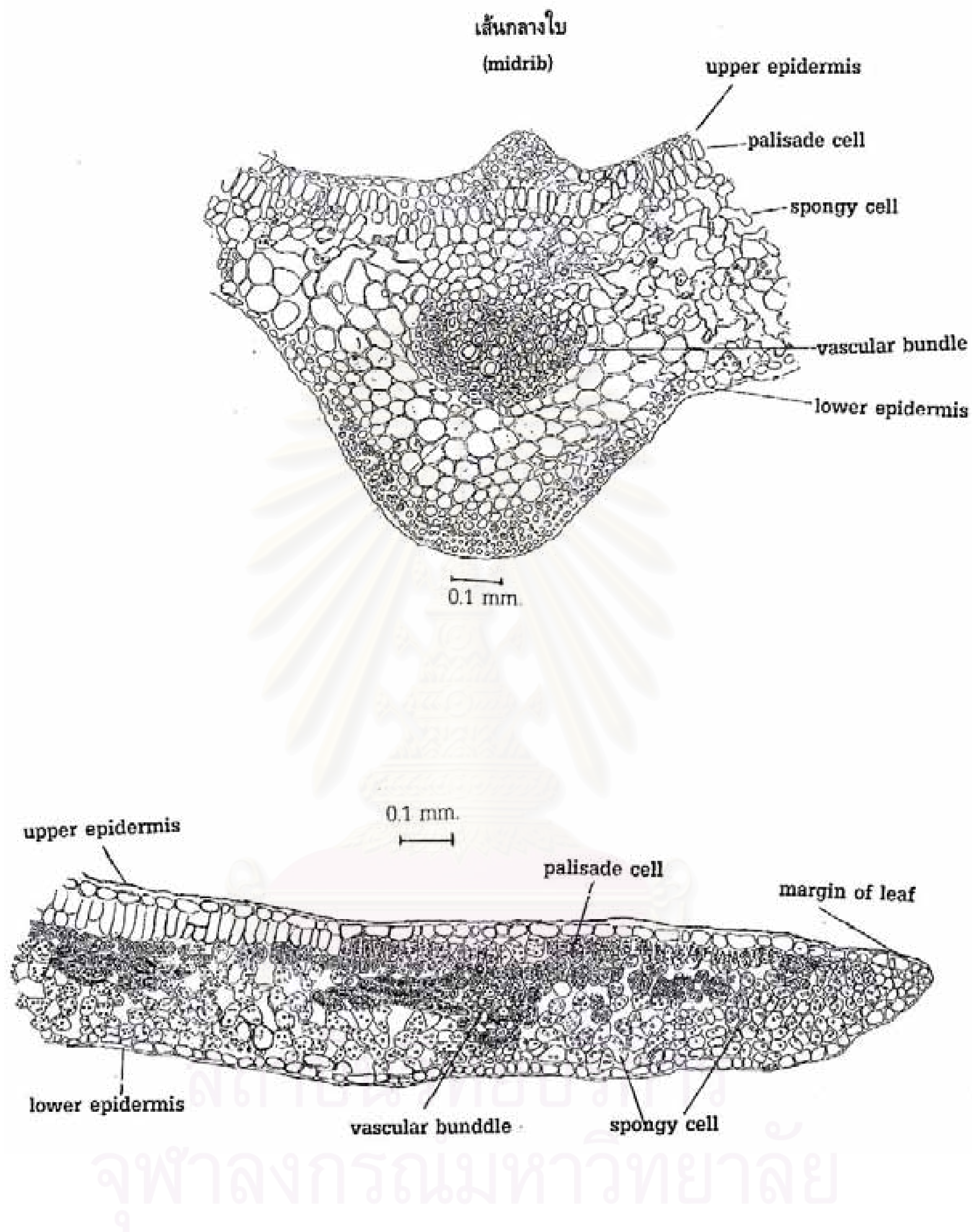

Figure 2.5 Transverse section of *Thunbergia laurifoilia Linn*. (Putiyanan et al., 2009)**.**

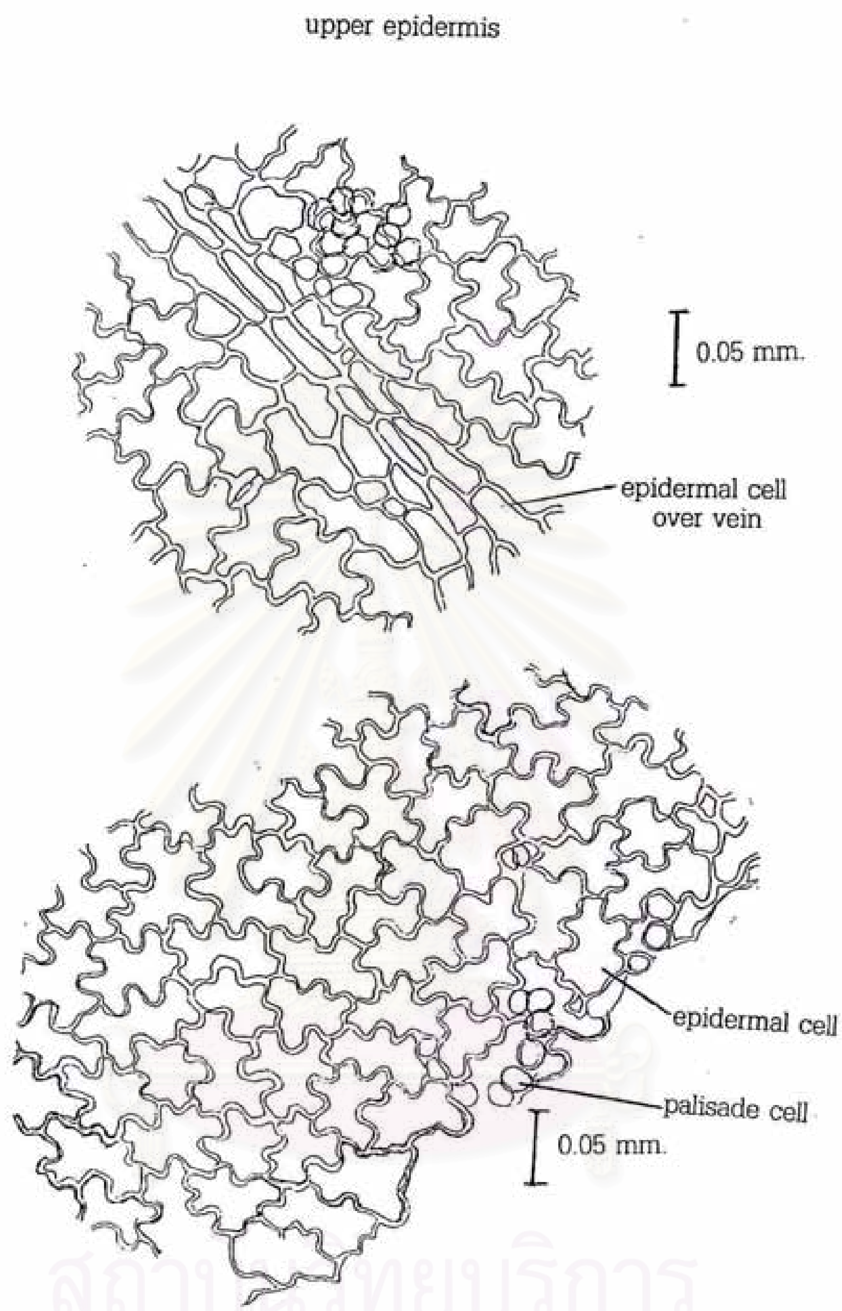

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Figure 2.6 Surface section of epidermal tissue of *Thunbergia laurifoilia Linn*. (upper epidermis) (Putiyanan et al., 2009)**.**

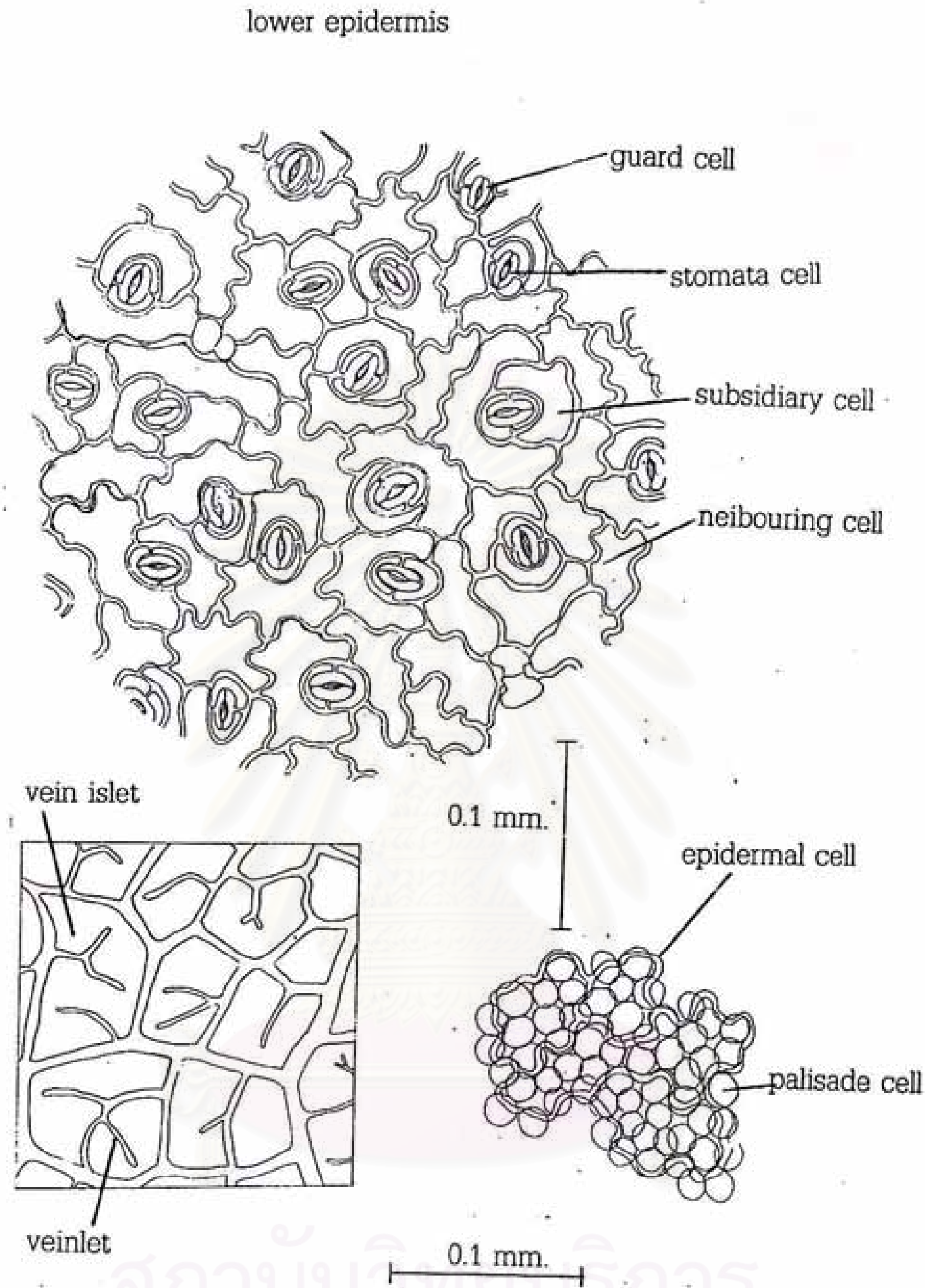

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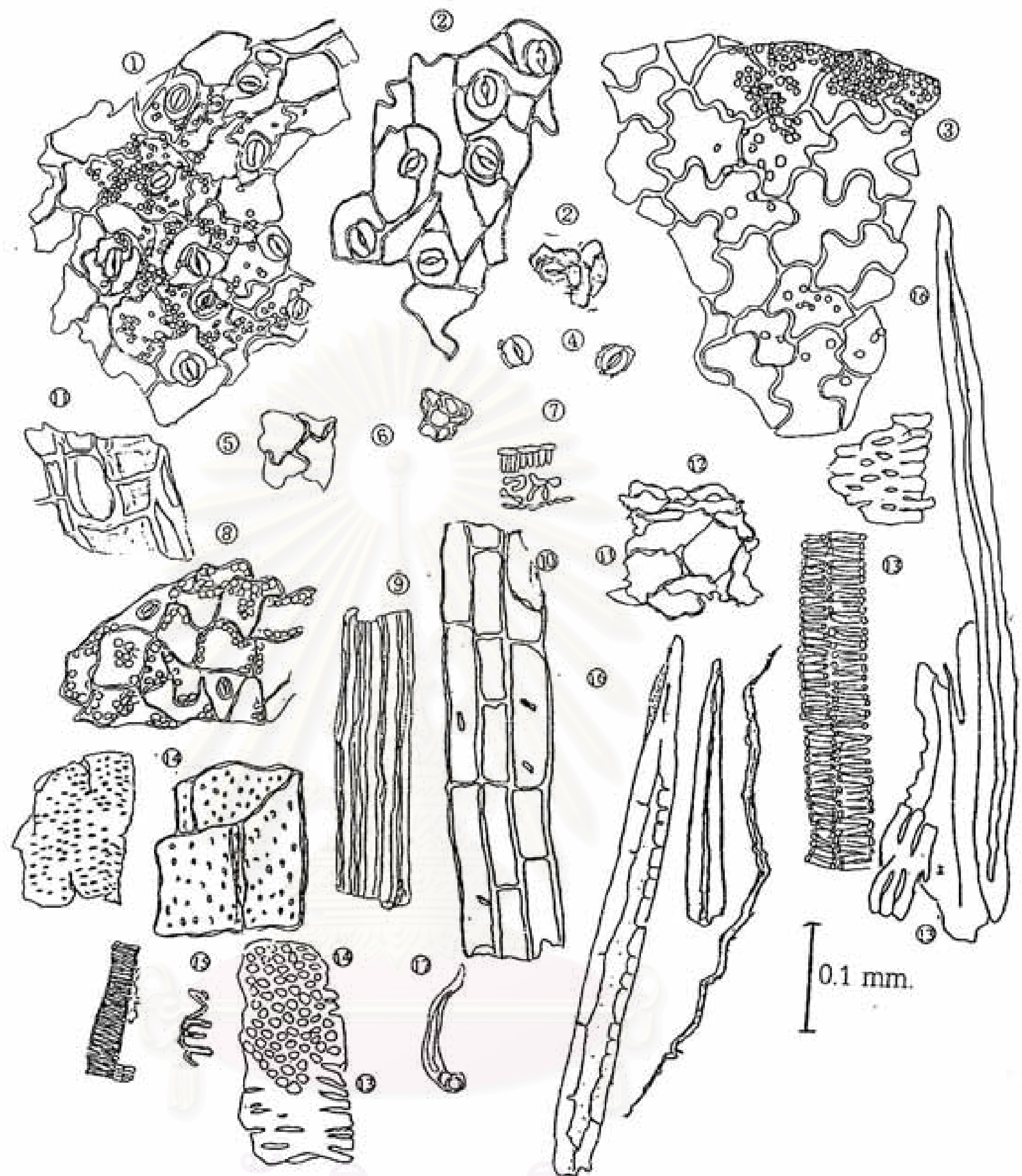
Figure 2.7 Surface section of epidermal tissue of *Thunbergia laurifoilia Linn.* (lower epidermis) (Putiyanan et al., 2009)**.**



① epidermal tissue with anomocytic stoma and chloroplast
② epidermal tissue with anomocytic stoma
③ lower epidermis with chloroplast
④ guard cell
⑤ epidermal cell
⑥ epidermal cell with cutin
⑦ transverse section of leaf
⑧ parenchyma cell with starch grains
⑨ bundle of fiber
⑩ parenchyma cell over vein
⑪ parenchyma cell
⑫ epidermal cell in surface veiw
⑬ reticulate vessel
⑭ bordered vessel ⑮ spiral vessel
⑯ various shapes and size of fiber
⑰ trichomes

Figure 2.8 Powdered *Thunbergia laurifoilia Linn*. Leaf (Putiyanan et al., 2009).

2.1.4 Ecology and distribution

Thunbergia laurifolia Linn. can be found in evergreen, grove forest and indigenous pasture of Thailand, Malaysia, and India (ชุมพล, 1986). In Thailand, it can grow well in different areas with more distribution in north and north-eastern parts. Moreover, it is found mainly during winter and summer seasons.

2.1.5 Botanical characteristics

The botanical characteristics of *Thunbergia laurifolia Linn* can be described as follow 1) stem is climbing type, light green color and smooth skin 2) leaf is ovate figure and green color 3) flower is raceme type and purple color (ชุมพล, 1986).

2.1.6 Chemical constituents

Purnima and Gupta (1978) reported that *Thunbergia laurifolia Linn*. contains flavonoids including Apiginin, Apigenin-7-o-β-D-glucopyranoside and Delphinidin-3,5-di-o-β-D glucopyranoside and also contains methionine, glycine, serine and carotenoid (วีรยุทธ, 1979). Stigmasterol, β-sitosterol, α-spinasterol, and Δ^7–stigmastenol were also found in *Thunbergia laurifolia Linn* (ขวัญใจ, 1983; ขวัญสิริและคณะ, 1999). Kanchanapoom et al. (2002) reported that *Thunbergia laurifolia Linn.* has several substances such as 8-epi-grandifolic acid, 3'-o-β glucopyranosyl-stibericoside, benzyl β-glucopyranosyl-stibericoside,benzyl-β-(2'-o-β-glucopyranosyl) glucopyranoside, gradifloricacid, (E)-2-hexenyl β-glucopyrano- side, hexazanol. Other substances such as β-glucopyranoside,6-c-glucopyranoslyapigenin, and 6, 8–di-c-glucopyra-nosyl apigenin were found in *Thunbergia laurifolia Linn*. (วีระชัย, 2004).

2.1.7 Nutrient composition

The analysis of *Thunbergia laurifolia Linn*. showed that its leaves contain 22.55 % crude protein, 26.45 % crude fiber, 2.74 % crude fat, 6.35 % ash, 47.63 % nitrogen free extract, 36.16 % acid detergent fiber, 46.88 % neutral detergent fiber, 8.15 % lignin, 2.08 % potassium, 147.6 mg/% magnesium, 279.7 μg/% chromium, 0.78 mg/% copper, 27.1 μg/% iodine, 27.4 mg/% iron, 2.42 mg/% manganese, 5.58 mg/% selenium and 3.24 mg/% zinc (กรมปศุสัตว์, 2007).

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2.1.8 History and local traditional uses

Thunbergia laurifolia Linn. is used as traditional medicine in all South East Asian countries. It is indicated by many evidences that it has been developed by the ancient Indian Medical System (Ayurveda) (Sasisakulporn, 1981). The Ayurveda used the herb for alcohol detoxification treatment.

In Thailand, The herb was recorded on the stone pole of Wat Phra Chetuphon Vimolmonkkhalaram (วัดพระเชตุพนวิมลมังคลาราม) or Wat Phothi (วัดโพธิ์). The records described that the herb was used as anti-pyretic medicine which Thai people call "Ya-Khiow" (ยาเขียว). Furthermore, the people who like drinking use this herb before drinking alcohol so they will not get drunk easily. For these reasons, it can be assumed that *Thunbergia laurifolia Linn*. has anti-drunken or anti-alcohol property.

2.1.9 Different effects of *Thunbergia laurifolia Linn.*

There are several trails involving the effects of *Thunbergia laurifolia Linn*. as following.

2.1.9.1 Antidote for ethanol

Blended dried stem of *Thunbergia laurifolia Linn*. can be used as an antidote for ethanol. Blended, dried stems of *Thunbergia laurifolia Linn.* can be mixed together with rice washed water for the purpose of alleviating the ethanol toxicity (กรุงไกร, 1973).

The effect of the aqueous extract of *Thunbergia laurifolia Linn.* in antagonizing ethyl alcohol (17.5%) activity was studied on motor activity in rats. The result showed that *Thunbergia laurifolia Linn.* extract could inhibit the CNS suppression caused by ethanol in the short period, approximately 5 minutes (ชัชวดีและพาณี, 1978).

Chanawirat (2000) investigated the possible protective mechanism of *Thunbergia laurifolia Linn.* extract on ethanol-induced hepatotoxicity in mice. The result showed that *Thunbergia laurifolia Linn.* might be useful for counteracting the effect of alcohol and might be effective for treating hepatic injury.

2.1.9.2 Anti-insecticide, anti-herbicide and anti-toxicant

It was reported that *Thunbergia laurifolia Linn.* could alleviate suffering in rats receiving insecticide (ธีระและธำรงค์, 1978). It was also found that *Thunbergia laurifolia Linn.* could reduce toxic residue of insecticide in broilers (สะเทิม, 2003).

For the effect as antiherbicide, it was reported that *Thunbergia laurifolia Linn.* extract slightly reduced toxicity of paraquat in patients (สุนันทา, 1997; ภัทร์รวีและวลีพร, 1999). Also, the plant could relieve toxicity in rats caused by paraquat (สกุลรัตน์และคณะ, 1998) and toxicity of parathion in rats (สกุลรัตน์และธานี, 1999). Interestingly, Sasisakulporn (1981) reported that *Thunbergia laurifolia Linn*. has pesticide adsorbent property**.**

The possible antitoxic effect of *Thunbergia laurifolia Linn.* on toxicities induced by extract of *Pueraria mirifica* in male rats were investigated. The results showed that *Thunbergia laurifolia Linn.* significantly increased sperm density and inhibited reduction of micronuclei. However, effects of *Pueraria milifica* on red blood cell volume and body weight of rats were not be improved by *Thunbergia laurifolia Linn*. (กนกพรและคณะ, 2001; พัฒนพงษ์, 2003).

2.1.9.3 Anti-inflammation

It was reported that eight different steroids were found in *Thunbergia laurifolia Linn.* extract (ผการัตน์และละออง, 1997). Purified active constituents from *Thunbergia laurifolia Linn.* were seperated using column chromatography and preparative thin layer chromatography. The constituents of the extract exhibited some characteristics that might be proposed that their structural formulas were similar to those of the steroids (สุพรและคณะ, 1998).

In the anti-inflammatory study using carreenin-induced paw edema ethanol and hexane *Thunbergia laurifolia Linn.* extracts were significantly effective in reducing paw edema in mice ($p<0.01$) compared to the control group (สุพรและคณะ, 1998). The best results were found when skin permeation enhancer was added with the drug (กรกตและสุรางค์รัตน์, 2003). The development of the cream, lotion and gel bases was also included in this study, based on their good appearance, optimum pH and viscosity as well as good

stability. Another anti-inflammatory study was also tested using ethyl-phenylpropionate induced ear edema method. It is shown that only *Thunbergia laurifolia Linn*. gel at 20% and 30% concentrations possessed anti-inflammatory activity compared to the reference preparation. Other *Thunbergia laurifolia Linn.* creams showed no anti-inflammatory activity (สุพรและคณะ, 1999).

2.1.9.4 Antioxidant activity

The radical scavenging activity of *Thunbergia laurifolia Linn*. extract was tested with 2,2-diphenyl-1-picrythydrazyl radical (DPPH) using butylated hydroxyl toluene (BHT) and vitamin C as standards. EC_{50} of *Thunbergia laurifoia Linn.* was found to be 7.37. The EC_{50} of BHT and vitamin C were 18.73 and 5.05. It can be concluded that *Thunbergia laurifolia Linn.* extract showed the potent antiradical scavenging property (ศิริพลและสันติ, 2003).

2.1.9.5 Hepatoprotective activity

Scientific research in herbal medicine with hepatoprotective activity may be a great benefit as an alternative therapy in alcohol induced liver disease. Premyothin et al.(2005) used primary cultures of rat hepatocyte and rat as the in vitro and in vivo models to evaluate the hepatoprotective activity of aqueous extract from *Thunbergia laurifolia Linn* (TLE). Ethanol was selected as hepatotoxin and silymarin (SL) was the reference hepatoprotective agent. The results suggested that TLE and SL possess the hepatoprotective activity against ethanol induced liver disease in both primary cultures of rat hepatocyte and rats.

2.1.9.6 Blood glucose level

The effect of *Thunbergia laurifolia Linn*. on blood glucose level was studied. Wistar albino rats were used as the experimental animal. The experiment aimed to compare blood glucose level before and after receiving the extract from *Thunbergia laurifolia Linn.* in normal and diabetic groups. It was found that there was a reduction of blood glucose level in normal and diabetic groups (สุริยันต์, 1998).

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2.1.9.7 Gastrointestinal tract

The mechanism of *Thunbergia laurifolia Linn.* extract at cholinergic and histamic receptor of smooth muscle were determined in 92 preparations of isolated rat small intestine. It was found that atropine and diphenhydramine could not antagonize the stimulating effect of the extract on the smooth muscle. This effect is similar to the action of prostaglandin, bradykinin and substance P (วีระวรรณ, 1980).

2.1.10 Toxicity

Acute and 28 days repeated oral dose toxicity studies were performed using Spraque Dawley rats of both sexes. Bacteria *Salmonella typhimurium* strains TA98 and TA100 were used in this study of mutagenicity. The results revealed that the aqueous extract of *Thunbergia laurifolia Linn.* at a single oral dose of 10 g/kg body weight did not alter the general behavior and the feature of the visceral organ of rats. The aqueous extract of *Thunbergia laurifolia Linn*. at the concentrations between 2.5 - 20.0 milligrams per milliliter did not cause genomic mutation of bacteria (วิรวรรณและคณะ, 2003).

It was reported that the side effect of *Thunbergia laurifolia Linn.* extract (15%) caused depression of central and peripheral nervous system. Dosages of *Thunbergia laurifolia Linn*. extract which significantly depressed CNS were 0.5 and 1.0 ml/100 gm. High doses of *Thunbergia laurifolia Linn.* (0.2, 0.4 and 0.8 ml/100gm.) could depress neuromuscular junction for a long time. Dose that produces marked hypotensive effect was 120 mg/kg. Dose that caused the highest change of tonus and contractile force of intestine was 16mg/ml (ชัชวดีและคณะ, 1979).

2.2 Mycotoxins

2.2.1 General information

Mycotoxins are secondary metabolite produced by several fungi (Samapaudo et al., 2007). These fungi can grow well in feedstuff (such as corn and soybean) under the favorable condition for mycotoxins production. In addition, mycotoxins can be found in both human and animal feed. It is found mycotoxins are produced from *Aspergillus, Fusariums*, and *Penicillium* more than other fungal species. The metabolite can damage

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the function of liver and kidney which are the major detoxification organ. Moreover, it interferes with protein synthesis and suppresses the immune system.

2.2.2 Important mycotoxins in broiler industry

In broiler industry, major important mycotoxins are aflatoxin, fumonisins, and tricothecene which affect growth performance and physiological system.

2.2.2.1 Aflatoxin

Aflatoxin is produced from several types of *Aspergillus spp.* especially flavus and paraciticus. There are four major types: B1, B2, G1 and G2 as follow.

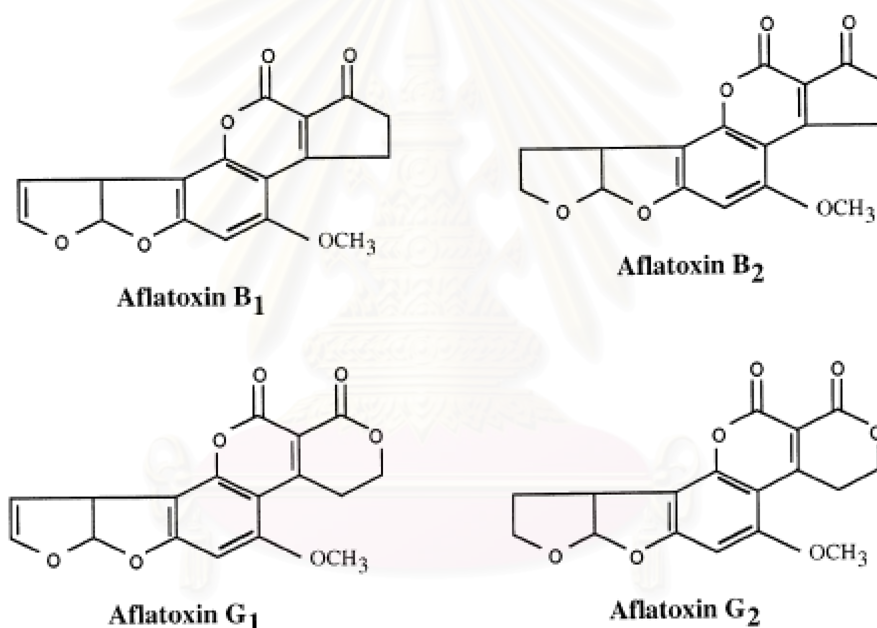

Figure 2.9 Types of aflatoxins (Sweeney and Dobson, 1998)

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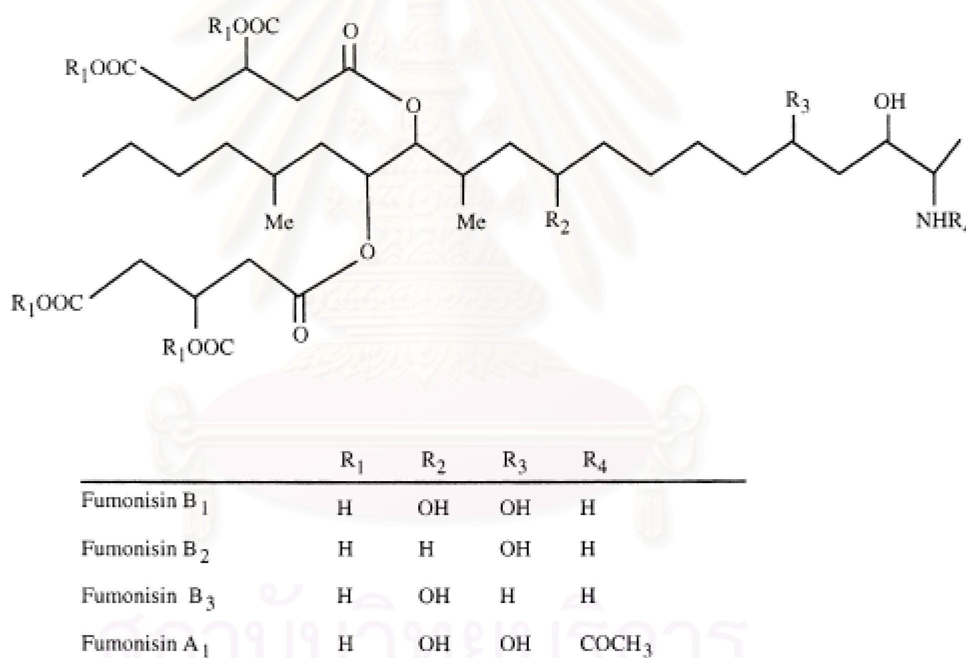
Aflatoxin B1 is found in broilers more than other species of animals. It is produced as a secondary metabolite by the mould *Aspergillus flavus*. In addition, it is a protein mutagen and hepato-carcinogen. Previous research has been indicated that aflatoxin B_1 is activated by hepatic cytochrome P450 (CYP450) enzyme system to produce intermediate, AFB_1-8,9-epoxide(AFBO). After that, the intermediate bind to nucleophilic site in DNA to form DNA adducts. In addition, it preferentially attacks guanine residues in DNA and forms the major adduct, 8, 9-dihydro-8-(N^7-guanyl)-9-hydroxy- AFB_1 adduct (AFB_1- N^7-Gua). The formation of AFB_1-DNA adducts is

regarded as the initiation of AFB_1-induced hepatocarcinogenesis (Zhang et al., 1991). In addition, the metabolic process of AFB_1 by cytochrome P450 also leads to an elevated production of intracellular reactive oxygen species (ROS). The impairment of ROS to cellular component, especially the formation of 8-hydroxydeoxyguanine (8-OHdG), attacks hydroxyl radical on C^8 position in guanine residues. Consequently, it contributes to the carcinogenicity of AFB_1 (Liu et al., 2001).

2.2.2.2 Fumonisin

Fumonisins are produced by fungi *Fusarium verticillioides* or *Fusarium moniforme and* other *Fusarium* species. There are at least 14 types but only 4 major types can be found as following



	R_1	R_2	R_3	R_4
Fumonisin B_1	H	OH	OH	H
Fumonisin B_2	H	H	OH	H
Fumonisin B_3	H	OH	H	H
Fumonisin A_1	H	OH	OH	$COCH_3$

Figure 2.10 Structure of some fumonisin (Sweeney and Dobson, 1998).

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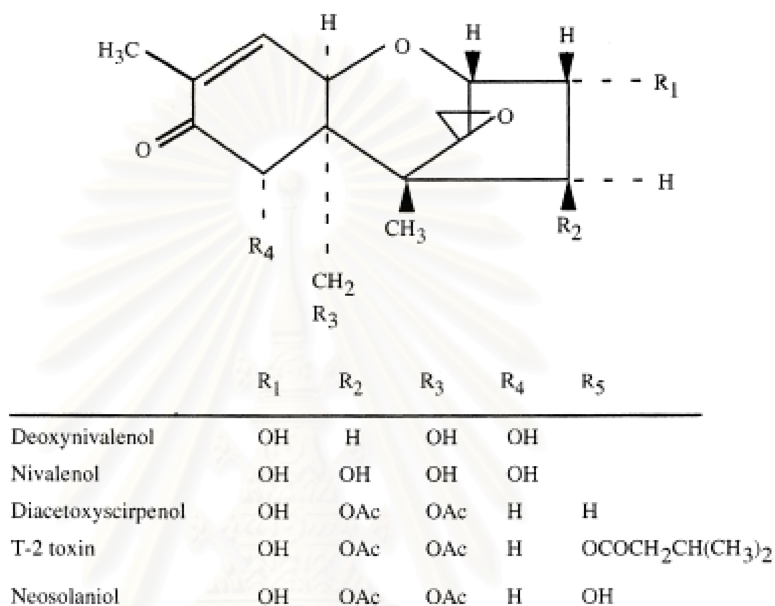
The most plentiful and potential fumonisins is fumonisin B1. The previous research showed that it can lead to leukoencephalomalacia in horse, pulmonary edema in pig, and liver and kidney toxicity in other species (Dragan et al., 2001). It also causes liver disease in horses and cardiac effects, pulmonary edema in swine. Furthermore, it causes esophageal cancer and neural tube defects in humans.

2.2.2.3 Tricothecene

Trichothecenes are secondary fungal metabolites produced by *Fusaruim poae* and *Fusarium sporotrichoides*. (Jacobsen and Coppock, 2007). The four major types can be always found as following



	R_1	R_2	R_3	R_4	R_5
Deoxynivalenol	OH	H	OH	OH	
Nivalenol	OH	OH	OH	OH	
Diacetoxyscirpenol	OH	OAc	OAc	H	H
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
Neosolaniol	OH	OAc	OAc	H	OH

Figure 2.11 Structure of some trichothecenes (Sweeney and Dobson, 1998)

Animals fed on trichothecene contaminated feed will refuse feed as the first sign. After that, weight loss, diarrhea as well as lethargy can be found. Then abdominal pain, teeth grinding, hemorrhages and bleeding from intestinal tract can occur. Moreover, the metabolite affects rapid cell division in all cells such as precursor cells that form the red and white blood cell. It also decreases feed consumption, growth performance and immune function (Sweeney and Dobson, 1998).

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2.2.3 Mycotoxin absorption, distribution and excretion

Mycotoxins can be absorbed to body in different ways such as gastrointestinal tract, respiratory tract and dermal application. Especially, gastrointestinal tract is the major absorptive area. The toxin can be absorbed in every part of the gut from mouth to large intestine (อนงค์, 2003).

Distribution of mycotoxins can be found in different parts of the body. The major cells and organs, where mycotoxins accumulate, are plasma protein, fat, liver, and kidney.

Mycotoxins excretion occurs in different way such as bile, gastrointestinal tract, respiratory tract, urinary tract and milk. The toxin is eliminated by glucoronide and sulfate form in bile and urine.

2.2.4 Effects of mycotoxins on broilers

2.2.4.1 Growth performance

Mycotoxins decrease feed consumption resulting in weight loss and increased feed conversion ratio of broiler. In addition, mycotoxins decrease the activity of several important enzymes involving the digestibility of carbohydrates, proteins, lipids, and nucleic acid in broiler (Abousadi et al., 2007). It also decreases protein and energy utilization of broiler (Verma et al., 2002). The critical toxic level of aflatoxin is 1.25 ppm, fumunisin is 2 ppm and tricothecene is 1-2 ppm. The acute toxic level of aflatoxin is 4 ppm, fumunisin is 8 ppm and tricothecene is 15 ppm (จักรกริศน์, 1997).

2.2.4.2 Free radical and reactive oxygen production

Mycotoxins cause free radical and reactive oxygen production. It damages several cells in the body particularly liver cells by react with phospholipids bilayer and then produces unsaturated fatty acids. Finally, the free radical production occurs in the cell and free radical is released to blood circulation (Atroshi et al., 2002). Moreover, Eraslan et al. (2005) reported that broilers fed mycotoxins contaminated-feed had decreased amount of superoxide dismutase, catalase and glutathione peroxidase enzymes but malondialdehyde concentration was increased.

2.2.4.3 Liver function

Liver is the major mycotoxin detoxification organ. Mycotoxins cause degenerative change, congestion and mild hemorrhages in liver (Sandhu et al., 1995). It also increases the amount of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT) and serum lipase but decreases serum amylase in rats fed on mycotoxins-contaminated feed (Balachandran and Ramarkrishman, 1988)

2.2.4.4 Immunity

Mycotoxins induce immunosuppression which relate to both natural and adaptive immunity. It decreases activities of natural killer cells, macrophages, T and B lymphocytes resulting in reduction of immunoglobulin, interferon and complement concentration (Surai and Mezes, 2005). Furthermore, Moura et al. (2004) reported that the admistration of ochratoxin increased the number of heterophil and monocyte, however, it decreases lymphocyte and eosinophil.

2.2.5 Different adsorbent for mycotoxin detoxification

There are several types of mycotoxin adsorbent. However, the four major types are well known as follow.

2.2.5.1 Activated charcoal

Activated charcoal is formed by pyrolysis of organic material which is a very porous non-soluble powder with a high surface to mass ratio. The previous study indicated that it is beneficial to acute poisoning or high amount of mycotoxins (Huwig et al., 2001).

2.2.5.2 Aluminocilicates

Aluminocilicates are used in several mycotoxin detoxification experiments. It consists of aluminates, silicates and some interchangeable ions, mainly alkali metal, and alkaline earth metal ions. It is documented that its effectiveness in mycotoxins adsorbent is better than other commercial adsorbent (Pasha et al., 2007).

2.2.5.3 Polymers

Cholestyramine is an anion exchange resin that use to adsorb mycotoxins in animal feed which is used to bind bile acids in gastrointestinal tract to reduce lipoproteins and cholesterol. It also reduces Ochratoxin in bile, blood and tissue (Huwig et al., 2001).

2.2.5.4 Yeast and products from yeast

The new biotechnology of adsorbent is an esterified glucomanan derived from *Saccharomyces cerevisiae*. It consists of several glucose molecules. Moreover, it is

a natural complex carbohydrate that enhances immune response by activating phagocytic cells which damage foreign cells (Stanley et al., 2004).

2.3 Antioxidant nutrients and mycotoxins

There are many herbs that has active compound such as phenolic compound and flavonoid as an antioxidant agent. The antioxidant agent interfere the formation of free radical induced by mycotoxins. It is assumed that antioxidant agent can protect the cell from mycotoxins toxicity (Atroshi et al., 2002). The mechanism is described as in Figure 2.12.

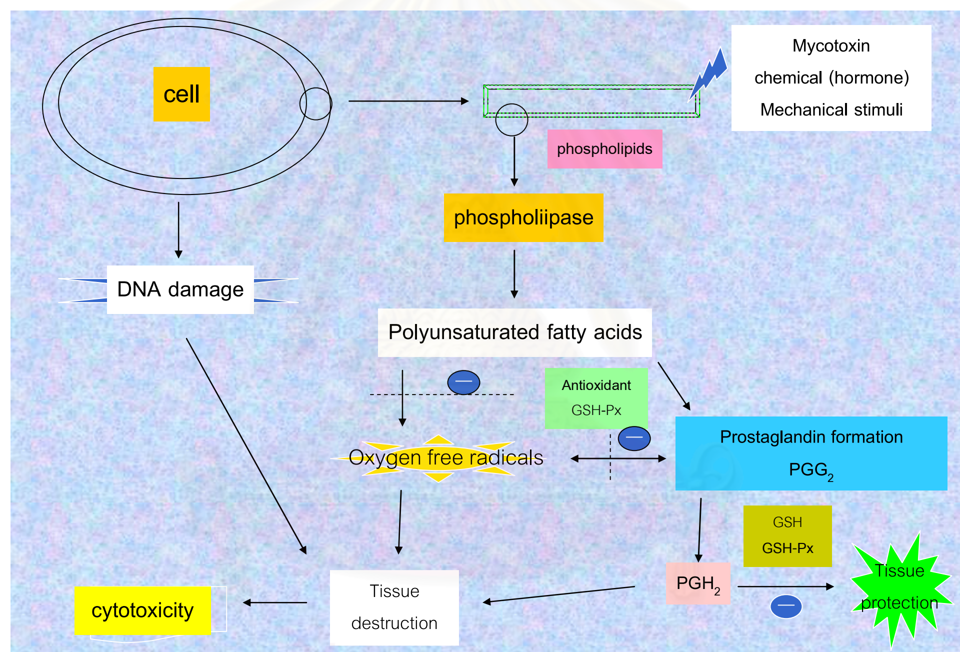

Figure 2.12 Possible complementary role of antioxidant enzymes (modified from Atroshi et al. 2002).

CHAPTER III
MATERIALS AND METHODS

This experiment was divided to two parts which were preliminary experiment and experiment.

3.1 Preliminary experiment (Part I)

The objective of the preliminary experiment was to evaluate two levels of *Thunbergia laurifolia Linn*. (TL) on growth performance, heterophil:lymphocyte ratio and plasma glucose concentration in broilers. The appropriate level of TL was chosen to use in the experiment.

3.1.1. Animals and management

The experiment was made in private broiler farm, Salaya district, Nakornpathom province. One hundred and fifty day-old, male, Arbor Acre broilers were divided into 3 treatment groups (five replicates of ten chicks each). Total body weights of all chicks in each group were similar.

Feed and water were given *ad libitum* for 42 days in all treatments.

3.1.2. Preparation of *Thunbergia laurifolia Linn* powder

Fresh whole plants of *Thunbergia laurifolia Linn* were obtained from Nan province. After washing, leaves and small stems were cut and dried in an oven at 50°C for 24 hours. The dried *Thunbergia laurifolia Linn* was grounded to fine particle using cutting mill. The powder was kept frozen at -20 °C. The major ingredients (phenolic compound and flavonoids) were determined.

Table 3.1 The proximate analysis of *Thunbergia laurifolia Linn.*

Proximate analysis	*Thunbergia laurifolia Linn*. crude powder
Dry matter (%)	89.77
Crude protein (%)	18.49
Crude fat (%)	4.35
Crude fiber (%)	8.26
Ash (%)	10.53

3.1.3. Diet

The chicks were fed on high-energy starter (day 1 to day 21), grower (day 22 to day 35), and finisher (day 36 to day 42) diets composed of corn and soybean meal as major ingredients. The diets are 1) basal diet (control group), 2) basal diet supplemented with 2% *Thunbergia laurifolia Linn*. powder (2% TL group) and 3) basal diet supplemented with 4% *Thunbergia laurifolia Linn*. powder (4% TL group). Experimental diets and *Thunbergia laurifolia Linn*. were proximately analyzed by the method described by Horwitz (2000).

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Table 3.2 Composition and nutrient content of diets in preliminary experiment

Ingredient	Amount (kg/100kg)		
	Starter*	Grower*	Finisher*
Corn 7.81%	51.56	56.58	62.79
Soybean meal 43.25%	38.71	33.02	27.16
Palm oil	5.47	6.20	5.92
L-lysine	-	-	0.002
DL-methionine	0.37	0.35	0.34
L-threonine	0.03	0.13	0.13
Mono-dicalcium phosphate	1.60	1.50	1.45
Limestone	1.34	1.29	1.21
Sodium bicarbonate 27%	0.17	0.17	0.17
Salt	0.31	0.30	0.30
Coccidostat	0.05	0.05	0.05
Mycotoxin binder	0.05	0.05	0.05
Choline chloride 60%	0.10	0.10	0.10
Premix	0.25	0.25	0.25
Proximate analysis			
Dry matter (%)	88.11	88.16	88.05
Metabolizable energy (kcal/kg)	3000.00	3100.00	3150.00
Crude protein (%)	21.00	19.00	17.00
Crude fat (%)	7.64	8.50	8.40
Crude fiber (%)	3.84	3.56	3.28
Calcium (%)	0.90	0.85	0.80
Phosphorus (%)	0.74	0.70	0.67

*The amount of 2 and 4% *Thunbergia laurifolia Linn.* were topped up onto each treatment feed except control.

3.1.4. Experimental protocol

The chicks were weighed at day 1, 21, and 42. The feed intake was recorded during day 0 to 21, and day 22 to 42. Ten chickens from each treatment group (two chickens per replicates) were randomly selected on day 21 and 42. On day 21 and 42, blood samples were collected to determine blood glucose and heterophil/lymphocyte ratio.

The experiment was performed as described in the experimental design (Table 3.3) and experimental protocol below.

Table 3.3 The description of treatments in preliminary experiment

Treatment	Description
1. Control	Basal diet
2. 2% TL	Basal diet + 2% *Thunbergia laurifolia Linn.*
3. 4% TL	Basal diet + 4% *Thunbergia laurifolia Linn*.

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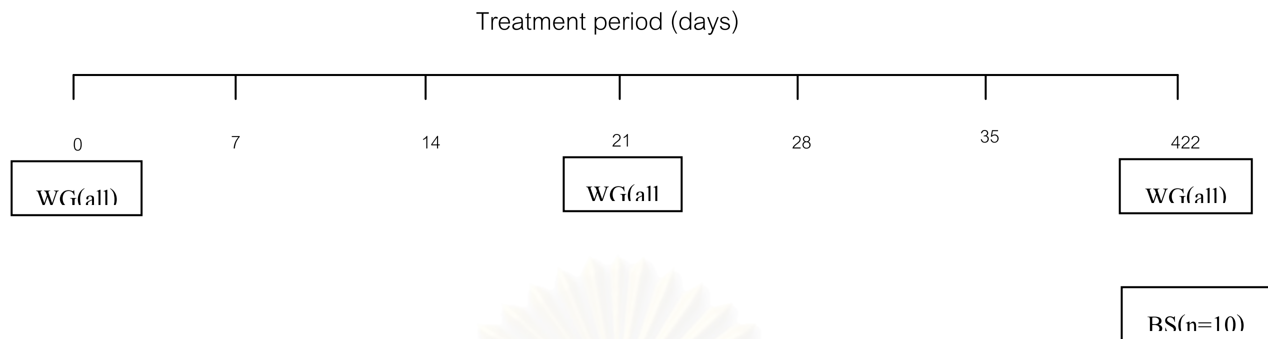
Protocol of preliminary experiment



WG = weighing (body weight and feed intake)

BS = Blood sampling

Parameters (preliminary experiment)

1. Growth performance
2. Blood glucose
3. Heterophil / Lymphocyte ratio

3.2 Experiment (Part II)

The TL supplement that had less negative effect on broiler growth performance at least was used in the experiment.

3.2.1. Animals and management

Total numbers of 450, day-old, male, Arbor Acre broilers were divided into 6 treatment groups (five replicates of fifteen chicks each). Total body weights of all chicks in each group were similar.

Feed and water were given *ad libitum* for 42 days in all treatments.

3.2.2 Preparation of *Thunbergia laurifolia Linn*. powder

The procedure was followed as in the preliminary experiment.

3.2.3 Preparation and analysis of mycotoxin-contaminated corn

Mycotoxins contaminated corn and glucomannan (mycotoxin binder) were purchased from local broiler farm and private company, respectively. Mycotoxin concentration was determined using ELISA methods (The RIDASCREEN® FAST test kit, Scimedex, USA). Three mycotoxins (aflatoxin, tricothecene and fumonisin) that have major impact on broiler performance were analyzed.

Table 3.4 Analysis of mycotoxins in corn powder

Mycotoxin	Amount
Aflatoxin	96.69 ppb
Fumonisin	1.42 ppm
Tricothecene	25.34 ppb
Ochratoxin	5.58 ppb

3.2.4. Diets

The chicks were fed on starter (day 1 to day 21), grower (day 22 to day 35), and finisher (day 36 to day 42) diets composed of corn and soybean meal as major ingredients.

Table 3.5 Mycotoxin concentration in broiler starter feed

Treatment	Mycotoxin		
	Aflatoxin (ppb)	Fumonisin (ppm)	Tricothecene (ppb)
T1	10.06	0.39	10.00
T2	6.62	0.45	7.58
T3	12.23	0.20	2.22
T4	3.2	0.22	9.57
T5	8.59	0.14	5.34
T6	10.76	0.10	0.00

3.2.5. Experimental protocol

Treatment 1. Control (n = 75) This group was fed on basal diet for the whole experimental period.

Treatment 2. Glucomannan from yeast (n = 75) This group was fed on basal diet supplemented with 1 % glucomannan (GCM).

Treatment 3. Mycotoxin (n = 75) This group was fed on diet mixed with mycotoxin contaminated corn for six weeks.

Treatment 4. Mycotoxin + 1% GCM (n = 75) This group was fed on diet used in treatment 3, supplemented with 1 % GCM, for six weeks.

Treatment 5. Mycotoxin + 2% TL (n = 75) This group was fed on diet used in treatment 3, supplemented with 2% TL, for six weeks.

Treatment 6. Mycotoxin + 2% TL + 1% GCM (n = 75) This group was fed on diet used in treatment 3, supplemented with 2% TL and 1% GCM, for six weeks.

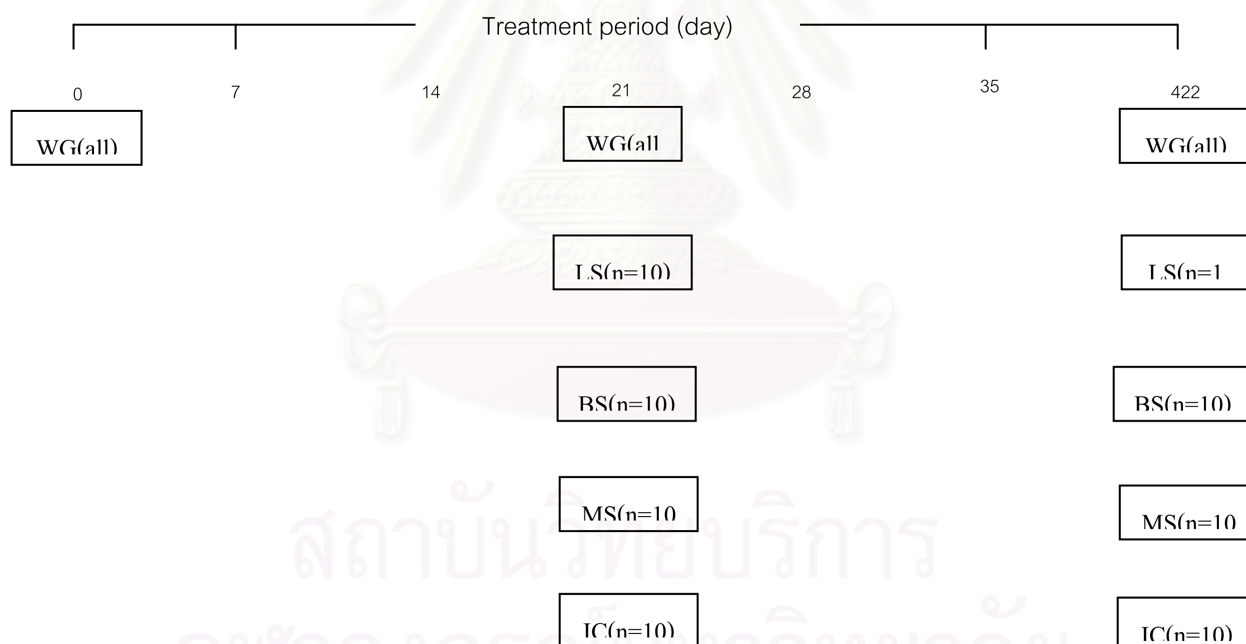
The broilers were weighed on day 1, 21, and 42. The feed intake was recorded during day 0 to 21, and day 22 to 42. Ten broilers from each treatment group (two chickens per replicates) were randomly selected and sacrificed using overdose intracardiac injection of pentobabital sodium on day 21 and 42. Jejunal mucosal samples were scraped from the mucosa layer using glass slide, wrapped with aluminum foil and stored at -70 °C until analysis. Jejunal mucosal scrapings were analyzed for maltase and sucrase activity (Dahlqvist, 1968). The ileal contents were collected and kept frozen at -20 °C until analysis of nutrient digestibility. Liver samples were collected to determine malondialdehyde, glutathione peroxidase, superoxide dismutase, catalase, and liver hispathology (H&E stain); blood samples were collected to determine blood glucose, heterophil / lymphocyte (H/L) ratio, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum albumin.

The experiment was performed as described in the experimental design (Table 3.5) and experimental protocol.

Table 3.6 The description of treatments in experiment

Treatments	Description
1 Control	Basal diet
2 Glucomannan from yeast	Basal diet + 1% GCM
3 Mycotoxins	Diet with mycotoxin contaminated corn
4 Mycotoxins + 1% GCM	Diet as in group 3 + 1% GCM
5 Mycotoxins + 2% TL	Diet as in group 3 + 2% TL
6 Mycotoxins + 2% TL + 1% GCM	Diet as in group 3 + 2% TL+ 1% GCM

Protocol of Experiment



WG = weighing (body weight and feed intake)

LS = liver sample collection

BS = blood sample collection

MS = mucosal scrapings

IC = collecting of ileal contents

Parameter (Experiment)

1 Growth performance
2 Ileal digestibility of nutrients
3 Intestinal disaccharidase activity
4 Liver glutathione peroxidase
5 Liver superoxide dismutase
6 Liver malondialdehyde
7 Liver catalase activity
8 Liver histopathology
9 Heterophil/lymphocyte (H/L) ratio
10 Blood glucose
11 Serum glutamate pyruvic transaminase (SGPT)
12 Serum glutamate oxaloacetate transaminase (SGOT)
13 Albumin

Table 3.7 Composition and nutrient content of diets in the starter period (experiment).

Ingredient	Amount (kg/100kg)					
	T1	T2	T3	T4	T5	T6
Corn 8.13%	52.85	50.74	-	-	-	-
Mycotoxin contaminated corn	-	-	52.85	50.74	50.39	48.74
Soybean meal 47.0%	31.57	31.95	31.57	31.95	31.12	31.57
Palm oil	5.24	5.97	5.24	5.97	6.07	6.97
Rapeseed meal 38.2%	3.00	3.00	3.00	3.00	3.00	3.00
Inra - corn gluten M 60.4	2.00	2.00	2.00	2.00	2.00	2.00
Ground *Thunbergia laurifolia Linn.*	-	-	-	-	2.00	2.00
Limestone 35.69%	1.73	1.72	1.73	1.72	1.72	1.72
Mono-.di-calcium phosphate	1.45	1.46	1.45	1.46	1.48	1.48
Glucomannan	-	1.00	-	1.00	-	1.00
BS-premix	0.50	0.50	0.50	0.50	0.50	0.50
Pellet binder	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.48	0.48	0.48	0.48	0.48	0.48
L-lysine HCl	0.33	0.32	0.33	0.32	0.35	0.35
DL-methionine	0.25	0.26	0.25	0.26	0.27	0.27
L-threonine	0.07	0.07	0.07	0.07	0.08	0.08
L-tryptophan	0.03	0.03	0.03	0.03	0.04	0.04
Proximate analysis						
Dry matter	88.27	87.52	88.27	87.52	88.46	87.52
Crude protein	22.00	22.00	22.00	22.00	22.00	22.00
Crude fat	7.779	8.433	7.779	8.433	8.597	9.233
Crude fiber	3.102	3.113	3.102	3.113	3.229	3.229
Calcium	1.000	1.000	1.000	1.000	1.000	1.000
Phosphorus	0.674	0.674	0.674	0.674	0.672	0.672
Metabolize energy (kcal/kg)	3,000.00	3,000.00	3,000.00	3,000.00	3,000.00	3,000.00

Table 3.8 Composition and nutrient content of diets in the grower period (experiment).

Ingredient	Amount (kg/100kg)					
	T1	T2	T3	T4	T5	T6
Corn 8.13%	58.03	55.90	-	-	-	-
Mycotoxin contaminated corn	-	-	58.03	55.90	55.54	53.03
Soybean meal 47.0%	24.84	25.22	24.84	25.22	24.41	24.84
Palm oil	5.58	6.31	5.58	6.31	6.42	7.58
Rapeseed meal 38.2%	4.00	4.00	4.00	4.00	4.00	4.00
Inra-corn gluten M 60.4	3.00	3.00	3.00	3.00	3.00	3.00
Ground *Thunbergia laurifolia Linn.*	-	-	-	-	2.00	2.00
Limestone 35.69%	1.59	1.59	1.59	1.59	1.59	1.59
Mono-,di-calcium phosphate	1.21	1.23	1.21	1.23	1.24	1.24
Glucomannan	-	1.00	-	1.00	-	1.00
BS-premix	0.50	0.50	0.50	0.50	0.50	0.50
Pellet binder	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.38	0.38	0.38	0.38	0.38	0.38
L-lysine HCl	0.20	0.19	0.20	0.19	0.22	0.22
DL-methionine	0.13	0.14	0.13	0.14	0.15	0.15
L-threonine	-	-	-	-	0.01	0.01
L-tryptophan	0.04	0.04	0.04	0.04	0.04	0.04
Proximate analysis						
Dry matter	88.21	87.45	88.21	87.45	88.39	87.45
Crude protein	20.00	20.00	20.00	20.00	20.00	20.00
Crude fat	8.237	8.891	8.237	8.891	9.059	9.737
Crude fiber	3.056	3.028	3.056	3.028	3.145	3.145
Calcium	0.900	0.900	0.900	0.900	0.900	0.900
Phosphorus	0.610	0.611	0.610	0.611	0.608	0.608
metabolize energy (kcal/kg)	3,100.00	3,100.00	3,100.00	3,100.00	3,100.00	3,100.00

Table 3.9 Composition and nutrient content of diets in the finisher period (experiment).

Ingredient	Amount (kg/100kg)					
	T1	T2	T3	T4	T5	T6
Corn 8.13%	55.36	53.23	-	-	-	-
Mycotoxin contaminated corn	-	-	55.36	53.23	52.86	50.36
Soybean meal 47.0%	22.52	25.90	25.52	25.90	25.09	25.09
Palm oil	7.72	8.45	7.72	8.45	8.56	9.45
Rapeseed meal 38.2%	4.00	4.00	4.00	4.00	4.00	4.00
Inra-corn gluten M 60.4	3.00	3.00	3.00	3.00	3.00	3.00
Ground *Thunbergia*	-	-	-	-	2.00	2.00
laurifolia Linn.	1.58	1.58	1.58	1.58	1.58	1.58
Limestone 35.69%	1.23	1.24	1.23	1.24	1.26	1.26
Mono-,di-calcium phosphate	-	1.00	-	1.00	-	1.00
Glucomannan	0.50	0.50	0.50	0.50	0.50	0.50
BS-premix	0.50	0.50	0.50	0.50	0.50	0.50
Pellet binder	0.35	0.35	0.35	0.35	0.35	0.35
Salt	0.11	0.12	0.11	0.12	0.13	0.13
L-lysine HCl	0.10	0.10	0.10	0.10	0.13	0.13
DL-methionine	-	-	-	-	-	-
L-threonine	0.03	0.03	0.03	0.03	0.04	0.04
L-tryptophan	-	-	-	-	-	-
Proximate analysis						
Dry matter	88.49	87.73	88.49	87.73	88.67	87.73
Crude protein	20.00	20.00	20.00	20.00	20.00	20.00
Crude fat	10.267	10.921	10.267	10.921	11.090	11.721
Crude fiber	3.031	3.003	3.031	3.003	3.120	3.003
Calcium	0.900	0.900	0.900	0.900	0.900	0.900
Phosphorus	0.611	0.611	0.611	0.611	0.608	0.608
metabolize energy (kcal/kg)	3,200.00	3,200.00	3,200.00	3,200.00	3,200.00	3,200.00

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3.3 Determination of mycotoxins in broiler feed

The starter feed samples were collected on day 14 of the experiment and they were sent to Chakmartin Intervirontech Ltd. (Bangkok, Thailand) to determine mycotoxin in broiler feed using ELISA technique. The RIDASCREEN®FAST Aflatoxin, Fumonisin, Trichothecene test kits (Scimedex, USA) were used to determine concentrations of aflatoxin, fumonisin and trichothecene.

The basis of the test is the antigen-antibody reaction. The micro titer wells were coated with captured antibodies directed against anti-aflatoxin, fumonisin or trichothecene antibodies. Aflatoxin, fumonisin or trichothecene standards or sample solution, aflatoxin, fumonisin or trichothecene enzyme conjugate and anti-aflatoxin, fumonisin or trichothecene antibodies were added. Free aflatoxin, fumonisin or trichothecene and aflatoxin, fumonisin or trichothecene conjugate competed for the aflatoxin, fumonisin or trichothecene antibody binding site (competitive enzyme immono-assay). At the same time, the aflatoxin, fumonisin or trichothecene antbodies were also bound by the immobilized capture antibodies. Any unbound conjugate was then removed in a washing step. Substrate/chromogen was added to the wells, bound enzyme conjugate converted the chromogen into a blue product. The addition of the stop solution leads to a change from blue to yellow. The measurement was made photomitrically at 450 nm. The absorbance was inversely proportional to the aflatoxin, fumonisin or trichothecene concentration in the sample.

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3.4 Determination of active ingredient in *Thunbergia laurifollia Linn*.

3.4.1 Extraction

Approximately 20 grams of TL were extracted by different solutions; Hexane, Methanol, Acetone, Ethanol and boiled water. Boiled water solution was collected after water was boiled for 30 minutes. The extractions of TL and different solutions were settled at room temperature for 72 hours. Afterward, the mixtures were filtered by Whatman No.1 filtrated paper. They were evaporated by a rotary evaporator (Eyela). The residues were removed into the tubes and were stored at 4 °C.

3.4.2 Total phenolic compound (Asami et al., 2003)

The amount of 0.02 grams of each extract was diluted in 1 ml of its solution. Then, 20 µl of the solution was put into the test tube and 100 µl of Folin Clocalteau's phenol reagent was added. The test tube was allowed to settle at room temperature for 5 minutes. Next, 300 µl of 2% Na_2CO_3 was added and the test tube was allowed to settle at room temperature for 2 hours. The bright-green color was used as an indicator to show that the sample was ready. The optical density was read at wavelength 765 nm against reagent blank using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path).

The standard curve was plotted using the gallic acid concentration at 0, 0.25, 0.5, 1, 2 mg/ml. The slope of curve was used to calculate the concentration of total phenolic compound.

3.4.3 Flavonoid I: flavavones (Chang et al., 2002)

The amount of 0.02 grams of extract was diluted in 1 ml of its solution. Consequently, 500 µl of the solution was put into the test tube and 1.5 ml of 95% of ethanol, 100 µl of 10% aluminium chloride, 100 µl of 1 M sodium acetate and 2.8 ml of distilled water were added in the tube. The test tube was allowed to settle at room temperature for 30 minutes. The bright-yellow color was used as an indicator to show that the sample was ready. Finally, the optical density was read at wavelength 415 nm against blank reagent by using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path).

The standard curve was plotted by using the quercetin concentration at 0, 6.25, 12.5, 25, 50 and 100 µg/ml. The curve's slope was used to calculate the concentration of flavavones.

3.4.4 Flavonoid II: flavones and flavanol (Chang et al., 2002)

The amount of 0.08 grams of each extract was diluted in 1 ml of its solution. After that, 500 µl of the solution was put into the the test tube and 1 ml of absolute methanol and 1 ml of 1% 2,4 dinitrophenylhydrazine were added in the tube. Then, the test tube was put in a 50 °C shaking water bath. Next, the test tube was allowed to settle at room temperature for 30 minutes. After that, amount of 25 ml of 1% potassium hydroxide in 70% ethanol was added in the tube and it was allowed to settle at room temperature for 2 minutes. Then 1 ml of the solution was removed to 1.5-microtube. The microtube was centrifuged at 1,000 g for 10 minutes. Then, 100 µl of supernatant was removed into the new test tube and 2.4 ml of distilled water was added. The orange-yellowed was used as an indicator to show that the sample was ready. The optical density was read at wavelength 495 nm against reagent blank by using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path).

The standard curve was plotted using the naringenin concentration at 0, 1,000, 2,000, 4,000 and 8,000 µg/ml. The curve's slope was used to calculate the concentration of flavones and flavanol.

3.5 Proximate analysis (Horwitz, 2000)

3.5.1 Dry matter and moisture (Horwitz, 2000)

Small foil dish was dried at 105 °C for 1 hour in a hot air oven. The dish was cooled in a desiccator and weighed. The dish was dried again until its weight was stable. Then, approximately 2 grams of feed or 1 gram digesta sample was weighed in the dried dish and dried at 105 °C for 8 hours. The samples were cooled in a desiccator. The dry matter of sample in the dish was weighed to examine whether the weight was stable (all water had been evaporated from the sample). Percentage of dry matter and moisture were calculated using the following equation:

$$\text{Dry matter (\%)} = \frac{a - b}{w} \times 100$$

where a = weight of dish and sample before drying

b = weight of dish and sample after drying

w = sample weight

moisture (%) = 100 - % dry matter

3.5.2 Analysis of ash (Horwitz, 2000)

Porcelain crucible was burned at 550 °C for 1 hour in Muffle furnace. The crucible was cooled in a desiccator and weighed. The crucible might be burned again until it was dried enough and its weight was stable. Afterward, approximately 2 grams of feed or 1 gram of digesta sample was weighed in the dried crucible and ashed at 550 °C for 8 hours until the ash became white or grey. The crucibles were then cooled in a desiccator. Finally, the ash in the crucible was weighed. Percentage of ash was calculated using the following equation:

$$\text{Ash (\%)} = \frac{b - a}{w} \times 100$$

where a = porcelain crucible weight

b = weight of porcelain crucible and ash after burned

w = sample weight

3.5.3 Analysis of crude fat (Horwitz, 2000)

Flat bottom flask was dried at 105 °C for 2 hours in a hot air oven. The flask was cooled in a desiccator and weighed. The flask was dried until its weight was stable. Then, approximately 2 grams of feed or 1 gram digesta sample was weighed in filter paper and put in extraction thimble. The thimble was plugged with cotton. Afterward, the samples were put in a soxhlet tube which was combined with a condenser and a flat bottom flask. Approximately 200 milliliters petroleum ether was put into the flask, the condenser and hot plate were turned on, and the sample was extracted approximately 16 hours. The thimble was taken out from soxhlet tube and all petroleum ether in the flask was evaporated on hot plate. The flask was dried at 105 °C for 2 hours in a hot air oven. Finally, the flask was cooled in a desiccator and weighed. The flask was dried until its weight was stable. Percentage of crude fat was calculated using the following equation:

$$\text{Crude fat (\%)} = \frac{b - a}{w} \times 100$$

where a = flat bottom flask weight

b = weight of flat bottom flask and ether extract after dried

w = sample weight

3.5.4 Analysis of crude fiber (Horwitz, 2000)

Approximately 2 grams of feed or 1 gram of digesta sample was weighed and put in a high cylinder-beaker. After that, 200 milliliters of warm 1.25 % sulfuric acid was put in the beaker and boiled on fiber digestion apparatus which was combined with condenser to control and make its concentration stable for 30 minutes. Solution was taken off from fiber digestion apparatus and filtered with linen cloth on Bucher funnel attached to suction pump. Acid was washed out from the residual on the cloth using distilled water. Then, the residual was transferred into the same beaker and approximately 200 milliliters of warm 1.25 % sodium hydroxide was put into the beaker and boiled on fiber digestion apparatus for 30 minutes. The solution was filtered with linen cloth on Bucher funnel again until all bases were washed out by distilled water. The residue was transferred into a glass crucible. The crucible was dried at 105 °C for 2 hours in hot air oven then cooled in a desiccator and weighed. The crucible was dried until its weight was stable. Finally, the crucibles were burned at 550 °C for 30 minutes in Muffle furnace until the ash became white or gray and cooled in a desiccator and weighed. The crucible was dried until its weight was stable. Percentage of crude fiber was calculated using the following equation:

$$\text{Crude fiber (\%)} = \frac{a - b}{w} \times 100$$

where a = weight of glass crucible and crude fiber before burned

b = weight of glass crucible and crude fiber after burned

w = sample weight

3.5.5 Analysis of crude protein (Horwitz, 2000)

Approximately 2 grams of feed or 1 gram of digesta sample was weighed on filter paper (nitrogen-free) and put in a Kjeldahl flask. Approximately 10 grams of catalyst mixture (7 grams of copper sulfate and 10 grams of potassium sulfate) and 15 milliliters of concentrated sulfuric acid were put in the flask. The flask was boiled on protein digestion apparatus in Fume hood for 3 hours until the solution became clear

green or blue. After that, the Flask was taken off from the apparatus and cooled. Approximately 30 milliliters of 4% boric acid was prepared in 500 milliliters-flask and then indicator mixture (1 part of bromocresol green and 5 parts of methyl red) was added into the flask. The 500 milliliters flask and Kjeldahl flask were put in protein distillation apparatus and distilled automatically using 45% sodium hydroxide and hot water for 4 minutes. Then, the 500 milliliters-flask was taken off from the apparatus and cooled in room temperature. Finally, the solution in flask was titrated by 0.5 N sulfuric acid until its solution became light pink. The added acid volume was assigned as V. Percentage of crude protein was calculated using the following equation:

$$\text{Crude protein (\%)} = \frac{1.4(V)N \times 6.25}{w}$$

where V = volume of sulfuric acid that used in titration

N = normal concentration of sulfuric acid

w = sample weight

3.5.6 Gross energy (Horwitz, 2000)

Gross energy of broiler feed was determined by Ballistic Bomb Colorimeter. The value was obtained from Thermocouple and Galvanometer system and was compared with known calorific value standard sample (Chulalongkorn University Science Equipment center, 2551).

3.6 Determination of nutrient digestibility and acid-insoluble ash

Celite, a source of acid-insoluble ash, was added in the feed as a marker for the determination of ileal digestibility of nutrients. Acid-insoluble ash was measured by the method described by Choct and Annisson (1992). First, sintered glass crucible (Pyrex®, England) was weighed, then 2 grams of diet and 1 or 0.5 gram of dried and grounded digesta samples were added to sintered glass crucible (Pyrex®, England), dried at 105 °C for 8 hours and weighed as dry matter sample. Later, the sample was ashed at 550 °C for 8 hours. After ashing the crucible was cooled, and boiled slowly in 4N HCl for 30 minutes on a hot plate in fume hood. The ash in crucible was washed with distilled water using suction pump, and dried at 105 °C. The ash residue in crucible was ashed and cooled in the same way again. Finally, the ash in crucible was dried at 105 °C for 6 hours,

the crucible was cooled in a desiccator and weighed while containing the ash. Percentage of acid-insoluble ash was calculated using the following equation:

$$AIA\ (\%) = \frac{Wf - We}{Ws} \times 100$$

Where Wf = weight of crucible with ash

We = weight of empty crucible

Ws = weight of sample (dry matter)

The percentage of ileal digestibility (ID) of nutrients eg. protein and fat was calculated using the following equation:

$$ID = 1 - \left(\frac{\text{(ileal nutrient (\%) / ileal acid insoluble ash (\%))}}{\text{(Diet nutrient (\%) / diet acid insoluble ash (\%)}} \right) \times 100$$

3.7 Calculation of the growth performance

In both experiments, the broilers were weighed when they were 0, 21, 42 days old. The feed intake was recorded during day 0-21, day 22-35 and day 36-42. Number and body weight of dead broilers were recorded for calculation of mortality rate and feed conversion ratio (FCR), respectively. Finally, feed conversion ratio (FCR) was calculated by total pen feed (gram) divided by body weight gain (gram).

Feed intake (g/b) = Total feed intake / final chick number

Daily feed intake (DFI, g/b/d) = Feed intake / days

Body weight gain (g/b) = Final body weight – Initial body weight

Average body gain (ADG, g/b/d) = Body weight gain / days

Mortality rate (%) = (Number of dead chicks / total chick numbers) $\times$100

Feed conversion ratio = Total pen feed / Total body weight gain
(FCR, kg feed intake/kg body weight gain)

$$\text{Feed conversion ratio (FCR, kg feed intake/kg body weight gain)} = \frac{\text{Total pen feed}}{\text{Total body weight gain}}$$

3.8 Determination of mucosal disaccharidase activity

Jejunal scraping samples were analyzed for brush-border disaccharidase activity (sucrase and maltase) using method described by Dahlqvist (1968) as modified by ขุนพล (2003).

The amount of 1 gram of jejunal mucosa scraping from broiler was homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with 4 ml of distilled water. After that, the sample was centrifuged at 3,000 g (Centrifuge, GLC-2B, SORVALL) for 10 minutes. The supernatant was used to determine disaccharidase activity.

The supernatant was diluted with distilled water (sucrase 1:100, maltase 1:1000). The test tube containing 50 µl of the diluted enzyme solution was placed in a water bath for 5 minutes. Next, 5 µl of substrate buffer solution was added (sucrose or maltose substrate solution). Then, the test tube was incubated in a water bath at 37 °C for 1 hour. After that, 1,000 µl of enzyme reagent (Glucose liquicolor, Human Gesllschaftnfur Biochemica and Diagnostica mbH, Germany) was added in to the tube. The test tube was then placed in a water bath at 37 °C for 5 minutes. The formed hydrogen peroxide reacted under catalysis of peroxidase with phenol and 4-aminophenazone to red-violet quinoneimine was used as an indicator to show that the sample was ready. The optical density was read at wavelength 500 nm against blank reagent using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path).

The standard curve was plotted using the glucose at 0, 5, 10, 20, 30, 40 and 50 mg%. The slope of the curve was used to calculate the concentrations of glucose.

Results were expressed as specific activity (unit per mg brush border protein). One unit was defined as disaccharidase that hydrolyzes 1 µmol of the substrate per minute under the experimental condition.

The disaccharidase activity was obtained by the following formula

$$\frac{a \times b}{n \times 1080} \quad \text{units/ml}$$

Where

a = μg glucose liberated in 60 minutes

b = dilution factor for enzyme solution

n = number of glucose molecules per molecule of disaccharide

(sucrose n = 1, maltose n =2)

3.9 Determination of liver antioxidant enzyme activity

3.9.1 Liver superoxide dismutase activity (Maccord and Fridovich, 1969)

The amount of 0.3 gram of liver sample was homogenized by hand homogenizer with 4.5 ml of 50 mM phosphate buffer, pH 7.8. The liver homogenate was centrifuged at 1,500 g, 4°C for 30 minutes. Then, the supernatant was diluted to 1:5 ratio and the enzyme activities in the resulting supernatant and protein concentration were determined.

First, 1.45 ml of solution A (xanthine sodium: cytochrome C, 1:10) was put into a plastic cuvette. Then, added 25 μl of phosphate buffer for blank, standard or sample. The optical density was read at wavelength 550 nm using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path) as a first value. Next, solution B (xanthine oxidase) was added into the cuvette. The increased absorbance was recorded every 1 minute for 3 minutes. Reading was made against the blank.

The standard curve was plotted by the superoxide dismutase at 0,20,40,60 and 80 U/100 μl. The slope of the curve was used to calculate the superoxide dismutase activity.

Results were defined by the amount of the enzyme required to inhibit the rate of reduction of cytochrome C by 50% in coupled system with xanthine and xanthine oxidase. The specific activity was expressed in unit per mg of liver protein.

3.9.2 Liver catalase activity (Aebi *et a*l., 1983)

The amount of 0.3 grams of liver sample was homogenized with 4.5 ml of enzyme buffer (100 ml phosphate buffer: 100 μl triton x), pH 7.0 by hand homogenizer. The liver homogenate was centrifuged at 1,500 g, 4 °C for 30 minutes. Then, the supernatant was diluted to 1:5 ratio. Next, the enzyme activity in the resulting supernatant and protein concentration were determined.

A 100 µl of phosphate buffer, standard or supernatant and 1.9 ml of phosphate buffer were put into a plastic cuvette. The optical density was read at wavelength 240 nm using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path) as a first value. Next, 1 ml of H_2O_2 was added into the cuvette. The decreased absorbance was recorded every 15 seconds for 1 minute. Reading was made against the blank.

The standard curve was plotted using the catalase at 0, 50,100, 150, 200 and 300 U/100 µl. The curve's slope was used to calculate the catalase activity.

Results were defined as 1 µmol of hydrogen peroxide decomposed per minute. The specific activity was expressed in unit per mg of liver protein.

3.9.3 Glutathione peroxidase (Bolcal *et al*., 2007)

The amount of 0.3 grams of liver sample was homogenized with 4.5 ml of 50 mM potassium phosphate buffer by hand homogenizer. The liver homogenate was centrifuged at 1,500 g, 4 °C for 30 minutes. Then, the supernatant was diluted to 1:5 ratio. Next, the enzyme activity in the resulting supernatant and protein concentration were determined.

The amount of 43 µl of supernatant was incubated with start reagent (25 µl of 8.4 mM NADPH in 1% $NaHCO_3$ and 48 µl of 2.2 mM of H_2O_2). The optical density was read at wavelength 340 nm using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path) as a first value. Next, 625 µl of 5.8 mM reduced glutathione in 50 mM phosphate buffer and 25 µl of 10 U/ml glutathione reductase in 50 mM phosphate buffer in 0.5 mM EDTA and 1mM NaN_3 were added.

The absorbance was read as the rate of disappearance of NADPH every 15 seconds for 1 minute. Reading was made against reagent blank.

One unit of glutathione peroxidase activity was defined as the amount of glutathione peroxidase that transformed 1 mmol NADPH to NADP per minute in experimental condition. The specific activity was expressed in mUnit per mg of liver protein.

The glutathione peroxidase activity was obtained by the following formula.

$$\text{GSH-px activity (mUnit)} = \left(\left(\Delta 340/\text{min}/\text{extinction coefficient}\right) \times D \times d \times \frac{(TV)}{SW}\right) \times SV$$

Where

Extinction coefficient = 0.0622

D = dilution factor

d = light path in cm

TV = total volume (ml)

SV = sample volume (ml)

SW = sample weight (mg)

3.10 Determination of malondialdehyde concentration (Ohkava et al., 1979)

The amount of 0.3 grams of liver sample was homogenized with 1.5 ml of 1.15% of potassium chloride phosphate buffer by hand homogenizer. The liver homogenate was centrifuged at 3,000 g, 4 °C for 30 minutes. Then, the malondialdehyde concentration and protein concentration were determined.

The amount of 300 µl of liver supernatant was put into a 15ml-plastic tube with cover. Then, 50 µl of 50 mM BHT, 50 µl of distilled water, 100 µl of 8.1% of SDS, 750 µl of 20 % acetic acid, pH 3.5 and 750 µl of o.5% TBA in 0.02 M NaOH were added. Next, the solution was mixed and boiled at 95 °C for 60 minutes. After that, it was cooled at room temperature for 10 minutes and 2 ml of butanol: pyridine (15:1) was added and mixed for 1 minute. Then, it was centrifuged at 5,000 rpm for 10 minutes and 1.7-1.8 ml of upper layer (pink) was removed into 2 ml-micro tube. The micro tube was centrifuged at 12,000 rpm for 10 minutes and 1.2 ml sample was removed into a microcuvette. The optical density was read at wavelength 532 nm by using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path). Reading was made against the blank.

The standard curve was plotted using the malondialdehyde at 0, 5, 10, 20, 40 and 60 nmol/ml. The curve's slope was used to calculate the concentration of malondialdehyde.

The specific concentration was expressed in nmol per mg of liver protein

3.11 Determination of liver and jejunal mucosa protein concentrations (Lowry et al., 1951)

Liver or jejunal mucosa scrapings were homogenized with phosphate buffer or distilled water by hand homogenizer of Homogenizer (GKH, GT MOTOR CONTROL,

GLAS-COL®). Next, the homogenated sample was used to determine the protein concentration.

The amount of 50 µl of the homogenated sample was put into the test tube. After that, 1.5 ml of fresh reagent (composed of 100 ml of 2% Na_2CO_3 in o.1 M NaOH, 1 ml of 1% of $CuSo_4.5H_2O$ and 0.5 ml of 2% Na-tartrate) was added into the tube. The test tube was allowed to settle at room temperature for 10 minutes. Next, 300 µl of Folin's reagent (1:1) was added into the test tube and it was allowed to settle at room temperature for 30 minutes. The strong-blue was used as an indicator to show that the sample was ready. The optical density was read at wavelength 650 nm using UV-VIS spectrophotometer (Shimadzu UV 1201, 1cm light path). Reading was made against the blank.

The standard curve was plotted using the bovine serum albumin (BSA) at 0, 20, 40, 60, 80 and 100 mg %. The curve's slope was used to calculate the concentration of total protein.

The specific concentration was expressed in mg per 100 ml (mg %).

3.12 Determination of blood samples

3.12.1 Determination of blood glucose

Blood glucose was measured by Glucometer (Accucheck II) (@2004 Roche Diagnostics, USA). The specific concentration was expressed in mg per 100 ml (mg%).

3.12.2 Determination of heterophil/lymphocyte ratio

The samples were sent to Veterinary Diagnostic Laboratory center (Bangkok, Thailand).

Blood were smeared in slide and then stained by Wright-Giemsa stain. Heterophil and ymphocyte ratio were counted using light microscope.

3.12.3 Determination of serum glutamate pyruvate and oxaloacetate transaminase

The samples were sent to Veterinary Diagnostic Laboratory center (Bangkok, Thailand).

Kinetic method (Human Gesllschaftnfur Biochemica and Diagnostica mbH, Germany) was used to determine of serum glutamate pyruvate and oxalozcetate transaminase activity according to the recommendation of IFCC (International Federation of Clinical Chemistry).

3.12.4 Determination of serum albumin

The samples were sent to Veterinary Diagnostic Laboratory center (Bangkok, Thailand).

Bromocresol green forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentrations in the sample.

3.12.5 Histopathology

The samples were sent to Chulalongkorn University livestock hospital (Nakornpathom, Thailand).

The liver samples were collected and preserved in 10% formalin. They were later processed, sectioned and stained with hematoxylin and eosin. The slides were examined for pathological change and parts of hepatic lobules were photographed. Two samples from each replicates (10 samples for each treatment) were read by a pathologist and descriptive report on liver histopathological changes was made.

The histopathology change was determined following Oriatali et al. (2005).

3.13 Data analyses

All data were presented as Mean ± S.E.M. The effects of treatment were analyzed using One-Way Analysis of Variance (ANOVA). Duncan's New Multiple Range test was used to compare the individual means. The level of statistical significant was set at $P<0.05$. The statistical program used for this analysis was Sigmastat v 2.01.

CHAPTER IV
RESULTS

4.1 Preliminary experiment

4.1.1 Active ingredient analysis

Effect of different solutions on percentage of yield of *Thunbergia laurifolia Linn.* (TL) is shown in Table 4.1 and Figure 4.1. There was no difference in percentage of yield when water, ethanol, methanol, acetone and hexane solutions were used as solvents.

The amount of total phenolic compound and flavonoid derivatives is shown in Table 4.2 and Figure 4.2. Water extract of TL solution had significantly ($p<0.05$) higher total phenolic compound than other groups. Likewise, phenolic compound concentrations were highest when boiled water was used as solvent.

Acetone extraction resulted in significantly ($p<0.05$) higher flavanones than other groups. In addition, no flavanones were detected when water and hexane were used as solvents

Acetone solution had significantly ($p<0.05$) higher total flavonoid concentration than other groups.

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Table 4.1 Effect of different solutions on % yield[1] of *Thunbergia laurifolia Linn*.

Solvents	Yield (%)
water	8.60±0.22
ethanol	6.20±0.25
methanol	3.36±0.34
acetone	1.45±0.02
hexane	0.84±0.13

[1] Mean ± S.E.M.

Table 4.2 Effect of different solvent on total phenolic and total flavonoid concentration of *Thunbergia laurifolia Linn*.

Parameters	Solvents				
	water	ethanol	methanol	acetone	hexane
Total phenolic Compound (mg/g)	5.91±0.15[a]	2.14±0.04[b]	2.18±0.06[b]	0.64±0.02[c]	0.00±0.00[d]
Flavones and Flavonol (mg/g)	0.22±0.01[a]	0.14±0.01[c]	0.13±0.00[c]	0.18±0.01[b]	0.00±0.00[d]
Flavanones (mg/g)	0.00±0.00[c]	2.69±0.08[b]	2.27±0.17[b]	3.29±0.12[a]	0.00±0.00[c]
Total flavonoid (mg/g)	0.22±0.01[c]	2.83±0.08[b]	2.40±0.17[b]	3.47±0.11[a]	0.00±0.00[d]

[1] Mean ± S.E.M.

[a,b,c,d] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

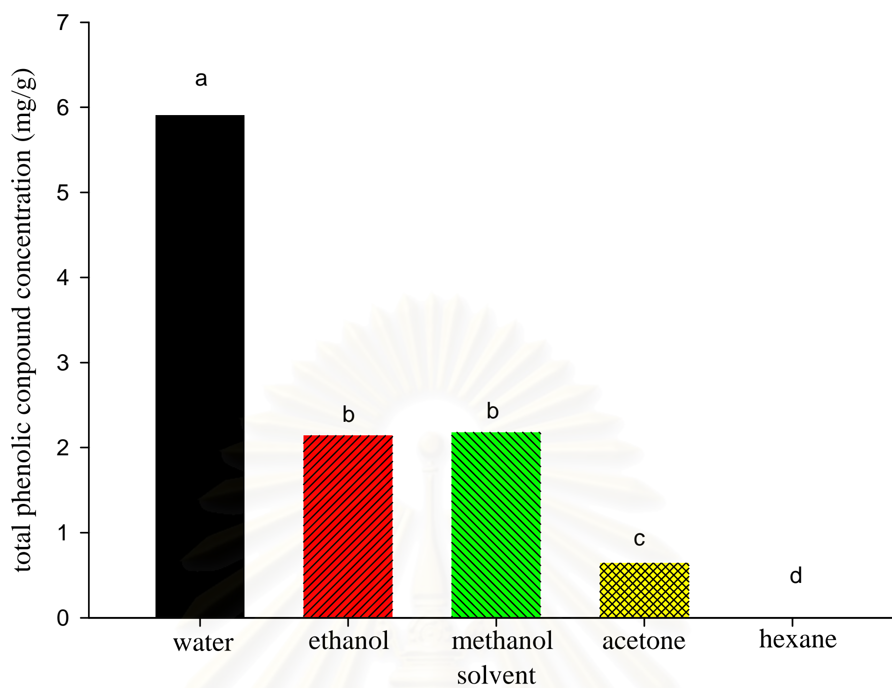

Figure 4.1 Effect of diffferent solventson total phenolic compound concentration of *Thunbergia Laurifolia Linn*. (preliminary experiment)

Each bar represents mean ± S.E.M. Values obtained from six replicates.
[a, b, c, d] Different superscripts mean significantly different ($P<0.05$)

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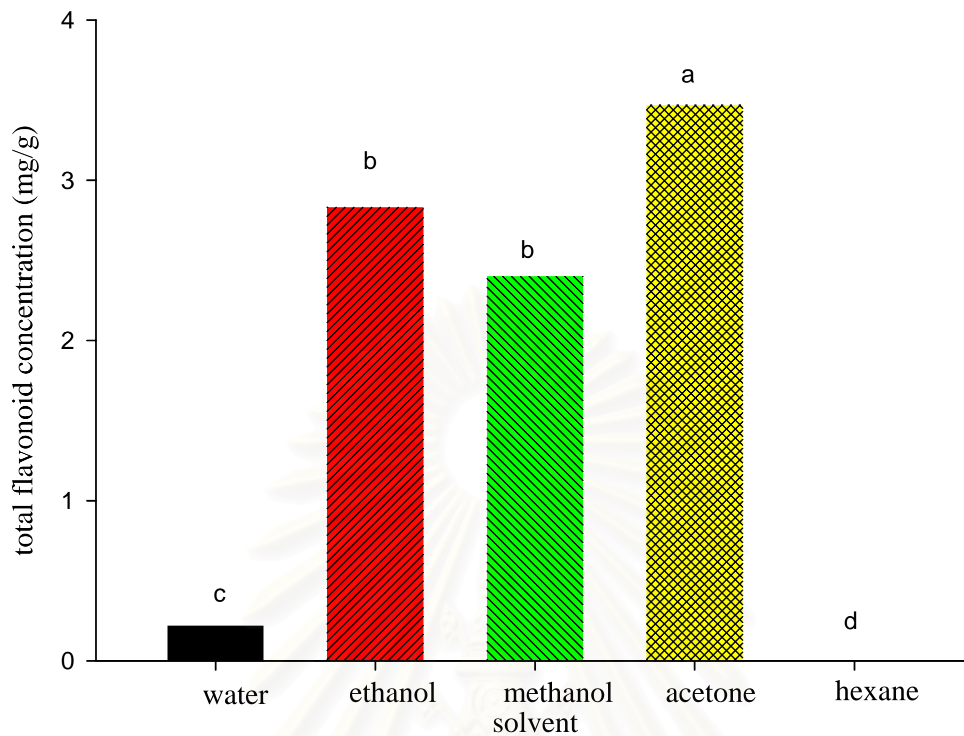

Figure 4.2 Effect of diffferent solvents on total flavonoid concentration of *Thunbergia Laurifolia Linn*. (preliminary experiment)

Each bar represents mean ± S.E.M. Values obtained from six replicates.
[a, b ,c, d] Different superscripts mean significantly different ($p<0.05$)

4.1.2 Growth performance

Growth performance of broilers during the starter period (day 1-21 of age) is shown in Table 4.3. It was demonstrated that there was no significantly difference in weight gain, average daily gain (ADG) and mortality rate among each group of the broilers. However, broilers in T2 (2% TL) and T3 (4% TL) group had significantly ($p<0.05$) higher daily feed intake (DFI) and feed conversion ratio (FCR) than T1 (control) group.

Table 4.3 Effect of treatments on growth performance[1] of the broiler during the starter period (Day 1-21).

Parameters	Treatmens[2]		
	T1	T2	T3
Initial BW (g/b*)	44.3±0.4	44.6±0.2	45.0±0.0
DFI (g/b*/d)	38.4±0.4[b]	45.1±0.9[a]	43.9±0.6[a]
ADG (g/b*/d)	32.5±0.7	32.0±0.3	31.2±0.3
FCR	1.12±0.02[a]	1.31±0.02[b]	1.32±0.02[b]

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 2% TL; T3: 4% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

Growth performance of broilers in grower-finisher period (day 22-42 of age) is shown in Table 4.4. There were no significantly difference in feed intake (FI), daily feed intake (DFI), weight gain, and average daily gain (ADG) and feed conversion ratio (FCR) among groups of broilers.

Table 4.4 Effect of treatments on growth performance[1] of the broiler during the grower-finisher period (Day 22-42).

Parameters	Treatments[2]		
	T1	T2	T3
Initial BW (g/b*)	726.0±14.4	716.9±5.5	700.0±5.5
DFI (g/b*/d)	135.0±4.2	150.5±6.4	135.2±3.8
ADG (g/b*/d)	69.6±1.8	72.5±2.5	64.5±1.7
FCR	1.35±0.02	1.38±0.02	1.42±0.02

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 2% TL; T3: 4% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

For the overall period of trial (day 1-42 of age), broilers in T1 (control) group and T2 (2% TL) group had significantly ($p<0.05$) higher final body weight than T3 (4% TL) (Table 4.5). Broilers in T2 (2% TL) group had significantly ($p<0.05$) higher daily feed intake (DFI) than other groups but they were not significantly different from T3 (4% TL) and T1 (control) group. Broilers in T2 (2% TL) group had significantly ($p<0.05$) higher average daily gain (ADG) than T3 (4% TL), however, it was not significantly different from T1 (control) group. Broilers in T3 (4% TL) group had significantly ($p<0.05$) higher feed conversion ratio (FCR) than T1 (control) group. On the other hand, when compared with T2 (2% TL) group, it was not significantly different.

Table 4.5 Effect of treatments on growth performance[1] of the broilers during overall period (Days 1-42).

Parameters	Treatments[2]		
	T1	T2	T3
Initial BW (g/b*)	44.3±0.4	44.6±0.19	45.0±0.00
Final BW (g/b*)	2,187.1±45.4[a]	2,238.3±53.3[a]	2,055.4±34.9[b]
DFI (g/b*/d)	92.1±2.5[b]	107.2±4.3[a]	95.9±2.7[b]
ADG (g/b*/d)	51.0±1.1[a]	52.2±1.3[a]	47.9±0.8[b]
FCR	1.82±0.03[a]	1.92±0.04[b]	1.98±0.03[b]

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 2% TL; T3: 4% TL

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

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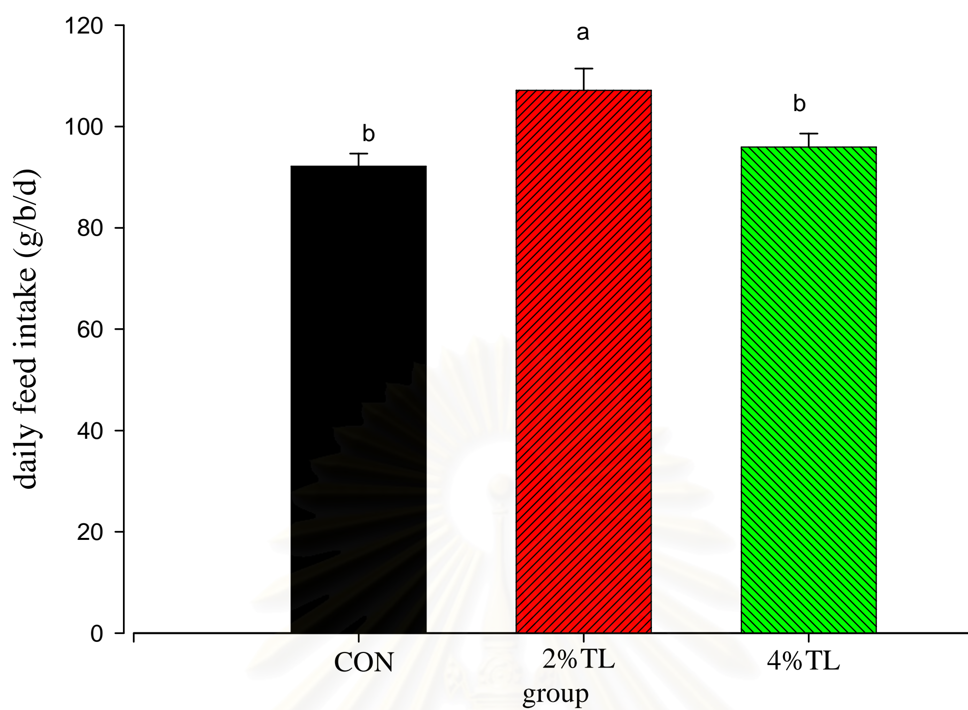

Figure 4.3 Effect of treatments on daily feed intake of broilers (week 1-6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

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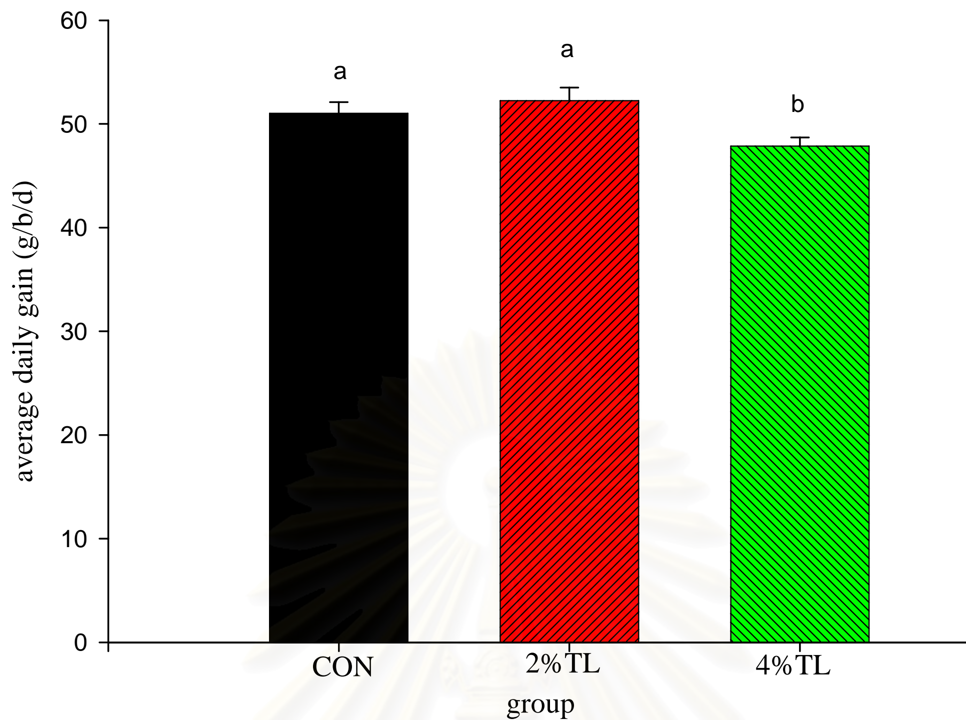

Figure 4.4 Effect of treatments on average daily gain of broilers (week 1-6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

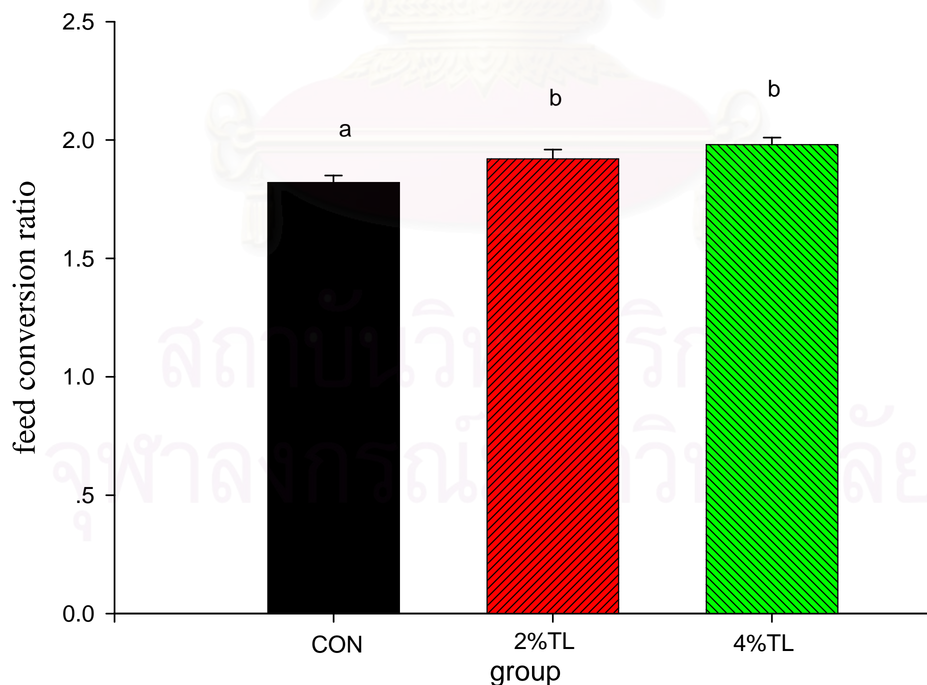

Figure 4.5 Effect of treatments on feed conversion ratio of broilers (week 1-6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

4.1.3 Blood glucose and heterophil/lymphocyte ratio

The blood glucose concentration and heterophil/lymphocyte ratio are demonstrated in Table 4.6.

At day 21 of age, there was no significantly difference in blood glucose among groups of broilers.

At day 42 of age, broilers in T1 (control) group had significantly ($p<0.05$) higher blood glucose concentration than T2 (2% TL) and T3 (4% TL) group. However, there was no significantly difference between (2% TL) and T3 (4% TL) group.

There was no significantly difference in heterophil:lymphocyte ratio of broilers in both starter period (day 1-21 of age) and grower-finisher period (day 22-42 of age)

Table 4.6 Effect of treatments on blood glucose[1] and Heterophil:Lymphocyte ratio[1] of broilers in each groups.

	Treatments[2]		
	T1	T2	T3
Blood glucose (mg/dl)			
Day 21	188.60±6.24	200.00±12.46	188.60±8.02
Day 42	219.70±6.51 [a]	196.80±3.15 [b]	199.40±8.23 [b]
Heterophil:Lymphocyte ratio			
Day 21	0.80±0.20	1.24±0.33	0.73±0.10
Day 42	0.82±0.20	1.30±0.19	1.38±0.36

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 2% TL; T3: 4% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

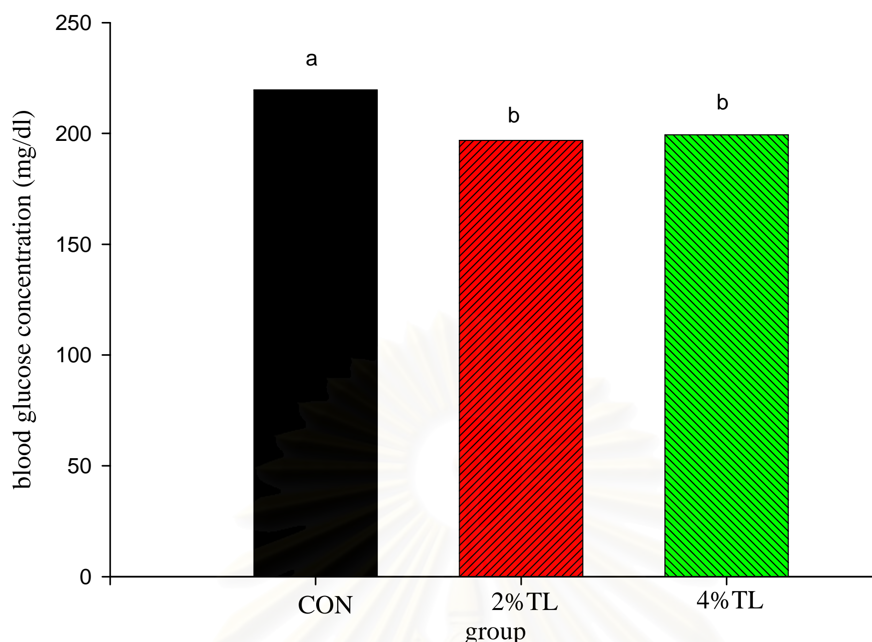

Figure 4.6 Effect of treatments on blood glucose concentrationof broilers (week 6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

4.2 Experiment

4.2.1 Active ingredient analysis

Effect of different solutions on yield percentage of *Thunbergia laurifolia Linn.* is shown in Table 4.7 and Figure 4.7. There was no difference on percentage of yield when water, ethanol, methanol, acetone and hexane solution were used as solvents.

Extraction using boiled water resulted in significantly ($p<0.05$) higher total phenolic compound and flavones and flavonol than other groups (Table 4.8 and Figure 4.8).

Acetone solution had significantly ($p<0.05$) higher flavanones than other groups. However, flavonone can not be detected when water and hexane solution were used as solvents. Likewise, acetone solution resulted in significantly ($p<0.05$) higher total flavonoid than other groups.

There were no extract of total phenolic compound and total flavonoid from TL when hexane was used as solvent.

Table 4.7 Effect of different solution %yield[1] of *Thunbergia laurifolia Linn*.

Solvents	Yield (%)
water	7.87±0.42
ethanol	4.88±0.17
methanol	2.81±0.25
acetone	1.03±0.02
hexane	0.96±0.08

[1] Mean ± S.E.M.

Table 4.8 Effects of different solutions on total phenolic and total flavonoid[1] of *Thunbergia laurifolia Linn*.

Parameters	Solvents				
	water	ethanol	methanol	acetone	hexane
Total phenolic Compound (mg/ml)	7.47±0.16[a]	1.96±0.08[b]	2.04±0.16[b]	1.15±0.06[c]	0.00±0.00[d]
Flavoned and Flavonol (mg/ml)	0.30±0.01[a]	0.19±0.00[c]	0.15±0.01[d]	0.24±0.01[b]	0.00±0.00[e]
Flavanones (mg/ml)	0.00±0.00[d]	3.14±0.09[b]	1.81±0.13[c]	4.14±0.26[a]	0.00±0.00[d]
Total flavonoid (mg/ml)	0.30±0.01[d]	3.34±0.09[b]	1.96±0.14[c]	4.37±0.26[a]	0.00±0.00[e]

[1] Mean ± S.E.M.

[a,b,c,d] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

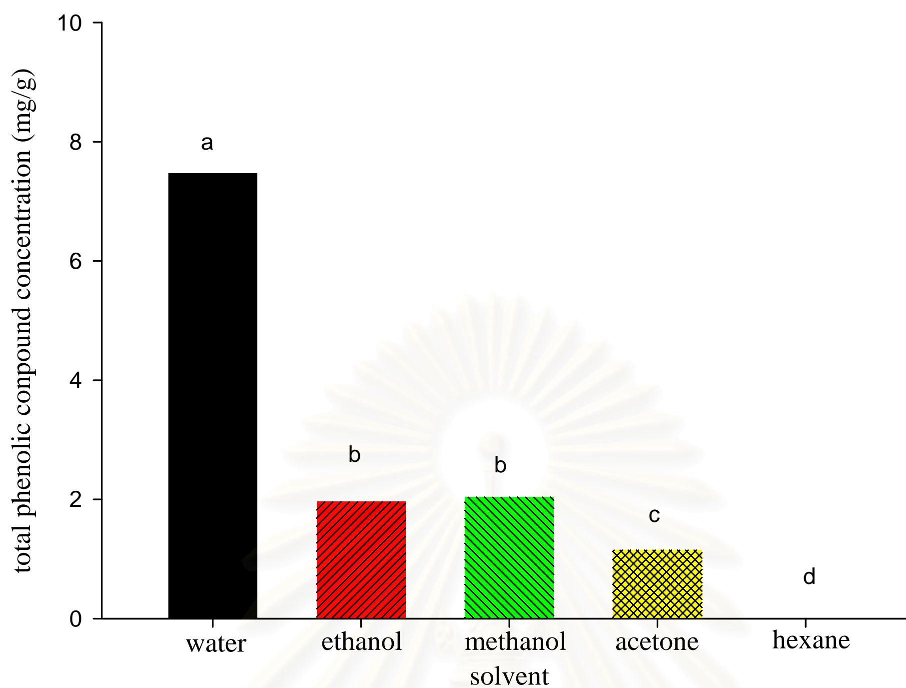

Figure 4.7 Effect of diffferent solvents on total phenolic compound concentration of *Thunbergia Laurifolia Linn*. (experiment)

Each bar represents mean ± S.E.M. Values obtained from six replicates.
[a, b,c,d] Different superscripts mean significantly different ($p<0.05$)

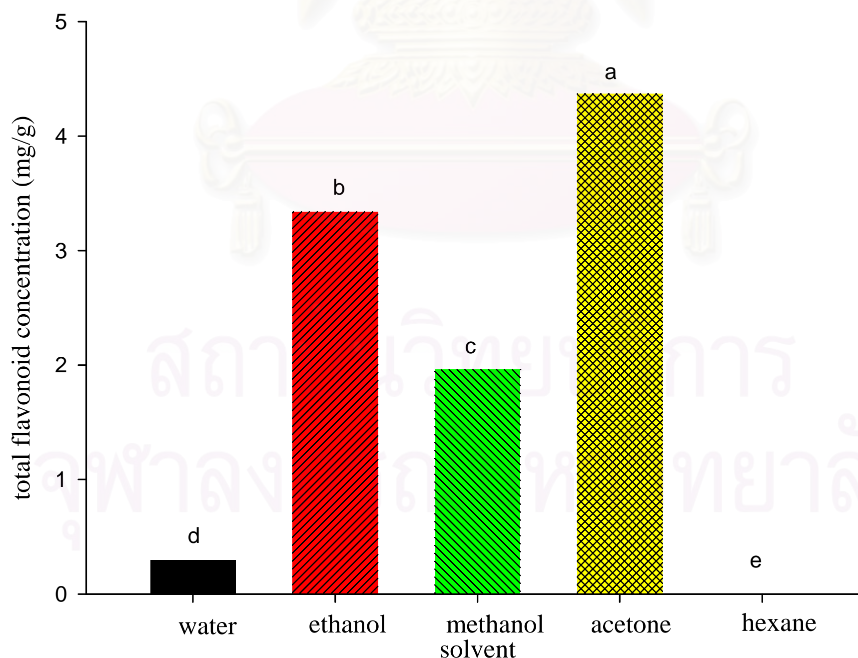

Figure 4.8 Effect of diffferent solvents on total flavonoid concentration of *Thunbergia Laurifolia Linn*. (experiment)

Each bar represents mean ± S.E.M. Values obtained from six replicates.
[a, b,c,d,e] Different superscripts mean significantly different ($P<0.05$)

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4.2.2 Growth performance

Growth performance of broilers during starter period (day 1-21 of age) is shown in Table 4.9. There was no significantly difference in weight gain, average daily gain (ADG), feed conversion ratio (FCR) and mortality rate among groups of broilers. However, broilers in T3 (mycotoxin) group had significantly ($p<0.05$) higher daily feed intake (DFI) than other groups.

Growth performance of broilers in grower-finisher period (day 22-42 of age) is shown in Table 4.10. There was no significantly difference in initial weight and mortality rate among groups. Broilers in T1 (control) group had significantly ($p<0.05$) lower daily feed intake (DFI) than other groups. Broilers in T2 (1% GCM) group had significantly ($p<0.05$) higher average daily gain (ADG) than T3 (mycotoxin) group but was not significantly difference from T1 (control), T4 (mycotoxin + 1% GCM), T5 (mycotoxin + 2% TL) and T6 (mycotoxin +1% GCM + 2% TL) groups. Broilers in T1 (control) group had significantly ($p<0.05$) better feed conversion ratio (FCR) than T3 (mycotoxin) group but was no significantly difference from T2, T4, T5 and T6 groups.

Data of growth performance in the overall period (day 1-42 of age) is depicted in Table 4.11. There was no significantly difference in initial weight, final weight, average daily gain (ADG) and mortality rate among groups. Broilers in T1 (control) group had significantly ($p<0.05$) lower daily feed intake (DFI) than other groups. Broilers in T3, T5 and T6 groups had significantly ($p<0.05$) higher feed conversion ratio than T1 (control) group but were not significantly different from T2 and T4 groups.

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Table 4.9 Effect of treatments on growth performance[1] (Day 1-21).

Starter period	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Initial BW (g/b*)	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4
DFI (g/b*/d)	51.7±0.6^{d}	53.3±0.0^{c}	54.5±0.0^{a}	53.9±0.0^{b}	53.9±0.0^{b}	53.9±0.00^{b}
ADG (g/b*/d)	34.0±0.5	34.0±0.7	35.1±0.3	34.3±0.4	33.5±0.7	34.2±0.2
FCR	1.52±0.03	1.57±0.03	1.55±0.01	1.57±0.02	1.61±0.03	1.58±0.01
Mortality rate (%)	0.0±0.0	3.8±2.5	0.0±0.0	0.0±0.0	1.3±1.3	1.3±1.3

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

^{a,b,c,d} Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

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Table 4,10 Effect of treatments on growth performance[1] of the broilers (Day 22-42).

Grower-Finisher period	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Initial BW (g/b*)	797.2±12.8	825.5±22.9	822.0±7.8	801.3±10.3	795.2±11.4	806.8±12.5
DFI (g/b*/d)	103.3±0.7^{b}	112.1±3.4^{a}	106.3±0.5^{a}	107.6±1.10^{a}	109.5±1.2^{a}	106.9±2.0^{a}
ADG (g/b*/d)	67.9±0.9^{ab}	70.7±2.7^{a}	63.1±1.0^{b}	68.2±1.5^{ab}	69.2±1.3^{ab}	65.9±1.3^{ab}
FCR	1.52±0.02^{a}	1.59±0.03^{ab}	1.69±0.02^{b}	1.58±0.05^{ab}	1.58±0.03^{ab}	1.62±0.02^{ab}
Mortality rate (%)	5.7±1.4	0.0±0.0	0.0±0.0	1.4±1.4	1.4±1.4	0.0±0.0

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

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Table 4.11 Effect of treatments on growth performance[1] of the broilers (Days 1-42).

Overall period	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Initial BW (g/b*)	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4	47.6±0.4
Final BW (g/b*)	2,019.8±13.1	2,011.4±27.3	1,951.3±21.8	2,031.7±30.8	2,017.3±31.1	1,966.4±10.3
DFI (g/b*/d)	71.5±0.4^{b}	73.9±0.6^{a}	74.2±0.2^{a}	74.5±0.5^{a}	74.6±0.5^{a}	73.4±0.3^{a}
ADG (g/b*/d)	47.9±0.3	47.7±0.6	46.3±0.5	48.1±0.7	47.8±0.7	46.6±0.2
FCR	1.49±0.02^{b}	1.55±0.01^{ab}	1.60±0.02^{a}	1.55±0.03^{ab}	1.56±0.02^{a}	1.58±0.01^{a}
Mortality rate (%)	5.0±1.3	3.8±2.5	0.0±0.0	1.3±1.3	2.5±1.5	1.3±1.3

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

* b = bird

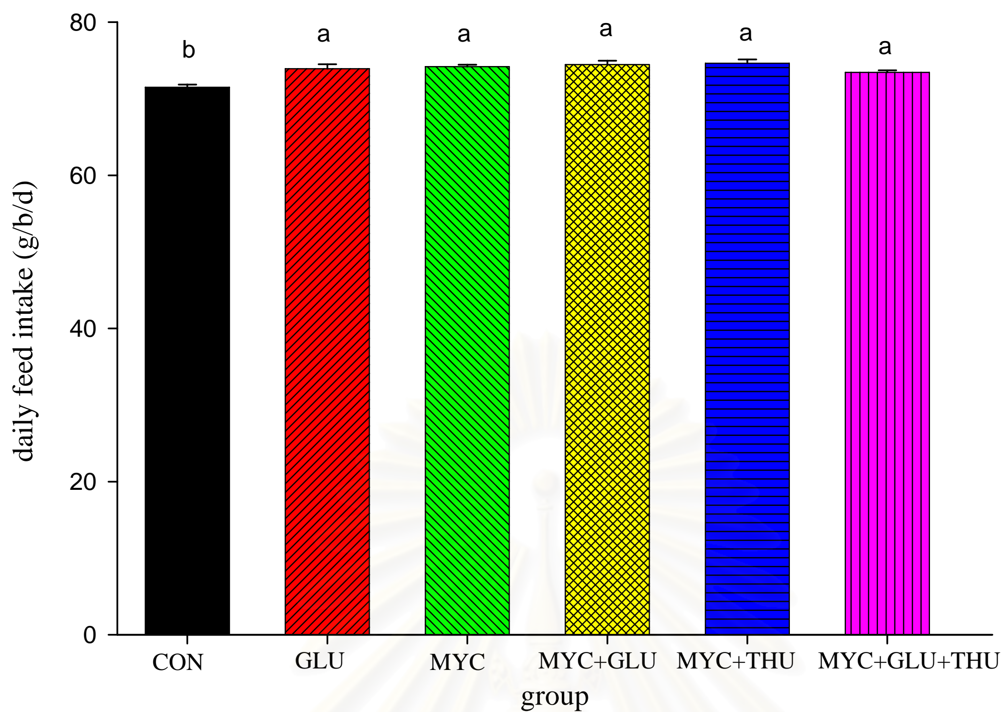

Figure 4.9 Effect of treatments on daily feed intake of broilers (week 1-6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.

[a, b] Different superscripts mean significantly different ($p<0.05$)

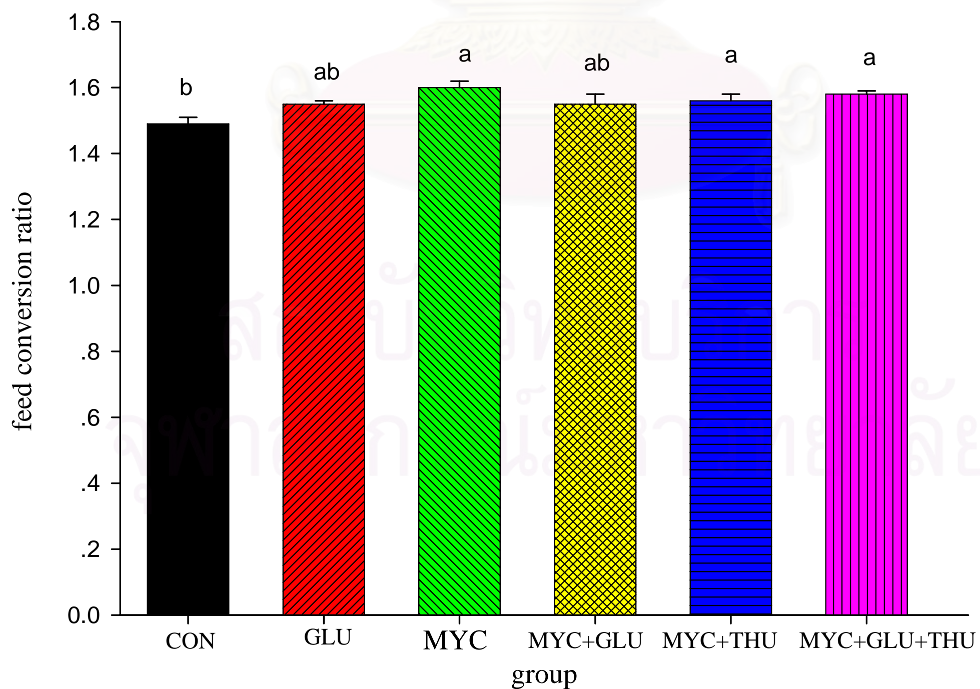

Figure 4.10 Effect of treatments on feed conversion ratio of broilers (week 1-6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.

[a, b] Different superscripts mean significantly different ($p<0.05$)

4.2.3 Nutrient digestibility

Ileal nutrient digestibility of broilers at day 21 and 42 of age is demonstrated in Tables 4.12 and 4.13, respectively. There was no effect of any treatments on protein, fat, fiber and energy digestibility during starter period.

During grower-finisher period, broilers receiving glucomannan in both T2 and T4 groups had significantly ($p<0.05$) lower protein digestibility than in T1 (control), T3 (mycotoxin), T5 and T6 groups. It is apparent that broilers in T5 and T6 groups had significantly ($p<0.05$) higher crude fat and crude fiber digestibility than other groups. Broilers in T5 and T6 groups had significantly ($p<0.05$) higher energy digestibility than T2 and T4 groups but were not significantly different from T1 and T3 groups.

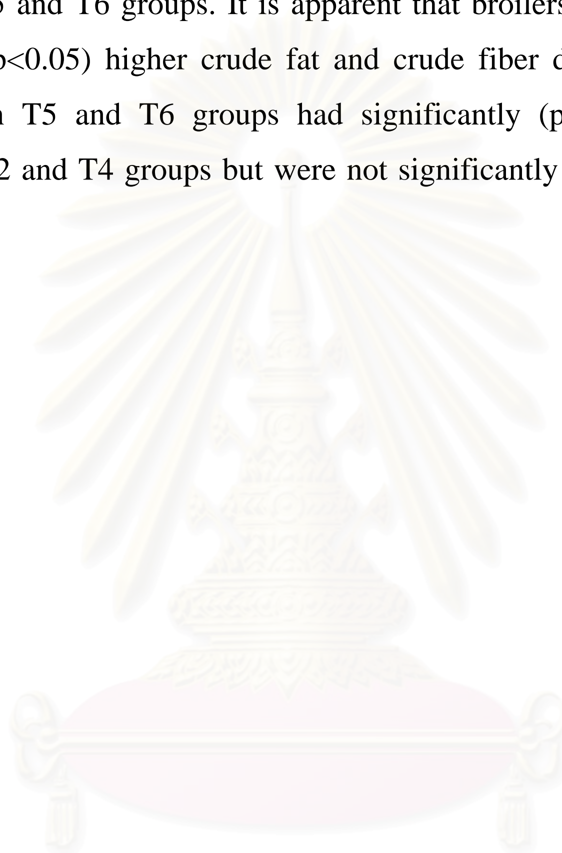

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Table 4.12 Effect of treatments on digestibility[1] of the broilers (Day 21).

Parameters	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Protein (%)	52.8±6.8	34.4±19.6	58.0±14.6	45.3±5.5	42.0±17.2	55.1±5.7
Fat (%)	72.2±4.1	55.0±13.7	73.4±7.3	69.1±3.4	66.5±9.9	78.6±2.6
Fiber (%)	57.6±6.5	48.6±15.8	67.4±10.3	50.1±4.7	64.9±10.8	73.6±2.6
Energy (%)	38.6±8.7	30.4±27.6	38.6±21.2	28.9±7.0	41.4±20.4	44.4±6.6

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

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Table 4.13 Effect of treatments on digestibility[1] of the broilers (Day 42).

Parameters	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Protein (%)	82.3±1.4[a]	77.3±3.3[b]	82.0±2.6[a]	73.0±3.4[b]	87.2±1.5[a]	88.4±0.7[a]
Fat (%)	89.9±0.6[b]	88.3±1.9[b]	90.8±1.2[b]	87.4±1.5[b]	94.5±0.6[a]	94.6±0.3[a]
Fiber (%)	38.3±4.4[b]	29.8±6.5[b]	35.6±8.8[b]	27.3±7.6[b]	59.3±5.1[a]	58.2±2.0[a]
Energy (%)	74.1±2.2[ab]	66.8±5.1[b]	74.4±3.7[ab]	64.7±4.3[b]	82.1±2.3[a]	83.0±1.0[a]

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

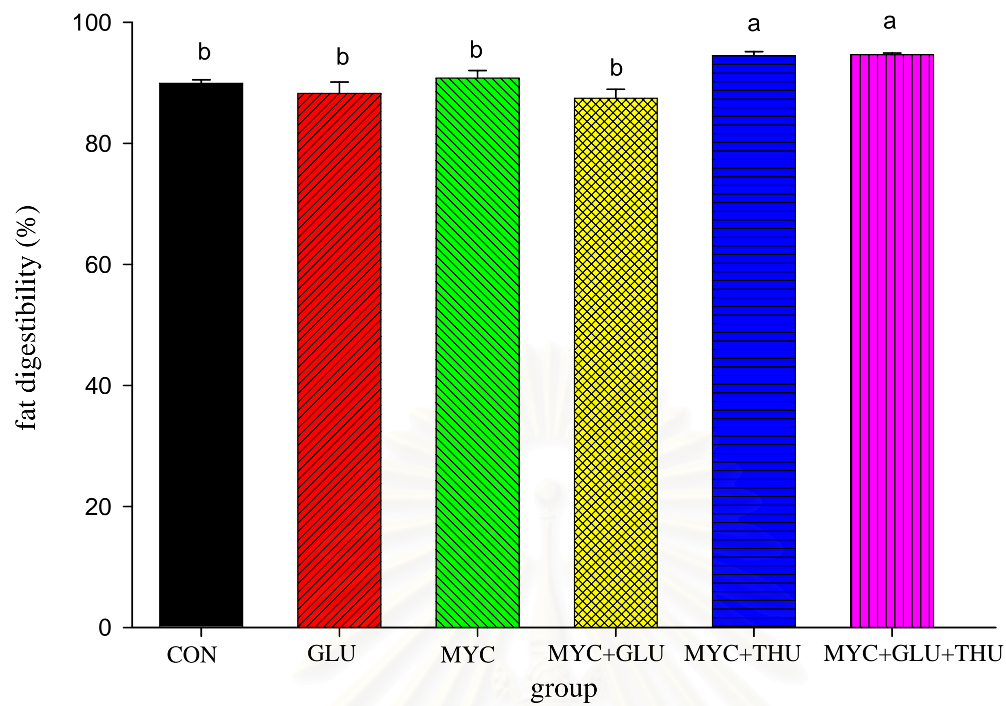

Figure 4.11 Effect of treatments on fat digestibility of broilers (week 6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

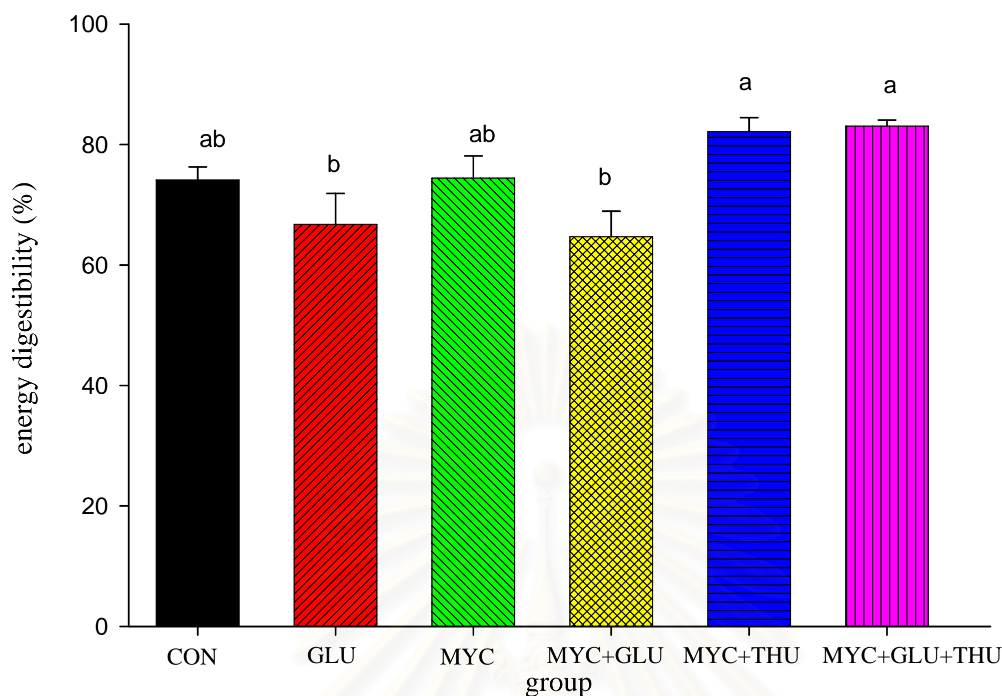

Figure 4.12 Effect of treatments on energy digestibility of broilers (week 6)
Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different (p<0.05)

4.2.4 Jejunal disaccharidase activity

The alteration in maltase and sucrase activity of jejunal mucosa is demonstrated in Table 4.14.

At day 21, maltase activity in T4 group was significantly (p<0.05) greater than T1, T3 and T6 groups but was not significantly different from T2 and T5 groups.

At day 42, it was found that the maltase activity was not significantly different among groups of broilers. In both day 21 and 42 of age, it was found that the sucrase activity was not significantly different among groups of broiler.

Table 4.14 Effect of treatments on jejunal disaccharidase activity [1] of the broilers.

Disaccharidase	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Maltase (U/mg protein)						
Day 21	443.5±37.8^{b}	497.1±33.0^{ab}	410.7±34.9^{b}	599.7±43.1^{a}	478.8±14.5^{ab}	497.3±31.8^{b}
Day 42	580.5±29.4	598.2±26.0	579.1±26.1	644.8±35.1	687.2±66.9	614.1±68.2
Sucrase (U/mg protein)						
Day 21	43.4±2.3	46.6±4.5	40.1±2.8	54.5±3.8	46.5±2.0	46.6±2.6
Day 42	57.2±1.6	57.6±2.5	59.2±4.2	63.6±4.9	66.8±7.9	61.5±6.5

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

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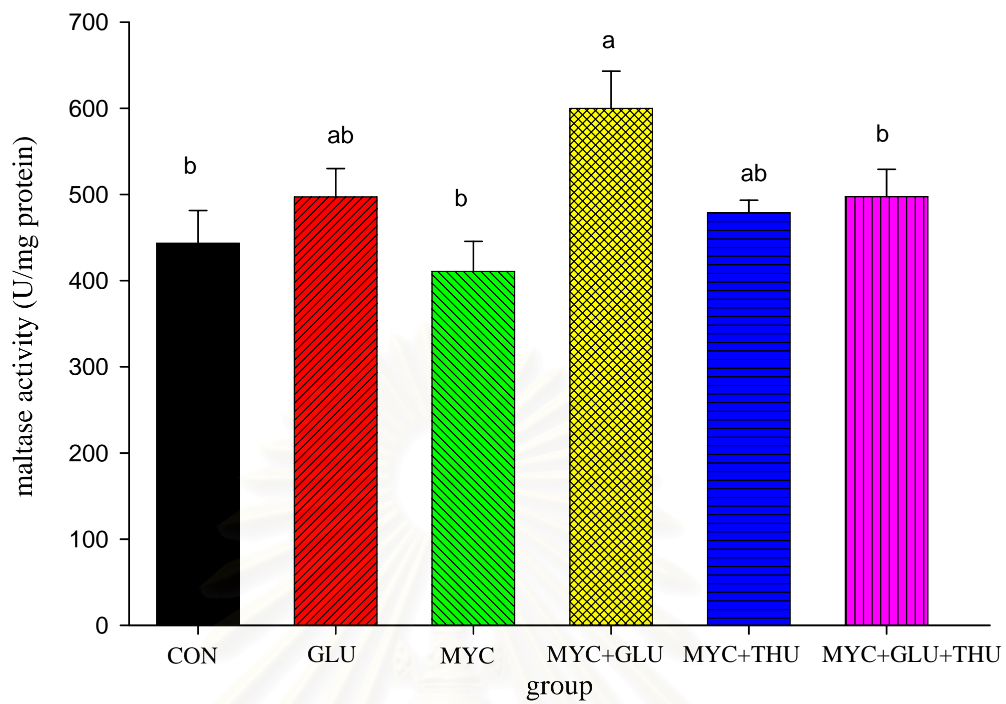

Figure 4.13 Effect of treatments on maltase activity of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

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4.2.5 Effect of treatments on antioxidant enzyme activity and MDA concentrations

The liver antioxidant enzyme activities and MDA concentrations of broilers at day 21 and day 42 are shown in Tables 4.15 and 4.16, respectively.

At day 21, broilers in T1 (control) group had significantly ($p<0.05$) lower superoxide dismutase activity than other groups.

At day 42, there was no effect of treatments on superoxide dismutase activity among groups of broilers. Likewise, in both days 21 and 42, there was no effect of treatments on catalase activity among groups of broilers.

At day 21 and 42, broilers in T4, T5 and T6 groups had significantly ($p<0.05$) higher glutathione peroxidase activity than T1, T2 and T3 groups. Broilers in T1 group had the lowest glutathione peroxidase concentrations among groups of broilers. Similar finding was demonstrated at day 42 of age which broilers in T1, T2 and T3 had significantly lower gluthathione peroxidase than T4, T5 and T6 groups. At day 21 of age, broilers in T6 group had significantly ($p<0.05$) higher MDA concentrations than T1 (control) group but was not significantly different from T2, T3, T4 and T5 groups. At day 42, there was no significantly difference in MDA concentrations among groups of broilers.

Table 4.15 Effect of treatments on antioxidant enzyme activity and malondialdehyde (MDA) concentrations[1] of the broilers (day 21).

Day 21	Treatments[2]					
	T1	T2	T3	T4	T5	T6
SOD (U/mg protein)	19.8±2.2^{b}	22.5±2.2^{a}	27.8±1.7^{a}	27.7±1.8^{a}	28.2±3.2^{a}	28.7±1.7^{a}
Catalase (U/mg protein)	100.0±8.1	87.9±8.3	115.0±4.3	115.0±9.4	102.2±4.3	100.4±6.0
GSH-px (mU/mg protein)	216.5±15.4^{c}	256.9±17.8^{b}	253.5±16.3^{b}	361.5±43.3^{a}	316.0±25.1^{a}	347.8±30.9^{a}
MDA (nmol/mg protein)	0.79±0.10^{b}	0.90±0.11^{ab}	0.92±0.13^{ab}	1.04±0.09^{ab}	1.08±0.07^{ab}	1.23±0.09^{a}

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

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Table 4.16 Effect of treatments on antioxidant enzyme activity and MDA concentrations[1] of the broilers (day 42).

Day 42	Treatments[2]					
	T1	T2	T3	T4	T5	T6
SOD (U/mg protein)	26.8±2.9	28.4±3.5	25.8±2.0	31.5±3.2	29.2±3.5	36.3±4.3
Catalase (U/mg protein)	129.5±12.4	127.9±5.2	150.9±6.7	126.6±13.4	147.5±6.2	119.3±8.9
GSH-px (mU/mg protein)	322.1±25.4[b]	290.6±22.9[b]	288.2±17.2[b]	391.7±26.6[a]	437.5±33.5[a]	389.6±23.1[a]
MDA (nmol/mg protein)	1.03±0.08	1.01±0.08	1.02±0.05	1.10±0.07	1.08±0.12	1.08±0.09

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

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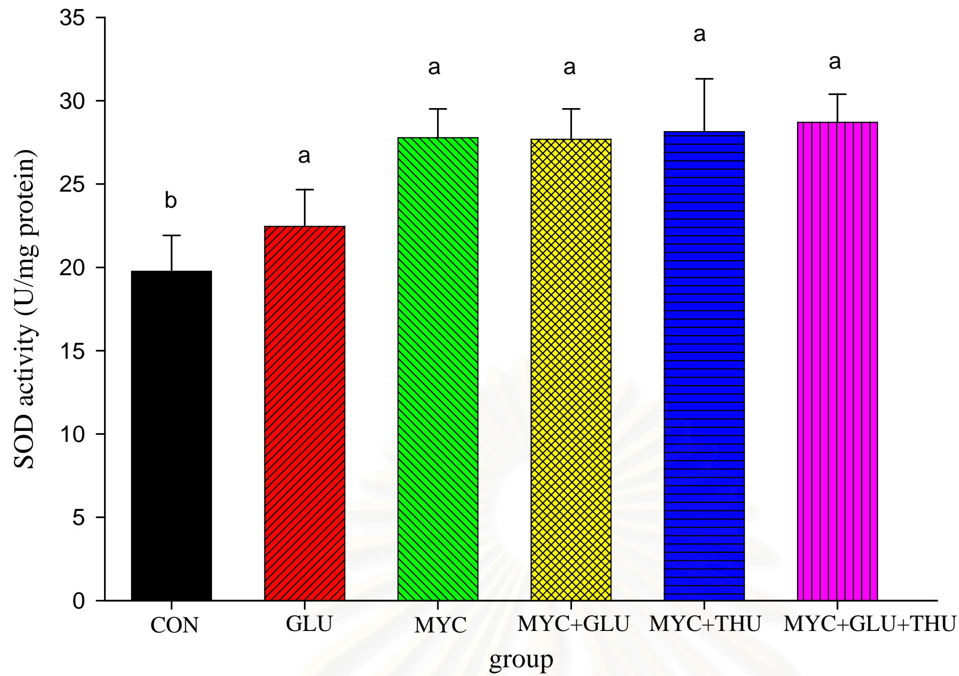

Figure 4.14 Effect of treatments on liver superoxide dismutase of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a,b] Different superscripts mean significantly different ($p<0.05$)

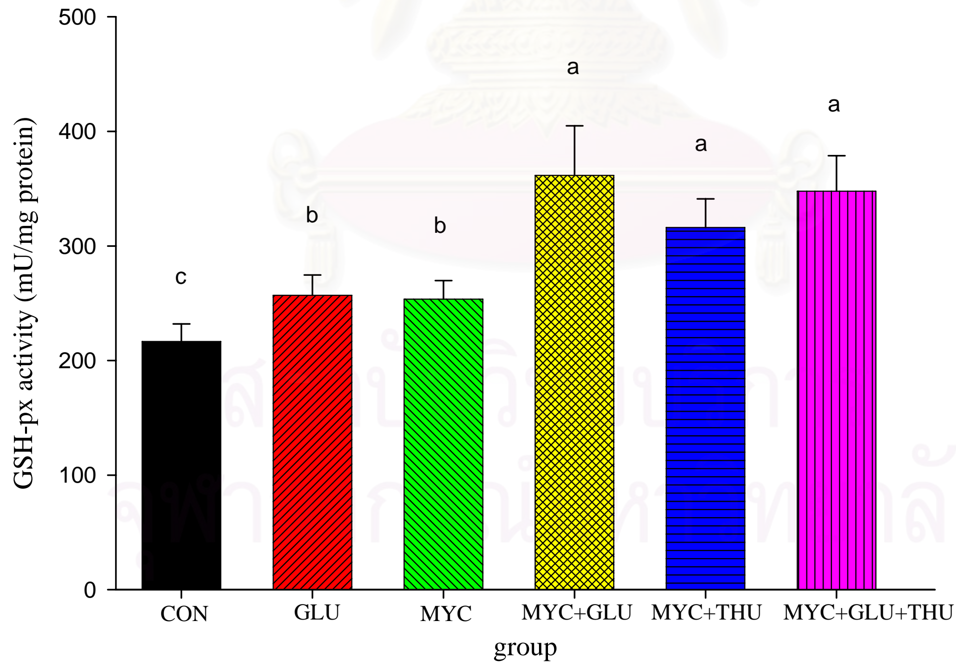

Figure 4.15 Effect of treatments on liver glutathione peroxidase of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b, c] Different superscripts mean significantly different ($p<0.05$)

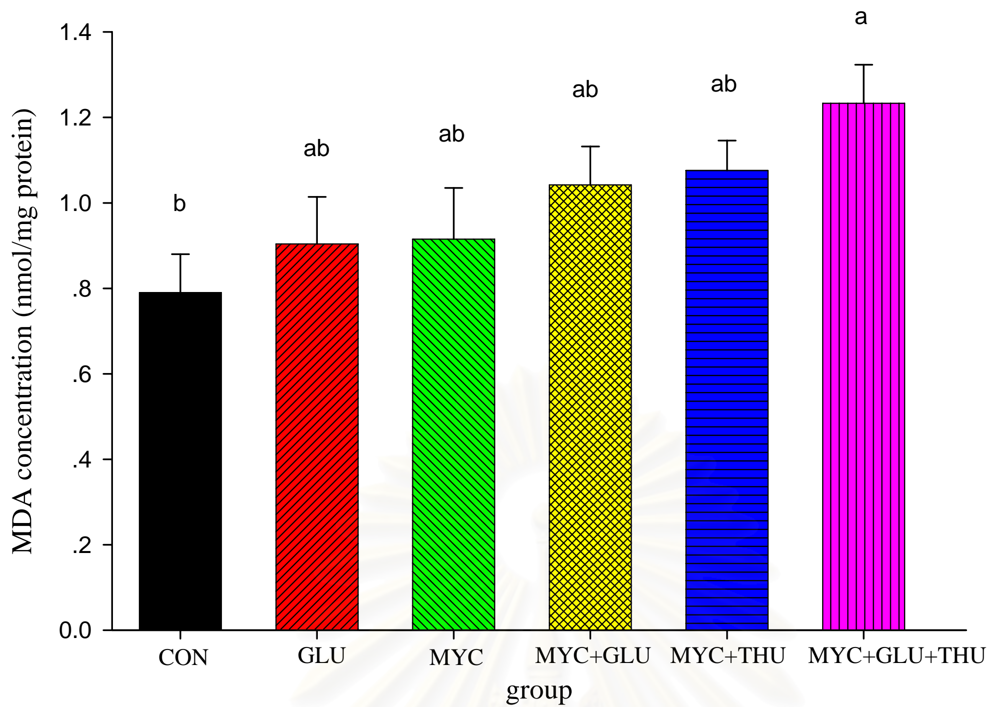

Figure 4.16 Effect of treatments on liver malondialdehyde of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

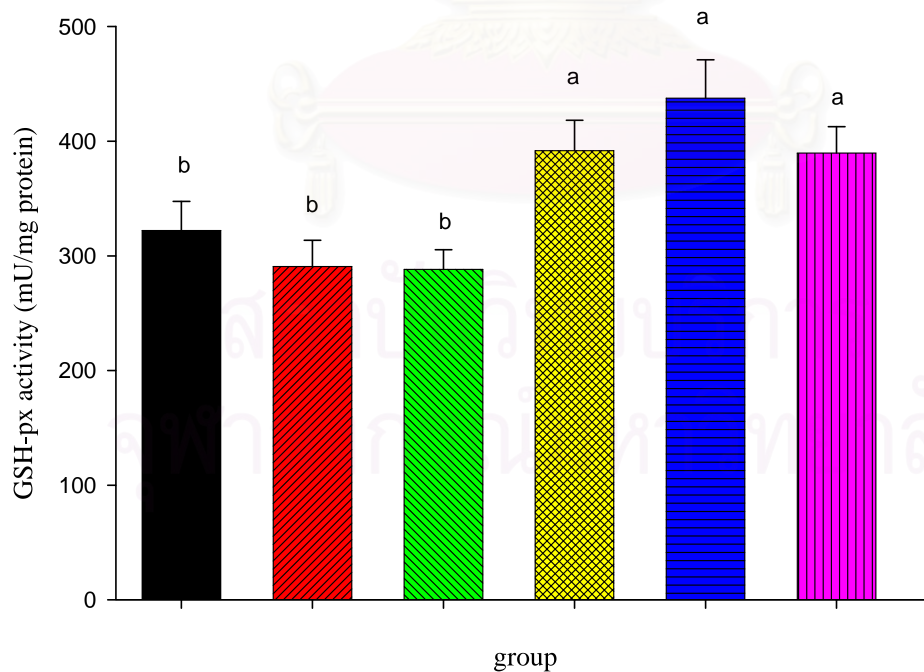

Figure 4.17 Effect of treatments on liver glutathione peroxidase of broilers (week 6)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

4.2.6 Blood and Serum chemistry analysis

4.2.6.1 Blood glucose concentrations and Heterophi: Lymphocyte ratio

The data on blood glucose concentrations and H/L ratio are shown in Table 4.17.

In both day 21 and 42 of age, there was no significantly difference in blood glucose concentrations among groups of broilers. In addition, the heterophil:lymphocyte ratio was not different among groups of broilers in both day 21 and 42.

4.2.6.2 Serum chemistry analysis

The serum SGPT, SGOT, serum albumin, and total protein concentrations are shown in Table 4.18.

There was no effect of treatments on serum glutamic oxaloacetate transaminase concentrations among groups of broilers in both day 21 and 42.

At day 21, Broilers in T1 (control) group had significantly ($p<0.05$) higher serum glutamic pyruvic transaminase concentrations than other groups. Broilers in T4 (1% glucomannan) group had significantly ($p<0.05$) higher serum albumin concentrations than other groups.

At day 42, there was no effect of treatments on serum glutamic pyruvic transaminase concentrations among groups of broilers. Broilers in T4 (mycotoxin +1% GCM), T5 (mycotoxin + 2% TL) and T6 (mycotoxim + 1% GCM + 2% TL) groups had significantly ($p<0.05$) higher serum albumin concentration than T2 (1% GCM) group but were not significantly different from T1 (control) and T3 (mycotoxin) groups.

Table 4.17 Effect of treatments on blood glucose concentrations and H:L ratio[1] of the broilers.

Parameters	Treatments[2]					
	T1	T2	T3	T4	T5	T6
Blood glucose (mg/dl)						
Day 21	244.00±5.26	227.80±5.34	234.20±8.05	234.40±6.63	228.80±8.10	224.20±5.51
Day 42	248.80±9.51	243.20±9.75	238.60±7.50	245.40±13.60	254.80±11.49	258.00±13.25
H:L ratio						
Day 21	1.69±0.29	2.46±0.60	1.46±0.21	3.65±1.06	2.71±0.39	2.64±0.30
Day 42	0.67±0.09	0.84±0.10	0.91±0.18	0.79±0.07	0.95±0.13	1.25±0.26

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

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Table 4.18 Effect of treatments on serum SGOT, SGPT and albumin concentrations [1] of the broilers.

Parameters	Treatments[2]					
	T1	T2	T3	T4	T5	T6
SGOT (IU/L)						
Day 21	200.8±13.0	202.1±13.2	216.8±15.4	191.9±6.1	210.3±5.8	199.7±7.6
Day 42	230.0±13.0	288.4±22.8	299.6±30.4	230.1±4.5	272.8±12.6	236.3±7.1
SGPT (IU/L)						
Day 21	27.0±2.1^{a}	16.3±2.1^{b}	18.8±1.5^{b}	17.2±1.9^{b}	16.4±2.6^{b}	10.9±0.9^{b}
Day 42	9.3±2.5	10.5±1.2	9.4±2.0	7.2±1.1	8.7±2.1	12.9±1.5
Albumin (g/dl)						
Day 21	1.30±0.04^{b}	1.51±0.05^{b}	1.46±0.02^{b}	1.72±0.10^{a}	1.44±0.02^{b}	1.38±0.09^{b}
Day 42	1.46±0.05^{ab}	1.36±0.02^{b}	1.48±0.05^{ab}	1.60±0.11^{a}	1.70±0.04^{a}	1.64±0.06^{a}

[1] Mean ± S.E.M.

[2] Treatments were T1: control; T2: 1% GCM; T3: mycotoxin; T4: mycotoxin + 1% GCM; T5: mycotoxin + 2% TL; T6: mycotoxin + 1% GCM + 2% TL.

[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$)

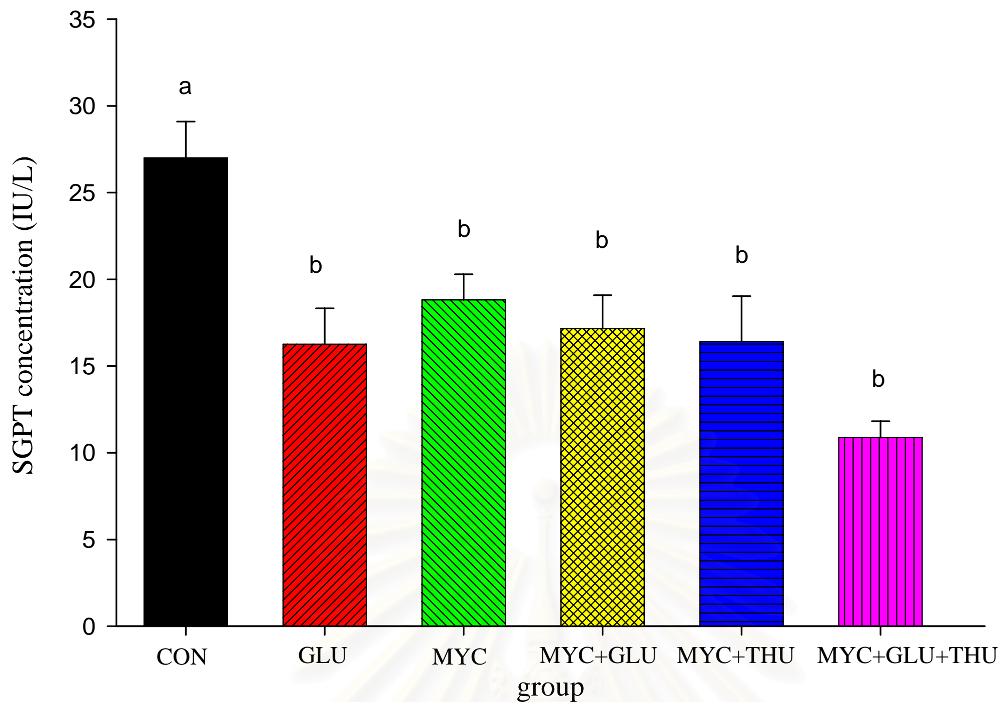

Figure 4.18 Effect of treatments on SGPT concentration of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

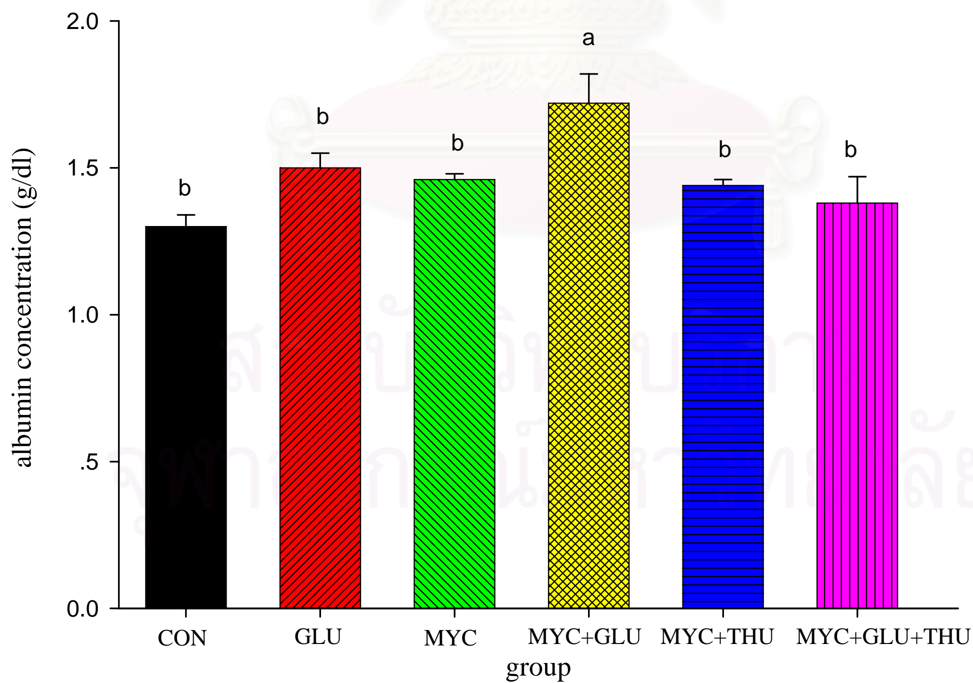

Figure 4.19 Effect of treatments on albumin concentration of broilers (week 3)

Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

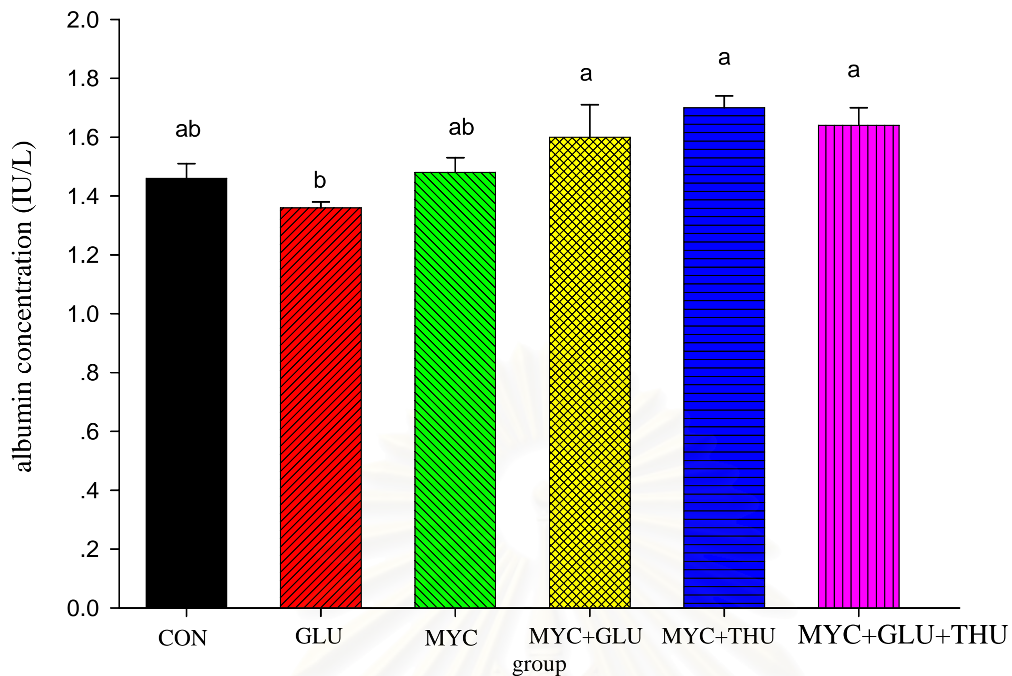

Figure 4.20 Effect of treatments on albumin concentration of broilers (week 6)
Each bar represents mean ± S.E.M. Values obtained from ten replicates.
[a, b] Different superscripts mean significantly different ($p<0.05$)

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4.2.7 Liver Histopathology

The pictures of H&E stained of liver samples are demonstrated in Figure 4.22. It is shown that the livers of T1 and T2 broilers had normal microscopic appearance. However, some hepatocyte swelling, hydropic degeneration, some hemorrhage and leukocyte infiltration were found in broilers receiving mixed mycotoxins (T3). The hemorrhage spots were still found in T4, T5 and T6 groups but the severity was less than those found in T3. No fatty degeneration, necroinflamation and leukocyte infiltration were found in these groups of broilers.

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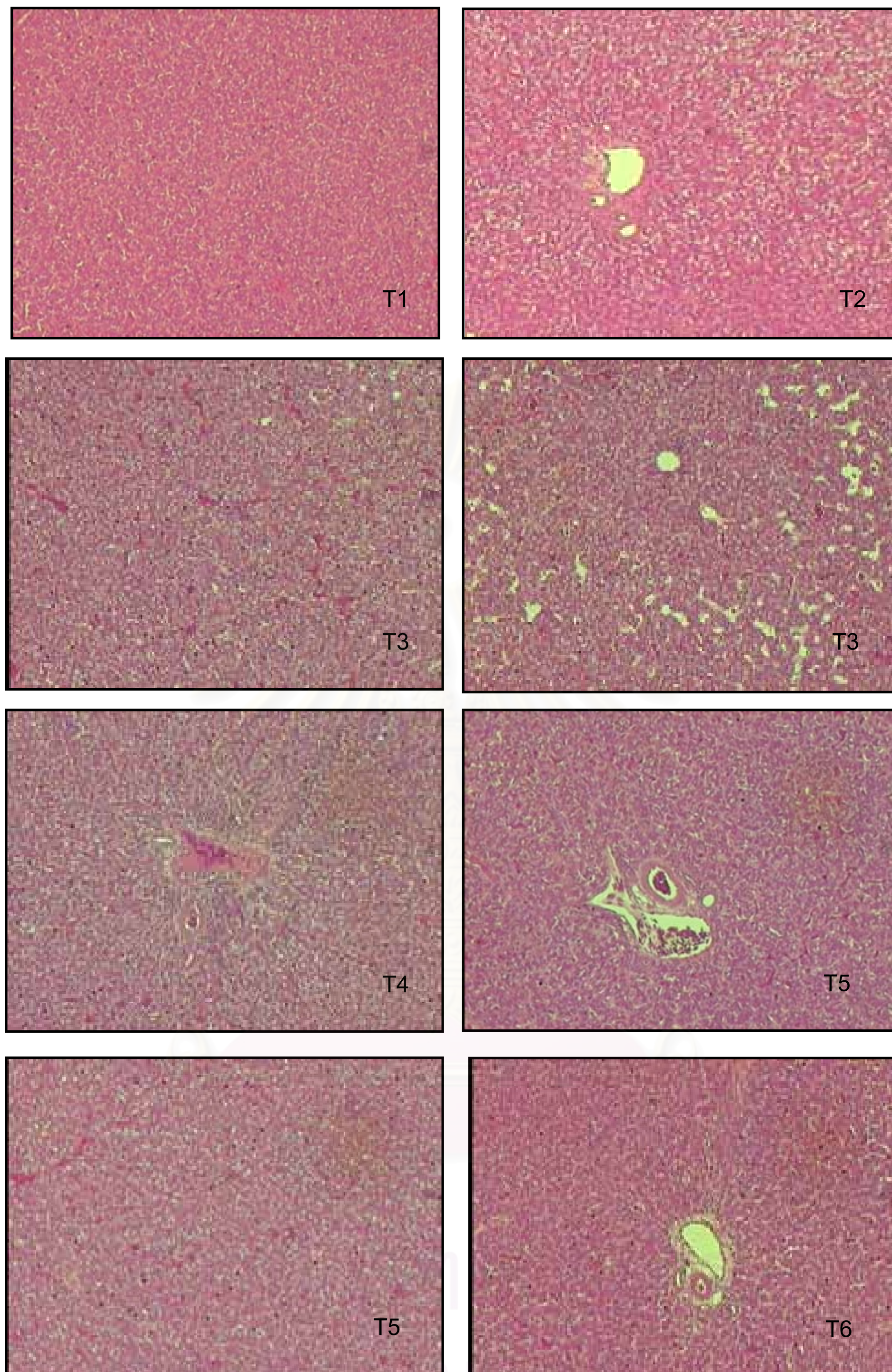

Figure 4.21 Examples of liver histopathological sections of each treatment.

CHAPTER V
DISCUSSION

5.1 Active ingredient analysis

The assessment of total phenolic compound and flavonoid concentrations is important to evaluate the quality of herb (Maisuthisakul et al., 2007). These active ingredients can affect body functions of both human and animal (Peterson and Dwyer, 1998). The most common number of phenolic compounds is flavonoids, cinnamic acid and lignin. The examples of phenolic compounds are flavones, flavonols, flavanones, flavanonols, anthocyanin and hydroxycinnamic acid etc. (Huwig, 2001).

The active ingredient in *Thunbergia laurifolia Linn.* (TL) has been detected and elucidated in several experiments such as apigenin which is flavonoid substance (Purima and Gupta, 1998), amino acids (วีระยุทธ์, 1979) and stimasterol which is also flavonoid substance (ขวัญใจ, 1983; ขวัญสิริและคณะ, 1999).. In the present study, it was found that TL had high concentrations of both total phenolic compound and flavonoids. It is shown that 5-7 mg/g of total phenolic compound was found when boiled water was used as solvent as well as 3-4 mg /g of total flavonoid when acetone was used as solvent. For these results, it was indicated that boiled water was the best solvent to extract phenolic compound in TL. On the other hand, acetone was the best solvent to extract flavonoid. Interestingly, the amount of total phenolic compound in this study was greater than previous study that TL from Nakorn Ratchasima contained 0.24 mg/g of total phenolic compound when boiled water was used as solvent (Oonsivilai et al., 2007). It was indicated that the amount of total phenolic compound depended on environmental factors. The herb used in this study was grown in Nan province. The soil and weather may be more appropriate for accumulation of phenolic compound than in Nakorn Ratchasima. Moreover, it was also greater than *Momordica chavantia*, the famous Thai medicial herb, which 6 mg/g of total phenolic compound and 0.3 mg/g of total flavonoid was detected (กมลทิพย์, 2007). On the contrary, both total phenolic compound and flavonoid were less than several Thai herbs such as *Antidesma velutinum Tulas.* (123 and 50 mg/g), *Cleisolyx operculatus var paniala* (Roxb.) (173 and 44 mg/g), *Eugenia siamensis Craib.* (180 and 50 mg/g), *Tamaridus indica Linn.* (134 and 41 mg/g), *Acacia catecho* (L.F.) wild (177 and 41 mg/g), *Areca catecho Linn.* (137 and 42 mg/g) and *Cassia fistula Linn.* (103 and 25 mg/g)

(Maisuthisakul et al., 2007). However, total phenolic compound in TL was higher than Polish medical herbs such as *Melisa officinalis* (0.013 mg/g), *Echimacea purpurea* (0.015 mg/g), *Acorus calamus* (0.012 mg/g), and *Polygonum aviculare* (0.011 mg/g) (Wojdylo et al., 2007). Moreover, total phenolic compound in TL was higher than Fijian medical herbs such as *Ipomoca batata* Var. (2mg/g), *Oringa oleifern* (2.6 mg/g) and *Colocasia escuenta* (1.2 mg) (Lako et al., 2007). In this study, crude powder was used because the antitoxic and antioxidant activities may result from different active ingredients which was clearly elucidated (Cai et al., 2004).

Interestingly, San and Chan (1987) reported that phenolic compound inhibited the activation enzyme of aflatoxin induced mutagenesis in *Salmonella typhimurium* strain TA 98 in suspension assay and the presence of rat liver microsome. It might activate a signaling cascade that resulted in the activation of detoxification enzymes involved in the elimination of chemical carcinogen. In addition, Peterson and Dwyer (1998) reported that flavonoid could act as antioxidant agent that inhibits lipid peroxidation system. Moreover, it inhibited CYP 450 activation which are enzyme that metabolize most drugs as well as mycotoxin in human or animal body.

From these results, it is indicated that phenolic compound in TL can be extracted using polar solvent such as boiled water. In contrast, flavonoid in TL was dissolved in non-polar sovent such as acetone. Similarly, Oonsivilai et al. (2007) found that total phenolic compound in TL was extracted more by boiled water than ethanol and acetone.

5.2 Preliminary study

The preliminary study was made to examine the appropriate level of TL supplement in the next experiment.

It is reported that 1.5 % TL crude powder could be supplemented in broiler feed and did not have aversive effect on broiler growth performance (สะเทิม, 2003). Therefore, 2 and 4 % supplementation was chosen in the preliminary experiment.

It is well established that many herbs improve growth performance of animals (Jang et al., 2007). Each herb has active ingredient which affect on weight gain, feed intake and feed utilization (Steenfeldt et al., 1998). Moreover, some herb can reduce blood glucose in diabetic animal as well as increase glucose utilization in normal animal (Yang et al., 2007). Furthermore, it reduces the stress which comes from the management condition in animal (Zulkifli et al., 2000; Lee et al. 2009).

In this study, on day 21, although both 2 and 4 % TL supplementation did not improve feed conversion ratio but they increased daily feed intake when compared with control group. It is possible that TL might increase feed appetite of chicks. Similarly, Jang et al. (2007) found that essential oil promoted total feed intake but not feed conversion ratio when birds are kept at optimal conditions. In addition, the essential oil might stimulate the secretion of pancreatic enzyme and then increased feed intake. However, both levels of TL increased feed conversion ratio. On day 42, there was no significant difference in daily feed intake and average daily gain among groups. For all periods, although the 2 % TL supplement did not improve feed conversion ratio, daily feed intake increased when compared with control and 4 % TL supplement groups. On the other hand, the 4 % TL supplement decreased average daily gain and increased feed conversion ratio. Therefore, 4 % TL supplement decreased growth performance when compared with other groups. It was reported that growth performance was not significantly significantly from basal diet group when 0-1.5 % TL powder was supplemented on broiler feed. However, the 0.5 and 1.0 % herb supplement tended to improve weight gain and feed intake (สะเทิม, 2003).

It was found that supplementation of 40 mg/ml fresh of *Thunbergia laurifolia linn***.** extract decreased blood glucose in normal rat as well as diabetic rat that received 600 mg/ml of dried leaf herb extract (สุริยนต์, 1998). It was suggested that TL may improve the glucose metabolism and utilization. In this study, on day 21, the blood glucose concentration was not significantly different among groups. However, on day 42, broiler received TL in both groups had significantly lower blood glucose concentrations than control. It is possible that TL may decrease blood glucose and result in appetite stimulation as shown in an increased feed intake. Similarly, Xie et al. (2009) found that American ginseng extract regulated uncoupling protein-2 (UCP-2), which resulted in a decrease ATP production and increase insulin production which stimulated cellular GLUT4 activation to promote metabolism. Jaiswal et al. (2009) found that *Moringa oleifern Linn.* aqueous leave extract had some direct effect on increasing the tissue utilization of glucose by inhibiting hepatic gluconeogenesis and absorption of glucose into the muscle and adipose tissue. Yang et al. (2007) reported that polysaccharide from *Opumtia monacanla* improved insulin sensitivity in peripheral insulin target tissues and suppression of hepatic glucose output. The mechanism how TL decreased blood glucose in conventional broiler chick should be further investigated.

It is well established that heterophil: lymphocyte ratio is relevant to the stress condition of animals (Gross and Siegel, 1983). The average range is 0.54-0.70 (Mitchell et al., 1992). However, it depended on age, sex, breed, management condition of broilers (Zulkifil et al., 2000; Han et al., 2007). In both day 21 and 42, heterophil: lymphocyte ratio in TL supplement group was not significantly different from control group.

In short, although 2% TL supplement tended to slightly reduce some growth performance parameter when compared with control group. However, it was not significantly different. In addition, 2% TL supplementation help to stimulate feed intake and reduced blood glucose in normal broilers. Therefore, 2% TL was appropriate to be used in the experiment.

5.3 Experimental study

Previous studies on the toxic effect of various mycotoxin in broiler involved supplementation of pure form of mycotoxin in feed (Atanda et al., 2007). The advantage of using pure form is that its accuracy of dosage can be used to observe the acute affect or to study the mechanism of the specific mycotoxins. However, this model could only be performed in a small flock of chicken because the pure mycotoxin is costly. In this experiment mouldy corn contaminated with mixed mycotoxins was obtained from broiler farm, thoroughly ground and mixed to homogeneous corn stock to be used in experimental group. The levels of aflatoxin, fumonisin and T-2 were analyzed using ELISA as shown in the result. The advantage of this model is to mimic the condition found in some broiler farm in Thailand. In general, mixed mycotoxins in feed result in the chronic effect of the growth performance and decreased production of chicks (Jozefiak et al. 2007). However, the limitation of this model is the control of equal concentration of mixed mycotoxins in each experimental group.

It is well known that mycotoxin reduce growth performance of broiler such as feed intake, weight gain and feed conversion ratio. Maurice et al. (1983) reported that mycotoxin resulted in growth depression, weight loss, jaundice and death. In addition, Hussein et al. (2001) found that mycotoxin reduced body weight and increased liver and kidney weights. However, mycotoxin toxicity was ameliorated by mycotoxin adsorbent such as bentonite, aluminosilicate and yeast (*Saccharomyces cerevisiae*) (Abousadi et al., 2007). In this study, on day 21, broiler in T3 group (mycotoxin) had significantly higher daily feed intake than other groups. It is possible that mycotoxins in corn reduced nutrient utilization or quality of feed thus resulting in the lower energy and protein content of the

feedstuff. Broilers in mycotoxin group (T3) had significantly lower average daily gain than other groups during days 22-42 of age. The FCR of chicks in this group was also higher than other groups. Similarly, Abousadi et al. (2007) reported that aflatoxin contaminated-feed induced nutrient deficiency and it disrupted activity of the digestive enzymes and the absorption of essential nutrients. Average daily gain, feed conversion ratio and mortality rate were not significantly different among groups. Pasha et al. (2007) reported that mycotoxin reduce growth and feed deficiency. It also caused liver and kidney damage as well as immune suppression and changed in relative organ weight. It was noticed that growth performance of T4 (mycotoxin + glucomannan), T5 (mycotoxin + TL) and T6 (mycotoxin + glucomannan + TL) was not significantly different from control group. Both glucomannan from yeast cell wall and TL powder ameliorated mycotoxin toxicity and improved growth performance of broilers. Santin et al. (2003) reported that yeast cell wall improves feed conversion ratio of broiler fed mycotoxin contaminated-feed. Razzaghi et al. (2008) proposed that phenylpropanoids in *Satureja nortensis L.* essential oil inhibit cytochrome P450 dependent enzyme name cypA in aflatoxin biosynthesis pathway. In addition, growth performance of T2 (glucomannan group) was not significantly different among groups. It could be concluded that glucomannan increased broiler performance that fed mycotoxin contaminated-feed. (Aravind et al., 2003; Jodynis et al., 2006). Santin et al. (2003) reported that addition of yeast cell wall to diet showed improvement in feed conversion ratio of bird fed with or without mycotoxin in diet at day 42 of age. For overall periods, it is demonstrated that mycotoxin caused growth depression such as feed intake and feed conversion ratio. However, There were no effect on mortality rate because the dose of mycotoxin used in this experiment was low. Lethal dose of afltoxin is 4 ppm, fumonisin is 8 ppm and tricothecene is 10-15 ppm (จักรกริศน์, 1997).

Verma et al. (2002) found that mycotoxin decreased protein and energy utilization in broilers. In this experiment, on day 21, mycotoxins did not have any impact on protein, fat, fiber, and energy digestibility of broilers. It is shown that the digestibility of nutrients in each group were low. This may be the result of a small amount of sample collected from the chicks at day 21 of age. On day 42, it was found that TL supplemented group (T5 and T6) had significantly higher fat and energy digestibility than other groups. It can be concluded that TL increased fat digestibility which resulting in energy digestibility. It was indicated that active ingredient such as phenolic compound and flavonoid in TL

might be responsible for improving digestive function. Lin et al. (2006) found that tradition Chinese medicine increased apparent digestibility of protein and fat. It might influence digestive process by enhancing enzyme activity and improving digestibility of nutrients. However, glucomannan supplementation decreased protein, fat and energy digestibility. It might be that glucomannan interfered nutrient digestibility.

Brush border membrane of the small intestine especially jejunum is the major source of disaccharidase production (Chotinsky et al. 2001). In this experiment, mycotoxins did not affect disaccharidase activity of broiler. On day 21, glucomannan improve maltase activity of broilers. Similarly, Promthong et al. (2004) reported that corn starch and cassava powder supplement intend to stimulate higher disaccharidase activities as the digesta continuously flowed further down the intestinal tract.

Atroshi et al. (2002) suggested that mycotoxins caused free radical formation. It damages several cell body particularly liver cells. In this study, mycotoxins increased malondialdehyde production. It also increased catalase activity. In contrast, it decreased superoxide dismutase and glutathione peroxidase activity on day 21. Similarly, Elaslan et al. (2005) reported that broiler fed mycotoxins contaminated-feed decreased the activity of superoxide dismutase, catalase and glutathione peroxidase. Interestingly, it was found that glucomannan and TL powder improved the activity of antioxidant enzyme possibly to defense free radical formation because malondialdehyde concentration was not significantly different among groups on day 42. In addition, TL powder supplement improved the activity of antioxidant enzyme glutathione peroxidase. Similarly, Huwig et al. (2001) recommended that many herbs have active ingredient such as phenolic compound and flavonoid as antioxidant agent which can stimulate glutathione peroxidase activity.

In this study, it was found that mycotoxins had no effect on blood glucose concentration in chicks. However, Maurice et al. (1983) reported that plasma glucose showed a linear increased with mycotoxin dosage. It might be ascribed to impair glucose utilization since mycotoxins had been shown to reduce the activities of the enzyme involved in glucose metabolism. In addition, TL did not reduce blood glucose among groups as found in the preliminary experiment.

Moura et al. (2004) found that the administration of ochratoxin increased the number of heterophils and monocyte. In contrast, it decreased number of lymphocyte and eosinophils. In this study, it was found that mycotoxins did not have any impact on Heterophil:Lymphocyte ratio of broiler which indicate the stress condition of broilers.

Mycotoxins caused degenerative change, congestion and mild hemorrhages in liver (Saudhu et al., 1995). It also increased the amount of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT) which indicates liver damage (Wang et al., 2008). However in this study, it was found that mycotoxins had no effect on serum SGOT, SGPT and albumin although some treatment groups had significantly higher SGPT (T1) but the level was within normal limits. Supplementation of both glucomannan and TL powder slightly increased albumin when compared to other groups but it was not significant. The histopathological lesion was also not obvious as seen in other findings. Only some hemorrhage and derangement of hepatic cells were found in liver of chicks receiving mixed mycotoxin. It is obvious possible that the level of each mycotoxin in this study was not high enough to cause microscopic changes. Chicks receiving glucomannan and TL supplementation had slight improvement in liver lesion when compared to mycotoxin group (T3). Similarly, Sareshkumar and Mishra. (2006) reported that flavonoid of *Pergulartia daemia* ehanolic extract possess hepatoprotective activity against CCl_4 intoxicity in rat.

In conclusion, *Thunbergia laurifolia Linn* reduced blood glucose concentrations in normal broilers. Mixed mycotoxins contamination in feed reduced growth performance of broilers at day 21-42 of age. TL and TL plus yeast glucomannan helped to ameliorate the adverse effect of mixed mycotoxin by improving nutrient digestibility and increasing antioxidant enzyme (glutathione peroxidase) activity. The level of mixed mycotoxin in this study slightly affects serum enzyme activity and histopathological changes.

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