ผลของใบบัวบกทดแทนยาปฏิชีวนะต่อคุณลักษณะการเจริญเติบโต ปริมาณเอนไซม์
จากเซลล์เยื่อบุผนังลำไส้เล็ก และการย่อยได้ของโภชนะในไก่เนื้อ

นายขุนพล พงษ์มณี



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสรีรวิทยาการสัตว์ ภาควิชาสรีรวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2546
ISBN 974-17-3911-7

# EFFECTS OF ASIATIC PENNYWORT (*CENTELLA ASIATICA* L. URBAN) LEAVES, A REPLACEMENT OF ANTIBIOTICS, ON GROWTH PERFORMANCE, MUCOSAL ENZYME ACTIVITIES OF THE SMALL INTESTINE AND NUTRIENT DIGESTIBILITY IN BROILER CHICKENS

Mr. Koonphol Pongmanee



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Animal Physiology
Department of Physiology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2003

ISBN 974-17-3911-7

| | |
|---|---|
| Thesis Title | Effects of Asiatic pennywort (*CENTELLA ASIATICA* L. URBAN) leaves, a replacement of antibiotics, on growth performance, mucosal enzyme activities of the small intestine and nutrient digestibility in broiler chickens. |
| By | Mr. Koonphol Pongmanee |
| Field of Study | Animal Physiology |
| Thesis Advisor | Assistant Professor Kris Angkanaporn, Ph.D. |
| Thesis Co-advisor | Associate Professor Suwanna Kijparkorn, M.S. |

---

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

..... Dean of Faculty of Veterinary Science
(Professor Narongsak Chaiyabutr, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Duangnarumon Prachankhadee, Ph.D.)

..... Thesis Advisor
(Assistant Professor Kris Angkanaporn, Ph.D.)

..... Thesis Co-advisor
(Associate Professor Suwanna Kijparkorn, M.S.)

..... Member
(Associate Professor Wara Panichkriangkrai, Ph.D.)

..... Member
(Assistant Professor Sutthasinee Poonyachoti, Ph.D.)

ขุนพล พงษ์มณี: ผลของใบบัวบกทดแทนยาปฏิชีวนะต่อคุณลักษณะการเจริญเติบโต ปริมาณเอนไซม์จากเซลล์เยื่อบุผนังลำไส้เล็ก และการย่อยได้ของโภชนะในไก่เนื้อ (EFFECTS OF ASIATIC PENNYWORT (*CENTELLA ASIATICA* L. URBAN) LEAVES, A REPLACEMENT OF ANTIBIOTICS, ON GROWTH PERFORMANCE, MUCOSAL ENZYME ACTIVITIES OF THE SMALL INTESTINE AND NUTRIENT DIGESTIBILITY IN BROILER CHICKENS) อ. ที่ปรึกษา: ผศ.น.สพ.ดร. กฤษ อังคนาพร, อ. ที่ปรึกษาร่วม: รศ. สุวรรณา กิจภากรณ์, 65 หน้า. ISBN 974-17-3911-7.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของใบบัวบกทดแทนยาปฏิชีวนะต่อคุณลักษณะการเจริญเติบโต ปริมาณเอนไซม์จากเซลล์เยื่อบุผนังลำไส้เล็ก และการย่อยได้ของโภชนะในไก่เนื้อ การทดลองที่ 1 เป็นการศึกษาเบื้องต้นเพื่อดูผลของใบบัวบกต่อคุณลักษณะการเจริญเติบโต ไก่ทดลอง 432 ตัว ถูกแบ่งเป็น 9 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐาน เป็นกลุ่มควบคุม กลุ่มที่ 2 4 6 8 ได้รับอาหารพื้นฐานที่ผสมผงใบบัวบกในระดับ 1.67 3.33 6.67 10.00 กรัมต่อกิโลกรัมอาหาร กลุ่มที่ 3 5 7 9 ได้รับอาหารพื้นฐานที่ผสมสารสกัดจากผงใบบัวบกในระดับ 0.40 0.80 1.60 2.40 กรัมต่อกิโลกรัมอาหาร ชั่งน้ำหนักไก่ทดลองทุกกลุ่มและบันทึกปริมาณอาหารที่กินในวันที่ 21 และ 42 ของการทดลอง การทดลองที่ 2 ศึกษาผลของบัวบก (ใบและลำต้น) ต่อคุณลักษณะการเจริญเติบโต ปริมาณเอนไซม์จากเซลล์เยื่อบุผนังลำไส้เล็ก และการย่อยได้ของโภชนะ ไก่ทดลอง 792 ตัว ถูกแบ่งเป็น 6 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐาน เป็นกลุ่มควบคุม กลุ่มที่ 2 4 ได้รับอาหารพื้นฐานที่ผสมผงบัวบกในระดับ 20 40 กรัมต่อกิโลกรัมอาหาร กลุ่มที่ 3 5 ได้รับอาหารพื้นฐานที่ผสมสารสกัดบัวบกในระดับ 3.6 7.2 กรัมต่อกิโลกรัมอาหาร กลุ่มที่ 6 ได้รับอาหารพื้นฐานที่ผสมยาปฏิชีวนะในระดับ 2.5 มิลลิกรัมต่อกิโลกรัมอาหาร ชั่งน้ำหนักไก่ทดลองทุกกลุ่มและบันทึกปริมาณอาหารที่กิน สุ่มไก่ทดลองมากลุ่มละ 6 ตัว ในวันที่ 21 และ 42 ของการทดลอง เก็บตัวอย่างอาหารที่ย่อยแล้วในลำไส้เล็กส่วนกลาง ส่วนปลาย และในไส้ตันมาวัดค่าพีเอช เก็บตัวอย่างเซลล์เยื่อบุผนังลำไส้เล็กส่วนกลางมาวัดระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่และเอนไซม์ที่ย่อยเปปไทด์ เก็บตัวอย่างเซลล์เยื่อบุผนังลำไส้เล็กส่วนปลายมาตรวจวัดปริมาณดีเอ็นเอและอาร์เอ็นเอ สุ่มไก่ทดลองกลุ่มละ 12 ตัว ในวันที่ 25 และ 46 ของการทดลอง เก็บตัวอย่างอาหารในลำไส้เล็กส่วนปลายมาตรวจหาค่าการย่อยได้ของโปรตีนและไขมัน

ในการทดลองที่ 1 ช่วง 21 วันแรก พบว่าไก่ทดลองที่ได้รับผงใบบัวบกในระดับ 1.67 และสารสกัดในระดับ 0.40 กรัมต่อกิโลกรัมอาหาร มีน้ำหนักตัวที่เพิ่มขึ้นและการเจริญเติบโตเฉลี่ยต่อวันมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($P<0.05$) อย่างไรก็ตามการเจริญเติบโตตลอด 42 วัน ของทั้ง 2 การทดลอง ในไก่ทดลองทุกกลุ่มไม่มีความแตกต่างกัน ในการทดลองที่ 2 พบว่าไก่ทดลองที่ได้รับบัวบกในรูปผงและสารสกัดทุกระดับมีแนวโน้มว่าค่าพีเอชในลำไส้เล็กส่วนต่างๆ ลดลง แต่ไม่มีผลต่อระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ ในขณะที่ไก่ทดลองที่อายุ 42 วัน ที่ได้รับผงบัวบก 40 กรัมต่อกิโลกรัมอาหาร สารสกัด 7.2 กรัมต่อกิโลกรัมอาหาร และยาปฏิชีวนะ มีระดับเอนไซม์ที่ย่อยเปปไทด์สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($P<0.05$) ส่วนค่าการย่อยได้ในวันที่ 25 พบว่าไก่ทดลองที่ได้รับสารสกัดบัวบกในระดับ 3.6 และ 7.2 กรัมต่อกิโลกรัมอาหาร มีค่าการย่อยได้ของโปรตีนมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($P<0.05$) ในขณะที่ไก่ทดลองที่ได้รับสารสกัดบัวบกในระดับ 3.6 กรัมต่อกิโลกรัมอาหาร มีค่าการย่อยได้ของไขมันมากกว่ากลุ่มอื่นๆ อย่างมีนัยสำคัญ ($P<0.05$) แต่ไม่แตกต่างจากกลุ่มควบคุม นอกจากนี้ยังพบว่าการเสริมบัวบกในอาหารไม่มีผลต่อการเปลี่ยนแปลงระดับของโปรตีน ดีเอ็นเอ และอาร์เอ็นเอ ของเซลล์เยื่อบุผนังลำไส้เล็ก

สรุปได้ว่า คุณลักษณะการเจริญเติบโตของไก่เนื้อในกลุ่มที่มีการเสริมบัวบกลงในอาหารไม่แตกต่างจากกลุ่มที่เสริมยาปฏิชีวนะ อย่างไรก็ตามการเสริมบัวบกมีผลบางส่วนต่อพีเอชในลำไส้ ระดับเอนไซม์ที่ย่อยเปปไทด์ และการย่อยได้ของโภชนะ ดังนั้นจึงต้องมีการศึกษาต่อไปว่าบัวบกสามารถใช้ทดแทนยาปฏิชีวนะในการผลิตไก่เนื้อได้หรือไม่

ภาควิชา สรีรวิทยา
สาขาวิชา สรีรวิทยาการสัตว์
ปีการศึกษา 2546

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

## 4375553531: MAJOR ANIMAL PHYSIOLOGY
KEY WORDS: ASIATIC PENNYWORT/ GROWTH PERFORMANCE/ DNA/ RNA/ MUCOSA/ DIGESTIBILITY/ BROILER

KOONPHOL PONGMANEE: EFFECTS OF ASIATIC PENNYWORT (*CENTELLA ASIATICA* L. URBAN) LEAVES, A REPLACEMENT OF ANTIBIOTICS, ON GROWTH PERFORMANCE, MUCOSAL ENZYME ACTIVITIES OF THE SMALL INTESTINE AND NUTRIENT DIGESTIBILITY IN BROILER CHICKENS. THESIS ADVISOR: ASSISTANT PROFESSOR KRIS ANGKANAPORN, Ph.D., THESIS CO-ADVISOR: ASSOCIATE PROFESSOR SUWANNA KIJPARKORN, M.S. 65 pp. ISBN 974-17-3911-7.

The objectives of this investigation were to study the effect of Asiatic pennywort (*Centella asiatica* L. Urban) leaves, a replacement antibiotics, on growth performance, mucosal enzyme from the small intestine and nutrient digestibility in broilers. In experiment 1, a preliminary study on the effect of Asiatic pennywort leaves on growth performance was examined. Four hundred and thirty two broilers were allocated into 9 treatments: T1, basal diet (control); T2, T4, T6, T8, basal diet plus crude powder of Asiatic pennywort leaves at the level of 1.67, 3.33, 6.67, 10.00 g/kg feed; T3, T5, T7, T9, basal diet plus crude extract of Asiatic pennywort leaves at the level 0.40, 0.80, 1.60, 2.40 g/kg feed. Body weight and feed intake were measured at days 21 and 42 of the experiment. In experiment 2, the effect of Asiatic pennywort (leaves and stem) on growth performance, brush border enzymes from the small intestine and nutrient digestibility were studied. Seven hundred and ninety two broilers were allocated into 6 treatments: T1, basal diet (control): T2, T4, basal diet plus crude powder of Asiatic pennywort at the level 20, 40 g/kg feed; T3, T5, basal diet plus crude extract of Asiatic pennywort 3.6, 7.2 g/kg feed; T6, basal diet plus antibiotic 2.5 ppm. At days 21 and 42 of the experiment, body weight and feed intake were measured, six broilers in each group of each period were randomed selected and the contents of jejunum (J), ileum (I) and caecum (CE) were collected for pH determination. Jejunal mucosa were collected for the determination of disaccharidase and peptide hydrolase activities. Ileal mucosa were collected for the determination of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) concentrations. At days 25 and 46 of the experiment, ileal digesta from twelve broilers in each treatments were randomed collected for protein and fat digestibility.

At day 21 of experiment 1, it was found that the broilers supplemented with 1.67 g/kg feed of crude powder and 0.40 g/kg of crude extract of Asiatic pennywort had significant ($P<0.05$) higher weight gain and average daily gain than those of the control group. However, growth performance determined at day 42 of the experiment were not different among treatment. In the experiment 2, it was found that Asiatic pennywort given regardless of forms and doses tended to decrease pH in various parts of the small intestine, but did not affect the disaccharidase enzyme activities. While, at day 42, it was found that the broilers supplemented with 40 g/kg feed of crude powder, 7.2 g/kg feed of crude extract of Asiatic pennywort and antibiotic had significant ($P<0.05$) higher peptide hydrolase activities than that of the control group. For digestibility at day 25, it was found that the broilers supplemented with 3.6 and 7.2 g/kg feed of crude extract of Asiatic pennywort had significant ($P<0.05$) higher protein digestibility than that of the control group. While, the broilers supplemented with 3.6 g/kg feed of crude extract of Asiatic pennywort had significant ($P<0.05$) higher fat digestibility than those of the other groups, except that of the control group. Moreover, it was found that the Asiatic pennywort supplementation did not affect protein, DNA and RNA concentrations of the small intestine.

In conclusion, growth performance of broilers in Asiatic pennywort supplementation groups were not significantly different from antibiotic group. However, Asiatic pennywort had some effects on intestinal pH, peptide hydrolase activities and nutrients digestibility, Further studied are required to examine whether Asiatic pennywort can be used to replace antibiotic growth promoter in poultry.

Department Physiology Student's signature……

Field of study Animal Physiology Advisor's signature……..……...

Academic year 2003 Co-advisor's signature……..……..……

# ACKNOWLEDGEMENT

I would like to express my deep gratitude to my advisor, Assistant Professor Dr. Kris Angkanaporn and my co-advisor, Associate Professor Suwanna Kijparkorn for their kind advice, guidance, helpful consultation and constant encouragement throughout this study.

My thanks also expressed to the thesis committee for their valuable suggestions; to the teachers of Department of Physiology and Department of Animal Husbandry for their valuable suggestions and helpful consultation.

My sincere and warm appreciation is expressed to Mr. Somchai Pondeenana, Miss. Siripen Komolvanich, Mr. Wanchai Yenpetch, Miss. Petcharat Nampibul, Miss. Sujin Sirisawat and Miss. Kanjana Jantarawiwat for their kind helps, provision of the facilities used in the experimental works and laboratory technical suggestions.

I wish to thank Associate Professor Chaiyo Chaichantippayut of Department of Phamacognosy, Faculty of Phamaceutical Science, Chulalongkorn University for the donation of asiaticoside (active ingredient standard of Asiatic pennywort) and to the Thailand research fund for funding this study.

Finally, I am deeply grateful to my family and my friends for their helps and kind encouragement throughout my study period.

# TABLE OF CONTENTS

Page

# LIST OF TABLES

Table Page

# LIST OF FIGURES

Figure Page

# ABBREVIATION

| | | |
|---|---|---|
| A | = | absorbance |
| ADG | = | average daily gain |
| AGPs | = | antibiotic growth promoters |
| AIA | = | Acid insoluble ash |
| ANOVA | = | Analysis of Variance |
| BSA | = | bovine serum albumin |
| BW | = | body weight |
| C | = | concentration |
| CE | = | caecum |
| cm | = | centimeter |
| Cys | = | cystine |
| DNA | = | deoxyribonucleic acid |
| EU | = | European Union |
| FCR | = | feed conversion ratio |
| g | = | gram |
| h | = | hour |
| I | = | ileum |
| IB | = | infectious bronchitis |
| ID | = | lieal digestibility |
| J | = | jejunum |
| kcal | = | kilocalorie |
| kg | = | kilogram |
| KOH | = | potassium hydroxide |
| L | = | liter |
| m | = | meter |
| ME | = | metabolizable energy |
| Met | = | methionine |
| mg | = | milligram |
| mg% | = | milligram percent |

| | | |
|---|---|---|
| ml | = | milliliter |
| mM | = | millimolar |
| mm | = | millimeter |
| N | = | Normal |
| ND | = | Newcastle disease |
| nm | = | nanometer |
| nmol | = | nanomole |
| No. | = | number |
| ppm | = | part per million |
| RNA | = | ribonucleic acid |
| rpm | = | round per minute |
| SD | = | standard deviation |
| U | = | unit |
| UV | = | ultraviolet |
| VRE | = | vancomycin-resistant enterococci |
| WG | = | weight gain |
| w/v | = | weight per volume |
| μl | = | microliter |
| μmol | = | micromole |

# CHAPTER I

# INTRODUCTION AND AIMS

Many years ago, the antibiotics are regularly administered to pigs, meat chickens, laying chickens, beef cattle, dairy cows and fish. Farm animals are given these antibiotics for two purposes: growth promotion (to make animals grow faster) and disease control and prevention. Antibiotics are widely used in animal farming to control conditions that are crowded, unhealthy and likely to lead to the spread of infections (Parker and Armstrong, 1987). In addition, this helps to minimize labour burden and times on animal management.

At present, there are public concern on using antibiotics in livestock production. Antibiotic growth promoters (AGPs) have led to many problems such as an emergence of bacterial resistant strain and antibiotic residues in animal products. People consumed such meat or products may be sick of these contaminated agents. Because the bacteria are resistant to the current antibiotics, efforts to treat illnesses may be ineffective.

Nowadays, the customers who consume animal products request the animal production with the absence of antibiotic growth promoters, due to some risk to human health for instance enterococci infection due to vancomycin resistant bacterial strain. Many countries are regulating the use of antibiotics in feed or setting up programs to reduce the overall use of antibiotics.

In 1999, the European Union (EU) banned four antibiotic growth promotants, namely virginiamycin, spiramycin, tylosin and zinc bacitracin, which are commonly used in animal feed around the world. The occurrence of vancomycin-resistant enterococci (VRE) in Australia has been linked with the use of glycopeptide avoparcin and consequently, the use of avoparcin and other glycopeptides as growth promotants in animal feed has been discontinued in Australia (Choct, 2001).

In July 4th, 2002, Thailand also bans four antibiotics, Nitrofurazone, Furazolidone, Dimetridazole and Ronidazole sold under 14 different trades in animal production. These antibiotics are carcinogenic substances which are risky for market meat consumption.

Due to these problems of antibiotic, many parts of the world are experimenting with alternative feed additives that may be used to alleviate the problems associated with the withdrawal of antibiotics from feed in livestock production.

Choct (2001) reported that the aid of a number of alternatives including organic acids, prebiotics (mainly oligosaccharide products), probiotics and feed enzymes, not only has the general health status of the poultry in Sweden been maintained, but also the growth rate and feed efficiency improved markedly.

Currently, some countries are interested in searching for new alternative feed additive from natural resources such as plants or herbs which are plentiful and not expensive. Many medicinal plant has been employed since prehistoric times. Those plants are continued to be used within the framework of folk medicine as an effective remedy. Using herbs as a substitute of antibiotics or chemical substances in feed for animal production, is not good only for health of animals and people, but also resulting in the reduction of antibiotics or chemical substances imported. It is a good way to save the money and promote the usage of available natural resources within country.



Asiatic pennywort (*Centella asiatica* L. Urban) is the herb of interest due to its wide range of medical properties and applications reported for treating illnesses or diseases. Many researches indicated that Asiatic pennywort elicited an immunostimulant action in human and mice (Farnsworth and Bunyapraphatsara, 1992; Brinkhaus *et al*., 2000). Moreover, Upadhyay *et al*. (1991) found that there was a significant effect of Asiatic pennywort on antistress activity in rats, which was similar to the work performed by Sarma *et al.* (1996). Furthermore, some researches reported that Asiatic pennywort can stimulate appetite and digestion (Ahmad *et al*., 1998; Brinkhaus

*et al.*, 2000). In addition, Asiatic pennywort has been used to prevent and treat gastric ulcers (Farnsworth and Bunyapraphatsara, 1992; Sarma *et al.*, 1995; Tan *et al.*, 1997; Cheng and Koo, 2000).

However, research involving the effect of Asiatic pennywort are investigated only in mice, rat and human, not in livestock. Therefore this study is the first report on the use of Asiatic pennywort in broilers. It is hypothetized that Asiatic pennywort affects intestinal functions particular in activation of digestive enzymes, alteration of intestinal pH, nutrient digestibility, and mucosal cell proliferation in broilers.

The objectives of the present study were to examine the effects of Asiatic pennywort in the form of crude powder and extract, substituting for antibiotic on growth performance, intestinal pH, peptide hydrolase and disaccharidase activities, intestinal DNA and RNA and ileal digestibility of nutrient of the broilers.

# CHAPTER II

# BACKGROUND INFORMATIONS

## Asiatic Pennywort

Asiatic pennywort is a medicinal plant that has been employed since prehistoric times. The therapeutic use of this herbal remedy with its wide range of applications has been well documented in South East Asia and India for centuries. This plant is continued to be used within the framework of folk medicine as an effective remedy (Brinkhaus *et al*., 2000).

## Names and synonyms

*Centella asiatica* (L.) Urban, a scientific name of Asiatic pennywort, belongs to the family Apiaceae (Umbelliferae) (Table 1). This medicinal plant has many common names such as Asiatic Pennywort or Indian Pennywort (English), Hydrocotyle Asiatique (French), Asiatischer Wassernable and Indischer Wassernable (German), Idrocotyle (Italian), Brahma-manduki and Brahmi-Buti (Hindi), Tsubo-Kusa (Japanese), and Tungchian and Luei Gong Gen (chinese) (Brinkhaus *et al*., 2000).

## Botanical Description

The plant is a perennial herb. The stems are long, creeping and rooting at the nodes. It has a smell that is reminiscent of tobacco leaves, and a mildly bitter taste. The leaves have long petioles arising rosette-like from a common base (the node), and the individual leaf rosettes are connected by slender aerial stolons (Figure 1).

The leaves are thin and soft, with palmate nerves, hairless or with only a few hairs, and measured about 2 to 5 cm in diameter. The leaf margin is crenate or slightly lobed. The petioles are between 5 and 15 cm in length, slender and hairless or bear

only a few scattered hairs. The short-pediceled umbels arise in the leaf axils. The 2 to 5 fruits of each umbel are enclosed within a pericarp comprising 1 to 2 cm-large elliptical bracts (Farnsworth and Bunyapraphatsara, 1992; Brinkhaus *et al.*, 2000)

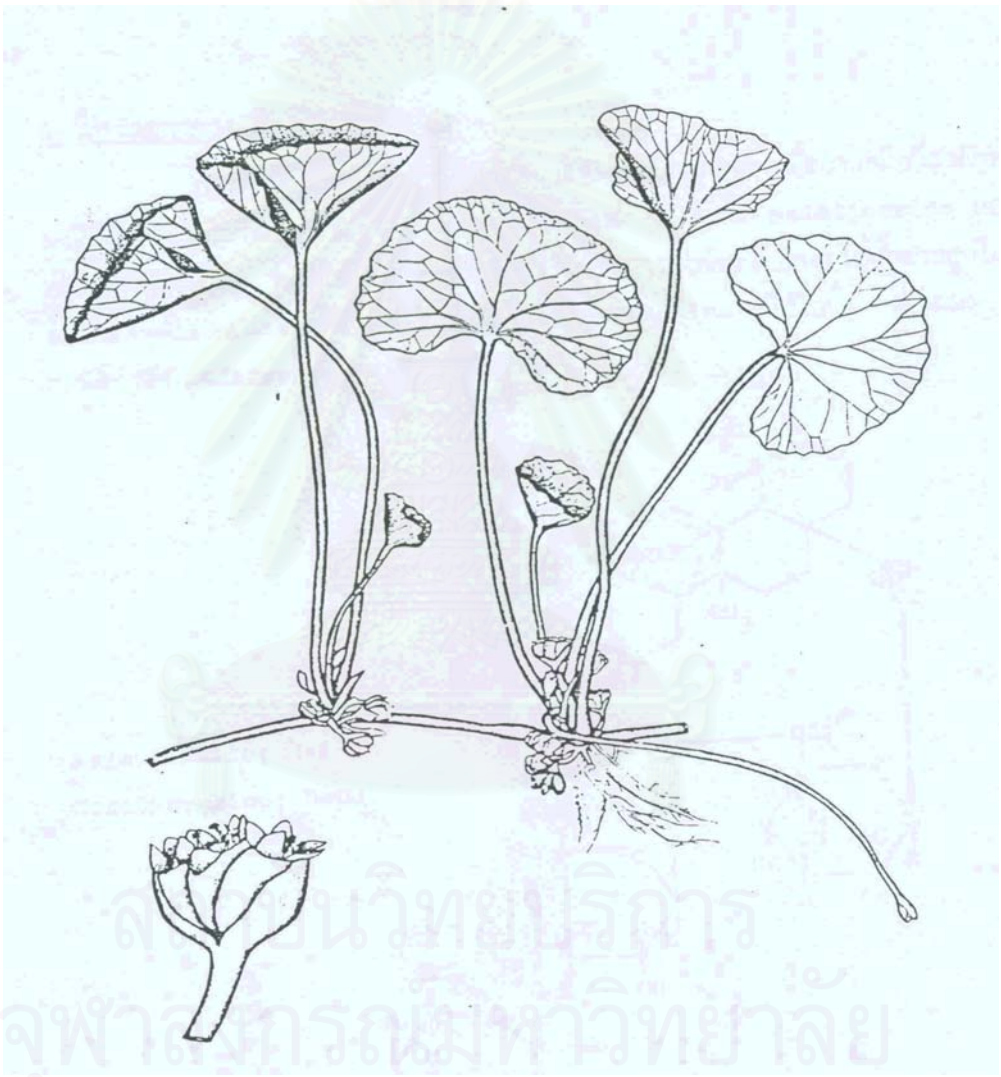


**Figure 1** Asiatic pennywort (*Centella asiatica*).

**Table 1** Systematic classification (Taxonomy) of *Centella asiatica* (Brinkhaus *et al*., 2000).

| Classification | Name |
|---|---|
| Kingdom | Eukaryota |
| Subkingdom | Embryophyta |
| Division | Spermatophyta |
| Subdivision | Angiospermae |
| Class | Dicotyledoneae |
| Subclass | Rosidae |
| Superorder | Aralianae |
| Order | Araliales (Umbelliflorae) |
| Family | Apiaceae or Umbelliferae |
| Subfamily | Hydrocotyle |
| Genus | Centella |
| Species | Centella asiatica |

## Ecology and Distribution

Asiatic pennywort is a tropical weed, found especially in wet places of tropical and subtropical regions (Farnsworth and Bunyapraphatsara, 1992). In addition, the plant is indigenous to the warmer regions of both hemispheres, including Africa, Australia, Cambodia, Central America, China, Columbia, Indonesia, the Lao People's Democratic Republic, Madagascar, Mexico, the Pacific Islands, South America, Thailand, southern United States of America, Venezuela, and Vietnam. It is especially abundant in the swampy areas of India, the Isalamic Republic of Iran, Pakistan, and Sri Lanka up to an altitude of approximately 700 m (Tan *et al*., 1997; Brinkhaus *et al*., 2000; WHO; 1999).

## Using Asiatic pennywort in folk medicine

Asiatic pennywort has been used in the framework of folk medicine for treating albinism, anemia, asthma, bronchitis, cellulite, cholera, measles, constipation, dermatitis, diarrhoea, dizziness, dysentery, dysmenorrhoea, dysuria, epistaxis, epilepsy, haemorrhoids, hepatitis, hypertension, jaundice, leukorhoea, nephritis, nervous disorders, neuralgia, rheumatism, smallpox, syphilis, toothache, urethritis, and varices; and as an antipyretic, analgesic, anti-inflammatory, and “brain tonic” agent. Poultices have been used to treat concussion, closed fractures, sprains, and furunculosis. However, uses described in folk medicine, are not supported by experimental or clinical data (WHO, 1999).

## Chemical constituents

According to many researches concerning chemical constituents of Asiatic pennywort, it was found that saponin glycosides or saponins (also called triterpenoids) are the major chemical constituents (Table 2). Saponins are composed of aglycone (genin or sapogenin) (Table 3) and glycone molecule. Moreover, Asiatic pennywort also contains polyacetylenes, sterols and lipid constituents, nitrogen containing constituents, flavonoids, and miscellaneous compounds. (Singh and Rastogi, 1968; Ramaswamy *et al*., 1970; Srivastava *et al*., 1997).

Although Asiatic pennywort contains a wide range of many substances (Table4), but many researches indicated that the triterpene glycosides sush as asiaticoside, asiatic acid, madecassic acid and madecassoside are the active ingredients of Asiatic pennywort (Figures 2 and 3) (Windholz *et al*., 1983; Conti *et al*., 1992; Marzaeki *et al*., 1994; Brinkhaus *et al*., 2000; Cheng and Koo, 2000; WHO; 1999).

**Table 2** Saponins isolated from *Centella asiatica* (Srivastava *et al*., 1997).

| Saponin | Constituents of saponin | Melting point(M.P.) |
|---|---|---|
| Asiaticoside | Asiatic acid, glucose, rhamnose | 230°-233° |
| Madecassoside | Madecassic acid, glucose, rhamnose | ND |
| Centelloside | Centellic acid, glucose, fructose | ND |
| Brahmoside | Brahmic acid, glucose, rhamnose, arabinose | 242° |
| Brahminoside | Brahmic acid, glucose, rhamnose, arabinose | 223° |
| Thankuniside | Thankunic acid, glucose, rhamnose | 239° |
| Isothankuniside | Isothankunic acid, glucose, rhamnose | 250° |
| Asiaticoside-A | 6β-Hydroxyasiatic acid, glucose, rhamnose | ND |
| Asiaticoside-B | Terminolic acid, glucose, rhamnose | ND |

ND = Not determined

**Table 3** Triterpenic acids isolated from *Centella asiatica* (Srivastava *et al*., 1997).

| Acid | M.P. |
|---|---|
| Asiatic acid | 241° |
| Madasiatic acid | ND |
| Brahmic acid | 293° |
| Isobrahmic acid | 263° |
| Thankunic acids | 314° |
| Isothankunic acid | 288° |
| Betulic acid | 308° |
| Centoic acid | 256°-261° |
| Centellic acid | ND |
| 6β-Hydroxyasiatic acid | 285°-288° |
| Terminolic acid | >300° |

ND = Not determined

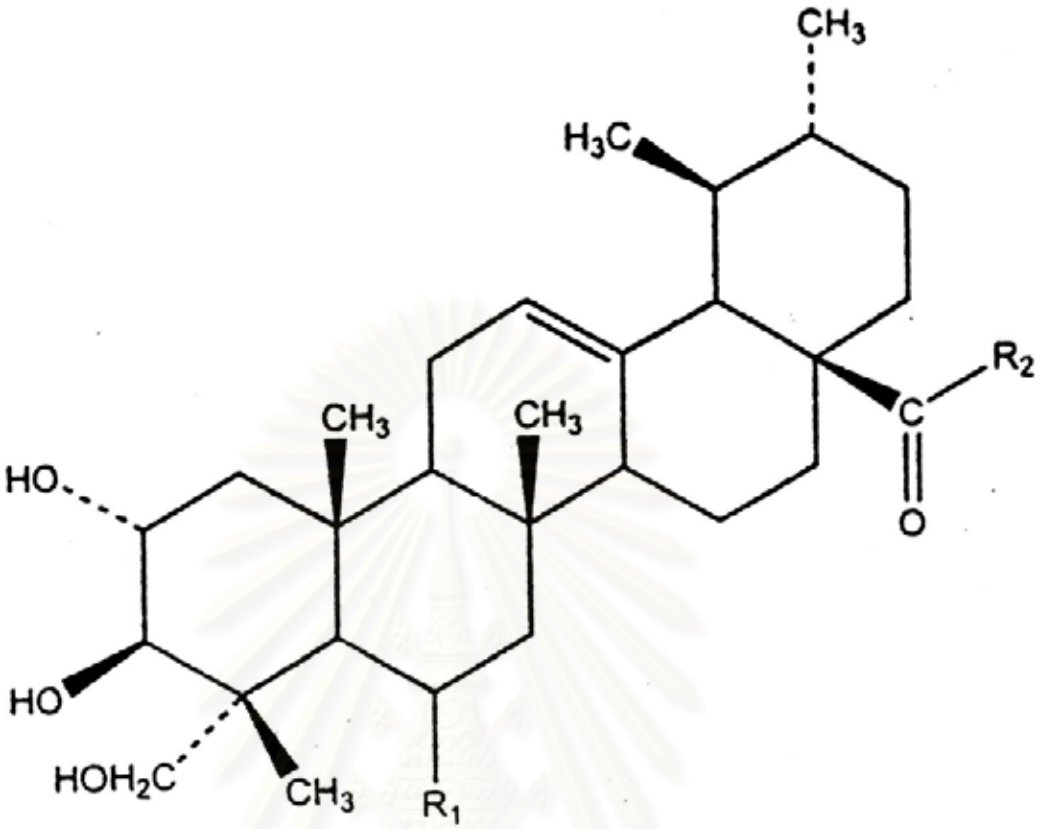
**Table 4** Centella asiatica: Main constituents with differences depending on country or origin and their classification into main groups (Brinkhaus *et al*., 2000).

| Main Groups | Constituents |
|---|---|
| Essential oil | |
| (0.1% of the plant) | Terpene acetate |
| | Germacrene |
| | Caryophyllene |
| | p-Cymol |
| | Pinene |
| Flavone derivatives | Quercetin glycoside |
| | Kaempferol, glycoside and in free form Astragalin |
| Sesquiterpenes | Caryophyllene |
| | Elemene and bicycloelemene |
| | Trans-farnesene |
| | Ermacrene D |
| Triterpenic steroids | Stigmasterol |
| | Sitosterol |
| Triterpenic acids | Asiatic acid |
| | 6-hydroxy asiatic acid |
| | Madecassic acid |
| | Madasiatic acid[1] |
| Triterpenic acid sugar esters | Betulinic acid |
| (= saponins or pseudosaponins) | Thankunic acid[3] |
| (1-8% depending on country or origin) | Isothankunic acid[3] |
| | Asiaticoside (major component) |
| | Asiaticoside A |
| | Asiaticoside B |
| | Asiaticoside A (Madecassoside) and B |
| | Braminoside[2] |
| | Brahmoside[2] |
| | Brahminoside[2] |
| | Thankuniside[3] |
| | Isothankuniside[3] |

[1] *Centella asiatica* from Madagascar

[2] *Centella asiatica* from India

[3] ***Centella asiatica*** **from Northeast India**




R1=H; R2=OH: Asiatic acid

R1=OH; R2=OH: Madecassic acid

R1=H; R2=glu-glu-rhamn: Asiaticoside

**Figure 2** Structure of asiatic acid madecassic acid and asiaticoside in *Centella asiatica* (Cheng and Koo, 2000).

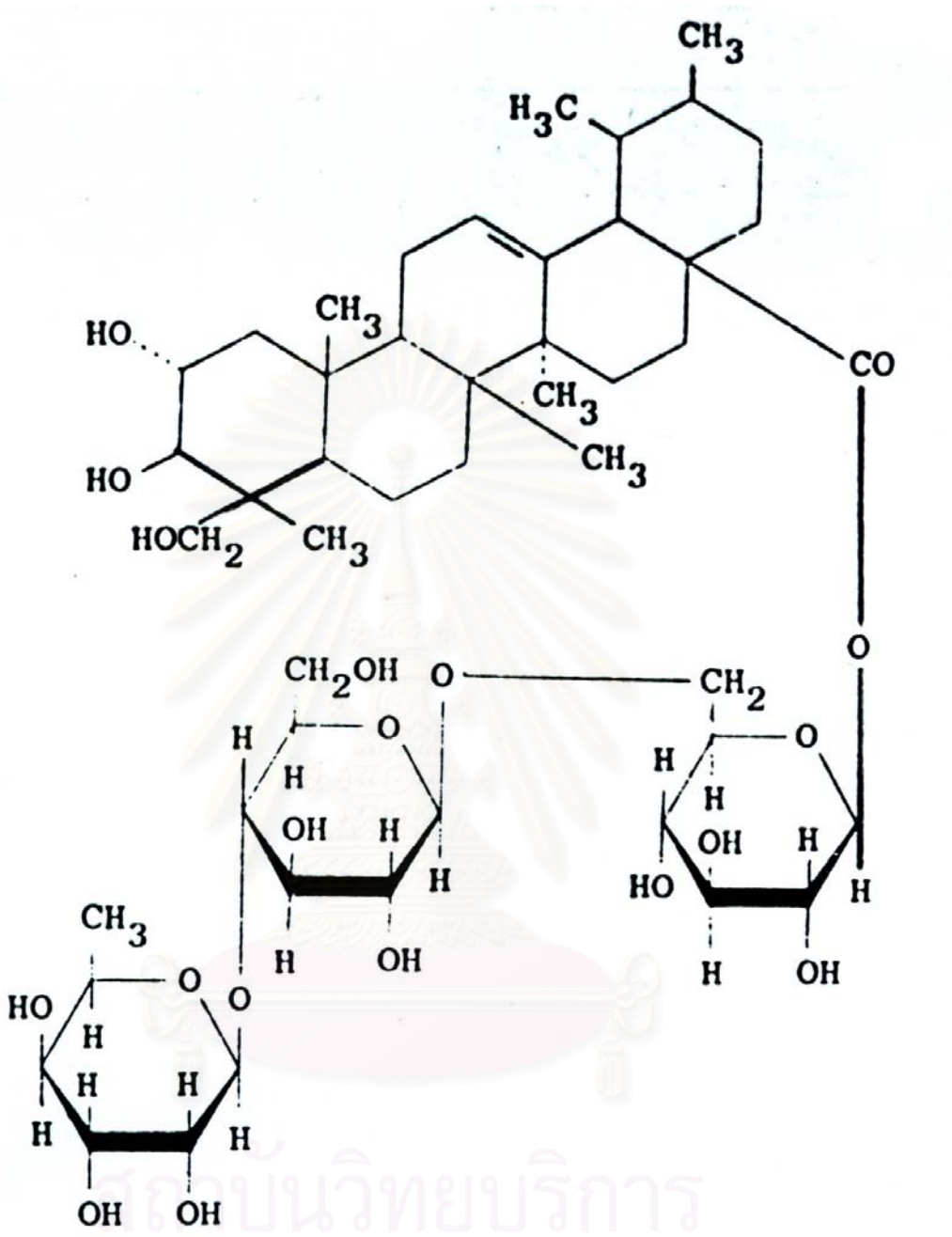



**Figure 3** Structure of asiaticoside, active principle of *Centella asiatica* (Windholz *et al*., 1983).

**Analysis of nutrient composition**

The analysis of Asiatic pennywort shows that 100 g of the leaves contain 34 kilocalories, 89.3 g water, 1.6 g protein, 0.6 g fat, 6.9 g carbohydrates, 2 g fiber, 1.6 g ash, 170 mg Ca, 30 mg P, 3.1 mg Fe, 414 mg K, 6.58 mg beta carotene, 0.15 mg thiamine, 0.14 mg riboflavin, 1.2 mg niacin, and 4 mg ascorbic acid (Brinkhaus *et al*., 2000). In addition, information from WHO (1999) reported that *Centella asiatica* has foreign organic matter not more than 2%, total ash not more than 19%, acid-insoluble ash not less than 6%, water-soluble extractive not less than 6%, alcohol-soluble extractive not less than 9.5%, and contains not less than 2% triterpene ester glycosides (asiaticoside and madecassoside).

The leaves of Asiatic pennywort, contain varieties of amino acids; alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and tyrosine (Srivastava *et al*., 1997; Brinkhaus *et al*., 2000).

**Effect of *Centella asiatica* on antistress activity**

Upadhyay *et al*. (1991) studied the effect of defatted alcoholic extract of *Centella asiatica* from the Mauritian and the Indian on antistress activity in male adult albino rats (Charles Forter strain). Results showed that the adrenocortical response to immobilization stress in Mauritian and Indian groups was significant when compared with control group. The plasma corticosterone levels were lower in Mauritian group than Indian group. They also found that the total glycoside content of *Centella asiatica* of Mauritian was higher than the Indian which is due to the environmental difference condition, especially the salinity of the soil.

Sarma *et al.* (1996) studied the effect of defatted ethanol 95% extracts of *Centella asiatica* on antistress activity in inbred male adult albino rats of the Charles Foster Strain. The result showed that this plant extract significantly reduced the stress-induced rise in circulating corticosterone levels in rats, thus preventing the activation of hypothalamo-adrenal-pituitary axis. Moreover, they found that the treated animals showed a near normal adrenal weight while the animals in restraint group had higher adrenal weight than that of the control group.

**Effect on gastrointestinal diseases**

In the area of folk medicine (Ayurveda) in India, *Centella asiatica* is continued to be a constituent of the Ayurveda diet used to treat dysentery, diarrhoea, colicky abdominal pain and indigestion. In addition, traditional healers in Indonesia also use the medicinal plant to treat gastric ulcers, gastritis and inflammatory diseases of the liver. In the northern Thailand and the Fiji Islands, this plant has been known as a tonic for use in diarrhoea. Furthermore, Asiatic pennywort was also used to stimulate appetite (Brinkhaus *et al*., 2000).

**Toxicology and tolerance**

In experimental animal studies (in the mouse), acute toxicity of Asiatic pennywort was not shown when 1 g/kg BW of an ethanolic 50% extract of *Centella asiatica* was administered. In the case of intramuscular injection in mice and rabbits, the toxic dose was approximately 40 to 50 mg/kg BW. For intraperitoneal injection, the maximum tolerated dose (MTD) in mice was 250 mg/kg BW (Farnsworth and Bunyapraphatsara, 1992; Brinkhaus *et al*., 2000).

# CHAPTER III

# MATERIALS AND METHODS

## Animals and management

A total of 1224, one day-old, mixed sex, Arbor Acre broilers were used in two experiments.

In experiment 1, 432 chicks were divided into 9 treatment groups (four replicates of twelve chicks each). Total body weights of all chicks in each group were similar. The chicks were raised on litter floor pens (the size of pen was 1.00 x 1.25 m per 12 chicks). All chicks received Newcastle-bronchitis vaccine (B1 Type, B1 Strain, Mass. and Conn. Types, Live virus) and infectious bursal disease vaccine (live virus) at day 7 and day 14, respectively.

The range of the temperature and relative humidity in the morning was at 29.06$\pm$1.76°C and 75.96$\pm$8.59%, respectively, and at noon was at 32.03$\pm$1.88°C and 64.06$\pm$9.18%, respectively.

In experiment 2, 792 chicks were divided into 6 treatment groups (six replicates of twenty-two chicks each). Total body weight of all chicks in each group were similar. The chicks were raised on litter floor pens (the size of pen was 1.45 x 1.90 m per 22 birds). All chicks received Newcastle-bronchitis vaccine (TAD IB/ND vac, $10^9$ $EID_{50}$ ND La Sota virus and $10^6$ $EID_{50}$ IB H 120 virus, Live virus) and infectious bursal disease vaccine (Izovac GUMBORO 3, virus strain Winterfield 2512/90: 10/2.7 EID-50) at day 7 and day 14, respectively.

The range of the temperature and relative humidity in the morning was at 24.20$\pm$2.25°C and 80.60$\pm$9.28%, respectively, and at noon was at 31.08$\pm$1.85°C and 56.09$\pm$2.67%, respectively.

Both experiments 1 and 2, all diets were given *ad libitum* and the chicks had free access to water for the duration of the experiment.

**Raw materials**

In experiment 1, 410 kg of fresh whole plant of Asiatic pennywort (*Centella asiatica* L.) were obtained from vegetable market in Bangkok between March-May 2002. After washing, leaves were cut and used to prepare leave powder and leave powder extract. Total 182.70 kg fresh leaves of Asiatic pennywort were obtained from 410 kg whole plant of Asiatic pennywort.

In experiment 2, 700 kg of fresh whole plant of Asiatic pennywort (*Centella asiatica* L.) were obtained from Ban Naung Sur-ah in Nakorn Pathom between October-November 2002. After washing, the whole plant of Asiatic pennywort were prepared to be used in the form of powder and powder extract.

**Preparation of Asiatic pennywort powder**

In experiment 1, fresh leaves of Asiatic pennywort were used in this experiment. The fresh leaves were dried by oven at 50°C for 24 h. They were milled to pass a 2 mm screen by Cutting Mill machine. Total 15.31 kg crude powder of Asiatic pennywort were obtained from total fresh leaves.

In experiment 2, fresh whole plant of Asiatic pennywort were used in this experiment. The preparation of Asiatic pennywort powder were similar to that in the experiment 1. Total 69.48 kg crude powder of Asiatic pennywort were obtained from fresh whole plant of Asiatic pennywort.

**Preparation of Asiatic pennywort powder extract**

In experiment 1, 5.81 kg of Asiatic pennywort leave powder in 58.10 L of 50% ethanol (1:10 w/v) were sonicated for 30 min and macerated at room temperature for 72 h. The extract was hand squeezed through a thin cloth and filtered pass Whatman filter paper No. 1, respectively. The filtrate was evaporated to remove ethanol, then kept frozen at –70°C and freeze-dried by lyophilizer (Labconco®, Kansas, USA) at –60°C. Total 1.39 kg crude extract of Asiatic pennywort were obtain from 5.81 kg of Asiatic pennywort leave powder.

In experiment 2, 34.33 kg of whole plant of Asiatic pennywort powder in 343.30 L of 50% ethanol (1:10 w/v) were macerated at room temperature for 72 h. The extract was hand squeezed through a thin cloth and filtered pass Whatman filter paper No. 1, respectively. The filtrate was evaporated to remove ethanol, then were dried in the oven at 50°C for 24 h. Total 6.11 kg crude extract of Asiatic pennywort were obtain from 34.33 kg whole plant of Asiatic pennywort powder.

**Diets**

Diets used in both experiments 1 and 2 were commercial corn-soybean meal based diet. Asiatic pennywort was included with the expense of rice bran in diet. The ingredients and composition of the basal diet are shown in Table 5. Experimental diets and Asiatic pennywort in both experiments were proximately analyzed (AOAC, 1990). The proximate analysis of Asiatic pennywort in both experiments are shown in Table 6.

In experiment 1, the nine treatments were randomly assigned to four replicates each according to the experimental design (Table 7). In experiment 2, the ingredients and composition of the treatments are shown in Table 8-9. The six treatments were randomly assigned to six replicates each according to the experimental design (Table 10). At days 21 to 25 and days 42 to 46, Celite, a source of acid-insoluble ash (AIA) was added to all diets (20 g/kg feed) as an indigestible dietary marker.

**Table 5** Ingredients and composition of the basal diet.

| Ingredient | Amount (kg/Ton diet) | |
|---|---|---|
| | Starter period (0-3 week) | Grower period (3-6 week) |
| Corn | 540.05 | 632.88 |
| Soybean meal | 350.88 | 224.14 |
| Fish meal | 26.90 | 57.62 |
| Palm oil | 35.00 | 35.00 |
| Corn gluten | 4.58 | 13.42 |
| Rice bran | 10.00 | 10.00 |
| Calcium carbonate | 10.99 | 8.40 |
| Mono-dicalcium phosphate (Hyperphos) | 6.63 | 3.08 |
| Salt | 2.44 | 2.70 |
| DL-methionine | 2.53 | 1.85 |
| L-lysine | 0.00 | 0.91 |
| Premix | 10.00[1] | 10.00[2] |
| Calculated analysis | | |
| Dry matter | 87.04 | 86.98 |
| Crude protein | 21.50 | 19.00 |
| Lysine | 1.18 | 1.07 |
| Met+Cys | 0.93 | 0.82 |
| Threonine | 0.82 | 0.73 |
| Tryptophan | 0.25 | 0.20 |
| Crude fat | 6.35 | 6.84 |
| Crude fiber | 3.76 | 3.27 |
| Ash | 5.48 | 5.07 |
| Calcium | 0.95 | 0.95 |
| Available phosphorus | 0.44 | 0.45 |
| Salt | 0.30 | 0.40 |
| ME (kcal/kg) | 3100.00 | 3200.00 |

[1]Supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 3,000 IU; α-tocopherol acetate, 15 mg; menadione,1.5 mg; thiamine, 1.5 mg; riboflavin, 5.5 mg; pyridoxine, 2.0 mg; cyanocobalamin, 0.01 mg; nicotinic acid, 25 mg; D-calcium pantothenate, 12 mg; folic acid, 0.5 mg; biotin, 0.01 mg; choline chloride, 250 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 8 mg; I, 0.5 mg; Co, 0.1 mg; Se, 0.1 mg.

[2]Supplied per kilogram of diet: retinol, 10,000 IU; cholecalciferol, 2,400 IU; α-tocopherol acetate, 12 mg; menadione,1.2 mg; thiamine, 1.2 mg; riboflavin, 4.4 mg; pyridoxine, 1.6 mg; cyanocobalamin, 0.01 mg; nicotinic acid, 20 mg; D-calcium pantothenate, 10 mg; folic acid, 0.4 mg; biotin, 0.01 mg; choline chloride, 250 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 8 mg; I, 0.5 mg; Co, 0.1 mg; Se, 0.1 mg.

**Table 6** The proximate analysis of Asiatic pennywort in both experiments.

| Proximate analysis | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | Crude powder | Crude extract | Crude powder | Crude extract |
| Dry matter | 93.00 | 94.61 | 94.83 | 93.26 |
| Crude protein | 24.92 | 18.92 | 19.46 | 15.51 |
| Crude fat | 3.31 | 4.18 | 2.57 | 0.55 |
| Crude fiber | 10.00 | 0.12 | 13.44 | 0.17 |
| Ash | ND | ND | 11.65 | 21.20 |
| Calcium | ND | ND | 0.74 | 0.13 |
| Phosphorus | ND | ND | 0.51 | 0.35 |
| Asiaticoside (%)* | 0.57 | 6.50 | 0.04 | 2.69 |
| Bulk density(g/ml) | 0.22 | 0.77 | 0.24 | 0.70 |

ND = not determined

*Asiaticoside concentrations were determined using High Performance Liquid Chromatography (HPLC).

**Table 7** The description of the treatments in experiment 1.

| Treatments | Description |
|---|---|
| 1. Control (T1) | Basal diet without antibiotic |
| 2. Crude powder 1 (T2) | Basal diet + Asiatic pennywort crude powder 1.67 g/kg feed |
| 3. Crude extract 1 (T3) | Basal diet + Asiatic pennywort crude extract 0.40 g/kg feed |
| 4. Crude powder 2 (T4) | Basal diet + Asiatic pennywort crude powder 3.33 g/kg feed |
| 5. Crude extract 2 (T5) | Basal diet + Asiatic pennywort crude extract 0.80 g/kg feed |
| 6. Crude powder 3 (T6) | Basal diet + Asiatic pennywort crude powder 6.67 g/kg feed |
| 7. Crude extract 3 (T7) | Basal diet + Asiatic pennywort crude extract 1.60 g/kg feed |
| 8. Crude powder 4 (T8) | Basal diet + Asiatic pennywort crude powder 10.00 g/kg feed |
| 9. Crude extract 4 (T9) | Basal diet + Asiatic pennywort crude extract 2.40 g/kg feed |

**Table 8** Ingredients and composition of the experimental diet in the starter period (Experiment 2).

| Ingredient | Amount (kg/Ton) | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Corn | 540.05 | 540.05 | 540.05 | 540.05 | 540.05 | 540.05 |
| Soybean meal | 350.88 | 350.88 | 350.88 | 350.88 | 350.88 | 350.88 |
| Fish meal | 26.90 | 26.90 | 26.90 | 26.90 | 26.90 | 26.90 |
| Palm oil | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Corn gluten | 4.58 | 4.58 | 4.58 | 4.58 | 4.58 | 4.58 |
| Rice bran | 10.0000 | 8.0000 | 9.6400 | 6.0000 | 9.2800 | 9.9975 |
| Asiatic pennywort | 0.00 | 2.00 | 0.36 | 4.00 | 0.72 | 0.00 |
| Antibiotic (avilamycin) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0025 |
| Calcium carbonate | 10.99 | 10.99 | 10.99 | 10.99 | 10.99 | 10.99 |
| Mono-dicalcium phosphate (Hyperphos) | 6.63 | 6.63 | 6.63 | 6.63 | 6.63 | 6.63 |
| Salt | 2.44 | 2.44 | 2.44 | 2.44 | 2.44 | 2.44 |
| DL-methionine | 2.53 | 2.53 | 2.53 | 2.53 | 2.53 | 2.53 |
| Premix | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Proximate analysis | | | | | | |
| Dry matter | 90.88 | 90.92 | 90.59 | 90.74 | 90.90 | 90.76 |
| Crude protein | 21.61 | 21.55 | 21.62 | 21.82 | 22.01 | 21.83 |
| Crude fat | 7.02 | 7.25 | 6.95 | 6.98 | 7.11 | 7.18 |
| Crude fiber | 2.74 | 2.80 | 2.52 | 2.66 | 2.30 | 2.73 |
| Ash | 5.64 | 5.68 | 5.88 | 5.47 | 5.64 | 5.66 |
| Calcium | 0.80 | 0.83 | 0.86 | 0.88 | 0.85 | 0.84 |
| Phosphorus | 0.89 | 0.83 | 0.86 | 0.88 | 0.83 | 0.84 |
| Gross energy (kcal/kg) | 4120.35 | 4157.65 | 4106.00 | 4079.65 | 4101.30 | 4125.90 |

**Table 9** Ingredients and composition of the experimental diets in the grower period (experiment 2).

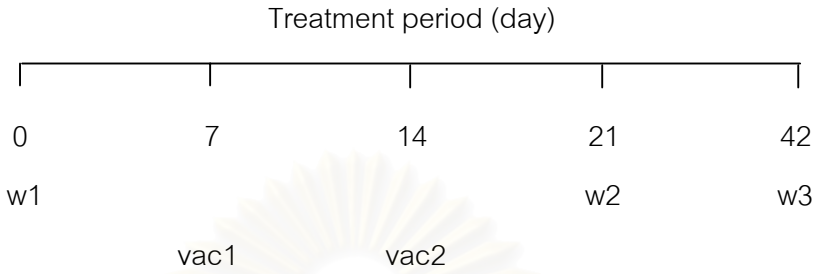
| Ingredient | Amount (kg/Ton) | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Corn | 632.88 | 632.88 | 632.88 | 632.88 | 632.88 | 632.88 |
| Soybean meal | 224.14 | 224.14 | 224.14 | 224.14 | 224.14 | 224.14 |
| Fish meal | 57.62 | 57.62 | 57.62 | 57.62 | 57.62 | 57.62 |
| Palm oil | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Corn gluten | 13.42 | 13.42 | 13.42 | 13.42 | 13.42 | 13.42 |
| Rice bran | 10.0000 | 8.0000 | 9.6400 | 6.0000 | 9.2800 | 9.9975 |
| Asiatic pennywort | 0.00 | 2.00 | 0.36 | 4.00 | 0.72 | 0.00 |
| Antibiotic (avilamycin) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0025 |
| Calcium carbonate | 8.40 | 8.40 | 8.40 | 8.40 | 8.40 | 8.40 |
| Mono-dicalcium phosphate (Hyperphos) | 3.08 | 3.08 | 3.08 | 3.08 | 3.08 | 3.08 |
| Salt | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 |
| DL-methionine | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 |
| L-lysine | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 |
| Premix | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Proximate analysis | | | | | | |
| Dry matter | 92.75 | 92.91 | 92.73 | 92.93 | 92.88 | 92.90 |
| Crude protein | 19.23 | 19.81 | 19.90 | 19.91 | 19.83 | 19.86 |
| Crude fat | 6.11 | 6.09 | 6.01 | 6.03 | 6.27 | 6.21 |
| Crude fiber | 2.18 | 2.56 | 2.14 | 2.28 | 2.08 | 2.31 |
| Ash | 4.75 | 4.87 | 4.62 | 4.88 | 4.78 | 4.71 |
| Calcium | 0.73 | 0.72 | 0.70 | 0.74 | 0.78 | 0.74 |
| Phosphorus | 0.86 | 0.87 | 0.87 | 0.82 | 0.85 | 0.85 |
| Gross energy (kcal/kg) | 4120.65 | 4094.05 | 4105.95 | 4048.40 | 4107.70 | 4105.25 |

**Table 10** The description of the treatments in experiment 2.

| Treatments | Description |
|---|---|
| 1. Negative control (T1) | Basal diet without antibiotic |
| 2. Crude powder 1 (T2) | Basal diet + Asiatic pennywort crude powder 20 g/kg feed |
| 3. Crude extract 1 (T3) | Basal diet + Asiatic pennywort crude extract 3.6 g/kg feed |
| 4. Crude powder 2 (T4) | Basal diet + Asiatic pennywort crude powder 40 g/kg feed |
| 5. Crude extract 2 (T5) | Basal diet + Asiatic pennywort crude extract 7.2 g/kg feed |
| 6. Positive control (T6) | Basal diet + antibiotic (Avilamycin) 2.5 ppm |

**Protocol of the experiment**

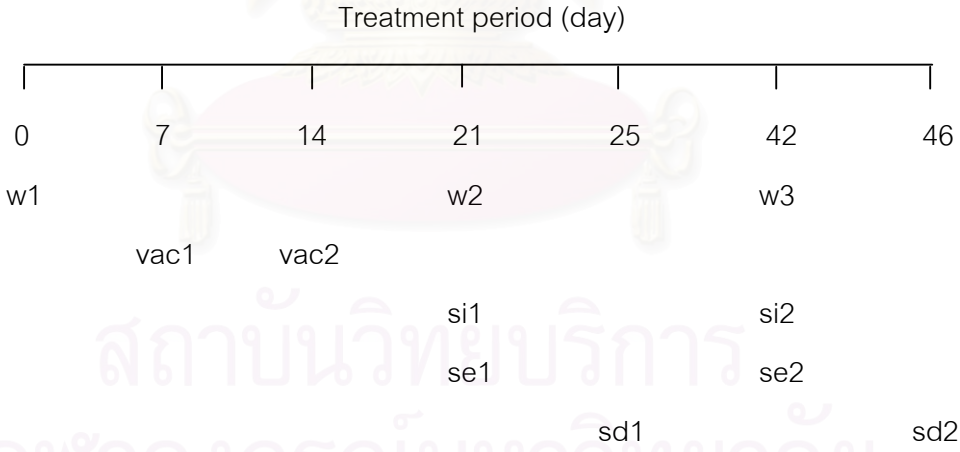
**Experiment 1.**




w = weighing (body weight and feed), vac = vaccination

**Figure 4** Diagram of the experiment 1.

**Experiment 2.**




w = weighing (body weight and feed), vac = vaccination, si = sampling for intestinal contents, se = sampling for enzymes activities, sd = sampling for digestibility

**Figure 5** Diagram of the experiment 2.

**Sample collection and Tissue preparation**

In experiment 1, there was no sample collected because only growth performance was studied.

In experiment 2, six broilers in each treatment group at 21 and 42 days old were randomly selected. They were sacrificed with intracardiac injection of pentobarbital sodium (120 mg/kg BW) using 21G, 1.5 inch needle. The abdomen was exposed and the whole intestine from duodenum to cloaca was removed. The intestinal section from the entry of pancreatic and bile duct to a section at Meckel's diverticulum was taken as the jejunal (J) part. The ileal (I) part was taken from Meckel's diverticulum to the ileocaecal junction (Figure 6). The contents of J, I and caecum (CE) were collected by gentle squeezing into plastic bottles. The pH of the contents were immediately measured using the pH meter (ORION, model 420A). The jejunal part and the ileal part were opened longitudinally, rinsed with ice cold saline and placed on a foam pad. Mucosal samples were scraped from the mucosa layer using a glass slide, wrapped in thin foil and stored at –70°C until analysis. Jejunal mucosal scrapings were analyzed for the peptide hydrolase activities (Nicholson and Kim, 1975) and disaccharidase activities (Dahlquist, 1968). Ileal mucosal scrapings were analyzed for the DNA concentration (Burton, 1956; Giles and Myers, 1965), the RNA concentration (Flek and Begg, 1954) and the ratios of RNA: DNA, RNA: protein and protein: DNA (Berseth *et al*., 1983; Simmen *et al*., 1990).

In addition, twelve broilers from each treatment group at 21 and 42 days old were randomly selected. These broilers were fed on diet containing Celite as an indigestible marker. They were sacrificed with intracardiac injection of pentobarbital sodium (120 mg/kg BW) using 21G, 1.5 inch needle. The abdomen was exposed and the ileum was removed. The contents in the ileum were collected by gentle squeezing into plastic bottles. The ileal contents from chickens in each replicate were pooled together due to the small amount of the contents. The ileal contents were kept frozen at –20°C until analysis of nutrient digestibility.

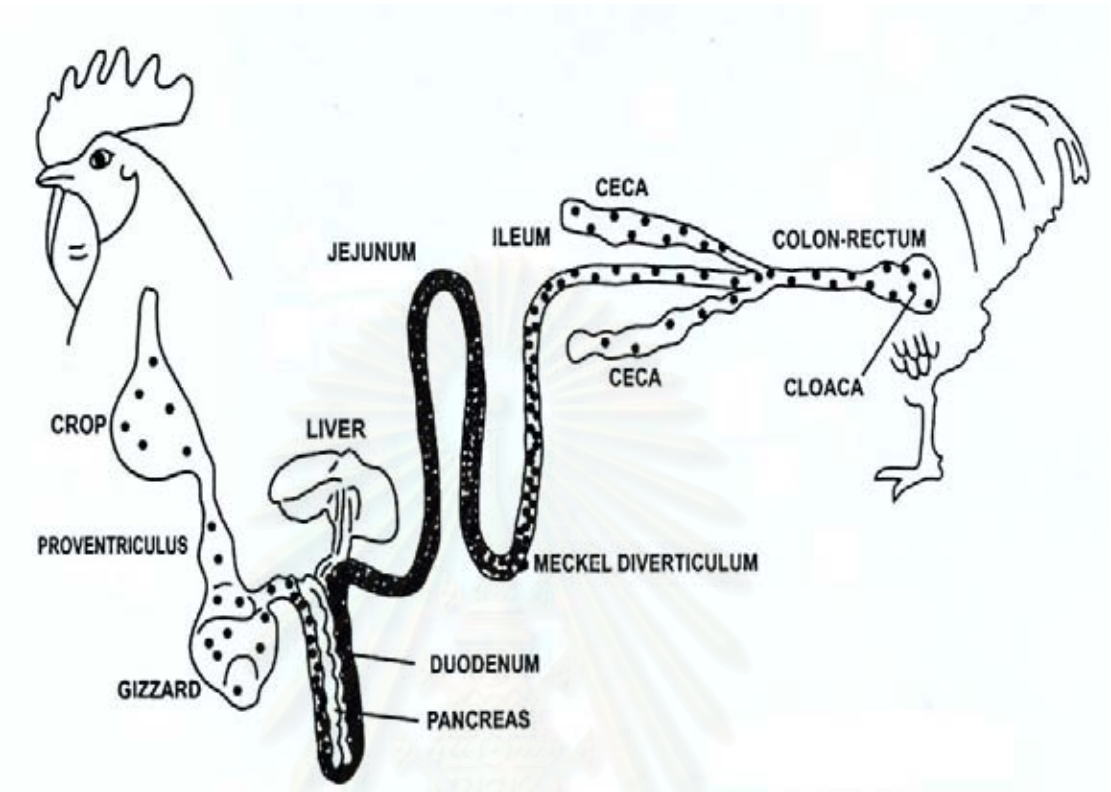



Figure 6 The alimentary canal of chicken (Gauthier, 2002).

## Determination of brush border protein concentrations

### Tissue preparation

Total protein concentrations in jejunal and ileal mucosa were determined using Lowry method (1951). Jejunal mucosal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of distilled water weight by volume (w/v). Then the homogenated samples were diluted 40 times. Ileal mucosal scrapings were homogenized with twenty parts of distilled water weight by volume (w/v). Then the homogenated samples were diluted 10 times.

### Protein concentration assay procedure

The test tubes containing 100 μl of the homogenated samples were added with 3.0 ml of fresh reagent[1], allowed to settle at room temperature for 10 min. Folin reagent (300 μl) was added into the solution, left for 30 min at room temperature. The optical density was read at the wavelength at 650 nm against blank using UV-VIS spectrophotometer (Shimudsu UV 1201) (1 cm light path).

### Standard solution

The standard curve was plotted using the bovine serum albumin (BSA) at 0, 20, 40, 60, 80 and 100 mg%. The slope of the curve was used to calculate the concentrations of protein in the jejunal and ileal mucosa.

[1]



---

[1] Fresh reagent consisting of 50 ml of 2% $Na_2CO_3$ in 0.1 M NaOH, 0.5 ml of 1% $CuSO_4 \bullet 5H_2O$, and 0.5 ml of 2% Na-tartrate.

**Determination of one step peptide hydrolase activities**

**Tissue preparation**

Mucosal scrapings from the jejunum of 72 male broilers were used as the source of enzyme. Mucosal jejunal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with nine parts of 14% glycerol (w/v) and diluted 30 times for enzyme preparation.

*The one step assay procedure*

The peptide substrate, glycyl-L-phenylalanine 5 μmoles was dissolved in 0.5 ml of 50 mM Tris-HCl buffer, pH 8.0. This solution was added with 25 μl of enzyme preparation and 1.0 ml of L-amino acid oxidase reagent (LAOR). Then the solution was mixed and placed in water bath at 37°C for 20 min, 0.74 ml of 50% sulfuric acid was added to stop the reaction. The added sulfuric acid was found to produce the optimal color development. Absorbance of the purple color produced was measured at the wavelength at 530 nm using UV-VIS spectrophotometer (Shimudzu UV 1201) (1 cm light path). Readings were made against a reagent blank consisting of 0.5 ml of 50 mM Tris-HCl buffer, 25 μl of 14% glycerol (the enzyme diluent), 1.0 ml of LAOR, and 0.74 ml of 50% sulfuric acid.

**Standard solution**

For routine analyses of enzyme activities against glycyl-L-phenylalanine, the standard curve was plotted using L-phenylalanine at 0, 20, 40, 60, 80, and 100 nmoles dissolved in 0.5 ml of 50 mM Tris-HCl buffer, 25 μl of 14% glycerol, and 1.0 ml of LAOR, incubated in water bath at 37°C for 20 min before addition of sulfuric acid. The slope of the curve was used for the calculation of phenylalanine concentrations .

### Determination of intestinal disaccharidase activities

#### Tissue preparation

Mucosal scrapings from the jejunum of male broilers were used as the sources of enzyme. Mucosal jejunal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of distilled water weight by volume (w/v). The mucosa and water were well chilled with crushed ice for at least 5 min before and during homogenization. The samples were centrifuged at 3,000 rpm (Centrifuge, GLC-2B, SoRVALL®) for 10 min to remove the large debris of the cells.

#### Disaccharidase activities assay procedure

An aliquot of the sample was adjusted with different dilution factors for the appropriate disaccharidase activities. Suitable dilutions of the different activities were maltase 1: 1,000 and sucrase 1: 100. The test tubes containing 10 μl of the diluted enzyme solution were placed in a water bath at 37°C for 3 min. Then 10 μl of the substrate buffer solution (maltose or sucrose) was added and mixed. After incubated in 37°C for 60 min, 1,000 μl of enzyme reagent (Glucose liquicolor, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany) was added and mixed. These tubes were placed in a water bath at 37°C for 5 min. The optical density was read at the wavelength at 500 nm using UV-VIS spectrophotometer (Shimudsu UV 1201) (1 cm light path). Reading were made against the reagent blank consisting of 20 μl of distilled water and enzyme reagent.

#### Standard solution

For routine analyses of disaccharidase activities, the standard curve was plot using the glucose at 0, 10, 20 and 30 mg%. The slope of the curve was used for calculating the concentration of glucose.

Results were expressed as specific activity (units per gram brush border protein). One unit (U) was defined as the activity that hydrolyzed 1 micromole (μmole) of the substrate per minute under the experiment condition. The disaccharidase activity was obtained by the following formular:

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

where a = microgram of glucose liberated in 60 minutes

d = dilution factor for enzyme solution

n = number of glucose molecules per molecule of disaccharide (for maltase, n=2 and sucrase, n=1)

**Determination of tissue deoxyribonucleic acid (DNA)**

**Tissue preparation: extraction of DNA from tissue**

Ileal mucosa were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with 2.5 N $HClO_4$ to the final concentration of 0.25 N $HClO_4$ and centrifuged at 3000 rpm (Centrifuge, GLC-2B, SoRVALL®) for 10 min after chilling for 30 min. The precipitate is broken up with a glass rod and stirred with 0.5 ml of 0.5 N $HClO_4$. A further 3.5 ml of 0.5 N $HClO_4$ was added and the suspension was heated at 70°C for 15 min with occasional stirring. After centrifuging, the supernatant is decanted into a 10 ml of graduated tube. The precipitate was re-extracted in the same way with a further 3 ml of 0.5 N $HClO_4$, the two extracts were mixed and the volume was measured. A portion of extract was used for the diphenylamine reaction (Burton, 1956).

**DNA concentration assay procedure**

The diphenylamine reaction is created by adding 2 ml of 4% diphenylamine in glacial acetic acid to 2 ml of the test solution (extract of DNA from ileal mucosa) followed by 0.1 ml of aqueous 1.6 mg/ml acetaldehyde. After incubation at 30°C overnight, the optical density difference at the wavelength at 595-700 nm (spectrophotometer, Shimudsu UV-160 A, double beam) were read against the blank (Giles and Myers, 1965).

**Standard solution**

The calf thymus DNA were used as the standard DNA. A stock solution was prepared by dissolving 0.4 mg/ml DNA in 5 mM of NaOH. From this solution, working standard were prepared every 3 weeks by mixing a measured volume of the stock standard solution with an equal volume of 1 N $HClO_4$ and heating at 70°C for 15 min. Both standard solution were stored in the refrigerator.

**Determination of tissue ribonucleic acid (RNA)**

Five milliliters of 105-fold homogenate of ileal mucosa were transferred to a centrifuge tube and 2.5 ml of iced cold of 0.6 N $HClO_4$ added. After mixing the solution and allowing to stand 10 min at 10°C, the precipitate was centrifuged at 3,000 rpm (Centrifuge, GLC-2B, SoRVALL®) for 10 min and washed twice with 5 ml of cold 0.2 N $HClO_4$. Excess acid was drained off, 4 ml of 0.3 N KOH added, and the mixture incubated at 37°C for 1 h. The alkaline solution was cooled in ice and 2.5 ml of cold 1.2 N $HClO_4$ added. After allowing the precipitate to flocculate, it was centrifuged down at 3000 rpm for 10 min and washed twice with cold 0.2 N $HClO_4$. Supernatant was made up to a suitable volume in 0.1 N $HClO_4$ for absorbancy measurements using ultraviolet absorption (spectrophotometer, Shimudsu UV-160 A, double beam). Selecting the two wavelengths 260 nm and 232 nm for ileal mucosa RNA, the result were determined in terms of μg RNA per ml solution.

$$C_{RNA} = 3.40\ A\ 260\ nm - 1.44\ A\ 232\ nm$$

**Determination of nutrients digestibility**

**Determination of acid-insoluble ash**

Celite was added as a marker for the determination of ileal digestibility of nutrients. Acid-insoluble ash was measured as described by Choct and Annison (1992). Two gram of diet and 1 gram of digesta samples from drying and grounding were weighed into sintered glass crucibles (Pyrex®, England), dried at 105°C for 24 h and ashed at 550°C for 8 h. After ashing the crucibles were cooled, and boiled slowly in 4 N HCl for 30 min on a hot plate in fume hood. The ash in crucibles was washed with distilled water using suction pump, and dried at 105°C for 6 h. The ash residues in crucibles were ashed and boiled in the same way. Finally, the ash in crucibles were dried at 105°C for 6 h, the crucibles were cooled in a desiccator and weighed while containing the ash. Percentage acid-insoluble ash was calculated using the following equation (Van Keulen and Young, 1977):

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

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 (protein and fat) was calculated using the following equation:

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

**Calculation of the growth performance**

In both experiments (1 and 2), the broilers were weighed at 0, 21 and 42 days old. The feed intake was recorded between days 0 to 21 and days 22 to 42. Number of dead broilers and body weight were recorded mortality rate and the feed conversion ratio (FCR) were calculated.

Weight gain (WG, g) = Final body weight – Initial body weight

$$\text{Average daily gain (ADG, g/day)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake}}{\text{Final body weight} - \text{Initial body weight}}$$

**Statistical analysis**

All data are presented as means $\pm$ SD. The effect of treatment were analyzed using one-way Analysis of Variance (ANOVA). If there were any significant effect ($F<0.05$), Duncan's New Multiple Range Test was used to compare the individual means. The level of significant difference was set at $P<0.05$ (Steel and Torrie, 1960).

# CHAPTER IV

# RESULTS

## Experiment 1

### 1.1 Effect of Asiatic pennywort supplementation on growth performance.

The effect of various treatments on growth performance of broilers is shown in Table 11. During the starter period (days 0-21), it was found that the broilers in T2 and T3 had significant ($P<0.05$) higher weight gain than that of the T1 (control group) (Figure 7). In addition, the results showed that the average daily gain in T3 was significantly ($P<0.05$) higher than that of the control group.

During the grower period (days 22-42), it was found that the weight gain, feed intake, average daily gain and feed conversion ratio were not significant difference among groups of the broilers.

For the overall period of the trial (days 0-42), there was no effect of any treatment on the weight gain, feed intake, average daily gain and feed conversion ratio among groups of the study.

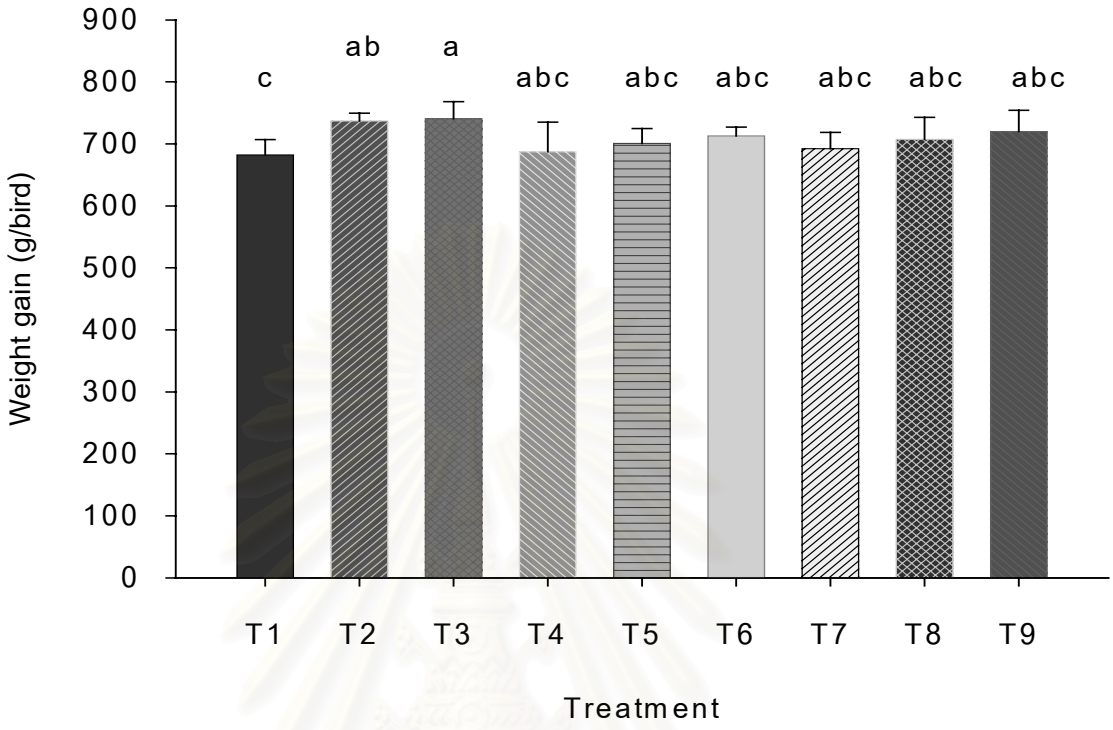
**Table 11** Effect of Asiatic pennywort supplementation on growth performance [1] of the broilers (Experiment 1).

| Item | Treatment [2] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
| **Weight gain (g/bird)** | | | | | | | | | |
| Day 0 — Day 21 | 682.29±24.53 [c] | 736.72±12.74 [ab] | 740.62±27.49 [a] | 687.29±47.64 [abc] | 700.83±23.99 [abc] | 712.80±14.35 [abc] | 692.03±26.36 [abc] | 707.08±35.88 [abc] | 719.79±34.29 [abc] |
| Day 22 – Day 42 | 1189.58±21.92 | 1254.97±74.87 | 1280.00±149.54 | 1221.88±30.31 | 1242.88±101.67 | 1242.99±85.08 | 1318.03±237.66 | 1206.25±127.95 | 1213.07±85.59 |
| Day 0 – Day 42 | 1871.88±14.60 | 1991.69±63.17 | 2020.62±126.04 | 1909.17±67.73 | 1943.71±106.08 | 1955.80±83.67 | 2010.06±218.72 | 1913.34±134.84 | 1932.86±110.74 |
| **Feed intake (g/bird)** | | | | | | | | | |
| Day 0 — Day 21 | 1232.09±58.68 | 1237.29±33.50 | 1224.38±53.16 | 1240.52±14.66 | 1245.10±12.42 | 1230.63±37.65 | 1180.34±59.00 | 1245.94±15.26 | 1247.08±27.12 |
| Day 22 – Day 42 | 2612.60±66.31 | 2614.02±105.81 | 2626.49±56.62 | 2591.04±41.40 | 2556.04±20.48 | 2623.26±26.72 | 2567.12±121.58 | 2593.13±105.51 | 2543.86±63.08 |
| Day 0 – Day 42 | 3844.69±113.53 | 3851.31±78.46 | 3850.86±76.97 | 3831.56±49.34 | 3801.15±26.96 | 3853.88±50.09 | 3755.46±171.98 | 3839.06±111.58 | 3790.94±83.34 |
| **Average daily gain (g/bird/day)** | | | | | | | | | |
| Day 0 — Day 21 | 32.49±1.17 [b] | 35.08±0.61 [ab] | 35.27±1.31 [a] | 32.73±2.27 [ab] | 33.38±1.14 [ab] | 33.94±0.68 [ab] | 32.95±1.26 [ab] | 33.67±1.71 [ab] | 34.27±1.63 [ab] |
| Day 22 – Day 42 | 56.64±1.04 | 59.76±3.57 | 60.95±7.12 | 58.18±1.44 | 59.18±4.84 | 59.19±4.05 | 62.76±11.31 | 57.44±6.09 | 57.76±4.08 |
| Day 0 – Day 42 | 44.57±0.34 | 47.42±1.50 | 48.11±3.00 | 45.46±1.62 | 46.28±2.53 | 46.56±1.99 | 47.86±5.21 | 45.56±3.21 | 46.02±2.64 |
| **Feed conversion rate** | | | | | | | | | |
| Day 0 — Day 21 | 1.80±0.04 | 1.72±0.04 | 1.69±0.07 | 1.81±0.12 | 1.78±0.05 | 1.73±0.06 | 1.78±0.02 | 1.76±0.08 | 1.74±0.05 |
| Day 22 – Day 42 | 2.20±0.08 | 2.11±0.13 | 2.10±0.18 | 2.12±0.07 | 2.10±0.10 | 2.12±0.14 | 2.04±0.33 | 2.18±0.16 | 2.15±0.06 |
| Day 0 – Day 42 | 2.06±0.06 | 1.96±0.08 | 1.94±0.08 | 2.01±0.06 | 1.98±0.07 | 1.98±0.08 | 1.94±0.21 | 2.02±0.08 | 1.99±0.05s |
| **Mortality (%)** | | | | | | | | | |
| Day 0 — Day 21 | 0.00 | 2.08 | 2.08 | 0.00 | 0.00 | 2.08 | 4.17 | 0.00 | 0.00 |
| Day 22 – Day 42 | 0.00 | 2.13 | 2.13 | 0.00 | 0.00 | 2.13 | 4.35 | 2.08 | 0.00 |
| Day 0 – Day 42 | 0.00 | 4.17 | 4.17 | 0.00 | 0.00 | 4.17 | 8.33 | 2.08 | 0.00 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 1.67 g/kg; T3: crude extract 0.40 g/kg; T4: crude powder 3.33 g/kg; T5: crude extract 0.80 g/kg; T6: crude powder 6.67 g/kg; T7: crude extract 1.60 g/kg; T8: crude powder 10.00 g/kg; T9: crude extract 2.40 g/kg.

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




[a, b, c] Different superscript mean significantly difference (P<0.05).

**Figure 7** Effect of treatments on weight gain (g/bird) in broilers 0-21 days of age.

## Experiment 2

### 2.1 Effect of Asiatic pennywort supplementation on growth performance.

The effect of various treatments on growth performance of broilers are depicted in Table 12. Both starter and grower periods (days 0-21 and days 22-42), there were no effect of any treatment on the weight gain, feed intake, average daily gain and feed conversion ratio among groups in each period.

At the overall period (days 0-42), there were no significant differences in weight gain, feed intake, average daily gain and feed conversion ratio among groups of the broilers. However, the weight gain and average daily gain in T2 and T4 groups, although were lower than those of the T3 and T5 groups but were not significant different. Moreover, it was found that feed conversion ratio in T2 and T4 groups were higher than those of the T3 and T5 groups but there were no significant differences.

### 2.2 Effect of Asiatic pennywort supplementation on the pH of the intestinal content in various parts of the small intestine.

The changes in pH of the intestinal content at the jejunum, ileum and caecum were measured (Table 13). At days 21 and 42, there were no significant differences in jejunal pH, ileal pH and caecal pH among groups of the broilers. However, it was found that the pH of jejunum and ileum in T2, T3, T4 and T5 groups were slightly lower ($P>0.05$) than those of the T1 and T6 groups. Moreover, the pH of the jejunum, ileum and caecum in T5 group tended to be lower ($P>0.05$) than those of the other groups but there was no significant difference (Figures 8-10).

**Table 12** Effect of Asiatic pennywort supplementation on growth performance [1] of the broilers (Experiment 2).

| Item | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| **Weight gain (g/bird)** | | | | | | |
| Day 0 – Day 21 | 680.52±67.86 | 671.02±20.09 | 703.26±24.19 | 639.80±40.54 | 692.76±51.78 | 691.20±53.23 |
| Day 22 – Day 42 | 1454.30±76.44 | 1449.22±48.76 | 1452.39±61.80 | 1460.96±65.09 | 1421.67±95.45 | 1438.64±103.14 |
| Day 0 – Day 42 | 2134.82±105.19 | 2120.24±51.38 | 2155.65±56.99 | 2100.75±47.31 | 2114.44±140.98 | 2129.84±146.95 |
| **Feed intake (g/bird)** | | | | | | |
| Day 0 – Day 21 | 987.12±59.49 | 1041.67±45.74 | 1054.17±59.01 | 1006.44±19.25 | 986.74.86±61.78 | 1016.29±75.07 |
| Day 22 – Day 42 | 2745.47±118.75 | 2767.98±76.71 | 2751.56±79.75 | 2733.28±72.32 | 2724.33±173.77 | 2725.44±118.03 |
| Day 0 – Day 42 | 3732.59±151.85 | 3809.65±97.69 | 3805.73±101.49 | 3739.72±84.04 | 3711.07±220.94 | 3741.72±163.92 |
| **Average daily gain (g/ bird/day)** | | | | | | |
| Day 0 – Day 21 | 32.40±3.23 | 31.95±0.96 | 33.49±1.15 | 30.47±1.93 | 32.99±2.46 | 32.91±2.53 |
| Day 22 – Day 42 | 69.25±3.64 | 69.01±2.32 | 69.16±2.94 | 69.57±3.10 | 67.70±4.54 | 68.51±4.91 |
| Day 0 – Day 42 | 50.83±2.50 | 50.48±1.22 | 51.32±1.36 | 50.02±1.13 | 50.34±3.36 | 50.71±3.50 |
| **Feed conversion ratio** | | | | | | |
| Day 0 – Day 21 | 1.49±0.08 | 1.55±0.10 | 1.52±0.10 | 1.59±0.11 | 1.46±0.06 | 1.52±0.09 |
| Day 22 – Day 42 | 1.88±0.11 | 1.91±0.08 | 1.90±0.07 | 1.87±0.06 | 1.91±0.02 | 1.90±0.06 |
| Day 0 – Day 42 | 1.76±0.08 | 1.80±0.08 | 1.78±0.08 | 1.78±0.03 | 1.77±0.02 | 1.78±0.06 |
| **Mortality rate (%)** | | | | | | |
| Day 0 – Day 21 | 2.27 | 0.00 | 1.52 | 0.76 | 3.03 | 2.27 |
| Day 22 – Day 42 | 3.64 | 2.63 | 4.46 | 2.65 | 3.64 | 6.31 |
| Day 0 – Day 42 | 5.30 | 2.27 | 5.30 | 3.03 | 6.06 | 7.58 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 2 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 25 ppm.

**Table 13** Effect of Asiatic pennywort supplementation on the intestinal pH [1] in various parts of the small intestine.

| Intestinal part | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Jejunum (J) | | | | | | |
| Day 21 | 6.33±0.19 | 6.48±0.20 | 6.45±0.23 | 6.34±0.24 | 6.08±0.30 | 6.48±0.28 |
| Day 42 | 6.210±.31 | 5.97±0.48 | 5.97±0.23 | 5.93±0.66 | 5.74±0.77 | 6.11±0.44 |
| % Change | 2.48±1.91 | 6.10±3.03 | 7.38±2.49 | 4.72±3.18 | 1.06±0.60 | 4.02±0.81 |
| Ileum (I) | | | | | | |
| Day 21 | 7.62±0.61 | 7.59±0.50 | 7.38±0.53 | 7.56±0.43 | 7.06±0.80 | 7.59±0.38 |
| Day 42 | 7.31±0.35 | 6.65±0.92 | 6.47±1.03 | 6.50±1.24 | 6.21±0.90 | 7.45±0.54 |
| % Change | 5.80±1.81 | 12.11±1.72 | 9.35±4.11 | 6.12±1.05 | 10.63±4.62 | 3.46±2.15 |
| Caecum (CE) | | | | | | |
| Day 21 | 6.93±0.31 | 6.82±0.64 | 6.68±0.30 | 6.60±0.31 | 6.17±0.61 | 6.56±0.52 |
| Day 42 | 6.45±0.42 | 6.43±0.59 | 6.31±0.10 | 6.54±0.52 | 6.04±0.63 | 6.30±0.65 |
| % Change | 8.27±2.54 | 9.14±4.52 | 2.27±0.52 | 2.30±2.63 | 8.66±7.96 | 4.74±2.80 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

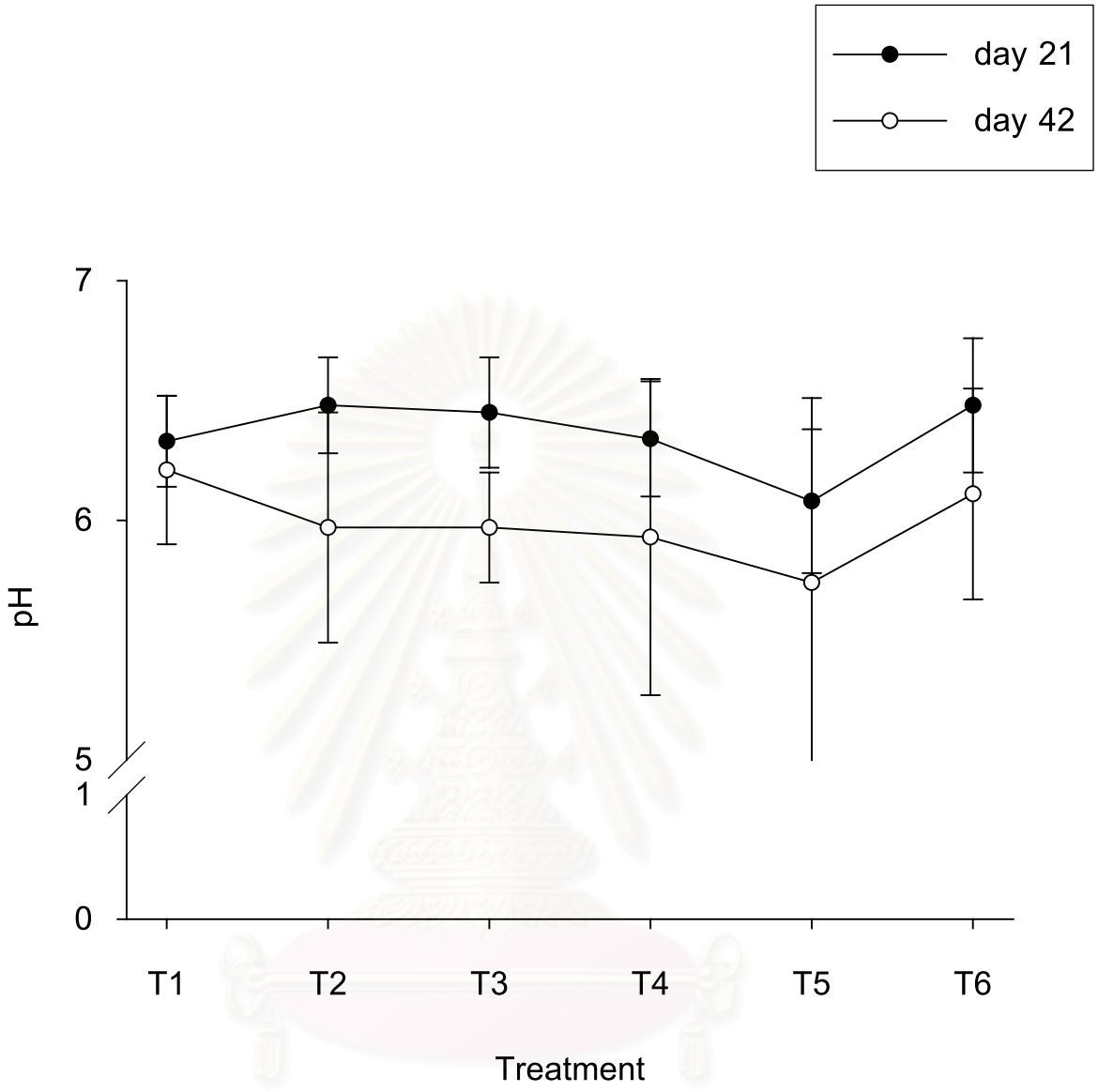



**Figure 8** The pH of jejunal content of broilers at days 21 and 42 of the experiment.

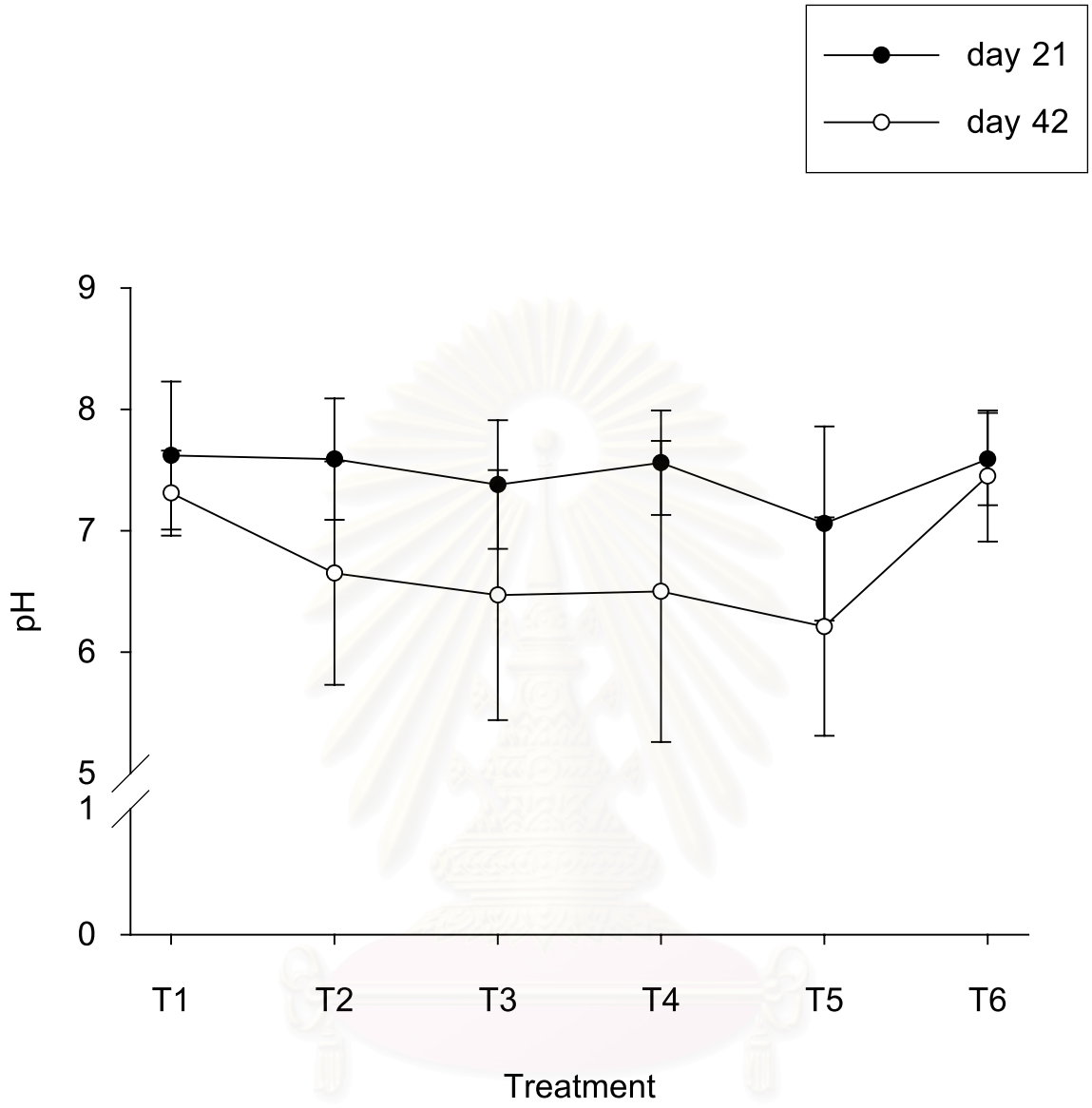



**Figure 9** The pH of ileal content of broilers at days 21 and 42 of the experiment.

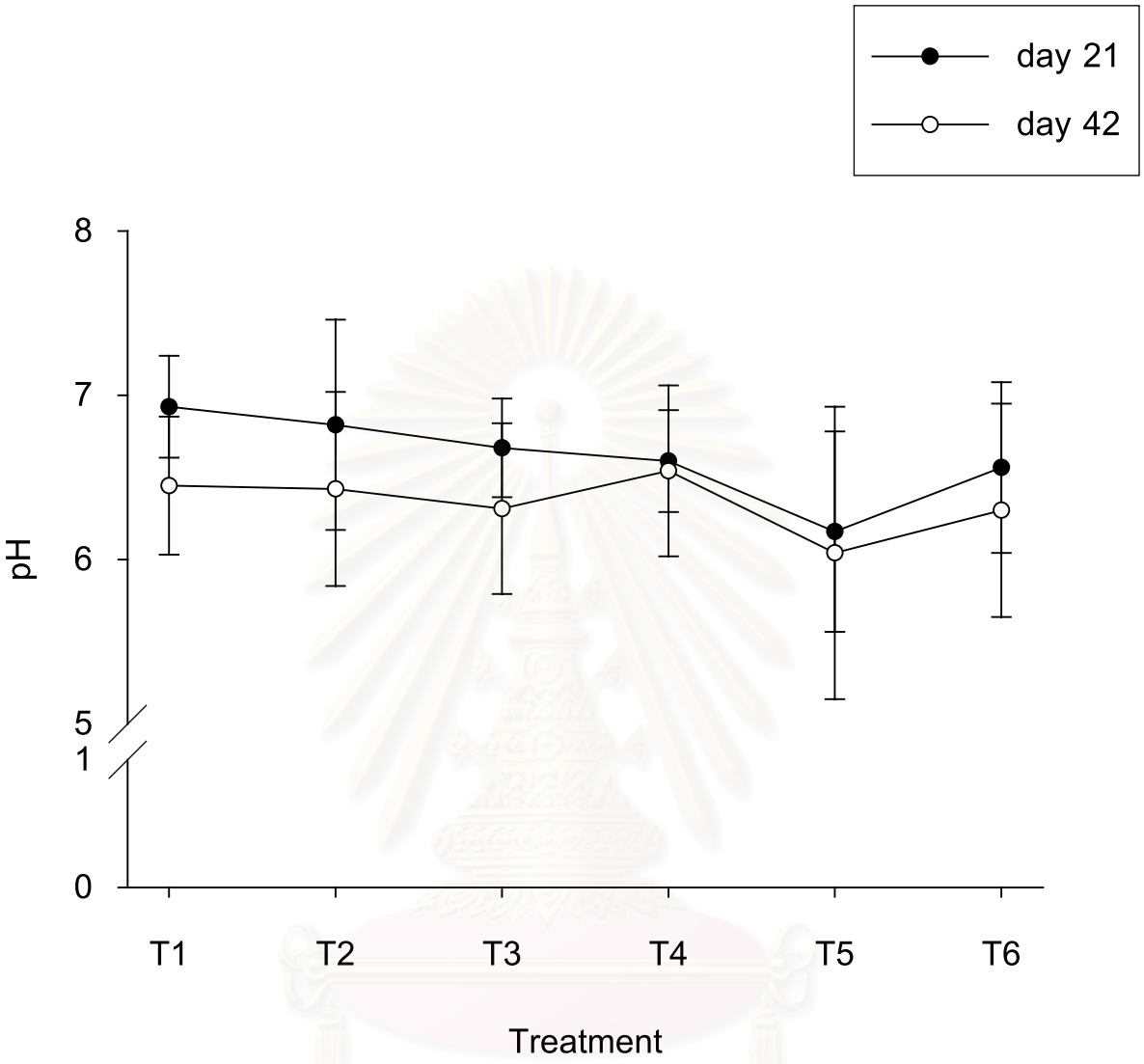



**Figure 10** The pH of caecal content of broilers at days 21 and 42 of the experiment.

**2.3 Effect of Asiatic pennywort supplementation on the protein concentrations of the small intestinal mucosa.**

The protein concentrations of small intestinal mucosa of the broilers are depicted in Table 14. At days 21 and 42, it was found that the protein concentrations of small intestinal mucosa were not significant different among groups of the broilers.

**2.4 Effect of Asiatic pennywort supplementation on the concentrations of the DNA, RNA, ratios of RNA: DNA, RNA: Protein and Protein: DNA of the ileal mucosa.**

The concentrations of DNA and RNA are demonstrated in Table 15. There was no significant difference in concentrations of DNA, RNA and the RNA: DNA, RNA: Protein and Protein: DNA ratios among groups of the broilers.

**2.5 Effect of Asiatic pennywort supplementation on the peptide hydrolase activities in jejunal mucosa.**

The alteration in peptide hydrolase activities of jejunal mucosa are shown in Table 16. At day 21, the peptide hydrolase activities in jejunal mucosa was no significant different from each others.

At day 42, the peptide hydrolase activities in T6 group were significantly higher ($P<0.05$) than those of the other groups. It was found that the broilers in T5 group had significantly higher ($P<0.05$) jejunal peptide hydrolase activities than those of the control, T2 and T3 groups. In addition, the results showed that the peptide hydrolase activities in T4 group were significantly higher ($P<0.05$) than those of the control and T2 groups (Figure 11).

**Table 14** Effect of Asiatic pennywort supplementation on protein concentrations [1] (mg/g wet weight tissue) of the small intestine.

| | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Jejunum (J) | | | | | | |
| Day 21 | 22.35±9.35 | 25.57±9.72 | 20.83±3.52 | 26.82±9.30 | 21.69±6.48 | 27.69±11.63 |
| Day 42 | 26.72±3.12 | 22.03±2.97 | 26.87±7.74 | 26.92±5.62 | 22.66±3.72 | 25.71±8.76 |
| Ileum (I) | | | | | | |
| Day 21 | 35.86±20.52 | 29.52±11.23 | 45.12±19.06 | 28.35±10.42 | 28.98±8.60 | 29.20±15.75 |
| Day 42 | 24.53±5.09 | 23.58±2.79 | 25.12±6.13 | 20.26±4.72 | 22.99±3.04 | 23.20±5.84 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

**Table 15** Effect of Asiatic pennywort supplementation on DNA, RNA concentrations (mg/g wet weight tissue) and ratios of RNA: DNA, RNA: Protein and Protein: DNA[1] of the ileal mucosa.

| | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| DNA | | | | | | |
| Day 21 | 0.22±0.15 | 0.23±0.18 | 0.40±0.10 | 0.27±0.18 | 0.16±0.07 | 0.18±0.12 |
| Day 42 | 0.89±0.13 | 0.97±0.22 | 0.83±0.22 | 0.90±0.23 | 0.83±0.20 | 1.03±0.27 |
| RNA | | | | | | |
| Day 21 | 0.53±0.47 | 0.46±0.15 | 0.88±0.28 | 0.52±0.32 | 0.53±0.23 | 0.41±0.12 |
| Day 42 | 0.85±0.26 | 0.82±0.29 | 0.86±0.20 | 0.78±0.13 | 0.82±0.22 | 0.93±0.34 |
| RNA: DNA ratio | | | | | | |
| Day 21 | 2.24±0.80 | 3.40±2.23 | 1.92±1.04 | 2.50±1.83 | 3.76±1.73 | 2.59±0.80 |
| Day 42 | 0.99±0.47 | 0.84±0.21 | 1.05±0.10 | 0.95±0.37 | 1.00±0.24 | 1.04±0.70 |
| RNA: Protein ratio | | | | | | |
| Day 21 | 0.01±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 |
| Day 42 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 | 0.04±0.01 |
| Protein: DNA ratio | | | | | | |
| Day 21 | 179.56±53.37 | 189.37±109.95 | 103.22±50.20 | 144.88±84.28 | 197.68±63.22 | 166.17±26.68 |
| Day 42 | 28.60±10.48 | 24.97±3.96 | 30.80±3.73 | 24.65±10.06 | 28.84±7.57 | 25.43±14.35 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

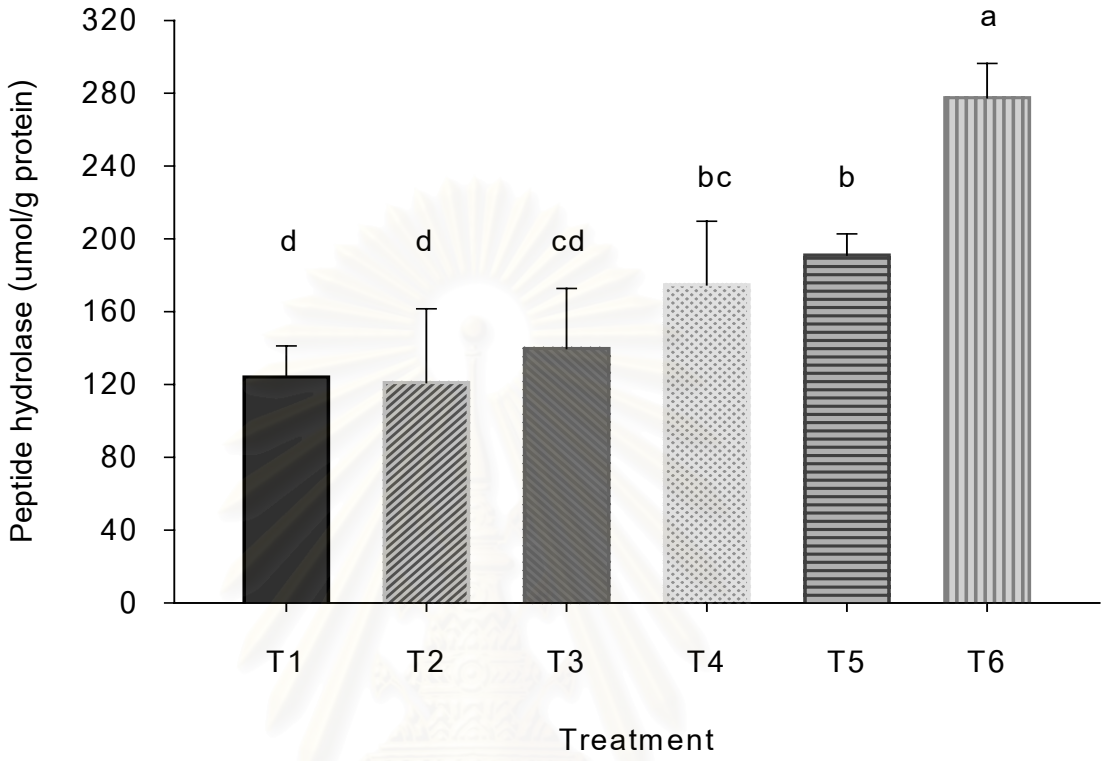
**Table 16** Effect of Asiatic pennywort supplementation on peptide hydrolase activities [1] (μmol/g mucosal protein) in jejunal mucosa.

| Day | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Day 21 | 221.38±91.87 | 202.48±120.55 | 282.08±23.98 | 188.86±101.42 | 264.84±65.52 | 239.62±75.16 |
| Day 42 | 124.13±17.08 $^{d}$ | 121.11±40.55 $^{d}$ | 139.79±32.99 $^{cd}$ | 174.82±34.90 $^{bc}$ | 191.14±11.62 $^{b}$ | 277.61±18.74 $^{a}$ |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

[a,b,c,d] Mean in the same row with different superscripts differed significantly ($P<0.05$).




[a, b, c, d] Different superscript mean significantly difference (P<0.05).

**Figure 11** Effect of treatments on peptide hydrolase activities (μmol/g mucosal protein) of jejunal mucosa in the broilers age 42 days.

## 2.6 Effect of Asaitic pennywort supplementation on the disaccharidase activities of jejunal mucosa.

### 2.6.1 Maltase activities

The alteration in maltase activities of jejunal mucosa are depicted in Table 17. At day 21, the Asiatic pennywort supplementation did not affect the maltase activities, while T6 (antibiotic group) had significantly ($P<0.05$) higher activities than that of the control group (Figure 12).

At day 42, there was no significant difference in maltase activities of jejunal mucosa among groups. The maltase activities in T2 group was tend to be slightly higher ($P>0.05$) than those of the others.

### 2.6.2 Sucrase activities

The sucrase activities of jejunal mucosa are shown in Table 17. At day 21, there was no significance difference in the sucrase activities among groups. The sucrase activities in T6 group was tend to be slightly higher ($P>0.05$) than those of the others.

The sucrase activities among group at day 42, were not different. The sucrase activities in T2 group was tend to be slightly higher than those of the other groups but it was not statistically different.

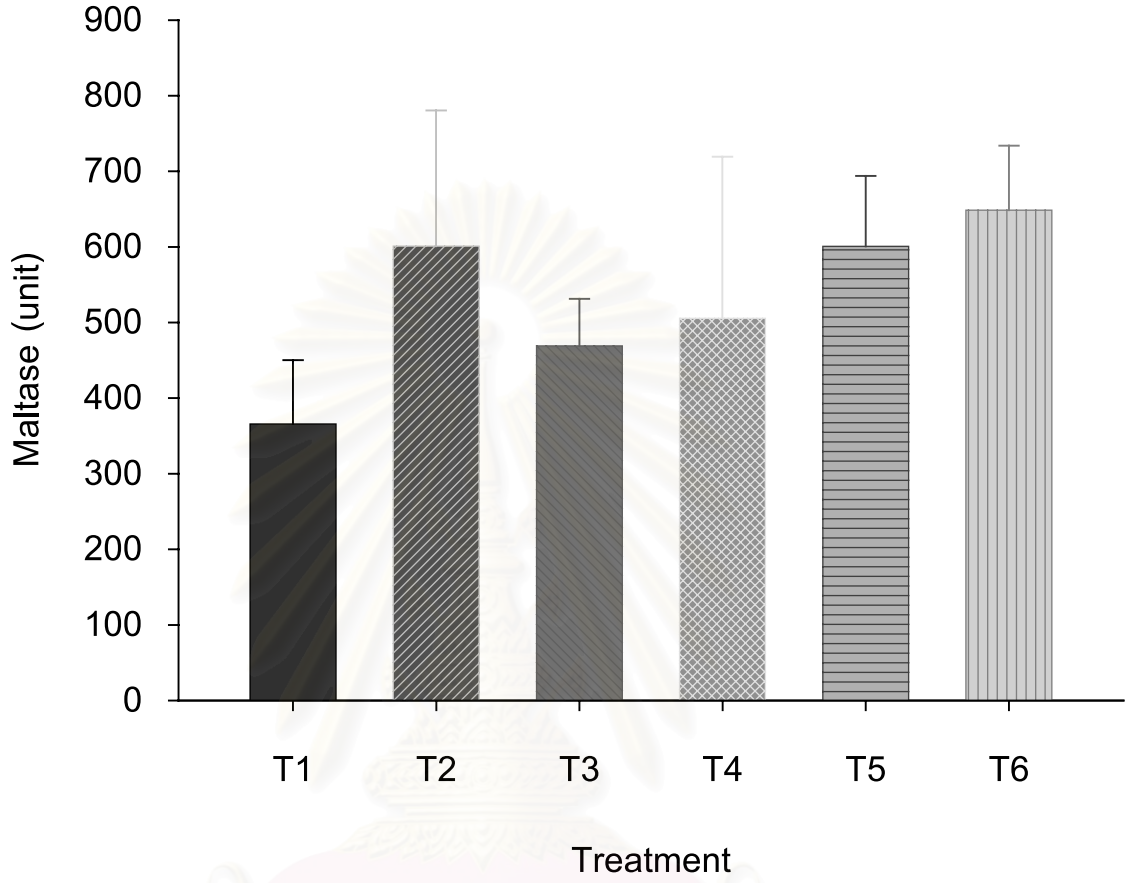
**Table 17** Effect of Asiatic pennywort supplementation on jejunal disaccharidase activities [1] (units/g protein) in jejunal mucosa.

| Enzyme | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Maltase | | | | | | |
| Day 21 | 365.71±84.61 [b] | 600.81±179.63 [ab] | 468.96±62.30 [ab] | 505.35±213.85 [ab] | 600.64±93.22 [ab] | 648.56±85.51 [a] |
| Day 42 | 402.61±123.53 | 536.93±124.11 | 512.10±161.23 | 443.31±69.47 | 371.62±80.12 | 395.21±43.53 |
| Sucrase | | | | | | |
| Day 21 | 90.72±17.55 | 86.57±16.46 | 78.68±10.43 | 59.97±12.17 | 76.97±8.92 | 91.17±24.46 |
| Day 42 | 81.30±9.45 | 122.63±34.35 | 97.74±39.62 | 79.50±22.76 | 79.16±7.05 | 85.34±12.41 |

[1] Mean ± SD.

[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

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




[a, b] Different superscript mean significantly difference (P<0.05).

**Figure 12** Effect of treatments on maltase activities (units/g mucosal protein) of jejunal mucosa in the broilers age 21 days.

**2.7 Effect of Asiatic pennywort supplementation on ileal digestibility of nutrient.**

**2.7.1 Ileal digestibility of protein.**

The ileal digestibility of protein of the broilers are demonstrated in Table 18. At day 25, it was found that the ileal digestibility of protein in T2 and T3 groups were significantly ($P<0.05$) higher than those of the T1, T4 and T6 groups. In addition, the broilers in T5 group had significantly ($P<0.05$) higher ileal digestibility of protein than that of the T4 group, likewise, the ileal digestibility of protein in T3 group tended to be higher than that of the T2 group (Figure 13).

At day 46, there was no effect of treatments on the ileal digestibility of protein among groups of the broilers.

**2.7.2 Ileal digestibility of fat.**

The fat digestibility at ileum of the broilers are depicted in Table 15. At day 25, it was found that the ileal digestibility of fat in T3 group was significantly ($P<0.05$) higher than those of the others except that of the T1. Furthermore, when the supplementation was at low level, it was showed that the broilers in T3 group had significantly ($P<0.05$) higher ileal digestibility of fat than that of the T2 group. At the high level of supplementation, it was found that the ileal digestibility of fat in T5 group tend to be higher than that of the T4 group but there was not of statistical significant (Figure 14).

At day 46, it was found that the ileal digestibility of fat in broilers were no significant different among groups.

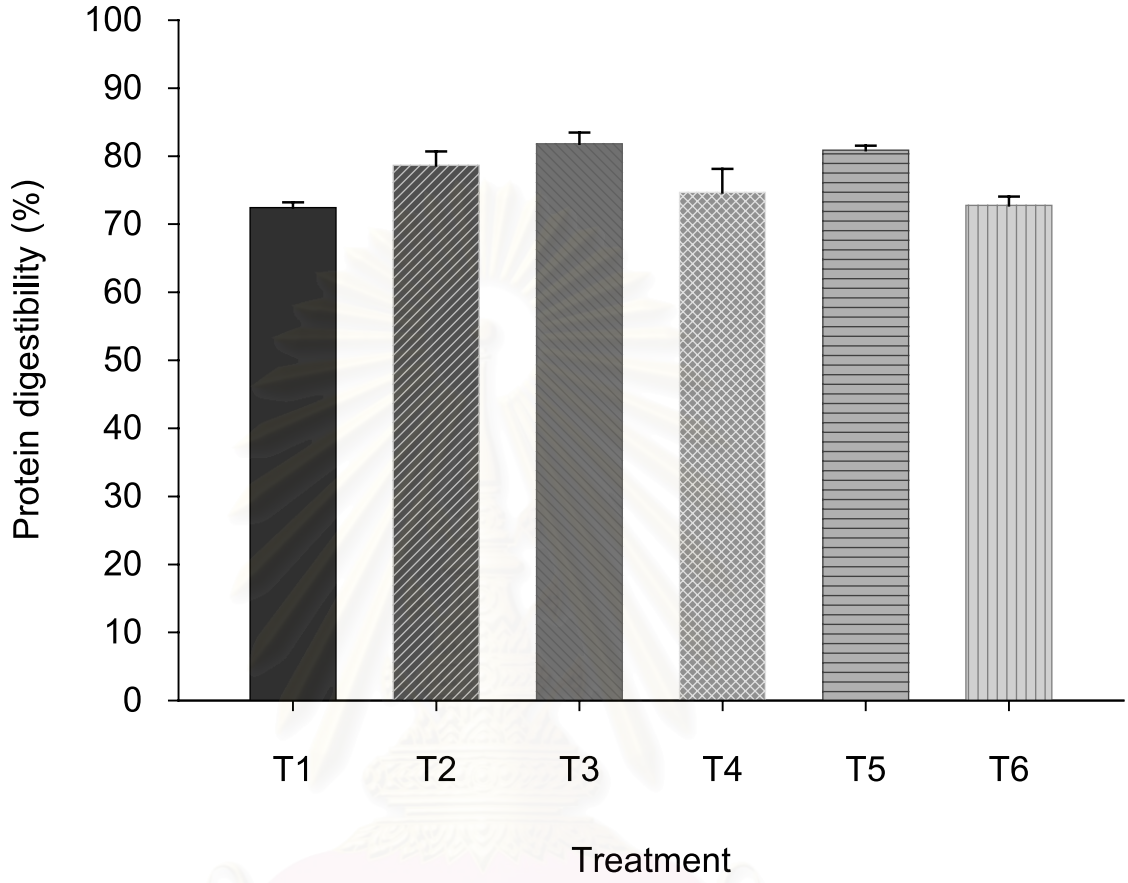
**Table 18** Effect of Asiatic pennywort supplementation on ileal digestibility of nutrient [1] (%, dry matter basis) of the broilers.

| Nutrient | Treatment [2] | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Protein | | | | | | |
| Day 25 | 72.43±0.80 [b] | 78.65±2.06 [a] | 81.83±1.65 [a] | 74.66±3.49 [b] | 80.87±0.68 [a] | 72.78±1.29 [b] |
| Day 46 | 76.60±1.43 | 69.87±3.03 | 73.56±3.82 | 68.85±4.50 | 72.89±5.67 | 71.18±3.75 |
| Fat | | | | | | |
| Day 25 | 83.52±2.04 [ab] | 83.71±1.69 [b] | 86.88±1.62 [a] | 80.32±1.76 [b] | 81.95±1.70 [b] | 81.69±0.24 [b] |
| Day 46 | 86.58±1.62 | 82.86±2.81 | 84.17±3.08 | 83.97±2.49 | 82.54±2.33 | 83.52±2.12 |

[1] Mean ± SD.

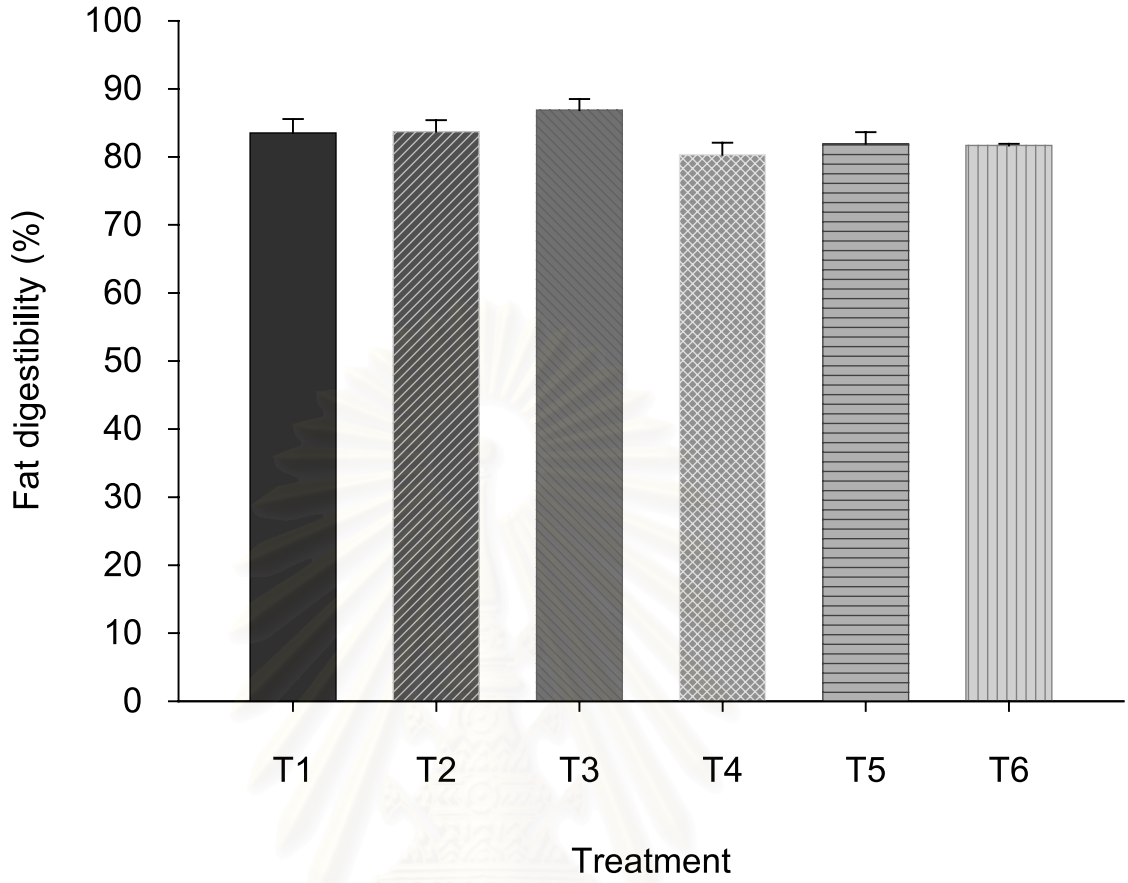
[2] Treatments were T1: control; T2: crude powder 20 g/kg feed; T3: crude extract 3.6 g/kg feed; T4: crude powder 40 g/kg feed; T5: crude extract 7.2 g/kg feed; T6: antibiotic 2.5 ppm.

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




[a, b] Different superscript mean significantly difference (P<0.05).

**Figure 13** Effect of treatments on ileal digestibility of protein (%, dry matter basis) in the broilers age 25 days.




[a, b] Different superscript mean significantly difference (P<0.05).

Figure 14 Effect of treatments on ileal digestibility of fat (%, dry matter basis) in the broilers age 25 days.

# CHAPTER V

# DISCUSSION

**Effect of Asiatic pennywort on various parameters.**

Asiatic pennywort was supplemented to the broilers to examine its effects on various parameters compare to those of the control. In experiment 1, Asiatic pennywort leaves and its extracts were used. The description of the treatments in experiment 1 are depicted in Table 7 (Chapter III).

Initially, experiment 1 was designed to preliminary examine the level of Asiatic pennywort that affect growth performance. There was no study that previously discussed on the applicable dose in broilers. It is known that the first step of research with herbal plants is to find an appropriate level to be used in the experimental animal. However, the results in experiment 1 did not fulfill our objective since there was no significant difference of growth performance among groups. This may be due to the low inclusion of Asiatic pennywort. Therefore, in experiment 2, the higher level of Asiatic pennywort was used and the entire Asiatic pennywort (leaves and stem) were included as described in Table 10 (Chaper III). The objective of experiment 2 was more in depth to the function of small intestine and the overall growth performance.

**Effect on growth performance.**

At the overall period, the result showed that supplementation of Asiatic pennywort to the broilers did not significantly affect the growth performance of broilers in both experiments (Tables 11 and 12). However, results in the starter period (Experiment 1) showed that 1.67 g/kg crude powder and 0.40 g/kg crude extract of Asiatic pennywort slightly improved weight gain and average daily gain.

For feed conversion ratio, although Asiatic pennywort did not significantly affect feed conversion ratio but broilers supplemented with the crude extract tended to have lower feed conversion ratio than that of the crude powder. It is possible that crude extract had 11.40 times more asiaticoside than that of the crude powder in experiment 1 and the increment was up to 67.25 times in experiment 2.

**Effect on pH of the intestinal contents**

There was no effect of treatments on pH of the intestinal contents (Table 13). There were trends of decreasing pH in jejunum, ileum and caecum in Asiatic pennywort supplementation groups, especially in 7.2 g/kg feed crude extract of Asiatic pennywort supplementation. The reduction of intestinal pH might be due to an acidifier action of Asiatic pennywort, especially those of the crude extract. Asiatic pennywort consists of the saponins containing sapogenins (aglycone molecule), for instance asiatic acid, madecassic acid, brahmic acid, thankunic acid, 6β-hydroxyasiatic acid and terminolic acid (Brinkhaus *et al*., 2000). It is proposed that these acids might be helpful to decrease intestinal pH. The pH reduction effect of Asiatic pennywort was well shown in jejunum and ileum than caecum.

It was found that the percentage of reduction of intestinal pH in Asiatic pennywort supplement groups was higher than that of the control group. These showed that the pH in various intestinal parts at day 42 were lower than those of day 21. It is possible that decreasing of the pH in various intestinal parts at day 42 can be useful in studies focusing on the reduction of pathogenic bacteria and other microorganisms involved in meat hygiene such as Salmonella spp., *Campylobacter jejuni* in the intestine of broilers (Van Der Wielen *et al*., 2000). The acidic environment in the small and large intestine were hostile to these pathogenic bacteria and prevented their attachment to the mucosal cells (Corrier *et al*., 1995). However, the mechanism of Asiatic pennywort in reducing intestinal pH was still unknown and further investigation was needed.

**Effect on intestinal tissue development**

Effects of Asiatic pennywort on intestinal tissue development are shown in Tables 14 and 15. Tissue development was examined by three parameters comprising protein, DNA and RNA concentrations. The intestinal mucosal DNA and RNA concentrations represented the tissue growth and protein synthesis, respectively.

The ratio of RNA: DNA indicated the intestinal tissue activity while RNA: protein ratio showed the ribosomal capacity and protein: DNA ratio reflected the mucosal cell size. Tissue development was basically studied in early postnatal period than in the mature period of animals. (Berseth *et al*.,1983; Simmen *et al*., 1990). Therefore, we cannot find any significant changes in almost all of the parameters.

In this study, there were no changes of protein, DNA and RNA concentrations. There were no changes of RNA: DNA, RNA: protein and protein: DNA ratios in each period of the experiment. It is possible that Asiatic pennywort had no effect on intestinal tissue development or the level of active ingredient in Asiatic pennywort in experiment 2 was not sufficient to stimulate the intestine development.

The protein concentrations in jejunum and ileum in the present study ranged from 20 to 40 mg/g tissue. While, the DNA concentrations in ileum was 0.2 to 1.0 mg/g tissue. These values were lower than those in the other reports (Palo *et al*., 1995; Uni *et al*., 1995; Uni et al., 2001). Uni *et al*. (1998) reported that the protein concentrations in jejunum and ileum were approximately 90 to 100 mg/g tissue. The DNA concentrations was 10 to 20 mg/g tissue. The lower of values might be due to different strains of broilers, errors of the method and technique used in the laboratory.

**Effect on jejunal enzymes activities.**

In the present study, activities of peptide hydrolase and disaccharidase containing maltase and sucrase in various treatments were examined. It was found that peptide hydrolase activities in T4 and T5 groups (crude powder 40 g/kg feed and crude extract 7.2 g/kg feed) were slightly higher than those of the T2, T3 groups (crude powder 20 g/kg feed and crude extract 3.6 g/kg feed) and the control group. It is possible that the T4 and T5 may contain some substances which might encourage activities of peptide hydrolase. The supplement of Asiatic pennywort did not increase disaccharidase activities. On the contrary, supplement of antibiotic avilamycin, resulted in a significant increase in jejunal peptide hydrolase activities at day 42 and maltase activities at day 21 of the experiment. Our results especially the peptide hydrolase activities were similar to those of Collinton *et al*. (1990) who reported that the inclusion of antibiotic had significant effects on the development of peptide hydrolase activities such as tripeptidase.

The maltase and peptide hydrolase, brush border hydrolytic and proteolytic enzymes were part of the cellular role in carbohydrate and protein digestion. It may possibly, reflect the improvement in digestion of these substrates. However, other luminal enzymes such as amylase, trypsin and pepsin should be taken into account in this circumstance.

**Effect on nutrient digestibility**

One of our objectives was to determine whether Asiatic pennywort affected ileal digestibility of nutrients comprising protein and fat.

In case of protein digestibility, it was found that broilers receiving crude extract of Asiatic pennywort at the level 3.6 g/kg feed (T3) had significantly ($P<0.05$) higher protein digestibility than that of the control group. In addition, the results showed that broilers received crude extract had higher protein digestibility than that of the crude

powder of Asiatic pennywort supplementation. It is proposed that increased protein digestibility was not only resulting from the peptide hydrolase activities in the jejunum but also the effect of increased enzyme activities in proventiculus. There are two mechanisms underlying this alteration. Firstly an active ingredient in Asiatic pennywort may directly activate the oxyntic cells to secrete both HCl and pepsinogen. The other mechanism is the effect of acid molecules (sapogenins) in Asiatic pennywort such as asiatic acid, madecassic acid that can indirectly activate the transformation of pepsinogen to pepsin.

Likewise, fat digestibility in broilers receiving crude extract of Asiatic pennywort at level 3.6 g/kg feed (T3) was significantly ($P<0.05$) greater than that of the control group. There are two possible mechanisms underlying an increase in fat digestibility caused by Asiatic pennywort. The first mechanism is involving substances in Asiatic pennywort that may assist lipase activities in jejunum and secondly, it may activate bile secretion from gall bladder. Which helps to emulsify fat droplet and make them more available for lipase.

However, the result showed that protein and fat digestibility increased only at days 25 of age but there was no change at days 46 of age. It is possible that the growth of broilers in the first 3 weeks was marked. They eat more diet to support growth which was higher than at 6 weeks of age. The more digestion of feed occurred. The more digestive enzymes and bile needed. Therefore the effect of Asiatic pennywort was clearly shown only in the growing period.

In conclusion, the present study demonstrated that supplementation of Asiatic pennywort in both crude powder and crude extract did not significantly promote disaccharidase activities in jejunum and intestinal tissue development in normal broilers growth. However, Asiatic pennywort had some effects on growth performance, intestinal pH, peptide hydrolase activities and nutrients digestibility. The growth performance of broilers supplemented with Asiatic pennywort was slightly better than those of the given antibiotic. In the present study, the growth performance in Asiatic pennywort

supplementation groups were not significantly different from the antibiotic group. Further studied are required to examine whether Asiatic pennywort can be used to replace antibiotic growth promoter in poultry. The overall results showed that the crude extract tended to be more appropriate for use than crude powder due to its higher amount of active ingredient. Crude powder may contain variety of constituents which some may have an effect on utilization of nutrient for instance anti-nutritional factors. The mechanism underlying the improved digestibility of nutrients and acidic effect of Asiatic pennywort should be further studied. It is also interested whether Asiatic pennywort will be beneficial in broilers under stress or during infection.

# REFERENCES


AOAC, 1990. Method of analysis. Association of Official Analytical Chemists. Washington, DC: Association of Official Analytical Chemists.

Ahmad, I., Mehmood, Z., and Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62: 183-193.

Berseth, C.L., Lichtenberger, L.M., and Morriss, F.H. 1983. Comparison of the gastrointestinal growth-promoting effects of rat colostrum and mature milk in newborn rats invivo. Am. J. Clin. Nutr. 37: 52-60.

Brinkhaus, B., Lindner, M., Schuppan, D. and Hahn, G. 2000. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine. 7(5): 427-448.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315-322.

Cheng, C.L., and Koo, M.W.L. 2000. Effect of *Centella asiatica* on ethanol induced gastric mucosal lesions in rats. Life Science. 67: 2647-2653.

Choct, M. 2001. Alternatives to in-feed antibiotics in monogastric animal industry. ASA Technical Bulletin. AN30-2001.

Choct, M., and Annison, G. 1992. The inhabitation of nutrient digestion by wheat pentosans. Br. J. Nutr. 67: 123-132.

Collington, G.K., Parker, D.S., and Armstrong, D.G. 1990. The influence of inclusion of either an antibiotic or probiotic in the diet on the development of digestive enzyme activity in the pig. Br. J. Nutr. 64: 59-70.

Conti, M., Cristoni, A., and Magistretti, M.J. 1992. Activity of delphinidin on microvascular damage models in rodents. Phytother. Res. 6: 99-103.

Corrier, D.E., Nisbet, D.J., Scanlan, C.M., Hollister, A.G., and Deloach, J.R. 1995. Control of salmonella typhimurium Colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria. Poult. Sci. 74: 916-924.

Dahlqvist, A. 1968. Assay of intestinal disaccharidases. Anal. Biochem. 22: 99-107.

Farnsworth, N.R., and Bunyapraphatsara, N. eds. 1992. Thai medicinal plants. Bangkok, Prachachon. Bangkok. pp. 111-114, 328-330.

Fleck, A, and Begg, D. 1954. The estimation of ribonucleic acid using ultraviplet absorption measurements. Biochem. Biophys. Acta. 108: 333-339.

Gauthier, R. 2002. Intestinal health, the key to productivity (The case of organic acids). Precongreso Cientifico Avicola IASA, XXVII Convencion ANECA-WPDC, pp. 1-14. Puerto Vallarta, Jal. Mexico, 30 de Abril 2002.

Giles, K.W., and Myers, A. 1965. An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature. 206: 93.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R. 1951. Proteinmeasurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Marzaeki, P., Mutiawati, Y. and Komarawinata, D. 1994. Isolation and identification of asiaticoside from *Centella asiatica* Linn. Proceeding of the 15th ASIAN Congress of Phamaceutical Sciences.

Nicholson, J.A., and Kim, Y.S. 1975. A one-step L-amino acid oxidase assay for intestinal peptide hydrolase activity. Anal. Biochem. 63: 110-117.

Palo, P.E., Sell, J.L., Piquer, F.J., Soto-Salanova, F., and Vilaseca, Lluis. 1995. Effect of early nutrient restriction on broiler chickens. 1. Performance and development of the gastrointestinal tract. Poultry Sci. 74: 88-101.

Parker, D.S., and Armstrong, D.G. 1987. Antibiotic feed additives and livestock production. Proc. Nutr. Soc. 46: 415-421.

Ramaswamy, A.S., Periyasamy, S.M., and Basu, N. 1970. Phamacological studies on *Centella asiatica* Linn. J. Res. Ind. Med. 4(2): 160-173.

Sarma, D.N.K., Khosa, R.L., Chansauria, J.P.N., and Sahai, M. 1995. Antiulcer activity of *Tinospora cordifolia* Miers and *Centella asiatica* Linn extracts. Phytother. Res. 9: 589-590.

Sarma, D.N.K., Khosa, R.L., Chansauria, J.P.N., and Sahai, M. 1996. Antistress activity of *Tinospora cordifolia* and *Centella asiatica* extracts. Phytother. Res. 10: 181-183.

Simmen, F.A., Cera, K.R., and Mahan, D.C. 1990. Stimulation by colostrum or mature milk of gastrointestinal tissue development in newborn pigs. J. Anim. Sci. 68: 3596-3603.

Singh, B., and Rastogi, R.P. 1968. Chemical examination of *Centella asiatica* Linn-III. Phytochem. 7: 1385-1393.

Srivastava, R., Shukla, Y.N., and Kumar, S. 1997. Chemistry and pharmacology of *Centella asiatica*: A review. J. Med. Aro. Sci. 19: 1049-1056.

Steel, R.G.D., and Torrie, J.H. 1960. Principle and Procedures of Statistic. N.Y: McGraw-Hill Book co., Inc.

Tan, P.V., Njimi, C.K., and Ayafor, J.F. 1997. Screening of some African medicinal plants for antiulcerogenic activity: Part 1. Phytother. Res. 11: 45-47.

Uni, Z., Gal-Garber, O., Geyra, A., Sklan, D., and Yahav, S. 2001. Changes in growth and function of chick small intestine epithelium due to early thermal conditioning. Poultry Sci. 80: 438-445.

Uni, Z., Ganot, S., and Sklan, D. 1998. Posthatch development of mucosal function in the broiler small intestine. Poultry Sci. 77: 75-82.

Uni, Z., Noy, Y., and Sklan D. 1995. Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poultry Sci. 36: 63-71.

Upadhyay, S.C., Khosa, R.L., Sharma, D.N., Kumar, M., and Chansauria, J.P.N. 1991. Total glycoside content and antistress activity of Indian and Mauritius *Centella asiatica* – A comparison. Ind. Drugs. 28(8): 388-389.

Van Der Wielen, P.W.J.J., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A.P., Van Knapen, F. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66(6): 2536-2540.

Van Keulen, J., and Young, B.A. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44(2): 282-287.

WHO. 1999. WHO monographs on selected medicinal plants. Volume 1. Malta: WHO Graphics.

Windholz, M., Budavari, S., Blummetti, R.F., and Otterbein, E.S. 1983. The merck index: An encyclopedia of chemicals, drugs, and biologicals. 10th ed. New Jersey: Merck.

# BIOGRAPHY

Mr. Koonphol Pongmanee was born on January 21, 1978 in Chiang Rai, Thailand. He graduated from the Faculty of Agriculture, Chiang Mai University. He received Bachelor degree of Science of the Agriculture in 2000.