INSTITUTE OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION

EFFECTS OF *ANDROGRAPHIS PANICULATA* (BURM. F.) NEES ON PERFORMANCE, MORTALITY AND COCCIDIOSIS IN BROILER CHICKENS

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LIST OF ABBREVIATIONS

%	percent
°C	degree Celsius
ACE	angiotensin converting enzyme
ADG	average daily gain
ANOVA	analysis of variance
AP	*Andrographis paniculata*
$BaCl_2$	Barium chloride
C.	*Clostridium*
cm	centimeter
CRD	completely randomized design
CTC	chlortetracycline
DA	14-deoxy-11
DC	doxycycline
DDA	12-didehydroandrographolide
DLD	Department of Livestock Development
DRC	Diagnosis Research Center
E.	*Eimeria*
E. coli	*Escherichia coli*
EHEC	enterohemorrhagic escherichia coli
ETEC	enterotoxigenic escherichia coli
EU	European Union
FAO	Food and Agriculture Organization
FCR	feed conversion ratio
g	gram
g/day	gram per day
g/kg po	gram per kilogram per oral
g/kg/day	gram per kilogram per day
g/l	gram per liter
GF 254	silica gel
GOT	glutamine oxaloacetic transaminase

GPT	glutamine pyruvic transaminase
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
hr	hour
hRf	100 x Rf
HUS	hemolytic uremic syndrome
i.e.	for example
iNOS	inducible nitric oxide syndrome
IR	irradiation ray
kg	kilogram
l	litre
LD_{50}	lethal dose of 50% of treated target animals
LSD	least significant different
m	metre
M	molar
ME	metabolisable energy
MeOH	methanol
mg	milligram
mg/dl	milligram per decilitre
mg/kg BW	milligram per kilogram body weight
mg/kg ip	milligram per kilogram intra peritoneal
mg/ml	milligram per millilitre
MHA	Mueller-Hinton Agar
MIC	minimal inhibitory concentration
min	minute
MJ/kg	Megajoule per kilogram
ml	mililitre
ml/kg	milligram per kilogram
mm	millimetre
MR	mortality rate
MRLs	maximum residue limits
n	number
Na_2So_4	sodium sulphate

NaOH	Sodium hydroxide
nm	nanometre
NO	nitric oxide
OTC	oxytetracycline
P.	*Pasteurella*
pH	hydrogen ion concentration
ppm	parts per million
Rf	retardation factor or relative front, distance between start line and substance zone divided by the distance between the start line and the mobile phase front
S.	*Salmonellae*
S. aureus	*Staphylococcus aureus*
S. spp.	*Salmonellae* species
SBP	systolic blood pressure
SEM	standard error of the mean
spp.	species
TC	tetracycline
TCM	Traditional Chinese Medicine
TCs	tetracycline antibiotics
THM	Thai Herbal Medicine
TLC	thin layer chromatography
UV	ultra violet ray
V.	*Vibrio*
w/v	weight by volume
WHO	World Health Organisation

1. INTRODUCTION

In Thailand, broiler production plays an important role in income generation of farmers. To improve broiler production, some farmers use antibiotics including chlortetracycline, tetracycline, viginiacin, spiramycin, tylosin phosphate, zinc bacitracin and avopacin. The excessive use of these antibiotics has led to contamination of Thai broiler meat. Therefore, poultry meat from Thailand has been banned from European and Japanese markets. Moreover, there is now a world tendency to produce "natural" food which is free from chemicals, the so called "Green Products".

Attempts to use medicinal plants in broiler production instead of antibiotics have been made by both farmers and researchers. However, only few trials have been carried out so far (BUNYAPRAPHATSARA, 2000). One of the medicinal plants that seems promising is *Andrographis paniculata* (AP), a shrub found throughout Southeast Asia. It is a well known medicinal plant commonly used in humans as an immune system booster. Also, it is very widely used in China, for healing common colds, inflammations and diarrhea (MPRI, 1999). In Thai Pharmacopoeia it is described for treating common colds, pharyngotonsillitis and sore throat (THAI PHARMACOPOEIA, 1995). The plant has some medicinal properties against diarrhea and dysentery in humans (THANANGKUL and CHAICHANTIPYUTH, 1985). In Europe and in South America, people use this plant for common colds and sore throats (RESEARCH REVIEW, 1997). Its main active compound is thought to be andrographolide, a diterpenoid lactone (TANG and EISENBRANDT, 1992).

Coccidiosis is one of the most expensive and most common poultry diseases in Thailand. It is commonly treated with anti-coccidial drugs in the feed. AP is now used by some farmers as a feed additive to broilers to avoid the use of these drugs. Although there are claims that the AP leaf supplement reduced mortality among broilers, there is no information about the real effect of this plant on broiler performance and coccidiosis. Also, the amounts of this plant to be mixed into broiler feed so as to prevent disease is also lacking. To solve this problem a trial on the effect of different levels of AP in broiler feed was conducted. The antibacterial properties of AP leaves were also tested because other serious poultry diseases in Thailand such as salmonellosis and colibacillosis are caused by bacteria.

The goal of this research work is to test the possibilities of improving broiler performance and reducing mortality using AP leaf preparations as feed additives as opposed to the conventional use of antibiotics.

The objectives of this study were as follows:

1. To analyze *Andrographis paniculata* powder preparation according to the quality specifications of Thai Herbal Medicine.

2. To determine the antibacterial activity of aqueous and alcohol extracts of *Andrographis paniculata* leaf powder using disk agar diffusion method with antibacterial susceptibility test.

3. To determine the effect of varying levels of *Andrographis paniculata* leaf powder in broiler feed on coccidiosis, broiler performance and mortality.

4. To compare the effect of varying leaves of *Andrographis paniculata* leaf powder and chlortetracycline additives on broiler performance and mortality.

The plan in Figure 1.1. form the basis of the experimental investigations.

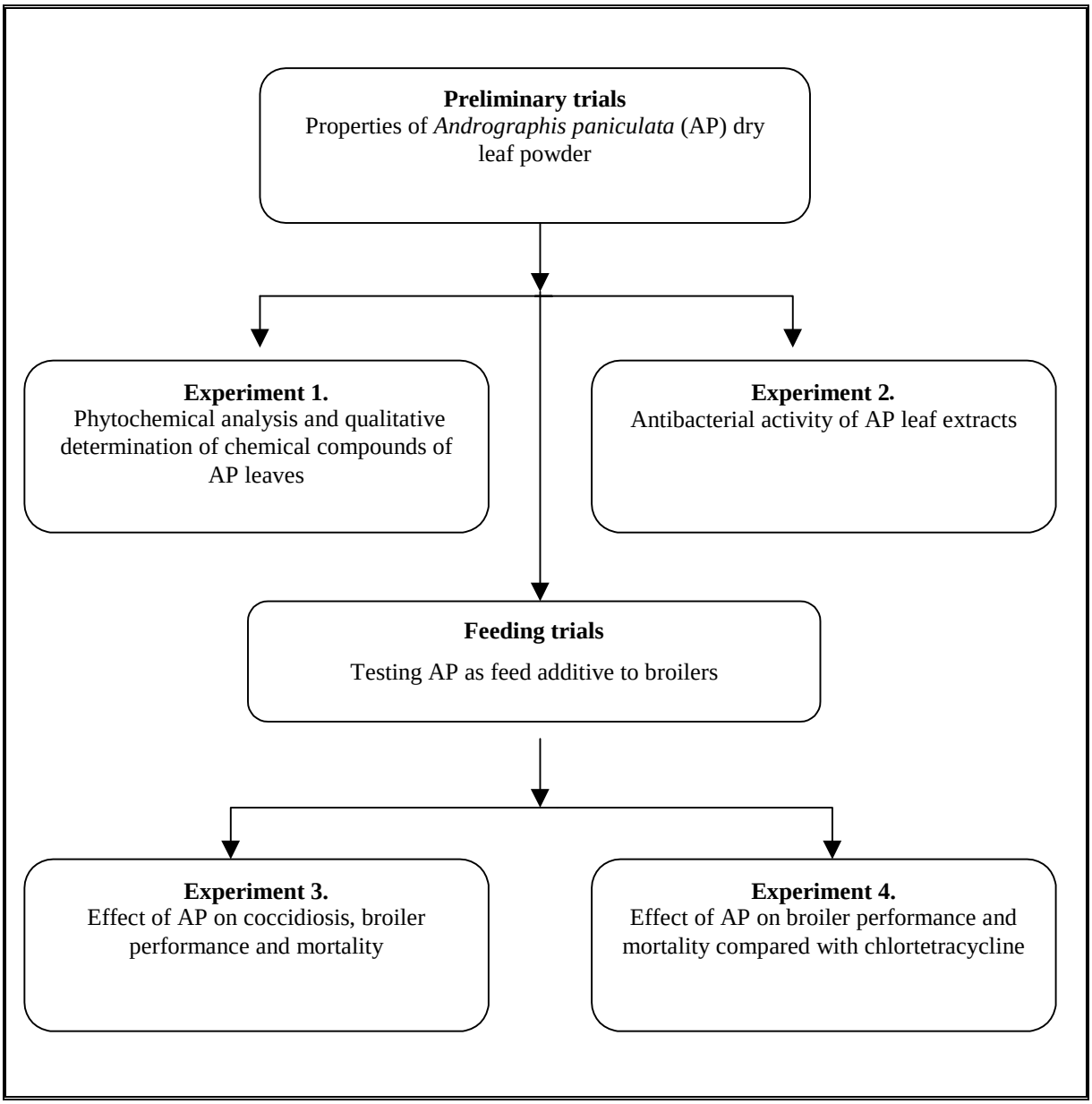


Figure 1.1. Plan of investigation

2. LITERATURE REVIEW

2.1. Botanical characteristics of *Andrographis paniculata* (AP) plant

Andrographis paniculata (AP) belongs to the family of *Acanthaceae.* This family includes many species which are meant to have medical properties. In Pakchong Research Station Garden, Nakhornradchasema Province, Thailand, many Thai medicinal plants have been collected and also medicinal plants of the family *Acanthaceae* including: *Andrographis paniculata* (Burm. f.) Nees (MPRI, 1999), *Acanthus ebrateatus* Vahl, *Barleria lupulina* Lidl., *Clinacanthus nutans* (Burm. f.) Lindau, *Justicia betonica* Linn., *Rhinacanthus nasutus* (L.), *Ruellia tuberosa* Linn. and *Thunbergia laurifolia* L. (Table 2.1.) (HANCHANLERD et al., 1994).

Andrographis paniculata (Burm. f.) Nees is a well known plant with many names. Scientific synonym are *Andrographis paniculata* Wall ex. Nees (DMPRD, 1990) and *Justicia paniculata* Burm. f. The English name is King of Bitter and other names are Chiretta, The Creat, Creyat Root, Halviva, Kariyat, Green Chiretta and Kreat (RUENGRUNGSRI and TUNTIWAT, 1994; MAUNWONGYATHI, 1994). AP is a 0.3 - 1.0 m high erect annual plant, which grows wild and abundantly in Southeast Asia: in India, Pakistan, Sri Lanka, Indonesia, China and Thailand. Its stem is dark green, 2 - 6 mm in diameter, quadrangular with longitudinal furrows and wings at angles of the younger parts (Figure 2.1). The leaves are opposite, decussate, lanceolate, up to 8 cm long and broad, glabrous, margin entire, venation pinnate; the petiole is very short. The flowers are small with bilabial corollas. The fruits are small 2-celled odorless capsules which taste intensely bitter. AP is found in evergreen, pine and deciduous forests and along roadsides. It can grow in all types of soil and it is the only plant that grows on what is called “serpentine soil” formations. This type of soil contains metals such as aluminum, copper and zinc. AP's ability to grow in such a harsh environment may explain its wide distribution. The plant has been observed to grow luxuriously in mild humid locations with tropical temperature and high rainfall (DMPRD, 1990; MPRI, 1999).

The local names of AP vary depending on region. For instance in central plain region in Thailand, it is called Num-Lai-Pung-porn or Fah-Tha-Laai-Joan. In Songkla province in Southern region it is called Ya-Gun-Ngoo. In Roi-Ed province in northeast region it is called

Sam-Sib-Dee. In Pattalung province in southern region it is called Fah-Sa Tan. In Potaram District, Ratchaburi province, it is called Kei-Tai-Yai-Klum and in Ya-La province in the southern region, it is called Mek-Tha-Laai (THAI PHARMACOPOEIA, 1997; MAUNWONGYATHI, 1994). In China it is called Kee-Pang-He, Chung-Sim-Noi, Jek-Keing-He, Kong-Chuil and See-Pang-Kee (HUANG, 1993; MANJUNATH, 1948).

Table 2.1. Medicinal plants of the family *ACANTHACEAE* that have been collected in Pakchong Research Station Garden in Thailand and their traditional use

Plant species	Characteristic	Seasonal blooming	Traditional use
1.*Acanthusebrateatus* Vahl	Shrub	Rainy season	All stem and seeds: healing of wounds, worm protection, fresh leaves for healthy hair
2.*Andrographis paniculata* (Burm. f.) Nees.	Perennial shrub	All year	Leaves and stems: healing sore throat and diarrhea
3. *Barleria lupulina* Lindl.	Shrub	Rainy season	Blended leaves: healing inflammation after insect bites
4.*Clinacanthus nutans* (Burm.f.) Lindua	Shrub	In January	Fresh leaves: healing burnings and scalds
5. *Justicia betonica* Linn.	Shrub	All year	No information
6. *Rhinacanthus nasutus* (L.)	Small Shrub	In January	Leaves and roots: healing skin diseases Roots are antiseptic
7. *Ruellia tuberosa* Linn.	Perennial shrub (long life)	Rainy season	Roots: reducing toxicity, healing urine tract inflammation
8. *Thunbergia laurifolia* L.	Shrub	Rainy season	Leaves: healing stomachache

Source: HANCHANLERD et al. (1994).

Figure 2.1. *Andrographis paniculata* (Burm. f.) Nees plant
Source: MPRI (1999).

2.2. Chemical composition of AP

The Sichuan Chinese Herb Research Institute reported that Andrographolide was first isolated from Andrographis by BOORSMA in 1896. It is a crystal compound with a very bitter taste with colorless crystalline appearance. In 1911 CORTER identified this compound as lactone (SCHRI, 1973).

DENG et al. (1982) reported that there are four lactones in *Andrographis paniculata,* including deoxyandrographolide (Andrographis A), which was also identified by

SANGALUNGKARN et al. (1990) and GARCIA et al. (1980), andrographolide (Androghraphis B), neoandrographolide (Andrographis C) and deoxydidehydro-andrographolide (Andrographis D) which were also identified by DHAMMA-UPAKORN et al. (1992). Andrographolide and total lactone are the common names used in clinics for the active ingredients (DENG et al., 1982).

The other medicinal chemicals are also bitter principles: diterpenoids viz. Deoxyandrographolide, -19-D-glucoside which have been isolated from the leaves (TECHADAMRONGSIN et al., 1999).

The active chemical constituents of *Andrographis paniculata* which have been identified so far include diterpene lactones (TANG and EISENBRANDT, 1992) and flavonoids (ZHU and LIU, 1984; KUROYANAGI et al., 1987). The main diterpenoids that have been isolated from *Andrographis paniculata* are 14-deoxyandrographis (DA, $C_{20}H_{30}O_4$, late crystal from methanol) and 14-deoxy-11,12-didehydroandrographolide (DDA, $C_{20}H_{28}O_4$, needle crystal) as reported by BALMAIN and CONNOLLY (1973) and KONGKATHIP (1995). MATSUDA et al. (1994) found that DDA has hydroxyl, alpha, beta-unsaturated gamma-lactone and exo-methylene groups in its chemical structure. This is very similar to that of DA, with the exception of a double bond at C-11 and 12 (Figure 2.2).

The concentration of these diterpene lactones depend both on growing region and season. *Andrographis paniculata* appears to grow best in the tropical and subtropical areas of China and Southeast Asia. The leaves contain the highest amount of the active components and the stems contain the lowest amount (MPRI, 1999) but SHARMA et al. (1992) found the seeds contain the lowest amount. The highest concentration of the active components is found just before the plant blooms, making early fall the best harvesting time. In those parts of Asia where *Andrographis paniculata* is sold commercially as medicine, a variety of laboratory methodologies are used to ensure a standardized level of andrographolides: thin-layer chromatography, ultraviolet spectrophotometry, liquid chromatography and volumetric and colorimetric techniques (QCMMPM, 1992; MPRI, 1999).

The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals. The leaves contain the highest amount of andrographolide (2.39%), the medically most active

phytochemical in the plant, while the seeds contain the lowest amount (RESEARCH REVIEW, 1997).

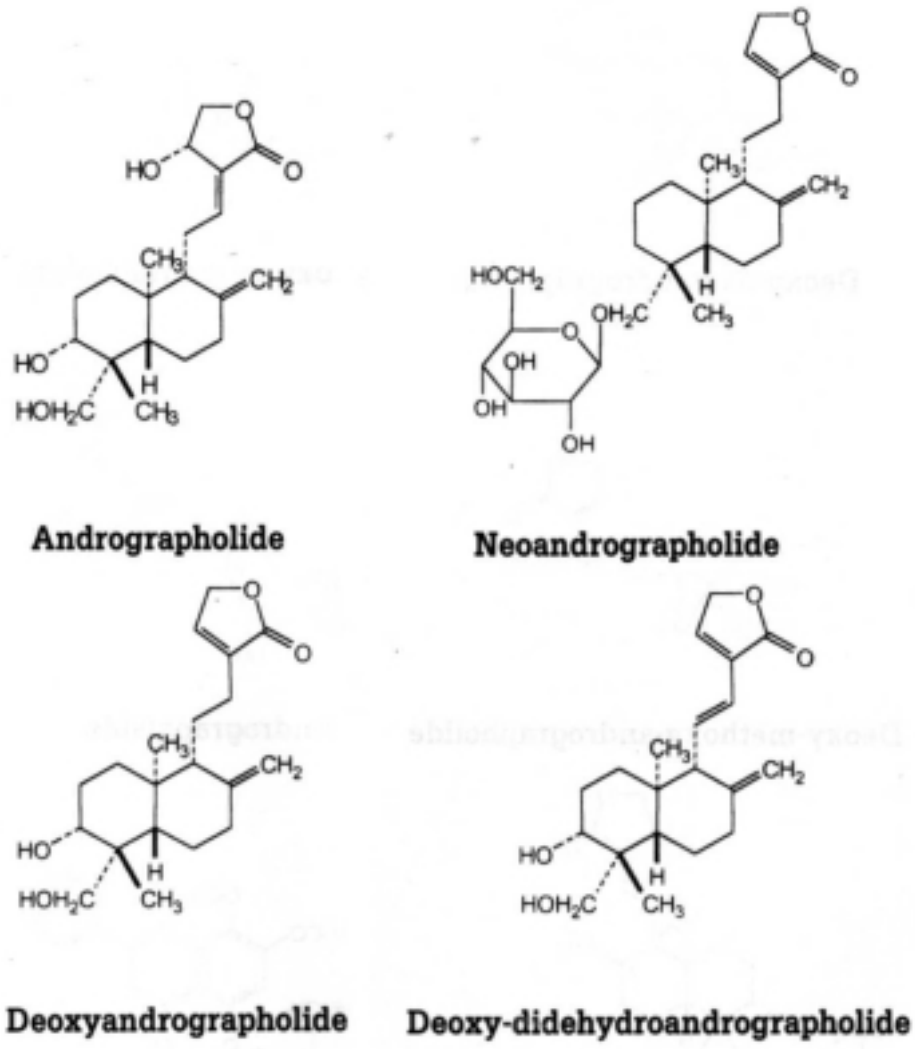


Figure 2.2. Chemical construction of the main active compounds in AP leaves
Source: MPRI (1999).

RAJANI et al. (2000) reported a simple and rapid method for isolation of andrographolide from the leaves of AP. It involves extraction of the leaf powder by cold maceration in a 1:1 mixture of dichloromethane and methanol and isolation of andrographolide directly from the resulting extract by recrystallisation. The identity of the compound is confirmed through IR, UV, mass and melting point and co-chromatography with a reference standard on TLC. The purity of the compound is confirmed by TLC, UV absorption spectrum, HPLC and differential scanning calorimetry, the latter gave the melting point of andrographolide as 235.3°C.

2.3. Production of AP leaves

For the production of a good quality drug from AP leaves not only good laboratory methods have to be assured, the raw material should also reach the factory in a good condition (TECHADAMRONGSIN et al., 1999). Each of the following four steps are of importance for the product quality: cultivation, harvesting, post- harvest handling, packaging and storage.

2.3.1. Cultivation

The seed should be mature and less destroyed by insects. About 1 table spoon of seed (6.5 - 7g) has 7,000 seeds (Figure 2.3.). Normally AP seeds should have a germination rate of not less than 85% (DMPRD, 1990). In a study in Thailand in 1996 on the cultivation of the AP plant to improve its quality as medicinal plant, it was found that AP plant grow at different rate depending on the season. If AP is cultivated in June the production of leaves (fresh matter) was higher than if it was cultivated in July, November, October and August, respectively. Also it is suggested that the AP plant can be grown in all types of soil but it should be avoided to cultivate it in soil that is flooded or wet all year and also on serpentine soil it will give very low production (KASETKLANGKLUNG, 1996).

Figure 2.3. Seeds of AP plant
Source: MPRI (1999).

2.3.2. Harvesting

The harvesting should be performed from the beginning of the flowering period until around 50% blooming (DECHATIWONGSE NA AYDHYA et al., 1988) and when the plant have an age of 110 - 150 days (SUWANBAREERAK and CHAICHANTIPYUTH, 1991). This period will give highest percent of active ingredients (diterpene lactones) in AP (Table 2.2.). The plant will bloom fast or slow depending on the environmental situation (PROMTO et al., 1997). By harvesting the stem should be cut 5 - 10 cm above ground level to allow for renewed growth for the next harvest.

Table 2.2. Percent total diterpene lactones in AP after harvesting at different growth periods

Sample	Period of harvesting	Total diterpene lactones (%)		
		Aerial part	Leaf	Stem
1.	Before Blooming	6.09	6.80	2.28
	Blooming	7.31	9.81	2.06
	Young fruit	5.29	6.79	3.93
	Mature fruit	4.80	6.44	4.67
2.	Before Blooming	7.05	9.54	3.02
	Blooming	9.79	12.52	5.81
	Young fruit	6.72	7.25	6.25
	Mature fruit	5.54	6.63	6.89

Source: MPRI (1999).

2.3.3. Post-harvest handling method

After harvesting the plants should be cleaned in water and cut in pieces at the size of 3 - 5 cm. Drying can be done by using dehydration or hot air oven at 50°C for 8 hours and after that 40 - 46°C until the material is dried properly (MPRI, 1999).

2.3.4. Packaging and storage

The dried material can be stored in airless plastic bags. If it is only a small amount it can be kept in a dry clean glass. It should be kept in a clean cool place. It is important not to store it

more than 1 year because the percent of total diterpene lactones will decrease up to 25% after 1 year (Table 2.3).

Table 2.3. Percent total diterpene lactones in the aerial part of AP after different periods of storage

Sample	Period of storage (Month)	Diterpene lactones (%)	Diterpene lactones decreased (%)
1	0	9.25	0.00
	3	9.13	1.30
	6	8.56	7.46
	9	7.99	13.62
	12	7.09	23.35
2	0	3.85	0.00
	3	3.58	7.01
	6	3.33	13.50
	9	3.18	17.40
	12	2.92	24.16
3	0	6.20	0.00
	3	6.03	2.74
	6	5.02	19.03
	9	4.18	32.58
	12	4.11	33.70
4	0	7.68	0.00
	3	7.32	4.69
	6	7.08	7.81
	9	6.78	11.72
	12	5.63	26.69
5	0	6.86	0.00
	3	6.44	6.12
	6	6.12	10.79
	9	5.78	15.74
	12	5.04	26.53

SOURCE: MPRI (1999).

2.3.5. Yield of *Andrographis paniculata*

AP leaves can be found on markets in every region of Thailand. A farmer in Karnjanaburi Province has cultivated AP plants for 3 years. He harvests up to 1000 kg dried leaves per Rai (1,600 m^2) per year. He sells the dry leaves for up to 100,000 Baht (2,500 Euro) per year at the market in Nakornpathom Province and at the local market (CHAIWONGKEART, 1997).

2.4. The use of AP as a traditional medicinal plant

AP or "King of Bitters" is a traditional medicinal plant. It has been frequently used for centuries to successfully treat upper respiratory tract infections, fever, sore throat, herpes and it also reduce inflammation and stop diarrhea (TECHADAMRONGSIN et al., 1999). Clinical experience with the use of this herb is reported in contemporary and ancient Chinese writings (WHO, 1978). In Traditional Chinese Medicine (TCM), AP is an important "cold property" herb, it is used to rid the body of heat, as in fevers and to dispel toxins from the body (OMPD, 1989; BENSKY and GAMBLE, 1993). DENG et al. (1982) also described that AP has been widely used in Chinese medicine as an anti-inflammatory and antipyretic drug for the treatment of cold, fever and laryngitis. It also has a reputation as a potent folk medicine in the treatment of diabetes and hypertension (AHMAD and ASMAWI, 1993). Chewing the fresh leaves is claimed to be effective against hypertension. YEUNG et al. (1987) reported that AP had phamacological properties which include antibacterial, immunological, antivenin and antithrombotic properties. A hepatoprotective effect of AP was reported by HANDA and SHARMA (1990), while antifertility activities were reported by AKBARSHA et al. (1990). In a coloured atlas of the Chinese materia medica specified in pharmacopoeia of the People ' s Republic of China in 1995 it is reported that AP can act to remove heat, counteract and induce subsidence of swelling and it is indicated that AP can heal influenza with fever, sore throat, ulcers in the mouth or on the tongue, acute or chronic cough, colitis, dysentery, urinary infections with difficult painful urination, carbuncles and venomous snack bite (ACACMSP, 1995).

In China, AP was used in different forms for example as tablet or injection: In tablet form it has different names: Kang Yang tablets, Chuanxinlian tablets and Chuanxinlian antiphologistic Pills. The injection forms are Yamdepieng, Chuanxinlian Ruangas injection (MAUNWONGYATHI, 1994).

The plant is also well known in the Indian Pharmacopoeia. It is prominent in at least 26 Ayurvedic formulas. In Scandinavian countries AP is also used extensively to prevent and treat common colds (RESEARCH REVIEW, 1997). Research conducted in the 80´s and 90´s has confirmed that AP properly administered, has a surprisingly broad range of pharmacological effects, some of them extremely beneficial (MPRI, 1999).

In Thailand the utilization of the AP plant as a medicinal herb has a long history of successful use in the treatment of numerous conditions. One suggestion is to fill 500 mg AP leaf powder in capsules and eat 2 capsules four times per day to reduce diarrhea or sore throat (DMPRD, 1990). Another suggestion is to use AP leaf powder to reduce cough and sore throat AP leaf powder is made into pills of which 3 - 6 pills should be consumed 4 times per day after meal and before bed time (OMPD, 1989). To reduce inflammations from injuries 1 handful of AP leaves, 3 crystals of salt are blended with half teacup of drinking alcohol and half teaspoon water. The filtrate is to drink and the fiber residue should be put on the wound and covered with clean bandage (PMPPHC, 1996). In Thailand it is believed that AP can be used as internal and external drug. As internal drug it can be used for dysentery, intestinal inflammation, common cold, tonsillitis, sore throat and influenza. As external drug it can be used for healing fire burns and scalds using fresh leaves blended with vegetable oil and placed on the wound, the same method can be used for healing itching (MAUNWONGYATHI, 1994). In Thai Medicinal Plants Magazine it is suggested that an extract of boiled roots can be effective against *Staphylococcus aureus*, an extract made by boiling all stem with methanol can be effective against *Proteus vulgaris* and stem and leaves blended into powder can be effective against the *Shigella* bacteria but it is not effective against cholera. Also an ethanol extract of AP leaves is effective against *Staphylococcus aureus*, while hot water extract is not effective. An ethanol extract made by boiling is effective to *E. coli* (BUNYAPRAPHATSARA, 2000).

The knowledge of the benefits of the AP plant for human health has also led to its use in livestock production. On several farms AP leaves or mixtures of AP and other leaves are fed to animals. Farmers believe that mixing AP leaves into chicken feed reduce mortality especially based on digestive tract and respiratory tract diseases (CHAIWONGKEART, 1997).

2.5. Physiological and pharmacological properties of AP leaves

Andrographis paniculata has been extensively studied, mainly during the last half of the 20th century, concentrating on its pharmacological composition, safety, efficacy and mechanisms of action (MPRI, 1999).

2.5.1. Effect of AP leaves on inflammation and fever

AP is used as a folk medicine for reducing inflammation. DUTTA and SUKUL found in 1982 that 3 of the effective ingredients in AP leaf powder are effective in reducing inflammation: deoxyandrographolide, andrographolide and neoandrographolide (DUTTA and SUKUL, 1982). In other studies it was found that AP extracted with alcohol (SAWASDIMONGKOL et al., 1990; CHANTASUTRA and LIMPAPANICHKUL, 1989), AP extracted with water (SAWASDIMONGKOL et al., 1990) and AP extracted with chloroform (CHANTASUTRA and LIMPAPANICHKUL, 1989) reduced inflammation. In China it has been reported that andrographolide has some beneficial effects as an anti-inflammatory agent (CHIOU et al., 2000; SHEN et al., 2000), whereas DENG et al. (1982), who studied in rats and mice concluded that all four lactones have anti-inflammatory and anti-pyretic effects. Since the herb showed no effect in treating infected animals when the adrenal gland of the animals were totally removed, they suggested that andrographis might exert its anti-inflammation effect through stimulation of the adrenal gland.

In Thailand rats were given injections with carrageenan (agent stimulating inflammation) to study the anti-inflammatory effect of 500, 1250, 2500 mg AP water extract/kg BW. The result showed that water extract of AP effectively reduced the paw volume in rats treated with AP whereas the control group and a group treated with indomethacin (anti-inflammatory drug) did not obtain these results (Figure 2.4.).

The ability of AP to lower fever has been demonstrated independently in several laboratories. Rat studies done in China have shown that andrographolide, neoandrographolide and dehydroandrographolide can lower fever produced by different fever-inducing agents, such as

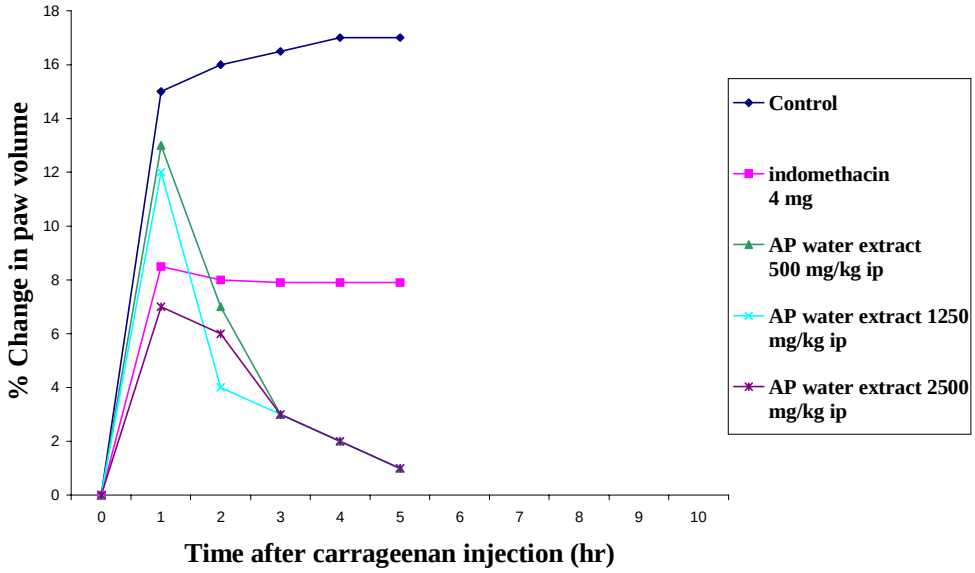


Figure 2.4. Anti-inflammatory effect of water extract of AP in rats
Source: Modified after DMPRD (1990).

bacterial endotoxins of *Pneumococcus spp.*, hemolytic *Streptococcus*, *P. multocida* and the chemical 2, 4-dinitrophenol (HUANG, 1993).

Sichuan Provincial Herb Institute in China used Andrographis A, B and C to treat 84 cases (50 male and 34 females) of 4 to 55 years old flu patients in 1973. None of the patients whose temperature ranged from 38°C to 40°C took antibiotics before they were treated in the hospital. The patients were divided into three groups and given Andrographis A, B and C, respectively. The total effective rate was 83.3%. However, antibiotics were added to the treatment for 14 infective cases (SCHRI, 1973).

In India researchers tested AP to determine whether it could reduce fever (MADAV et al., 1995). Fever was induced in rats. There was a reduction in rectal body temperature for 30, 100 and 300 mg of andrographolide/ kg BW. While the analgesic (painkilling) activity of andrographolide extracted from AP was weak compared to aspirin, the antipyretic (fever-reducing) activity of 300 mg of andrographolide /kg BW was comparable to that of 300 mg aspirin.

In another study, AP extracts were found to produce results comparable to 200 mg of aspirin/kg BW (VEDAVATHY and RAO, 1991). The researchers also established that there

was a wide margin of safety in using AP extract, an indication of the lack of toxicity. In Thailand, further studies on rabbits showed that the antipyretic effect of 2.5 g 85% ethanolic extract of AP /kg BW was as effective as 300 mg/kg of aspirin (Figure 2.5.).

2.5.2 Effect of AP leaves on the common cold

The effectiveness of *Andrographis paniculata* extract compared against a placebo, to lessen the symptoms associated with common cold was measured in a patients group of 158 male and female adults (CACERES et al., 1999). The effects of AP were measured on days 0, 2 and 4 of the treatment. On day 2, the patient group taking AP displayed alleviation of several of the associated symptoms, and on day 4, the same group was shown to have significant reductions in all of the symptoms as compared with the placebo group. CACERES et al. (1999) and HANCKE et al. (1995) concluded that AP had a high degree of effectiveness in reducing the prevalence and intensity of the symptoms in uncomplicated common cold beginning at day two of treatment. No adverse effects were reported.

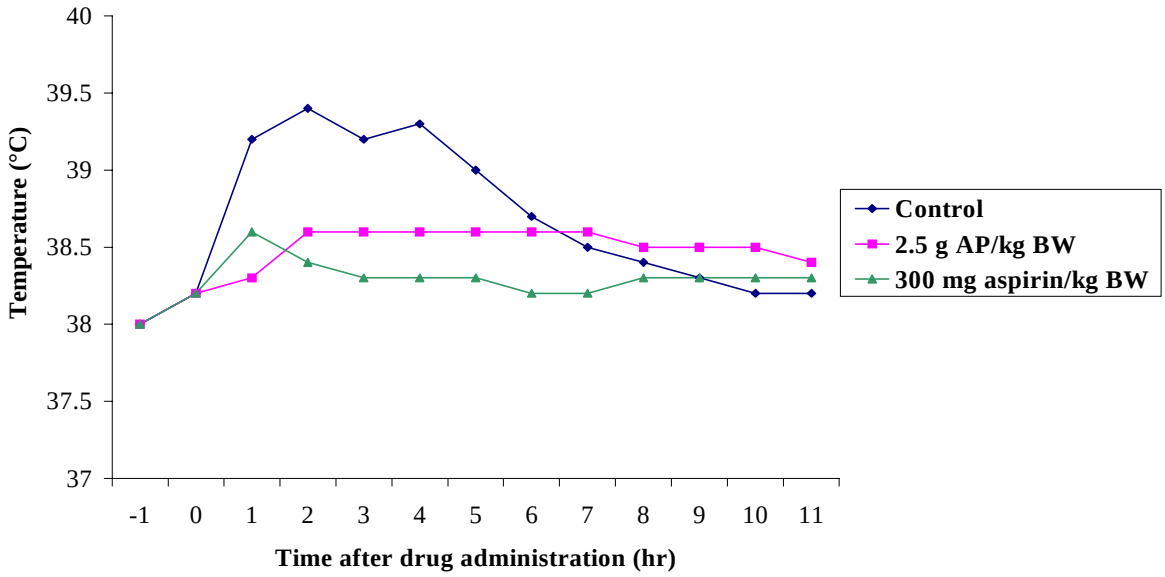


Figure 2.5. Antipyretic effect of 85% ethanolic extract of AP in rabbits
Source: Modified after DMPRD (1990).

In another study conducted by the same group (CACERES et al., 1997), students in a rural school were given either placebo or a dose of 200 mg a day of Kan Jang, a formulation of AP from the Swedish Herbal Institute. The number of colds occurring over a three month period was observed. After 1 month no significant difference was found. However after 3 months,

significant benefits were reported. The Kan Jang group was 2.1 times less likely to catch a cold than the placebo group. They had a rate of incidence of 30%, compared to 62% in the placebo group.

2.5.3. Effect of AP leaves on diarrhea and intestinal tract

Diarrhea diseases are one of the top ten causes of death worldwide and are a leading cause of death in children in developing countries, especially among those that are under five years of age. The use of antibiotics is producing antibiotic-resistant strains of bacteria. There are many drugs used to relieve the symptoms of diarrhea (kaolin-pectin, loperamide, bismuth, lomotil etc.), but they may have undesirable side effects (SINDERMSUK, 1993). An inexpensive and easily obtained herbal remedy would benefit many, especially people in developing countries where diarrhea disease is almost catastrophic. Experiments in animals demonstrate that AP can prevent or stop diarrhea. Extracts of AP have been shown to have significant effect against the diarrhea associated with *E. coli* infections (DUKE and AYENSU, 1985). The AP components andrographolide and neoandrographolide showed comparable activity to loperamide (Imodium), the most common anti-diarrheal drug. GUPTA et al. (1990) reported, that the active ingredients against diarrhea are andrographolide and deoxyandrographolide.

At Ramatipbodee Hospital in Bangkok, AP was tested against diarrhea by people. 1 g AP leaf powder in capsule was given every 12 hours for 2 days to the patients who got diarrhea. After 2 days it was found that AP leaf powder gave better results than tetracycline, but AP leaf powder can not heal cholera better than tetracycline (THANANGKUL and CHAICHANTIPYUTH, 1985). In one study, acute bacterial diarrhea in patients was treated with a total dose of 500 mg andrographolide divided over three doses per day for six days (2.5 to 3.0 mg/kg BW). This regimen was combined with rehydration. There were 66 cures among 80 treated patients, an 82.5% cure rate. Seven additional patients responded favorably to the treatment and only seven patients (8.8%) did not respond. The effectiveness of the treatment was confirmed by laboratory tests of stool samples (YIN and GUO, 1993). In another study, AP was used to treat 1,611 cases of bacterial dysentery and 955 cases of diarrhea with overall effectiveness of 91.3% (CHTURVEDI et al., 1983). In a pharmacological research institute in Shanghai, China, a study on 165 dysentery patients was conducted in 1973, 165 patients were given *Andrographis paniculata* tablets equal to the amount of 15.6 g crude powder per day. Twenty-eight patients were given Fluroxone, a common drug used to treat dysentery. The

result showed the effective rate of *Andrographis paniculata* was 75.2% and the effective rate of Fluroxone was 71.4% (SCHRI, 1973).

It has been believed that AP was effective against bacterial dysentery and diarrhea because it has antibacterial activities and PLEUMJAI and SITHISOMWONGES (1990) also found in *in-vitro* trials that AP extract with 70% and 80% ethanol can kill bacteria that cause diarrhea for example *E. coli* and *V. cholerae*. Other studies could not confirm this effect (SINDERMSUK, 1993). However, the andrographolides are very effective in stopping diarrhea. How this is accomplished is not completely understood at present.

In one study on mice it was found that 50% and 85% alcohol extract of AP leaves powder were effective in reducing intestinal tract movements (SAWASDIMONGKOL et al., 1990; THAMAREE et al., 1985). CHOUDHURY and PODDAR (1985) compared the effect of andrographolide and Kalmegh (*Andrographis paniculata)* extract on intestinal brush-border membrane-bound hydrolases. They suggest that both andrographolide and Kalmegh accelerate intestinal digestion and absorbtion of carbohydrate by activating the intestinal disaccharidases.

2.5.4. Effect of AP leaves on respiratory system

Andrographis paniculata was used to treat cough and sore throat by THAMLIKITKUL and his staff in 1991. They compared AP leaf powder with Paracetamol and found that 6 g AP leaf powder per day after three days reduced fever and sore throat better than Paracetamol or 3 g AP leaf powder per day. However, after seven days the healing process among those 3 treatments was not different (THAMLIKITKUL et al., 1991). In 1996, the use of AP leave powder and Penicillin V was compared for healing sore throat. The results show that there were no significant difference in recovery rate between the groups of patients. More than 75% of patients were cured and among the rest more than 15% getting better (SUBJAREUN, 1996).

In 1972, the Chinese Herb Research Institute used Andrographis A (deoxyandrographolide) and Andrographis C (neoandrographolide) to treat 24 cases of upper respiratory tract infection, acute tonsilitis and bronchitis. The average recovery time was about 4 to 7 days (SCHRI, 1973).

Andrographis paniculata has further been used to treat tonsillitis, respiratory infections, and tuberculosis. In one study, AP was used to treat 129 cases of acute tonsilltis. Sixty-five percent of patients responded to the therapy (AKBARSHA et al., 1990). The same authors used andrographolide to treat 49 pneumonia patients. Thirty five cases were found to show positive changes and nine patients completely recovered.

Tuberculosis is usually treated with the antibiotic rifampin. When used alone, rifampin therapy still results in 22.5% of patients dying. In a study using an injectable solution of 2.5% andrographolide given so as to provide 50 to 80 mg/kg body weight per day for two months, results were improved. Of seventy cases of tubercular meningitis, 30% of patients were considered cured with a fatality rate of 8.6% (ZOHA et al., 1989). The combination of andrographolide plus rifampin resulted in a 2.6 fold decrease in fatality rates.

2.5.5. Effect of AP leaves on cardiovascular system

AMROYAN et al. (1999) found an inhibitory effect of andrographolide from *Andrographis paniculata* on the biosynthesis of eicosanoids and a platelet-activating factor. They indicated that andrographolide has a mechanism of action different from the action of non-steroidal anti-inflammatory drugs. Most likely this mechanism is associated with the cardiovascular and antithrombotic activity of AP. ZHANG and TAN (1996) found a hypotensive activity of aqueous extract of AP in rats and they indicated that the aqueous extract of AP lowers the systolic blood pressure (SBP) of spontaneously hypertensive rats possibly by reducing circulating angiotensin-converting enzyme (ACE) in the plasma as well as by reducing free radical levels in the kidneys. In further studies by ZHANG and TAN (1997); ZHANG et al. (1998) and TAN and ZHANG (1998) on cardiovascular activity of 14-deoxy-11 (DA),12-didehydroandrographolide (DDA, diterpenoids of AP) in rats, they found that, DA and DDA decreased significantly the mean arterial pressure and heart rate of anaesthetized rats. Both DA and DDA caused relaxation of the isolated rat thoracic aortae through activation of the nitric oxide (NO) production in endothelial cells. Also CHIOU et al. (1998) indicated that andrographolide inhibits nitrite synthesis by suppressing expression of inducible nitric oxide synthase (iNOS) protein in vitro, and this inhibition of iNOS synthesis may contribute to the beneficial haemodynamic effects of andrographolide in endotoxic shock.

Researchers at the Tongi Medical University in China have demonstrated that AP given to dogs one hour after development of myocardial infection decreased the damage that occurred to the heart muscle (ZHAO and FANG, 1991). Such damage occurs after the blood supply is restored to the muscle. This is due to a sudden influx of oxygen (which produces free radicals that damage tissue) and abnormally high amounts of calcium. In subsequent studies at the same university, the researchers demonstrated by electrocardiography that abnormal changes in heart readings were prevented by pretreatment with AP. Also, clumping of platelets was inhibited and clotting that could cause infarction was not induced (ZHAO and FANG, 1991). An added effect of AP was that it activated fibrionolysis, the natural process in the body that dissolves clots (HUANG, 1993).

2.5.6. Effect of AP leaves on nervous system

Many compounds do not penetrate the blood-brain barrier. However, andrographolide does so and concentrates in the brain and particularly in the spinal cord (WEIBO, 1995). Several studies have shown that AP products have a sedative effect. In mice given barbital as anesthesia along with AP, the animals became sedated more quickly and the anesthesia lasted longer. Also, it was possible to give less of the anesthesia if it was given along with AP (DENG, 1978). The studies indicate that AP products may act at the barbital receptors in the brain.

2.5.7. Effect of AP leaves on liver and gall bladder protection

In Ayurvedic medicine 26 different formulations containing AP are used to treat liver disorders. AP were tested by RANA and AVADHOOT (1991) and KAPIL et al. (1993) for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride, alcohol, or other toxic chemicals. These chemicals damage the liver by causing lipid peroxidation (CHOUDHURY and PODDAR, 1984). This is a process whereby free radicals (reactive molecules) produced by the chemical attack and destroy cellular membranes that surround liver cells. When the AP compounds were given to animals three days before the toxic chemicals, there was a significant protective effect in the liver. This effect was attributed to the antioxidant ability of the AP compounds, which was as effective as silymarin (another plant antioxidant from milk thistle).

Hepatoprotective effect of andrographolide was studied on acute hepatitis induced in rats by a single dose of galactosamine (800 mg/kg, ip) paracetamol (3 g/kg, po). Hepatoprotective activity was monitored by estimating the serum transaminases (GOT and GPT), alkaline phosphatase and bilirubin in serum, hepatic triglycerides and histopathological changes in the livers of experimental rats. The result confirmed the *in vivo* hepatoprotective effect of andrographolide against galactosamine or paracetamol-induced hepatotoxicity in rats (HANDA and SHARMA, 1990). VISEN et al. (1993) found also that andrographolide can protects rat hepatocytes against paracetamol-induced damage and further that andrographolide was more potent than silymarin (a standard hepatoprotective agent).

Infective hepatitis is an acute inflammatory condition of the liver. It is often followed by liver cirrhosis and may progress to coma and death. In a study from India, twenty cases of infective hepatitis (hepatitis A) in men and women were treated with a decoction of AP (Kalmegh) equivalent to 40 g of the crude compound for over twenty-four days. In all twenty patients, the yellowing of the conjunctiva of the eyes and the urine returned to normal coloration. 90% of the patients regained their appetite and 83% had relief from general depression. Overall, 80% of the patients were considered cured and 20% improved based on biochemical tests and changes in symptoms (CHTURVEDI et al., 1983). In a similar study in China, 83% of 112 cases of hepatitis were successfully treated (DENG, 1978).

The andrographolides present in AP are potent stimulators of the gallbladder function. They have been shown to produce a significant increase in bile flow (SHUKLA et al., 1992), bile salts, and bile acids (HOLT and COMAC, 1998). These increases are beneficial and result in enhanced gallbladder function. Use of AP might, therefore, decrease the probability of gallstone formation and might also aid fat digestion (HOLT and COMAC, 1998). When a chemical, paracetamol, was given to animals pretreated with andrographolide, the usual decrease in bile production seen with this chemical was prevented. In this case, andrographolide was more potent than silymarin (SHUKLA et al., 1992). The andrographolides also prevented decreases in the amount of bile that are caused by acetaminophen toxicity (HOLT and COMAC, 1998).

2.5.8. Effect of AP leaves on other diseases

AP extract has also been found to possess a certain anti-ulcerogenic activity. Among patients it reduced the development of ulcers by 31%, while the standard ulcer drug cimetidine had an 85.43% reduction rate. Andrographolide caused a significant decrease in total stomach acidity and acid stomach juice secretion, without the cost and side effects associated with other ulcer therapy.

Leptospirosis is a disease caused by the bacterium *Leptospira interogens*. Infection with this organism results in fever, hemorrhagic lesions, central nervous system dysfunction and jaundice. Several studies have shown efficacy in approximately 80% of patients treated with deoxyandrographolide, andrographolide, and neoandrographolide tablets (SCARG, 1976).

2.6. Possible negative side effects of AP leaves

Extraction is usually performed using ethanol and liquid extracts or tinctures are the most common form of dispensing the product. When consumed, andrographolides appear to accumulate in organs throughout the viscera. In one study, after 48 hours, the concentration of labeled andrographolide was 20.9% in brain; 14.9% in spleen; 11.1% in heart, 10.9% in lung; 8.6% in rectum; 7.9% in kidney; 5.6% in liver; 5.1% in uterus; 5.1% in ovary and 3.2% in intestine (ZHENG, 1982). Absorption and excretion is rapid 80% is removed within eight hours via the kidney (urine) and the gastrointestinal tract. Ninety percent is eliminated within forty eight hours.

BURGOS et al. (1997) studied the possible testicular toxicity of AP dried extract evaluated in male rats for 60 days. They found no toxicity with the treatment of 20, 200 and 1000 mg/kg during 60 days by evaluation of reproductive organ weight, testicular histology, ultrastructural analysis of Leydig cells and testosterone level after 60 days of treatment. They concluded that AP dried extract did not produce subchronic testicular toxicity effect in male rats.

In mice that received oral extracts of AP (10 g/kg BW) once a day for seven days, none of the mice died (HUANG, 1978). This very high amount did produce decreased activity and general lethargy. Heart, kidney, liver and spleen were found to be normal in these animals.

When 500 mg/kg of AP were given daily for ten days to mice, there was no effect on growth, appetite or stool production. The animal were energetic and results of complete blood counts were normal. In rabbits given intravenous andrographolide (10 mg/kg), there were no abnormal cardiovascular responses. Liver enzyme tests and heart, liver, kidney and spleen were normal in these animals (CCZSDJ, 1975). In another tests for toxicity, rats or rabbits received 1 g/kg of andrographolide or neoandrographolide orally for seven days. This amount did not affect body weight, blood counts, liver or kidney function or other important organs (YIN and GUO, 1993). Also extract of 20 g leaves with 600 ml water administered at the rate of 10 ml/kg BW produced no abnormality in rats and rabbits (CHANTASUTRA and LIMPAPANICHKUL, 1989). Neither did 2 g/kg BW of AP leaves powder, 2.4 g/kg BW of Alcoholic extracted AP and 3 g/kg BW of AP leaves powder produce any abnormalities in any sex of mice (GEORGE and PANDALAI, 1949).

In another study in Thailand no toxicity that caused more than half of the animal died (LD_{50}) was found when 15 g of 50% alcoholic extract /kg BW was given orally, more than 15 g/kg BW was given subcutaneous or 14.98 g/kg BW was injected in the stomach (DHAMMA-UPAKORN and CHAICHANTIPYUTH, 1989). In a study of PANOSSIAN et al. (1999), they found that a therapeutic dose of AP extract cannot induce progesterone-mediated termination of pregnancy in rats. However, AKBARSHA and MURUGAIAN (2000) found that it had an effect as a male reproductive toxin when used as therapeutic. Also they considered the possible prospective use of andrographolide in male contraception.

In traditional Chinese medicine (TCM) and in systems of healing in Thailand and India AP has long been perceived as safe (MPRI, 1999). Formal toxicological studies in animal models and human clinical trials confirmed that andrographolide and other members of this AP family of compounds have very low toxicity. To test the subchronic toxicity of AP, 24 rats were over 6 months administered 0, 0.12, 1.2 and 2.4 g/kg BW/day which mean doses equal to 1, 10, and 20 times that used for human doses (6 g/day/person). The growth rate and body weight of the rats were normal. Also no abnormalities were found in blood serum, inner organs, testis and ovary (DMPRD, 1990). The result is shown in figure 2.6.

Rarely, people who use AP experience dizziness and heart palpitations (MPRI, 1999). As with all herbs, some people will have an allergic reaction to AP (MPRI, 1999). The other side effect, as discussed above, is anti-fertility (ZOHA et al. 1989). In general, evidence to date

indicates that andrographolides are naturally occurring compounds with low toxicity when used appropriately.

2.7. Main poultry diseases of economic importance in Thailand

Thailand is an agricultural country. Besides other agricultural products, livestock and livestock products play an important role in national income and become more significant year by year. The government promotes livestock production with the aim to increase export as it has been stated in the National Development Plan (CHAISRISONGKRAM, 1992).

Within the livestock sector, poultry raising seem more attractive than production of other animals and the progress in poultry farming has brought about a surplus of poultry meat

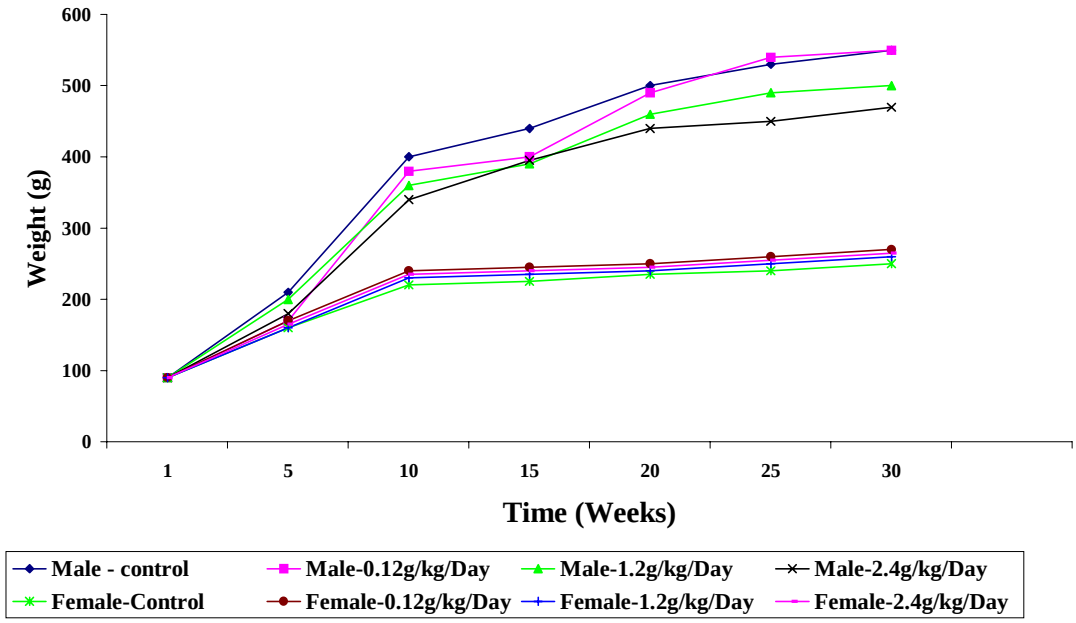


Figure 2.6. Subchronic toxicity study of AP in rats
Source: Modified after DMPRD (1990).

which has led the government to attempt export of poultry meat. Thailand has exported chicken meat since December 1973 (YSR, 1992). Increasing industrialization of the poultry production has led to that the export of poultry meat and poultry products has increased every year (POULTRY INTERNATIONAL, 1998). However, poultry raising in Thailand still has

problems with poultry diseases which lower the productivity. Diseases of major economic importance are coccidiosis, salmonellosis, colibacillosis and fowl cholera.

2.7.1. Coccidiosis

Coccidiosis remains one of the most expensive and common diseases of poultry in Thailand. Infection cause decreased growth rate and if severe, mortality (POONSUK, 1993).

Cause

In most cases, broiler flocks are infected with 5 or more species of *Eimeria* (MUANGYAI, 1991). A recent survey showed that all farms in Thailand are contaminated with Eimerian oocysts (POONSUK, 1987). Recently, *Eimeria tenella* has become the most pathogenic and important species, followed by *Eimeria necatrix* (MUANGYAI et al., 1987). SOULSBY (1968) collected data on the species that have been reported in the domestic chicken:

Eimeria acervulina	TYZZER, 1929
Eimeria brunetti	LEVINE, 1942
Eimeria hagani	LEVINE, 1938
Eimeria maxima	TYZZER, 1929
Eimeria mivati	EDGAR and SIEBOLD, 1964
Eimeria necatrix	JOHNSON, 1930
Eimeria praecox	JOHNSON, 1930
Eimeria tenella	(RAILLIET and LUCET, 1891; FANTHAM, 1909)

Transmission

Coccidiosis should be regarded as ubiquitous in poultry management, since even under the extreme conditions of experimental work, it is difficult to avoid infection completely for any length of time. Essentially, the clinical disease entity depends on the number of oocysts ingested by individual birds (FERNANDO, 1982). After ingestion of the oocysts the outer cover is broken down in the stomach of the birds and the freed sporozoit move to the intestinal wall where they multiply. New oocysts are formed which leave the animal with the faeces. The life cycle of *Eimeria spp.* in chicken is presented in Figure 2.7. Oocysts persist in litter. If the environmental hygiene is poor, the number may be very large and this is

particularly so with *E. tenella* which has a high biotic potential. Where young birds are placed on heavily contaminated litter, deaths may occur within a few days and up to 100% may die.

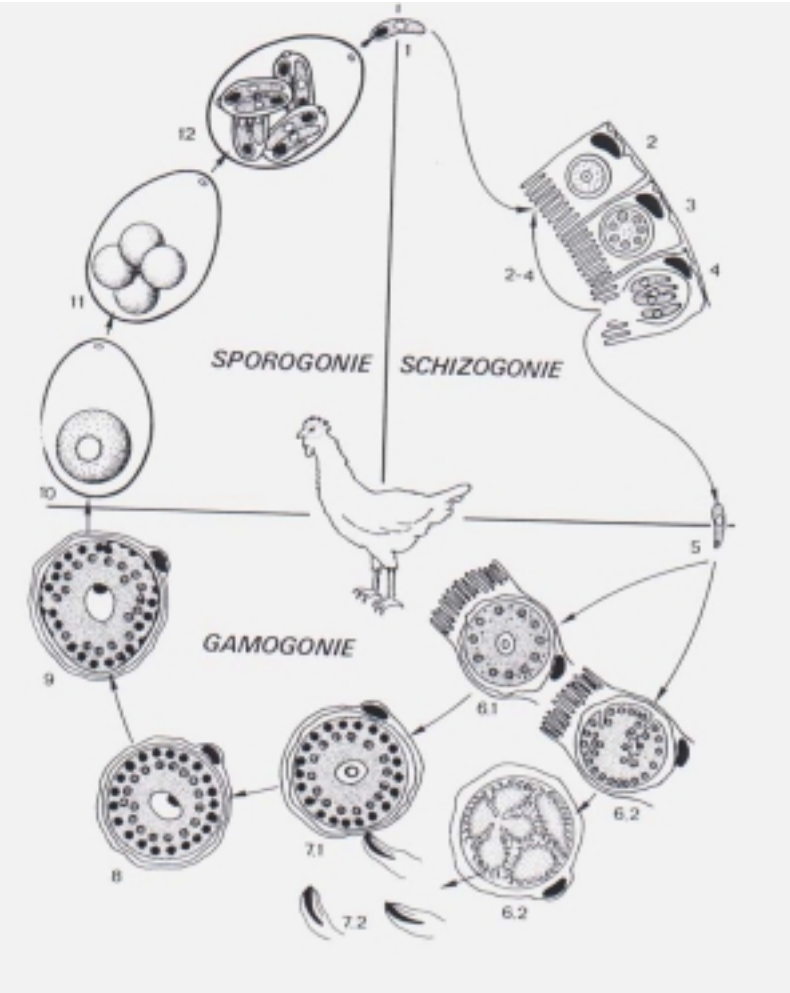


Figure 2.7. Life cycle of *Eimeria spp*. in chicken

1. Sporozoit (Oral uptake in oocyst)
2.-4. Schizonts produces mobile Merozoit in epithelial cells of the gut. This process can be repeated
5. Merozoit is tranformed into Gamete
6.1. Female Gamete; is called Macrogamete
6.2. Male Gamete is called Microgamete
7.1. Fertile Macrogamete
7.2. Fertile Microgamete, mobil with flagella
8. Zygote
9. The cell wall of oocysts is formed by the fusion of wall forming bodies with the zygote
10. Non sporulated oocysts leave the animal with the faeces
11.-12. Spolulation with formulation of 4 sporocysts with 2 sporozoits outside the animal (Sporulated oocysts which are infectious)
Source: Modified after MEHLHORN and PIEKARSKI (1995).

The environment is being contaminated continuously, even from immune birds, though the initiation of an outbreak depends upon factors which allow oocysts to sporulate and remain viable (MUANGYAI, 1991). For sporulation oocysts require moisture and warmth and they survive best in shaded, moist conditions. Poorly maintained litter houses may well supply such needs and excessive numbers of sporulated oocysts may be found in poorly kept quarters (SOULSBY, 1968).

Principle symptoms

In Thailand coccidia are found in all chicken from 4 - 10 weeks of age. Depending on the amount of oocysts intake, primary signs might not be obvious. Lightly infected birds might not look ill but the feed intake will be lowered, feed efficiency less and the birds loose weight. Layers will have lower egg production. By severe infection the birds sit, look ill, get hemorrhagic diarrhea and might die after 12-15 days.

Lesions

Diagnosis of coccidiosis in chicken is best done by post mortem examination of birds (POONSUK, 1993). Diagnosis on faecal examination may lead to quite erroneous results. For example the major pathology is produced before oocysts are shed in the faeces (e.g. *E. tenella*) and the presence of large numbers of oocysts may not necessarily indicated a serious pathogenic condition. With *E. acervulina*, which has a high biotic potential, comparatively larger numbers of oocysts are shed per oocyst given than, for example with *E. necatrix* (SUKSAITHAISHANA, 1989). Moreover, the accurate identification of the oocysts of various poultry coccidia is not easy. Mistakes can be avoided by post mortem examination (SOULSBY, 1968). The location of the major lesions gives a good indication of the species of coccidia concerned. For example the haemorrhagic lesions in the caecum would suggest *E. tenella* (MUANGYAI, 1991). A diagram of the location of lesions for 9 species of poultry coccidia is given in Figure 2.8.

Lesion scoring, where the lesions in intestinal tract of poultry are determined, is one way of diagnosing the severity of this disease. The lesions and infections of the organ are given scores from 1 - 4, 1 for no lesion found, and 4 for servere lesions (JOHNSON and REID, 1970).

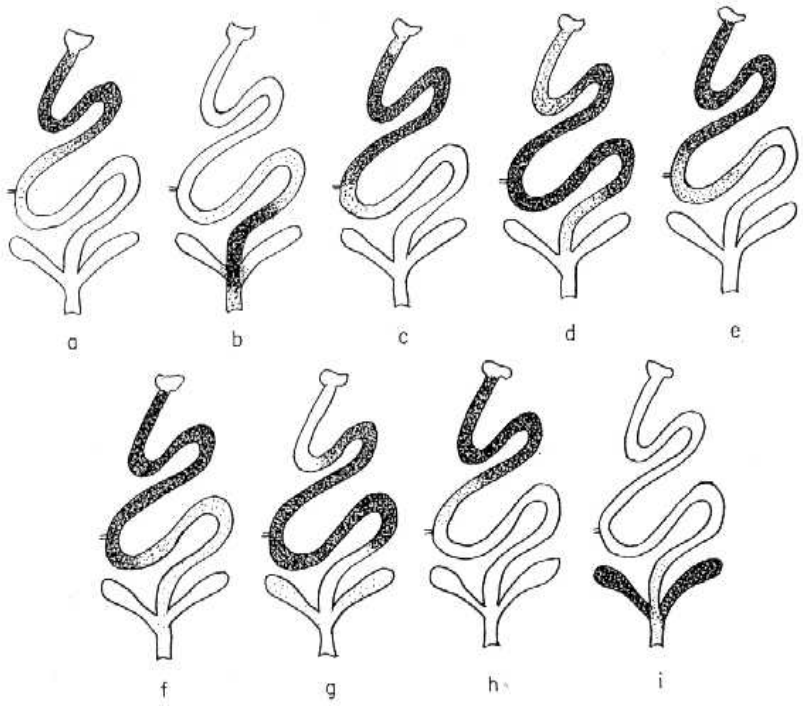


Figure 2.8. Diagrammatic representation of the location of lesions of 9 species of poultry coccidia: a. *E. acervulina*; b. *E. brunetti*; c. *E. hagani*; d. *E. maxima*; e. *E. mivati*; f. *E. mitis*; g. *E. necatrix*; h. *E. praecox*; i. *E. tenella*
Source: Adapted from REID (1964).

Treatment

As treatment sulfadimerazine 2 g/l drinking water or sulfaquinoxaline 0.25 g/l drinking water for 3 days twice at 2 days interval is suggested (SAY, 1995). In Thailand the medicine is freely available in the local pharmacies.

Prophylaxis

In broilers, turkeys and layer coccidiostats replacements such as amprolium: 0.012% in mash feed up to 12 weeks prevent clinical disease. Anticoccidial drug susceptibility tests has shown that sulfa dimethoxine are more effective compared to others coccidiostats as preventive medication (FRAZIER, 1987). TANAWONGNUVET and MUANGYAI (1990) found that trimethoprim mix with sulfaquinoxaline sodium with 1:5 in 500 ppm for 4 - 5 day was very effective for coccidiosis infections with *E. tenella*. However, some isolated coccidia species showed complete or partial resistance to some kinds of drugs. Due to problems of drug resistance, shuttle and rotation programs are used. In breeder broiler, mainly live attenuated

vaccine is given via the drinking water at 7 - 10 days of age. However, there is also some use of coccidiocidal water treatment at peak challenge age 4 - 10 weeks. Good litter management helps reduce this challenge. However, oocysts are resistant to most disinfectants (MUANGYAI et al., 1985). Coccidiostats that are promoted and general used in Thailand are listed in Table 2.4.

Table 2.4. Promoted and general used coccidiostats in Thailand

General name	Trade name
Amprolium	Amprol
Decoquinat	Decox
Furazolidone	nf-180
Monensin	Coban
Nicarbazin	Nicar
Sulfa dimethoxine	Rofenoid
Sulfa quinoxaline	Sulquin
Salinomycin	Coxistac

Source: POONSUK (1993).

However, use of coccidiostat drugs in broiler chicken in the near premarket period should be considered carefully. The proper type of drugs should be selected and the prescribed premarketing withdrawal period, normally between 3 - 7 days, to avoid residues of drugs in the chicken meat should be observed (MUANGYAI et al., 1990).

2.7.2. Salmonellosis

Salmonellosis remains of importance to the poultry industry both through the risk of direct commercial losses by infection of poultry flocks and due to the risk of transmission to humans. It is a common disease in the digestive system caused by enteropathogens which can cause diarrhea in animals as well as in humans (POONSUK, 1993).

Cause

Salmonellosis is an infection caused by *salmonellae. Salmonellae* has been known to cause illness for over 100 years. *Salmonellae* infections are increasing in Thailand. There are many

different kinds of *Salmonellae*. In Thailand *Salmonellae* serotype *Enteritidis* which can cause diarrhea are the most common. *Salmonellae* serotype *Salmonellae typhimurium* can cause Pullorum disease and Paratyphoid infection in poultry.

Pullorum can cause problems in breeder chicken and in layer hen. Infection of this disease can reduce the egg laying rate with 7 - 10% (POONSUK, 1993). During the last decade, the causative agent of Typhoid in poultry (*Salmonellae gallinarum*) has not been detected in Thailand.

Transmission

Salmonellae pass with the faeces of infected animals to other animals or people. They are usually transmitted to humans by foods contaminated with animal faeces. Contaminated foods usually look and smell normal (POONSUK, 1993).

Principle symptoms

Most persons infected with *Salmonellae* develop diarrhea, get fever and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons the diarrhea may be so severe that patient needs to be hospitalized. In these patients, the salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. In chicken *Salmonellae* can cause diarrhea and in layer hen reduce egg production (POONSUK, 1993)

Lesions

In poultry the disease cause inflammation of the digestive tract which might spread to the blood system. Also inflammation in liver, spleen, kidneys (GROVES, 1986) and ovaries are seen (POONSUK, 1987).

Treatment

Salmonellosis is treated with antibiotics as chloramphenicol, neomycin and sulfaquinoxaline in doses of 100 - 300 ppm in feed or drinking water for 7 days, then the treatment is withdrawn for 3 days after which it is repeated twice for 3 days (POONSUK, 1993).

Prophylaxis

In Thailand pullorum disease has been controlled with pullorum testing and with antigen prepared by the Department of Livestock Development (DLD) (CHAISRISONGKRAM, 1992).

2.7.3. Colibacillosis

Colibacillosis is a common bacterial diarrhea, which causes economic losses in animal production within chickens, swine, pigs and also in cattle. It can spread to human where it also causes diarrhea (POONSUK, 1993).

Cause

Harmless strains of *E. coli* normally can be found widespread in nature, including in the intestinal tracts of humans and other vertebrates (POONSUK et al., 1986). Disease causing types are a frequent cause of both intestinal and urinary-genital tract infections. Several different types of *E. coli* can cause diarrhea. A particularly dangerous type is referred to as enterohemorrhagic *E. coli* (EHEC). EHEC strains produce toxins that are structurally similar and have effects like those produced by *Shigella* dysenteriae bacteria (NIAID, 2000).

Enterotoxigenic *E. coli* (ETEC) is an important cause of bacterial diarrhea. Infection with ETEC is the leading cause of travelers' s diarrhea and a major cause of diarrhea in underdeveloped nations, especially in children (CDC, 2000).

Transmission

Cattle are known to carry EHEC, but other domestic and wild animals and birds also can harbor these bacteria. Heat destroys EHEC and its toxins. To avoid infection from *E. coli* with the food only thoroughly cooked meat and meat products should be eaten, ground beef should be cooked to an internal temperature of 72°C, unpasteurized juices should be avoided and uncooked fresh fruits and vegetables should be thoroughly washed (NIAID, 2000).

ETEC is transmitted by food or water contaminated with animal or human faeces (CDC, 2000).

Principle symptoms

In chicken symptoms are found in different organs and are given different names, for example: Airsac disease, Coli disease, Coli septicemia and Coligranuloma (POONSUK, 1993). In some cases inflammations in face and eyes of broiler chicken (facial cellulitis) are found (GROSS, 1957).

Lesions

The toxins can damage the lining of the intestine, cause anemia, stomach cramps and bloody diarrhea, and a serious complication called hemolytic uremic syndrome (HUS), which can lead to kidney failure. In North America, HUS is the most common cause of acute kidney failure in children, who are particularly susceptible to this complication (NIAID, 2000).

SAITANOO et al. (1986) found that the disease often caused lesions in the airsac of broiler chicken, which has given its the name airsac disease. SAITANOO (1989) made further studies to confirm the former results in 64 broiler farms and found that 54 farms had outbreaks of colibacillosis whereas only one farm had mycoplasmosis. The lesions were airsaculitis, paricarditis around heart, peneumonitis and inflammation of intestinal tract. Very serious lesions on the airsac were found by up to 94% of the broiler.

Treatment

E. coli bacteria have many mutations. It can be found throughout the environment and also inside animals. POONSUK et al. (1986) reported sensitivity of *E. coli* from broiler from central region in Thailand to antibiotics. They found that *E. coli* was sensitive to ampicillin, chloramphenicol, kanamycin, neomycin sulfametoxazone but resistant to tetracycline, streptomycin and sulfa groups. They suggested to send the ill birds to laboratories for diagnosis and use antimicrobial susceptibility testing before treatment of the sick animals.

Prophylaxis

Vaccine to prevent *E. coli* is available but not efficient in Thailand because *E. coli* often mutate. Major means of prevention from this disease is therefore hygienic housing management. Also stress in the flock should be reduced. As efficient prevention ascorbic acid 330 microgram /kg BW can also be given in the drinking water at 23 - 28 days of age (POONSUK, 1993).

2.7.4. Fowl cholera

Fowl cholera is an acute infectious disease of poultry, gamebirds, waterfowl and wild birds. The mortality in the flock is usually severe. A chronic form of the disease may occur following an acute outbreak (STANLEY and VANHOOSER, 2001).

Cause

Pasteurella multocida is a gram negative bipolar staining bacterium, it is an important veterinary and opportunistic human pathogen. It can cause diseases such as fowl cholera in domestic and wild birds, bovine haemorrahagic septicaemia and porcine atrophic rhinitis (HUNT et al., 2000)

CHAISRISONGKRAM (1992) reported that fowl cholera is encountered in both chickens and ducks in Thailand. This disease causes higher economic losses in duck farms than chicken farms due to higher morbidity and mortality by ducks. There are also problems of resistance against treatment drugs in some farms in Thailand.

In Taiwan fowl cholera is the most common poultry disease and also economic importance (SUNG and LIN, 1992) which mainly hits ducks and geese. In 1990 a total of 53,836 birds were reported to be infected with fowl cholera and 23,928 of them died (TSAI, 1992).

Transmission

Infected birds will excrete the bacteria in ocular and nasal exudate. Survivors can be carrier of the pathogen. Rats and cats serve as reservoirs infecting birds via bites or faecal and urine contamination of litter and drinking water. Local aerosol spreading can also contaminate the drinking water. This is the most common method of transmission from bird to bird (STANLEY and VANHOOSER, 2001).

Principle symptoms

The disease occurs in several forms, for example in preacute form, where there are few clinical signs and suddenly birds are found dead in good condition though often with blood at the mouth and nostrils. In acute form affected birds are severely depressed and often cyanosed. In chronic form birds are less severely affected but may show respiratory disease (CHAISRISONGKRAM, 1992).

Lesions

Clinical signs are gross lesions as solid lungs, parboiled liver and widespread hemorrhages. In acute disease signs and lesions are minimal. Diagnosis is based on isolation of the bacteria from liver or heart blood (STANLEY and VANHOOSER, 2001).

Treatment

Treatment can be done with antibiotic: streptomycin 15 mg/kg live weight orally. Use of antibiotics such as salfadimethoxine, tetracyclines, erythromycin or penicillin will decrease mortality within the flock (STANLEY and VANHOOSER, 2001).

Prophylaxis

Means of prophylaxis are sanitation, rodent and predator control and proper disposal of dead birds. Immunity can be induced in broiler breeder chickens by killed vaccine however, this only induce immunity to homologous serotypes (ROBERS and HEDDLESTON, 1977).Commercial vaccines are available to aid the control of fowl cholera within a flock (CHAISRISONGKRAM, 1992). However, vaccination is not recommend until fowl cholera becomes a problem on the premises (STANLEY and VANHOOSER, 2001).

2.8. Use of antibiotics in animal production

From chapter 2.7. it can be seen that antibiotics are used frequently in modern agriculture practice: both to prevent disease, and in higher dosage forms, for the treatment of individual animals for specific disease conditions. Apart from this, antibiotics are also used on a herd basis as additives to feed and water to promote weight gain and improve feed conversion efficiency. Such usage may lead to problems with residues in agricultural products and to environmental contamination.

2.8.1. Use of tetracycline in animal production

Tetracycline antibiotics (TCs) which are derived from *Streptomyces spp.* have broad spectrum activity against many gram-positive and -negative bacteria and are especially effective against *Staphylococcus, Steptococcus, Pneumococcus, Gonococcus, Vibrio, Rickettsia, Chlamydia* and *Mycoplasma*. TCs are actively transported into the cells of susceptible bacteria where they exert a bacteriostatic effect by inhibiting protein biosynthesis after binding to the 30 S ribosomal subparticle. Since the first member of the tetracycline family, chlortetracycline (CTC) was discovered in 1948 (DUGGER, 1948), eight TCs have become commercially available. Of these, oxytetracycline (OTC), tetracycline (TC), CTC and doxycycline (DC) are commonly applied to food-producing animals (including honeybees) as drugs and feed additives because of their broad spectrum activity and cost effectiveness (HISAO et al., 1995).

2.8.2. Antibiotic residues in animal meat

The residues of antibiotics in animal meat can enter human food and increase the risk of ill health in persons who consume products from treated animals. To solve this problem, the inspection of agricultural products for unacceptable residues is one of the most important duties of public health.

The 36th Joint meeting of Food and Agriculture Organization (FAO) and World Health organization (WHO) expert committee on food additives meeting in 1990 established "maximum residue limits" (MRLs) for oxytetracycline of 600 microgram/kg in kidney; 300 in liver; 100 in muscle; 100 in milk; 200 in egg and 100 in fat for all species for which residue depletion data were provided (cattle, swine, sheep, chicken, turkey and fish) (JECFA, 1990). The 45th Joint FAO/WHO expert committee on food additives meeting in 1995 allocated the same MRLs, except for milk where also MRLs for chlortetracycline and tetracycline additional to those previously allocated to oxytetracycline at the 36th meeting where established: 100 microgram/kg for muscle (cattle, pig, poultry), 300 for liver (cattle, pig, sheep, poultry), 600 for kidney (cattle, pig, sheep, poultry) and 200 for eggs (poultry) (JECFA, 1995).

Still residues are found in animal products. AL-GHAMDI et al. (2000) found residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. They collected chicken muscle, liver and egg samples from 33 broiler and 5 layer farms over a period of two years. Antibiotic residue positive samples were identified in the products of 23 (69.7%) broiler and 3 (60%) layer farms. Also WILLIAMA (1999) reported, that shipments of pork from the United States to other countries have been rejected because of the alleged presence of residues of tetracycline.

BUNYAPRAPHATSARA (2000) reported that in Thailand the excessive use of antibiotics including virginiacin, spiramycin, bacitracin and avopacin leads to residue contamination of the meat products. This has led to the ban of meat by EU and Japan.

2.8.3. Problems of antibiotic resistant microorganisms in animal production

Some bacteria become resistant to antibiotics (HISAO et al., 1995). LOPES et al. (1979) found that broiler chicks which had received tetracycline and chloramphenicol in drinking water had antibiotic resistant *E. coli* in the intestine. OHYA and SATO (1983) found also that feeding of diets containing antibiotics to broiler chicken may possibly affect the stability of the intestinal microflora. MOLITORIS et al. (1986) described that *streptococcaceae* in faeces of broilers which were fed diets with chlortetracycline were resistant to antibiotics. CHAISRISONGKRAM (1992) reported that on some duck farms in Thailand there were problems of resistance against antibiotic drugs for against fowl cholera.

HISAO et al. (1995) reported that antibiotics and antimicrobial compounds in food have effect on the human gut flora and the residues of them may give rise to resistant pathogens in the human gastrointestinal tract or that adventitious organisms may gain a hold.

3. EXPERIMENT 1

PHYTOCHEMICAL ANALYSIS AND QUALITATIVE DETERMINATION OF CHEMICAL COMPOUNDS OF *ANDROGRAPHIS PANICULATA* LEAVES

3.1. Objective

The objective of this experiment was to analyse the quality of the sample of AP leaves to assure that they contained sufficient active compounds which determine the quality, and which may contribute to the biological effect.

3.2. Materials and methods

This experiment was conducted at Division of Medicinal Research and Development Laboratory, Department of Medical Sciences, Bangkok, Thailand under supervision of a chemist and pharmacist. *Andrographis paniculata* Nees (AP) leaves were bought from local markets around Nakornphatom Province. The leaves were identified by a botanist from Kasetsart University. One kg fresh AP leaves were dried in an automatic oven at 60°C overnight after which the dry leaves were ground to powder.

The phytochemical compounds of this powder was analysed by colour test (preliminary test) and thin layer chromatography (confirmatory test). Both analyses were supported by chemists and pharmacists at Medicinal Plant Research Institute (MPRI), Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand.

3.2.1. Preliminary test

To 1 g of powdered AP leaves, 20 ml of ethanol were added, boiled in a water bath and filtered. 300 mg of decolourising charcoal were added to the filtrate, then it was stirred and again filtered (solution A) (MPRI, 1999).

Method 1. With Kedde reagent

To 1 ml solution A 2 drops were added of a 2% w/v solution of 3,5-dinitrobenzoic acid in methanol (Kedde A) and 2 drops of a 5.7 % w/v of potassium hydroxide in methanol (Kedde B). A colour change to a purplish red colour indicate that the sample has active compounds like AP (Figure 3.1.) (MPRI, 1999).

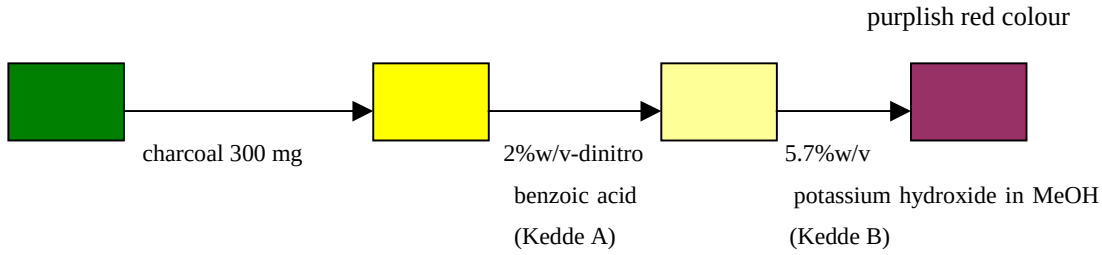


Figure 3.1. Diagram of colour test by using Kedde A and Kedde B (MPRI, 1999).

Method 2. With ethanolic potassium hydroxide

To confirm the preliminary test a second colour test was performed. To 1 ml of solution A were added several drops of ethanolic potassium hydroxide until its showed a red colour. Then it was set aside for 10 to 15 minutes. A colour change to yellow indicate that the sample has active compounds like AP (Figure 3.2) (MPRI, 1999).

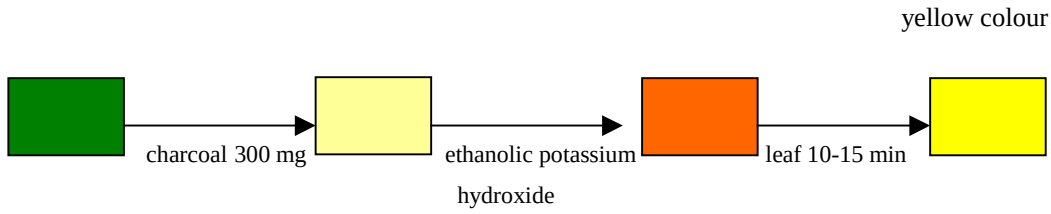


Figure 3.2. Diagram of colour test by using ethanolic potassium hydroxide (MPRI, 1999)

3.2.2. Confirmatory test

Thin layer chromatography analysis was done after the colour test to confirm the AP identity. 1 g of powdered AP leaves was boiled with 20 ml of ethanol in a water-bath for 5 minutes, then 300 mg of decolourising charcoal were added, stirred and filtered. The filtrate was evaporated under reduced pressure until dryness and the residue dissolved in 1 ml of warm ethanol (80%). As standard 2 mg of andrographolide, 2 mg of neoandrographolide and 4 mg of dehydroandrographolide were dissolved each in 1 ml of ethanol by using adsorbent silica gel GF254 and mobile phase chloroform (absolute ethanol 85:15). 5 microliter were used for

each spot. Migration path was 15 cm (ascending). After quenching by UV radiation (lambda=254 nm) detection was made by spraying with 2% w/v 3,5-dinitrobenzoic acid in methanol (Kedde A) and excess of 5.7% w/v potassium hydroxide in methanol (Kedde B) (MPRI, 1999).

3.2.3. Determination of chemical contents

Foreign matter

100 g of AP leaves were sampled according to the specification described in Thai Pharmacopoeia (1987).The leaves were placed on a flat plate, after which the foreign matter was selected under magnifying glass and weighed. Foreign matter as percentage of the initial weight was calculated as the foreign matter content (MPRI, 1999).

Water content

5 g of AP leaves powder were weighed and dried in the oven at 105°C until constant weight. The loss in weight of the powder as percentage of the initial weight was calculated as the water content in AP sample (MPRI, 1999).

Acid-insoluble ash

2-4 g of AP powder were burnt in crucible at 450°C. The ash was boiled with 25 ml 2 M HCl for 5 minutes. Then it was filtered through filter paper, the residue was washed with hot water until the water became neutral. The filtered ash and filter paper was placed in a crucible, dried and then burnt at 500°C until constant weight. The loss in weight of the powder as percentage of the initial weight was calculated as acid-insoluble ash in the AP sample (MPRI, 1999).

85% ethanol extractives

5 g of AP powder were together with 100 ml of 85% ethanol put in a glass bottle, sealed and kept for 24 hr at room temperature. After 6 hr the bottle was shaken. The solution was filtered fast and 25 ml of the filtered solution filled in a wide glass. This was dried in the oven at 105°C after which the percentage of 85% ethanol extractives were calculated (MPRI, 1999).

Water extractives

Water extractives were determined following the same method as 85% ethanol extractives, only the solvent was changed to water with chloroform (MPRI, 1999).

Total lactones or diterpene lactones

The method to identify the active constituents (diterpene lactones) of AP powder is presented in Figure 3.3. (MPRI, 1999).

1 g of AP leaves was boiled with 50 ml of 85% ethanol with reflux in water bath for 2 hr after which it was filtered. The residue was washed with 85% ethanol until the solvent appeared clear. All portions of the solvent used for washing were collected in an Erlenmeyer flask. 1ml of basic lead acetate solution was filled in the solution and left for 15 minutes. Again it was filtered and washed with 85% ethanol until the solvent appeared clear. The effluent solution was again collected and mixed together with the former. 1ml of 25% Na_2So_4 was added drop wise and the bottle shaken between each drop, then it was left for 1 hr. After that 0.5 g of charcoal was added and the bottle boiled with reflux in water bath for 10 minutes. Then it was filtered through a Büchner funnel which contained 0.5 g smoothly adjusted charcoal. The residue was washed with hot ethanol and the filtrate and wash solutions mixed together. 20 ml of distilled water were added and the solution left to cool down. Then 2 - 3 drops of phenolphthalein were added and the solution adjusted to neutral with 5 ml of 0.1 M NaOH. This was again boiled with reflux in water bath for 30 minutes and again left to cool down. After that it was titrated with 0.05 M of HCl. A blank sample was made as test. The percentage of the total diterpene lactones was calculated from the dry matter weight of AP leaves. 1ml 0.1 M of NaOH solution is equal to 35.04 mg andrographolide.

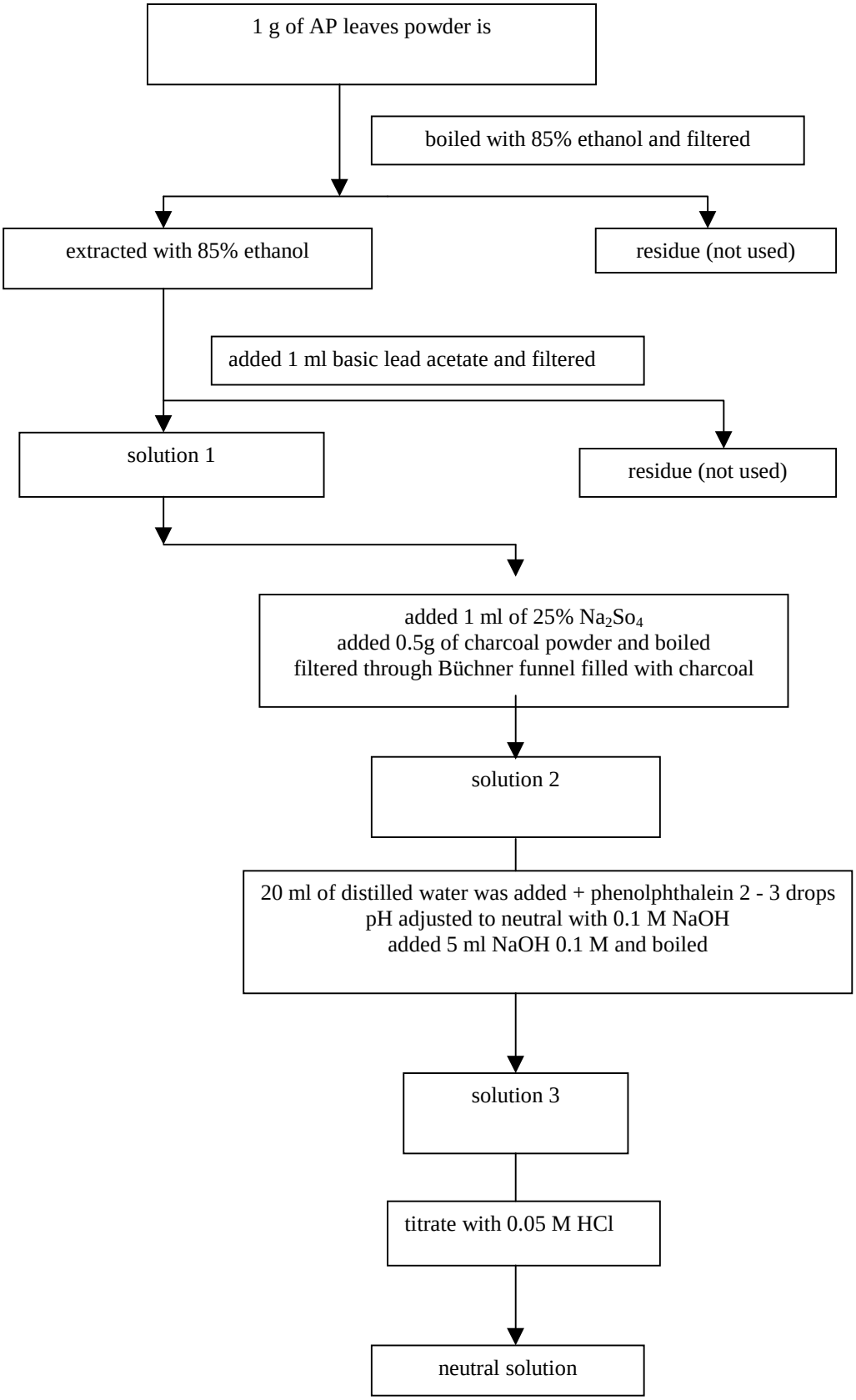


Figure 3.3. Method to identify the active constituents (diterpene lactones) of AP powder (MPRI, 1999)

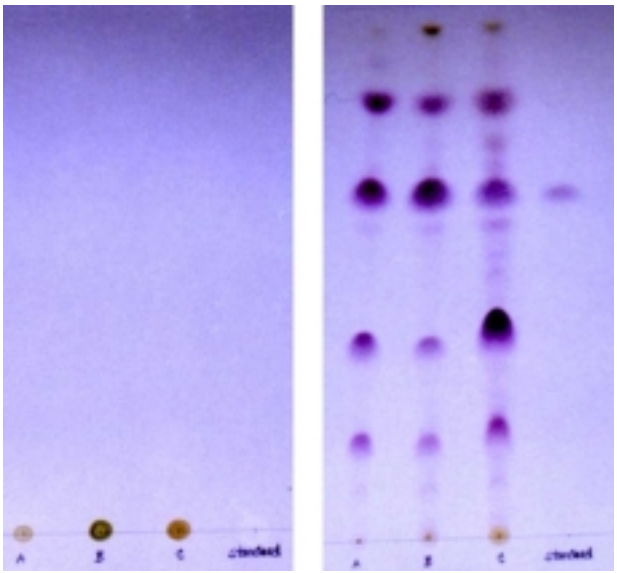
3.3. Results

3.3.1. Results of colour test

The result of colour test was that the colour of the AP solution changed to purplish red after the chemical solvents was added according to the first method. By the second method, the solution of the AP sample changed to yellow after the chemicals were added.

3.3.2. Results of confirmatory test

The result of the thin layer chromatography (Figure 3.4.) are given in Table 3.1.



A B

Figure 3.4. Thin layer chromatography of AP leaves powder before placed in chromatographic tank (A) and after development (B) a - c: AP solvent sample, standard: andrographolide

3.3.3. Results of chemical content of AP

The following results were obtained: foreign matter 0%, water 8.58%, acid-insoluble ash 1.39%, 85% ethanol extractives 18.10% and active constituents (total diterpene lactones calculated in andrographolide) 7.30%.

Table 3.1 Thin layer chromatography of AP

Diterpene lactones	*hRf Values	Development	
		UV_{254}	Kedde A/Kedde B
Lactone type 1	1 - 5	-	Strong purplish
Lactone type 2	11 - 15	opacity	Purplish
Lactone type 3	18 - 22	opacity	Purplish
Neoandrographolide	28 - 32	-	Strong purplish
Lactone type 5	49 - 51	-	Purplish
Andrographolide	52 - 56	opacity	Strong purplish
Lactone type 7	57 - 59	opacity	Purplish
Lactone type 8	66 - 68	-	Purplish
Dehydroandrographolide	69 - 71	opacity	Strong purplish

*hRf = 100 Rf; Rf (retardation factor or relative front, distance between start line and substance zone divided by the distance between the start line and the mobile phase front

3.4. Discussion

The colour tests are normally used in Thailand in the laboratories of the government offices which have the duty to test and control the quality of medicinal plants or other phytomedical laboratories. The pros of using colour tests are that these are quite easy, cheap and one can very rapidly know the result. However, they are primary tests which only show the presence of compounds which reacts chemically like the active compounds of AP. To prove that the sample contain AP further tests in the laboratory are necessary.

The thin layer chromatographic test showed that the AP plant sample had the same hRf values (line spots and colour) as the diterpene lactones standards which were used for comparison in this test. So it can be stated that the plant sample contains the three active compounds of AP plants: dehydroandrographolide, andrographolide and neoandrographolide. The TLC method is used to confirm the result from the colour test by the same offices which do the colour test.

In all analysed constituents chemical content of the AP plant sample were better than required in the THM quality specifications as shown in Table 3.2. Quality control is important. To get the good quality of medicinal plants for humans or as feed additive for animals many factors should be considered.

Table 3.2. Quality of AP leaves sample compared with standard quality requirements of Thai Herbal Medicine (THM)

Parameter	AP leaves sample (%)	THM (%)
Foreign matter	0.00	< 2.00
Water content	8.58	< 11.00
Acid-insoluble ash	1.39	< 2.00
85% Ethanol extractives	18.10	> 13.00
Water extractives	23.85	> 18.00
Active constituents*	7.30	> 6.00

* total diterpene lactones calculated in andrographolide

This study used AP plant material that was cultivated in June, the beginning of the rainy season. The good quality confirm the results of KASETKLANGKLUNG (1996) who reported that plants cultivated in June give the highest yield of leaves and the highest content of active constituents.

The AP plant sample was harvested at the beginning of blooming in October, 2000. DECHATIWONGSE NA AYDHYA et al. (1988) reported that to get the best quality of AP plants they should be harvested in the period of the beginning until 50% of blooming. This period will give the highest percent of active constituent. Also SUWANBAREERAK and CHAICHANTIPYUTH (1991) reported that when the AP plant have an age of 110 - 150 days it will have the mature age and yield the highest amount of active compounds.

The AP plant sample was dried in the hot air oven at 60°C to assure that the plant sample was dried properly and to be able to store it for use in the feeding trial. MPRI (1999) reports that the proper temperature to use in the oven are between 45 - 50°C, if the plant is to be stored a long time temperatures up to 60°C can be used.

The AP plant sample was used for feeding trial in February, 2001 after only 4 months storage time. The quality test was done in May, 2001 after the feeding trial. Medicinal Plant Research Institute in Thailand reported that the good quality of AP plant depends on the period of storage. After 1 year of storage the active constituents will be decreased about 25 - 50% (MPRI, 1999). However, since in the present study the chemical content was determined after the feeding trial, the content of active ingredients is not over estimated.

3.5. Conclusions

The plant material tested contains the three active compounds of AP plants: dehydroandrographolide, andrographolide and neoandrographolide and the chemical content of the sample was better than required in the THM standard specification.

4. Experiment 2.

ANTIBACTERIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACTS

4.1. Objective

The objective of this experiment was to determine the antibacterial activity of *Andrographis paniculata* (Burm. f.) Nees extract with different solvents.

4.2. Materials and methods for experiment 2.1.

This experiment was carried out in the laboratory of Diagnosis and Research Centre, Department of Livestock Development, Wang Tong District, Phitsanulok Province, Thailand under the supervision of a team of veterinarians.

4.2.1. Preparation of AP leaves powder

AP leaves powder (andrographolide 7.30%) that was already tested for quality in experiment 1 was used in this experiment.

4.2.2. Extraction of AP by maceration method

Based on the standard method for chemical analysis described by CUILEI (1984) AP leaves powder was extracted in a series of three different solvents: distilled water, 70% alcohol and 85% alcohol. The antibacterial activity of each AP leaves solvent was analysed by using agar disk diffusion method as antibacterial susceptibility test (NCCLS, 1993).

AP leaves powder was placed in flasks and added solvent either distilled water, 70% alcohol or 85% alcohol (1:10 w/v). The flasks were placed in a shaking water bath at 50°C for 48 hr after which the solvents were filtered through filter paper (residue was not used). The extract solutions were put in a rotary evaporator and evaporated at 50°C until dry residues were left. These residues were weighed.

4.2.3. Preparation of disk papers with AP extract

Disk papers with a 5.8 mm diameter, were sterilised and dried. Then the dry extract prepared as described in 4.2.2. was dissolved again with the same solvent and diluted with distilled water in proportions of 1:10, 1:100, 1:1000 (w/v) to determine which of these concentrations have a inhibitory effect on bacterial growth. 0.03 ml AP extract were added to each paper. After which this was placed in an oven and dried at 50°C overnight.

4.2.4. Preparation of bacteria

4 samples of bacteria (Figure 4.1.) from the Laboratory of Diagnosis and Research Centre , Department of Livestock Development, Wang Tong District, Phitsanulok Province, Thailand were used in this experiment: *S. typhimulium*, *S. spp.*, *E. coli* ATCC 25922 (standard), *E. coli* (from chicken). The bacteria were grown in Petri dishes with Mueller-Hinton Agar (MHA). The inoculum suspensions were compared with McFarland standard No. 0.5. After that the bacteria were spread over the surface of the MHA disks using sterilised cotton buds.

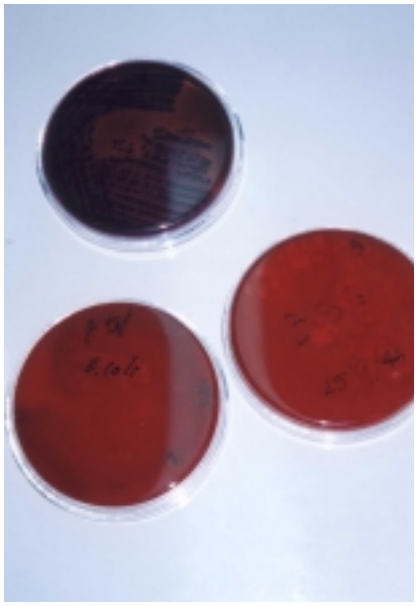


Figure 4.1. Disks with *S. typhimulium* and *E. coli*

4.2.5. Preparation of disk diffusion test with AP extract

The papers with AP extract in different rations that were prepared under 4.2.3. were placed on the MHA disks on which the bacteria were spread (4.2.4.) and the disks incubated at 37°C for

24 hr. After that the diameters of inhibition zones (clear zones) around the papers were measured.

Result were measured according to RUENGSAKUL (1987) for medicinal plants as:

Resistant:	< 6 mm	(-)
Susceptible:	intermediate susceptible: 6 - 10 mm	(+)
	moderate susceptible: 11 - 15 mm	(++)
	susceptible: 16 - 20 mm	(+++)

4.3. Materials and methods for experiment 2.2.

This experiment was conducted after Experiment 2.1. was evaluated. It used the same method as Experiment 2.1. but the number of samples was changed to 14 samples with 3 types of bacteria: *Salmonellae* (sample 1 - 3), *E. coli* (sample 4 - 10) and *P. multocida* (sample 11 - 14) and only the concentration 1:10 w/v of the different AP extracts was used. As control Streptomycin 2 mg/ml was used.

Results for medical plants were recorded as under experiment 2.1. whereas results for the antibiotic streptomycin was recorded according to ARUNRUEK (1994) as:

Resistant:	< 11 mm	(-)
Susceptible:	> 11 mm	(+)

4.4. Results

In experiment 2.1. none of the used concentrations 1:10, 1:100 and 1:1000 of AP aqueous extract (Table 4.1.) showed any antibacterial effect on *S. typhimrium*, *S. spp.*, *E. coli* ATCC and *E. coli* (from chicken). Only the concentration 1:10 of 70% and 85% of alcoholic extract of AP showed moderate to intermediate activity to *S. typhimurium* with inhibition zones of 12 and 10 mm respectively. The minimum inhibitory concentration (MIC) of 70% and 85% alcoholic extract of AP was 1:10 according to this test. The lower concentrations of 1:100 and 1:1000 did not influence the bacteria growth.

Table 4.1. Susceptibility results of agar diffusion test with different AP extracts and concentrations for different diarrhea bacteria

Types of bacteria	**Aqueous extract of AP**			**70% alcoholic extract of AP**			**85% alcoholic extract of AP**		
	1:10	**1:100**	**1:1000**	**1:10**	**1:100**	**1:1000**	**1:10**	**1:100**	**1:1000**
S. typhimurium	-	-	-	**++ 12**	-	-	**+ 10**	-	-
S. spp.	-	-	-	-	-	-	-	-	-
E. coli ATCC (25922)	-	-	-	-	-	-	-	-	-
E. coli (from chicken)	-	-	-	-	-	-	-	-	-

Notes: for medicinal plants: Resistant: - = < 6 mm; Susceptible: + = 6 - 10 mm, ++ = 11 - 15 mm, +++ = 16 - 20 mm

Results of experiment 2.2. are given in Table 4.2. The aqueous extract of AP 1:10 showed no antibacterial activity towards any of the bacteria tested. Also 70% and 85% alcoholic extract of AP 1:10 showed no antibacterial activity towards any of the *Salmonellae* and *E. coli* strains of bacteria tested. However, these two extracts of AP showed antibacterial activity towards three of the four tested *P. multocida* strains. Six of the seven tested *E. coli* strains, one of the two tested *S. spp.* and one of the four tested *P. multocida* showed resistance to the Streptomycin (Figure 4.2.).

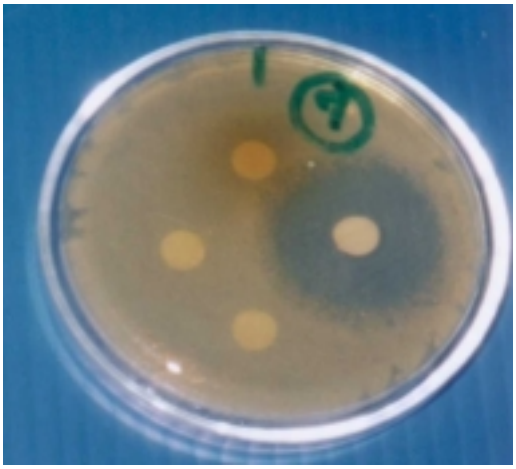


Figure 4.2. Reactions of disk susceptibility test, 3 negative, 1 positive (halo)

Table 4.2. Susceptibility results of agar diffusion test with different AP extracts for different diarrhea bacteria

Type of bacteria	**Aqueous extract of AP 1:10**	**70% alcoholic extract of AP 1:10**	**85% alcoholic extract of AP 1:10**	**Streptomycin 2 mg/ml**
1. *S. spp.* (Lampang province)	-	-	-	**+ 20 ***
2. *S. typhimurium* (Institute)	-	-	-	**+ 18**
3. *S. spp.* (Phitsanulok)	-	-	-	**- 6**
4. *E. coli* 25922	-	-	-	-
5. *E. coli* (chicken)	-	-	-	-
6. *E. coli* (chicken)	-	-	-	-
7. *E. coli* (pig)	-	-	-	-
8. *E. coli* (pig)	-	-	-	-
9. *E. coli* (deer)	-	-	-	**+ 20**
10. *E. coli* (duck)	-	-	-	**- 7**
11. *P. multocida* (buffalo tissue)	-	**++ 12**	**+ 10**	**+15**
12. *P. multocida* (buffalo liver)	-	**+ 6**	**+ 6**	**- 6**
13. *P. multocida* (beef tissue)	-	**+ 6**	**+ 7**	-
14. *P. multocida* (beef heart)	-	-	-	**+ 20**

Notes for medicinal plants: Resistant: - = < 6 mm; Susceptible: + = 6 - 10 mm, ++ = 11 - 15 mm, +++ = 16 - 20 mm

*For Streptomycin (control): Resistant: - = < 11 mm; Susceptible: + = > 11 mm

4.5. Discussion

In experiment 2.1 and 2.2 the antibacterial activities of AP leaves extracts were determined using the agar diffusion test method. ARUNRUEK (1994) describes in her book about sensitivity tests, that the agar dilution test is quite difficult because the medicine must be diluted several times to treat each sample, so it takes a long time and is more expensive than the agar diffusion test which gives comparable rapid results. Therefore, in this experiment the agar diffusion test was chosen. Agar diffusion is meant to be an excellent method to quickly

determine anti-microbial properties (ROVINSKY and CIZADLO, 1998). In the disk diffusion susceptibility test, disks containing known amounts of an antimicrobial agent are placed on the surface of an agar plate that has been inoculated confluently with a standardised suspension of a strain of bacteria. The antimicrobial agent diffuses into the medium causing a zone of inhibition of growth of the strain around the disk corresponding to the susceptibility of the strain to that agent. Interpretative zone diameters have been established to permit classifying an isolate as belonging to the susceptible, intermediate resistance or resistant categories of susceptibilities to an antimicrobial agent (CDCD, 2000), but this method has also a few sources of error. Among this method are clerical error in recording data, reader error in measuring zone diameters and contamination or changes in the bacterial strain being tested. Also agar diffusion may not give an accurate picture of the effectiveness of an antibiotic within a living organism. Microbes may show *in vitro* sensitivity to an antibiotic, with little or no sensitivity to it *in vivo* (ROVINSKY and CIZADLO, 1998).

In experiment 2.1, the AP aqueous extract had no activity in any type of diarrheal bacteria. However, the 70% and 85% alcoholic extract in concentration 1:10 showed high activity to *S. typhimurium*. The inhibition zone (clear zone) on *S. typhimurium* in 70% alcoholic extract was 12 mm and 10 mm in 85% alcoholic extract. However, the lower concentrations showed no activity on any bacteria.

In a similar experiment using agar dilution method PLUEMJAI (1992) studied the antibacterial activity of 70% and 80% alcoholic extracts from AP leaves against diarrhea and respiratory tract bacteria. She found that both alcoholic extracts showed high activity. However, at the same value of the minimum inhibitory concentration (MIC), the 85% alcoholic extract which contained 8.30 mg of andrographolide showed significant antibacterial activity against *E. coli, S. kerfeld, S. typhimulium, V. cholerae* 01 and *S. dysenteriae*, whereas the 70% alcoholic extract showed less antibacterial activity.

The result of PLUEMJAI (1992) are similar to the results of experiment 2.1. However, the experiment 2.2 differs from her results since here neither of the alcoholic extracts 70% and 85% showed any activity to *Salmonellae* and *E. coli*. They only showed high activity in *P. multocida*. The 70% alcoholic extract showed higher antibacterial activity in *P. multocida* from buffalo tissue than 85% alcoholic extract, but 85% alcoholic extract showed higher antibacterial activity than 70% alcoholic extract in sample from beef tissue. A reason for these

different results may be due to the different methods that were used. A possible reason could be the different content of active constituents of AP leaves in the studies. This study used AP leaves with 7.30% of andrographolide, the most effective active constituent of AP plant but PLUEMJAI used plant material with 6.77% andrographolide in 70% alcoholic extract and 8.30% andrographolide in 85% alcoholic extract. Another reason for the different result are the different source of bacteria that were used in the experiments. In this experiment all bacteria were from the tissue of different animals that had been sent for disease diagnosis at the Diagnosis Research Center in Phitsanulok Province, Thailand whereas PLUEMJAI got all bacteria samples from human patients of Siriracha Hospital in Bangkok, Thailand. Some bacteria from the animals may already have been treated by antibiotics. This may have led to resistance in some strains.

Streptomycin used as control in experiment 2.2 showed high activity in *Salmonellae, P. multocida* but was not active in *E. coli* bacteria. 70% and 85% AP alcoholic extract had antibacterial activity on *P. multocida* but not on any other bacteria. *P. multocida* strains tested were from buffalo and this may give different result as when bacteria from poultry are tested. So further studies should be established. If it proves effective in poultry strains, it could be good to give this plant as a feed additive in poultry production to prevent fowl cholera.

However, also if the AP plant has no antibacterial activity killing diarrhea bacteria, it might relieve the diarrhea symptoms. YIN and GUO (1993) found that a dose of 500 mg per day for six day of andrographolide was effective on acute bacterial diarrhea in human patients. CHTURVEDI et al. (1983) found also an overall effectiveness of 91.3% of AP leaves on bacterial dysentery and diarrhea and THANANGKUL and CHAICHANTIPYUTH (1985) found that AP had effect on curing diarrhea and bacillary dysentery in the group of patients in Ramatipbodee Hospital in Bangkok, Thailand. DHAMMA-UPAKORN et al. (1992) and SAWASDIMONGKOL et al. (1990) found that the AP extract has an effect to reduce the movements of the smooth muscle in stomach and intestinal tract in human.

4.6. Conclusions

Though active constituents in AP leaves have the activity to reduce the symptoms of diarrhea and dysentery diseases in human this effect doesn't seem to be an antibacterial effect. Further test should be made for poultry.

5. Experiment 3.

EFFECT OF *ANDROGRAPHIS PANICULATA* ON COCCIDIOSIS, BROILER PERFORMANCE AND MORTALITY

This experiment was conducted at the poultry research farm, Phichit College of Technology and Agriculture, Phichit, Thailand. The feeding trial was accomplished in approximately 2 months, while the laboratory analyses were conducted after the feeding trial.

5.1. Objectives

The objective of this experiment were as follows:
1. to determine the effects of AP leaves powder mixed in the broiler feed on coccidiosis,
2. to determine the effects of AP leaves powder mixed in the broiler feed on broiler performance,
3. to determine the effects of AP leaves powder mixed in the broiler feed on broiler mortality rate.

5.2 Materials and methods

5.2.1 Animals and housing

105 one day old broilers, bought from C.P. Company Limited in Phitsanulok Province were used. All chickens were raised in coops on deep litter of rice husk under station management for 6 weeks. During the first week the chicken were kept together and brooder lamps were supplied for all chickens.

5.2.2. Design of experiment

After one week the chickens were randomly divided into 5 groups (5 treatments) under a completely randomised design (STEEL and TORRIE, 1981). Each treatment had three replications with 7 chickens per replication.

The chickens were managed under the same conditions with the same bedding as that used by farmers. This should give the same coccidiosis pressure as on other farms. However, to assure that the chicken were heavily infected, coccidia were collected from a flock of domestic chicken where coccidiosis was diagnosed by a veterinarian at the Diagnosis and Research Center in Phitsanulok Province. The bedding of these chicken with faeces from 7 days were collected and used as inoculum. After the experimental animals reached three weeks of age, coccidia were spread into the bedding of all coops (Figure 5.1.) after which water was sprayed to assure quick development of coccidia. After one week samples of faeces from all coops were collected and brought to the Diagnosis and Research Center for coccidia test.

When the chickens were 2, 4 and 6 weeks of age, blood was collected and glucose, total protein, albumin and globulin levels determinded. When the broiler reached 6 weeks, they were slaughtered for lesion scoring and carcass evaluation.

Figure 5.1. After 3 weeks coccidia were spread on bedding of the coops

5.2.3. Feed and feeding procedure

All chickens were fed *ad libitum* with a basal diet of formulated feed divided in two phases: starter phase (0-3 weeks) with 23.20% crude protein content and the finisher phase (4-6 weeks) with 21% crude protein content (Table 5.1.). The nutrient content of the feed as analysed by proximate analysis is given in Table 5.2. This feed was added AP leaf meal

supplement at different levels: 0% (control group without AP), 0.1%, 0.2%, 0.3% and 0.4% (Figure 5.2.) after the first week.

Table 5.1. Feed composition of broiler feed in the experiment

Ingredient	Starter phase	Finisher phase
Rice grain, polish & broken	45.50	46.65
Rice bran	10.00	15.00
Soybean meal	33.00	27.00
Fish meal	6.00	6.00
Dicalcium phosphate	1.00	1.00
Shell meal	1.25	1.25
Palm oil	2.00	2.00
Normal salt	0.50	0.50
Vitamin-mineral mix(Premix)	0.50	0.50
Methionine	0.25	0.10
Total	100.00	100.00

Table 5.2. Feed ingredients in diet of starter phase (0-3 weeks), finisher phase(4-6 weeks) on dry matter basis

Feed composition	Starter phase	Finisher phase
Crude protein (%)	23.20	21.00
Ether extract (%)	4.40	6.62
Crude fibre (%)	2.99	3.39
Ash (%)	7.73	7.77
Nitrogen free extract (%)	61.68	61.22
Calcium (%)	2.00	1.95
Phosphorus (%)	0.73	0.76
Metabolisable energy (MJ/kg)*	11.53	11.22

* Calculated based on NRC (1994).

Figure 5.2. AP leaves powder supplement were mixed at different levels into the broiler feed

5.2.4. Measurements

5.2.4.1. Average daily gain (ADG)

The initial body weight of experimental animals was recorded per group. Weight of all birds were measured weekly at 07.00 am before feeding after which ADG for each replication were calculated per week as follows:

$$\text{ADG} = \frac{\text{Final group weight (g) - inital weight (g)}}{\text{Total number of birds}}$$

5.2.4.2. Feed conversion ratio (FCR)

Feed consumption of each group of broiler were measured every week. Feed conversion ratios were determined weekly to evaluate effect of diets:

$$\text{FCR} = \frac{\text{Feed consumed (g)}}{\text{Final weight (g) - initial weight (g)}}$$

5.2.4.3. Mortality rate

Death of broilers were recorded throughout the experiment. Post mortem examinations of dead birds were done at the Department of Animal Science, Phichit College of Agriculture

and Technology, Phichit Province or at the Diagnosis and Research Centre (DRC), Department of Livestock Development, Phitsanulok Province, Thailand. Mortality rate per week was calculated as:

$$\%\ \text{Mortality} = \frac{\text{Total number of dead birds}}{\text{Initial number of birds}} \times 100$$

5.2.4.4. Blood clinical chemistry values

6 broilers were sampled from each treatment (2 per replication) to test their blood values. 1ml of blood was collected per broiler by the veterinarian staff from DRC at the end of week 2, 4 and 6 (Figure 5.3.-5.4.). The blood samples were tested for glucose, total protein, albumin and globulin levels in the serum. Glucose was tested using GOD-PAO method, total protein was determined by the Biuret method (Enzymatic colorimetric test, method without deproteinisation), albumin was tested by BCG method (Photometric Colorimetric Test). All methods are described in Human Gesellschaft für Biochemica und Diagnostica mbH (1998). Globulin levels were calculated as the difference between total protein level and albumin level.

Figure 5.3. Blood sampling in a 2 weeks old broiler

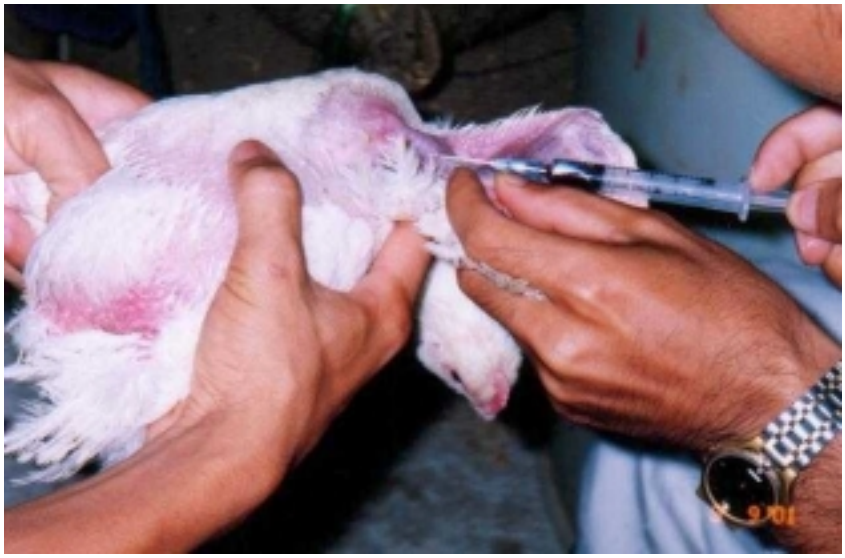


Figure 5.4. Blood sampling in a 4 weeks old broiler

5.2.4.5. Lesion scoring in intestinal tract and carcass evaluation

After all chickens were slaughtered at 6 weeks of age, 9 broilers from each treatment (3 per replication) were sampled randomly for lesions scoring and carcass evaluation. Their intestines were frozen at -15°C. The frozen intestines were brought to the DRC for diagnosis. The lesions and infections of the intestines were given scores from 1 - 4, 1 for no lesion found, and 4 for severe lesions (JOHNSON and REID, 1970).

The live weight, slaughtered weight without feathers, carcass weight without inner organs, inner organs (total inner organs as they are removed by slaughtering including intestinal content) and liver weight were measured and all data recorded.

5.3. Data analysis

The collected data were analysed using analysis of variance procedure (SAS, 1986). Least significant different test was used to compare differences between treatment means.

5.4. Results

Coccidia were found in faeces of broiler chickens in all coops 1 week after infection. A microscopical picture of oocystes found is shown in Figure 5.5.

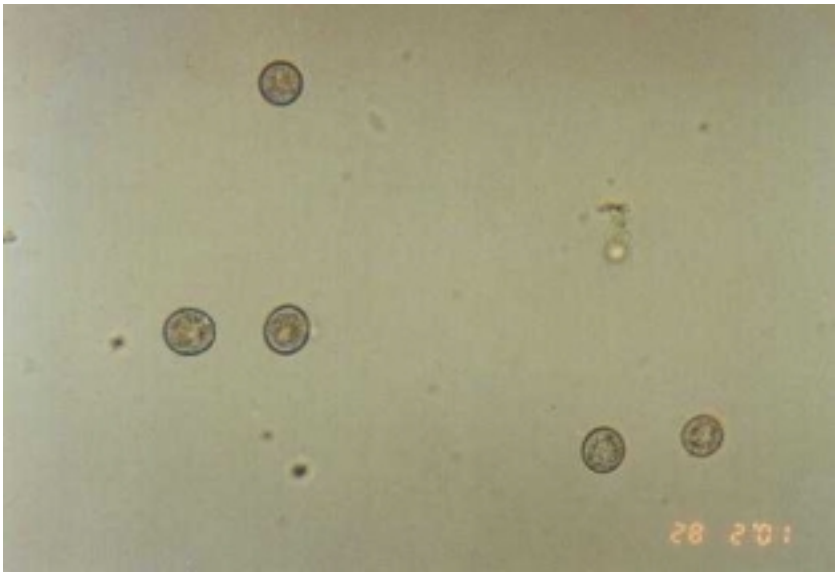


Figure 5.5. Picture of oocystes of coccidia found in faeces of broiler chicken 1 week after infection

The chickens which had developed well during the first week under the brooder lamps showed later clear signs of coccidiosis infection. Some birds looked ill, the appetite was lowered, the birds sat still instead of moving around and some birds developed hemorrhagic diarrhea (Figure 5.6.).

Figure 5.6. Broilers raised under coccidiosis infection at 6 weeks of age

5.4.1. Average daily gain

The result indicated no significant differences ($p>0.05$) in average daily gain (Table 5.3.) of the broiler between the different groups.

Table 5.3. Average daily gain (ADG) of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Group	Week						Average daily gain	Average gain/ bird
	1	2	3	4	5	6		
1	13.69	19.83	26.59	28.01	30.60	25.22	23.99	1007.58
2	13.46	17.61	23.33	37.24	43.49	33.81	27.81	1182.65
3	11.23	17.46	23.37	44.25	38.80	39.76	29.17	1224.11
4	12.32	19.32	27.70	30.91	45.52	36.58	28.72	1206.52
5	12.17	18.05	29.60	31.51	41.83	48.57	30.28	1272.13
SEM[1/]	2.32	5.81	38.29	65.14	196.35	89.73	137.20	

[1/] Standard error of the mean (n = 21)

Average daily gain ranged from 13.69 – 30.60 g/broiler/day in group 1 and from 13.46 – 43.45 in group 2, 11.42 – 44.25 in group 3, 12.32 – 45.52 in group 4 and 12.17 – 48.57 in group 5 (Table5.3.). Even though there were no significant differences among the groups there was a tendency for group 5 to have the highest ADG in week 6, followed with group 3, 4, 2 and 1 with 48.57, 39.76, 36.58, 33.81 and 25.22 g/broiler/day respectively.

5.4.2. Feed conversion ratio

The result indicated no significant differences ($p>0.05$) in feed conversion ratio when differences among treatment means were compared (Table 5.4.).

Mean feed conversion ratio for 1 kilogram weight gain of broilers ranged from 1.22 – 2.69 in group 1, 1.16 – 2.23 in group 2, 1.21 – 2.17 in group 3, 1.17 – 2.22 in group 4 and from 1.20 – 2.05 in group 5. There was a tendency in week 6 for group 5 to have the lowest feed conversion ratio of 2.05, followed by group 3, 4, 2 and 1 with FCRs of 2.17, 2.22, 2.23 and 2.69 respectively. The control group had the highest FCR when compared with other groups.

Table 5.4. Feed conversion ratio (FCR) of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Group	Week						FCR
	1	2	3	4	5	6	
1	1.22	1.44	1.51	1.75	2.44	2.69	1.84
2	1.16	1.43	1.48	1.65	1.99	2.23	1.65
3	1.21	1.42	1.48	1.63	2.05	2.17	1.65
4	1.17	1.30	1.39	1.63	1.80	2.22	1.58
5	1.20	1.41	1.42	1.59	1.76	2.05	1.57
SEM[1/]	0.03	0.03	0.06	0.02	0.18	0.06	0.17

[1/] Standard error of the mean (n = 21)

5.4.3. Mortality rate

The results indicated significant differences across treatments ($p<0.05$) in mortality rates of broilers (Table 5.5.). Group 5 showed better ($p<0.05$) performance than group 1 and 2, but there were no significant differences ($p>0.05$) with groups 3 and 4. In groups 1 and 2 there were no significant difference ($p>0.05$) but group 1 was significantly different from groups 3, 4 and 5. There were no significant difference among groups 2, 3 and 4 but in group 2 there was significant difference from group 5. In groups 3, 4 and 5, there were no significant differences among groups. In total the broiler mortality rate was 42.85 % in group 1, 33.33 % in group 2, 19.04 in group 3 and 4, and 0 % in group 5.

Table 5.5. The mortality rates of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Group	Rations of *A. paniculata* (%)	Mean of mortality rate (%)
1	0	42.85^{a}
2	0.1	33.33^{ab}
3	0.2	19.04^{bc}
4	0.3	19.04^{bc}
5	0.4	0^{c}

LSD (0.05) 23.24; SEM: 163.29

[a,b,c] Means within the same column not having at least one common superscript are significant different ($P<0.05$)

5.4.4. The lesion scoring

There were significant differences ($p<0.05$) in lesion scoring among treatments. The lesion scoring done (Table 5.6.) to evaluate coccidiosis infestation after slaughter at 6 weeks amounted to 3.33 for group 1 which was the highest score. It was 2.17, 1.67, 1.50 and 1.33 for group 2, 3, 4 and 5 respectively. The results indicated no significant difference ($p>0.05$) in lesion scoring between group 1 and 2. Also lesions among groups 2, 3, 4 were not significantly different ($p>0.05$). However, between group 1 and groups 3, 4 and 5, there were significant differences ($p<0.05$). An intestine with coccidiosis lesions is shown in Figure 5.7.

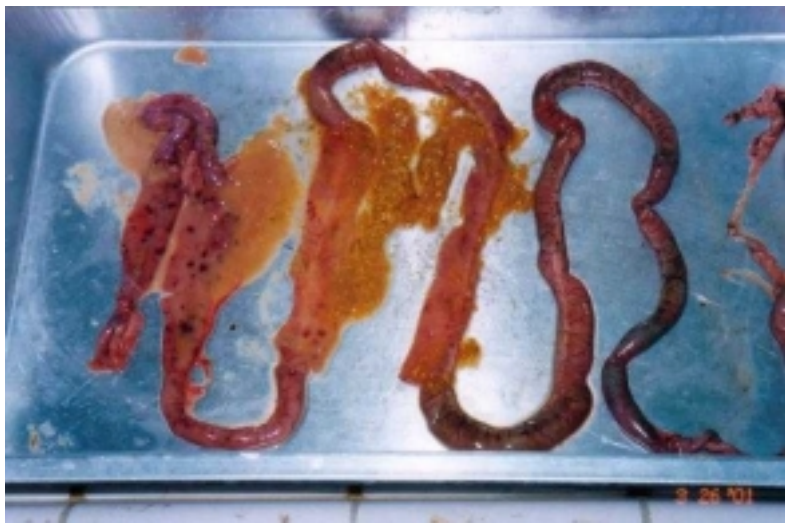


Figure 5.7. Intestinal tract of a broiler slaughtered at 6 weeks with coccidiosis lesions

Table 5.6. The lesion scoring on intestines of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Group	Rations of *A. paniculata* (%)	Mean lesion scoring*
1	0	3.33[a]
2	0.1	2.17[ab]
3	0.2	1.67[b]
4	0.3	1.50[b]
5	0.4	1.33[b]

LSD (0.05) 1.24; SEM: 0.47

[a,b,c] Means within the same column not having at least one common superscript are significant different ($P<0.05$)

* Diagnosis of coccidiosis by lesion scoring ranging from 0 – 4 (Johnson and Reid, 1970);
0: found no lesions; 1: found few lesions; 2: found medium lesions; 3: found much stronger lesions;
4: found strongest lesions

5.4.5. Blood clinical chemistry values

Blood clinical chemistry values of broiler chickens, namely glucose, total protein, albumin and globulin levels in blood serum were analysed at DRC. The result indicated that there were significant differences ($p>0.05$) among groups (Table 5.7.).

Table 5.7. Results of blood clinical chemistry analyses of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Parameters	**Week**	**Group**					**SEM**[1/]
		1	**2**	**3**	**4**	**5**	
Glucose (mg/dl)	2	204.85	193.49	200.18	220.18	181.94	951.44
	4	284.10	311.22	276.48	293.12	287.34	3258.21
	6	262.99	217.90	210.40	222.64	246.19	593.99
Total protein (mg/dl)	2	2.35	2.23	2.56	2.41	2.50	0.11
	4	3.51	4.05	4.25	3.76	4.78	0.48
	6	3.67	3.30	3.49	3.46	4.09	0.41
Albumin (mg/dl)	2	1.07	0.97	0.92	0.99	1.10	0.04
	4	1.43	1.07	1.37	1.22	1.25	0.05
	5	1.98	1.46	1.39	1.51	1.56	0.15
Globulin (mg/dl)	2	1.26	1.25	1.64	1.42	1.49	0.04
	4	2.08	2.98	2.87	2.54	3.52	0.54
	6	1.68	1.84	2.10	1.95	2.53	0.26

[1]/ Standard error of the mean (n = 6)

Glucose level ranged from 181.94 - 220.18 mg/dl in group 1 to group 5 in week 2, 276.48 - 311.22 mg/dl in week 4 and 210.40 - 262.99 mg/dl in week 6. Total protein level ranged from 2.23 - 2.56 mg/dl in group 1 to group 5 in week 2, 3.51 - 4.78 mg/dl in week 4 and 3.30 - 4.09 mg/dl in week 6. Albumin level ranged from 0.92 - 1.10 mg/dl in group 1 to group 5 in week 2, 1.07 - 1.43 mg/dl in week 4 and 1.39 - 1.98 mg/dl in week 6. Globulin level ranged from 1.25 - 1.64 mg/dl in group 1 to group 5 in week 2, 2.08 - 3.52 mg/dl in week 4 and 1.68 - 2.53 mg/dl in week 6.

There were no significant differences ($p>0.05$) in glucose level, total protein, albumin and globulin levels in blood serum among groups 1 to 5 in week 2, 4 and 6.

5.4.6. Carcass characteristics

The results indicated that there were no significant differences ($p>0.05$) in carcass characteristics among groups (Table 5.8.). At 6 weeks of age, the live weight ranged from 1240.33 - 1496.50 g/broiler in group 1 to group 5. Slaughter weight without feathers ranged from 1091.95 - 1313.49 g/broiler, carcass weight without inner organs ranged from 845.97 - 1006.42 g/broiler, weight of inner organs ranged from 226.82 - 250.82 g/broiler and liver weight ranged from 37.65 - 46.28 g/broiler in group 1 to group 5 respectively. However, even if there were no significant differences ($p>0.05$) on carcass characteristics among the groups, there was a tendency for group 5 to have the highest and group 1 the lowest weight for carcass characteristics on live weight, slaughtered weight without feathers, carcass weight without inner organs and liver weight. For inner organs group 2 had the highest weight and group 1 the lowest weight.

Table 5.8. Carcass characteristics of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal supplement after infection with coccidiosis

Variable/gram	**Group**					
	1	**2**	**3**	**4**	**5**	**SEM[1/]**
Life weight	1240.33	1256.67	1330.17	1439.51	1496.50	55405.41
Slaughtered weight without feathers	1091.95	1125.37	1171.20	1272.29	1313.49	38950.49
Carcass weight without inner organs	845.97	871.06	919.94	998.32	1006.42	26298.31
Inner organs	226.82	251.84	247.12	250.82	250.47	616.52
Liver	37.65	41.38	42.25	44.25	46.28	31.64

[1/] Standard error of the mean (n = 9)

5.5. Discussion

Before the chickens arrive, the normal practice of farmers is to clean the housing and prepare clean bedding. For this experiment the housing was cleaned and sprayed with antiseptic drugs before the chickens came. During the first few days the chicken still have immunity from the mother via the egg against coccidiosis but at quite low level (MUANGYAI, 1991) which help them to overcome the stress of transport and getting accustomed to the new housing. After 2-3 weeks the chickens grow at a very high rate and they produce a lot of faeces. If the farmers

will not clean the bedding or turn it over, the bedding becomes moist and ammonia gas production increases. This stimulates outbreaks of diseases (TUNCHO, 1995). Under normal management practices some farmers care less to these things due to extra cost for new bedding or more work to turn over the old bedding. To simulate the optimum conditions for coccidia growth these malpractices were copied and bedding was not changed during the experiment causing unhealthy conditions after 2-3 weeks of raising. MUANGYAI (1991) reported, that in Thailand coccidiosis especially from *E. tenella* is always found in chicken at the age of 3-5 weeks, 1-3 weeks later also *E. necatrix* is found. However, to assure the presence of coccidia in this experiment, coccidia were given into the bedding after 3 weeks. In Thailand coccidiosis is always found more in the rainy season than in other seasons because of the high humidity (MUANGYAI et al., 1990). At the time this experiment began it started to rain giving optimum conditions for coccidia growth.

The AP experimental plant was used as feed supplement for broiler at levels of 0.1, 0.2, 0.3 to 0.4%. These levels were chosen based on a former study by SUJIKARA (2000) who used AP plant supplement to domestic chicken. For his chicken the best levels of AP leaves powder, which gave the lowest mortality rates and best economic result was 0.18%. To have a safe margin, levels up to double the amount of his dose were used (0.4%).

Average daily gain (ADG) and feed conversion ratio (FCR) and carcass characteristics were not significantly different between groups whether they received AP or not. However, increased levels of AP tended to increase average daily gain and reduce feed conversion ratio.

The mortality rate (MR) of broiler was significantly different between groups that got AP feed supplements and the control group. The control group had the highest mortality rate (42.85%) after infection with coccidiosis. Group 5 that got 0.4% AP had the lowest MR (0%). This is in agreement with the observations of SUJIKARA (2000) who also reported lower mortality rates after supplementation of AP leaves powder, though clinical disease and mortality may occur in heavy coccidiosis infections especially in broiler, layers or breeders (POONSUK, 1993).

All measured blood clinical chemistry values in this experiment were in the normal range of chickens of the same breed in Thailand (SONTORNCHAT and THEERAPHAN, 2000).

Different methods can be used to differentiate the coccidia species (MUANGYAI, 1991). In this experiment the lesion scoring technique (JOHNSON and REID, 1970) was used. This method can determine the virulence and species of coccidia infection based on the lesions of the inner organs especially the lesions in the intestinal tract. This method gives better diagnosis than other methods for example counting of oocystes in litter or faeces of chicken which can only classify one specie, *E. maxima*, that has the biggest size (30.5 micron x 20.7 micron) and brown cell wall of oocysts (MUANGYAI, 1991).

In this experiment most of the lesions appeared in the duodenum. It was bloody along the duodenum loop, haemorrhagic syndrome or pin point syndrome. Some lesions were found in the jejunum, and to some extend in ileum and very few in caecum. This means that the chicken were infected with coccidia of *E. acervulina* (MUANGYAI, 1991). This was not surprising since when coccidiosis in broiler is found either in subclinical or clinical form always *E. tenella* and/or *E. acervulina* are involved. Moreover MUANGYAI (1991) reported that *E. acervulina* is always followed by *E. maxima* and *E. tenella*, which were not identified in the present study. *E. acervulina* causes gut damage and leads to mortality through necrotic enteritis in broilers (POONSUK, 1993) which gives the reason for the high mortality rates in this study.

The control group had the highest lesion score (3.33%) and group 5 with no mortality had the lowest score (1.33). It indicates that AP plants effectively reduced disease virulence and mortality rate in the coccidiosis infected broiler, but it can not cure the disease. The best level of AP supplement in this experiment was 0.4% which is higher than the level measured by SUJIKARA (2000). The reason may be that different breeds of chicken or that other plant materials were used in the two experiments. SUJIKARA used AP powder in domestic chicken which have more resistance to the diseases that occur in Thailand than the broiler chicken of imported breed that were used in this experiment. A higher level of AP powder may improve the performance even more.

5.6. Conclusions

According to this study 0.4 % *A. paniculata* as feed additive in broiler feed can reduce the very serious impact of coccidiosis. If promoted to smallholder farmers it will improve the productivity.

6. Experiment 4.

EFFECT OF *ANDROGRAPHIS PANICULATA* ON BROILER PERFORMANCE AND MORTALITY COMPARED WITH CHLORTETRACYCLINE

This experiment was conducted at the poultry research farm, Phichit College of Agriculture and Technology, Phichit, Thailand. The feeding trial was accomplished in approximately 2 months, while the laboratory analyses were conducted after the feeding trial.

6.1. Objectives

The objectives of this experiment were as follows:
1. To assess whether AP leaf powder or chlortetracycline as feed additive causes changes in broiler performance,
2. To assess whether AP leaf powder or chlortetracycline as feed additive causes changes in broiler mortality rate.

6.2. Materials and methods

6.2.1. Animals and housing

240 one day old broilers were bought from C.P. Company Limited in Phitsanulok Province. All chickens (240) were raised in coops on deep litter of rice husk under station management for 6 weeks. During the first week the chicken were kept together and brooder lamps were supplied for all chicken.

6.2.2. Design of experiment

After one week the chickens were randomly divided into 6 groups (6 treatments) under a completely randomised design (STEEL and TORRIE, 1981). Each treatment had four replications with 10 chickens per replication.

When the chickens were 2, 4 and 6 weeks of age, blood was collected and glucose, total protein, albumin and globulin levels tested. When the broilers reached 6 weeks, they were slaughtered for carcass evaluation and for microbiological findings in inner organs.

6.2.3. Feed and feeding procedure

All chickens were fed *ad libitum* with a balanced diet fed in two phases: starter phase (0 - 3 weeks) with 23.20% crude protein content and finisher phase (4 - 6 weeks) with 21% crude protein content (Table 5.1.). The nutrient content of the feed as analysed by proximate analysis is given in Table 5.2. AP leaf meal additive at different levels was added to this feed: 0% (control group), 0.1%, 0.2%, 0.3%, and 0.4% of AP leaves, that were tested for quality in experiment 1 (Andrographolide 7.30%) for group 1 to 5 respectively. For group 6, 50 mg of chlortetracycline (CTC) per kg of feed was used (Figure 6.1.). Water was freely available.

Figure 6.1. Chlortetracycline was mixed in feed used in treatment 6

6.2.4. Measurements

6.2.4.1. Average daily gain (ADG)

The initial body weight of experimental animals was recorded per group. Weight of all birds were measured weekly at 07.00 am before feeding after which ADG for each replication were calculated per week as follows:

$$\text{ADG} = \frac{\text{Final group weight (g) - inital weight (g)}}{\text{Total number of birds}}$$

6.2.4.2. Feed conversion ratio (FCR)

Feed consumption of each group of broilers was measured every week. Feed conversion ratios were determined weekly:

$$\text{FCR} = \frac{\text{Feed consumed (g)}}{\text{Final weight (g) - initial weight (g)}}$$

6.2.4.3. Mortality rate

Death of broilers was recorded throughout the experiment. Post mortem examinations of dead birds were done at the Department of Animal Science, Phichit college of Agriculture and Technology, Phichit Province or at the Diagnosis and Research Centre (DRC), Department of Livestock Development, Phitsanulok Province, Thailand. Mortality rate per week was calculated as:

$$\%\,\text{Mortality} = \frac{\text{Total number of dead birds}}{\text{Initial number of birds}} \times 100$$

6.2.4.4. Blood clinical chemistry values

8 broilers were sampled from each treatment (2 per replication) to test their blood clinical chemistry values. 1 ml blood was collected per broiler by the veterinarian staff from DRC at the end of week 2, 4 and 6. The blood samples were tested for glucose, total protein, albumin and globulin levels in the serum. Glucose was tested using GOD-PAO method, total protein was determined by the Biuret method (Enzymatic colorimetric test, method without deproteinisation), albumin was tested by BCG method (Photometric Colorimetric Test). All methods are described in "Human Gesellschaft für Biochemica und Diagnostica mbH" (1998). Globulin levels were calculated as the difference between total protein level and albumin level.

6.2.4.5. Microbiological findings in inner organs and carcass evaluation

After all chickens were slaughtered at 6 weeks of age, 8 broilers from each treatment (2 per replication) were sampled for microbiological findings in inner organs. Liver, kidneys, heart

were frozen at -15°C and brought to the DRC for diagnosis. 20 broilers from each treatment (5 per replication) were sampled for carcass evaluation. The live weight, slaughter weight without feathers, carcass weight without inner organs, inner organs and liver weight were measured and all data recorded.

6.3. Data analysis

The collected data were analysed using analysis of variance procedure (SAS, 1986). Duncan`s new multiple range test was used to compare differences between treatment means.

6.4. Results

6.4.1. Average daily gain

The result indicated no significant differences ($p>0.05$) in average daily gain of the 6 weeks growth period (Table 6.1.) between the groups.

Table 6.1. Average daily gain (ADG) of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal or chlortetracycline additive.

Group	Week						Average daily gain	Average gain/ bird
	1	2	3	4	5	6		
1	12.29	20.57	28.03	45.35	46.42	33.92	31.09	1305.99
2	13.57	21.24	28.74	54.10	45.71	44.64	34.66	1456.00
3	13.78	21.35	28.21	53.37	44.28	48.21	34.86	1464.40
4	13.92	22.06	29.82	52.49	39.63	51.78	34.95	1467.90
5	14.21	21.81	31.60	50.71	52.13	53.57	37.33	1568.21
6	13.13	21.28	25.71	50.36	38.56	44.61	32.28	1355.76
SEM[1/]	0.11	2.48	6.30	27.58	50.78	70.18	240.93	

[1/] Standard error of the mean (n = 40)

Average daily gain ranged from 12.28 – 46.42 g/broiler/day in group 1 and from 13.57 – 54.10 in group 2, 13.78 – 53.37 in group 3, 13.92 – 52.49 in group 4 and 14.21 – 53.37 in group 5, and 13.13 – 50.36 in group 6. The average daily weight from group 1 – 6 were 31.09, 34.66, 34.86, 34.95, 37.3 3 and 32.28 respectively. ADG ranged from 12.29 -1 4.21, 20.57 -

22.06, 25.71 - 31.60, 45.35 - 54.10, 38.56 - 52.13 and 33.92 - 53.57 in week 1 to 6 respectively. Even though there were no significant differences among the mean ADGs of the groups there was a tendency for group to have the highest ADG, followed by groups 4, 3, 2, 6 and 1 with respective ADGs of 37.33, 34.95, 34.86, 34.66, 32.28 and 31.09 g/broiler/day. A similar pattern was observed within the separate weeks.

6.4.2. Feed conversion ratio

The results indicated no significant differences ($p>0.05$) in feed conversion ratio between the treatment groups (Table 6.2.). Mean feed conversion ratio ranged from 1.50 – 2.69 in group 1, 1.35 – 2.27 in group 2, 1.34 – 2.10 in group 3, 1.33 – 1.98 in group 4, 1.33 – 1.82 in group 5 and from 1.45 – 2.39 in group 6. Mean FCR from group 1 to 6 were 1.97, 1.71, 1.66, 1.62, 1.57 and 1.80 respectively.

Table 6.2. Feed conversion ratio (FCR) of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal or chlortetracycline additive

Group	Week						FCR
	1	2	3	4	5	6	
1	1.50	1.66	1.74	1.92	2.36	2.69	1.97
2	1.35	1.44	1.58	1.73	1.94	2.27	1.71
3	1.34	1.41	1.56	1.71	1.89	2.10	1.66
4	1.33	1.43	1.47	1.73	1.80	1.98	1.62
5	1.33	1.41	1.45	1.68	1.76	1.82	1.57
6	1.45	1.58	1.60	1.78	2.05	2.39	1.80
SEM[1/]	0.002	0.002	0.002	0.004	0.013	0.032	0.10

[1/] Standard error of the mean (n = 40)

FCR ranged from 1.33 - 1.50, 1.41 - 1.66, 1.45 - 1.74, 1.67 - 1.92, 1.76 - 2.36 and 1.82 - 2.69 in week 1 to 6 respectively. Even though there were no differences ($p>0.05$) in mean FCR, there was a tendency for group 5 to have the best feed conversion ratio of 1.57, followed by groups 4, 3, 2, 6 and 1 with FCRs of 1.62, 1.66, 1.71, 1.80 and 1.97 respectively. The control group and the group supplemented with CTC had the worst FCRs when compared with the other groups.

6.4.3. Mortality rate

The result indicated that there were significant differences across treatments ($p<0.05$) in mortality rates (Table 6.3.). The mean broiler mortality rates were 32.50 % in group 1, 20.00 % in group 2, 12.50% in group 3 and 10.00% in group 4, 7.50% in group 5 and 15.00 % in group 6. Group 1 (control) had the highest mortality rate (32.50%), followed by groups 2, 6, 3, 4, and 5 with mortality rates of 20, 15, 12.50, 10, and 7.5 % respectively. The broilers in group 5 showed better ($p<0.05$) performance in terms of mortality rate (MR) than the broilers in group 1, 2 and 6 but there were no significant differences ($p>0.05$) from broilers in groups 3 and 4. In group 1, MR was higher ($p<0.05$) than in other groups. Between group 2 and 6, there were no difference ($p>0.05$) but MR of group 2 was significant higher ($p<0.05$) than of group 4, 5 and 6. Among groups 3, 4 and 5, there were no significant differences ($p>0.05$) but their MRs were lower ($p<0.05$) than for groups 1 and 2.

Table 6.3. Mortality rates of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal or chlortetracycline additive

Group	Rations of *A. paniculata* (%)	Mean of mortality rate (%)
1	0.0	32.50^{a}
2	0.1	20.00^{b}
3	0.2	12.50^{cd}
4	0.3	10.00^{cd}
5	0.4	7.50^{d}
6	chlortetracycline	15.00^{bc}

SEM (standard error of the mean): 18.05

^{a,b,c} Means within the same column not having at least one common superscript are significant different ($p<0,05$)

Post mortem analysis on dead birds showed that the dead birds suffered from Colibacillosis between week 4 and 6.

6.4.4. Blood clinical chemistry values

The result indicated that there were no significant differences ($p>0.05$) in the means of glucose, total protein, albumin and globulin levels in serum (Table 6.4.).

Table 6.4. Effect of AP or chlortetracycline on blood clinical chemistry values of glucose, total protein, albumin and globulin levels in serum

Parameters	Week	Group						SEM[1/]
		1	2	3	4	5	6	
Glucose (mg/dl)	2	228.69	258.40	246.15	232.74	276.23	290.83	1209.73
	4	215.21	204.55	228.57	205.77	243.56	211.95	881.53
	6	239.53	209.40	201.99	221.82	219.88	208.97	338.62
Total protein (mg/dl)	2	3.20	3.35	2.80	3.10	2.46	2.93	0.42
	4	3.11	3.25	3.23	3.88	3.03	3.32	0.19
	6	2.97	3.63	3.11	3.88	3.51	3.59	0.20
Albumin (mg/dl)	2	0.97	1.19	1.03	1.01	1.05	1.02	0.03
	4	1.66	1.68	1.50	1.59	1.55	1.64	0.02
	5	1.78	1.72	1.68	2.12	1.53	1.34	0.18
Globulin (mg/dl)	2	2.23	2.16	1.77	2.09	1.41	1.91	0.24
	4	1.45	1.57	1.73	2.29	1.48	1.68	0.16
	6	1.19	1.91	1.43	1.76	1.98	2.25	0.32

[1/] Standard error of the mean (n = 8)

Glucose levels ranged from 228.68 – 290.83 mg/dl in group 1 to group 5 in week 2 (Table 6.1), 204.55 – 243.56 mg/dl in week 4 and 201.99 – 239.53 mg/dl in week 6. Total protein level ranged from 2.46 – 3.35 mg/dl in group 1 to group 5 in week 2, 3.03 – 3.88 mg/dl in week 4 and 2.97 – 3.88 mg/dl in week 6. Albumin levels ranged from 0.97 – 1.19 mg/dl in group 1 to group 5 in week 2, 1.50 - 1.68 mg/dl in week 4, and 1.34 – 2.12 mg/dl in week 6. Globulin levels ranged from 1.41 – 2.23 mg/dl in group 1 to group 5 in week 2, 1.45 – 2.29 mg/dl in week 4 and 1.19 - 2.25 mg/dl in week 6. No special trend could be observed among the values.

6.4.5. Carcass characteristics

The result indicated that there were no significant differences ($p>0.05$) in the mean carcass characteristics of broilers between each group (Table 6.5.). Live weight at 6 weeks of age ranged from 1414.50 - 1503.50 g/broiler in group 1 to 5 (Table 6.2). Slaughter weight without feathers ranged from 1224.52 - 1319.62 g/broiler. Carcass weight without inner organ ranged from 1009.53 - 1073.19 g/broiler. Weight of inner organs ranged from 191.42 – 216.06 g/broiler. Liver weight ranged from 42.62 – 49.94 g/broiler in group 1 to 5. No special trend could be observed among the values.

Table 6.5. Carcass characteristics of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal or chlortetracycline additive

Variable/gram	Group						SEM[1]/
	1	2	3	4	5	6	
Live weight	1414.50	1433.50	1451.50	1460.75	1503.50	1419.50	5472.54
Slaughter weight without feathers	1251.57	1283.66	1264.26	1224.52	1319.62	1254.35	3696.95
Carcass weight without inner organs	1025.99	1033.12	1058.00	1009.52	1073.19	1029.33	4646.08
Inner organs	207.85	191.42	200.13	212.10	216.06	195.83	180.62
Liver	48.61	42.62	45.44	45.19	49.94	44.54	35.94

[1]/ Standard error of the mean (n = 20)

6.4.6. Microbiological findings in inner organs

The microbiological examination revealed inner organs (heart, kidneys and liver) had *E. coli* and/or *Staphylococcus spp*. in all groups (Table 6.6.). In group 1 (control) 4 of the 8 samples were contaminated, 2 samples with *E. coli* and 2 samples with *Staphylococcus spp.* in heart and kidneys. In group 2 (0.1% AP) also 5 of 8 samples were contaminated, 4 samples with *E. coli* and 1 sample with *Staphylococcus spp.* in heart, liver, kidneys. In group 3 (0.2% AP) 6 of 8 samples were contaminated, 4 samples with *E. coli* and 2 samples with *Staphylococcus spp*. in heart and kidneys. In group 4 (0.3% AP) 2 of 8 samples were contaminated, 2 samples with *E. coli* and *Staphylococcus spp.* in heart, liver and kidneys. In group 5 (0.4% AP) 3 of 8 samples were contaminated with *E. coli* in heart and kidneys. In group 6 (chlortetracycline with 50 mg/1kg DM of feed) 2 of 8 samples were contaminated, 1 sample with *E. coli* and 1 sample with *Staphylococcus spp.* in kidneys.

Table 6.6. Microbiological findings in inner organs of broilers fed diets with varying levels of *Andrographis paniculata* leaf meal or chlortetracycline additive

Treatment/Replication	Organs	Type of micro-organism
T_1R_1	heart, kidney	*E. coli*
T_1R_1	heart	*Staphylococcus spp.*
T_1R_2	-	
T_1R_2	kidneys	*Staphylococcus spp.*
T_1R_3	-	
T_1R_3	-	-
T_1R_4	kidney	*E. coli*
T_1R_4	-	
T_2R_1	heart, liver, kidney	*E. coli*
T_2R_1	kidney	*Staphylococcus spp.*
T_2R_2	-	-
T_2R_2	-	
T_2R_3	kidney	*E. coli*
T_2R_3	-	
T_2R_4	kidney	*E. coli*
T_2R_4	kidney	*E. coli*
T_3R_1	kidney	*E. coli*
T_3R_1	-	-
T_3R_2	kidney	*Staphylococcus spp.*
T_3R_2	heart, kidney	*Staphylococcus spp.*
T_3R_3	kidney	*E. coli*
T_3R_3	-	-
T_3R_4	kidney	*E. coli*
T_3R_4	kidney	*E. coli*
T_4R_1	-	-
T_4R_1	-	-
T_4R_2	-	-
T_4R_2	-	
T_4R_3	-	-
T_4R_3	heart, kidney	*E. coli*, *Staphylococcus spp.*
T_4R_4	-	-
T_4R_4	liver, kidney	*E. coli*, *Staphylococcus spp.*
T_5R_1	kidney	*E. coli*
T_5R_1	-	-
T_5R_2	-	-
T_5R_2	-	-
T_5R_3	kidney	*E. coli*
T_5R_3	-	-
T_5R_4	-	-
T_5R_4	heart	*E. coli*
T_6R_1	-	-
T_6R_1	-	-
T_6R_2	kidney	*Staphylococcus spp.*
T_6R_2	kidney	*E. coli*
T_6R_3	-	
T_6R_3	-	-
T_6R_4	-	
T_6R_4	-	-

6.5. Discussion:

The experimental plant AP was used in different levels as feed additive for broilers and compared with the use of chlortetracycline (CTC). The mean average daily weight gain (ADG) as well as mean feed conversion ratio (FCR) of the groups indicated no significant differences ($p>0.05$) whereas the mortality rates of the groups were significant different. ($p<0.05$). The result indicates that group 1 without AP as feed additive had the highest mortality rate and group 5 that got 0.4% of AP had the lowest mortality rate also if compared with group 6 that got CTC.

STUTZ and LAWTON (1984) found effects of diet and antimicrobials on growth, feed efficiency, intestinal *C. perfringens* and ileal weight of broiler chicks. The result supports the concept of antimicrobials as growth permittants and provide further evidence for *C. perfringens* as reason for growth depression. SHAFEY and McDONALD (1991) found that antibiotic supplement did not improve the performance of chickens but increased the digestibility of most amino acids in chickens fed on diets. This is especially true for diets with high calcium and high available phosphorus contents (KOBLAND et al., 1987).

The results indicated no significant differences in carcass characteristics of broilers among the groups. The same is true for the results of blood values. By the microbiological examination of the inner organs in group 3 *E. coli* and *Staphylococcus spp*. were found in organs in six of eight animals, followed with group 2, 1, 5, 4 and 6. That could indicate that 0.3 and 0.4% AP as feed supplement can reduce infections from bacteria. The 0.4 % only *E. coli* was found in organs of three of eight animals whereas at 0.3% AP level both *E. coli* and *Staphylococcus spp.* was found in organs of two animals. For the group that used CTC bacteria were found in two out of eight samples. That means AP at 0.3% and 0.4% levels can reduce bacterial infection as good as CTC.

However, due to the lower mortality rate of group 5 that got 0.4% of AP supplement when compared with group 6 with used CTC antibiotic, the use of AP as feed supplement can be recommended for chicken raising. It may also be better for human consumer if farmer use AP instead of CTC since CTC leaves antibiotic residue in tissues of treated animals as pointed out by AL-GHAMDI et al. (2000). They found, in samples of chicken muscle, liver and eggs collected from 33 broiler and 5 layer farms in the eastern province of Saudi Arabia over a

period of two years antibiotic residues in products from 23 (69.7%) broiler and 3 (60%) layer farms. Also 87% and 100% of the samples from broiler farms which contained antibiotic residue were positive for at least one tetracycline compound in raw muscle and liver respectively. Furthermore, in 82.6% and 95.5% of these samples tetracycline compounds were found in excess of the permissible maximum residue limit (MRL) in raw muscle and liver. They recommended that concern was needed and more attention should be given to withdrawal times. They identified a need for more strict regulations for use of antimicrobial drugs in poultry production. So far no studies are available on eventual residues of AP active components in chicken meat.

It has been pointed out in many studies that the use of antibiotics of the tetracycline group including chlortetracycline that was used in this experiment can cause resistance of bacteria in the animal body. MOLITORIS et al. (1986) found that the use of chlortetracycline in the diets of chicken caused resistance of broiler faecal *streptococci*. Mutations leads to distribution of resistant strains of bacteria. LOPES et al. (1979) found that tetracycline and chloramphenicol in feed of broiler in a eight weeks period selected for a resistant population of *E. coli*. OHYA and SATO (1983) found also effects of dietary antibiotics on intestinal microflora in broiler chicken. They suggested that antibiotics used as feed additives may possibly affect the stability of the intestinal microflora in chicken. ELWINGER et al. (1998) found effects of antibiotic growth promoters and anticoccidials on growth of *C. perfringen* in the caeca and also on broiler performance. They reported that under good hygienic conditions it is not necessary to use antibiotic growth promoters to increase bird performance when the diets are supplemented with an anticoccidial with antibacterial effects.

The use of antibiotic in animal diets has some risk for human health if the withdrawal period is not long enough. Moreover the use of antibiotic lead to an increasing number of resistant strains of bacteria that will cause problems to the human health in the future. AP leaves could possibly replace the use of antibiotic in the poultry production. However, up to now there is too little knowledge of possible negative effects.

6.6. Conclusions

According to this study the use of 0.4% AP leaves as feed supplement can replace the use of antibiotics in poultry production.

7. GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE WORK

7.1. General discussion

The use of *Andrographis paniculata* (AP) as a medicinal plant against diarrhea, intestinal and respiratory problems in human and in animal has been known for long. However, there is still lack of scientific information regarding its properties, active compounds and possibilities of induced side effect in broiler chickens. The objective of this study was to determine the effects of administration of varying levels of AP leaf powder on broiler performance, mortality and coccidiosis and to compare the use of different levels of AP powder with those of CTC. In addition, the antibacterial activity of AP leaves (aqueous and alcohol extract) against *Salmonellae*, *E. coli* and *P. multocida* was identified.

The oral administration of AP leaf powder at 0.4% as feed additive for broilers reduced the mortality rate and lesions in the intestinal tract of broilers with coccidiosis. Probably, a higher dosage of the AP leaf could have led to a higher broiler performance such as average daily gain or feed conversion ratio. The AP leaf powder administration at week 6 demonstrated better chicken growth performance than in the control group. The lesion score of broilers with coccidiosis infection demonstrated less virulence or symptoms of disease damage to gastro-intestinal inner organs.

A significant difference ($p<0.05$) was observed among groups. Mortality was 42.85% in the control group and 33.33, 19.04, 19.04 and 0% in the 0, 0.1, 0.2, 0.3 and 0.4% AP groups respectively. Moreover, this study also tended to reduce the virulence of coccidiosis infections in the group that used AP leaves powder. The lesion scoring showed significant differences between both groups. The highest score, indicating the highest virulence of the disease was seen in the control group, followed by groups 2, 3, 4 and 5 respectively with the scores of 3.33, 2.17, 1.67, 1.50 and 1.33. DUKE and AYENSU (1985) reported similar findings in an experiment on animals that demonstrated that AP can prevent or stop diarrhea. The AP extracts have been shown to have significant effect against diarrhea associated with *E. coli* infection. The active compounds that are called diterpene lactones including the main active compound called andrographolide have shown comparable activity to loperamide (the most common anti diarrhea drug). SAWASDIMONGKOL et al. (1990) and THAMAREE et al. (1985) found that 50% and 85% alcoholic extracts were effective in reducing intestinal tract

movements in rats. SITHISOMWONGSE et al. (1989) and PLEUMJAI and SITHISOMWONGSE (1990) also found that 70% and 80% AP ethanol extract can kill bacteria that cause diarrhea for example *E. coli* and *V. cholerae*. GUPTA et al. (1990) reported that the active ingredients in AP leaves against diarrhea are andrographolide and deoxyandrographolide. THANANGKUL and CHAICHANTIPYUTH (1985) reported that AP leaves powder can reduce the amount of stool of the patients who got diarrhea and bacteria dysentery. The result was confirmed by YIN and GUO (1993) who tested AP properties against acute bacterial diarrhea in patients with 82.5% cure rate. Its effect on bacterial dysentery was studied by CHTURVEDI et al. (1983) with overall effectiveness of 91.3%. That demonstrates that AP has properties to reduce the symptoms of diarrhea.

No activities of AP against *E. coli* was seen in this study. This is different from other studies like PLEUMJAI (1992) who tested human bacteria in her experiment. However, in this experiment bacteria from animal were used. These bacteria strains may be resistant since the farmers used antibiotic drugs to treat the animal before the animals died and tissue was send for examination. This idea is supported by the result that these bacteria were also resistant towards streptomycin. POONSUK (1993) reported, that *E. coli* mutates quickly and it is very difficult to treat with the same drugs.

Antibiotic drugs are available in the local pharmacies in Thailand. Farmers can buy and treat their animals freely. That causes many problems. First, all bacteria may become resistant to the antibiotic drugs which make it difficult to treat animal diseases with the same drugs and same dose as usual (POONSUK, 1993). The risk on human health is another problem. Still a high percentage of the produced meat have residues of antibiotics (HISAO et al., 1995). This was the reason to investigate possibilities of AP plant to replace the antibiotic drugs. This study revealed that the AP leaf powder at 0.4% gave better results with lower mortality rate compared with the control and antibiotic group. The 0.4% AP had the lowest mortality rate (7.5%) followed by 0.3%, 0.2% AP groups, antibiotic group, 0.1% AP and control group with 10%, 12.50%, 15%, 20% and 32.50% respectively. There were significant differences between the group that received 0.4% AP supplement and control group ($p<0.05$).

The biological response and the properties of AP active constituents and the effect of AP leaves could be different across regions. These differences could be due to different types of

soil, fertilisation, harvesting period, drying method, storage method, climate or season and variety of AP plant.

The negative side effect and the toxicity effect associated with consumption of AP leaves was also investigated. MPRI (1999) gives the indication, contraindication and warning that AP leaves are not to be used in case of patients who have sore throat from infection by *Streptococcus* group A, preceding bacterial kidneys inflammation, neck ulcer or high fever (LEELARASAMEE et al. 1990). Persons who use AP plant might get stomach-ache, diarrhea or headache. They should change to use other medicine (PMPPHC, 1996). In many studies it was found that AP leaves have low toxicity. ZHENG (1982) found that the absorption and excretion is rapid, 80% is removed within 8 hr via the kidneys and digestive tract, 90% is eliminated within 48 hr. This indicates AP's low residual toxicity. BURGOS et al. (1997) studied the possible testicular toxicity of dried extract of AP in rats by giving 20, 200 and 1000 mg AP dried extract/kg BW and found no toxicity. SITTHISOMWONGSE et al. (1989) who studied the acute toxicity of AP, administered 50% AP ethanol extract to rats orally, with the highest dose 15 g AP/kg BW. They also found no toxicity. To test the subchronic toxicity of AP, they gave 0.12, 1.2 and 2.4 g AP powder/kg BW to rats equal to 1, 10 and 20 times of human dose. The growth rate of the rats were normal and no toxicity was found in the blood system neither in the inner organs. The results of these studies indicates that AP powder at the rates used for humans is safe and of low toxicity.

7.2. Recommendations for future work

Further studies are necessary regarding:

1. the agronomic factors which might influence the content of active constituents in AP leaves,
2. the different contents of active constituents in the different arial parts of AP plants and quality control of plant material,
3. feeding of AP supplement to day old chickens,
4. the effect of AP on other poultry diseases i.e., fowl cholera and salmonellosis,
5. the action of AP on other diseases i.e., in respiratory system, cardiovascular system, liver or gallbladder and its anti-inflammatory protection properties,
6. the metabolism and biochemical reactions of AP active constituents in the body,

7. efficacy, toxicity and accumulation of AP constituents in tissue to minimise negative side effects.

8. CONCLUSIONS

70% and 85% alcohol extract of the AP leaves with 7.30% andrographolide in concentrations of 1:10 showed in disk diffusion susceptibility tests high activity to the diarrheal bacteria *S. typhimurium* and *P. multocida*, whereas the aqueous extract of AP leaves had no activity.

The administration of 0.4% AP leaves with 7.30% andrographolide as supplement in broiler feed reduced the mortality rate of broilers and caused the lowest lesion scoring of the coccidiosis infection in the intestinal tract compared to the control group.

The administration of AP leaves as supplement in broiler feed can replace chlortetracycline as feed supplement since no significant differences in broiler performance was found when AP and antibiotic supplement were compared. Thereby, the use of AP plant can reduce the risk of antibiotic residues in chicken meat.

The administration of AP leaves for 42 days in broiler chickens revealed no toxicity effect in broilers. However, before using AP as a medicinal plant in animal production, efficacy and toxicity confirmation tests are required, eventual residues accumulation should be investigated and quality control of AP plants be considered.

9. SUMMARY

Andrographis paniculata (Burm f.) Nees (AP) is a shrub found throughout India and other Asian countries (China, India, Java and Thailand) sometimes called “India Echinacea”. It has been used historically in epidemics, including the Indian flu epidemic in 1919, during which AP was credited with stopping the spread of the disease. It is well known for its active compound andrographolide which is used as medicine for humans. On the other hand, AP is long known in traditional Asian medicine as an immune system booster. AP is said to have beneficial effects on various modifying functions and ailments ranging from degenerative disease to the common cold. This investigation was set up to elucidate the properties of active compound and effect of AP leaves on broiler performance, mortality and coccidiosis in broiler chicken and also to determine the antibacterial activity of AP on diarrheal bacteria such as *Salmonellae*, *E. coli*, and *P. multocida.*

Four experiments were conducted in Phichit, Thailand using 345 one day old broiler chicks.

Experiment 1.

Experiment 1 was set up to analyze and to identify the phytochemical compounds and chemical contents of AP using color test as preliminary test. Thin layer chromatography was used as confirmatory test to identify main active constituents. Moisture, total ash, acid insoluble ash and solvent extractives were also determined. In the preliminary test with Kedde reagent, the colour changed to purple red and in the test with ethanolic potassium hydroxide the colour changed to red and afterwards it changed to yellow. In this way it can be determined that the AP plant sample contain diterpene lactones as active constituents. In the confirmatory test the chromatogram of the AP plant sample had the same hRf values as the diterpene lactones standards. So it can be stated that the plant sample contains the three active compounds. All results of the chemical analysis were better than required in the THM quality specifications.

Experiment 2.

This experiment was carried out to determine the antibacterial activity of *Andrographis paniculata* (Burm. f.) Nees as AP aqueous extract, 70% alcoholic extract, 85% alcoholic extract of AP leaves on bacterial agents of diarrhea. In experiment 2.1, the 70% and 85% alcoholic extract with the concentration 1:10 showed high activity on *S. typhimurium* whereas the aqueous extract showed no activity on the bacteria. Also in experiment 2.2., the 70% and 85% alcoholic extract showed high antibacterial activity against *P. multocida* but not on other types of bacteria. The same result was observed with aqueous extract. *E. coli* and one strain of *P. multocida* were resistant to streptomycin which was used as control. Thus, the potency of AP as antibacterial agents against diarrhea is promising.

Experiment 3.

The third experiment was conducted to elucidate the effect of AP leaves powder mixed in the broiler feed on coccidiosis, the growth performance, feed conversion ratio and broiler mortalities and to establish the proper ration to mix AP leave powder in broiler feed. One hundred and five broiler chickens were divided into five groups and raised in deep litter system under station management for 6 weeks. They were fed a balanced diet added 0% (control group) 0.1%, 0.2%, 0.3%, and 0.4% of AP leaf meal. In every group average daily gain (ADG), feed conversion ratio (FCR) and mortality were measured. Every 2 weeks blood samples were collected and analyzed for glucose, total protein, albumin and globulin levels. As the chickens reached three weeks of age, coccidia were spread to the bedding. After 1 week the faeces were checked and coccidia found in every group. After 6 weeks the chicks were slaughtered, the intestines collected and diagnosis for infection of coccidiosis with lesion scoring were made. Also carcass characteristics were collected. Data were collected and analyzed according to analysis of variance (ANOVA) and least significant test was done on differences between groups. The study showed that there were no differences ($p>0.05$) in average daily gain, feed conversion ratio, blood clinical chemistry values and carcass characteristics of broilers but differences ($p<0.05$) in mortality rates of broilers in group 1 and group 3, 4, 5 and difference ($p<0.01$) between group 1 and group 5, and difference ($p<0.05$) in lesion scoring between group 1 and group 5.

Experiment 4.

Experiment 4 was carried out to examine the effect of AP leaves meal on broiler performance and mortality and to establish the proper levels of AP leaves meal supplement. 240-broiler chickens were divided into six groups and raised in coops in deep litter system under station management for 6 weeks. They were fed a balanced diet added 0% (control group) 0.1%, 0.2%, 0.3%, 0.4% of AP leaf meal. For group 6, the feed was mixed with chlortetracycline (50 mg/ 1 kg of feed). In every group average daily gain (ADG), feed conversion ratio (FCR) and mortality rates were measured. Every 2 weeks blood samples were collected and analyzed for glucose, total protein albumin and globulin. After 6 weeks the chicks were slaughtered. Carcass characteristics and microbiological findings in inner organs were analyzed. Data were collected for analysis according to ANOVA and Duncan´s new multiple rang test. The study showed no significant differences in mean of average daily gain, feed conversion ratio, blood clinical chemistry values and carcass characteristic among all treatment groups. However, there were significant differences in mortality rate between group 1 (control) and other groups. Group 5 that received 0.4% AP as feed additive, showed lower mortality rate than group 6 that received chlortetracycline. Thus, AP leaves can be used as feed additive to replace antibiotics due to lower mortality rate. This will reduce the risk of antibiotic residue in chicken meat that can occur when antibiotics are used in poultry production.

10. REFERENCES

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11. APPENDIX

Appendix 1. Mean of temperature (°C) and mean of relative humidity (%) in broiler housing during 6 weeks in experiment 3

Week	Temperature/Time				Relative humidity/Time			
	06.00	**12.00**	**18.00**	**24.00**	**06.00**	**12.00**	**18.00**	**24.00**
1	23.00	30.28	24.92	24.71	90.00	66.71	62.28	90.00
2	23.28	30.85	25.14	25.14	90.71	64.42	63.85	91.42
3	23.64	32.14	30.42	25.57	92.85	58.00	68.42	89.85
4	24.71	31.21	29.85	26.21	92.14	64.42	73.31	88.14
5	23.78	27.78	26.50	24.92	94.28	78.14	83.00	89.42
6	26.14	33.71	31.14	27.28	89.85	58.42	67.14	87.28

Appendix 2. Mean of temperature (°C) and mean of relative humidity (%) in broiler housing during 6 weeks in experiment 4

Week	Temperature/Time				Relative humidity/Time			
	06.00	**12.00**	**18.00**	**24.00**	**06.00**	**12.00**	**18.00**	**24.00**
1	22.42	33.00	22.85	23.14	90.00	74.28	85.85	83.28
2	22.28	32.00	29.57	24.28	90.00	66.14	73.42	86.57
3	23.71	33.28	31.14	25.85	88.85	61.71	69.00	89.14
4	24.85	30.71	29.28	26.00	89.00	68.14	75.00	83.57
5	23.85	29.85	27.85	25.28	89.00	76.14	80.71	90.42
6	26.42	33.28	29.85	26.71	88.71	65.85	77.57	85.57

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