การประเมินประสิทธิผลของยาต้านจุลชีพสำหรับการรักษาและควบคุมโรค PORCINE PROLIFERATIVE ENTEROPATHY

นางสาวชนันดา ระพนาไพรวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ภาควิชาอายุรศาสตร์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EVALUATION OF ANTIMICROBIAL EFFICACY FOR TREATMENT AND CONTROL OF PORCINE PROLIFERATIVE ENTEROPATHY

Miss Chanunda Raphanaphraiwan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2013

Thesis Title	EVALUATION OF ANTINICROBIAL EFFICACY FOR TREATMENT AND CONTROL OF PORCINE PROLIFERATIVE ENTEROPATHY
By	Miss Chanunda Raphanaphraiwan
Field of Study	Veterinary Medicine
Thesis Advisor	Suphot Wattanaphansak, Ph.D.
Thesis Co-advisor	Associated Professor Chaidate Inchaisi, Ph.D.
Thesis Co-advisor	Pornchalit Assavacheep, Ph.D.

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

.....Dean of the Faculty of Veterinary Science
(Professor Mongkol Techakumphu, D.V.M., Doctorat de 3^{e} cycle)

THESIS COMMITTEE

.....Chairman
(Associate Professor Supol Leungyosluechakul, Ph.D.)

.....Thesis Advisor
(Suphot Wattanaphansak, Ph.D.)

.....Thesis Co-advisor
(Assistant Professor Chaidate Inchaisri, Ph.D.)

.....Thesis Co-advisor
(Pornchalit Assavacheep, Ph.D.)

.....Examiner
(Assistant Professor Thanis Damrongwatanapokin, Ph.D.)

.....External Examiner
(Associate Professor Boonmee Sunyasootcharee)

ชนันดา ระพนาไพรวรรณ : การประเมินประสิทธิผลของยาต้านจุลชีพสำหรับการรักษาและควบคุมโรค PORCINE PROLIFERATIVE ENTEROPATHY. (EVALUATION OF ANTIMICROBIAL EFFICACY FOR TREATMENT AND CONTROL OF PORCINE PROLIFERATIVE ENTEROPATHY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อ.น.สพ.ดร.สุพจน์ วัฒนะพันศักดิ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.น.สพ.ดร.ชัยเดช อินทร์ชัยศรี, อ.น.สพ.ดร.พรชลิต อัศวชีพ, 86 หน้า.

การศึกษานี้เป็นการศึกษาแรกในประเทศไทยที่รายงานเกี่ยวกับประสิทธิผลของการใช้ยาต้านจุลชีพผสมอาหารเพื่อป้องกันและควบคุมการติดเชื้อแบคทีเรียลอโซเนีย อินทราเซลลูราลิส (*LI*) วัตถุประสงค์การศึกษานี้คือการประเมินผลของยาต้านจุลชีพในการยับยั้งปริมาณเชื้อ *LI* ที่แพร่ออกมาในมูลสุกร สมรรถนะการเจริญเติบโต และผลทางด้านเศรษฐกิจในสุกรรุ่นของฟาร์มที่มีความชุกของโรคนี้ประมาณ 30% การศึกษานี้ใช้สุกรทั้งหมด 448 ตัว เริ่มศึกษาที่สุกรอายุ 11 สัปดาห์ สุกรแต่ละตัวได้รับการระบุเครื่องหมาย และถูกแบ่งออกเป็น 5 กลุ่มๆละ 89 - 90 ตัว กลุ่มศึกษา 5 กลุ่มได้แก่ กลุ่ม T1 กินอาหารไม่ผสมยา กลุ่ม T2 กินอาหารผสมยา tiamulin (Triamulox®) 100 พีพีเอ็ม กลุ่ม T3 กินอาหารผสมยา tiamulin 100 พีพีเอ็ม กลุ่ม T4 กินอาหารผสมยา lincomycin และ spectinomycin (LincoSpectin880®) 88 พีพีเอ็ม และ กลุ่ม T5 กินอาหารผสมยา tylosin 110 พีพีเอ็ม ในสัปดาห์แรกของการศึกษา สุกรทุกกลุ่มกินอาหารไม่ผสมยา จากนั้นให้แต่ละกลุ่มกินยาผสมอาหารตามที่กำหนดไว้ต่อเนื่องนาน 3 สัปดาห์ ทำการชั่งน้ำหนักสุกรรายตัวทั้งก่อนและหลังการศึกษา สุ่มเก็บตัวอย่างมูลสุกรจำนวน 30 - 33 ตัวอย่างต่อกลุ่ม ความถี่อาทิตย์ละครั้งรวมทั้งหมด 5 ครั้งโดยตามเก็บจากสุกรตัวเดิม ตัวอย่างมูลสุกรที่เก็บนำมาตรวจหาปริมาณเชื้อ *LI* ที่แพร่ออกมาโดยใช้วิธี quantitative PCR

ผลการศึกษาที่ได้พบว่า ยาต้านจุลชีพที่ใช้ในการศึกษานี้ทุกกลุ่มสามารถลดปริมาณเชื้อ *LI* ที่แพร่ออก และลดจำนวนสุกรที่ให้ผลตรวจเป็นบวกลงอย่างมีนัยสำคัญ ($P < 0.05$) รวมทั้งลดจำนวนสุกรที่แพร่เชื้อออกมาในปริมาณมากได้ นอกจากนี้ ความสำเร็จของการศึกษาในเรื่องของการประเมินประสิทธิภาพทางด้านเศรษฐกิจของการใช้ยาต้านจุชีพ พบว่าการใช้ยาต้านจุลชีพมีความคุ้มค่าในเรื่องการลดความสูญเสียสมรรถนะการเจริญเติบโต และลดการแพร่เชื้อ *LI* ผลการศึกษายืนยันว่าการใช้ยาต้านจุลชีพบางตัวมีประสิทธิภาพในการลดความสูญเสียสมรรถนะการเจริญเติบโตโดยเฉพาะในสุกรที่มีสมรรถนะการเจริญเติบโตช้า ดังนั้น ผลของการใช้ยาต้านจุลชีพสามารถลดการแพร่เชื้อและการติดเชื้อ *LI* ในฟาร์มสุกร

ภาควิชา อายุรศาสตร์	ลายมือชื่อนิสิต .....
สาขาวิชา อายุรศาสตร์	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....
ปีการศึกษา 2556	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....
	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5475307931: MAJOR VETRINARY MEDICINE
KEYWORDS : ANTIMICROBIALS / ECONOMIC IMPACT / GROWTH PERFORMANCE / *LAWSONIA INTRACELLULARIS* / SWINE

CHANUNDA REPHANAPHRAIWAN: EVALUATION OF ANTIMICROBIAL EFFICACY FOR TREATMENT AND CONTROL OF PORCINE PROLIFERATIVE ENTEROPATHY. ADVISOR : SUPHOT WATTANAPHANSAK, Ph.D., CO-ADVISOR : ASST. PROF. CHAIDATE INCHAISRI, Ph.D., PORNCHALIT ASSAVACHEEP, Ph.D., 86 pp.

This study is the first report of *in vivo* antimicrobial efficacy for treatment and control of *L. intracellularis* infection in Thailand. The aim of this study is to quantify bacterial shedding in feces, growth performance and economic outcome after selected antimicrobial intervention in growing pigs of farm in which had prevalence 30%. Four hundred and forty-eight pigs with eleven weeks of age were used and identified individually using ear-tag. The animals were divided into 5 groups of 89-90 pigs. Five groups were comprised of T1: non-medicated feed group, T2: fed with tiamulin (Triamulox®) 110 ppm, T3: fed with tiamulin 110 ppm, T4: fed with lincomycin and spectinomycin (LincoSpectin880®) 88 ppm and T5: fed with tylosin 100 ppm. All groups were treated with non-medicated feed in the first week. Then, the T2-T5 groups were treated with medicated feed for 3 weeks. The T1 group was continuously fed with non-medicated feed until the end of study. Each pig was weighed both at the beginning and at the end of study. Fecal samples of 30-33 pigs per group were collected once a week throughout the experiment, totally 5 times, from the same pig. Quantification of bacterial shedding was measured by using a quantitative PCR. The results revealed that, subclinical form of *L. intracellularis* infection in growing pigs under field conditions, can be benefit from using selected antimicrobials which significantly reduce the amount of bacterial shedding, number of positive pigs and number of high shedding pigs. Moreover, our results showed the economic effectiveness from using antimicrobial to reduce the loss of growth performance and bacterial shedding in subclinical pigs. The results confirmed that the use of selected antimicrobials was efficient to reduce weight loss particularly in slow growth pigs. Therefore, antimicrobial can be confirmed to reduce amount of bacterial shedding and infection of *L. intracellularis* in swine farm.

Department : Veterinary Medicine Student's Signature .....
Field of Study : Veterinary Medicine Advisor's Signature .....
Academic Year : 2013 Co-advisor's Signature .....
Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my major advisor; Dr.Supot Wattanaphansak and my co-advisors: Asst.Prof.Dr.Chaidate Inchaisri and Dr.Pornchalit Assavachep. I deeply appreciate all my advisors kindness dedication and contribution. I am also grateful to expertise invaluable guidance and providing their valuable time to pursue the accomplishment of my thesis.

I would like to thanks my members of proposal and thesis committee: Assoc. Prof. Boonmee Sunyasootcharee, Assoc. Prof. Dr. Supol Leungyosluechakul, Asst. Prof. Dr. Thanis Damrongwatanapokin and Assoc. Prof. Dr. Niwat Chansiripornchai. All members support me to give thoughtful criticism and attention in order to achieve my thesis.

I would like to thanks my friends, including Supansa Tuanthap, Komparn Buapaichit and Jutarat Noiphinit. In addition, there are all scientists in laboratory of animal hospital at Nakornpathom province; Nathamon Yimpring, Yupaporn Lanumtiang, Wenika Kaenson and Ausanee Doung-in. All of their thoughtfulness to collect field samples and laboratory support has in my recognition for fast and efficient performances throughout this course.

I would like to thanks Nongnuch Saetang and her staffs for professional work in feed formulation and experience in field study. I would not have been able to finish my thesis without their excellent assistance.

I would like to take this opportunity to acknowledge a full scholarship by H.M. the King's 72nd Birthday Scholarship, Chulalongkorn University for providing financial and academic support for Master Degree in Veterinary Science. I am also thanks Zoetis (Thailand) Limited to support grant to conduct this study in field experiment.

Last but not the least, I would not have achieved this far without truly encourage from everyone in my family. My beloved parents; Bordin Raphanaphraiwan and Petchara Raphanaphraiwan, my nice brother; Distut Raphanaphraiwan and my lovely husband; Watchrapong Pramarn. All of them always provide me with their understanding, spirit and fulfilling meaning of my life.

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADG	=	Average daily gain
AEC-DMF	=	3-amino-9-ethyl-carbozol dissolved with N,N-dimethyformamide
aspA gene	=	Aspartrate ammonia-lyase gene
B. hyodysenteriae	=	*Brachyspira hyodysenteriae*
B. pilosicoli	=	*Brachyspira pilosicoli*
^{o}C	=	Degree Celsius
$CaCO_3$	=	Calcium carbonate
CD4+	=	Cluster of differentiation 4+
CD8+	=	Cluster of differentiation 8+
CI	=	Confidence interval
CMI	=	Cell-mediated immunity
CRP	=	C-reactive protein
CTC	=	Chlortetracycline
D	=	Day
DMEM	=	Dulbecco's modified Eagled medium
DNA	=	Deoxyribonucleic acid
DOC-ELISA	=	Sodium deoxycholate enzyme-linked immunosorbent assay
ELISA	=	Enzyme-linked immunosorbent assay
ELISPOT	=	Enzyme-linked immunosorbent spot
EU	=	European Union
FBS	=	Fetal bovine serum
FCR	=	Feed conversion ratio
FITC	=	Fluorescein isothiocyanate
H&E	=	Hematoxylin eosin staining
HI	=	Humoral immunity
HRP	=	Horseradish peroxidase
IBD	=	Inflammatory bowel disease
IFA	=	Indirect fluorescent antibody test
IgA	=	Immunoglobulin A
IgG	=	Immunoglobulin G

IgM	=	Immunoglobulin M
IHC	=	Immunohistochemistry staining
IPMA	=	Immunoperoxidase monolaer assay
IPX	=	Immunoperoxidase staining
k	=	Cohen's kappa coefficient
kg	=	Kilogram
km	=	Kilometre
L. intracellularis	=	*Lawsonia intracellularis*
LatA	=	Lawsonia autotransporter protein
LsaA	=	Lawsonia surface antigen
LPS-ELISA	=	Lipopolysaccaride enzyme-linked immunosorbent assay
mg	=	Milligram
MIC	=	Minimum inhibitory concentration
ml	=	Millilitre
M-PCR	=	Multiplex polymerase chain reaction
NE	=	Necrotic enteritis
PBS	=	Phosphate buffered saline
PCR	=	Polymerase chain reaction
PE	=	Proliferative enteropathy
PHE	=	Porcine hemorrhagic enteropathy
PIA	=	Porcine intestinal adenomatosis
PPE	=	Porcine proliferative enteropathy
ppm	=	Part per million
Q	=	Quartile
QAC	=	Quaternary ammonium compound
qPCR	=	Quantitative polymerase chain reaction
RI	=	Regional ileitis
SD	=	Standard deviation
So-ELISA	=	Sonicated enzyme-linked immunosorbent assay
THB	=	Thai Baht
Tm	=	Melting temperature

US	=	The United States
WHO	=	The World Health Organization
WS	=	Warthin-Starry silver staining
µg	=	Microgram
µl	=	Microlitre
µM	=	Micromolar
µm	=	Micrometre
x g	=	Times gravity

CHAPTER I
INTRODUCTION

Porcine proliferative enteropathy (PPE) or ileitis is an important enteric disease in swine industry worldwide. In many countries such as Canada, the United States, South Korea and Australia, the prevalence in swine herd varies from 75 to 100% (Lee et al., 2001; Marsteller et al., 2003; Paradis et al., 2007; Holyoake et al., 2010). The causative agent of PPE is an intracellular, gram-negative and curve shape bacteria called *Lawsonia intracellularis* (*L. intracellularis*) which is able to transmit among pigs via fecal-oral route. Clinical signs of PPE are divided into three forms which are acute, chronic and subclinical forms. All clinical forms relate to the severity of disease (McOrist and Gebhart, 2012). An acute form presenting as a severe case of PPE is characterized by bloody diarrhea with a high mortality in adult pigs whereas chronic form is presented as a mild case of PPE with clinical signs of loose or watery diarrhea, reduction in weight gain and feed efficacy particular finding in late nursery to finishing pigs (Lawson and Gebhart, 2000). For subclinical form, even though the infected pigs do not express clinical signs, it causes significant economic loss due to the negative impact on growth performances (Brandt et al., 2010; Paradis et al., 2012). The disease severity depends on the infectious dose of *L. intracellularis*. With the higher infectious dose of *L. intracellularis*, the symptoms of disease is more severe (Paradis et al., 2012; Pedersen et al., 2012a; Pedersen et al., 2012b). However, with a lower dose of infection, the severity of intestinal lesion may be less and simultaneously not cause to damage the growth rate (Paradis et al., 2005; Paradis et al., 2012; Pedersen et al., 2012b). In order to diminish the dose of infection and the spreading of disease in swine herd, reducing fecal shedding from carrier pigs and reducing bacteria in environment play an important role to control and reduce the severity of disease (Guedes et al., 2003; Riber et al., 2011).

In general, pigs which receive high bacterial load should be high in degree of disease severity and high in an amount of fecal shedding. However, the previous studies reported that the different inoculation doses of *L. intracellularis* organisms, the severity of lesions and pig performances did not clearly differ among infected pigs. Moreover, recent studies reported in the field study (Brandt et al., 2010; Pedersen et al., 2012b) that subclinical PPE pigs with fecal PCR positive and low amount of fecal shedding (3.3 $\log_{10}$ *L.*

intracellularis per gram feces) did not significantly affect the average daily grain (ADG). However, when the comparison study for the effect on pig performances was performed between the infected pigs with different inoculation doses and non-infected pigs, the significant differences of pig performance were found. Up to the present time, the relationship between the infectious doses of *L. intracellularis* and load of fecal *L. intracellularis* shedding has not yet studied.

Nowadays, *L. intracellularis* infection is generally controlled by antimicrobials such as lincomycin, tiamulin and tylosin (McEwen and Fedorka-Cray, 2002; Holyoake et al., 2009). Using antimicrobials is benefit not only to reduce clinical manifestations, morbidity, disease prevalence and pig performance loss caused by PPE but also reduce the problems from other endemic diseases in swine herds (Holyoake et al., 2009). However, some countries restrict or ban the use of some antimicrobials that can be cause of antimicrobial resistance among food animal and human pathogens (McEwen and Fedorka-Cray, 2002; Angulo et al., 2009).

Regarding to antimicrobial resistant development in *L. intracellularis*, little information is available and the results remain unclear due to lacking of the breakpoint establishment (Aarestrup et al., 2008). However, the preliminary results suggested that the extracellular and intracellular minimum inhibitory concentration (MIC) can be used as a guideline for antimicrobial resistant prediction (Aarestrup et al., 2008; Wattanaphansak et al., 2009a).

The *in vitro* MIC determination against *L. intracellularis* can be evaluated using the cell culture system. The MIC results can be used as a guideline for choosing the appropriate antimicrobial for controlling PPE (Wattanaphansak et al., 2009a; Yeh et al., 2011). Although some antimicrobials, such as lincomycin, provide a high *in vitro* MIC value, it showed a positive effect on the control of PPE under field condition (Alexopoulos et al., 2006; Wattanaphansak et al., 2009a). The different *in vitro and in vivo* outcomes might be resulted from pharmacodynamic property of each antimicrobial and the different in strain of *L. intracellularis* (McOrist et al., 1995c; Wattanaphansak et al., 2009a). Although the *in vitro* test studied have been conducted to determine the antimicrobial activities against *L. intracellularis*, the *in vivo* study is still required to confirm the antimicrobial activities when use in pigs (Wattanaphansak et al., 2009a; Yeh et al., 2011).

According to *in vivo* studies of the efficacy of antimicrobial against *L. intracellularis*, they were evaluated in multilateral aspects including clinical sign, fecal shedding, intestinal lesions and pig performances (Walter et al., 2001). The administration of antimicrobials for treatment and control of PPE can be applied in feed, water and injection to infected animals (McOrist et al., 1999b). In several *vivo* studies, the use of tiamulin in feed or the use of lincomycin-spectinomycin in water can decrease proliferative lesions, severity of clinical disease and fecal shedding of bacteria (McOrist et al., 2000; Walter et al., 2001). In those studies, the conventional PCR technique was used for detection of *L. intracellularis* fecal shedding as it was a parameter to determine antimicrobial efficacies. Although the PCR is a useful diagnostic test, it has some limitations. The conventional PCR cannot quantify the bacteria in fecal sample. It provides only presence or absence of *L. intracellularis* DNA. To date, the quantitative PCR assay which determines the quantity of organisms DNA had been developed and validated (Richter et al., 2010; Wattanaphansak et al., 2010a). Many studies show the advantages of quantitative PCR over conventional PCR in term of more sensitivity, less time consuming and less contamination problem (Lindecrona et al., 2002; Nathues et al., 2009).

The economic loss due to PPE had been reported (ref). A negative consequence of growth performance, feed efficiency and morbidity has been found in pigs with *L. intracellularis* infection even in subclinical infected pigs (ref). Therefore, focusing on treatment and control of PPE with antimicrobial are an important role to prevent pig performance and economic loss. However, an economic effectiveness of using antimicrobial to reduce growth performance loss and to reduce a number of infected pigs has still limited.

In Thailand, the evaluation of antimicrobial efficacy to reduce growth performances loss, and to reduce load of fecal shedding and a number of infected pigs in swine herd is lacking. Moreover, a report on the use of quantitative PCR technique to determine an amount of *L. intracellularis* shedding after antimicrobial intervention has limited. Therefore, the aims of this study are to evaluate the efficacy of selected in-feed antimicrobials against *L. intracellularis* infection in growing pigs under field conditions in terms of reducing amount of fecal bacterial shedding and reducing growth performance loss. In addition, this study also evaluates the cost-effectiveness of in-feed antimicrobial to reduce weight loss and to reduce number of infected pigs.

CHAPTER II
LITERATURE REVIEW

2.1 Introduction

Porcine proliferative enteropathy (PPE) or called “ileitis” is an important enteric disease in swine worldwide. PPE is commonly found in grower to finisher pigs. An infected pigs can show acute or chronic clinical signs but they can also show subclinical signs. Production and economic losses is negative consequential outcomes from *L. intracellularis* infection, including pig with subclinical disease (Paradis et al., 2005; Paradis et al., 2012). Therefore, control and treatment strategy are an important role to reduce the negative impact from *L. intracellularis* infection.

2.2 Etiology

In 1995, a new taxonomy of ileal symbiont intracellular organism was established and named “*Lawsonia intracellularis*” for an honor of G.H.K. Lawson who discovered this new agent (McOrist et al., 1995a). The taxonomic lineage which is categorized by Kingdom to species is Bacteria, Proteobacteria, Deltaproteobacteria, Desulfovibrionales, Desulfovibrionaceae, Lawsonia and Intracellularis (Gebhart et al., 1993; McOrist et al., 1995a). This obligate intracellular bacterium is a microaerophillic, curved to sigmoid shaped, gram negative, cell dependent and single flagellum. The size of this agent is approximately 1.25 to 1.75 μm long by 0.5 to 1.5 μm wide (Lawson et al., 1993; Kroll et al., 2005b). These intracellular organisms were cultured and inoculated to pigs with various doses of *L. intracellularis* organisms from low to high titers. The infected pigs were challenged with *L. intracellularis* showing proliferative enteropathy lesions in the ileum although the severity of lesions depended on dose of infection (McOrist et al., 1993). For isolation of this bacterium, the convention media cannot be used for growth and maintenance. Only cell culture system has been developed for *in vitro* culture and maintenance of this pathogen. Cell lines that have been used were a rat enterocyte cell line (IEC-18), a human fetal intestine (INT-407), a rat colonic adenocarcinoma (CRL 167), a pig kidney cells (PK-15) and a murine fibroblast-like McCoy cells (ATCC CRL 1696) (Lawson et al., 1993; Guedes and Gebhart, 2003a). Moreover, a specific atmosphere mixture of 8%

oxygen, 8.8% carbon dioxide and 83.2% nitrogen at 37^0C is needed for growing bacteria (Lawson et al., 1993) . To date, an original space bag with gas mixture of 10% hydrogen, 10% carbon dioxide and 80% nitrogen is adopted to be an alternative choice for cultivation of *L. intracellularis* (Vannucci et al., 2012).

2.3 Pathogenesis

The pathogenesis of *L. intracellularis* infection in pig was studied by using *in vitro* model of rat or pig enterocyte monolayer cells (McOrist et al., 1995b). The model was useful to investigate how intracellular bacterium entry into the enterocytes because the situation was similar to event occurred in pig infected with *L. intracellularis. In vitro* model, this intracellular bacterium accesses into the enterocytes by membrane-bound vacuoles three hours after infection (McOrist et al., 1995b). The entry process of bacterium into the target cell relate to cell activity and bacterial viability (Lawson and Gebhart, 2000). In addition, a study of gene expression of *L. intracellularis* determines a *Lawsonia* gene encoded a surface antigen called LsaA (for *Lawsonia* surface antigen). A property of the LsaA gene also associates with cell attachment and entry into enterocyte cell (McCluskey et al., 2002). After entry of bacterium to enterocyte cell, breakdown and loss of membrane-bound vacuoles go on to release bacterium free in the cytoplasm which is the next step happened within effected cell three hours after infection (McOrist et al., 1995b). Then, intracellular bacterium free in cytoplasm multiplies itself and releases from the infected cell by protrusion of cytoplasm two to six days after infection, depending on the virulent of the isolation and infectious dose of infection (McOrist et al., 1995b; Lawson and Gebhart, 2000). However, entrance and multiplication of proliferation process may need active mitotic division of intestinal crypts and differentiation with this bacterial growth (McOrist et al., 2006).

Pigs orally inoculated with mucosal homogenate were euthanized and sections of intestinal samples were examined by immunohistochemistry and fluorescence *in-situ* hybridization. *L. intracellularis* organisms were localized inside epithelial cells of many parts of intestine, including jejunum, ileum, caecum and colon. These bacteria were observed in laminar propria at 12 hours post inoculation. Later, inoculated pigs are observed development of intestinal lesions by discretion of group of bacteria in epithelium and laminar propria from 12 hours to 5 days post inoculation (MacIntyre et al., 2003; Boutrup et al., 2010). These results demonstrate that area of proliferation of *L. intracellularis* occurred

in both the crypt of epithelial cells and the laminar propria. Therefore, the laminar propria is suggested an active role in spreading of infection from crypt to crypt (Boutrup et al., 2010).

Although the preference site of *L. intracellularis* organism is unclear, this pathogen prefers to infect immature crypt of epithelial cells rather than mature of epithelial cell. *L. intracellularis* can stay within cell and stimulate cell division that might be a condition for bacteria multiplication and transmitted to another cell (Smith and Lawson, 2001).

Onset and incubation period of *L. intracellularis* infection in pigs was approximately 7 - 14 days. Fecal shedding starts on day 7 after challenging and has pattern as intermittent shedding. The level of immunoglobulin G (serum IgG) can be detected at day 14 after challenge. A peak of clinical signs was found around day 21 of the study. In addition, intestinal hyperplasia becomes normal around one month after inoculation (Guedes and Gebhart, 2003b; Guedes and Gebhart, 2003a)

2.4 Clinical signs

Pigs infected with *L. intracellularis* commonly show three clinical presentations. The first is an acute form of disease so-called “proliferative hemorrhagic enteropathy” (PHE). The second is chronic form so-called “porcine intestinal adenomatosis” (PIA). The last is subclinical form of disease.

The PHE is characterized by severe intestinal hemorrhage and sudden death from internal bleeding. Pale skin and blood in the intestinal lumen are often found in postmortem examination. The PHE may represent a progression of PIA however the exact explanation for hemorrhage is still unknown. The disease is usually found in late finishing pigs, gilts, breeding sows and boars. An acute form seems to be more common in pigs with high health status. Mortality is often high and the recovery pigs are carrier in infected herds (McOrist et al., 1992; Lawson and Gebhart, 2000; Jacobson et al., 2010).

The PIA is characterized by thickening of epithelial proliferation. The infected pigs show loose or watery diarrhea, anorexia, poor growth and feed conversion rate. No bloody diarrhea is found in this form of clinical sign. Infected pigs are commonly found in nursery and grower pigs. The mortality rate is low. The suffering pigs recovery within 3-4 weeks after infection (Lawson and Gebhart, 2000). Recent studies reported that clinical signs and gross lesions of pigs infected with porcine circovirus type 2 are similar to subacute or chronic form of PPE (Jensen et al., 2006; Opriessnig et al., 2007).

The subclinical form is indicated by presenting intestinal hyperplasia with few or no clinical signs in nursery to finishing stage of production (Paradis et al., 2005). Subclinical form is hardly detected and observed but omission of this form should not ignore because of negative impact on performance and economic observation (Paradis et al., 2005; Jacobson et al., 2010; Paradis et al., 2012). Moreover, subclinical pig becomes carrier or source of infection for other pigs in farm (Paradis et al., 2012). Some reports demonstrated that the amount of *L. intracellularis* which excreted from subclinical pigs was 2.2 X 10^3 organisms per gram feces. In contrast, pigs with PPE lesions can shed 5 X 10^4 to 7 X 10^8 *L. intracellularis*/gram of feces under field conditions (Pedersen et al., 2010). Therefore, the presence of subclinically and chronically infected animals in the herd seem to be the main factors of pathogen circulation in the farms (Guedes, 2004).

Degree of disease severity seemed to be related to infectious dose of *L. intracellularis* (Guedes et al., 2003; Paradis et al., 2005). Pigs challenged with high dose of *L. intracellularis* (5.4x10^{10} organisms) had shown not only significant increase in mortality rate and average gut lesion length but also decrease in average daily gain (ADG) when compared to pigs challenged with medium to low doses of *L. intracellularis* (5.4x10^9 – 5.4 x 10^8 organisms), respectively (Guedes et al., 2003). Moreover, low infectious dose of *L. intracellularis* generated an adverse impact on growth performance parameters when compared with unchallenged group (Guedes et al., 2003; Paradis et al., 2005). In contrast, a recent field study found that the ADG of subclinical ileitis pigs was not different from uninfected pigs as those pigs were identified and classified by using quantitative PCR (Pedersen et al., 2012b).

2.5 Macroscopic lesions

A lower part of intestinal tract especially in ileum is an affected area of *L. intracellularis* infection. However, lesions sometimes can be found in duodenum, jejunum, cecum and colon. Gross lesions consist of hyperemia, increasing of deep longitudinal and transverse folding and thickening of the mucosal wall. The intestinal lumen was filled with fresh blood or fibrinous blood clot in PHE case but not seen in PIA. Originate bleeding site is detected in the sub-surface capillary bed (Lawson and Gebhart, 2000). For *L. intracellularis* infection exception of PHE and PIA form, necrotic enteritis (NE) and regional ileitis (RI) are counted as pathological change of PPE. Pseudodiptheric membrane in NE

can be found in case of secondary bacterial infection and mucosal destruction (McOrist et al., 1992). Sudden death can be seen in pig with NE form as well (Lawson and Gebhart, 2000). Regional ileitis is referred to pigs recovered from stage of NE. The damaged mucosa is replaced by granulation tissue resulting in the thickening of intestinal wall, as called "hosepipe gut" (Lawson and Gebhart, 2000). Some natural *L. intracellularis* infection show mesenteric and inguinal lymphadenitis (Segalés et al., 2001).

2.6 Microscopic lesions

Microscopic lesions found that the normal villous and the intestinal crypt were replaced with hyperplasia of immature epithelial cells. The free *L. intracellularis* accumulated at the apical cytoplasm of enterocytes in the epithelium and laminar propria regions. Moreover, the intracellular bacteria were found in cytoplasm of mononuclear cells (macrophages) in laminar propria, submucosa, epithelial capillaries and lymphatics and ileacecal lymph node in case of PHE (Guedes and Gebhart, 2003a; MacIntyre et al., 2003). The crypt and villus hyperplasia of epithelial cells were enlarged and branched with proliferation of hyperchromatic pseudostratified tall columnar immature epithelial cells (McOrist et al., 1993; Kim et al., 2000; Ladinig et al., 2009). The goblet cells were absent from abnormal sites but immunocytological cells were presented (McOrist et al., 1992). Moderate infiltration of mononuclear lymphoid cells and polymorphonuclear leukocytes (eosinophil, mass cell, CD8+ T lymphocytes, IgM and IgA B lymphocytes and cell lysis into the laminar propria) were found in case of PHE while infiltration of CD8+ T cells was mild in case of PIA (McOrist et al., 1992). Macrophages were accumulated at hyperplastic lesions rather than infected site. In addition, the highest number of activated macrophages was found on day 14 after infection (MacIntyre et al., 2003). This finding suggested that macrophages can phagocytose and lyse the intracellular bacteria in cytoplasm. In contrast, the bacteria can multiply within macrophages because of evidence involving in an increase of bacterial number in macrophage cell line (MacIntyre et al., 2003; Ladinig et al., 2009).

Mesenteric lymph node and Peyer's patches exhibited moderate depletion of lymphocyte with granulomatous inflammation of lymph node parenchyma. This histological finding indicates granulomatous enteritis associated with *L. intracellularis* infection (Segalés et al., 2001; Machuca et al., 2012).

2.7 Transmission

In natural infection, infected pigs continuously shed the organisms via feces from 1 to 13 weeks after exposure (Guedes and Gebhart, 2003b). This intracellular organism can evade from the host immune response, resulting in long-term shedding of infected pigs (Kroll et al., 2005b). These results indicated that the shedding pigs are an important source of infection to new susceptible pig. Under field conditions, a naïve pig becomes infectious stage by ingesting of the *L. intracellularis* contaminated feces. Hence, fecal-oral route is the major transmission route of *L. intracellularis* from pig to pig (Kroll et al., 2005b). Fecal contaminated materials, such as boots and clothing, are also source of disease transmission within pig farms (McOrist et al., 1999b; Jordan et al., 2004). The environmental resistance and pathogenicity of this agent have been described (Collins et al., 2000). *L. intracellularis* in feces can survive up to 2 weeks in the wide range temperature (between 5 and 15 degree Celsius). When this feces inoculated into the naïve pigs, it can induce the proliferative enteropathy lesions at ileum, seum IgG response and fecal PCR positivity (Collins et al., 2000).

Vectors, such as rodent and fly, cause *L. intracellularis* infection in pig herds (Friedman et al., 2008; Collins et al., 2011; McOrist et al., 2011). In one study, the prevalence of *L. intracellularis* in wild rodent lived in pig herds with endemic PPE was 70.6% by using real-time PCR. Furthermore, a number of *L. intracellularis* excreted from some wild rats were estimated at 10^{10}/gram feces (Collins et al., 2011). In the same study, the experimental mice and rats were inoculated with *L. intracellularis*. The result showed that the bacteria can be shed via rodent feces 14-21 days after exposure (Collins et al., 2011). For mechanical vectors, houseflies and hover flies can be held and spread this pathogen within and between pig farms due to fly movement around the original site for 7 km (McOrist et al., 2011). These evidences indicate that rodents and flies are an important source of *L. intracellularis* reservoir in swine farm. Therefore, sanitation program to control and prevent vector should be executed in husbandry practice to reduce the risk of infection.

2.8 Prevalence

The prevalence of PPE in pig had been reported around the world. In Denmark, the bacterial agent had been detected in 93.7% of 79 farms by polymerase chain reaction (PCR) technique (Stege et al., 2000). In South Korea, one hundred percents of herds had

antibodies against *L. intracellularis* while 53% of 828 pigs had seropositivity by using indirect fluorescent antibody test (IFA) (Lee et al., 2001). In the United States, Marsteller et al. (2003) found that 75% of grower-finisher and 78% of breeding herd were seropositivity to *L. intracellularis* infection by using immunoperoxidase monolayer assay (IPMA). In the Australia, hundred percents of all pig herds had antibodies against *L. intracellularis* (Holyoake et al., 2010). In Russia, 86.5% of commercial pig farms were seropositive to *L. intracellularis* infection using enzyme-linked immunosorbent assay test (Kukushkin and Okovytaya, 2012). This information above suggested that PPE is an important enteric disease spreading throughout the world and it can be cause of negative impact on pig health and productions.

Based on seroprevalence of *L. intracellularis* infection, serological profile provides an useful information of seroconverted age and time of infection (Marsteller et al., 2003; Stege et al., 2004; Walter et al., 2004). For instance, the seroconversion of PE in swine in the United States could be detected at 18 – 24 weeks of age. This indicated that time of infection could occur at approximately 15 - 16 weeks of age (Just et al., 2001; Marsteller et al., 2003). In France and Spain, age of seroconversion depends on farm production system and status of breeding sows. In farrow to finish system with seropositive sows, seroconversion of growing pigs was found between 8 and 16 weeks of age. In farms with a multi-site system with seronegative sows, the seroconversion was found during 16 and 20 week-old pigs in the finisher period (Chouet et al., 2003). In Denmark, within herd seroprevalence of PPE was identified at 12 to 14 weeks of age (Stege et al., 2004). An advantage of knowing age of seroconversion is to determine time or age of *L. intracellularis* infection because immune system responses two weeks after infection (Knittel et al., 1998b; Walter et al., 2004). Moreover, detection of seroconversion relates to clinical and subclinical signs of PPE (Chouet et al., 2003). Hence, serological profile is not only providing disease prevalence but also determine the proper timing to intervene strategy against *L. intracellularis* infection in pig farm (Walter et al., 2004).

2.9 Risk factors

Risk factors associated with level of PPE prevalence in breeding unit and finishing unit are infectious status of sow/grower-finisher pigs, the use of antimicrobials, farm production systems and farm managements (Stege et al., 2000; Bronsvoot et al., 2001; Hammer, 2004; Mauch and Bilkei, 2004; Corzo et al., 2005b; Hands et al., 2010; Holyoake et al., 2010). For prevalence of PPE in breeding unit, the sow parity structure (> parity 3) with all in-all out system in farrowing unit are related to have a low seropositivity to *L. intracellularis* infection. In addition, a high seroprevalence of PPE in grower-finisher pigs was associated with high seroprevalence in breeding unit (Bronsvoot et al., 2001). For prevalence of post-weaning period, the high seropositivity of nursery to finisher pigs are strongly associated with high seroprevalence in breeding sow (Bronsvoot et al., 2001; Mauch and Bilkei, 2004).

Piglets from older sows seem to be less seropositive than piglets from gilts and younger sows. This is because the older sows excrete a low dose of pathogen organisms that do not sufficient to stimulate immune response in their offspring or do not protect their piglets with maternal immunity (Mauch and Bilkei, 2004). Furthermore, to decrease prevalence of *L. intracellularis* infection in grower-finisher pigs, other factors which are multi-site production system, all in-all out practice, antimicrobial in-feed administration, sufficient time space between batches, raising pigs on the concrete slate, efficacy of disinfectant, reducing pig density and stress of animals, cleaning pen and reducing moving and mixing between groups should be implemented (Stege et al., 2000; Bronsvoot et al., 2001; Corzo et al., 2005b; Paradis et al., 2007; Hands et al., 2010; Holyoake et al., 2010). Therefore, if risk factors can be controlled or managed, prevalence of PPE should be declined. Those results are a benefit to pig health and performances.

2.10 Immunity

After infection with *L. intracellularis*, both humoral immunity (HI) and cell-mediated immunity (CMI) were stimulated and activated after expose to the bacteria.

2.10.1 Immunoglobulin M (IgM)

IgM is an immune response in early stage of infection and have short live (Lawson et al., 1988). Serum IgM can be detected by immunofluorescence test when infected pig

develops proliferative intestinal lesions. While IgM can be detected in serum of affected pigs, serum IgG response is still weak (Lawson et al., 1988). Furthermore, a study on immunocytologic responses showed that PHE naturally infected pig can be found moderate accumulation of IgM in intestinal laminar propria, Peyer's patches and lymph nodes (McOrist et al., 1992). These results indicate that IgM is one of the antibodies responses to this bacterium. In older parity of sows, IgM is one of the components in colostrums (Mauch and Bilkei, 2004). Piglets from these sows delay seropositivity compared with piglets from gilts because of protection against disease infection in colostrums (Mauch and Bilkei, 2004). Therefore, IgM in colostrum is also protective immunity against *L. intracellularis* infection in piglets.

2.10.2 Immnnoglobulin A (IgA)

Accumulation of mucosal IgA called "local humoral immune response" at cytoplasm of enterocytes was found in pig with *L. intracellularis* infection (McOrist et al., 1992). Accumulation of IgA results from stimulating specific IgA production inside infected area or interrupting normal process of non-specific IgA production that normally releases onto luminal surface (McOrist et al., 1992). Several kinds of samples such as serum, feces and intestinal lavage sample can be used for detection of IgA (Guedes and Gebhart, 2004; Guedes and Gebhart, 2010; Cordes et al., 2012). However, IgA levels in both serum and feces were lower than serum IgG level because it has short half-life and lack of memory property of intestinal IgA (Guedes and Gebhart, 2004; Cordes et al., 2012). Therefore, local IgA response can be measured by using intestinal lavage from euthanized pigs (Cordes et al., 2012).

At present, the specific intestinal mucosal IgA which responses to *L. intracellularis* infection can be identified by the modified immunoperoxidase monolayer assay (IPMA) (Guedes and Gebhart, 2010). IgA against *L. intracellularis* infection can be found in the intestinal lavage from day 15 post inoculation of *L. intracellularis* pure culture or intestinal mucosal homogenate (Guedes and Gebhart, 2004; Guedes and Gebhart, 2010). These results indicated that local IgA may play important role as protection from *L. intracellularis* invasion, induction of intracellular proliferation and against re-infection of this pathogen (Guedes and Gebhart, 2010; Cordes et al., 2012).

2.10.3 Immnnoglobulin G (IgG)

Serum IgG is mainly antibody response to *L. intracellularis* infection after level of serum IgM declines (Smith and Lawson, 2001). Many researches revealed that serum immunoglobulin G (IgG) can be initially detected 2 weeks after pig exposed to *L. intracellularis* (Guedes et al., 2002c; Guedes and Gebhart, 2003b; Riber et al., 2011). The duration of serum IgG or level of antibody titers are depended on clinical form and severity of the disease (Guedes, 2004; Gebhart, 2008). In case of PHE outbreak or infected with pathogenic strain of *L. intracellularis*, IgG can persist for 12-13 weeks with high serum titer after exposure to this bacteria (Guedes et al., 2002c; Guedes and Gebhart, 2003b). While case of PIA or subclinical form, IgG show low level of immune response and stay only two to three weeks after infection (Guedes et al., 2002c). One half-life titer of serum IgG is declined every two to three weeks (Gebhart, 2008). Seropositive piglet which receives antibodies from its sow via colostrums can detect up to 5 weeks of age (Guedes et al., 2002c). Because *L. intracellularis* is an obligate intracellular bacterium living within apical cytoplasm of cell, detection of IgG and passive maternal immunity do not directly associate with protective immunity against *L. intracellularis* infection (Guedes et al., 2002c; Guedes and Gebhart, 2003b; Cordes et al., 2012). Therefore, measurement of IgG level is an useful information for timing of pigs exposure to *L. intracellularis*, not reflex animal status at the time of sampling (Guedes et al., 2002c; Guedes and Gebhart, 2003b). Furthermore, detection of IgG antibody is highly sensitivity diagnostic tool to determine an infected pig with or without clinical signs of PPE (Jensen et al., 2005).

2.10.4 Cell-mediated immunity (CMI)

Because *L. intracellularis* is an intracellular bacterium, CMI plays an important role as protective immune system to limit proliferation of enterocyte cells, prevent infection and re-infection of *L. intracellularis* (Smith et al., 2000; Guedes and Gebhart, 2003b; Go et al., 2005; Guedes and Gebhart, 2010; Cordes et al., 2012). Interferon-gamma in whole blood was initially detected 2 - 4 weeks after exposure to pure pathogenic isolation and the modified-live vaccine. It could be detected up to 13 weeks after exposure in both groups by using ELISPOT assay (Guedes and Gebhart, 2003b; Guedes and Gebhart, 2010). An importance of the interferon-gamma was demonstrated in transgenic mice model with deletion of interferon-gamma receptor as called interferon-gamma receptor knockout mice.

These results showed that interferon-gamma receptor knockout mice group had more severe lesions, including intracellular bacteria and proliferation of epithelial cell in ileum and colon than wild-type mice (Smith et al., 2000; Go et al., 2005). Moreover, infection rate of interferon-gamma receptor knockout mice was 100% from day 21-post inoculation onwards. In contrast, infection rate of wild-type mice was cleared between day 21 and 28 post inoculation (Smith et al., 2000). Furthermore, a high level of serum interferon-gamma was detected in infected mice at two weeks post infection and protect against re-infection with the same inoculation dose of *L. intracellularis* (Go et al., 2005).

The study of immunocytological responses in PIA and PHE forms showed evidence of mild infiltration of $CD8^+$ and moderate infiltration of $CD8^+$ in the intestinal laminar propria, respectively (McOrist et al., 1992). In addition, experimentally infected pig with twice inoculations with virulent strain of *L. intracellularis* had high fraction of $CD8^+$ lymphocytes and $CD4^+CD8^+$ double positive lymphocytes by intracellular staining and flow cytometry (Cordes et al., 2012). These results indicated that $CD8^+$ lymphocytes as local effector cells and $CD4^+CD8^+$ double positive lymphocytes as memory T cell were identified as the major interferon-gamma producing cells and play a role in protecting against infection and re-infection of *L. intracellularis* (Cordes et al., 2012).

2.10.5 Acute phase protein

Nowadays, detection of acute phase protein has been developed to demonstrate status of *L. intracellularis* infection in pig (Riber et al., 2011). The researchers showed response of two kinds of acute phase protein which are haptoglobin and C-reactive protein (CRP) in groups of infected pigs using a sandwich ELISA technique. The study revealed that serum haptoglobin was increased during days 14 - 21 after inoculation with infected intestines while increasing of serum CRB was responded at day 6 after inoculation. In contrast, both haptoglobin and CRP was not detected in group of re-inoculated pigs. These results might suggested that the acute phase protein from primary infection was protection against secondary infection with *L. intracellularis* (Riber et al., 2011).

Therefore, detection of serum haptoglobin and serum CRP can be used as a new alternative diagnostic tool combined with other serological techniques to confirm of disease infection (Riber et al., 2011).

2.11 Economic impact

The economic loss due to PPE resulted from a negative consequence of growth performance, feed efficiency, mortality and morbidity. It was estimated that total loss due to *L. intracellularis* infection in chronic form was approximately 20 - 40 THB per growing pig, depending on variable price of alive pig, feed and building (McOrist, 2005). However, this value might probably underestimate in case of subclinical with sub-optimal weight gain and improper of food digestibility (McOrist, 2005). In United Kingdom, a report was conducted to estimate the financial losses due to proliferative enteropathy in weaning pigs. The conclusion of the study clearly reported that all challenged groups showed reduction of the mean weight gain (9 - 13%), increased feed conversion ratio (6 - 25%), added the extra feed costs (approximately 50 – 300 THB), and increased the costs of the reduced space utilization due to the increased days to market (approximately 25 – 50 THB). In addition, the direct financial loss because of *L. intracellularis* challenged infection is approximately 100 – 350 THB per pig (McOrist et al., 1997b). In every years, the estimated financial loss from PPE to swine industry revealed cost approximately 600 million THB in the United States and 100 – 200 million THB in the United Kingdom (McOrist et al., 1997b; Guedes, 2004). Therefore, treatment and control of PPE are an important issue to prevent pig performance and economic loss in swine herd.

For economic point of view, several models such as partial budgeting, cost-benefit analysis, decision analysis, Monte-Carlo simulation model and dynamic programming have been applied to determine the efficacy of animal health program (Dijkhuizen et al., 1995). The cost-effectiveness analysis is a subset of cost-benefit analysis (Johannesson, 1995). Meanwhile, cost-benefit analysis measures the using monetary unit by including all costs and benefit outcomes in long-term disease control program (Dijkhuizen et al., 1995; Johannesson, 1995). The cost effectiveness analysis measures the health care effect related with a specific intervention cost in short-term disease control program which can be expressed and prioritized for the effective cost on change in health unit. For PPE, disease affects growing pig in a short period and the economical effectiveness to use in-feed antimicrobials to reduce growth performance loss and to reduce a number of infected pigs have still limited. Therefore, the cost effectiveness analysis is such a valuable technique to determine the effectiveness of intervention programs against *L. intracellularis* infection in term of the intervention cost on change in pig performance and in infected status unit.

2.12 Treatment and control

At present, control of PPE is generally used modified live vaccine or antimicrobials (Jacobson et al., 2010). Moreover, using an efficient disinfectant can reduce and kill *L. intracellularis* organisms in the environment (Collins et al., 2000; Wattanaphansak et al., 2009b; Wattanaphansak et al., 2010b).

2.12.1 Vaccination

A modified live vaccine of *L. intracellularis* is commercially available now as called Enterisol® Ileitis (Boehringer Ingelheim, Germany). Application of this oral vaccine requires specific considerations. Firstly, timing of application should be administered 3 – 4 weeks before onset of infection or before seroconvert detection (Walter et al., 2004). Secondly, antimicrobial must be free in feed or water for several days before and several days after vaccination. Therefore, it has been recommended that an optimal vaccination timing should be done during nursery phase rather than grower period (Walter et al., 2004). Efficacy of vaccine is proven by stimulating both HI and CMI immunological responses as pathogenic strain inoculated, but less violence (Guedes and Gebhart, 2003b). ADG was significantly increased in group of vaccination comparing with group of non-vaccination (McOrist and Smits, 2007; Bak and Rathkjen, 2009).

A commercial vaccine has been available since 2006, the use, however, of this modified live vaccine is limited in some countries. It is due to the cost of vaccine, experience potency and the requirement of antimicrobial free in the system when the vaccine is applied. Therefore, the efficacy of the modified live vaccine is not suitable to use in farm that required antimicrobials for managing many endemic diseases (Holyoake et al., 2009).

2.12.2 Antimicrobials

Main treatment and control of PPE often focus on antimicrobial application. Many antimicrobials have been recommended for use such as chlortetracycline, tylosin, tiamulin, lincomycin and the combination of lincomycin and spectinomycin (Holyoake et al., 2009; Jacobson et al., 2010). To make a decision for selecting antimicrobials against *L. intracellularis*, several factors should be considered including available of antimicrobials, cost, *in vitro* and *in vivo* antimicrobial activities.

For *in vitro* antimicrobial activity, little information is available about minimum inhibitory concentration (MIC) of antimicrobial against *L. intracellularis*. It is due to difficulty of primary isolation and maintenance of organisms in the complicated cell culture system (Wattanaphansak et al., 2009a). For intracellular organism, the MIC value is defined as the lowest concentration of antimicrobials which can inhibit 99% of *L. intracellularis* growth in a cell culture comparing to the antimicrobial-free control (McOrist et al., 1995c; Wattanaphansak et al., 2009a). Moreover, the MIC for *L. intracellularis* was expressed as intracellular and extracellular activity. The intracellular MIC was acted as the effect of antimicrobials on *L. intracellularis* after the bacteria had invaded into enterocytes. While, the extracellular MIC was shown as the effect of antimicrobials on *L. intracellularis* prior to entering into enterocytes (Wattanaphansak et al., 2009a).

For *L. intracellularis* isolated from US and European countries, previous study found that the most active antimicrobials for intracellular activity were carbadox, tiamulin and valnemulin with MIC of $\leq$ 0.5 µg/ml while chlortetracycline and tylosin presented moderated MIC at 0.125 - 64 µg/ml. The least antimicrobial activity was lincomycin with the MIC of 8 - >128 µg/ml. For extracellular MIC, only valnemulin was the most active against *L. intracellularis* with MIC of 0.125 - 4 µg/ml. Chlortetracycline, tiamulin and tylosin provided moderate activity with MIC of 1- >128 µg/ml. The highest extracellular MIC was lincomycin that presented MIC at 32 - > 128 µg/ml (Wattanaphansak et al., 2009a).

In addition, different strains of *L. intracellularis* can provide a different MIC value of antimicrobial. For instance, MIC of tylosin for two strains of *L. intracellularis* from South Korea was 0.125 – 0.5 µg/ml, whereas MIC of tylosin against US and EU isolation were 0.5 – >128 µg/ml (Wattanaphansak et al., 2009a; Yeh et al., 2011). Moreover, increasing of MIC value from same region was found that a 2010 isolation of South Korea had higher two to eight times than a 2002 isolation (Yeh et al., 2011). In contrast, *L. intracellularis* from the North America and European region showed no tendency of increasing MIC value over time (Wattanaphansak et al., 2009a).

The *in vitro* study is only a guideline for selection of antimicrobials. *In vitro* efficacy may not always relate to *in vivo* result. This effect is resulting from different pharmacodynamics of each antimicrobial (McOrist et al., 1995c). For example, lincomycin has the highest MIC when compared to other antimicrobials (Wattanaphansak et al., 2009a), but it had treatment effect to control PPE in grower pigs under field conditions (Alexopoulos

et al., 2006). Therefore, the *in vivo* test is still required to confirm the antimicrobial activity against *L. intracellularis.*

Several antimicrobials were reported *in vivo* activity against *L. intracellularis,* including tylosin, tiamulin, lincomycin, lincomycin-spectinomycin, josamycin and chlortetracycline (McOrist et al., 1996; McOrist et al., 1997a; Veenhuizen et al., 1998; McOrist et al., 1999a; McOrist et al., 2000; Bradford et al., 2004; Alexopoulos et al., 2006). Oral administration of tylosin to weaning pigs at 100 ppm for 21 days after challenged with a virulent *L. intracellularis* reduced clinical signs, microscopic lesion of PPE and improved daily gain when compared with untreated group (McOrist et al., 1997a). Moreover, oral administration of tylosin to growing pigs at 110 ppm for 21 days after a natural outbreak of PPE improved average daily gain and feed efficiency when compared with non-medicated group (ADG: 0.61 versus 0.53 and FCR: 2.39 versus 2.66, respectively) (Veenhuizen et al., 1998). Previous studies demonstrated that tylosin at concentration of 100-110 ppm in feed can be used for treatment and control of PE in nursery and growing pigs.

Administration of tiamulin at concentration of 150 ppm in feed for 21 days could prevent proliferative development of enterocyte and reduce abnormal clinical expression in challenged group. In addition, ADG of tiamulin treated group had 9% better than control group (McOrist et al., 1996). Further study demonstrated that tiamulin at concentration of 60 ppm in water for 5 days and at concentration of 35 ppm in feed for 28 days significantly decreased severity of clinical symptoms and fecal PCR positive ($P < 0.05$) (Walter et al., 2001). Therefore, tiamulin could be used for treatment and control of *L. intracellularis* as *in vivo* results showed good efficacy.

For use of lincomycin, it was demonstrated that lincomycin at the dose of 44 ppm and 110 ppm for 21 days could reduce clinical sign of PPE and incidence of diarrhea when compared to untreated group. Moreover, mortality rate in lincomycin 110 ppm treated group had significant lower than non-medicated (Winkelman et al., 2002). In finisher pigs, lincomycin in-feed at 110 ppm for 3 weeks showed significantly improvement of ADG and FCR when compared to non-medicated group ($P < 0.05$). Furthermore, lincomycin group could reduce fecal shedding of bacteria through feces. Alexopoulos et al (2006) found that once pigs were treated with lincomycin, the number of fecal PCR positive was reduced from 55% to 10% positivity. In contrast, the number of fecal PCR positive in the untreated group was increased from 55% to 60% at the end of the study. These *in vivo* studies indicated that

lincomycin could be used as an alternative antimicrobial for control and treatment of PPE in pigs.

For use of lincomycin and spectinomycin combination, it had been added in drinking water at concentration of 10 mg/kg for 14 days. The results found that diarrhea symptom could be stopped within 3-7 days after treatment and weight gain was significantly increased (P = 0.01) when compared to untreated group in PPE outbreak farm. Moreover, fecal shedding of *L. intracellularis* was not found in treated group when determined by PCR (McOrist et al., 2000). These results indicated that the combination of lincomycin-spectinomycin can be used in infected pigs in order to reduce the number of shedding pigs.

For use of josamycin, one kind of macrolides, pigs received in-feed josamycin 36 mg/kg and 50 mg/kg for 14 days have significant lower diarrhea score than non-medicated group. When comparing intestinal lesion between medicated and non-medicated pigs, it found a numerous of *L. intracellularis* organisms contained in intestine of non-medicated pigs using WS staining and modified Ziehl-Neelsen technique (Kyriakis et al., 2002a). These results indicated that josamycin is effective medication for control of PPE.

For use of chlortetracycline (CTC), it was showed that in-feed CTC at 300 or 600 ppm 4 days before challenged and continued to 21 days after challenged could prevent gross lesion and histological lesions of proliferative enteropathy. Moreover, pigs received CTC had 0.15 - 0.21 kg/day better growth performance and 0.12 - 0.36 lower FCR than non-medicated pigs (McOrist et al., 1999a). These results indicated that CTC can be used to control of *L. intracellularis* infection in pigs.

For use of in-feed medication with doxycycline, one kind of tetracycline derivative, it was applied to control *L. intracellularis* infection using 125 and 250 ppm for 14 day interval (Kyriakis et al., 2002b).The results showed that doxycycline 125 or 250 ppm gave a benefit to reduce diarrhea sign and improve ADG. Furthermore, intestinal sections from pigs with 250 ppm of doxycycline showed lower percent of *L. intracellularis* organisms comparing to non-medicated pigs (Kyriakis et al., 2002b). These advantages indicated that doxycycline can use for *L. intracellularis* infection and growth performance improvement.

In conclusion, the advantages of antimicrobial treatment involved not only reducing gross lesions and clinical signs but also preventing loss of pig performances such as ADG and FCR (McOrist et al., 1997a; Veenhuizen et al., 1998; McOrist et al., 2000). Moreover,

using antimicrobials, such as tiamulin, tylosin, chlortetracycline, lincomycin-spectinomycin and lincomycin for treatment and control of PPE could reduce the number of infected pigs and shedding of *L. intracellularis* in feces (McOrist et al., 2000; Lee et al., 2001). These results indicated that efficacy of antimicrobials could decrease bacterial shedding from shedder pigs resulting in reduction of number of *L. intracellularis* organisms shedding in the environment. In Thailand, antimicrobials including tiamulin, tylosin and lincomycin, widely use to treat and control against *L. intracellularis* infection but limited report of *in vivo* antimicrobial efficacy is published. Therefore, our results will confirm the antimicrobial efficacy when using in infected pigs under field conditions.

2.12.3 Disinfectants

L. intracellularis could survive in the environment for at least 21 days. Therefore, disinfectant is an important tool to reduce or kill harmful organisms circulating in the environment (Collins et al., 2000). Efficacies of some disinfectants were evaluated by measurement of *L. intracellularis* viability after exposure to different concentrations of disinfectants. These results found that the most effective disinfectants were 3% cetrimide®, Roccal-D Plus® quaternary ammonium (QAC), DC&R®, Synergize® (combination between QAC and aldehydes), Virkon-S® (oxidizing agents) and Stalosan® F (mixture of chemical and heavy metal) (Collins et al., 2000; Wattanaphansak et al., 2009b; Wattanaphansak et al., 2010b). Disinfectant application for cleaning procedure, firstly, all pigs and feces are removed. Washing pens by hot water to remove all organic materials. Then, effective disinfectant is applied before re-washing. Finally, empty pen is leaved at least 2 weeks before new batch coming (Collins et al., 2000).

For use disinfectants with maximum capacity, quality of diluted water and exposure time of disinfectants to material surfaces should be concerned. Effectiveness of some disinfectants was reduced dramatically when the disinfectants were diluted in water with high hardness (1000 ppm of $CaCO_3$) (Wattanaphansak et al., 2010b). Therefore, it is necessary to treat the hardness of water before mixing with the disinfectants. For the appropriate exposure time, a contact timing between the pathogen and disinfectants were recommended at least 10 minutes as a minimum requirement for most disinfectants (Wattanaphansak et al., 2010b). However, the effectiveness of disinfectant is better for increasing contact time and concentrations.

2.13 Diagnosis

Diagnostic techniques to detect pig with *L. intracellularis* infection can be divided into two major parts, postmortem and antemortem diagnosis. Postmortem assays were Hematoxylin and eosin (H&E) staining, Warthin-Starry silver staining and immunohistochemistry (IHC) staining. All of the postmortem testing requires a section of intestinal sample to detect specific lesions or a specific organism in target site. Main disadvantage of the postmortem technique was that it required euthanizing pig before examination. In contrast, the antemortem technique can be utilized in large scales of samples without euthanasia animals (Gebhart, 2008).

For antemortem examination, serological tests, including indirect fluorescent antibody test (IFA), immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA), and detection of bacteria in feces (polymerase chain reaction; PCR) were the common assays that had been used in the field. For the serological diagnosis, there were inexpensive, time effectiveness, simple to perform and high throughput (Jacobson et al., 2009). However, the most serological assays required to prepare and maintain of live bacteria as pure culture for use as source of antigen production (Guedes et al., 2002d). These limitations have an impact on capture of antigen and antibody complex, including variation between batch of antigen and cost of whole cell cultivation (Watson et al., 2011). A recent study found a new protein of *L. intracellularis*, called an autotransporter protein; LatA. Its characterization associates with virulence function and antigenicity of pathogen. Therefore, this new recombinant protein could be used as a candidate for serological ELISA (Watson et al., 2011).

2.13.1 Postmortem diagnosis

2.13.1.1 Hematoxylin and eosin staining (H&E)

H&E staining is a histopathological technique that used to demonstrate the proliferation of epithelial cells and cell abnormalities without specific to *L. intracellularis* infection. In addition, only case of severe proliferation showed an accuracy result (Kroll et al., 2005b). Agreement between H&E and PCR results was 53%, indicating low-moderate agreement (Huerta et al., 2003)

2.13.1.2 Warthin-Starry (WS) silver staining

The numerous of intracellular bacteria with curved or rod – shaped can be seen in infected enterocytes when the tissue section was stained with WS silver staining. However, both H&E and WS staining were not specific for *L. intracellularis* infection. False positive can be found with necrotic or autolysis samples (Guedes et al., 2002d). Therefore, WS provides trustworthy result only with obvious intestinal hyperplasia (Huerta et al., 2003).

Combinations between silver staining with alcian blue and hematoxylin-eosin are applied to increase sensitivity of histopathology technique because of the difference of property of the three stains. Alcian blue staining detects mucus-producing cell while H&E recognizes the alternative of affected tissue without interference of WS staining (Driemeier et al., 2002).

2.13.1.3 Immunohistochemistry staining (IHC)

IHC is a gold standard for diagnosis of PPE. IHC staining detected a specific pathogen in apical cytoplasm of enterocytes in tissue section. This technique can identify the organisms in both formalin-fixed tissue and paraffin-embedded tissue sections because of the high specificity of monoclonal antibody against the organisms (Kim et al., 2000). The early and late stage of infection can be differentiated by using IHC whereas H&E and WS cannot identify (Guedes, 2004). Besides, IHC can detect extracellular *L. intracellularis* in exudates or necrotic debris in superficial mucosa. Subclinical signs or recovery stage of *L. intracellularis* infection can be presented by IHC (Gebhart, 2008).

A comparative study among three types of tissue section staining found that sensitivity of H&E, WS and IHC was 36.8%, 50%, and 86.8%, respectively. These results indicated that IHC staining is more sensitive than H&E and WS staining due to the specificity of the monoclonal antibody binding to outer membrane of *L. intracellularis* (Guedes et al., 2002d). Another comparative study reported that the sensitivity of H&E, WS and IHC was 41, 34, and 66%, respectively. Therefore, the latter study confirmed that IHC technique had more sensitivity and can be used in case of lacking of PPE lesions (Ladinig et al., 2009).

2.13.2 Antemortem diagnosis

2.13.2.1 Indirect fluorescent antibody test (IFA)

IFA test was developed in order to detect a specific serum IgG by using an anti-swine IgG fluorescein isothiocyanate (FITC)-labeled as a conjugate for secondary antibody. When challenged pigs with pure culture of *L. intracellularis* were identified, sensitivity of the IFA from serum sample for detection of *L. intracellularis* infection was higher than fecal PCR at 90% and 47%, respectively. But, both tests showed perfect specificity (Knittel et al., 1998a). These results demonstrated that serum IFA is more sensitive than fecal PCR to identify infected pigs.

In addition, the IFA can be adapted to identify the pathogen in an intestinal tissue section as postmortem test. However, this technique is not popular because of a lack of available monoclonal antibody and limitation of only 5 μm intestinal tissue section, resulting to low sensitivity test (Jordan et al., 1999).

2.13.2.2 Immunoperoxidase monolayer assay (IPMA)

IPMA is a serological test that detected a specific serum IgG with an anti-swine IgG peroxidase-labeled conjugate as the secondary antibody. Advantages of the IPMA over the IFA test were that a stained plate could be kept for several months due to stability of the red color and it was easier to interpret by using an inverted light microscope (Guedes et al., 2002a). **Sensitivity** and specificity of the IPMA was 88.9% and 100%, respectively, at cutoff 1:30 of serum dilution. Furthermore, there were no cross reaction with serum from pigs infected with common enteric pathogens, such as *Brachyspira pilosicoli* and *Campylobacter* species (Guedes et al., 2002a).

To compare the IFA and the IPMA, Cohen's kappa coefficient (*k*) was 0.97 as almost perfect agreement when the serums form pigs inoculated with gut homogenate were tested (Guedes et al., 2002b; Viera and Garrett, 2005). In contrast, when testing in naturally infected pigs with PPE, a *k* at individual level between IFA and IPMA was 0.28 as fair agreement (Corzo et al., 2005a). The two previous studies gave different *k* value because of relating with laboratory work in different training and infecting with multiple pathogens in natural herds. However, a *k* of 0.55 as moderate agreement was calculated at herd level

(Corzo et al., 2005a). Therefore, the application of IPMA should use at herd level as like as IFA.

In addition, immunoperoxidase (IPX) staining with fecal samples could detect bacterial antigen as fecal PCR. Agreement between two assays on day 21 and 28 post infection was 67.5% and 72.7%, respectively. Furthermore, the IPX trended to be more sensitivity (> 90%) than fecal PCR because fecal inhibitor was not interfere in IPX staining. However, false positive could occur from non-specific background (Guedes et al., 2002d).

2.13.2.3 Enzyme-linked immunosorbent assay (ELISA)

ELISA is an assay that detects serological response from infected pigs like IFA and IPMA. However, ELISA is more powerful technique that a lot of sera samples can be performed at the same time by using automatic reader machine. Therefore, the outcomes were objective results and the ELISA preparation showed lower risk of bacterial contamination than the IFA and the IPMA (Boesen et al., 2005; Kroll et al., 2005a; Wattanaphansak et al., 2008). Many ELISA systems for diagnosis of *L. intracellularis* infection were developed and validated. Lipopolysaccharide (LPS) derived from bacteria (LPS-ELISA), outer membrane lipoprotein of bacteria extracted by sodium deoxycholate (DOC-ELISA) or sonicated pure culture of *L. intracellularis* (So-ELISA) were used as antigen coated on the ELISA plate (Holyoake et al., 1994; Boesen et al., 2005; Kroll et al., 2005a; Wattanaphansak et al., 2008). Moreover, blocking ELISA is a commercial diagnostic tool available in market for detecting serum responded to *L. intracellularis* infection (Jacobson et al., 2011). Sensitivity of those ELISAs was ranged from 89.8 – 99.5% and specificity was ranged from 99.3 – 100% but the blocking ELISA showed lower sensitivity at 79% and specificity at 93% (Holyoake et al., 1994; Boesen et al., 2005; Kroll et al., 2005a; Wattanaphansak et al., 2008; Jacobson et al., 2011). For this reason, variation of the results between countries, laboratory process and group of pigs may occur (Jacobson et al., 2011).

When compared to the IFA and the IPMA, observed agreement of the ELISA compared to the IFA and IPMA were 97.3 – 98.8% and 90.65 – 95.08%, respectively, depending on type of ELISA and source of samples (Boesen et al., 2005; Wattanaphansak et al., 2008). Generally, results from the ELISA test often generated more positive outcome than the IFA and the IPMA. This indicated that either ELISA was more sensitive or it was

less specificity than IFA and IPMA. For screening tests, ELISA can be used as an alternative tool for health monitoring program, epidemiology and timing of seroconversion against *L. intracellularis* infection.

2.13.2.4 Polymerase chain reaction (PCR)

PCR technique is used as a diagnostic tool for detecting fecal bacterial shedding but it cannot identify a carrier pig with *L. intracellularis* colonization without excretion (Kroll et al., 2005b). The PCR technique was first developed and used for *L. intracelluralis* detection in 1993 (Jones et al., 1993). The assay can identify status of infected pigs with or without disease manifestation (Jones et al., 1993; Jordan et al., 1999). For tissue sample, a detection limit of PCR is 10^1 *L. intracellularis* organisms whereas the detection limit for fecal samples is $10^2 – 10^5$ cells per gram feces (Jones et al., 1993; Pedersen et al., 2010). Based on review of validation data, PCR technique had diagnostic specificity from 50% to 100% and diagnostic sensitivity from 36%-100% (Pedersen et al., 2010). Factors that affecting the diagnostic performances were PCR inhibitors in samples, inactivate process of cell lysis during DNA extraction process (Kroll et al., 2005b), contamination of bacteria during samples collection or DNA extraction (Kroll et al., 2005a). Moreover, paraffin wax-embedded tissue section decreased the sensitivity of PCR comparing to fecal samples (Ladinig et al., 2009).

To reduce an impact of PCR inhibitors, a mimic molecule and a floatation method were developed to reduce an influence of the inhibitors (Jacobson et al., 2003a; Jacobson et al., 2009). A mimic was a DNA molecule that was designed to differentiate from the target template but it could be amplified by using the same primer at recognition sites. For instance, when a lot of PCR inhibitors found in fecal samples, the mimic and target DNA products could not be seen. In contrast, once only mimic product was found, it indicated that the target DNA was absent as negative PCR result. The mimic molecule could be used and acted as internal control and identified PCR inhibitors (Jacobson et al., 2003a). For a flotation technique, a principle of this method based on buoyant density of particles, such as PCR inhibitors, other microbes, and *L. intracellularis* organisms that could be separated after the centrifugation. Then, the specific gradient would be directly continued to perform conventional PCR. The results found that the buoyant density gradient centrifugation assay

showed the similar positive results as extraction by the commercial kit (Jacobson et al., 2009).

For comparison of WS stain, IFA, and PCR for diagnosis of PPE from intestinal sample, although PCR is more expensive assay, the sensitivity and the specificity are better than WS and IFA staining. Therefore, PCR is widely used assay for detection *L. intracellularis* pathogen (Jordan et al., 1999). In case of absence PPE lesions, fecal PCR was the most sensitivity (70%) comparing to WS and IHC from intestinal samples (34% and 66%, respectively). However, Ladining et al (2009) found that the specificity of fecal PCR was lower than specificity of WS and IHC (95%, 100%, and 100%, respectively) (Ladinig et al., 2009).

Once PCR and serological tests were used for diagnosis of PPE, the relationship between fecal shedding and serological response must be considered. Both experimental trials and field studies indicated that fecal shedding detected by fecal PCR could be found prior serological response 1-8 weeks after expose to bacteria, depending pathogen load and antimicrobial use (Guedes and Gebhart, 2003b; Hammer, 2004). If fecal PCR is positive but serological test negative, it implies that early stage of infection takes place and time of seroconversion is still not yet occurred or load of pathogen is not sufficient to stimulate detectable level of immune response. If fecal PCR is positive and serological test also positive, pig represents active infection stage. Meanwhile, if fecal PCR is negative but serolocial positive, pig may have previous exposure to *L. intracellularis* but no longer shedding or bacterial shedding are lower than detection limit of PCR. If fecal PCR is negative and serological negative, pigs may free from *L. intracellularis* infection at time of sampling (Gebhart, 2008).

2.13.2.5 Multiplex PCR (M-PCR)

A M-PCR had been developed to simultaneous detect the major three common enteric bacterial pathogens in grower to finisher stage, including *L. intracellularis*, *Brachyspira hyodysenteriae* and salmonella species from intestinal sample (Elder et al., 1997; Suh and Song, 2005). The M-PCR reduced time-consuming, labor, conserve reagents and cost for identification those pathogens (Elder et al., 1997; Suh and Song, 2005). Sensitivity and specificity of M-PCR was 100% when the mucosal scraping of specimens was tested and results were compared with standard culture for salmonellae and *B.*

hyodysenteriae and histopathological of affected tissue for *L. intracellularis* (Elder et al., 1997). However, sensitivity of M-PCR is decreased 10 times compared to conventional PCR because of PCR inhibitors, interference or combination of 3 primer sets (Suh and Song, 2005).

For M-PCR for *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *L. intracellularis,* Nathues et al (2007) found that detection limit of M-PCR was 10^4, 10^2 and 10^3 copies for *B. hyodysenteriae*, *B. pilosicoli* and *L. intracellularis*, respectively. Specificity of test was 99.8, 99.9 and 96.7% in the detection of *B. hyodysenteriae*, *B. pilosicoli* and *L. intracellularis*, respectively. Furthermore, Agreement identified by kappa index between M-PCR and isolation method for *B. hyodysenteriae*, *B. pilosicoli* was almost perfect while the agreement was only moderate between IFA for *L. intracellularis* and M-PCR (Nathues et al., 2007).

2.13.2.6 Real-time PCR or quantitative PCR (qPCR)

Unfortunately, limitation of PCR interpretation is determined only as presence or absence of nucleic acid in tested samples. At present, qPCR has been developed for quantification aspect. The qPCR provided many advantages including less time-consuming, less laboratory contamination (Lindecrona et al., 2002) and more sensitivity and specificity than conventional PCR (Lindecrona et al., 2002; Nathues et al., 2009). The first generation of qPCR was developed for detection of *L. intracellularis* in feces. Although the assay showed more sensitive and specific than conventional PCR, it could not provide the quantitative data (Lindecrona et al., 2002). To date, the use of qPCR for determination of quantity and quality of *L. intracelluaris* in porcine feces had been further developed and validated based on probe and SYBR green based (Nathues et al., 2009; Drozd et al., 2010; Richter et al., 2010; Wattanaphansak et al., 2010a). The probe-based technique provided more specificity than SYBR-green although probe-based technique had higher cost than SYBR-green (Wattanaphansak et al., 2010a). Detection limit of qPCR is approximately 10^3 *L. intracellularis* organisms per gram feces or 10^6 genomic equivalents of *L. intracellularis* per gram feces for SYBR-green and probed-based, respectively (Nathues et al., 2009; Wattanaphansak et al., 2010a). Quantification of bacterial agent in feces is useful information for study of disease transmission of disease, dynamic of bacterial shedding and comparing of antimicrobial efficacy (Wattanaphansak et al., 2010a).

In conclusion, qPCR technique is an appropriate tool to evaluate the amount of bacterial shedding after intervention with antimicrobial. It may be a new indicator for evaluating antimicrobial efficacy against *L. intracellularis* infection.

2.14 Public health

Based on current knowledge, PPE caused by *L. intracellularis* is not zoonosis (Michalski et al., 2006; Jacobson et al., 2007). There had been reported about human inflammatory bowel disease (IBD) with similar clinical signs to *L. intracellularis* infection. However, it was confirmed that *L. intracellularis* was not associated with human IBD (Michalski et al., 2006). Moreover, a survey of fecal PCR from sixty children who lived in the farm revealed no *L. intracellularis* infection (Jacobson et al., 2007).

CHAPTER III
METHODOLOGY

3.1 Farm study

This study was conducted in a one two-site system farm with 30% prevalence of *L. intracellularis* infection in growing-finishing period. Under diagnosis by farm veterinarian, routine health status records and clinical signs, swine dysentery and salmonellosis were not found in the study period. After weaning, only female pigs with 21 - 24 days of age were moved to finishing site and all pigs were stayed until slaughter. Weaned pigs were randomly allocated for 44 - 45 pigs/pen. All pigs were treated with general routine husbandry. Briefly, commercial feed was fed *ad libitum*. Pigs were freely to access drinking chlorinated water (3 - 5 ppm). The vaccination program was done routinely, for example, *Mycoplasma hyopneumoniae* vaccine at 3 weeks of age, foot and mouth vaccine at 8 and 12 weeks of age and classical swine fever vaccine at 8 weeks of age. To control internal parasites, flubendazole-medicated feed was used before the beginning of study.

3.2 Experimental design

This intervention was conducted in growing-finishing pigs at 11-15 weeks of age for 28 days. Sample size had been estimated with power of 80% and confidence interval (95% CI) under Win Episcope version 2.0 (University of Edinburgh, the United Kingdom). The expected difference between average of daily gain (ADG) of non-medicated feed and medicated feed was assumed about 7 - 15% based on previous reports (Veenhuizen et al., 1998; Alexopoulos et al., 2006), thus the estimated sample size was at least 55 samples per group. Furthermore, this study estimated the difference of the prevalence of *L. intracelluralis* infection between non-medicated and medicated feed that expected about 50% (Alexopoulos et al., 2006). Hence, at least 10 fecal samples per group were collected. With the main aims and above sample size calculation, 448 weaned pigs with 44-45 pigs per pens had been randomly selected into 5 experimental groups with totally 89-90 weaned pigs per group.

At day (D) 0 of the study, all pigs were located in a same house and identified using ear-tag, weighed individually and given non-medicated feed. At D7 to D28 of the study, experimental pigs in each group were fed different medications as follows: I) T1 - non-

medicated feed served as a control group, II) T2 - 100 ppm tiamulin (Triamulox®), III) T3 - 100 ppm tiamulin, IV) T4 - 88 ppm lincomycin and spectinomycin (LincoSpectin880®) and V) T5 - 110 ppm tylosin.

Before beginning of study, pigs in each group (T1 = 30, T2 = 30, T3 = 33, T4 = 31 and T5 = 32) were randomly selected. These experimental pigs were followed up weekly until D28 for fecal bacterial shedding by using the quantitative PCR. Totally, 780 fecal samples were collected during study period. This study was conducted under single blind technique.

3.3 Data collection

Several production parameters were collected in this study. At the individual level, weight of pig was recorded at D0 and D28. At the group level during study period, the amount of feed consumption, number of dead pigs, number of culled pigs and amount of all antimicrobials used were recorded. The dead and culled pigs during study period were weighed and recorded for the cause of dead and culling.

Economic values were based on farm recording system. The parameters including cost of selling alive pigs, cost of selling dead or culled pigs, cost of feed consumption, cost of all antimicrobials used and cost of feed medication were recorded for further economic evaluation.

3.4 Laboratory examination

3.4.1 Bacterial preparation for standard curve construction

L. intracellularis vaccine strain (Enterisol® Ileitis, Boehringer Ingelheim, the United States) was grown in McCoy cell as described by Guedes and Gebhart (2003). Briefly, McCoy cells were grown in Dulbecco's Modified Eagled Medium (DMEM; JR Scientific, Inc., the United States) with 7% fetal bovine serum (FBS; JR Scientific, Inc., the United States), 1% L-glutamine (JR Scientific, Inc., the United States) and 0.5% amphotericin B (JR Scientific, Inc., the United States) without antimicrobials. The next day, 30% confluent monolayer of McCoy cells were infected with *L. intracellularis* vaccine strain and incubated in tri-gas incubator with 8.8% carbon dioxide and 8% oxygen and 83.2% nitrogen at 37 ^{0}C

for 7 days. After incubation, the infected cells with bacteria were harvested by using cell scraper and stored in phosphate buffered saline (PBS) at -20 ^{0}C until further use.

To quantify final amount of pure culture bacteria, the bacteria were stained by modified immunoperoxidase assay and directly counted under an inverted light microscope as described by Guedes and Gebhart (2003). According to the protocol, 10 µl of each 10-fold dilution of *L. intracellularis* suspensions in PBS were coated on 15-well-multitest slide. Two replications of each dilution were fixed with cold acetone:methanol (1:1) and waited for air-dried in room temperature. The coated slide was rehydrated with distilled water for 20 minutes at room temperature. Then, two ml of diluted rabbit polyclonal antibody in 5% skim milk in PBS (1:5000) was added and placed in a moisture chamber in the 37 ^{0}C incubator for 1 hour. Next, two ml of diluted goat anti-rabbit IgG – HRP in 5% skim milk in PBS (1:2000) was added and placed in a moisture chamber in the 37 ^{0}C for 1 hour. Then, two ml of AEC-DMF solution (0.6 ml of AEC solution; (one tablet of 3-amino-9-ethyl-carbozole dissolved with 2.5 ml of N,N-Dimethylformamide; 9.5 ml of acetate buffer; 8 µl of 30% H_2O_2) was added to slide for 20 minutes at room temperature. Finally, the slide was washed with tap water for 3 times and waited until dry. The quantification of redden bacteria in each well were directly counted and calculated for the final amount of *L. intracellularis*. The selected well comprised of 50-500 *L. intracellularis* organisms. An average of the two replications was calculated and used for setting the standard curve on quantitative PCR method.

3.4.2 Bacterial DNA extraction

For DNA extraction from pure culture bacteria, DNeasy blood & tissue kit (QIAGEN, Maryland, the United States) was used. The extraction was followed by manufacturer recommendation. Briefly, the suspension of *L. intracellularis* in PBS was thawed at room temperature. One ml of suspension was centrifuged for 10 minutes at 5000 X g. The supernatant was discarded. The pellet was resuspended in 180 µl Buffer ATL and added 20 µl proteinase K. The solution was mixed thoroughly by vortexing and incubated at 56 ^{0}C for 1 hour for complete lysis of tissue. After that, 200 µl of Buffer AL and 200 µl of ethanol (96-100%) were added and mixed thoroughly again by vortexing. The mixture was pipetted into the DNeasy Mini spin column and placed in a 2 ml collection tube. The tube was centrifuged at 14,462 x g for 1 minute and discharged flow-through. 500 µl Buffer AW1 were added to the DNeasy Mini spin column and centrifuged for 1 minute at 4,722 x g. Next, 500

µl Buffer AW2 were added to the DNeasy Mini spin column and centrifuged for 3 minutes at 14,462 x g to dry the DNeasy membrane. Finally, 200 µl Buffer AE were added onto the DNeasy membrane and centrifuged for 1 minute at 4,722 x g to elute DNA yield. For maximum DNA yield, the elution step was repeated once, but only the first elute of 200 µl were used in quantitative PCR method to perform standard curve.

3.4.3 Standard curve construction

Bacterial DNA from pure culture extracted as mentioned above was used to construct standard cure. A serial 10-fold dilution of standard curve was prepared by making dilution of 10-fold series of *L. intracelluaris* DNA in molecular grade distilled water. Briefly, 45 µl of distilled water were added to each microcentrifuge tube for 6 tubes. Then, 5 µl of stock DNA of *L. intracelluaris* were added to the first microcentrifuge tube to give a dilution of 10^{-1}. Using a new pipette, 5 µl of the first dilution were added to the second tube to yield a dilution of 10^{-2}. The dilution was repeated until a dilution of 10^{-6}. 5 µl of 10-fold dilution from 10^{-1} to 10^{-6} were used for quantitative PCR method as positive control to quantify the amount of *L. intracelluaris* in unknown fecal samples. Quantification of *L. intracellularis* organisms in standard curve at dilution of 10^{-1} was determined by back calculating from the first elute of bacterial DNA pure culture to 5 µl volumes of DNA pure culture which was a final DNA volume for quantitative PCR.

3.4.4 *L. intracellularis* DNA detection from fecal sample

All fecal samples were extracted for bacterial DNA by using PSP® Spin Stool DNA kit (STRATEC Molecular GmbH, Berlin, Germany). The extraction method was followed by manufacture's manual. Briefly, every fecal sample was weighed at 200 mg into 2 ml microcentrifuge tube. 1,200 ml of Lysis buffer P were added and incubated at 95^{0}C for 10 minutes. Then, 5 Zirconia beads II were added to sample and vortexed for 2 minutes to optimize prelysis buffer. The homogenate sample was centrifuged at 10,625 x g and transferred the supernatant to the InviAdsorb-Tube to bound PCR inhibitors. All undissolved particles and PCR inhibitors were removed by centrifuging at 14,462 x g for 3 minutes. Next, 25 µl proteinase K and 200 µl Binding Buffer P were added to ensure high yield bacterial DNA. Then, the whole mixture was transferred to the RTA Spin Filter and centrifuged at 7,378 x g. The filtrated was discarded, 500 µl of Washing Buffer I and 700 µl of Washing Buffer II were put into the filter column to wash and remove all impurities. Finally, to elute

purified bacterial DNA, 100 µl of low-salt buffer (Elution Buffer D) were added directly into the spin filter column, incubated at room temperature for 1 minute and centrifuged at 7,378 x g for 1 minute. The eluted total DNA was stored immediately at -20^{o}C until usage.

3.4.5 SYBR green quantitative PCR condition

Bacterial DNA samples extracted as described above were thawed at 4^{0}C. The procedure of quantitative PCR method was modified from Wattanaphansak et al. (2010). Briefly, the quantitative PCR was conducted by using a specific nucleotide sequence designed from the aspartrate ammonia-lyase (*aspA*) gene. The expected PCR product of this gene has 162 base pairs and shows melting temperature (*Tm*) peak at 79^{0}C. The sequences of primers are forward: 5'-GCTGTGGATTGGGAGAAATC-3' and reverse: 5'-CAAGTTGACCAGCCTCTGC-3'. The detection based on SYBR green dye (SYBR® Select master mix, AppliedBiosystems, California, the United States). The 20 µl PCR reaction consisted of 10 µl SYBR® Select master mix, 0.3 µl of 0.15 µM forward and reverse primer, 4.4 µl distilled water, and 5 µl template. One run of quantitative PCR in 96-well plate consisted of negative control using distilled water as template, positive control of each serial dilution (10^{-1} to 10^{-6}) as template and unknown DNA fecal samples. All samples were tested in triplicate in the same run. Amplification and qualification of the PCR product were performed by a StepOnePlus Real-time PCR System (AppliedBiosystems, California, the United States). The quantitative PCR condition was set at 50^{0}C for 2 minutes for UDG activation, 95^{0}C for 2 minutes and followed by 40 cycles of 95^{0}C for 15 seconds, 60^{0}C for 1 minute and 72^{0}C for 30 second. The fluorescence data was collected at the end of each elongation phase and was recorded continuously during melting curve analysis period using StepOne™ software version 2.1 (AppliedBiosystem, California, the United States). The amplification, signal detection and quantitative analysis were used automatic threshold setting by quantitative PCR machine. Sample that had given specific *Tm* at 78-79^{0}C was counted as positive test result and used for analysis.

3.4.6 Calculation of the amount of *L. intracellularis* in feces

An amount of *L. intracellularis* in 0.2 gram feces collected from quantitative PCR machine was brought to formula for calculation of the total amount of *L. intracellularis* in one gram of feces. The amount of *L. intracellularis* in each sample was calculated by using the

average amount of bacteria from three wells of each sample. Then, the amount of *L. intracellularis* in one gram of feces was done by using the formula of the average amount of *L. intracellularis* x 100 x 3 where factor 100 was total volume of elute DNA / volume of DNA per PCR well / 0.2 gram of feces and factor 3 was expected volume of DNA that lost during the DNA extraction protocol.

3.5 Statistical analysis

Two major aspects of statistical analysis were performed to analyze the antimicrobial efficacy on the reduction of bacterial shedding and the reduction of loss in growth performance. Quantification of *L. intracellularis* organisms from laboratory result was transformed to $\log_{10}$ before further analysis. Average daily gain (ADG) of each individual pig was calculated from the difference between weight at D28 and D0 divided by total number of observation days (28 days). ADG of dead pig was excluded for statistical analysis. Pig production performances such as morbidity rate, mortality rate, FCR, average daily feed intake (kg) were calculated. Weight distribution at D0 and D28 of experimental pigs were displayed.

For the reduction of bacterial shedding, this analysis composed of two steps. The first step was to analyze the antimicrobial efficacy on the amount of bacterial shedding. The descriptive statistic for the amount of $\log_{10}$ *L. intracellularis* organisms per gram feces was calculated. Due to the distribution of the amount of bacteria was not normal distribution, non-parametric test was used to compare the amount of bacterial shedding between groups and between days of study. Within the same day, Kruskal Wallis test was used to analyze the different among groups while Mann-Whitney U test was used for the pairwise between group comparisons. Within the same group, the related sample test with Friedman was used to analyze the difference among days of study while Wilcoxon Signed Rank test was used for the pairwise between days of study.

For the second step, the antimicrobial efficacy on the percentage of positive pigs was determined. The generalized linear mixed model with logistic regression model for repeated measurement on days of study was used. The infectious status of pig (positive or negative) was used as a dependent variable to determine the association with independent variables. Independent variables for this aspect included days of study and experimental groups. Moreover, a number of positive pigs were grouped to the different categories

depending on the amount of bacterial shedding in order to determine the effect of antimicrobial on the amount of bacterial shedding in positive pigs. To divide a distribution into a given number of groups of equal frequency, the amount of bacterial shedding was grouped quarterly into low level ($\leq$3.193 $\log_{10}$), low-medium level (>3.193 $\log_{10}$ to $\leq$3.402 $\log_{10}$), high-medium level (>3.402 $\log_{10}$ to $\leq$3.698 $\log_{10}$) and high level (>3.698 $\log_{10}$). The number of pigs with different categories of the amount of bacterial shedding was presented.

To evaluate the antimicrobial efficacy on growth performance, ADG was used as dependent variable to analyze the association with weight at D0, experimental groups, the amount of bacterial shedding at D0 and D28 and infectious status of pig at D28. Moreover, a total amount of bacteria, a number of positive times per pig and a number of times per pig with the amount of *L. intracellularis* >3.402 $\log_{10}$ were also generated and used as independent variables in analysis. During study period, the total amount of bacterial shedding was calculated by summing up of $\log_{10}$ *L. intracellularis* from each observation day. For each observation day, a number of times given a positive result were counted and classified to 0, 1, 2, 3 or 4 times while a number of times with the amount of *L. intracellularis* >3.402 $\log_{10}$ (50^{th} percentiles) were counted to be 0, 1, 2 or 3 times per study period. Association between ADG and all independent variables were tested using the generalized linear mixed model with linear regression. However, the previous study has shown that the antimicrobial efficacy on *L. intracellularis* affected less on ADG when medicated-feed was intervened to unspecific pigs (Veenhuizen et al., 1998). Thus, in this study, the analysis was further into specific pigs with high ADG or low ADG pigs. To evaluate the association of antimicrobial efficacy within specific pigs, the logistic regression model was performed by using the status of specific pigs with high ADG or low ADG as dependent variable. ADG of all experimental pigs was categorized into 2 groups with low ADG pigs (ADG < 0.75 kg/day) and other when the model was tested for the effect of independent variables on the percentage of low ADG pigs. When the model was tested for the effect of independent variables on the percentage of high ADG pigs, ADG of pigs were categorized to pigs with high ADG (ADG > 0.93 kg/day) and other. Independent variables in both models included weight at D0 and experimental groups.

In addition, to evaluate the antimicrobial efficacy on ADG of specific pigs, two models of the linear regression model were performed in slow growth pigs and in fast growth pigs. Pigs that grown slower than the lower quartile (Q1) were selected for the first

model and pigs that grown faster than the upper quartile (Q4) were selected for the second model. Weight at D0 and experimental groups were included in both analyses as independent variables.

For each statistical model, a univariable analysis was firstly performed to check the association between dependent variable and each independent variable. Pen was added as random effect in each model. The potential independent variable which associated significantly with dependent variable ($P < 0.15$) with no correlation with other independent variables ($r < 0.4$) was included in multivariable analysis. Confounding variable was also assessed when variable was excluded and changed the effect on the remained variable > 20%. In the final model, two way interaction between independent variables was checked and estimated mean with pairwise contrasts for categorical data and a constant mean for continuous data were designed. Furthermore, the least significant difference was calculated for multiple comparisons. For the final step, the model diagnosis was visually tested by using scatter plot between predicted value and Pearson residual.

All statistical analysis was tested by SPSS version 20 (IBM Crop., NY, the United States). The significant difference was assessed at 0.05 level using two-tails *P* - value and 95% CI level.

3.6 Economic analysis

All parameters involved total cost and total income during this study were listed. Total cost comprised of cost of purchase weaned pig (1,450 THB per pig) and total cost of raising pigs until the end of study. Total cost of raising pigs included cost of feed (14.99 THB/kg), cost of feed medication per ton of feed (T2=420 THB, T3=300 THB, T4=704.67 THB and T5=485.03 THB) and cost of antimicrobials used per sick pig. The incomes were from selling alive pigs at 15 weeks of age (65 THB per kg of live weight pig) and selling dead pig or culled pigs with the price depending on farm decision.

To evaluate the invention costs on health effects, the cost-effectiveness analysis was used to evaluate economic outcomes after implementation for treatment and control PPE. The cost-effectiveness to reduce 1 kg of weight loss from *L. intracellularis* infection and the cost-effectiveness to reduce 1% of positive pig after intervention of medicated feeds were determined. By comparison to non-medicated group, the cost-effectiveness of medicated group to reduce 1 kg of weight loss from non-medicated group was calculated from the

extra cost per pig divided by the extra weight per pig. Moreover, the cost-effectiveness to reduce 1% of positive pigs from non-medicated group was calculated from the extra cost per pig divided by the reduction in percentage of positive pigs. Subsequently, the costs per health unit were ranked among four medicated groups.

The net return per pig was also determined from the difference of the total income after selling pigs at the end of study and the total cost from the beginning to the end of study.

CHAPTER IV
RESULTS

4.1 Production performance

Production performances of experimental pigs are shown in Table 4.1. At the beginning of study, the average of weight in an experimental group was not significantly different between groups while the distribution of weight varied from 30 to 60 kg. At the end of study, T1 group had a little lower weight compared with other groups.

Table 4.1 Production performances of each group; T1=Non-medicated feed, T2=Tiamulin (Triamulox®), T3=Tiamulin, T4=Lincomycin and spectinomycin (LincoSpectin880®) and T5= Tylosin

Production performance	T1	T2	T3	T4	T5
Number of pigs at D0	89	89	90	90	90
Average weight at D0±SD	43.6±5.6	43.8±4.6	44.2±4.8	43.9±5.5	44.2±4.5
(minimum–maximum)	(30-60)	(33-55)	(35-56)	(30-57)	(33-54)
Number of pigs at D28	89	88	90	89	90
Average weight at D28±SD	66.9±7.3	67.7±6.7	69±6.5	67.6±7.7	67.8±6.6
(minimum–maximum)	(50-86)	(59-82)	(50-84)	(50-84)	(50-86)
FCR	2.309	2.304	2.302	2.350	2.348
Average daily feed intake(kg)	1.93	1.96	2.04	1.97	1.98
Morbidity rate	0	0	1.11	2.22	0
Mortality rate	0	1.12	0	1.11	0

The weight distributions at the beginning and at the end of study are shown in Figures 4.1 and 4.2. At the end of study, the weight distributions of pigs in T2, T3, T4 and T5 groups tended to shift to the right side more than T1 group. This result indicates that a part of population of T2, T3, T4 and T5 groups had better growth performance than T1 group during study period.

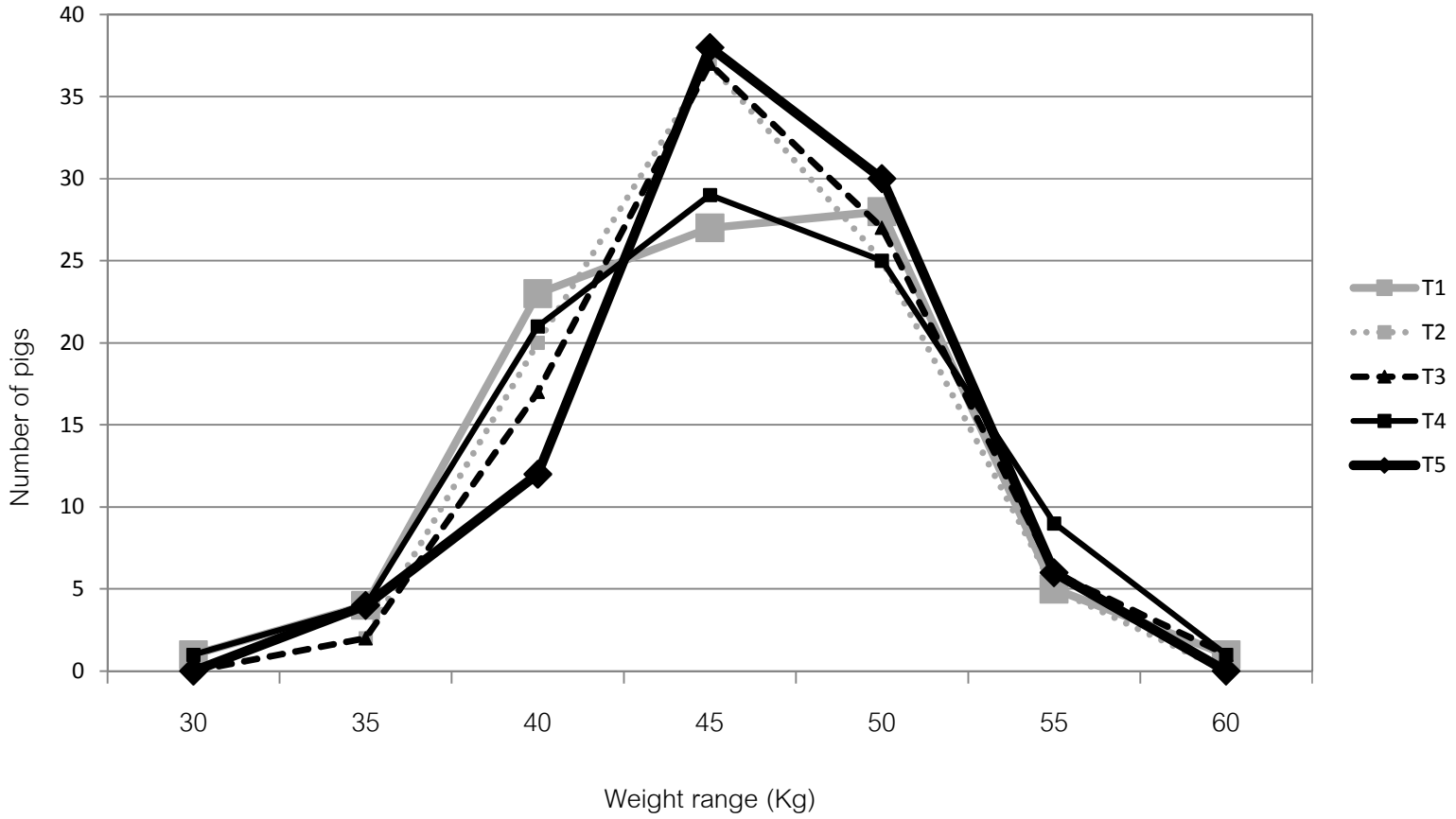

Figure 4.1 Demonstration of weight distribution of pigs in experimental groups at the beginning of study; T1=Non-medicated feed; n=89, T2=Tiamulin (Triamulox®); n=89, T3=Tiamulin; n=90, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=90 and T5=Tylosin; n=90

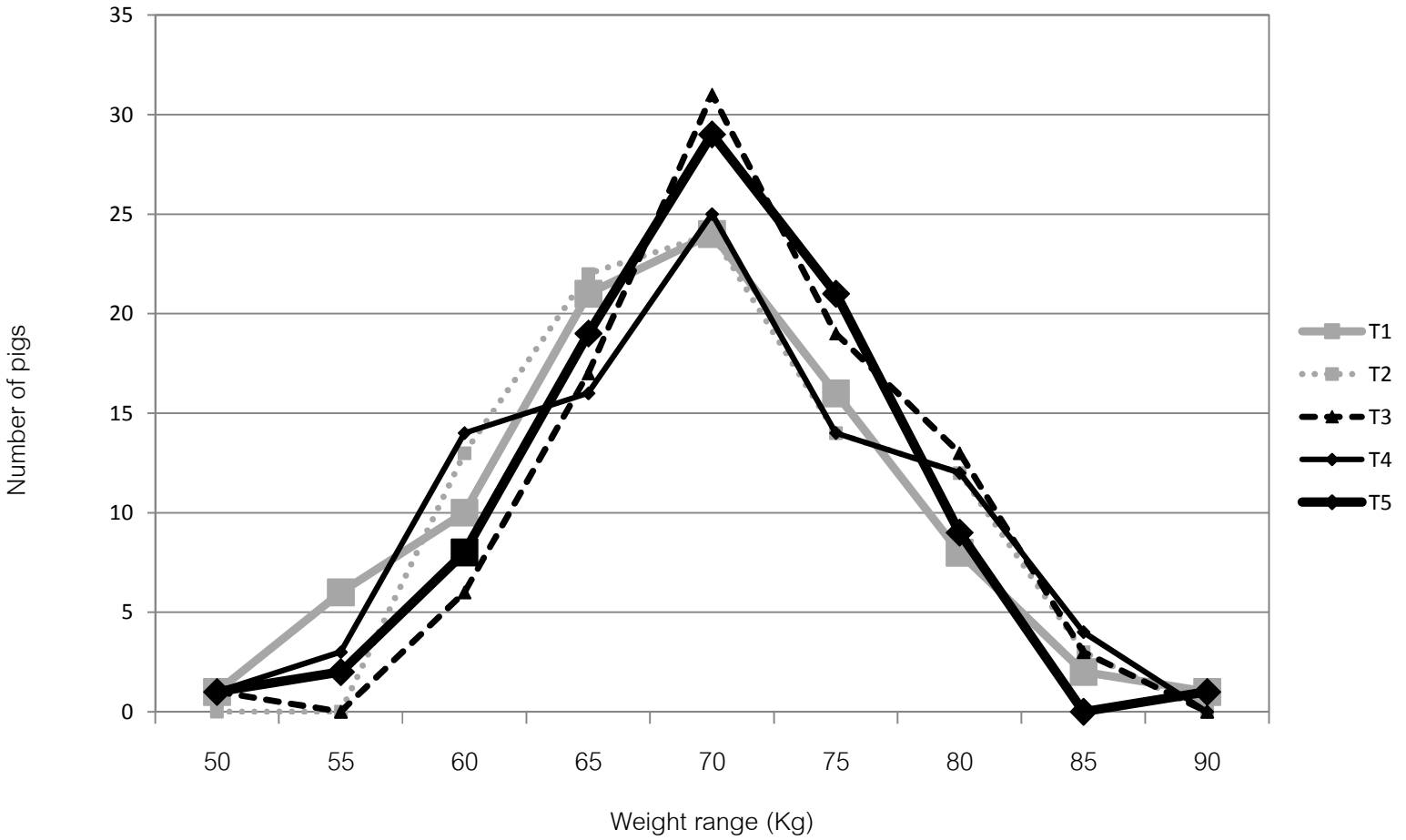

Figure 4.2 Demonstration of weight distribution of pigs in experimental groups at the end of study; T1=Non-medicated feed; n=89, T2=Tiamulin (Triamulox®); n=88, T3=Tiamulin; n=90, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=89 and T5=Tylosin; n=90

4.2 Antimicrobial efficacy

4.2.1 Bacterial shedding

The descriptive statistic of the amount of $\log_{10}$ *L. intracellularis* shedding is shown in Table 4.2 and Figure 4.3. At D0, D7 and D14, the amount of bacterial shedding within the same day was not found a significant difference among groups. In contrast, the amount of bacterial shedding of T2, T3, T4 and T5 groups was significant lower than T1 group at D21 and D28. Within the same group in different days of study, the amount of bacterial shedding of T1 group decreased significantly only for the first two weeks. After the second week, the amount of bacterial shedding of pigs in T1 group rose up to the same level as the beginning of study. Comparison with D0, the amount of bacterial shedding of pigs in T2, T3, T4 and T5 groups reduced significantly after intervention of medicated feed from D7 to D28.

Table 4.2 Descriptive statistic of the amount of $\log_{10}$ *L. intracellularis* organisms per gram feces; T1=Non-medicated feed, T2=Tiamulin (Triamulox®), T3=Tiamulin, T4=Lincomycin and spectinomycin (LincoSpectin880®) and T5=Tylosin

Group (n)	Average, median,(minimum – maximum) of the amount of $\log_{10}$ *L. intracellularis*				
	D0	D7	D14	D21	D28
T1 (n=30)	1.72, 2.74 (0–3.52)^{a}	0.6, 0 (0–3.73)^{b}	0.60, 0 (0–3.84)^{b}	1.64, 1.22 (0–3.71)^{aA}	1.45, 0 (0–3.98)^{aA}
T2 (n=30)	1.77 , 1.4 (0–5.15)^{a}	0.66, 0 (0–3.52)^{b}	0.73, 0 (0–4.78)^{b}	0.22, 0 (0–3.31)^{bB}	0.37, 0 (0–4.16)^{bB}
T3 (n=33)	2.23 , 0 (0–5.68)^{a}	0.25, 0 (0–3.3)^{b}	0.74, 0.00 (0.00–3.52)^{b}	0.45, 0 (0–3.19)^{bB}	0.68, 0 (0–3.52)^{bB}
T4 (n=31)	1.92, 0 (0–6.1)^{a}	0.52 , 0 (0–3.47)^{b}	1.18, 0 (0–4.05)^{ac}	0.87, 0 (0–5.56)^{bcB}	0.56, 0 (0–3.75)^{bcB}
T5 (n=32)	1.86 , 0 (0–4.1)^{a}	0.51 , 0.00 (0–3.77)^{b}	0.52, 0 (0–3.6)^{b}	0.57 , 0 (0–4.02)^{bB}	0.95, 0 (0–3.86)^{bB}

The statistical analysis was performed by non-parametric test. a, b and c are the significant difference ($P \leq 0.05$) between days of study within an experimental group while A, B and C are the significant difference ($P \leq 0.05$) between experimental groups within a day of study.

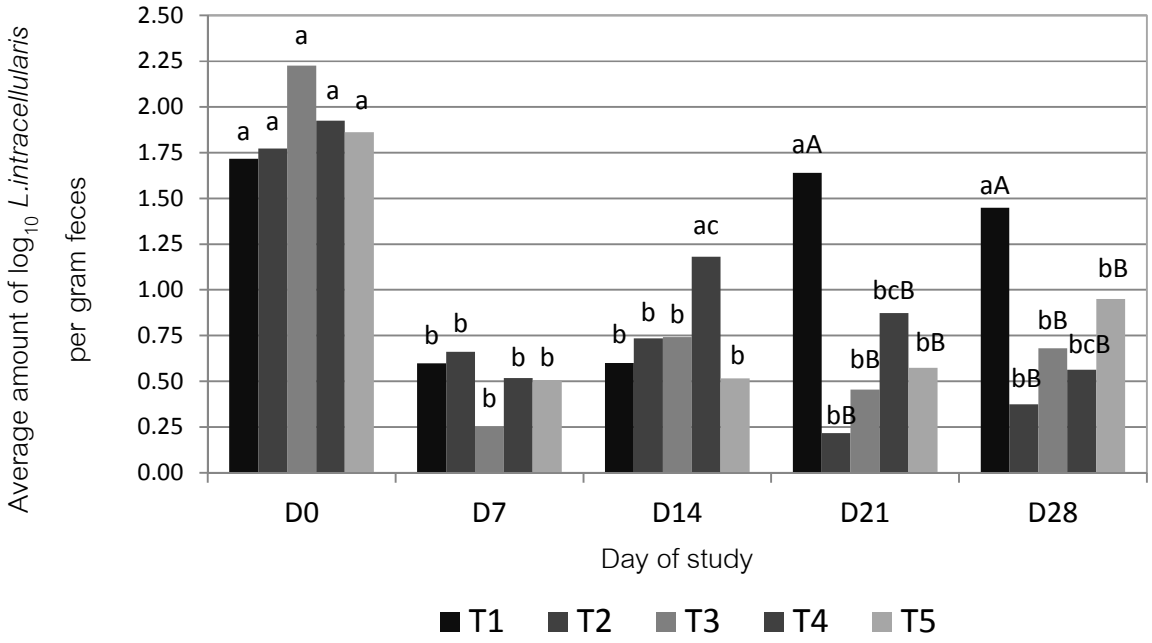

Figure 4.3 The average amount of $\log_{10}$ *L. intracellularis* organisms per gram feces; T1=Non-medicated feed; n=30, T2=Tiamulin (Triamulox®); n=30, T3=Tiamulin; n=33, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=31 and T5=Tylosin; n=32. The statistical analysis was performed by non-parametric test. a, b and c are the significant difference ($P \leq 0.05$) between days of study within an experimental group while A, B and C are the significant difference ($P \leq 0.05$) between experimental groups within a day of study.

Association between variables and positive pigs in univariable and multivariable analysis is shown in Tables 4.3 and 4.4. In univariable analysis, days of study and experimental groups were both statistically significant association with the percentage of positive pigs. The two influential variables were added into multivariable analysis. In multivariable analysis, the percentage of positive pigs in different days of study and experimental groups are shown in Figure 4.4. All groups had a significant decrease of

positive pigs in the first and second weeks of study. After the second week, percentage of positive pigs in T1 group rose up to the beginning level while the percentage of positive pigs in T2, T3, T4 and T5 groups remained stable. Within a day of study, percentage of positive pigs of all groups was not significantly different at D0, D7 and D14. In contrast, the different percentage of positive pigs of T2, T3, T4 and T5 groups was significant lower than T1 group on D21 and the different percentage of positive pigs of T2 and T4 groups was significant lower than T1 group on D28.

Table 4.3 Analysis of associated variables for positive pigs using the univariable analysis with repeated measure logistic regression in the generalized linear mixed model, n=780

Independent variable	Coefficient	Standard error	*P* - value
Day of study			< 0.01
D0	1.231	0.247	
D7	-0.453	0.285	
D14	-0.074	0.268	
D21	-0.074	0.268	
D 28	Ref		
Experimental group			0.01
T1=Non-medicated feed, n=150	0.592	0.257	
T2=Tiamulin (Triamulox®), n=150	-0.267	0.286	
T3=Tiamulin, n=165	-0.117	0.272	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=155	0.114	0.268	
T5=Tylosin, n=160	Ref		

Table 4.4 Analysis of associated variables for positive pigs using the multivariable analysis with repeated measure logistic regression in the generalized linear mixed model, n=780

Independent variable	Coefficient	Odds Ratio (Lower – Upper)	*P* - value
Intercept	-0.938		
Day of study			< 0.01
D0	1.063	2.90 (1.01-8.28)	
D7	-0.748	0.47 (0.14-1.63)	
D14	-0.748	0.47 (0.14-1.63)	
D21	-0.748	0.47 (0.14-1.63)	
D28	Ref		
Experimental group			0.05
T1=Non-medicated feed, n=150	0.533	1.7 (0.58-5.01)	
T2=Tiamulin (Triamulox®), n=150	-1.259	0.28 (0.07-1.2)	
T3= Tiamulin, n=165	-0.374	0.69 (0.22-2.18)	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=155	-0.711	0.49 (0.14-1.71)	
T5=Tylosin, n=160	Ref	-	
Interaction			
Day*group			0.18

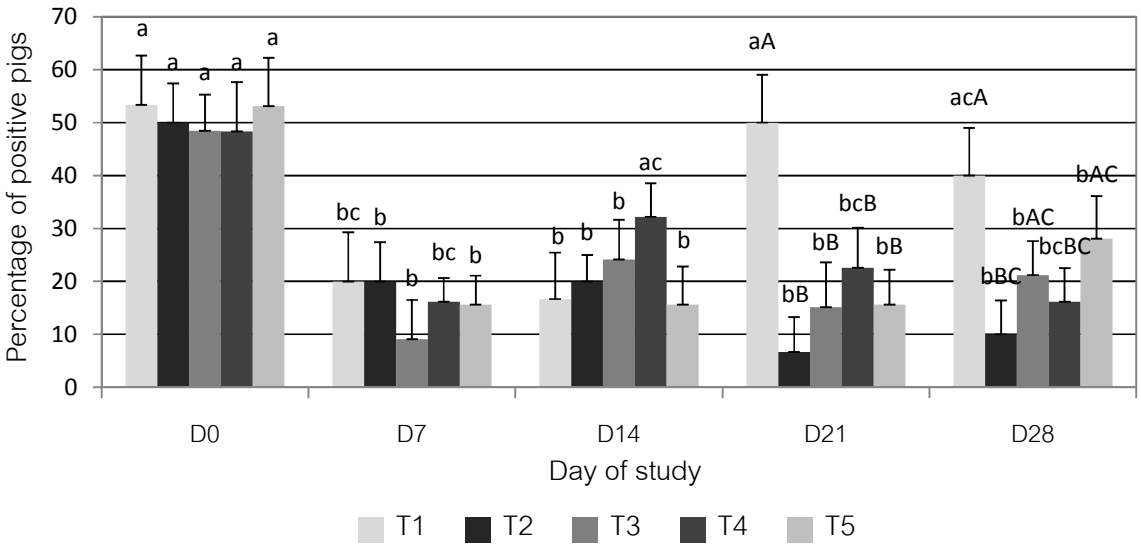

Figure 4.4 The antimicrobial efficacy on the percentage of *L. intracellularis* positive pigs (y-axis) during day of study (x-axis); T1=Non-medicated feed; n=150, T2=Tiamulin (Triamulox®); n=150, T3=Tiamulin; n=165, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=155 and T5=Tylosin; n=160. a, b and c are the significant difference ($P \leq 0.05$) between days of study within an experimental group while A, B and C are the significant ($P \leq 0.05$) difference between experimental groups within a day of study.

The percentage of positive pigs in each different category of the amount of *L. intracellularis* organisms shedding is shown in Table 4.5. Before the beginning of study, percentages of positive pigs were not different among groups but the amount of bacterial shedding of pigs in T2, T3, T4 and T5 groups were 10 - 33% of pigs higher than 3.698 $\log_{10}$ bacteria (Q4) while the amount of bacterial shedding of all pigs in T1 group were lower than 3.698 $\log_{10}$ bacteria (Q4). However, during the last week of study, 40% of pigs in T1 group became positive for *L. intracellularis* and 20% of pigs in this group shed the amount of bacteria in feces higher than 3.698 $\log_{10}$ bacteria (Q4). In contrast, the percentage of positive pigs in T2, T3, T4 and T5 groups decreased to 10-28% after the first week of study. Moreover, within the same period, 6-16% of pigs shed bacteria lower than 3.698 $\log_{10}$

bacteria (Q4) and at the last week of study, only 3% of pigs were detected bacterial shedding higher than 3.698 $\log_{10}$ bacteria (Q4).

Table 4.5 The percentage of negative and positive pigs in different categories of the amount of bacterial shedding in each group at D0, D7, D14, D21 and D28

Experimental group (n)	Day of study	% Negative pigs	% Positive pigs			
			Q1[1]	Q2[1]	Q3[1]	Q4[1]
T1=Non-medicated feed (n=30)	0	46.67	16.67	23.33	13.33	0
	7	80	13.33	6.67	0	0
	14	83.33	0	3.33	6.67	6.67
	21	50	16.67	13.33	16.67	3.33
	28	60	3.33	6.67	10	20
T2=Tiamulin (Triamulox®) (n=30)	0	50	6.67	13.33	20	10
	7	80	3.33	13.33	3.33	0
	14	80	6.67	0	0	20
	21	93.33	0	6.67	0	0
	28	90	0	0	6.67	3.33
T3=Tiamulin (n=33)	0	51.52	0	9.09	6.06	33.33
	7	90.91	6.06	3.03	0	0
	14	75.76	15.15	6.06	3.03	0
	21	84.85	15.15	0	0	0
	28	78.79	9.09	3.03	9.09	0
T4=Lincomycin and spectinomycin (LincoSpectin880®) (n=31)	0	51.61	9.68	16.13	3.23	19.35
	7	83.87	6.45	6.45	3.23	0
	14	67.74	3.23	0	12.90	16.13
	21	77.42	6.45	6.45	6.45	6.45
	28	83.87	0	3.23	9.68	3.23
T5=Tylosin (n=32)	0	46.88	6.25	12.50	25	9.38
	7	84.38	9.38	6.25	0	0
	14	84.38	6.25	6.25	3.13	0
	21	84.38	0	3.13	3.13	9.38
	28	71.88	6.25	9.38	9.38	3.13

[1] indicates the amount of *L. intracellularis* organisms shedding per gram feces from low to high bacterial organisms, including Q1 ($\leq$ 3.193 $\log_{10}$), Q2 (>3.193 $\log_{10}$ to $\leq$ 3.402 $\log_{10}$), Q3 (> 3.402 $\log_{10}$ to $\leq$ 3.698 $\log_{10}$) and Q4 (> 3.698 $\log_{10}$)

4.2.2 Growth performance

The results of univariable analysis of the association between ADG and the amount of *L. intracellularis* shedding are demonstrated in Table 4.6. The independent variables involved with the amount of bacterial shedding did not relate significantly with ADG except weight at D0 ($P < 0.01$).

Because no association between ADG and the amount of *L. intracellularis* shedding was found, only weight at D0 and experimental groups of all pigs was tested for the association with ADG. Results of each independent variable involved with ADG of all pigs in univariable model are shown in Table 4.7. The ADG of all pigs showed significant association with weight at D0 ($P < 0.01$) and experimental group ($P = 0.15$). Thus, both significant associated variables were added into multivariable model. In multivariable analysis, ADG of all experimental pigs depended on the weight at D0 ($P < 0.01$) and the experimental group was not significant association with ADG (Table 4.8). However, when the pairwise comparisons were tested between groups, the estimated mean of ADG was 0.836, 0.854, 0.883, 0.845 and 0.843 kg/day for T1, T2, T3, T4 and T5 groups, respectively (Figure 4.5). The estimated mean of ADG in T3 group was found significantly higher than in T1 group (Figure 4.5). Although ADG of all medicated groups were not significant difference from non-medicated group, ADG in all medicated groups were higher 7 – 47 g/day than non-medicated group.

The association between antimicrobial efficacy and percentage of low ADG pigs (ADG < 0.75 kg/day) was analyzed in univariable and multivariable model as shown in Tables 4.9 and 4.10. In univariable analysis, the percentage of low ADG pigs significantly associated with weight at D0 ($P = 0.01$) and experimental group ($P = 0.03$). After adjustment for other variables in multivariable analysis, percentage of pigs with low ADG decreased significantly ($P = 0.01$) with increasing weight at the beginning of study. Moreover, percentage of low ADG pigs was significant lower in T3 group than in T1 and T5 groups. The percentage of low ADG pigs in T2 and T4 groups were not significant difference from T1, T3 and T5 groups (Figure 4.6).

Regarding to percentage of high ADG pigs (ADG > 0.93 kg/day), none of associated variables were found significantly in univariable analysis (data not shown) except for weight at D0. Weight on the beginning of study associated positively with the percentage of pigs with high ADG.

The association between ADG in slow growth pigs and two independent variables is shown in Table 4.11. The results showed that the ADG was significant association with experimental group ($P < 0.01$) while ADG of those pigs did not related to weight at D0 ($P = 0.63$). Estimated mean of ADG in slow growth pigs was presented in Figure 4.7. Even in slow growth pigs without the effect of weight at the beginning on ADG, ADG in T3 group was the significant highest growth.

When ADG in fast growth pigs of each experimental group were considered for analysis, the associations between other variables, including experimental groups and weight at D0, with ADG were not found significantly in univariable analysis (data not shown). Therefore, no further analysis is needed.

Confounding variable was not found in all models of analysis in this study. The estimation of scattered plot of Pearson residual against predicted values in all models was acceptable for underlying assumption of linear model.

Table 4.6 Analysis of associated variables for average daily gain in pigs tested for infectious status using the univariable analysis with linear regression in the generalized linear mixed model, n=156

Independent variable	Coefficient	Standard error	*P* - value
An amount of $\log_{10}$ *L.intracellularis*/gram feces			
D0	0.001	0.006	0.84
D28	-0.005	0.008	0.51
D0 to D28	0.001	0.003	0.83
A number of positive results from D0 to D28			0.25
none of infection	-0.031	0.084	
1 time	-0.091	0.083	
2 times	-0.074	0.083	
3 times	-0.032	0.089	
4 times	Ref		
Infectious status at D28			0.52
negative result	0.017	0.027	
positive result	Ref		
A number of time with the amount of *L. intracellularis* > 3.402 $\log_{10}$			0.67
none of time	-0.034	0.083	
1 time	-0.038	0.083	
2 times	0.007	0.088	
3 times	Ref		
Weight at D0	0.007	0.002	< 0.01
Experimental group			0.28
T1=Non-medicated feed, n=30	-0.045	0.036	
T2=Tiamulin (Triamulox®), n=30	-0.006	0.036	
T3=Tiamulin, n=33	-0.032	0.035	
T4=Lincomycin and spectinomycin LincoSpectin880®), n=31	-0.021	0.035	
T5=Tylosin, n=32	Ref		

Table 4.7 Analysis of associated variables for average daily gain in all experimental pigs using the univariable analysis with linear regression in the generalized linear mixed model, n=446

Independent variable	Coefficient	Standard error	*P* - value
Weight at D0	0.006	0.001	< 0.01
Experimental group			0.15
T1=Non-medicated feed, n=89	-0.01	0.021	
T2=Tiamulin (Triamulox®), n=88	0.009	0.021	
T3=Tiamulin, n=90	0.04	0.021	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=89	0.001	0.012	
T5=Tylosin, n=90	Ref		

Table 4.8 Analysis of associated variables for average daily gain in all experimental pigs using the multivariable analysis with linear regression in the generalized linear mixed model, n=446

Independent variable	Coefficient	Standard error	*P* - value
Intercept	0.603	0.06	
Weight at D0	0.005	0.001	< 0.01
Experimental group			0.18
T1=Non-medicated feed, n=89	-0.007	0.021	
T2=Tiamulin (Triamulox®), n=88	0.011	0.021	
T3=Tiamulin, n=90	0.04	0.021	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=89	0.002	0.021	
T5=Tylosin, n=90	Ref		

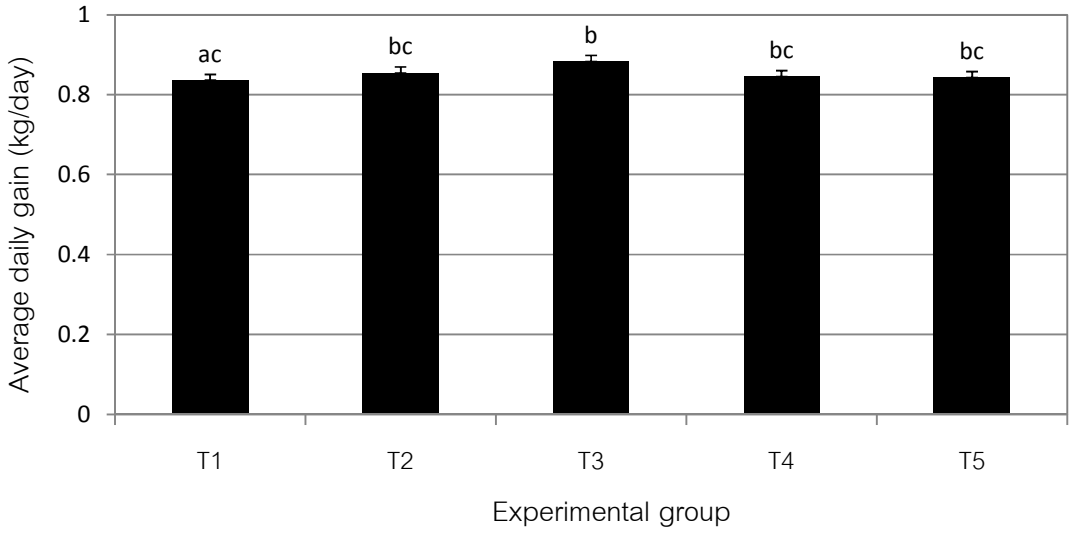

Figure 4.5 The antimicrobial efficacy on the average daily gain (kg/day) of all pigs (y-axis) in the experimental groups (x-axis); T1=Non-medicated feed; n=89, T2=Tiamulin (Triamulox®); n=88, T3 =Tiamulin; n=90, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=89 and T5=Tylosin; n=90. a, b and c are the significant difference ($P \leq 0.05$) between experimental groups.

Table 4.9 Analysis of associated variables for the percentage of pigs with low ADG (ADG < 0.75 kg/day) using the univariable analysis with logistic regression in the generalized linear mixed model, n=446

Independent variable	Coefficient	Standard error	*P* - value
Weight at D0	-0.065	0.024	0.01
Experimental group			0.03
T1=Non-medicated feed, n=89	0.18	0.334	
T2=Tiamulin (Triamulox®), n=88	-0.418	0.362	
T3=Tiamulin, n=90	-1.068	0.413	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=89	-0.361	0.358	
T5=Tylosin, n=90	Ref		

Table 4.10 Analysis of associated variables for the percentage of pigs with low ADG (ADG < 0.75 kg/day) using the multivariable analysis with logistic regression in the generalized linear mixed model, n=446

Independent variable	Coefficient	Odds Ratio (Lower-Upper)	*P - value*
Intercept	1.768		
Weight at D0	-0.063	0.94 (0.89-0.98)	0.01
Experimental group			0.03
T1=Non-medicated feed, n=89	0.136	1.15 (0.59-2.23)	
T2=Tiamulin (Triamulox®), n=88	-0.448	0.64 (0.31-1.31)	
T3=Tiamulin, n=90	-1.081	0.34 (0.15-0.77)	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=89	-0.389	0.68 (0.33-1.38)	
T5=Tylosin, n=90	Ref	-	

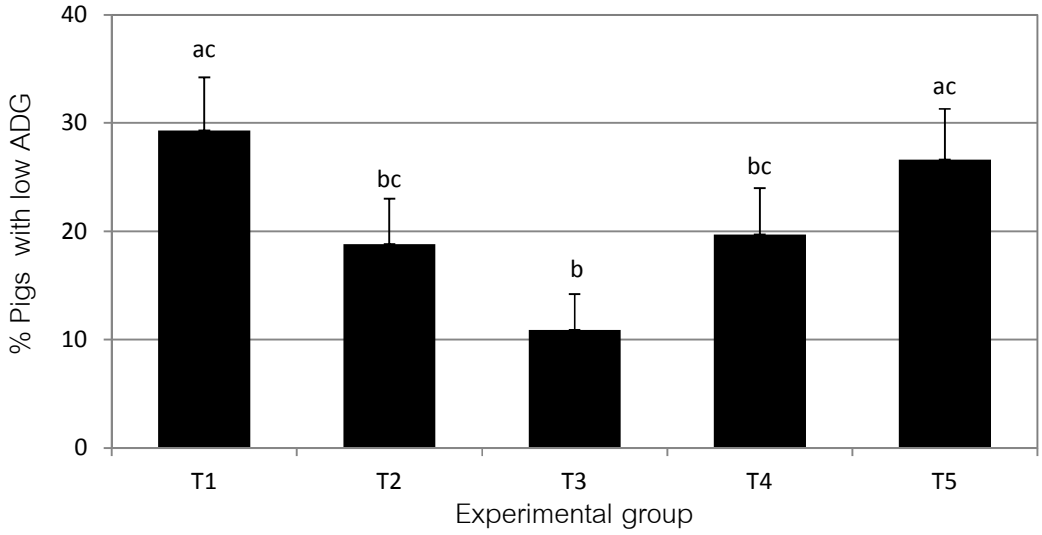

Figure 4.6 The antimicrobial efficacy on the percentage of pigs with low ADG (ADG < 0.75 kg/day) (y-axis) in the experimental groups (x-axis); T1=Non-medicated feed; n=89, T2=Tiamulin (Triamulox®); n=88, T3 =Tiamulin; n=90, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=89 and T5=Tylosin; n=90. a, b and c are the significant difference ($P \leq 0.05$) between experimental groups.

Table 4.11 Analysis of associated variables for ADG in slow growth pigs of each group using the univariable analysis with linear regression in the generalized linear mixed model, n=133

Independent variable	Coefficient	Standard error	*P* - value
Weight at D0	0.001	0.001	0.63
Experimental group			< 0.01
T1=Non-medicated feed, n=27	0.004	0.019	
T2=Tiamulin (Triamulox®), n=26	0.023	0.019	
T3=Tiamulin, n=33	0.093	0.018	
T4=Lincomycin and spectinomycin (LincoSpectin880®), n=23	0.009	0.018	
T5=Tylosin, n=24	Ref		

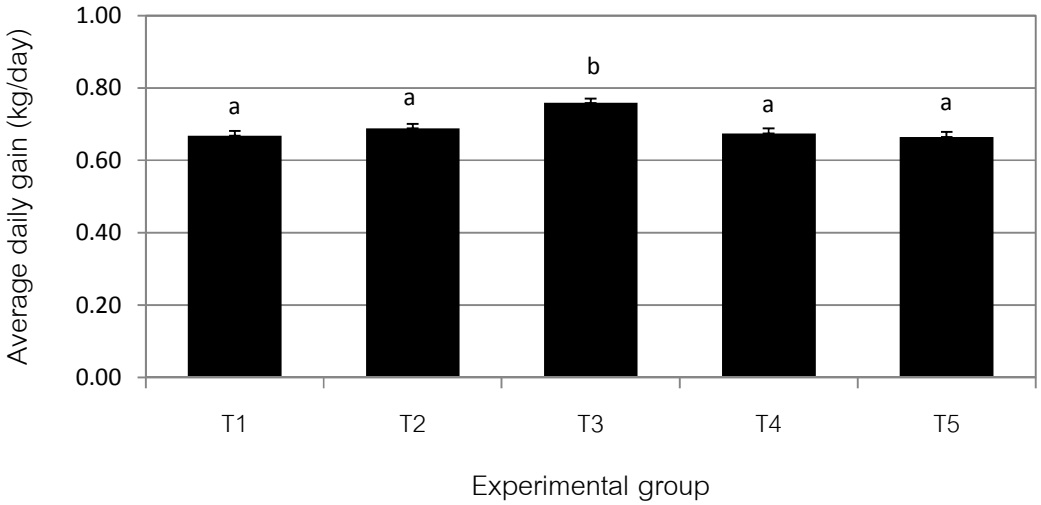

Figure 4.7 The antimicrobial efficacy on average daily gain (kg/day) of slow growth pigs (y-axis) in experimental groups (x-axis); T1=Non-medicated feed; n=89, T2=Tiamulin (Triamulox®); n=88, T3 =Tiamulin; n=90, T4=Lincomycin and spectinomycin (LincoSpectin880®); n=89 and T5=Tylosin; n=90. a and b are the significant difference ($P \leq 0.05$) between experimental groups.

4.3 Economic result

During the study period, two pigs died without gastrointestinal disorder. Therefore, cost and income of death pigs were excluded from analysis. The economic outcomes are shown in Table 4.12.

Comparison with non-medicated group, use of medicated feed reduced weight loss and reduced percentage of positive pigs. The cost in T2, T3, T4 and T5 groups that was paid extra from non-medicated group were 38.26, 63.36, 58.66, and 50.41 THB per pig, respectively. These costs, in T2, T3, T4 and T5 groups compared with non-medicated group, reduced economic loss for 52.37, 137.30, 50.39 and 60.02 THB per pig and also reduced the percentage of positive pigs for 30, 18.79, 23.87 and 11.87%, respectively (Figures 4.8 and 4.9).

Of the overall cost of raising pigs in this study, the cost of feed used was the majority while the income was only from selling alive pigs (Table 4.12). The cost of raising pigs in all medicated groups was higher than non-medicated group. The difference between these cost in medicated groups was higher approximately 38 – 63 THB/pig than in non-medicated group. However, the net return per pig compared with other groups was low in T1 and T4 groups (Table 4.12).

Comparison with other groups, the average weight per pig at the end of study in T3 group was the highest while the average weight per pig in T1 group was the lowest (Table 4.12). For the cost-effectiveness to reduce 1 kg of weight loss, T3 group was the most efficient payment option. The payment extra from non-medicated group to reduce 1 kg of weight loss in T3 group was only 30.03 THB/kg. To compare with T1 group, the ranking of the cost-effectiveness for extra payment to reduce 1 kg of weight loss, from the best to worst, was T3, T2, T5, and T4 groups, respectively.

At the end of study, the percentage of positive pigs of all medicated groups was lower 11-30% than non-medicated group (Table 4.12). The extra cost of T2 group from non-medicated group was most effectively to reduce 1% of positive pigs for only 1.28 THB per pig. To compare with T1 group, the ranking of the cost-effectiveness for extra payment to reduce 1% of positive pigs, from the best to worst, was T2, T4, T3 and T5 groups, respectively.

Table 4.12 The economic outcomes of each experimental group; T1=Non-medicated feed, T2=Tiamulin (Triamulox®), T3 =Tiamulin, T4=Lincomycin and spectinomycin (LincoSpectin880®) and T5=Tylosin

Economic outcome	T1	T2	T3	T4	T5
Number of pigs	89	88	90	89	90
A-Cost of purchased weaned pig (THB)	129,050	127,600	130,500	129,050	130,500
B-Cost of raising pigs(THB)					
B1-Cost of feed used	71,942.4	72,469.98	76,888.44	73,661.82	74,865.06
B2-Cost of feed medication	0	2,030.78	1,539	3,463.22	2,422.73
B3-Cost of antimicrobials used	0	0	25.30	38.33	0
Total cost of raising pigs per group (B1+B2+B3)	71,942.4	74,500.76	78,452.74	77,163.37	77,287.79
Total cost of raising per pig	808.34	846.6	871.7	867.00	858.75
Extra cost per pig* (THB)	0	38.26	63.36	58.66	50.41
C-Total cost (THB)(A+B)	200,992.4	202,100.76	208,952.74	206,213.07	207,787.79
Income (THB)					
D-Total income per group (selling of alive pigs at 15 weeks age)	387,010	387,270	403,715	391,495	396,760
Total income per pig	4,348.43	4,400.80	4,485.72	4,398.82	4,408.44
Reduction in economic loss per pig*	0	52.37	137.30	50.39	60.02

Table 4.12 continued

Economic outcome	T1	T2	T3	T4	T5
Total weight at the end of study (kg)	5,954	5,958	6,211	6,023	6,104
Average weight per pig at the end of study (kg)	66.90	67.70	69.01	67.67	67.82
Reduced weight loss per pig* (kg)	0	0.8	2.11	0.77	0.92
Cost-effectiveness to reduce 1 kg of weight loss* (THB)	0	47.83	30.03	76.18	54.8
Ranking of cost-effectiveness to reduce 1 kg of weight loss	-	2	1	4	3
Percentage of positive pigs at the end of study	40	10	21.21	16.13	28.13
Reduction in percentage of positive pigs*	0	30	18.79	23.87	11.87
Cost-effectiveness to reduce 1% of positive pigs* (THB)	0	1.28	3.37	2.47	4.25
Ranking of cost-effectiveness to reduce 1% of positive pigs	-	1	3	2	4
Net return (THB)					
per group (D-C)	186,017.6	185,169.24	194,762.26	185,281.93	188,972.21
per pig	2,090.09	2,104.2	2,164.03	2,081.82	2,099.69

* The value of each medicated group was compared with non-medicated group

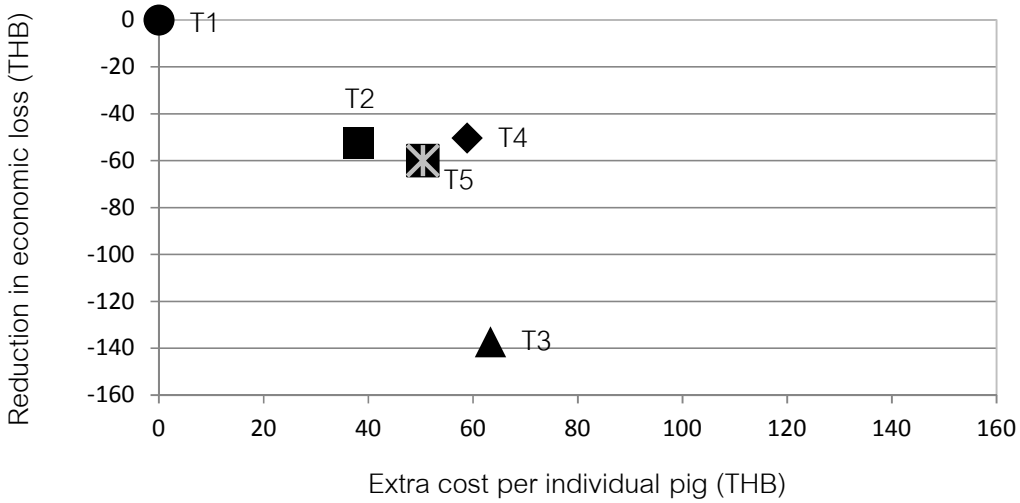

Figure 4.8 The cost per an individual pig (THB) in medication groups to pay extra from non-medicated group to reduce economic loss; T1=Non-medicated feed, T2=Tiamulin (Triamulox®), T3=Tiamulin, T4=Lincomycin and spectinomycin (LincoSpectin880®) and T5=Tylosin

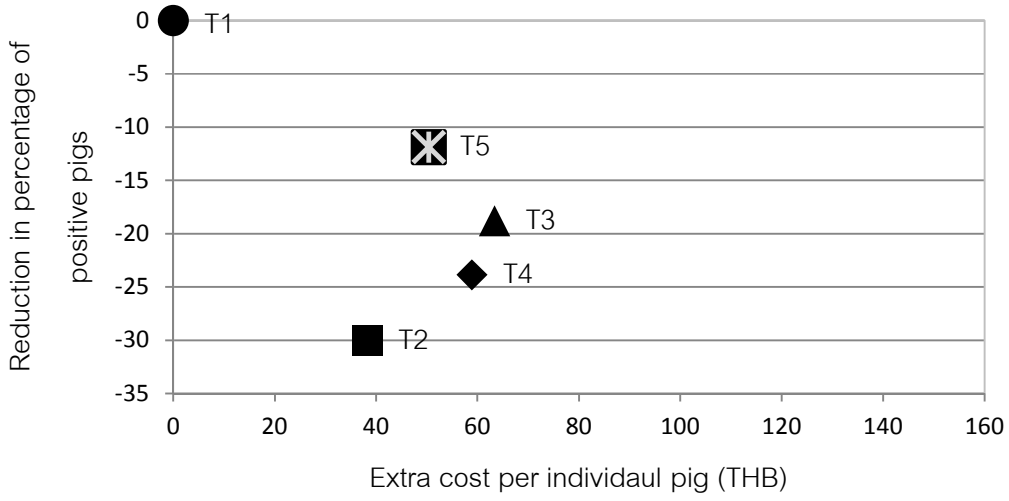

Figure 4.9 The cost per an individual pig (THB) in medication groups to pay extra from non-medicated group to reduce percentage of positive pigs; T1=Non-medicated feed, T2=Tiamulin (Triamulox®), T3=Tiamulin, T4=Lincomycin and spectinomycin (LincoSpectin880®) and T5=Tylosi

CHAPTER V
DISSCUSSIONS

In this study, the medicated feeds containing tiamulin (Triamulox®) 100 ppm, tiamulin 100 ppm, lincomycin and spectinomycin (LincoSpectin880®) 88 ppm or tylosin 110 ppm and non-medicated feed were fed to growing pigs in order to evaluate the economic efficacy of antimicrobials on the *L. intracellularis* shedding and growth performance under field conditions. The study outcome following feed medication was observed in a subclinical PPE farm in which experimental pigs did not show diarrhea. Therefore, the outcome was mainly focused on the reduction of growth retardation and bacterial shedding due to subclinical PPE. The study outcome is discussed with respect to analysis of an amount of bacterial shedding, growth performance and economic impact as following.

Bacterial shedding

As in the results of this study after feeding medication, antimicrobials reduced the quantity of bacterial shedding. Moreover, the quantity of bacterial shedding in positive pigs of all medicated groups was lower than the non-medicated group. In general, the antimicrobials accumulate in cytoplasm of enterocytes of ilium that is also the infected site of *L. intracellularis* (McOrist et al., 1997a). Although selected antimicrobials in this study are different drug group, these antimicrobials affect at the same target site as 50S ribosome of bacteria. After binding of antimicrobial to target site, the inhibition of bacterial replication and less fecal shedding occur resulting from the blocking of bacterial protein synthesis (McOrist et al., 1996; McOrist et al., 1997a; McOrist et al., 2000; Walter et al., 2001; Alexopoulos et al., 2006).

This study shows the decrease of percentage of positive pigs after intervention by antimicrobial. There are consistent to the other studies of examining antimicrobial efficacy in field experiments (Lee et al., 2001; Alexopoulos et al., 2006). Nevertheless, the other experiments revealed undetectable fecal PCR positive pigs after antimicrobial treatment (McOrist et al., 2000; Walter et al., 2001). It would be possible that the different percentage of positive pigs may result from the use of different methodologies and detection techniques. In this study, the experiment was performed in natural condition whereas the other study (Walter et al., 2001) was performed in the laboratory experiment. The study in

natural environment may increase chance of re-infection while the study in laboratory environment can control hygiene and re-infection better than in natural condition. In the context of the detection techniques, the quantitative PCR was used in this study while the conventional PCR was carried out in the other (McOrist et al., 2000; Walter et al., 2001). It has been known that the quantitative PCR is more sensitive than the conventional PCR (Lindecrona et al., 2002; Nathues et al., 2009). The advantage of this study is to combine between natural condition and sensitivity diagnostic technique. Thus, our results can be guideline to choose appropriate technique for control and treatment PPE.

Moreover, the results found that the quantity of bacterial shedding and percentage of positive pigs in all groups decreased during the first two weeks of study. This phenomenon may result from the shedding pattern of bacteria and immune system. According to the review of Guedes and Gebhart (2003), the *L. intracellularis* shedding was intermittent pattern. The intermittent pattern is clearly in non-medicated group with a lower number of bacterial shedding and percentage of positive pigs in the first two weeks of study before rising up again at the third to the fifth week of study. On the other hand, when the antimicrobial was intervened in medicated groups, the intermittent pattern was blocked and the quantity of bacterial shedding and a number of positive pigs remained at the low level until the end of study. However, although the percentage of positive pigs at the end of study in T2 and T5 groups was not significant lower than in non-medicated group, the effect of antimicrobials in T2 and T5 groups clearly shows lower in the quantity of bacterial shedding and lower in a number of high shedding pigs than in non-medicated group.

Based on the detection of *L. intracellularis* in feces at 5 - 6 weeks of age, before the beginning of study, thirty percent of pigs were positive by PCR. This result indicates that the experimental pigs were in the high possibility to develop protective immunity against *L. intracellularis* infection (Collins and Love, 2007; Cordes et al., 2012). Some pigs might become negative by their protective immune. Nevertheless, without using antimicrobial, pigs at the third and the fourth week of study might be re-infected and bacteria might circulate again as in a non-medicated group.

.

Growth performance

The relation between ADG and an amount of *L. intracellularis* shedding was not found in our study. The amount of bacterial shedding in most experimental pigs was approximately 3 $\log_{10}$organisms/gram feces throughout the study period. Pigs with shedding less than 3.3 $\log_{10}$ *L. intracellularis* in gram feces can be counted on subclinical form of PPE (Pedersen et al., 2010). In subclinical PPE pigs, ADG was not different from non-infected pigs, although the growth rate was slightly lower than normal (Brandt et al., 2010; Pedersen et al., 2010). Furthermore, the previous study found that growth rate of infected pigs decreased when the amount of fecal shedding was over 6 $\log_{10}$ *L. intracellularis* organisms per gram of feces (Johansen et al., 2013). During our study period, all clinical signs of PHE or PIA such as bloody or watery diarrhea, reducing feed intake and poor growth rate were not observed and the amount of fecal shedding of all pigs in our experiment were not over 6 $\log_{10}$ *L. intracellularis* organisms per gram feces. Therefore, the PPE in all experimental pigs was in a subclinical form of *L. intracellularis* infection with low amount of bacterial shedding which was less impact on growth performance loss.

In some experimental challenge studies, pigs that were inoculated with various doses of low to high level of homogenate mucosal from infected pigs with PPE did not differ statistically in ADG, feed intake and FCR among challenged groups. However, those parameters were only significantly different between infected groups and uninfected group (Paradis et al., 2005; Paradis et al., 2012). Until now, the relationship between the amount of *L. intracellularis* infection and the amount of *L. intracellularis* shedding has not yet reported. Generally, pig that received more bacterial load should shed more bacteria in feces. Furthermore, level of disease severity in infected pig also depends on immune system and age of pig (Riber et al., 2011). Riber et al (2011) reported that age-susceptibility of *L. intracellularis* infection is during weaning period which has higher severity of disease in term of weight reduction than in growing period. In our study, the experiment was performed in growing pigs. Therefore, it was possible that the impact of infection on ADG was less at this age.

The study shows that pig weight at the beginning of study significantly associated with ADG. The heavy weight at the beginning was the better weight out at the end which is the same result as a previous report (Pedersen et al., 2012b). The heavy weight pigs at the early of growing period may reflex to their good health and strong immunity in nursery pigs.

In contrast with the light weight pigs, they may be low weight and weak immunity as the main clinical characteristic of subclinical PPE (Paradis et al., 2005; Paradis et al., 2012).

The study also shows that the experimental group significantly impacted on ADG, particularly in slow growth pigs. It indicates that efficacy of antimicrobials can reduce weight loss from subclinical PPE especially in slow growth pigs. The ADG in slow growth pigs did not associate with weight at the beginning. Thus, the influence of antimicrobial is more important to reduce weight loss in those pigs. This result supports a previous study regarding benefit of using antimicrobial to reducing light weight pigs from *L. intracellularis* infection (Holyoake et al., 2009). According to our result, it suggests that reducing weight variation in the same pen is very important in order to provide a special care and treatment for light weight pigs and improve efficiency of antimicrobial use.

When focusing on the efficacy of each medicated feed in low ADG pigs, the number of low ADG pigs in T3 group was significantly lower than in non-medicated group (Figure 4.6). Moreover, the ADG of slow growth pigs in T3 group was significantly higher than in non-medicated group (Figure 4.7). This *in vivo* evidence can be explained by the results *in vitro* studies showing the lower intracellular and extracellular MIC of tiamulin than tylosin and lincomycin against *L. intracellularis* isolated from US, EU and South Korea (McOrist et al., 1995c; Wattanaphansak et al., 2009a; Yeh et al., 2011). Moreover, although active ingredient of T2 and T3 groups was the same, the results of two groups were slightly different. These might be due to the difference in drug formula and drug concentration (80% and 10% tiamulin concentration for T2 and T3, respectively).

Economic outcome

During the study period, two pigs in T2 and T4 groups died at day 5 and day 18 after feeding medication. Before death, these pigs showed individually depression with no watery or bloody diarrhea. The causes of death were attributed to other causes rather than *L. intracellularis* infection. Regarding to the economic point of view, economic loss due to death on this occasion is a little and has less impact on our study when consider for all costs per group.

This is the first report that shows cost-efficient assessment for using antimicrobial for treatment and control of *L. intracellularis* infection. When focusing on reducing economic loss from *L. intracellularis* infection compared with non-medicated group, T3 group was the

first rank among medicated groups. As in the analysis, T3 provided the lowest extra cost to reduce 1 kg weight loss.

For reducing 1% of positive pigs from non-medicated group, T2 group was the first rank among medicated groups. As in the analysis, T2 provided the lowest extra cost to reduce 1% of positive pigs from non-medicated group.

The extra cost of T4 group to reduce weight loss from non-medicated group cannot compensate the economic loss from subclinical PPE. However, T4 group still provided the low extra cost from non-medicated group at the second rank among medicated groups to reduce 1% positive pig.

Based on the results, decision to choose antimicrobial to reduce economic loss from subclinical PPE depends on situation. For example, the farm with high prevalence of *L. intracellularis* infection, the high efficiency antimicrobials, such as T2 group (tiamulin (Triamulox®)) is the better choice to reduce infection rate. For the farm with low level of *L. intracellularis* prevalence and requiring to reduce economic loss from this disease, the antimicrobial such as T3 group (tiamulin) that provides the reduction of weight loss is a good alternative option.

However, our study decides the economic outcomes of pig performance at 15 weeks of age. When the decision is changed to at the market age as 25 weeks old, economic outcomes might change (McOrist, 2005). For instance, the lowest net return can be found in T4 group because a total income at pig with 15 weeks of age cannot be compensated for the high cost of feed medication. When measurement of net return is changed to at 25 weeks of age, a net return of T4 group may turn to a higher level than non-medicated group. This speculation may be true because *L. intracellularis* prevalence in a non-medicated group was significantly higher than in T4 group at the end of study. According to the previous study, the presence of *L. intracellularis* and poor performance herd are significant association even in subclinical pigs (Jacobson et al., 2003b). Therefore, almost half of positive pigs in non-medicated group might be resulted lower in weight gain and lower in total income at the market age. Unfortunately, this study cannot collect data set at the market weight because some experimental pigs were sold to be breeding pigs. The evaluation of the market weight is needed in a further study to demonstrate the production performances and economic impact from using antimicrobials for control and treatment PPE in growing pigs.

In conclusion, *in vivo* antimicrobial efficacy against *L. intracellularis* infection can significantly reduce the amount of bacterial shedding and a number of infected pigs. Moreover, the number of high shedding pigs also decreased after antimicrobial intervention. These indicate that, in farm with high prevalence of *L. intracellularis* infection, antimicrobial can be effective alternation to reduce shedding and infection. For the cost-effectiveness consideration, the use of all antimicrobials provides the efficient cost to reduce 1% of positive pig from non-medicated group. In addition, antimicrobial tend to reduce growth performance loss from subclinical PPE particularly in slow growth pigs. A selected efficient medication treatment for specific pigs as for low weight pigs might be an alternative option to reduce production loss from subclinical PPE in growing pigs.

References

Aarestrup FM, Oliver Duran C and Burch DG. 2008. Antimicrobial resistance in swine production. Anim Health Res Rev. 9(2): 135-148.

Alexopoulos C, Tassis PD, Kyriakis CS, Tzika ED, Papatsiros V and Kyriakis SC. 2006. First experience on the effect of in-feed lincomycin for the control of proliferative enteropathy in growing pigs. J Vet Med. 53(3): 157-162.

Angulo FJ, Collignon P, Powers JH, Chiller TM, Aidara-Kane A and Aarestrup FM. 2009. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clin Infect Dis. 49(1): 132-141.

Bak H and Rathkjen PH. 2009. Reduced use of antimicrobials after vaccination of pigs against porcine proliferative enteropathy in a Danish SPF herd. Available from: http://www.actavetscand.com/content/51/1/1.

Boesen HT, Jensen TK, Moller K, Nielsen LH and Jungersen G. 2005. Evaluation of a novel enzyme-linked immunosorbent assay for serological diagnosis of porcine proliferative enteropathy. Vet Microbiol. 109(1-2): 105-112.

Boutrup TS, Boesen H, Boye M, Agerholm JS and Jensen TK. 2010. Early pathogenesis in porcine proliferative enteropathy caused by *Lawsonia intracellularis*. J Comp Pathol. 143(2-3): 101-109.

Bradford JR, Winkelman N, Moreira F and Elfring G. 2004. Evaluation of lincomycin in drinking water for treatment of induced porcine proliferative enteropathy using a swine challenge model. Vet Ther. 5: 239-250.

Brandt D, Kaim U, Baumgartner W and Wendt M. 2010. Evaluation of *Lawsonia intracellularis* infection in a group of pigs in a subclinically affected herd from weaning to slaughter. Vet Microbiol. 146(3-4): 361-365.

Bronsvoot M, Norby B and Bane DP. 2001. Management factors associated with seropositivity to *Lawsonia intracellularis* in US swine herds. J Swine Health Prod. 9(6): 285-292.

Chouet S, Prieto C, Mieli L, Veenhuizen M and McOrist S. 2003. Patterns of exposure to *Lawsonia intracellularis* infection on European pig farms. Vet Rec. 152(1): 14-17.

Collins A, Fell S, Pearson H and Toribio J. 2011. Colonisation and shedding of *Lawsonia intracellularis* in experimentally inoculated rodents and in wild rodents on pig farms. Vet Microbiol. 150(3-4): 384-388.

Collins A and Love R. 2007. Re-challenge of pigs following recovery from proliferative enteropathy. Vet Microbiol. 120(3): 381-386.

Collins A, Love R, Pozo J, Smith S and McOrist S. 2000. Studies on the ex vivo survival of *Lawsonia intracellularis*. Swine Health Prod. 8(5): 211-215.

Cordes H, Riber U, Jensen TK and Jungersen G. 2012. Cell-mediated and humoral immune responses in pigs following primary and challenge-exposure to *Lawsonia intracellularis*. Available from: http://www.veterinaryresearch.org/content/43/1/9.

Corzo CA, Friendship R, Dewey C and Blackwell T. 2005a. Comparison of 2 serologic tests for the diagnosis of porcine proliferative enteropathy. Can Vet J. 46(5): 433-435.

Corzo CA, Rajic A, Friendship R and Dewey C. 2005b. Risk factors associated with seropositivity to *Lawsonia intracellularis* in Alberta finishing swine. Am Assoc Swine Veterinarians. 373-374.

Dijkhuizen A, Huirne R and Jalvingh A. 1995. Economic analysis of animal diseases and their control. Prev Vet Med. 25(2): 135-149.

Driemeier D, Faccini GS, de Oliveira RT, Colodel EM, Traverso SD and Cattani C. 2002. Silver staining combined with alcian blue and hematoxylin-eosin for the detection of *Lawsonia intracelullaris* in swine proliferative enteropathy. Acta Histochem. 104(3): 285-287.

Drozd M, Kassem II, Gebreyes W and Rajashekara G. 2010. A quantitative polymerase chain reaction assay for detection and quantification of *Lawsonia intracellularis*. J Vet Diagn Invest. 22(2): 265-269.

Elder RO, Duhamel GE, Mathiesen MR, Erickson ED, Gebhart CJ and Oberst RD. 1997. Multiplex polymerase chain reaction for simultaneous detection of *Lawsonia intracellularis*, *Serpulina hyodysen teriae*, and Salmonellae in porcine intestinal specimens. J Vet Diagn Invest. 9(3): 281-286.

Friedman M, Bednář V, Klimeš J, Smola J, Mrlík V and Literák I. 2008. *Lawsonia intracellularis* in rodents from pig farms with the occurrence of porcine proliferative enteropathy. Lett Appl Microbiol. 47(2): 117-121.

Gebhart C. 2008. *Lawsonia intracellularis*: Diagnosis update. Am Assoc Swine Veterinarians. 493-497.

Gebhart CJ, Barns SM, Mcorist S, Lin G-F and Lawson GH. 1993. Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. Int J Syst Bacteriol. 43(3): 533-538.

Go YY, Lee JK, Ye JY, Lee JB, Park SY, Song CS, Kim SK and Choi IS. 2005. Experimental reproduction of proliferative enteropathy and the role of IFN-gamma in protective immunity against *Lawsonia intracellularis* in mice. J Vet Sci. 6(4): 357-359.

Guedes R. 2004. Update on epidemiology and diagnosis of porcine proliferative enteropathy. J Swine Health Prod. 12(3): 134-138.

Guedes R and Gebhart CJ. 2003a. Comparison of intestinal mucosa homogenate and pure culture of the homologous *Lawsonia intracellularis* isolate in reproducing proliferative enteropathy in swine. Vet Microbiol. 93(2): 159-166.

Guedes R and Gebhart CJ. 2003b. Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of *Lawsonia intracellularis*. Vet Microbiol. 91(2): 135-145.

Guedes R, Gebhart CJ, Deen J and Winkelman NL. 2002a. Validation of an immunoperoxidase monolayer assay as a serologic test for porcine proliferative enteropathy. J Vet Diagn Invest. 14(6): 528-530.

Guedes R, Gebhart CJ, Winkelman NL and Mackie-Nuss RA. 2002b. A comparative study of an indirect fluorescent antibody test and an immunoperoxidase monolayer assay for the diagnosis of porcine proliferative enteropathy. J Vet Diagn Invest. 14(5): 420-423.

Guedes R, Winkelman N and Gebhart C. 2003. Relationship between the severity of porcine proliferative enteropathy and the infectious dose of *Lawsonia intracellularis*. Vet Rec. 153(14): 432-433.

Guedes RMC and Gebhart CJ. 2004. Progression of *Lawsonia intracellularis* infection and mucosa immune response in pigs. Am Assoc Swine Veterinarians. 439-440.

Guedes RMC and Gebhart CJ. 2010. Evidence of cell-mediated immune response and specific local mucosal immunoglobulin (Ig) A production against *Lawsonia intracellularis* in experimentally infected swine. Can J Vet Res. 74(2): 97-101.

Guedes RMC, Gebhart CJ, Armbruster GA and Roggow BD. 2002c. Serologic follow-up of a repopulated swine herd after an outbreak of proliferative hemorrhagic enteropathy. Can J Vet Res. 66(4): 258-263.

Guedes RMC, Gebhart CJ, Winkelman NL, Mackie-Nuss RAC, Marsteller TA and Deen J. 2002d. Comparison of different methods for diagnosis of porcine proliferative enteropathy. Can J Vet Res. 66(2): 99-107.

Hammer JM. 2004. The temporal relationship of fecal shedding of *Lawsonia intracellularis* and seroconversion in field cases. J Swine Health Prod. 12(1): 29-32.

Hands I, McOrist S, Blunt R and Lawrence K. 2010. Current infection patterns of porcine proliferative enteropathy in Great Britain and the Republic of Ireland. Vet Rec. 167(9): 343-344.

Holyoake P, Collins A, Donahoo M, Lising R and Emery D. 2009. Identifying obstacles to reducing the use of antibiotics to control porcine proliferative enteropathy. Aust Vet J. 87(1-2): 33-34.

Holyoake P, Cutler R, Caple I and Monckton R. 1994. Enzyme-linked immunosorbent assay for measuring ileal symbiont intracellularis-specific immunoglobulin G response in sera of pigs. J Clin Microbiol. 32(8): 1980-1985.

Holyoake P, Emery D, Gonsalves J, Donahoo M and Collins A. 2010. Prevalence of antibodies to *Lawsonia intracellularis* in pig herds in Australia. Aust Vet J. 88(5): 186-188.

Huerta B, Arenas A, Carrasco L, Maldonado A, Tarradas C, Carbonero A and Perea A. 2003. Comparison of diagnostic techniques for porcine proliferative enteropathy (*Lawsonia intracellularis* infection). J Comp Pathol. 129(2-3): 179-185.

Jacobson M, CK T, ISTRUP H, Benz M, NDER CBF, Riedler J and Schram-Bijkerk D. 2007. Survey on the occurrence of *Brachyspira* species and *Lawsonia intracellularis* in children living on pig farms. Epidemiol Infect. 135(6): 1043-1045.

Jacobson M, Englund S and Ballagi-Pordány A. 2003a. The use of a mimic to detect polymerase chain reaction–inhibitory factors in feces examined for the presence of *Lawsonia intracellularis*. J Vet Diagn Invest. 15(3): 268-273.

Jacobson M, Fellström C and Jensen-Waern M. 2010. Porcine proliferative enteropathy: An important disease with questions remaining to be solved. Vet J. 184(3): 264-268.

Jacobson M, Hård af Segerstad C, Gunnarsson A, Fellström C, de Verdier Klingenberg K, Wallgren P and Jensen-Waern M. 2003b. Diarrhoea in the growing pig-a comparison of clinical, morphological and microbial findings between animals from good and poor performance herds. Res Vet Sci. 74(2): 163-169.

Jacobson M, Norling B, Gunnarson A and Aspan A. 2009. Flotation—a new method to circumvent PCR inhibitors in the diagnosis of *Lawsonia intracellularis*. Int J Microbiol. doi:10.1155/2009/410945.

Jacobson M, Wallgren P, Nordengrahn A, Merza M and Emanuelson U. 2011. Evaluation of a blocking ELISA for the detection of antibodies against *Lawsonia intracellularis* in pig sera. Available from: http://www.actavetscand.com/content/53/1/23.

Jensen TK, Vigre H, Sørensen V and Møller K. 2005. Naturally acquired *Lawsonia intracellularis* infection in pigs studied from weaning to slaughter by indirect immunofluorescence antibody test and polymerase chain reaction on faeces. Res Vet Sci. 79(2): 93-98.

Jensen TK, Vigre H, Svensmark B and Bille-Hansen V. 2006. Distinction between porcine circovirus type 2 enteritis and porcine proliferative enteropathy caused by *Lawsonia intracellularis*. J Comp Pathol. 135(4): 176-182.

Johannesson M. 1995. The relationship between cost-effectiveness analysis and cost-benefit analysis. Soc Sci Med. 41(4): 483-489.

Johansen M, Nielsen M, Dahl J, Svensmark B, Bækbo P, Kristensen CS, Hjulsager CK, Jensen TK, Ståhl M and Larsen LE. 2013. Investigation of the association of growth rate in grower-finishing pigs with the quantification of *Lawsonia intracellularis* and porcine circovirus type 2. Prev Vet Med. 108(1): 63-72.

Jones G, Ward G, Murtaugh M, Lin G and Gebhart C. 1993. Enhanced detection of intracellular organism of swine proliferative enteritis, ileal symbiont intracellularis, in feces by polymerase chain reaction. J Clin Microbiol. 31(10): 2611-2615.

Jordan DM, Knitted JP, Schwartz KJ, Roof MB and Hoffman LJ. 2004. A *Lawsonia intracellularis* transmission study using a pure culture inoculated seeder-pig sentinel model. Vet Microbiol. 104(1-2): 83-90.

Jordan DM, Knittel JP, Roof MB, Schwartz K, Larson D and Hoffman LJ. 1999. Detection of *Lawsonia intracellularis* in swine using polymerase chain reaction methodology. J Vet Diagn Invest. 11(1): 45-49.

Just SD, Thoen CO, Thacker BJ and Thompson JU. 2001. Monitoring of *Lawsonia intracellularis* by indirect serum immunofluorescence assay in a commercial swine production system. J Swine Health Prod. 9(2): 57-64.

Kim J, Choi C, Cho W and Chae C. 2000. Immunohistochemistry and polymerase chain reaction for the detection of *Lawsonia intracellularis* in porcine intestinal tissues with proliferative enteropathy. J Vet Med Sci. 62(7): 771-773.

Knittel J, Jordan D, Schwartz K, Janke B, Roof M, McOrist S and Harris D. 1998a. Evaluation of antemortem polymerase chain reaction and serologic methods for detection of *Lawsonia intracellularis*-exposed pigs. Am J Vet Res. 59(6): 722-726.

Knittel J, Jordan D, Schwartz K, Janke B, Roof M, McOrist S and Harris D. 1998b. Evaluation of antemortem polymerase chain reaction and serologic methods for detection of *Lawsonia intracellularis*-exposed pigs. Am J Vet Res. 59(6): 722.

Kroll JJ, Eichmeyer M, Schaeffer M, McOrist S, Harris D and Roof M. 2005a. Lipopolysaccharide-based enzyme-linked immunosorbent assay for experimental use in detection of antibodies to *Lawsonia intracellularis* in pigs. Clin Diagn Lab Immunol. 12(6): 693-699.

Kroll JJ, Roof MB, Hoffman LJ, Dickson JS and Hank Harris D. 2005b. Proliferative enteropathy: a global enteric disease of pigs caused by *Lawsonia intracellularis*. Anim Health Res Rev. 6(2): 173-197.

Kukushkin S and Okovytaya T. 2012. Seroprevalence of *Lawsonia intracellularis*, *Mycoplasma hyopneumoniae* and PCV2 in commercial pig farms in Russia. Vet Rec. 171(5): 126-126.

Kyriakis S, Alexopoulos C, Saoulidis K, Lekkas S, Miliotis C and Sauveroche B. 2002a. The effect of josamycine on the control of ileitis in weaned piglets under field conditions. J Vet Pharmacol Ther. 25(4): 279-284.

Kyriakis S, Bourtzi-Hatzopoulou E, Alexopoulos C, Kritas S, Polyzopoulou Z, Lekkas S and Gardey L. 2002b. Field evaluation of the effect of in feed doxycycline for the control of ileitis in weaned piglets. J Vet Med. 49(7): 317-321.

Ladinig A, Sommerfeld-Stur I and Weissenböck H. 2009. Comparative evaluation of diagnostic methods for *Lawsonia intracellularis* infection in pigs, with emphasis on cases lacking characteristic lesions. J Comp Pathol. 140(2-3): 140-148.

Lawson G and Gebhart C. 2000. Proliferative enteropathy. J Comp Pathol. 122(2-3): 77-100.

Lawson G, McOrist S, Jasni S and Mackie R. 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance *in vitro*. J Clin Microbiol. 31(5): 1136-1142.

Lawson G, McOrist S, Rowland A, McCartney E and Roberts L. 1988. Serological diagnosis of the porcine proliferative enteropathies: implications for aetiology and epidemiology. Vet Rec. 122(23): 554-557.

Lee SW, Kim TJ, Park SY, Song CS, Chang HK, Yeh JK, Park HI and Lee JB. 2001. Prevalence of porcine proliferative enteropathy and its control with tylosin in Korea. J Vet Sci. 2(3): 209-212.

Lindecrona R, Jensen TK, Andersen P and Møller K. 2002. Application of a 5′ nuclease assay for detection of *Lawsonia intracellularis* in fecal samples from pigs. J Clin Microbiol. 40(3): 984-987.

Machuca MA, Cappuccio JA, Quiroga MA, Insarralde L, Arrospide S and Perfumo C. 2012. Granulomatous enteritis associated with porcine proliferative enteropathy. Braz J Vet Pathol. 5(1): 25-30.

MacIntyre N, Smith D, Shaw D, Thomson J and Rhind S. 2003. Immunopathogenesis of experimentally induced proliferative enteropathy in pigs. Vet Pathol. 40(4): 421-432.

Marsteller TA, Armbruster G, Bane DP, Gebhart CJ, Muller R, Weatherford J and Thacker B. 2003. Monitoring the prevalence of *Lawsonia intracellularis* IgG antibodies using

serial sampling in growing and breeding swine herds. J Swine Health Prod. 11(3): 127-130.

Mauch C and Bilkei G. 2004. Effect of sow parity on the transmission of *Lawsonia intracellularis* to piglets. Vet Rec. 155(17): 532-532.

McCluskey J, Hannigan J, Harris JD, Wren B and Smith DGE. 2002. LsaA, an antigen involved in cell attachment and invasion, is expressed by *Lawsonia intracellularis* during infection *in vitro* and *in vivo*. Infect Immun. 70(6): 2899-2907.

McEwen SA and Fedorka-Cray PJ. 2002. Antimicrobial use and resistance in animals. Clin Infect Dis. 34(Supplement 3): S93-S106.

McOrist S. 2005. Defining the full costs of endemic porcine proliferative enteropathy. Vet J. 170(1): 8-9.

McOrist S, Blunt R and Gebhart CJ. 2011. Pig-associated *Lawsonia intracellularis* in various on-farm dipterous fly stages. J Swine Health Prod. 19(5): 277-283.

McOrist S and Gebhart CJ. 2012. Proliferative Enteropathy. Disease of Swine. 10th ed. In: Zimmerman, J.Karriker, L. Ramirez, A. Schwartz, K. Stevenson, G. Wiley-Blackwell. pp.811-820.

McOrist S, Gebhart CJ, Boid R and Barns SM. 1995a. Characterization of *Lawsonia intracellularis* gen. nov., sp. nov., the obligately intracellular bacterium of porcine proliferative enteropathy. Int J Syst Bacteriol. 45(4): 820-825.

McOrist S, Gebhart CJ and Bosworth BT. 2006. Evaluation of porcine ileum models of enterocyte infection by *Lawsonia intracellularis*. Can J Vet Res. 70(2): 155-159.

McOrist S, Jasni S, Mackie R, Berschneider H and RowlandG HK A. 1995b. Entry of the bacterium ileal symbiont intracellularis into cultured enterocytes and its subsequent release. Res Vet Sci. 59(3): 255-260.

McOrist S, Jasni S, Mackie R, MacIntyre N, Neef N and Lawson G. 1993. Reproduction of porcine proliferative enteropathy with pure cultures of ileal symbiont intracellularis. Infect Immun. 61(10): 4286-4292.

McOrist S, MacIntyre N, Stokes C and Lawson G. 1992. Immunocytological responses in porcine proliferative enteropathies. Infect Immun. 60(10): 4184-4191.

McOrist S, Mackie RA and Lawson G. 1995c. Antimicrobial susceptibility of ileal symbiont intracellularis isolated from pigs with proliferative enteropathy. J Clin Microbiol. 33(5): 1314-1317.

McOrist S, Morgan J, Veenhuizen M, Lawrence K and Kroger H. 1997a. Oral administration of tylosin phosphate for treatment and prevention of proliferative enteropathy in pigs. Am J Vet Res. 58(2): 136-139.

McOrist S, Muller Wager A, Kratzer D and Sjösten C. 2000. Therapeutic efficacy of water-soluble lincomycin-spectinomycin powder against porcine proliferative enteropathy in a European field study. Vet Rec. 146(3): 61-65.

McOrist S, Shearn M and Morgan J. 1999a. Control of porcine proliferative enteropathy by oral administration of chlortetracycline. Vet Rec. 144(2): 48-49.

McOrist S, Smith S and Green L. 1997b. Estimate of direct financial losses due to porcine proliferative enteropathy. Vet Rec. 140(22): 579-581.

McOrist S, Smith S and Klein T. 1999b. Monitored control programme for proliferative enteropathy on British pig farms. Vet Rec. 144(8): 202-204.

McOrist S, Smith S, Shearn M, Carr M and Miller D. 1996. Treatment and prevention of porcine proliferative enteropathy with oral tiamulin. Vet Rec. 139(25): 615-618.

McOrist S and Smits R. 2007. Field evaluation of an oral attenuated *Lawsonia intracellularis* vaccine for porcine proliferative enteropathy (ileitis). Vet Rec. 161(1): 26-28.

Michalski C, Di Mola F, Kümmel K, Wendt M, Köninger J, Giese T, Giese N and Friess H. 2006. Human inflammatory bowel disease does not associate with *Lawsonia intracellularis* infection. Available from: http://www.biomedcentral.com/1471-2180/6/81.

Nathues H, Holthaus K and Grosse Beilage E. 2009. Quantification of *Lawsonia intracellularis* in porcine faeces by real-time PCR. J Appl Microbiol. 107(6): 2009-2016.

Nathues H, Oliveira C, Wurm M, Grosse Beilage E and Givisiez P. 2007. Simultaneous detection of *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Lawsonia intracellularis* in porcine faeces and tissue samples by multiplex-PCR. J Vet Med. 54(9): 532-538.

Opriessnig T, Meng XJ and Halbur PG. 2007. Porcine circovirus type 2–associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest. 19(6): 591-615.

Paradis MA, Gebhart CJ, Toole D, Vessie G, APM D, Winkelman NL, Bauer SA, Wilson JB and McClure CA. 2012. Subclinical ileitis: Diagnostic and performance parameters in a multi-dose mucosal homogenate challenge model Ileitis J Swine Health Prod. 20(3): 137-141.

Paradis MA, Gottschalk M, Rajic A, Ravel A, Wilson JB, Aramini J, McClure CA and Dick CP. 2007. Seroprevalence of *Lawsonia intracellularis* in different swine populations in 3 provinces in Canada. Can Vet J. 48(1): 57-62.

Paradis MA, Mckay RI, Wilson JB, Vessie GH, Winkelman NL and Gebhart CJ. 2005. Subclinical ileitis produced by sequential dilutions of *Lawsonia intracellularis* in a mucosal homogenate challenge model. Am Assoc Swine Veterinarians. 189-191.

Pedersen K, Ståhl M, Guedes RM, Angen Ø, Nielsen J and Jensen T. 2012a. Association between faecal load of *lawsonia intracellularis* and pathological findings of proliferative enteropathy in pigs with diarrhoea. BMC Vet Res. 8(1): 198.

Pedersen KS, Holyoake P, Stege H and Nielsen JP. 2010. Diagnostic performance of different fecal *Lawsonia intracellularis*—specific polymerase chain reaction assays as diagnostic tests for proliferative enteropathy in pigs: a review. J Vet Diagn Invest. 22(4): 487-494.

Pedersen KS, Skrubel R, Stege H, Angen Ø, Ståhl M, Hjulsager C, Larsen LE, Nielsen JP, Back H and Kendall A. 2012b. Association between average daily gain, faecal dry matter content and concentration of *Lawsonia intracellularis* in faeces. Available from: http://www.actavetscand.com/content/54/1/58.

Riber U, Cordes H, Boutrup TS, Jensen TK, Heegaard PMH and Jungersen G. 2011. Primary infection protects pigs against re-infection with *Lawsonia intracellularis* in experimental challenge studies. Vet Microbiol. 149(3): 406-414.

Richter B, Ladinig A, Nedorost N and Herbert W. 2010. A TaqMan quantitative polymerase chain reaction assay for the detection of *Lawsonia intracellularis* in fecal and tissue samples from pigs. J Vet Diagn Invest. 22(1): 70-73.

Segalés J, Fernández-Salguero J, Fructuoso G, Quintana J, Rosell C, Pozo J, De Arriba M, Rubio P and Domingo M. 2001. Granulomatous enteritis and lymphadenitis in Iberian pigs naturally infected with *Lawsonia intracellularis*. Vet Pathol. 38(3): 343-346.

Smith D and Lawson G. 2001. *Lawsonia intracellularis*: getting inside the pathogenesis of proliferative enteropathy. Vet Microbiol. 82(4): 331-345.

Smith DGE, Mitchell SC, Nash T and Rhind S. 2000. Gamma interferon influences intestinal epithelial hyperplasia caused by *Lawsonia intracellularis* infection in mice. Infect Immun. 68(12): 6737-6743.

Stege H, Jensen TK, Møller K, Baekbo P and Jorsal S. 2000. Prevalence of intestinal pathogens in Danish finishing pig herds. Prev Vet Med. 46(4): 279-292.

Stege H, Jensen TK, Møller K, Vestergaard K, Baekbo P and Jorsal S. 2004. Infection dynamics of *Lawsonia intracellularis* in pig herds. Vet Microbiol. 104(3): 197-206.

Suh DK and Song JC. 2005. Simultaneous detection of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and Salmonella spp. in swine intestinal specimens by multiplex polymerase chain reaction. J Vet Sci. 6(3): 231-237.

Vannucci FA, Wattanaphansak S and Gebhart CJ. 2012. An alternative method for cultivation of *Lawsonia intracellularis*. J Clin Microbiol. 50(3): 1070-1072.

Veenhuizen MF, Mowrey DH, Moore GM and Watkins LE. 1998. Evaluating a natural outbreak of porcine proliferative enteropathy and treatment with tylosin in the grow-finish phase. Swine Health Prod. 6: 67-72.

Viera AJ and Garrett JM. 2005. Understanding interobserver agreement: the kappa statistic. Fam Med. 37(5): 360-363.

Walter D, Gebhart C, Kroll J, Holck J and Chittick W. 2004. Serologic profiling and vaccination timing for *Lawsonia intracellularis*. J Swine Health Prod. 12(6): 310-313.

Walter D, Knittel J, Schwartz K, Kroll J and Roof M. 2001. Treatment and control of porcine proliferative enteropathy using different tiamulin delivery methods. J Swine Health Prod. 9(3): 109-116.

Watson E, Clark EM, Alberdi MP, Inglis NF, Porter M, Imrie L, Mclean K, Manson E, Lainson A and Smith DGE. 2011. A novel *Lawsonia intracellularis* autotransporter protein is a prominent antigen. Clin Vaccine Immunol. 18(8): 1282-1287.

Wattanaphansak S, Asawakarn T, Gebhart CJ and Deen J. 2008. Development and validation of an enzyme-linked immunosorbent assay for the diagnosis of porcine proliferative enteropathy. J Vet Diagn Invest. 20(2): 170-177.

Wattanaphansak S, Gebhart CJ, Anderson JM and Singer RS. 2010a. Development of a polymerase chain reaction assay for quantification of *Lawsonia intracellularis*. J Vet Diagn Invest. 22(4): 598-602.

Wattanaphansak S, Singer RS and Gebhart CJ. 2009a. *In vitro* antimicrobial activity against 10 North American and European *Lawsonia intracellularis* isolates. Vet Microbiol. 134(3-4): 305-310.

Wattanaphansak S, Singer RS and Gebhart CJ. 2010b. Evaluation of *in vitro* bactericidal activity of commercial disinfectants against *Lawsonia intracellularis*. J Swine Health Prod. 18(1): 11-17.

Wattanaphansak S, Singer RS, Isaacson RE, Deen J, Gramm BR and Gebhart CJ. 2009b. *In vitro* assessment of the effectiveness of powder disinfectant (Stalosan® F) against *Lawsonia intracellularis* using two different assays. Vet Microbiol. 136(3): 403-407.

Winkelman NL, Crane JP, Elfring GD, Kratzer D, Meeuwse DM, Dame KJ, Buckham SL and Gebhart CJ. 2002. Lincomycin-medicated feed for the control of porcine proliferative enteropathy (ileitis) in swine. J Swine Health Prod. 10(3): 107-112.

Yeh JY, Lee JH, Yeh HR, Kim A, Lee JY, Hwang JM, Kang BK, Kim JM, Choi IS and Lee JB. 2011. Antimicrobial susceptibility testing of two *Lawsonia intracellularis* isolates associated with proliferative hemorrhagic enteropathy and porcine intestinal adenomatosis in South Korea. Antimicrob Agents Chemother. 55(9): 4451-4453.

APPENDICES

APPENDIX A
LABORATORY RESULTS

Quantitative PCR technique in this study was modified from Wattanaphansak et al (2010a). All performance of quantitative PCR, such as dissociation curves, amplification plot and efficiency of standard curve, showed the same pattern of results as previous study (Wattanaphansak et al., 2010a).

A.1 An amount of *L. intracellularis* from pure culture

Initial amount of *L. intracellularis* pure culture from the duplication which was stained by immunoperoxidase monolayer assay were 344 and 301 x 10^4 organisms per 10-µl well. The final amount of *L. intracellularis* for use as standard curve was the average of 3.22 x 10^8 per ml.

A.2 An amount of *L. intracellularis* DNA

To plot the standard curve, the DNA was extracted from 1000 µl 3.22 x 10^8organisms/ml with an assumption of 100% DNA yield. The final elution volume was made up to 200 µl. A total 5 µl DNA was used for quantitative PCR analysis, which could be equal to 8.05 x 10^6 *L. intracellularis* organisms.

A.3 Standard curve construction

A serial ten-fold dilution of DNA *L. intracellularis* pure culture was made for 6 times to construct standard curve. Therefore, the range of DNA template amount for PCR was 8.05 x 10^1 to 8.05 x 10^6 *L. intracellularis* organisms per dilution. To assure a specific target gene amplification, the dissociation curve of every dilution displayed a single melting temperature peak at 78 - 79 ^{o}C (Figure A.10). Standard curve performance was assessed by using linear range efficiency. The efficiency of all plate runs was 95-105% with R^2 value of 0.99 - 1 (Figure A.11). In addition, the comparison between Ct and the amount of organisms showed that each of the dilution increased, Ct value was decreased for 4 cycles.

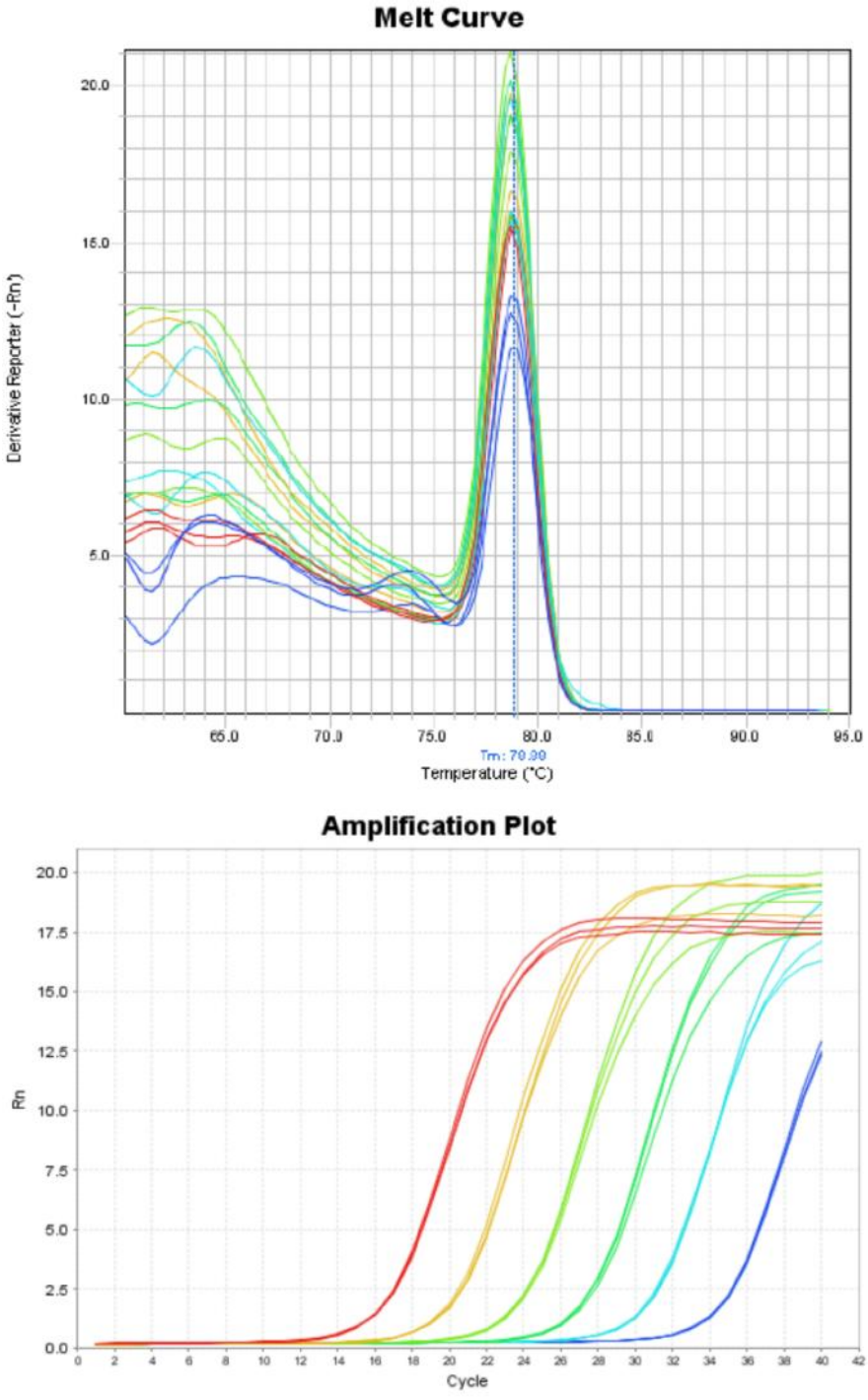

Figure A.10 Dissociation curves and amplification plot of a serial ten-fold dilution of *L. intracellularis* DNA of 8.05 x 10^6 to 8.05 x 10^1 showed a single melting temperature

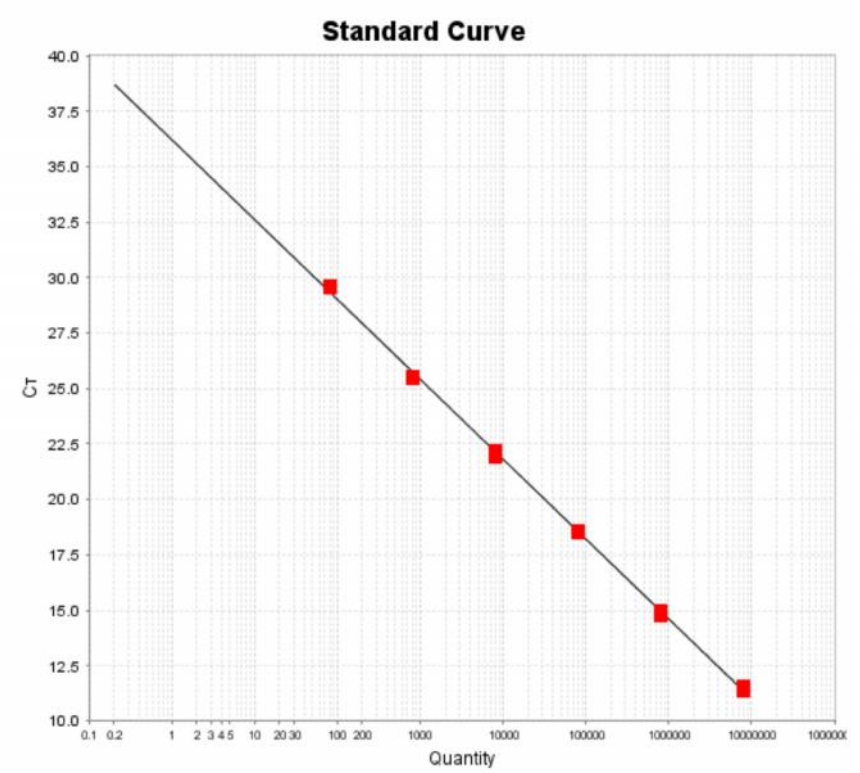

Figure A.11 Performance evaluation of standard curve of a serial ten-fold dilution of *L. intracellularis* DNA from 8.05 x 10^{1} to 8.05 x 10^{6} organisms

APPENDIX B
RESULT OF GENERALIZED LINEAR MIXED MODEL

Model analysis of generalized linear mixed model was carried out to determine association of dependent variables (antimicrobial efficacy) with independent variables as describe bellowed.

B.1 Generalized linear mixed model with logistic regression

$$\text{Logit } (P / 1\text{-}P) = \beta_0 + \beta_i X_i + \ldots + \varepsilon + \alpha$$

where as

P = probability of dependent variables such as infectious status of pig (positive or negative) in day of study of experimental group, pig with low ADG (ADG < 0.75 kg/day) in each experimental group and pig with high ADG (ADG > 0.93 kg/day) in each experimental group

β_0 is estimated intercept value

β_i is coefficient value of independent variables such as day of study, experimental group and weight at D0

X_i is independent variable such as day of study, experimental group and weight at D0

ε is estimated value of model deviation

α is estimated value of pen as random effect

B.2 Generalized linear mixed model with linear regression

$$Y = \beta_0 + \beta_i X_i + \ldots + \varepsilon + \alpha$$

where as

Y is dependent variable such as ADG of all pig, ADG of slow growth pig and ADG of fast growth pig

β_0 is estimated intercept value

β_i is coefficient value of independent variables such as weight at D0 and experimental group

X_i is independent variables such as weight of pig at D0 and experimental group

ε is estimated value of model deviation

α is estimated value of pen as random effect

BIOGRAPHY

Miss. Chanunda Raphanaphraiwan (Mrs. Chotika Pramarn) was born in December 21, 1979 in Bangkok, Thailand. She graduated from Kasetsart University with the second class honor of Veterinary Medicine in 2003. After graduation, she started to work as quality assurance veterinarian in swine farm (farrow to finish farm) of Betagro Hybrid International Co,.Ltd. since 2003 – 2007. Then, she moved to the second company named as Elanco Animal Health A Division of Eli Lilly Asia, Inc. Thailand Branch as swine technical advisor in 2007 - 2010. Before broaden her education, her last responsibility in Elanco was sale and technical advisor in 2010 - 2011. In her work experiences, she has concentrated in health monitoring, farm management and product experiment. With her high experiences and qualification in swine practice, she was received a full grant by H.M. the King's 72nd Birthday Scholarship, Chulalongkorn University, Bangkok, Thailand in academic year of 2011. She studied in Master of Science Program of Veterinary Medicine, Chulalongkorn University during 2011-2013.