EFFECT OF DIETARY MICROENCAPSULATED ORGANIC ACID AND ESSENTIAL OIL ON SOW PERFORMANCE AND NURSERY PIGLET GROWTH PERFORMANCE AND FECAL BACTERIA

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ผลของการเสริมกรดอินทรีย์และสารสกัดน้ำมันที่ห่อหุ้มด้วยไมโครแคปซูลในอาหารต่อประสิทธิภาพของแม่สุกรและประสิทธิภาพการเจริญเติบโตและแบคทีเรียในอุจจาระในสุกรอนุบาล

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Thesis Title	EFFECT OF DIETARY MICROENCAPSULATED ORGANIC ACID AND ESSENTIAL OIL ON SOW PERFORMANCE AND NURSERY PIGLET GROWTH PERFORMANCE AND FECAL BACTERIA
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เอพริเลีย ริชกี้ รีอาดานี : ผลของการเสริมกรดอินทรีย์และสารสกัดน้ำมันที่ห่อหุ้มด้วยไมโครแคปซูลในอาหารต่อประสิทธิภาพของแม่สุกรและประสิทธิภาพการเจริญเติบโตและแบคทีเรียในอุจจาระในสุกรอนุบาล. (EFFECT OF DIETARY MICROENCAPSULATED ORGANIC ACID AND ESSENTIAL OIL ON SOW PERFORMANCE AND NURSERY PIGLET GROWTH PERFORMANCE AND FECAL BACTERIA) อ.ที่ปรึกษาหลัก : อนงค์นาฏ อัศวชีพ, อ.ที่ปรึกษาร่วม : พรชลิต อัศวชีพ ,กฤษ อังคนาพร

ศึกษาผลการเสริมกรดอินทรีย์และสารสกัดน้ำมันห่อหุ้มด้วยไมโครแคปซูล (MOE, Porcinate+) ในอาหารต่อประสิทธิภาพของแม่สุกรและประสิทธิภาพการเจริญเติบโตและประชากรแบคทีเรียในอุจจาระลูกสุกรอนุบาล ในการทดลองที่ 1 แม่สุกรอุ้มท้องจำนวน 320 แม่ ในช่วงก่อนคลอด 1 สัปดาห์จนถึงหย่านม ถูกสุ่มแบ่งเป็น 2 กลุ่ม ได้แก่ กลุ่มควบคุมที่กินอาหารปกติ จำนวน 160 แม่ และกลุ่มทดลองที่กินอาหารปกติเสริมด้วย MOE ขนาด 2 กิโลกรัมต่อตันอาหาร จำนวน 160 แม่ โดยในการทดลองแบ่งออกเป็น 4 ซ้ำ ในแต่ละซ้ำจะมีแม่สุกร 40 แม่ในกลุ่มควบคุมและ 40 แม่ในกลุ่มทดลอง ผลการทดลองพบว่าแม่สุกรทั้งสองกลุ่มมีความหนาไขมันสันหลังและคะแนนรูปร่างลดลงในช่วงหย่านม โดยที่แม่กลุ่มทดลองมีการเสียความหนาไขมันสันหลังและคะแนนรูปร่างน้อยกว่าแม่กลุ่มควบคุม พบว่าร้อยละของแม่สุกรที่มีแผลหลุมที่หัวไหล่ในกลุ่มทดลองน้อยกว่ากลุ่มควบคุม รวมทั้งคะแนนแผลหลุมหัวไหล่ด้วย โดยพบความสัมพันธ์เชิงบวกระหว่างความหนาไขมันสันหลังและคะแนนรูปร่าง ในการทดลองที่ 2 ลูกสุกรอนุบาลจำนวน 2800 ตัว ถูกสุ่มแบ่งออกเป็นกลุ่มควบคุม (1400 ตัว) ได้รับอาหารปกติของฟาร์ม และกลุ่มทดลอง (1400 ตัว) ได้รับอาหารปกติเสริมด้วย MOE ขนาด 2 กิโลกรัมต่อตันอาหารในช่วงอายุ 28 - 42 และขนาด 1 กิโลกรัมต่อตันอาหารในช่วงอายุ 43 - 56 วัน ตามลำดับ ผลการศึกษาพบว่าอัตราการเจริญเติบโตต่อวัน อัตราการกินอาหารต่อวันและอัตราแลกเนื้อของลูกสุกรในกลุ่มทดลองมีค่าที่ดีกว่ากลุ่มควบคุม พบว่าจำนวนประชากรแบคทีเรียรวม เชื้อโคลัยฟอร์ม เชื้ออีโคลัยและเชื้อแลคโตบาซิลลัสในอุจจาระลูกสุกร รวมทั้งอัตราส่วนของเชื้อแลคโตบาซิลลัสต่อประชากรแบคทีเรียรวม อัตราส่วนของเชื้อแลคโตบาซิลลัสต่อเชื้อโคลัยฟอร์มในช่วงต้นและช่วงกลางของงานทดลองมีแนวโน้มจะไม่พบความแตกต่างทางสถิติระหว่างกลุ่มควบคุมและทดลอง แต่ในช่วงท้ายของการทดลองที่อายุ 56 วัน พบว่าจำนวนประชากรแบคทีเรียรวม เชื้อโคลัยฟอร์ม เชื้ออีโคลัยและเชื้อแลคโตบาซิลลัสในอุจจาระลูกสุกรของกลุ่มทดลองมีค่าสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ แต่ไม่พบความแตกต่างทางสถิติของอัตราส่วนของเชื้อแลคโตบาซิลลัสต่อประชากรแบคทีเรียรวม อัตราส่วนของเชื้อแลคโตบาซิลลัสต่อเชื้อโคลัยฟอร์มระหว่างทั้งสองกลุ่ม สรุปผลการทดลองว่าการเสริมด้วยส่วนผสมของกรดซิตริก กรดฟูมาริก กรดซอร์บิกและส่วนผสมของสารสกัดน้ำมัน ยูจีนอล ไธมอลและวานิลินที่ห่อหุ้มด้วยไมโครแคปซูลในอาหารช่วยทำให้ประสิทธิภาพแม่สุกรได้ดีขึ้น และเพิ่มน้ำหนักหย่านมและสมรรถภาพการใช้อาหารของลูกสุกรอนุบาลได้

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The effect of feed supplementation with microencapsulated organic acids and essential oils (MOE, Porcinate+) in feed was investigated according to sow and nursery pig growth performances, and fecal bacteria population. *Experiment 1:* healthy three hundred and twenty sows during its late gestation period (7 days before farrowing) until the weaning phase (28 days after farrowing) were randomly divided as control group (160 sows) which was fed with basal diet, and treatment group (160 sows), which was fed with basal diets supplemented with 2 kilograms (kg) of MOE in 1 ton of feed. Each of 4 replications was composed of 40 sows in control and 40 sows in treatment groups. The results showed that sow back fat (BF) thickness and body condition score (BCS) were significantly decreased during late gestation phase until weaning within groups. However, BCS and BF thickness in treatment group were significantly higher compared to the control group at wean. The percentage of shoulder ulcer and score during lactation were lower in treatment group compared to control group. Furthermore, the positive correlation between BF to BCS tends to be found (p=0.01, r=0.362). Weaned pig numbers and weight were significantly increased in the treatment group compared to the control group. *Experiment 2:* two thousand and eight hundred weaned pigs were randomly divided into two groups; the control group (1400 pigs) which was fed with basal feed without MOE), and the treatment group (1400 pigs) which was fed with basal feed + MOE 2 kg/1 ton between 28 - 42 days old, and basal feed + MOE 1 kg/1 ton between 43 - 56 days old, respectively. The outcome revealed that average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were statistically better in treatment than control groups. At the beginning and middle of the experiment, average number of coliform bacteria, *E. coli*, and *Lactobacillus spp*. in feces, including L/T and L/C ratios tended to have no significant difference between treatment than control groups. However, at the end of experiment (56 days of age), all bacterial population of both groups seemed to be statistically different, except the L/T and L/C ratios. In conclusion, feed supplementation with microencapsulated citric, fumaric, malic, and sorbic acid as organic acid mixture and eugenol, thymol, and vanillin as essential oil mixture offers better sow performance, increasing weaning pig weight gain and feed efficiency.

Field of Study:	Veterinary Science and technology	Student's Signature .....
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		Co-advisor's Signature .....
		Co-advisor's Signature .....

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“Verily, with every hardship, comes ease” 94: 5-6.

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CHAPTER I. INTRODUCTION

IMPORTANCE AND RATIONALE

Antibiotic use in intensive swine producing industries in the last fifty years has been widely used for treatment, prevent and control diseases. Concerns about the possibility of bacteria acquiring drug resistance gene might affect humans either derived from meat, or environment to humans (Papatsiros et al., 2012). Because of these negative aspects, some countries in Europe have attempted to ban the use of antibiotic in livestock animals since 2006 and US FDA also restricted the use of antibiotic in animal feed (Castanon, 2007; FDA, 2018).

Restriction of antibiotic use certainly affects health and production of pigs. During pig production cycle, decrease of feed intake in productive sow due to farrowing stress does not only affect colostrum production, but also reduce sow body fat (Revell et al., 1998). Reduction of sow colostrum production results in lower body weight, poor health, and subsequently higher mortality rate of suckling pigs (Schnier et al., 2019). Apart from stress derived from sows, weaning stage of piglets is critical for their survival due to the abrupt change of feed and the environmental shift which potentially leads to postweaning stress and diarrhea (Coffey et al., 2000). Many research works have been addressed on the ways to reduce diarrhea in weaned pig regarding to intestinal microbiota modification to reduce numbers of pathogenic bacteria such as *Escherichia coli*, or *Salmonella spp.* and increase some

beneficial bacterial population (Partanen and Mroz, 1999; Scharek et al., 2005; Muhl and Liebert, 2007; Dai et al., 2010; Tuoi et al., 2016). Balancing microbial population in gastrointestinal tract of pig positively affect growth performance by improving nutrient digestibility and reducing diarrhea incidence. The lower diarrhea during weaning stage the higher survival during growing-to-finishing stage. Attempts to reduce antibiotic use in feed motivate searching alternative products, including organic acid supplementation under term of acidifier.

A blend of organic acid (OA) and essential oil (EO) in swine feed has been purposely used as additives to reduce diarrhea. Their ability to reduce stomach pH, or control the pathogenic bacteria by suppressing nutrient transport and enzymes through bacterial cell wall (Partanen and Mroz, 1999; Vondruskova et al., 2010), causing antimicrobial activity, and aromatic properties to attract pig voluntary feed intake are promising future to production industries (Franz et al., 2010; Li et al., 2018; Xu et al., 2018). Using latest technology, microencapsulated form of OA and EO deliberately delivers the substances to target site in gastrointestinal tract (Balasubramanian et al., 2016). Lipid as protection matrix delays OA and EO dissolvement along gastrointestinal tract depending on acidity level (*pKa*) inside the tract (Piva et al., 2007).

Even though plenty of research has been attempted to prove that OA and EO blend could improve growth performance and fecal microflora in pig, there are very limited amount research the effect of dietary microencapsulated organic acid and

essential oil on growth performance and fecal bacterial population in farrowing sows and nursery pigs.

OBJECTIVES OF STUDY

This research was aimed to investigate the effect of dietary microencapsulated organic acid and essential oil on sow performance, and nursery piglet growth performance, and fecal bacterial population.

HYPOTHESIS

Microencapsulated organic acid and essential oil supplementation in feed can improve sow farrowing performance, and nursery piglet growth performance, and fecal bacterial population.

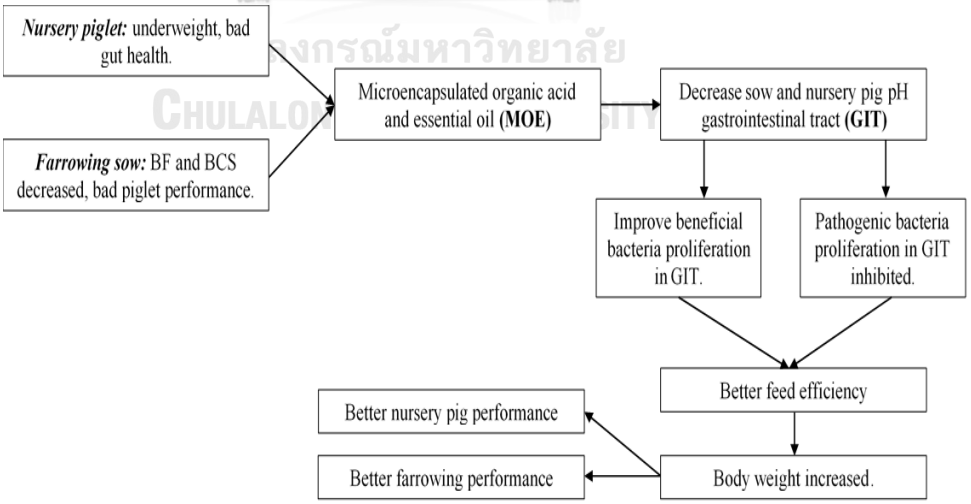

Figure 1 Conceptual framework.

CHAPTER II. LITERATURE REVIEW

A. NURSERY PIGS

1. Gastrointestinal tract development in nursery pigs.

The diarrhea problem due to gastrointestinal tract infection is often found during weaning period of pigs (Pluske et al., 2018). To minimize such problem, sow colostrum should be given to her offspring within 24 hours after give birth. The colostrum contains immunoglobulin (Ig), majorly IgG which mobilized from maternal blood circulation to colostrum, followed by IgA, and IgM. Colostrum contains several necessary components for piglet growth and survival including passive immunity against infection (Park. et al., 2013). After 24 hours, colostrum composition then becomes mature milk. Milk composition contains high lactose, which after consumed by the piglet, has been converted to lactic acid along its digestion process in stomach with help of lactic acid bacteria (Fu and Mathews, 1999). The pH value of sow colostrum is 5.7 at parturition then rises to 6.0 at day one, while sow milk pH range is neutral to slightly alkaline depending on psychological condition, or type of feed given, and equilibrium of calcium in micellar phase (Hurley, 2015). Piglet stomach secretes very small amount of gastric juice (HCl), but since piglet consumes milk which composed of high lactose, lactic acid bacteria such as *lactobacilli sp.* will convert lactose into lactic acid and this lactic acid will decrease pH level of piglet stomach (Cranwell and Titchen, 1974; Cranwell et al., 1976; Thaela et al., 1998). Piglet in weaning phase tend to have low level acidity in stomach which may cause

dysbiosis of pathogenic and beneficial bacteria due to stress and abrupt feed change (Heo et al., 2013). Also, weaning stress could induce the release of corticotrophin-releasing factor (CRF), that effect on depletion of mucus secretion in intestinal mucosa which lead to bacterial antigens adhesion and reducing ability of intestine to absorbs water in lumen (Moeser. et al., 2007; Rodiño-Janeiro et al., 2015).

2. Dysbiosis in nursery pigs

Dysbiosis is a term for disruption of pathogenic and beneficial bacteria balance inside mammal gut caused by several factors, for example; stress and feed composition change (Gresse et al., 2017). Balance of pathogenic and beneficial bacteria amounts in pig gut have association with health and growth performance. Under conditions of higher pathogenic enterobacteria such as Enterotoxigenic *E. coli* (ETEC), *Clostridium perfringens, Campylobacter spp., Fusobacteria spp.,* or *Salmonella typhimurium* in the infected gut, and lower desirable bacteria such as *Lactobacillus spp.* or *Bifidobacterium spp.* intestinal ability to absorbing nutrients and water may disturbed due to damaging of intestinal villi, and causing diarrhea (Gaskins., 2000; Guevarra et al., 2019; Patil et al., 2020). Correct this matter, many approaches have been done, for example, adding feed additives such as essential oil and organic acid to destroy acid-sensitive harmful bacteria, disrupt intracellular pH level of acid-sensitive bacteria, and promote beneficial bacteria growth which are mostly lactic acid bacteria (Partanen and Mroz, 1999; Yang et al., 2019). In weaning

transition, intestinal infection and inflammation contribute to transient drop of feed intake so-called anorexia. As adaptive reaction of the piglet is production of reactive oxygen such as nitric oxide which rapidly converted to nitrate when released in intestinal lumen which cause the growth of Enterobacteriaceae that encodes for nitrate reductase genes, leads to inhibited gut beneficial bacteria growth (Yang et al., 2016; Guevarra et al., 2019).

3. Lactic acid bacteria

Lactic acid bacteria (LAB) have ability to ferment carbohydrate to produce lactic acid as end product (Robergs. et al., 2018). Bacterial morphology in general is non-spore and non-respiring rod or cocci, and gram positive (Axelson., 2004). Axelson. (2004) report mentioned that carbohydrate has been fermented by LAB in two pathways: (1) glycolysis pathway in which the product is specifically lactic acid under standard conditions (excessive of sugar, under anaerobic condition), distinguished by homofermentative bacteria, and (2) another fermentation pathway of phosphoketolase, in which results in ethanol, acetate, and CO_2 as by product can be distinguished as heterofermentative bacteria. Mostly, LAB have homofermentative characteristic. Guan and Liu (2020) reported that under acidic conditions intracellular pH of acid-tolerant bacteria rapidly declined, but still maintained at higher level than extracellular pH. This means that extracellular pH reaching certain point, cell effort to maintaining intracellular pH has been greater, and need larger amount of ATP to

make neutralization of intracellular pH. Such occurrence generates pH homeostasis destroyed, leads to damaging bacteria metabolism, therefore bacteria growth is inhibited or death. Furthermore, bacterial cell membrane has a great role on maintaining LAB intracellular environment. Bacterial adaptative defense due to the change of environmental condition such as extreme acidity, higher temperature, or chemicals could be involved by altering fluidity of fatty acid layer in membrane cell (Wang et al., 2018).

4. Fecal microbiota in swine

Increase number of LAB have many benefits in swine GIT physiology. For example,Yang et al. (2015) report that oral administration of LAB in neonatal pig could enhance intestinal barrier function and reduce amount of *Escherichia spp.* and *Clostridium spp*. In weaning pig, LAB inclusion in diet could relieve weaning stress, reduce diarrhea, and promote growth during and after weaning. *Lactobacillus johnsonii* supplementation in sow diet could improve sow performance, litter weight at birth and weaning phase, increasing secretion of IgA in sow and piglet intestines, enhancing IgG level in serum and reducing alanine aminotransferase.

Sun et al. (2019) revealed that using 16S rRNA sequencing to assess fecal microbiota during suckling and early weaning periods in diarrheic and non-diarrheic piglet groups and concluded that four major phyla in piglet stool are *Firmicutes, Proteobacteria, Bacteroidetes,* and *Fusobacteria.* Within group comparison, the

largest group found in diarrheic piglet harbored *Firmicutes* and *Proteobacteria*, while in non-diarrheic group, *Firmicutes* and *Bacteroidetes* seemed to be two predominant phyla. More than 90% of bacteria in pig intestine were *Firmicutes* and *Bacteroidetes,* and up to 40% *Proteobacteria* were found in the Ileum. At genus level*, Provetella, Blautia, Oscillibacter,* and *Clostridium* were identified from fecal sample in Duroc, Landrace, and Yorkshire pig. Firmicutes is a phyla consisting of very large classes; *Bacilli, Clostridia,* and *Erisypelotrichi*. Proteobacteria is a phyla consisting of five classes; *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria,* and *Epsilonproteobacteria. Escherichia* and *Salmonella* are members in *gammaproteobacteria* class. *Bacteroidetes* classes are; *Bacteroidia, Flavobacteria,* and *Sphingobacteria. Prevotella* is included in this phyla under *Bacteroidia* class (Garrity, 2005; Kersters et al., 2006; Ludwig et al., 2009; Thomas et al., 2011; Isaacson and Kim, 2012; Guevarra et al., 2019).

5. Post weaning diarrhea (PWD) in nursery pigs

Diarrhea in weaned piglet is a serious health problem with approximately 20–30% mortality occurring during 1–2 months postweaning (Rhouma et al., 2017). During this period, piglet exhibits clinical signs as decreased feed intake, diarrhea, dehydration, weakness, and weight loss, and if not being treated well, may result in death. Feed change, poor management and physiological status are contributed to piglet diarrhea (Rhouma et al., 2017). Feed change and stress are recognizable factors

to cause a higher number of pathogenic bacteria and proliferation inside GIT due to pH increase. *Escherichia coli* is mostly found in GIT tract and its proliferation has been proved by many researchers as main culprit of diarrhea in weaned piglet. In human and mammal intestines, seven major diarrheagenic *E. coli* pathotypes are; enteropathogenic *E. coli (EPEC)*, enterotoxigenic *E. coli (ETEC),* shiga toxin producing *E. coli (STEC)* such as enterohemorrhagic *E. coli (EHEC),* enteroinvasive *E. coli (EIEC),* enteroaggregative *E. coli (EAEC),* diffusely adherent *E. coli (DAEC),* and adherent invasive *E. coli (AIEC)* (Tran et al., 2018). *ETEC* could produce adhesins which can induce adherence of bacteria and production of enterotoxin on the intestinal epithelium. Almost 87.8% from 563 weaned pigs were *ETEC* with alpha hemolytic causing PWD. Fimbriae is a virulent factor which can adhere the toxins of *ETEC* to luminal epithelia. F18 and F4 fimbriae are usually detected in piglet PWD, but F4 usually detected in suckling and weaned pigs, while F18 can be detected only in weaned pigs (Klemm, 1985; Fairbrother et al., 2005). *E. coli* fimbriae or other surface antigens interact to specific receptor at extracellular matrix of host target cell and induce intestinal lesions due to damaging of enterocyte microvilli and changing of cytoskeletal structure and absorbing surface decrease. These matters finally cause diarrhea and growth performance retardation (Tran et al., 2018).

B. SOW

1. Farrowing, Lactation, and Backfat

Farrowing is a condition where pregnant sow giving birth during days 114 – 116 of gestation. Poor farrowing condition and gestation sow management have a deleterious consequence in lactation period. Reduction of sow weight is commonly observable due to decreased voluntary feed intake. From this circumstance, attempt to increasing feed intake during gestation might help sow for increasing body reservation in lactation period, if the nutrient needed is adequate and reproductive disorder may be avoidable. However, insufficient body fat reservation during gestation prone to negative impact on sow appetite during lactation, which low appetite causing nutrient intake deficiency and negative effect on subsequent reproductive function in the next production cycle (Revell et al., 1998; Prunier et al., 2001). Because of this matter, sow will lost body weight during 2 – 3 weeks of lactation and will recover afterwards (Eissen et al., 2000). Eissen et al. (2003) reported that primiparous sows with high lactation feed intake have smaller impact on backfat and bodyweight loss, and reduced possibility of prolonged weaning to estrus interval.

Backfat (BF) is considered to be one of important parameters to decide a good breeding ability of sow (Roongsitthichai and Tummaruk, 2014). Indeed, under the field trial, to examine BF precisely is not easy and convenient as a traditional method: body conditioning score (BCS). However, BCS assessment can be varied depending on personnel experience and judgement. Fitzgerald et al. (2009)

previously related BF and BCS scoring and figured out a guideline as shown in Table 1.

Table 1 Backfat Guidelines used to categorize sows into a 5- and 9- point BCS and the subsequent distribution by BCS.

BCS	Backfat, mm			
	BCS 5		BCS 9	
	Minimum	Maximum	Minimum	Maximum
1	0	10	0	7.5
1.5	-	-	7.5	10
2	10	15	10	13
2.5	-	-	13	15
3	15	23	15	19
3.5	-	-	19	23
4	23	30	23	27
4.5	-	-	27	30
5	30	-	30	-

Modified from Fitzgerald et al. (2009)

C. ORGANIC ACIDS AND ESSENTIAL OIL AS SUPPLEMENTATION IN SWINE FEED

1. Organic acids

Organic acids (OA) are weak acids, which could be partly dissociated, and have antimicrobial activity at pKa 3.0 - 5.0 (Khan et al., 2015). Previously, OA were used to the reduction in number of *Salmonella sp., Clostridium perfringens,* and *Eschericia coli* growth in GIT, but mainly in stomach and small intestine (Lückstädt and Mellor, 2011). Undissociated form of OA penetrates semi-permeable membrane of the bacterial cell wall and dissociates in cytoplasm which have neutral pH, then proton (H+) is released and cause pH reduction inside bacteria cell. pH reduction impede enzymatic reaction of glycolysis and nutrient transport, leading to failure of pH normalization effort in cell, because of energy deprivation (Mroz et al., 2006; Pearlin et al., 2020) as demonstrated in figure 2.

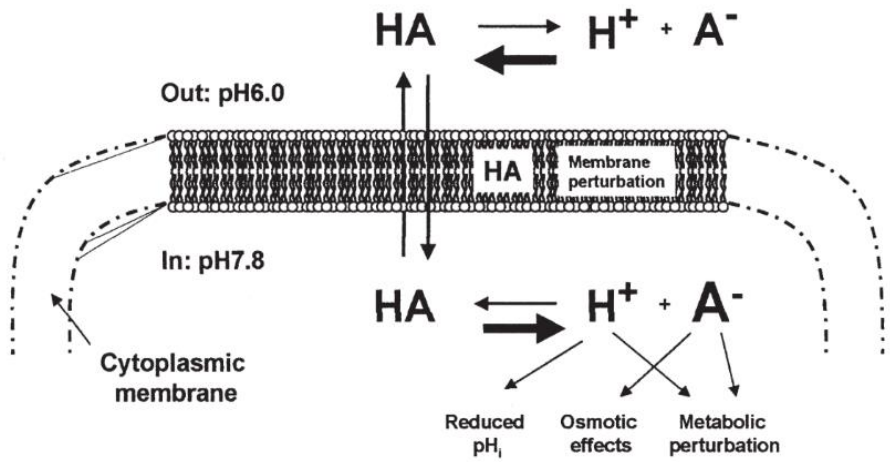

Figure 2 Mechanism of weak organic acid as antibacterial agent.
(Hirshfield et al., 2003).

Basically, OA is classified into three groups according to chemical structure difference: short chain fatty acids (SCFA) which improve intestinal morphology and decrease intestinal inflammation, (2) medium chain fatty acid (MCFA) which have higher antimicrobial potency due to higher pKa than SCFA in hindgut, and (3) tricarboxylic acids (TCA) which involved in energy metabolism and could improve gut morphology also barrier functions, and some other acid used as antifungal or antimould (Mroz et al., 2006; Rossi et al., 2010; Zentek et al., 2011; Tugnoli et al., 2020)

Table 2 Properties of tricarboxylic acid class and its antimicrobial activity.

Category	Acid	Molecular Formula	pKa	Antimicrobial activity
Tricarboxylic Acid (TCA)	Citric	$C_6H_8O_7$	3.13	*E. coli*
	Fumaric	$C_4H_4O_4$	3.02	*E. coli*
				Clostridia sp.
	Malic	$C_4H_6O_5$	3.4	*E. coli*
				Yeast
Other	Sorbic	$C_6H_8O_2$	4.76	*E. coli*
				Salmonella sp.
				Yeast, mould, fungi

Modified from Papatsiros et al. (2012); Tugnoli et al. (2020)

2. Essential oils

Essential oil (EO) is plant extracted product which can be used as appetite stimulant, aromatic compound, antimicrobial, anti-inflammatory, antioxidants, also, gastric, and pancreatic juice production enhancer (Xu et al., 2018). In swine production, EO commonly used as antibacterial agent mixed in feed as additive, though, most EO are more effective to inhibit the growth of gram-positive bacteria than gram negative bacteria inside the gut. Some EO such as thymol (terpenes) and eugenol (phenylpropenes) have damaging ability on gram negative bacteria due to due disruption of membranes and cause a loss of cellular integrity. Therefore, this trait could affect cell membrane and energy metabolism disruption which may lead to cell death or delay bacterial growth (Cetin-Karaca, 2011; Omonijo et al., 2018), as shown in Figure 3.

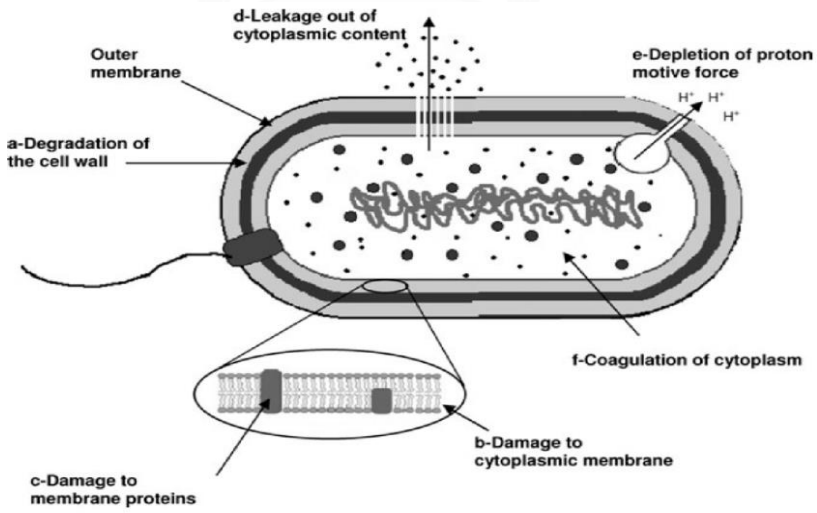

Figure 3 Mechanism of gram-negative bacteria disrupted by essential oil. (Cetin-Karaca, 2011).

D. MICROENCAPSULATION OF ORGANIC ACID AND ESSENTIAL OIL AS FEED ADDITIVE IN SWINE FEED

The synergism of microencapsulating organic acids and essential oil pose their antimicrobial ability in gut by damaging the membrane cell and disrupting metabolism of acid-sensitive bacteria, which lead to reduce number of acid-sensitive bacteria (Xu et al., 2018). Microencapsulation is a technique to coat antimicrobial agent (organic acid, essential oil, or others) with protective layer such as lipid to delay absorption of specific compound along gastrointestinal tract and can be dissolved due to some specific circumstances such as pH or temperature then released the contained compound (Piva et al., 2007; Vasisht, 2014; Callegari et al., 2016).

E. PREVIOUS STUDY OF MICROENCAPSULATED ORGANIC ACIDS AND ESSENTIAL OIL

Research on the use of essential oil or organic acid effect in sows and nursery pigs has been reported, but there are only a few works have been focused on effect of organic acid and essential oils microencapsulation *in vivo* in sow and nursery pigs. For example, Balasubramanian et al. (2016) previously supplemented microencapsulating citric acid, sorbic acid, vanillin and thymol in sows and suckling pigs, showing the significantly beneficial effect on body weight change of sows before and after farrowing, and weaning, with respect to slight body weight loss in lactation period with the increase of MOE supplementation. The average daily gain (ADG) of

suckling piglets was significantly increased. Also, the number of diarrheal piglets was decreased with better fecal score than those of non-supplemented pigs. Recently, Choi et al. (2020) reported that MOE supplementation in weaned piglet following by *ETEC* challenged could lower diarrhea score. This was in accordance with a previous study by Cho and colleagues showing that supplementing feed with MOE (citric acid, sorbic acid, vanillin, and thymol combination) in finishing pigs have significantly greater body weight gain and ADG than control group (Cho et al., 2014).

Recent studies above provided similar beneficial trends on MOE supplementation in pig feed with significant effect on pig performance. Therefore, our main idea in this study was to determine whether the addition of 0.2% microencapsulated eugenol, thymol, and vanillin as a mixture of essential oils, and citric, fumaric, malic, and sorbic acid as a mixture of organic acids in the feed of sows and weaning pigs could have a significant effect in growth performance, farrowing performance, and fecal bacterial population.

CHAPTER III. MATERIALS AND METHODS

Animal ethics and protocol

Prior to beginning of the field investigation, animal use (experiments 1 and 2) and protocols have been reviewed and approved by Institutional animal care and use committee (IACUC no. 2031084) and institutional biosafety committee (IBC no. 2031052), Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Experiment 1: Effect of MOE on sow performance.

A. Experimental animals and management

Healthy three hundred and twenty farrowing sows (Large white x Landrace) located in a commercial farrowing-to-finishing farm with 6500 sows on production, in Nakhon Nayok Province, Thailand was used as experimental animals. Four batches of each 80 sows were randomly divided into two groups; group one was the control group where the sows received corn – soybean meal basal diet without any feed additives, and group two was the treatment group where the sows received similar basal diet as control group + 2 kilograms (kg) of MOE/ton of feed. Lactation feed was given to pregnant sows 7 days before farrowing, until 28 days after farrowing. The sows were kept in an individual pen in a closed/clean house with evaporative cooling system, and separated water nipple and manual feeder. Farrowing pen was made of metal bar and T-bar slate flooring. All animals were in the same environmental and management conditions to avoid bias during study.

B. Experimental design

a. Feed and feedings

Own-producing feed under standard procedure of GMP feed mill was used as a basal diet for sow. The sows were divided into two feed groups: the control group (feed without MOE) and the treatment group (feed + 2 kg of MOE in one ton of feed). Water was freely accessed. The MOE (Porcinate+), a commercially registered product of JEFO Nutrition Inc. (Canada), containing citric acid, fumaric acid, malic acid, and sorbic acid as organic acid mixture and eugenol, thymol, and vanillin as essential oil mixture, was used.

C. Sample collection and analysis

Sow feed samples were collected once for proximate analysis at the beginning of experiment to determine the nutrient composition. The feed was analyzed by using proximate analysis procedure with respect to ash, moisture, crude protein, crude fiber, phosphorus, calcium, and fat (AOAC., 2016). Dry matter was measured using oven to reduce the moisture in feed and using a furnace to ash. Crude protein (CP) was tested using Kjeldahl method, whereas crude fiber was analyzed by raw fiber extractor, and phosphorus was analyzed by spectrophotometry.

Sow performance was determined by Backfat (BF), Body Condition Score (BCS), litter born alive, weaned litter size and weaned litter weight. BCS was determined by observing the sow body size with scores 1 to 5, where score 1 was

emaciated and 5 was overly fat. BF was determined by using an ultrasound probe at P2 position (6–8 cm away from body midline at the last rib curve) (Roongsitthichai and Tummaruk, 2014). BCS and BF were recorded at the beginning (seven days before farrowing) and last day of the experiment (at weaning). Shoulder ulcer was also measured at the same time as body condition evaluation. Scoring was divided into score 0-4, where score 0 = no lesion or scarring of skin over the tuber of the scapula, score 1 = no current lesion but previous scarring of skin over the tuber of the scapula, score 2 = skin is reddened over the tuber of the scapula, score 3 = broken skin over the tuber of the scapula <2.3 cm in diameter, and score 4 = broken skin over the tuber of the scapula >2.3 cm in diameter. Litter born alive was recorded at farrowing day. The numbers and weight of weaned piglets were recorded at day 28 of lactation (weaning). The experimental design is illustrated in Figure 4.

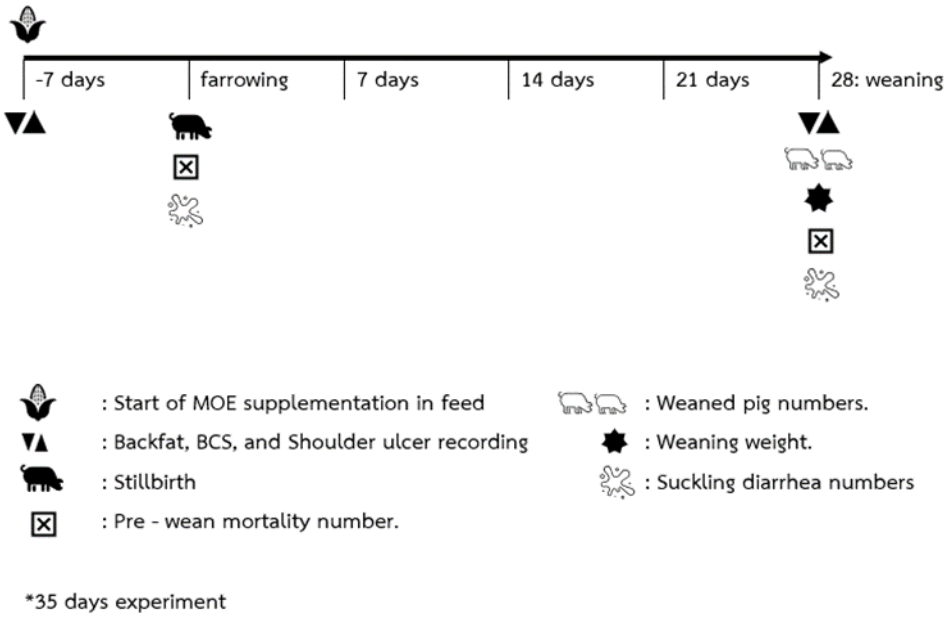

Figure 4 A summary of sow experiment.

D. Statistical Analysis

Performances in sow were analyzed using student *t-test* and paired *t-test*. BCS, BF correlation was using Pearson's method in IBM SPSS statistic 22 (Allen et al., 2014). The statistically significant level was set at $p < 0.05$.

Experiment 2: Effect of MOE on nursery piglet.

A. Experimental animals and Management

Healthy two thousand and eight hundred, Large white x Duroc x Landrace, weaned piglets (28 days old) were used. All animals were randomly divided into two groups as control group (1400 piglets fed with basal feed without MOE) and treatment group (1400 piglets fed with feed phase I (basal feed containing 2 kg MOE/ton) between 28-42 days old, and feed phase II (basal feed with 1 kg MOE/ton)

between 42-56 days old. Weaning piglets were randomly allotted in the pen with solid cement base with water bowl and feeder. Feed and water were provided ad libitum. All experimental animals were allocated in a completely randomized design.

B. Experimental Design

a. Feed and Feedings

Own-producing feed under standard procedure of GMP feed mill was used as a basal diet. The weaning piglet was divided into two groups as control (feed without MOE) between 28-56 days old, treatment feed I (feed + MOE 2 kg/ton of feed) between 28-42 days old, and treatment feed II (feed + MOE 1 kg/ton of feed) between 43-56 days old as mentioned in Figure 5. Water and feed were given ad libitum. The MOE (Porcinate+) given to the treatment group was described elsewhere in the sow experiment.

C. Feed and fecal collection

a. Feed collection

Feed samples was collected for proximate analysis (AOAC., 2016) at the beginning of experiment to determine the nutrient composition of experimental diets (control feed, nursery I feed, and nursery II feed). The weight of given and refused feed was recorded to determine the feed conversion ratio (FCR) and average of daily feed intake (ADFI).

b. Feed Analysis

Feed samples were collected once for proximate analysis at the beginning of experiment to determine the nutrient composition of experimental diets. The feed was analyzed by using proximate analysis procedure in ash, moisture, crude protein, crude fiber, phosphorus, calcium, and fat (AOAC., 2016). Dry matter was measured using oven to reduce the moisture in feed and using a furnace to ash. Crude protein (CP) was tested using Kjeldahl method. Crude fiber was analyzed by raw fiber extractor. Phosphorus was analyzed by spectrophotometry.

c. Fecal collection

A hundred and eighty fecal samples were collected 3 times at 28 days old (beginning, 42 days old (middle), and 56 days old (the end of experiment), for 10 samples each time. Approximately, one to two grams of feces were taken from the rectum and then transferred into plastic bags to determine number of total bacteria, coliform and E. coli, and Lactobacillus spp.

D. Fecal bacteria score assessment

a. Total Plate Count (TPC)

One gram of sample was weighed, mixed, and centrifuged until homogenized with PBS and 10-fold serial diluted to obtain 10^{-4} to 10^{-8} dilution. Then, 1 ml of sample suspension was transferred into plate count agar (PCA) (Difco™, France) and cultured with pour-plate method. The cultured PCA plate was then incubated at

37°C in an incubator for 24 hours. Feces sample dilution procedure and bacteria enumeration procedure was performed following FAO manual of food quality control (Andrews., 1992).

b. Coliform culture method

One gram of sample was weighed, mixed, and centrifuged until homogenized with PBS and 10-fold serial diluted to obtain 10^{-2} to 10^{-6} dilution. Then, 1 ml of sample suspension was transferred into Violet Red Bile Lactose (VRBL) (Merck™, Germany) and cultured with the pour-plate method. The VRBL plate was cultured at 37°C in an incubator for 24 hours. Further biochemical assessment could be tested on Indole, Methyl red, Voges Proskauer, and Citrate media (IMVPC) with interpretation (++--) or (-+--), considered as *E. coli* (Feng et al., 2018).

c. Escherichia coli culture method

One gram of fecal sample was weighed, mixed, centrifuged and suspended with PBS and 10-fold serial diluted to obtain 10^{-1} to 10^{-5} dilution. Then, 1 ml of sample suspension was transferred into MacConkey agar (OXOID™) and cultured with spread-plate method. The MCA plate was cultured at 37°C in an incubator for 24 hours.

d. Lactic acid bacteria culture method

One gram of sample was weighed, mixed, and centrifuged until homogenized with PBS and 10-fold serial diluted to obtain 10^{-3} to 10^{-7} dilution. Then, 1 ml of sample suspension were transferred into deMann Rogosa (MRS) agar (OXOID™) and cultured with the pour-plate method. The MRS plate was cultured at 37°C in an incubator for 48 hours.

For all methods, the overnight culture plates with colony number ranged 25–250 colonies were counted using CFU/gram method, as described in ISO (1998) :

$$\text{CFU/gram} = \frac{\text{Sum of colonies counted on the successful plates contains 25 – 250 colonies.}}{\text{Volume of inoculum applied in a dish x dilution factor of sample.}}$$

E. Growth performance analysis

The parameters used as nursery pig performance in this study included average daily gain (ADG) and feed conversion ratio (FCR). Weight gain was calculated based on weight at 28 and 56 days old. FCR and ADG was calculated using this following formula:

FCR = ADFI/ ADG

ADG (grams/day) = Weight gained/ total day of experiment

$$\text{ADFI (grams/day)} = \frac{\text{Feed offered – feed refused}}{\text{Total day of experiment}}$$

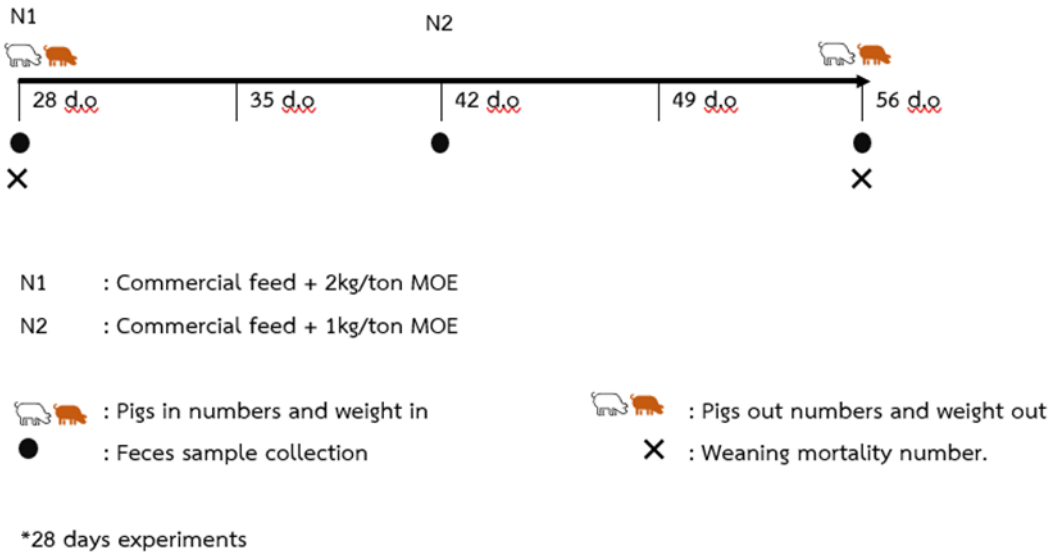

Figure 5 Nursery pig experiment summary.

F. Statistical Analysis

Growth performance in nursery pigs were analyzed using student *t* – test and the amount of fecal bacterial population (CFU/ gram) was compared using student *t* – test. Significance level set to $P < 0.05$.

CHAPTER IV. RESULTS

Under field conditions in a commercial farrowing-to-finishing farm, the impact of MOE supplementation in feed of sows and weaning pigs was carefully evaluated with respect to sow performances, pig growth performances and fecal bacterial population.

Experiment 1: Sow experiment.

A. Feed analysis

The feed chemical compositions in this study were analyzed by proximate analysis. The results are shown in Table 3.

Table 3 Chemical composition analysis of control and treatment feed in sow experiment.

Nutrients	Group		Standard**	Source
	Control	Treatment*		
Gross energy (Kcal/kg)	4072	4070	no data	Automatic Bomb Calorimeter; Leco model AC – 500
Moisture (%)	7.83	7.90	no data	
Crude fat (%)	7.20	7.80	no data	
Ash (%)	6.83	6.60	no data	
Phosphorus (%)	0.57	0.60	0.54 - 0.65	(NRC, 2012)
Calcium (%)	2.01	1.93	0.63 - 0.76	(NRC, 2012)
Crude protein (%)	15.27	14.96	16.30 - 19.20	(NRC, 1998)
Crude fiber (%)	5.67	5.31	no data	

***Based on NRC nutrient requirements of lactating sow (90% dry matter)*

**The treatment group feed is basal feed + 2 kg/ton microencapsulated organic acids and essential oil*

B. Effect of dietary microencapsulated organic acid and essential oil on sow performance

As shown in Table 4, supplementation of MOE in sow feed unveiled that average backfat thickness and BCS were statistically decreased from late gestation phase (7 days before farrowing) to weaning (28 days after farrowing), both in control (17.71 ± 2.43 and 15.01 ± 1.68 mm, respectively) and treatment groups (18.46 ± 2.38 and 16.23 ± 2.46 mm, respectively). Further analysis revealed that average backfat loss of treatment group (2.14 ± 0.87 mm) was significantly lowered than that of control group (2.70 ± 1.10 mm). BCS before farrowing and at wean of control group (3.09 ± 0.42 and 2.84 ± 0.35, respectively) and those of treatment group (3.15 ± 0.43 and 2.93 ± 0.47, respectively) showed a similarly declined trend. BCS loss in treatment group was lower than control group (0.26 ± 0.25 and 0.23 ± 0.33, respectively). Furthermore, the correlation between BF to BCS has positively associated (p=0.01, r=0.362). Weaning to service interval showed no significant difference in both groups. The average of shoulder ulcer score in treatment group was significantly lower compared to control group (1.35 ± 1.30 and 0.91 ± 1.16, respectively). Moreover, the shoulder ulcer number percentage of treatment group was apparently significantly lower compared to control group (11.39 ± 2.43 and 15.34 ± 2.87, respectively).

Analysis of stillbirth number between two groups shows significantly higher in treatment group than in control group (19.11 ± 8.35 and 14.86 ± 3.11, respectively). Interestingly, average weaned pig number of treatment group (504.81 ± 77.14) was statistically larger than that of control group (478.30 ± 36.94). Similar extent was found with average litter weight of which the treatment group (6.11 ± 0.34 kg) was bigger than that of control group (5.90 ± 0.32 kg) ($p<0.05$). Average of suckling pig diarrhea number was significantly lower in treatment group than that in control group (57.52 ± 17.07 and 75.47 ± 23.29, respectively) and the percentage of suckling pig diarrhea number was significantly lower in treatment group (1.26 ± 0.37) compared to control group (1.65 ± 0.51) ($p<0.05$).

Table 4 Effect of MOE on sow performances.

Sow performances	Group			
	Control (n = 160)		Treatment (n = 160)	
	Before[1]	After[2]	Before[1]	After[2]
Sow body performance				
BF (mm)	17.71 ± 2.43^{x}	15.01 ± 1.68$^{y, a}$	18.46 ± 2.38^{x}	16.23 ± 2.46$^{y, b}$
BCS	3.09 ± 0.42^{b}	2.84 ± 0.35$^{a, x}$	3.15 ± 0.43^{b}	2.93 ± 0.47$^{a, y}$
BF loss (mm)	-2.70 ± 1.10^{b}		-2.14 ± 0.87^{a}	
BCS loss	-0.26 ± 0.25^{b}		-0.23 ± 0.33^{a}	
Shoulder ulcer (%)	15.34 ± 2.87^{b}		11.39 ± 2.43^{a}	
Shoulder ulcer score	1.35 ± 1.30^{b}		0.91 ± 1.16^{a}	
Feed Intake (kg.)	5.40 ± 0.08^{a}		5.51 ± 0.12^{b}	
Sow reproductive performance				
Weaning to service interval (days)	3.91 ± 1.02		4.01 ± 0.87	
Piglet performance				
Stillbirth number	14.86 ± 3.11^{a}		19.11 ± 8.35^{b}	
Stillbirth (%)	0.31 ± 0.06^{a}		0.40 ± 0.17^{b}	
Suckling diarrhea number	75.47 ± 23.29^{b}		57.52 ± 17.07^{a}	
Suckling pig diarrhea (%)	1.65 ± 0.51^{b}		1.26 ± 0.37^{a}	
Pre-wean mortality (%)	4.06 ± 0.72		4.00 ± 1.09	
Weaned pig number	478.30 ± 36.94^{a}		504.81 ± 77.14^{b}	
Weaned weight/ litter (kg)	5.90 ± 0.32^{a}		6.11 ± 0.34^{b}	

Means in each row with different superscripts were statistically significantly difference *(p<0.05)*.

^{x,y} the comparison within group before and after, ^{a,b} the comparison between control group to treatment group, [1] 7 days before farrowing, [2] 28 days after farrowing.

Experiment 2: Nursery pig experiment.

A. Feed analysis

Basal feed supplemented with and without MOE 2 kg/ton **(N1)**, or MOE 1 kg/ton **(N2)** was analyzed. This contained approximately crude protein 18%, with gross energy 4099-4130 Kcal/kg, crude fat 7.05-7.41%, crude fiber 2.90-3.35%, moisture 8.24-8.71%, ash 5.77-6.21%, phosphorus 0.62%, and calcium 1.4-2.0%. The details are shown in Table 5.

Table 5 Chemical composition analysis of control and treatment feed in nursery pig experiment.

Nutrients	Group			Standard**	Source
	Control	Treatment*			
		N1	N2		
Gross energy (Kcal/kg)	4099	4124	4130	no data	Automatic Bomb Calorimeter, Leco model AC – 500
Moisture (%)	8.65	8.71	8.24	no data	
Crude fat (%)	7.05	7.27	7.41	no data	
Ash (%)	6.14	5.77	6.21	no data	
Phosphorus (%)	0.62	0.62	0.62	0.70 – 0.60	(NRC, 2012)
Calcium (%)	1.40	1.63	2.00	0.85 – 0.70	(NRC, 2012)
Crude protein (%)	18.38	18.59	17.8	23.7 – 18.0	(NRC, 1998)
Crude fiber (%)	2.90	3.35	3.18	no data	

**Based on NRC (2012) of nursery pigs with body weight ranged 5 – 25 kg when feed allowed ad libitum (90% dry matter)*

***The results of gross energy were determined by Automatic Bomb Calorimeter; Leco model AC – 500*

***N1** treatment = MOE 2 kg/ton of basal feed, control = basal feed, 28 – 42 days old piglets*

***N2** treatment = MOE 1 kg/ton of basal feed, control = basal feed, 43 – 56 days old piglets*

B. Effect of dietary microencapsulated organic acids and essential oil on nursery pig growth performances

Average daily feed intake (ADFI) was separately analyzed during 28 – 42 and 43 - 56 days old. The result showed that ADFI of control group in N1 period appeared to be higher than treatment group (603.21 ± 21.30 and 586.51 ± 34.98 g/d, respectively). Interestingly, in N2 period that of treatment group was higher than control group (617.04 ± 37.63 and 563.67 ± 53.89 g/d, respectively. This showed a better performance of treatment pigs.

Average daily gain (ADG) was also statistically significant higher in treatment group (427.96 ± 47.84 and 424.55 ± 2.66 g/d, respectively) than those of control group (401.03 ± 23.03, 397.49 ± 23.66 g/d, respectively).

Feed conversion ratio (FCR) of treatment group in N1 phase (1.49 ± 0.04) was significantly lesser than those of control group (1.50 ± 0.03). Furthermore, statistically significant lower FCR observed in control group (1.41 ± 0.05) than in treatment group (1.45 ± 0.08) in N2 phase ($p>0.05$). The details are shown in Table 6.

Table 6. Effect of MOE on nursery pig performances.

Parameters	N1 (28 – 42 days old) *		N2 (42 – 56 days old) **	
	control	treatment	control	treatment
Pig-in numbers	363.50 ± 0.50[a]	363.00 ± 1.00[b]	406.02 ± 3.00[a]	400.00 ± 0.00[b]
Pig-out numbers	360.50 ± 0.50[b]	354.03 ± 3.00[a]	403.06 ± 5.00[b]	388.50 ± 0.50[a]
Weight in (kg)	5.66 ± 0.07[a]	5.85 ± 0.24[b]	5.54 ± 0.15[b]	5.30 ± 0.00[a]
Weight out (kg)	22.74 ± 1.65[a]	23.02 ± 2.09 [b]	21.86 ± 2.60[a]	24.07 ± 1.65[b]
Mortality %	0.83 ± 0.00	0.82 ± 0.05	1.04 ± 0.26	0.83 ± 0.25
ADG (g)	401.03 ± 23.03[a]	427.96 ± 47.84[b]	397.49 ± 23.66[a]	424.55 ± 2.66[b]
ADFI (g/day)	603.21 ± 21.30[b]	586.51 ± 34.98[a]	563.67 ± 53.89[a]	617.04 ± 37.63[b]
FCR	1.50 ± 0.03[b]	1.49 ± 0.04[a]	1.41 ± 0.05[a]	1.45 ± 0.08[b]

Means in each row with different superscripts were statistically significantly difference *(p<0.05)*.

**** N1** treatment = MOE 2 kg/ton of basal feed, control = basal feed, 28 – 42 days old piglets*

***** N2** treatment = MOE 1 kg/ton of basal feed, control = basal feed, 43 – 56 days old piglets*

C. Effect of dietary microencapsulated organic acid and essential oil on fecal bacteria population

The effect of MOE supplementation in nursery pig feed on selected fecal bacterial population was investigated as shown in Table 7. At beginning of experiment (28 days of age), only average total bacteria number of treatment (8.03 ± 0.65 CFU/g) was significantly lower than that of control group (8.37 ± 0.54 CFU/g) with statistical difference of $p<0.05$. Average number of other bacterial populations, including coliform bacteria, *E. coli*, and *Lactobacillus spp*., was not statistically significant difference between treatment and control groups. However, these appeared to be lower number in treatment than control groups. Further analysis of the ratio of L/T and L/C also did not pose any statistical difference.

At middle of experiment (42 days of age), all bacterial population parameters of both groups seemed not to be statistically different. Interestingly, these appeared to be higher number in treatment than control groups. At the end of experiment (56 days of age), only total bacteria number of treatment (7.27 ± 0.57 CFU/g) was significantly higher than that of control group (6.43 ± 1.42 CFU/g). Similar extent was observable for the average numbers of coliform bacteria, *E. coli*, and *Lactobacillus spp*. of treatment group (5.68 ± 1.46, 4.95 ± 1.79, and 6.33 ± 0.75, respectively) were significantly greater than control group (3.79 ± 2.48, 3.22 ± 2.41, and 5.44 ± 1.24, respectively) ($p<0.05$). Additional investigation on the ratio of L/T and L/C did not show statistical difference between control and treatment groups.

Table 7 Effect of MOE supplementation in feed on fecal bacterial population in nursery pig experiment.

Fecal bacterial population (log10 CFU/g)	**Groups**					
	28 days		42 days		56 days	
	Control*	treatment	control	treatment	control	treatment
Total bacteria	8.37 ± 0.54[b]	8.03 ± 0.65[a]	7.04 ± 0.80	7.10 ± 0.77	6.43 ± 1.42 [a]	7.27 ± 0.57 [b]
Coliform bacteria	4.88 ± 2.09	4.65 ± 1.79	4.33 ± 1.42	4.46 ± 1.33	3.79 ± 2.48 [a]	5.68 ± 1.46 [b]
E. coli	3.97 ± 2.66	3.47 ± 2.50	2.72 ± 2.14	3.42 ± 1.72	3.22 ± 2.41 [a]	4.95 ± 1.79 [b]
Lactobacillus spp.	7.81 ± 0.74	7.56 ± 0.59	5.90 ± 1.29	6.25 ± 0.89	5.44 ± 1.24 [a]	6.33 ± 0.75 [b]
Bacterial population ratio						
L/T	0.93 ± 0.06	0.94 ± 0.07	0.86 ± 0.07	0.88 ± 0.08	0.86 ± 0.21	0.87 ± 0.07
L/C	1.77 ± 0.89	2.06 ± 1.34	1.37 ± 0.47	1.39 ± 0.54	1.08 ± 0.90	1.21 ± 0.44

* Control (commercial feed without MOE) from 28 - 56 days old, treatment feed I (commercial feed + MOE 2 kg/ton of feed) at 28 days old to 42 days old, and treatment feed II (commercial feed + MOE 1 kg/ton of feed) at 43 days old to 56 days old.

L/C = *Lactobacillus spp.*/Coliform ratio

L/T = *Lactobacillus spp.*/Total plate count ratio.

[a,b,] Means in each row with different superscripts were statistically significantly difference *(p<0.05)*

CHAPTER V. DISCUSSION AND CONCLUSION

In this study, effect of microencapsulated citric, fumaric, malic, and sorbic acid as organic acids combined with eugenol, thymol, and vanillin as essential oil on performances of sows and nursery pigs were investigated under field conditions. The study outcome in sow experiment revealed a better body condition including BF thickness and BCS of MOE-supplemented than non-supplemented groups. Similarly, difference of BF and BCS loss at wean compared to before farrowing period, appeared to be also much more improved in MOE supplemented group than control. Supportive evidence to this finding is reflected by the examination of shoulder ulcer score of MOE supplemented sows, which appeared to be lower than non-supplemented ones. Due to the fact that reduction in backfat thickness and body score are common found in multiparous sow, lower feed intake in lactating period directly causes loss of backfat thickness and BCS due to mobilization of body fat and protein reserve (De Rensis et al., 2005; Schenkel et al., 2010; Kim et al., 2015). Furthermore, supplementing phenols such as thymol in sow feed could improve nutrient absorption in intestines due to efficacy of stimulating organic and microbial digestion, also, as antioxidant agent, improving sow backfat thickness and body condition score in weaning phase, suggesting more body reserve gain during lactation period (Allan and Bilkei, 2005). In an earlier report suggested that pregnant sow had elevated oxidative stress during gestation and lactation periods. This could lead to

the excessive production of reactive oxygen species (ROS) in blood, which causes insulin signaling cascade that leads to insulin resistance (Berchieri-Ronchi et al., 2011). The primary role of insulin is to control glucose homeostasis by stimulating glucose transport into muscle and adipose cells, while reducing hepatic glucose production via gluconeogenesis and glycogenolysis. Insulin regulates lipid metabolism by increasing lipid synthesis in liver and fat cells while inhibiting lipolysis. (Rains and Jain, 2011). Furthermore, insulin resistance could negatively (lesser) effect on lactating sow feed intake (Weldon et al., 1994). That means, when the ROS is increased in blood, lipolysis in sow will occur, causing reduction of body weight and back fat thickness during lactation. Tan et al. (2015) reported, adding 15 mg/kg oregano essential oil (carvacrol and thymol) into multiparous sow diet could increase feed intake in the third week of lactation period. This report supports the result in this study, suggesting that supplementing essential oil could improving feed intake, increasing backfat and body condition score, and reduce backfat loss due to balanced ROS. However, blood profile was not determined in this study to confirm effect of MOE addition in late gestation - lactation diet on sow ROS and blood plasma level.

In this study, MOE supplementation in sow feed had no significant effect on piglet born alive number in both groups. This is because the combined product has no related effect to number of pigs fertilized. To achieve a better born alive, it requires to have an appropriate timing and technique of insemination and good

management during the first month of gestation. This, however, remains further scientific evidence and explanation to link between MOE effect and piglet born alive number. Similar finding to our results was a former report that administration of organic acid blends did not show significant results in increasing the number of live piglets (Balasubramanian et al., 2016). In this study, stillbirth numbers in treatment groups were significantly higher, an evidence of Maes et al. (2004) unveiled that thin sow with lower backfat thickness condition at the end of gestation phase tended to have a higher percentage of stillbirth. Indeed, the weight of weaned pigs seemed to be affected by MOE, due to increasing of backfat thickness and body condition score. The more mobilizing body reservation of sow, the higher attempt to maximize the milk production and the bigger pig weight at wean (Mullan and Williams, 1989; Allan and Bilkei, 2005).

Increased number of weaning pigs from sow supplemented MOE in this study may be associated with intake of sow colostrum in which passive immunity and energy for growth and thermoregulations during first 24 hours are relatively more concentrated than milk (Zurbrigg, 2006; Quesnel and Farmer, 2019). Furthermore, adding essential oils such as thymol could increase T-lymphocytes number in blood and milk, which may indicate efficacy of thymol as an immunostimulant for the suckling pig, thus improving piglets survival until weaning phase (Ariza-Nieto et al., 2011). Devi et al. (2016) also suggested that supplementing 0.2% protected organic acid could improve white blood cell counts in suckling pig.

In this study, the weaning to service interval of sows was reported to be not different in both groups. This is in accordance with a previous report by Balasubramanian et al. (2016), Lavery et al. (2019), and Thaker and Bilkei (2005) If feed intake during lactation is maximized, the probability of sow live weight loss from late gestation to weaning will be reduced, and weaning to service interval will thereafter not be prolonged .

Effect of dietary MOE supplementation on nursery pig growth performance was further examined. It is obviously suggestion that weaning pigs had better performances such as ADG, ADFI, and FCR than control group. Earlier, Yang et al. (2018) reported that mixture of essential oils and organic acids consists of cinnamaldehyde (15%), thymol (5%), citric acid (10%), sorbic acid (10%), malic acid (6.5%) and fumaric acid (13.5%) can increase ADG of 21 - 49 days old, crossbred piglets (Duroc × Landrace × Yorkshire). In addition, Upadhaya et al. (2016) also documented that giving 0.2% protected organic acids (MCFA and composite organic acids) were able to improve performance and ADG in growing pigs. Results related to the effect of giving essential oils and organic acids to improve the performance of weaning pigs were also displayed by Diao et al. (2014), Li et al. (2012) and Zeng et al. (2014). Nonetheless, Lee et al. (2007) and Manzanilla et al. (2004) published similar reports that the administration of a single acidifier or blended acidifier did not have a positive effect on the growth performance of pigs. This inconsistence may be due to the beneficial effect of microencapsulation of the blend of our study product in term

of prolonging the undissociated form of acids to be attentionally dissociated in the pig hind gut.

Related parameters such like FCR of MOE-supplemented group was also improved in the N1 period (28 – 42 days old) Yan et al. (2010) revealed that supplementation of essential oils (thyme, rosemary, and oregano extracts) had a better FCR. Another report by Hanczakowska et al. (2013) demonstrated that application of a mixture of formic, propionic and caprylic acid in the feed of pigs aged 35 - 56 days can significantly increase feed efficiency. This result can be again due to microencapsulation effect of acid and oil in this study that help to dissolves and be effective in the lower part of gut. FCR of pig during N2 diet (43 - 56 days old) did not differ between both groups. Luise et al. (2020) previously suggested a related factor of this may be associated with the dose and duration of the acidifier supplementation.

The effect on fecal bacterial population following dietary supplementation with MOE was investigated. Significant difference of total bacteria at the beginning of experiment (28 days old) and the end of experiment (56 days old), but not on day 42. The high number of bacterial counts at the beginning of the weaning phase is due to many factors in weaning pigs, such as stress due to separation from sows, resulting in loss of immunity transfer from sows via milk, environmental stress, the feeding pattern shift, and types of feed change. Such periods of stress can result in an imbalance of the gastrointestinal (GI) microbiota, which allows opportunistic

pathogens to proliferate and cause GI disturbances (Perez-Gutierrez, 2010). A similar opinion was shared by Partanen and Mroz (1999) that in the early post-weaning phase, pigs often have an overgrowth of pathogenic bacteria such coliform due to the high gastric pH resulting from an increase in the amount of undigested feed entering the GIT. In this study, markedly increased number of coliform bacteria, *E. coli*, and *Lactobacillus spp*. in feces of MOE-supplemented pigs, compared to non-supplemented pig, were also observable on day 56. In contrast, no significant difference of these fecal bacteria counts between both groups on days 28 and 42 was demonstrated. This impact at the end of experiment may require adequate length of MOE supplementation to amend the bacterial population in pig feces. Evidence of Suiryanrayna and Ramana (2015), who reviewed that adding 1.5% of citric or 1.5% of fumaric acid in feed does not significantly affect pH, or microflora in the contents of stomach, jejunum, caecum, or lower colon of weaning pigs, and, does not affect positively on *lactobacillus spp.* proliferation. However, active form of organic acids supplemented will be dissociated at different locations of pig gut and depended on pKa at active site. Activity of microencapsulation form of acids is desired to occur in the lower part of enteric tract.

The increased number of *Lactobacillus spp.* in this study is similar to results from Lan and Kim (2018), which examined supplementation of 0.2% of OA blend (fumaric, citric, malic, capric, caprylic acid, and kaolin) in suckling piglet and showed an increased amount of *Lactobacillus spp.* and reduced amount of *E. coli.* Zeng et

al. (2014) similarly reported that adding a combination of essential oil (0.025% cinnamaldehyde and thymol) slightly increased the number of *Lactobacillus sp.* and reduced *E. coli* number in feces of weaning pig. The increasing number of *Lactobacillus spp.* in feces can be affected by the increase the acidity level in the gastrointestinal tract of pig supplemented with addition of MOE in feed. When the pH of the gastrointestinal tract decreases towards an acidic direction, this will cause damage to the cell walls of coliform bacteria which are sensitive to acid, which in turn inhibits the process of proliferation of bacteria that are sensitive to acid (Wang et al., 2018; Guan and Liu, 2020). Knarreborg et al. (2002) explained in a previous reported that *in vitro* simulation of the growth of lactic acid bacteria vs. coliform to mimic major environments of stomach and proximal part of piglet small intestines displayed that a population of coliform cannot grow in stomach contents. The *Lactobacillus* population and L:C ratio of MOE-supplemented pigs in this study revealed a trend of higher number and ratio than non-supplemented pigs. This finding indicates that MOE supplementation provides gut environment conditions to be suitable for beneficial bacteria to be grown and offers a better health and growth performance of pigs.

CONCLUSION AND SUGGESTIONS

The results from this study support the conclusion that:

a. MOE supplementation can increase sow performances.

b. MOE supplementation can improve feed efficiency and better average daily gain of nursery piglet.

c. 0.2% supplementation of microencapsulated citric acid, fumaric acid, malic acid, and sorbic acid as organic acid mixture and eugenol, thymol, and vanillin as essential oil mixture in feed slightly affect beneficial fecal bacterial population.

With respect to our conclusions and knowledges that have been collected above, some aspects are needed to be further investigated to obtain an overall picture of the research as below.

1. Measurement of the pH level change in the gastrointestinal tract after MOE administration to display the activity of MOE.

2. Microscopic structure of the intestinal lumen linings to represent the gut health conditions.

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