Effect of Colistin and Liquid Methionine with Capsaicin Supplementation in Diets on Growth Performance and Intestinal Morphology of Nursery Pigs

Thitima Jarupan, Chaowit Rakangthong, Chaiyapoom Bunchasak, Theerawit Poeikhampha, and Pudcharaporn Kromkhun
Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
Email: Thitima.Jarupan@outlook.co.th, fagrcwr@ku.ac.th

***Abstract*—This study was conducted to evaluate comparative effect of Colistin and Liquid methionine (LMA) with Capsaicin supplementation in diets on growth performances and intestinal morphology of nursery pigs. Seventy-two crossbred pigs (Duroc x Large White x Landrace; initial weight 6.76±0.22 kg) were divided into three groups with six replications of eighteen piglets each. There were three dietary treatment: 1) basal diet (control) 2) basal diet with anitibiotic (Colistin 40 ppm of diet) and 3) basal diet with 0.2% LMA with Capsaicin. The result show that the supplemental of 0.2% LMA with Capsaicin significantly improve feed conversion ratio of pigs during pre-starter period ($p<0.01$). Adding Colistin and LMA with Capsaicin to diets can increase villous in the duodenum and ileum ($p<0.01$) and the villous height:crypt depth ratio in duodenum and jejunum. The supplement of LMA with Capsaicin significantly increase villous height and may lead to absorb nutrient for growth in pigs. In conclusion, this study indicates that adding LMA with capsaicin in diets can reduce antibiotic in diets of nursery pigs.**

***Index Terms*—colistin, liquid methionine, capsaicin, growth performance, small intestinal morphology, nursery pigs**

I. Introduction

Weaning the piglets under modern commercial conditions inflicts stress on pigs and associated with marked changes in gastrointestinal tract (GIT) physiology, microbiology and immunology [1]. Antibiotic feed additives as growth promoters have long been supplemented to pig feed to stabilize the intestinal microbial flora and improve growth performance and prevent some specific intestinal pathologies. Colistin is one of the few cationic antimicrobial peptides commercialized in both human and veterinary. Colistin has been extensively used orally since the 1960s in food animals and particularly in swine for the control of Enterobacteriaceae infections. In the present, bacteria have mechanism of resistance to colistin for *Salmonella* spp. and *Escherichia coli.*, due to the importance of these two bacteria in both swine and human health [2]. The European Union implemented a full ban on in-feed antibiotics usage in livestock diets in 2006. Currently, however, the livestock industry is focusing greater attention towards addressing public concern for environmental and food safety [3], then using antibiotic as a growth promotor is prohibited. To replace dietary antibiotic, many alternative feed additives, such as probiotic, prebiotic, antioxidant, acidifier and herbal extract, have been investigated [4].

Liquid methionine (LMA) with capsaicin are alternative feed additive to reduce antibiotic in feeds. Liquid Methionine's chemical structure is similar to that of methionine. However, instead of an amine group, the analogue has a hydroxyl group in its place. LMA can converted into L-methionine in liver. Once absorbed, L-methionine is directly incorporated into protein for growth. Because liquid methionine has a hydroxyl group instead of an amine group, it is an organic acid. Kaewtapee et al. (2010) [5] has shown that methionine hydroxy analogue or liquid methionine (LMA) provides acidifying effects of organic acids. Organic acids in the diet can enhance growth performance and modulate intestinal microbiota in pigs. Since derivatives of LMA, such as taurine, cystathionin or glutathione, play a key role in intestinal epithelial antioxidant function [6], and the antioxidants are essential compounds for host defense against oxidative stress and immunity [7].

Herb or phytogenic have been used for strengthening the taste, food preservation or medical purpose. Phytogenic feed additives are often claimed to improve flavor and palatability of feed, thus, enhancing production performance. capsaicin is the active ingredient of chili peppers and gives them the characteristic pungent flavor. Capsaicin and related compounds form a naturally occurring chemical group called capsaicinoids. Moreover, the pepper family is a good source of vitamin C and E [8] as well as provitamin A and carotenoids compounds with holding well antioxidant properties [9]. Moreover, Capsaicinoid with phenolic acid obtained from red fruits were characterized by high antioxidant activity [10] and the use of herbs and spices in piglet nutrition can reduce the incidence of

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infections. LMA with capsaicin interest feed additive to supplemented in feed for prevent bacteria resistant in swine.

The objectives of this study were to evaluate the influence of comparative colistin and LMA with capsaicin in diets on growth performance and small intestinal morphology in nursery pigs.

II. Materials and Methods

This study was conducted at the Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok and Thailand. Experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

A. Animals and Managements

A total of seventy-two crossbred barrows (Duroc x Large White x Landrace; initial weight 6.76±0.22 kg) were randomly allotted into three treatments and six replicates in each treatment (four pigs/pens). The average body weight of each replication were homogenized and balanced. During six weeks experimental periods, an evaporative cooling system was used to control air ventilation and temperature. Feed were offered as *ad libitum* and water were provided by water nipples.

B. Experimental Diets

Experimental diets were divided into two phases as Pre-starter period (after wean-14 days) and Starter period (15-42 days). All nutrients of basal diet were formulated according to National Research Council (2012). Composition of basal diet is given in Table I. Pigs in treatment 1 were fed a basal diet. Treatment 2 and 3 were the same as treatment 1 but supplement with antibiotic (40 ppm Colistin sulfate) and 0.05% LMA with 2.5 ppm capsaicin, respectively.

C. Growth Performance

Initial body weight (d 1) and final body weight (d 41) were measured. Three were two phases as Pre-starter period (14 days) and Starter (28 days) and two times measurement of body weight (BW) and body weight gain (BWG). Average daily feed intake (ADFI) was recorded daily on a per pen basis. Consequently, feed conversion ratio (FCR) were calculated per pen basis.

D. Gastrointestinal pH

At the end of trial, six pigs per treatment were put down. The digesta contents from stomach, duodenum, jejunum, ileum, cecum, colon and rectum were immediately collected for the determination of pH. Moreover, diet were sampled 10g from each test diet and from each test diet and mixed with 90 mL distilled water (pH 7) for 10 min at room temperature. The pH in diet and digesta samples were directly measured with a pH meter (IQ Scientific Instruments, Inc., Carlsbad, CA, USA).

TABLE I. Composition of the Basal Diet

Ingredient	Pre-starter	Starter
Broken Rice	40.00	39.18
Corn	15.93	21.19
Fish meal	3.00	1.50
Soybean oil	1.42	2.23
Soybean Meal (48% CP)	25.74	23.73
Soybean Full Fat	5.00	0.00
Rice Bran Defatted	0.00	5.00
Milk Whey Permeate	5.00	3.00
L-Lysine HCL	0.50	0.52
L-Theronine	0.16	0.17
DL-Methionine	0.22	0.21
Salt	0.35	0.35
Calcium Carbonate	0.60	0.75
Monodicarcalcium	0.87	0.87
Premix	0.43	0.43
Choline Choride 60%	0.08	0.07
Sodium bicarbonate	0.50	0.60
Corn cob	0.20	0.20
Calculated chemical composition		
Metabolizable energy (MJ/kg)	3400.00	3350.00
Protein (%)	21.00	18.50
Fat (%)	4.27	4.20
Calcium (%)	0.80	0.70
Total Phosphorus (%)	0.66	0.65
Available phosphorus (%)	0.40	0.34
Salt (%)	0.54	0.51
Lysine (%)	1.31	1.19
Methionine (%)	0.49	0.45
Threonine (%)	0.73	0.67
Lactose(%)	4.25	2.55

[a]Premix content; vitamin A (retinyl acetate) 5 MIU, vitamin D_3 (cholecalciferal) 1.2 MIU, vitamin E (DL-a-tocopheryl acetate) 4000 IU, vitamin K_3 (menadione) 0.6 g, vitamin B_1 (thiamine) 0.8 g, vitamin B_6 (pyridoxine) 1.2 g, vitamin B_{12} (cyanocobalamin) 25 mg, nicotinic acid 5 g, pantothenic acid 3.76 g, folic acid 0.2 g, d-biotin 0.036 g, Fe ($FeSO4ÆH2O$) 20 g, Cu ($CuSO_4$ $5H_2O$) 4 g, Mn (MnO) 24 g, Zn (ZnO) 20 g, Co($CoCO_3$) 0.08 g, Se (Na_2SeO_3) 0.04 g, carrier (grinded corn cob) added to 1 kg.

E. Morphology of Small Intestine

Morphology of the duodenum, jejunum and ileum was evaluated with a light microscope. The tissues were removed and immediately fixed in 10% neutral buffered formalin, and then carefully embedded in paraffin. For each paraffin block, at least 10 sections of 7 µm thickness were prepared, and then stained with hematoxylin-eosin for histological evaluation. In the morphological evaluation of the small intestine, villous height, crypt depth and villous height:crypt depth ratio were measured. The measurement of villous height (from the tip of the villous to villous-crypt junction) and crypt depth (from the villous-crypt junction to the lower limit of the crypt) were recorded as the mean of 10 fields for specimen.

F. Statistical Analysis

Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed using Duncan's new multiple range test (SAS Institute, 2004) [11].

III. Results

A. Growth Performance

The effect of comparative colistin and LMA with Capsaicin on growth performance was shown in Table II. The results showed that there was no significant difference in final weight, body weight, average daily gain, feed intake ($p>0.05$). However, supplemental LMA with capsaicin in diets result in better feed conversion ratio (FCR) of piglets in pre-starter period (1-14 days)than of control and colistin groups ($p<0.05$). During starter period (15-42 days), dietary treatments did not influence productive growth performance ($p>0.05$).

TABLE II. Effects of Comparative Colistin and LMA with Capsicin in Diet on Growth Performances in Pig

Item	Treatment			p-value	SEM
	Control	Colistin	LMA& capsaicin		
Pre-starter period					
Initial Body weight (kg)	6.79	6.76	6.74	0.95	0.05
BW (kg)	10.90	10.94	10.72	0.83	0.15
BWG (kg)	4.13	4.17	3.98	0.80	0.11
ADFI (kg/day)	0.36	0.38	0.33	0.19	0.01
ADG (kg/day)	0.29	0.29	0.28	0.80	0.01
FCR	1.24^{a}	1.28^{a}	1.17^{b}	**<0.01**	0.01
starter period					
Initial Body weight (kg)	10.90	10.94	10.72	0.83	0.15
BW (kg)	28.07	28.73	27.85	0.58	0.34
BWG (kg)	17.16	17.79	17.14	0.47	0.23
ADFI (kg/day)	0.95	0.96	0.98	0.73	0.01
ADG (kg/day)	0.61	0.63	0.61	0.46	0.01
FCR	1.56	1.51	1.61	0.11	0.01

[a,b] Means in the same row with different superscripts differ significantly ($p<0.01$)

B. pH in Gastrointestinal Tact

The pH in gastrointestinal tract of pigs is shown in Table III, pH in gastrointestinal tract did not impact on pH in stomach, ceacum, duodenum, jejunum, ileum, colon and rectum.

TABLE III. Effects of Comparative Colistin and LMA with Capsicin in Diet on pH in Gastrointestinal Tract of Pigs

Item	Treatment			p-value	SEM
	Control	Colistin	LMA& capsaicin		
Stomach	3.59	3.67	3.31	0.78	0.21
Caecum	5.53	5.68	5.41	0.47	0.09
Duodenum	4.75	5.28	5.16	0.33	0.15
Jejunum	5.55	5.69	5.64	0.81	0.09
Ileum	5.97	5.97	6.24	0.47	0.10
Colon	5.72	5.80	5.66	0.78	0.08
Rectum	6.21	6.18	6.18	0.96	0.05

C. Small intestinal morphology

The effect of comparative colistin and LMA with Capsaicin on small intestinal morphology is shown in Table IV. Supplementations colistin and LMA with Capsaicin significantly increased villous height in the duodenum and ileum ($p<0.01$). In addition, LMA with Capsaicin significantly increase crypt depth more than control and Colistin group ($p<0.01$). Colistin and LMA with Capsaicin supplementation in diets enhanced villous height and crypt depth ratio of Duodenum ($p=0.01$) and Jejunum ($p<0.05$).

TABLE IV. Effects of comparative Colistin and LMA with Capsicin in diet on small intestinal morphology in pigs.

Item	Treatment			p-value	SEM
	Control	Colistin	LMA& capsaicin		
Villous height (μm)					
Duodenum	448.66^{B}	520.164^{A}	557.53^{A}	**<0.01**	16.07
Jejunum	346.64	362.73	394.59	0.19	10.85
Ileum	260.81^{B}	293.11^{A}	296.46^{A}	**<0.01**	4.20
Crypt depth(μm)					
Duodenum	333.73	320.55	328.81	0.28	3.38
Jejunum	275.55^{A}	246.05^{B}	264.43^{A}	**<0.01**	3.87
Ileum	185.46	191.02	198.92	0.08	2.52
Villous:Crypt					
Duodenum	1.35^{b}	1.62^{a}	1.70^{a}	**0.01**	0.05
Jejunum	1.25^{b}	1.47^{a}	1.49^{a}	**<0.05**	0.04
Ileum	1.41	1.53	1.49	0.06	0.02

[A,B] Means in the same row with different superscripts differ significantly ($p<0.01$)

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

IV. Discussion

Generally, weaning pigs from the sow is one of the most stressful events in life that can contribute to

intestinal and immune system dysfunctions that result in reduced growth performance, feed intake and immunity particularly during the first week after weaning [12]. In our study, 0.2% of LMA with Capsaicin supplementation can improve gut health in pigs. Therefore, the combination of LMA with Capsaicin in diets improve FCR during pre-starter (1-14 days of experimental period), while did not significantly affect the performance during starter period (15-42 days of experimental period). Keawtapee et al. (2009) [4] indicated that adding LMA to drinking water that improved growth performance. It may come from indirect effect such its derivative (cysteine) which plays a key role in intestinal epithelial antioxidant function as a precursor for taurine and glutathione [6] and/or its function as acidifier. Moreover, Capsaicin absorbed form the gut lumen is almost completely metabolized before reaching the general circulation [13]. Capsaicin and other herb enhance the synthesis of bile acids in the liver and their excretion in bile, what beneficially effects the digestion and absorption of lipids. Most of the prelisted spices stimulate the function of pancreatic enzymes (lipases, amylases and proteases), some also increase the activity, extracts from herbs and spices accelerate the digestion and shorten the time of feed/food passage through the digestive tract [14], [15]. Windich et al. (2008) [16] also indicate that spices helped to increase the absorption of essential nutrients, hence improving the growth of the animals.

Addition of organic acids into pig diets decreased gastric pH [17], resulting in a longer retention time in the stomach (Walsh et al., 2004), consequently enhancing digestion and the absorption of nutrients throughout the gastrointestinal tract. But in this study, compare colistin and LMA with Capsaicin in diets not affect gastric pH, which was similar to Mathew *et al.* (1991) [18] and Risley *et al.* (1992) [19] who reported that supplementation of organic acids in diet did not change the pH in the gut, this may be cause by retention time and contents of the stomach [20] and physiological homeostasis in tract.

The main role of duodenum is to complete the first phase of digestion and after food is broken down in the duodenum, it moves to jejunum, where the inside wall absorb nutrients. The ileum is final section of small intestine and large surface area both for the absorption of enzyme molecules. The function of ileum is mainly to absorb vitamin B12, bile salts and any product from digestion that were not absorbed by the jejunum. In current study, the result of increase villous height in duodenum and jejunum by colistin and LMA with Capsaicin supplementation group. This may be because LMA is diffuse via the villous of ileum [21]. Moreover, Capsaicin helped host to protect against gastrointestinal mucosal injury [22]. In the present study, LMA with Capsaicin increase crypt depth in jejunum. However, decrease of crypts depth was found in supplementation group. Honda (2013) [23] found that the number of cells present in the mucosal lamina propria of the intestinal tract was drastically decrease in mice which were administered antibiotics to. Since ratio of villous height to crypt was significantly increase by supplement colistin and LMA with Capsaicin. This means that both colistin and LMA with Capsaicin may promote morphology of small intestine in difference way.

V. CONCLUSION

In summary, this study that supplementation of LMA with Capsaicin can improve feed conversion ratio of pigs during pre-starter period. In addition, supplementing both colistin and LMA with Capsaicin can improve small intestinal morphology of pigs. Thus, supplementations of LMA with Capsaicin is another choice for reduce antibiotic in nursery pigs.

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Thitima Jarupan, Department of Animal Science, Kasetsart University.

She obtained her Bachelor of Science (Agriculture) and Master of Science (Agriculture) in Chiang Mai University and Kasetsart University, respectively.

Choawit Rakangthong, Ph.D., Department of Animal Science, Kasetsart University.
He got his Bachelor of Science (Agriculture-Animal Science) in 2002 from Walailak University, Nakorn Sri Thamaraj, Thailand. Then, he obtained the Master of Science (Agriculture) in 2005 and Doctor of Philosophy (Animal Science) in 2012 from Kasetsart University, Bangkok, Thailand.
His selected publication and scientific credential are as follows:
S. Saree, C. Bunchasak, C. Rakangtong, J. Sakdee, N. Krutthai, and T. Poeikhampha, "Comparative effects of corn-based diet and phase-fed cassava-based diet on growth rate, carcass characteristics and lipid profile of meat-type ducks," *Asian-Australasian J. Animal. Science*, vol. 30, no. 6, pp. 843-848, 2017.
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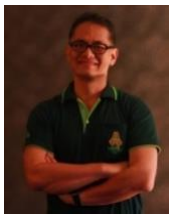

Chaiyapoom Bunchasak, Ph.D., Assoc. Prof., Department of Animal Science, Kasetsart University
He got his Bachelor of Science (Agriculture) in 1991 from Khon Kaen University, Khon Kaen, Thailiand. Then, he obtained the Master of Agriculture (Animal Science) in 1995 and Doctor of Philosophy (Agricultural Science) in 1998 from Gifu University, Japan.
His selected publication and scientific credential are as follows:
C. Bunchasak, Y. Ratchadapornvanitch, and J. Thiengtham, "Comparative effects of supplemental DL-2-hydroxy-4-[methylthio] butanoic acid and DL-methionine in diet on egg production and quality in laying hens," *The Journal of Poultry Science*, vol. 30, no. 6, pp. 843-848, 2012.
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Theerawit Poeikhampha, Ph.D. Asst. Prof., Department of Animal Science, Kasetsart University.
Dr. Theerawit Poeikhampha has been the Associate Director of Academic Affairs, Department of Animal Science, Kasetsart University and his research is a multidisciplinary strategy to research, develop and optimize on the animal nutrition technologies. The recent projects have included feed ingredients and feed additives, exogenous enzyme and enzyme's matrix calculation, amino acid metabolism and cell function, micronutrients in drinking water, feed formulation and feed manufacturing technology for increase the productivity of farm animal, especially in the tropical condition.
He obtained his Bachelor of Science (Agriculture), Master of Science (Agriculture) and Doctor of Philosophy (Animal Science) in 2002, 2004 and 2010, respectively.
His selected publication and scientific credential are as follows:
E. Sritiawthai, S. Sakulthai, J. Sakdee, C. Bunchasak, C. Kaewtapee, and T. Poeikhampha, "Effect of protein level and dietary energy on production, intestinal morphology and carcass yield of meat duck during starter phase of 14 days," J. Applied Sci., vol. 13, pp. 315-320, 2013.
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Pudcharaporn Kromkhun, Ph.D., Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University.

She graduated from Nippon Veterinary and Life Science University, Japan, and obtained her Doctor of Veterinary Medicine in Kasetsart University, Bangkok, Thailand.

Her selected publication and scientific credential are as follows:

M. Terada, *et al.*, "Plasma prolactin concentrations and copulatory behavior after salsolinol injection in male rats," *Reproductive Medicine and Biology*, vol. 9, no. 4, pp.205-209, 2010.

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