Effect of Colistin and Liquid Methionine with Capsaicin Supplementation in Diets on Blood Chemical and Intestinal Bacteria of Nursery Pigs

Theerawit Poeikhampha, Chaowit Rakangthong, Chaiyapoom Bunchasak, Thitima Jarupan, and Wiriya Loongyai
Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
Email: agrtrw@ku.ac.th

***Abstract*—This study was conducted to determine comparative effect of supplemented Colistin and Liquid Methionine (LMA) with Capsaicin in diets on blood chemical and bacteria population in caecum 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 results indicated that supplemental of Colistin in diets significantly decreased lymphocyte ($p<0.05$), increased of neutrophil and neutrophil:lymphocyte ratio ($p<0.05$) compared with control group. In addition, supplementing both Colistin and LMA with Capsaicin can increased population of Domain bacteria and *Lactobacillus* sp. in caecum of nursery pigs. In conclusion, this study indicates that supplementing of LMA with capsaicin in diets can reduce antibiotic in diets of nursery pigs.**

***Index Terms*—colistin, liquid methionine, capsaicin, blood chemical, bacteria population, nursery pigs**

I. Introduction

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 [1]. Therefore, weanling pigs usually showed a malabsorption syndrome characterized by villus atrophy, digestive enzyme disorderand pathogenic bacterial over-growth, which resulted in poor growth performance and increased mortality [2].

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 belongs to the family of polymyxins, cationic polypeptide, with broad-spectrum activity against Gram-negative bacteria, including most species of the family Enterobacteriaceae. In recent years, however, public concern over development of resistant pathogenic strains and antibiotic residue in animal products has led to pressure to search for alternative materials or methods to antibiotics in animal industry. 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 chemical structure is similar to that of methionine. However, instead of an amine group, the analogue has a hydroxyl group in its place. Because liquid methionine has a hydroxyl group instead of an amine group, it is an organic acid. Moreover, LMA can converted into L-methionine in liver. Methionine is considered as the second or third limiting amino acid in diets for modern nursery pigs [5], and it plays several roles such as an initiating amino acid in protein synthesis and as the principal biological methylating agent in the body [6]. Since derivatives of LMA, such as taurine, cystathionin or glutathione, play a key role in intestinal epithelial antioxidant function [7], and the antioxidants are essential compounds for host defense against oxidative stress and immunity [8].

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 [9] as well as provitamin A and carotenoids compounds with holding well antioxidant properties [10]. In addition, Capsaicin has recently attracted considerable attention its strong antimicrobial and anti-virulence activity. Supplementing of Capsaicin in diets can reduce the incidence of infections. LMA with capsaicin interest feed additive to supplemented in feed for prevent bacteria resistant in pigs.

The objectives of this study were to evaluate the influence of comparative colistin and LMA with capsaicin in diets on blood chemical in nursery pigs.

Manuscript received January 20, 2018; revised March 20, 2018.

II. MATERIALS AND METHOD

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). 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 0.2% of 0.05% LMA with 2.5 ppm capsaicin, respectively.

C. Blood Chemical

On the final day of experiment, six pigs per treatment were selected from each group and 3 ml blood sample was collected to a microfuge tube without anticoagulants via the jugular vein. Samples were centrifuged at 2500 g for 10 min at room temperature within 1h after collection.

Serum was stored at -20 ℃ until analysis for total protein and blood urea nitrogen by commercial test kit (Assay kit; HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Total protein concentration in the serum was analyzed by Biuret method by BCG-method (photometric colourimetric colorimetric test), and serum blood urea nitrogen concentration was analyzed by urea liuid colour method (enzymetic colourimetric test).

D. Bacterial population in caecum

1) DNA extraction

Sample of equivalent volume to 400 mg of digesta were preserved in ethanol and precipitated by centrifugation (13000 g for 5 min). DNA in the precipitate was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK). The recommended lysis temperature was increased to 90 ℃ and an incubation step with lysozyme was added afterwards (10 mg/ml, 37 ℃, 30 min) to 90 improve bacterial cell rupture. The DNA was stored at – 80 ℃ until analysis.

2) Quantitative PCR

To quantify Domain bacteria, *Lactobacillus* sp. and *Escherichia coli* different primers were used F-5'CGGYCCAGACTCCTACGGG3' (forward) and R-5'TTACCGCGGCTGCTGGCAC3' (reverse) for Domain bacteria [11] For *Lactobacillus* sp.: F-5'AGCAGTAGGGAATCTTCCA3' (forward) and R-5'CACCGCTACACATGGAG3' (reverse) [12] and for *Escherichia coli*: F-5' GCGAAAACTGTGGAATTGGG3' (forward) and R-5' TGATGCTCCATAACTTCCTG3' (reverse) [13]. The oligonucleotides were adapted from published specific primers or probes using the Primer Express Software to qPCR recommendations (Applied Biosystems, CA, USA). The different primers were also checked for their specificity using the database similarity search program nucleotide-nucleotide BLAST and the absence of amplification of porcine DNA was tested empirically by PCR using the DNA extracted from digesta in caecum pigs. The PCR cycles were 95 ℃ for 60 second, 30 cycles of 95 ℃ for 60 second, 63 ℃ for 30 second and 72 ℃ for 8 minutes. The PCR product was digested with 5 U MboI enzymes (Promega Co., Madison, WI) in a final volume of 10 µl. The digestion pattern separated using electrophoresis on 1.8 % agarose gel in 0.5X of TAE buffer. The gel was stained with ethidium bromide and visualized under UV light

E. 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) [14].

III. RESULTS AND DISCUSSION

A. Blood Chemical

The effects of comparative Colistin and LMA with Capsicin in diet on Blood urea nitrogen (BUN), total protein, titer of swine fever, White blood cell (WBC), Neutrophil, Lymphocyte and Neutrophil:Lymphocyte ratio in blood are presented in Table I. There were no significant difference in WBC, BUN, total protein and titer of swine fever among treatment ($P>0.005$). However, supplementing Colistin in diets significantly decreased Lymphocyte in blood ($P<0.05$), increased Neutrophil and Neutrophil:Lymphocyte ratio ($P<0.05$) compared with control group. Supplementation different between Colistin and LMA with Capsaicin supplementations on these parameters.

In our study indicates that the supplemental Colistin and LMA with Capsaicin in diets had no significant effect on WBC, BUN, total protein and titer of swine fever. However, Krutthai *et al*. (2015) [15] demonstrated that BUN was commonly used as an end point for evaluation of the effect of amino acid supplementation in diets on nitrogen balance of pigs, and LMA supplementation significantly decreased BUN and increased albumin in blood. BUN level is also an indicator of kidney injury [16]. In addition, Capsaicin has been shown to decrease BUN [17]. Paraksa (2011) [18] were found Capsaicin has been shown to decrease BUN. Due to Capsaicin has antioxidant function. Shimeda et al.

(2005) [19] indicated that Capsaicin can decrease BUN or increase excrete urea in kidney.

TABLE I. EFFECTS OF COMPARATIVE COLISTIN AND LMA WITH CAPSICIN IN DIET ON BLOOD CHEMICAL IN PIGS

Item	Treatment			p-value	SEM
	Control	Colistin	LMA& capsaicin		
BUN (mg/dl)	5.03	6.73	7.23	0.37	0.64
Total Protein (g/dl)	4.32	4.17	3.85	0.20	0.11
Titer of swine fever	24.00	22.67	24.00	0.99	4.68
WBC	22.66	18.31	16.24	0.16	1.39
Neutrophils (%)	20.33^{b}	33.17^{a}	30.17^{ab}	**<0.05**	2.26
Lymphocyte (%)	77.50^{a}	64.50^{b}	67.00^{b}	**<0.05**	2.25
N/L	0.27^{b}	0.55^{a}	0.46^{ab}	**<0.05**	0.05

^{a,b} Means in the same row with different superscripts differ significantly (p<0.05)

The circulatory and migratory properties of white blood cells have evolved to allow efficient surveillance of tissues for infectious pathogens and rapid accumulation at sites of injury and infection [20]. White blood cells have five types, which are neutrophils, lymphocytes, monocytes, eosinophils and basophils. Lymphocytes continually patrol the body for foreign antigen by recirculating from blood through tissue into lymph and back to blood. Timothy (1994) [20] indicated that the traffic signals for lymphocyte recirculation and for neutrophil and monocyte localization in inflammation were strikingly similar at the molecular level.

The amount of lymphocytes has been basically indicated by the percentage of white blood cells. Generally, if neutrophils increase, the percentage of lymphocytes will be lower. There are many factors affecting amount of neutrophil, such as infection, inflammation, drug and stress. Normally, the proportion of neonatal lymphocytes and lymphocytes of weaning pigs is approximately 18-73% and 13-70%, respectively [21]. In many instances, the increased number of neutrophils is a necessary reaction by the body, as it tries to heal or ward off invading microorganisms or foreign substances. Infections by bacteria, viruses, fungi, and parasites may all increase the number of neutrophils in the blood. Injury cause an increase in the number and activity of neutrophils. Some drugs also lead to an increased number of neutrophils in the blood [21]. In term of stress, McGlone *et al*. (1993) [22] reported that the number of neutrophils in pigs which had been transported for 4h were increased. In agreement with the present study, amount of lymphocyte was significantly declined, while increase of neutrophils was observed by Colistin or LMA with Capsaicin groups. Consequently, ratio of neutrophils and lymphocyte was significantly increased by Colistin supplementation. Moreover, supplementation of LMA with Capsaicin tended to similar effect as the Colistin.

In the present study, since all piglets were kept under good management conditions, and there was no outbreak or infections, it can be implied that the significant different of neutrophil, lymphocyte and neutrophil:lymphocyte ratio should be strongly influenced by Colistin and LMA with Capsaicin supplementation. Therefore, the supplementation of Colistin and LMA with Capsaicin may increase the defensing mechanism to bacteria via the stimulation of neutrophil production, although amount of lymphocyte was decreased.

B. Bacteria Population in Caecum

The effects of comparative Colistin and LMA with Capsicin in diet on bacteria population in caecum of pigs is show in Table 2. Supplementations Colistin and LMA with Capsaicin significantly increased population of Domain bacteria and *Lactobacillus* sp. in caecum of pigs. However, population of *Escherichia coli* (*E. coli*) did not difference in all groups (P<0.05).

TABLE II. EFFECTS OF COMPARATIVE COLISTIN AND LMA WITH CAPSICIN IN DIET ON BACTERIA POPULATION IN CAECUM OF PIGS.

Item	Treatment			p-value	SEM
	Control	Colistin	LMA & capsaicin		
Domain bacteria (log CFU/g)	9.68^{B}	11.04^{A}	11.03^{A}	**<0.01**	0.19
Escherichia coli (log CFU/g)	12.23	11.08	10.22	0.19	0.44
Lactobacillus sp. (log CFU/g)	13.40^{B}	17.06^{A}	16.22^{A}	**<0.01**	0.48

^{A,B} Means in the same row with different superscripts differ significantly (p<0.05)

In practice, feedstuffs and drinking water contaminated with *E. coli* have been reported to adversely affect animal health [23]. In the present study, indicates that the supplemental Colistin and LMA with Capsaicin in diets had no significant effect on *E. coli* population. However, both supplemental of Colistin and LMA with Capsaicin significantly increased Domain bacteria and *Lactobacillus* sp. population in caecum of pig. Similarly, Fuller (1977) [24] demonstrated that acidic conditions stimulate growth of lactobacillus bacteria in the gut. likely, this study found a significant effect of LMA on bacteria population in the caecum. In addition, several In vitro studies showed strong antimicrobial activity of Capsaicin against Gram negative and Gram positive bacteria. From the results the combination LMA with Capsaicin can increased *Lactobacillus* sp. population in caecum of pig be equivalent to supplemental Colistin.

IV. CONCLUSION

In summary, this study that supplementation of LMA with Capsaicin in diets can stimulation of neutrophil for defensing mechanism to bacteria, as same as Colistin. In addition, supplementing both Colistin and LMA with Capsaicin can increased Domain bacteria and *Lactobacillus* sp. population in caecum of pig. Thus, supplementations of LMA with Capsaicin is another choice for reduce antibiotic in nursery pigs.

ACKNOWLEDGMENT

This research was supported from the Graduate Program Scholarship from the Graduate School, Kasetsart University.

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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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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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Wiriya Loongyai, Ph.D., Asst. Prof., Department of Animal Science, Kasetsart University

She got her B.Sc., Biotechnology (Second Class Honors) in the year 2002 from Faculty of Science, Prince of Songkla University, Hat Yai, Songkla 90122, Thailand and Ph.D., Biotechnology in 2006.

Her selected publication and scientific credential are as follows:

W. Loongyai, B. Wiriya, and N. Sangsawang, "Detection of *Salmonella* and *Escherichia coli* in egg shell and egg content from different housing systems for laying hens," International Journal of Poultry Science, vol. 10, no. 2, pp. 93-97, 2011.

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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.