Effects of Liquid Methionine and Capsaicin Supplementation in Diets on Growth and Intestinal Morphology of Broilers

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***Abstract*—The experiment was conducted to investigate the effects of Liquid Methionine (LMA) and capsaicin in broiler. In the first study, an in vitro study was conducted investigating the antimicrobial efficacy of the combination between LMA and capsaicin against clinical isolates and reference laboratory strains of Escherichia coli ATCC25922. The minimum inhibitory concentration for Escherichia coli ATCC25922 was 0.125 %. The second study, a total of 192 commercial male broiler chicks was used. Birds were raised for 38 days in high ambient temperature and relative humidity in an open house. The chicks were divided into 2 groups in 6 replications with 16 chicks each in a completely randomized design for study the effects of supplementation of LMA 0.05% and capsaicin 2.5 ppm supplementation in diets on growth and intestinal morphology of broiler reared in open housed condition. The results indicated that broilers fed with LMA combination capsaicin, had higher final average daily gain (g) (P<0.05). However, the dietary treatments not influenced the feed intake, body weight and intestinal morphology (P>0.05). Therefore, the concluded that supplementation with LMA and capsaicin lead to improve the growth performance in live body weight of broiler reared in open housed condition.**

***Index Terms*—minimum inhibitory concentration (MIC), Liquid Methionine (LMA), capsaicin, broiler, open housed condition**

I. INTRODUCTION

The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents visible growth of a bacterium. This is in difference to the minimum bactericidal concentration (MBC) which is the concentration resulting in microbial death as defined by the inability to re-culture bacteria. The closer the MIC is to the MBC, the more bactericidal the compound [1]. Escherichia coli (E. coli), a seemingly ubiquitous Gram-negative bacterium, is best known for its ability to cause food-borne outbreaks [2]. E. coli is a common intestinal bacterium of mammals. Most E. coli are harmless, but some induce various diseases; thus, the species is considered an opportunistic pathogen. The strain ATCC25922 is a commonly used quality control commonly used quality control for antibiotic susceptibility testing.

Thailand is one of the world's leading broiler meat producing countries and located in tropical zone, the temperature is around 30 - 35 ℃. High ambient temperatures and high relative can result in significant economic loss due to reducing feed consumption, growth rate feed conversion, macrophage activity and survivability of poultry [3]. Virden and Kidd (2009) [4] reported that heat stress exposure may result in impaired digestibility of major nutrient and various essential amino acids. Consequently in order to improve live performance of broilers under heat stress we need to improve their access to limiting nutrients and decrease feed heat increment [5].

Methionine is usually the first limiting amino acid in broiler diets. The supplemental sources of methionine most often used are DL-methionine and Liquid Methionine (LMA) which is classified as an organic acid. In addition, Poosuwan et al. (2007) [6] demonstrated that the minimum inhibitory concentration of LMA for E. coli was 0.24% v/v in water, and this level promoted growth performance and tended to reduce E. coli in the gastrointestinal tract and consequently improved growth performance of broiler.

The restriction in the use of antibiotics to increase the growth rate of livestock or to improve the efficiency of animal feed use. According to Royal Thai Government Gazette (2015), any kinds of antibiotics, used to increase the growth rate of livestock or to improve the efficiency of animal feed use, are not allowed in animal feed, any kinds of antibiotics, used to increase the growth rate of livestock or to improve the efficiency of animal feed use, are not allowed in animal feed. It is accepted that organic acids are the most promising alternative to antibiotics.

Herbs, spices, and various plant extracts have received increased attention as possible alternatives to antibiotic growth promotants, since they are considered as natural products [7]. Capsaicin (8-methyl-N-Vanilly-6-nonenamide) is the active substance responsible for the irritating and pungent effects of various species of hot pepper. Capsaicin has emerged as a relatively selective neurotoxin for small-diameter sensory neurons [8]. It's active compounds are alkaloids, fatty acids volatile oil, rutin (flavonoids), high flavonoid content makes it a good

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antioxcidant [9]. Several studies [10] have shown that plant extracts containing a mixture of capsaicin, cinnamaldehyde and carvacrol improved the growth performance of broiler.

Therefore, the objectives of this study were to investigate the effects of combination of LMA and capsaicin supplementation in diet on growth performances and intestinal morphology of broiler reared in open housed condition

II. Materials and Method

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

A. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined following clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing; sixteenth informational supplement (M100- S16, vol.26 NO.3) and clinical and laboratory standards institute method for dilution antimicrobial susceptibility test for bacteria that growth aerobically; approve standard seventh edition (M7-A7, vol.26 NO.2). Positive control agents by amikacin and ofloxacin Negative control agent 0.5% dimethylsulfoxide (DMSO) (final concentration). Assay description The Gram-negative bacteria; E. coli (ATCC 25922) are grown in tryptic soy agar (TSA) at 37 ℃ for overnight. A single colony is inoculated in Mueller Hinton Broth (MHB) and incubated in a rotary shaker 200 rpm at 37 ℃ for 30 min. Cells at a logarithmic growth are harvested and diluted to 2.5 x105 CFU/ml in MHB prior to assay. This assay is performed in 384-well plate in triplicate. Each well is added with 5 µl of sample (or positive or negative control agents) and 45 µl of cell suspension. Blank wells are added with 5 µl of 5%DMSO and 45 µl media. Plates are then incubated at 37 ℃ for 14 hours. Bacterial growth is observed by OD600 measurement using microplate reader. The OD units of test wells are subtracted with mean OD units of blank wells before calculation. Percent of bacterial inhibition is calculated by the following equation:

$$\% \text{ Inhibition} = [1- (ODT/ ODC)] \times 100 \qquad (1)$$

B. Animals and Managements

A total of 192 one-day-old male chicks (Ross 308 strain) were obtained from a commercial hatchery. The birds were randomly assigned according to their initial body weights to with and without LMA 0.05% and capsaicin 0.25 ppm supplementation groups and each group was replicated by 6 pens and each pen consisted of 16 birds. The management and vaccination were provided according to commercial practices. Water and feed were offered ad libitum during 38 days of feeding trial. The water supply in each pen was equipped with bell from 1-10 days and nipple form 11-38 days. All birds will be raised in open housed condition.

C. Experimental design and diet

In order to study the effects of combination of LMA and capsaicin supplementation in diets. Chicks were fed a standard corn-soybean meal based diet. In this experiment, three phase feeding program was used with a starter diet from 1 to 10 days, a grower diet from 11 to 24 days and a finisher diet from 25 to 38 days. All birds were fed a starter diet until 10 days of age and followed by a starter, grower and finisher diet from day 1-10, day 11-24 and 25-38 days of age; respectively. Feed ingredients and nutrients composition of experimental diets are shown in Table I and Table II. The diets were formulated to meet the nutrients requirement of strain recommendations, contained 23 % CP and 3,025 ME kcal/kg in starter period, 21 % CP and 3,150 ME kcal/kg in grower period and 19 % CP and 3,200 ME kcal/kg in finisher period. The experiment was conducted between March and April, 2014, Bangkok, Thailand. During the experiment, house temperature and humidity were measured three times a day (07.00 am 1.00 pm and 05.00 pm).

TABLE I. Composition of the Experimental Diets

Items	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-38 days)
Corn Thai	48.806	52.475	58.600
Lard oil	6.217	7.599	7.198
Soybean meal (46.5 % CP)	39.155	34.850	29.515
L-Lysine HCL 78%	0.239	0.150	0.138
DL-Methionine	0.323	0.262	0.226
L-Threonine	0.096	0.054	0.044
Monodicalciumphosphate21	2.239	1.969	0.813
Calcium carbonate	1.534	1.278	0.241
Salt	0.277	0.283	0.288
PX Broiler[1]	0.550	0.530	0.400
Treatment (LMA+Cap)	0.350	0.350	0.350
Choline Chloride 75%	0.231	0.200	0.187
Total	100	100	100

[1]Vitamin and mineral premix content (per kilogram of feed): Vitamins: A 4 MIU, D 0.64 MIU, E 24,000 IU, K3 1.4 g, B1 0.6 g, B2 0.3 g, B6 0.75 g, B12 14 mg, nicotinic acid 20 g, pantothenic acid 10 g, folic acid 0.44 g, biotin 0.04 g, choline 60 g; Minerals: Fe 45 g, Cu 40 g, Mn 15 g, Zn 40 g, Co 0.2 g, I 0.4 g, Se 0.06 g

D. Growth Performance

All chicks were individually weighed at the start and the end of the experiment (38 day of age) and feed intake (of bird in each pen were measured according feeding period. Mean body weight (BW), weight gain and feed intake (FI) were calculated in order to calculation of feed conversion ratio (FCR) and average daily gain (ADG).

E. Morphology of Small Intestine

Morphology of small intestine histology of the duodenum, jejunum and ileum tissue was studied by light microscope in accordance with Nunez et al. (1996) [11]. The tissues were taken and immediately fixed in 10% neutral buffer formalin, and then carefully embedded in paraffin. For each specimen, at least 10 sections of 7 µm thickness were prepared. Tissues were then stained with haematoxylin and eosin for histological evaluation. The morphology of the small intestines in this study included villous height, crypt depth Measurements of villous height from the tip of the villous to the villous–crypt junction and crypt depth from the villus–crypt junction to the lower limit of the crypt were recorded as the mean of 10 fields for each specimen.

TABLE II. CALCULATED NUTRIENT OF EXPERIMENTAL DIET

Items	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-38 days)
ME. for Poultry (Kcal/Kg)	3,025	3,150	3,200
Protein (%)	23	21	19
Fat (%)	8.499	9.915	9.653
Fiber (%)	3.338	3.197	3.062
Calcium (%)	1.050	0.900	0.850
Total Phosphorus (%)	0.800	0.729	0.684
Avail. Phosphorus (%)	0.500	0.450	0.420
Salt (%)	0.320	0.320	0.320
Arginine (%)	1.485	1.356	1.202
Isoleucine (%)	0.952	0.874	0.781
Lysine (%)	1.429	1.246	1.101
Methionine + Cystine (%)	1.026	0.920	0.834
Methionine (%)	0.664	0.582	0.522
Threonine (%)	0.948	0.839	0.750
Tryptophan (%)	0.335	0.304	0.266
Valine (%)	1.052	0.972	0.880
Choline (%)	1,600.000	1,500.000	1,400.000

F. Statistical analysis

Data were analyzed as a completely randomized design using the ANOVA procedures of SAS (Statistical Analysis System, Version 9.0, 2002). The model used was as follows:

$$Yij = \mu + \tau i + \varepsilon ij \quad (2)$$

Where,
Yij= dependent variable
μ = overall mean effect
τi= fixed effect of treatments
I = with and without LMA 0.05% and capsaicin 0.25 ppm supplementation
εij= residual experimental error with N (0, σ2)

The significance of the differences between the treatment group means for each parameter was evaluated using the Duncan's New Multiple Range Test (DMRT). Probabilities of $P < 0.05$ were taken to indicate significant differences. All statistical analyses were computed in accordance with the method of Steel and Torrie (1980).

III. RESULTS

In this study, the minimum inhibitory concentration assay is a technique used to determine the lowest concentration of a LMA and capsaicin against. The lower level concentration in this study of activity that can be detected Escherichia coli ATCC25922 was 0.125%.

A. Environmental Conditions and Growth Performances

During the 38 days experimental period, the average daily lowest ambient temperatures were 28.10±1.20, 28.50±1.61 and 27.60±1.50 ℃ while the highest were 36.30±1.25, 36.60±1.08 and 35.60±1.70 ℃ during the starter, grower and finisher stages, respectively. On average, the lowest and highest temperature during the trial were 28.10±1.48 and 36.20±1.42 ℃ (Fig. 1), respectively. The corresponding average relative humidity readings were 91.00±2.21, 89.60±3.39 and 88.00±4.52±%. Thus, the average relative humidity during the experiment was 89.40±3.76% (Fig. 2). The results indicated, of the experimental on growth performances of broiler are presented in Table III.

At the end of the experiment, the dietary treatments not influenced the BW FI ADG and FCR (P>0.05). However, ADG (Finisher period) was the parameter affected by treatment diet, broilers fed with LMA combination capsaicin (P<0.05).

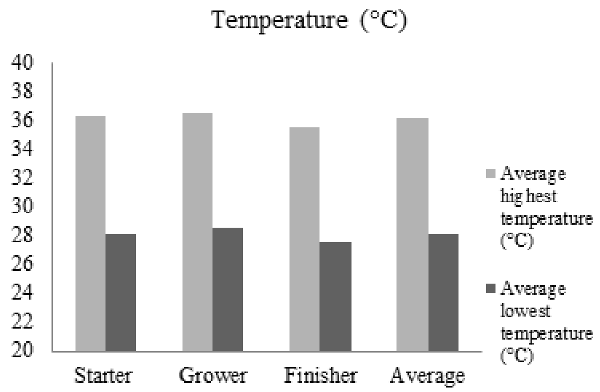

Figure 1. Average temperature during experimental period.

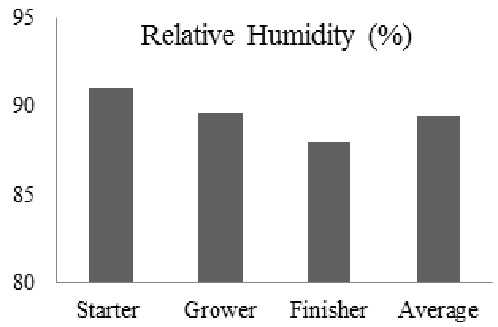

Figure 2. Average relative humidity during experimental period.

TABLE III. EFFECTS OF LMA AND CAPSAICIN SUPPLEMENTATION IN DIET ON GROWTH PERFORMANCE OF BROILER REARED IN OPEN HOUSED CONDITION (1-38 DAYS)

Item	control	LMA+Cap	P-value	SEM
Starter				
Initial BW (g)	45.02±0.05	45.03±0.03	0.07	0.01
BW (g)	328.91±5.79	333.68±9.86	0.33	2.34
FI	368.75±5.80	371.25±5.56	0.46	1.61
ADG (g)	28.39±0.58	28.87±0.99	0.33	0.23
FCR	1.30±0.03	1.29±0.04	0.52	0.01
Grower				
BW (g)	1292.34±52.83	1305.70±38.75	0.63	12.91
ADG (g)	68.82±3.59	69.43±2.66	0.74	0.87
FI	1371.98±44.98	1335.93±62.96	0.28	15.97
FCR	1.43±0.08	1.38±0.09	0.32	0.02
Finisher				
BW (g)	2459.94±145.00	2583.73±74.03	0.09	36.77
ADG (g)	83.40±7.15^{B}	91.29±3.90^{A}	0.03	1.98
FI	2237.31±133.88	2215.91±115.52	0.77	34.57
FCR	1.93±0.23	1.76±0.06	0.08	0.06
Starter-Finisher				
Initial BW (g)	45.02±0.05	45.03±0.03	0.07	0.01
ADG (g)	63.55±3.82	66.81±1.95	0.09	0.97
FI	3978.13±148.46	3923.09±165.72	0.56	44.89
FCR	1.65±0.12	1.55±0.05	0.06	0.03

^{AB} means in the same row with different superscripts differ significantly (P<0.05)

B. *Morphology of Small Intestine*

Effects of combination of LMA and capsaicin supplementation in diet on intestinal morphology of broiler reared in open housed condition is presented in Table 4. Adding LMA and capsaicin did not significantly affect villous height and crypt depth of the small intestines.

TABLE IV. EFFECTS OF COMBINATION OF LMA AND CAPSAICIN SUPPLEMENTATION IN DIET ON INTESTINAL MORPHOLOGY OF BROILER REARED IN OPEN HOUSED CONDITION (1-38 DAYS)

Item	control	LMA+Cap	P-value	SEM
Villus height (μm)				
Duodenum	1086.23±153.75	1113.34±117.47	0.75	39.93
Jejunum	930.08±123.45	934.90±63.32	0.95	35.78
Ileum	629.16±172.62	649.86±106.23	0.81	39.57
Crypt depth (μm)				
Duodenum	210.21±40.06	185.52±30.22	0.29	11.01
Jejunum	142.91±45.74	160.38±25.83	0.58	13.92
Ileum	131.32±50.01	135.09±32.43	0.88	11.62

Effects of combination of LMA and capsaicin supplementation in diet on intestinal morphology of broiler reared in open housed condition is presented in Table 4. Adding LMA and capsaicin did not significantly affect villous height and crypt depth of the small intestines.

IV. DISCUSSION

Broiler chickens are homeotherms that can live comfortably only in a relatively narrow zone of thermoneutrality and the optimal ambient temperature for efficient production for broiler chickens is 20℃ [12]. The high relative humidity and the big gap between the day and the night temperatures of this trial indicated the level of environmental stress that the birds were subjected to Sosnowka-Czajka et al. (2005) [13] reported that an ambient temperature range of 18-21℃ generally supports optimal growth of animals. The high ambient temperature and high relative humidity conditions in experimental housing were stressful and had a negative effect on growth performance. Thus, in our study where the average ambient temperature was 36.20±1.42 ℃ during the day, there was a marked tendency for growth performance to be affected by heat stress. As reported by Daghir (2009) [14] and Sosnowka-Czajka et al. (2005) [13], the physiologic and behavioral responses of broilers are detrimentally affected by hot environmental conditions. They found out that for every 1℃ increase in ambient temperature above 30℃, there was a corresponding decrease in feed intake of 2.43 g per bird.

A combination of LMA 0.05%, with either capsaicin 2.50 ppm, had higher final ADG (g) significant (P<0.05). An increase in performance of broiler in this group could have been due to positive effects of LMA Willemsen et al. (2011) [15] reported that methionine supplementation partially prevented the growth-depressing effects of chronic heat exposure compared with LMA supplementation. It can be inferred that LMA is more efficient in alleviating high temperature-induced oxidative damage because of a more favorable reduced glutathione (GSH) / total GSH ratio. Knight et al., (1994) [16] found that the uptake of DLM by intestinal epithelial cells was reduced in chicks exposed to short periods of heat stress. Dibner et al. (1992) [17] reported that total epithelial uptake of 14C-LMA (diffusion plus energy dependent and independent uptake) is reduced by 34 % in the intestines of acute heat stress chicks.

Villous atrophy may be followed by an increase in the depth of the crypts [18],[19] in this study, however, the villus height and depth of crypts was not significantly affected. For the effects of LMA and capsaicin in broiler diet is increase height of villus but not significantly, therefore, we suggest that LMA may affect small intestinal morphology via three mechanisms: (i) LMA directly stimulates cell proliferation and/or cell number as a precursor of protein synthesis, (ii) high derivatives of LMA such as taurine or glutathione which is an antioxidant, protect villous from damage caused by oxidative stress in the small intestines [20]. There is evidence that LMA has broader antimicrobial activities and might be used to inhibit pathogenic bacterial contamination in diet or drinking water. Poosuwan et al. (2007) [6] demonstrated that the MIC for E. coli of LMA was 0.24% v/v, and they also showed that adding LMA to drinking water around 0.025–0.100 % significantly decreased the pH of drinking water, promoting growth performance and tended to reduce E. coli in the gastrointestinal tract of broiler chicks.

V. Conclusion

It can be concluded that supplementation with LMA and capsaicin in diet lead to improve the growth performance in live body weight of broiler reared in open housed condition.

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