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Original Article

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Abstract

This study was conducted to evaluate the effects of adding liquid DL-methionine hydroxyl analog free acid (LMA) to drinking water on the growth performance and gastrointestinal tract in broiler chicks during 1-42 days of age. The chicks were divided into 5 groups as follows, Group 1 (negative control); chicks received a methionine (Met) deficient diet without LMA supplementation in drinking water. Group 2, 3, 4 and 5 were diets supplemented with DL-methionine (DLM) that meet total sulfur amino acids (TSAA) requirement, then each group was graded level with LMA in drinking water at 0%, 0.025%, 0.05% and 0.1%, respectively. Average daily gain (ADG) and feed conversion ratio (FCR) were significantly improved by DLM supplementation in diet. Furthermore, adding LMA at 0.025% and 0.050% to drinking water showed better growth performance than that of DLM supplementation group (p<0.01). Met deficient significantly induce low consumption of feed and water (p<0.05). The pH of drinking water was significantly reduced by LMA supplementation (p<0.001), consequently water consumption of 0.1% LMA in drinking water group was significantly lower than that of the Met sufficient diet group (0.0% LMA). However, the acidity of drinking water did not affect to acid-base value in the gastrointestinal tract, and population of *E. coli* or *Lactobasillus* spp in the ceacum. Adding LMA 0.05% in drinking water significantly increased villous height, villous surface area and crypth depth in the duodenum and jejunum (p<0.01). Concentration of propionic acid and total volatile fatty acid (total VFAs) were increased (p<0.001). The result indicate that adding LMA in drinking water 0.05% can be used as Met source and an acidifier to achieve maximal growth performance of broiler chicks via the improvement of gut morphology.

Keywords: broiler chicken, gastrointestinal tract, liquid DL-methionine hydroxy analog free acid, production performance

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Introduction

Antibiotics have been used to promote growth rate, FCR and diminishing the risk of disease (Gropp et al., 1992), while the concerning has extended of bacterial resistance and cross-resistance to antibiotic used in animal and human therapy is increasingly (Barton, 1998; Khachatourians, 1998). Therefore, alternative feed additives such as probiotic, prebiotic, herbal compounds and organic acid are considered to cope with following problems of antibiotics withdrawal. In addition, sanitary water is necessary for farm animals in order to achieve maximal performance since a lot of pathogenic bacteria including *Campyrobacter* spp., *E. coli, Salmonella* spp. and *Clostridium* spp. can be multiply in dirty water, and cause a great number of diseases which retard growth performance. Induce acidity in drinking water generally inhibit the pathogenic bacteria growth. Due to an acidifier's function of liquid DL-Methionine hydroxy analog free acid (LMA), Dibner and Buttin (2002) reported that LMA reduces pH of gastrointestinal tract (GIT) and shows anti-microbial benefits. In poultry, Xia et al. (2004) reported that supplementation of organic acids in diets promote villous height in the duodenum and jejunum of small intestine, while there is few reports focus on the effects of supplemental acidifiers in drinking water on small intestine morphology.

Methinonine (Met) is typically the first limiting amino acid in most corn-soy based diet of broiler chicken. The supplemental synthetic sources of Met are usually provided as powder form [DL-Methionine (DLM)] or liquid form of analog [LMA (88% active substance); dissociated constant (pKa) is between 3 and 4] (Dibner and Buttin, 2002). Bio-efficacy of LMA compared with DLM in diets on growth performance has been intensively reported (Thomas et al., 1991; Esteve-Garcia and Austic, 1993; Huyghebaert, 1993; Rostagno and Barbosa, 1995; Maenz and Engele-Schaan, 1996; Esteve-Garcia and Llaurado, 1997; Lemme et al., 2002). However, effect of LMA in drinking water on the improvement of production performance is not well understood. Reduction of pH in drinking water via LMA supplementation may be an alternative tool to improve the production performance. Moreover, derivatives of LMA such as taurine and glutathione can be used as antioxidant in gut (Shoveller et al., 2005).

However, there were less numerous reported effects of LMA in drinking water on growth performance (Damron and Flunker, 1992; Damron and Goodson-Williams, 1987; Baker, 1977), particularly there is no information published on the effect of adding LMA in drinking water on the population of microorganism in the digestive tract and small intestine morphology of poultry. Therefore, this study was conducted to evaluate the effects of LMA supplementation in drinking water on production performance and gastrointestinal tract of broiler chickens at 42 days of age.

Materials and Methods

Animal, Diets and Drinking Water: The study was completely randomized design. A total of 750 Ross 308 male broilers were obtained from a commercial hatchery on day 0 post-hatching. The chicks were allocated into 5 groups; each group replicated 6 times with 25 chicks each. They were kept, maintained and treated in compliance with the accepted standards for the human treatment of animals. The experimental design and procedures have been approved by the Authors' Institutions' Ethic Committee. The chicks were kept in floor pens (100x200 cm) located in an evaporative cooling house system; hence each chick had approximately 800 cm^2 of floor space. The temperature was set at 32ºC at first day and then was declined by 1ºC every 3 days until a final temperature of 25ºC was reached. The average house temperature and relative humidity (RH) were 25.7 ºC and 78.8%, respectively. The lighting management and vaccinations were provided according to the commercial practice. Water and feed were offered *ad libitum* throughout the experiment.

Feed formulation and calculated nutrients composition are shown in Table 1. The chicks were fed with two types diets consisted starter and grower as commercial recommendation (except negative control group). Diets were fed in crumble form and pellet form at starter (1-21 days old of age) and grower (22-42 days old of age) period, respectively. All of the experimental diets contained no growth factors, coccidiostats, exogenous enzymes, or antibiotics.

In term of drinking water, LMA solutions were batch-mixed daily in a large plastic box, and provide *ad libitum* to each pen and individually through plastic tubing and commercial watering nipples. Feed and water intake were recorded daily. The treatments were received differential diet and water as follows; 1) Met deficient diet (negative control group; TSAA was lower than commercial requirement recommendation) without DL-LMA in drinking water, 2) DLM supplementing diet according to commercial recommendation (meet TSAA requirement) without LMA in drinking water, 3) DLM supplementing diet with LMA 0.025% in drinking water, 4) DLM supplementing diet with LMA 0.050% in drinking water, and 5) DLM supplementing diet with LMA 0.10% in drinking water.

Measurements: Data on the following flock variables were collected: weight gain, feed and water intake, FCR, and mortality rate. The chicks will be individually weighted at 1 and 42 days of age. Each chick of each pen (6 birds/treatment) were randomly selected and sacrificed by asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2) for 1.5-2.0 min (Bunchasak et al., 2006). The carcasses were immediately opened and the entire of GI tract was removed and section for pH measurement. The pH of digesta from each portions of the GI tract was measured directly by pH meter (I.Q. scientific Instrument).

Sample Procedures: Intestinal samples were collected at 42 days of age to determine the populations of pathogenic bacteria (*E. coli*) and beneficial bacteria (*Lactobacillus* spp.) in the ceacum. Six birds (one per replication) were randomly selected from each group and killed by asphyxiation method. The dead chicks

were immediately opened and the entire ceacum removed aseptically. The cut sections of the ceaca were legated with a nylon suture.

Bacteriological Examinations: Approximately 5 g of the ceacal contents was mixed with 45 ml of peptone water and blended by stomacher (Stomacher Lab Blender 400, Seward Medical, United Kingdom) for 3 min. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in peptone water. The samples from the ceacum were diluted to 10^{-9}. For each dilution, 0.1 ml was inoculated on agar plate. The plate media are MRS agar (DifcoTM, Becton, Dickinson and Company, USA) for lactobacilli, and MacConkay agar (Britania Laboratories, Argentina) for *E.coli*. All the plates were incubated at 39°C. Total numbers of bacterial colonies were counted at the end of each incubation period. The MRS agar plates were incubated anaerobically for 24 h in anaerobic jars. MacConkay agar plates were incubated aerobically for 24 h in accordance with Brown (2005) and Biagi et al. (2006).

Table 1 Formulas and composition of basal diets

Ingredient	Amount			
	Starter		Grower	
	Negative group	Positive group	Negative group	Positive group
Corn 7.90%	45.825	45.825	50.600	50.600
Soybean meal 43.69%	42.000	42.000	36.470	36.470
Soybean oil	7.625	7.625	8.450	8.450
L-lysine HCl	0.000	0.000	0.035	0.035
DL-Methionine	0.000	0.300	0.000	0.210
L-Threonine	0.000	0.000	0.030	0.030
MDCP (Ca 16%, P 21%)	2.000	2.000	1.150	1.150
Limestone 38.67%	1.150	1.150	2.000	2.000
Sodium bicarbonate 27%	0.300	0.300	0.300	0.300
Salt	0.250	0.250	0.250	0.250
MTB100	0.050	0.050	0.050	0.050
Premix	0.500	0.500	0.500	0.500
Corn starch	0.300	0.000	0.21	0.000
Total	100.000	100.000	100.045	100.045
Chemical composition				
Energy (ME kcal/kg)	3099.963	3099.963	3204.761	3204.761
Crude Protein (%)	22.054	22.054	20.093	20.093
Lysine (%)	1.213	1.213	1.082	1.082
Methionine (%)	0.312	0.612	0.287	0.497
Total sulphur amino acid (%)	0.650	0.950	0.598	0.808
Calcium (%)	0.904	0.904	0.620	0.620
Available Phosphorus (%)	0.570	0.570	0.380	0.380

Premix: Broiler premix consisted of vitamin A 2,400,000 IU, vitamin D 480,000 IU, vitamin E 4,000 IU, vitamin K 0.49 g, vitamin B_1 0.38 g, vitamin B_2 0.998 g, vitamin B_6 0.398 g, vitamin B_{12} 0.004 g, nicotinic acid 0.60 g, pantotenic acid 2.956 g, choline chloride 80 g, folic acid 0.196 g, biotin 0.01 g, Cu 1.80 g, Fe 7.75 g, I 0.15 g, Mn 12 g, Zn 9 g, Se 0.20 g, carrier media to meet1 kg.

***Small Intestine Morphology*:** Segments of approximately 2 cm were taken from the midpoint of the duodenum (duodenum) and from the midpoint between the point of bile duct entry and Meckel's diverticulum (jejunum). Segments were gently flushed twice with normal saline (0.9% NaCl) to remove the intestinal contents and were fixed in fresh 10% buffered formaldehyde. All samples were dehydrated, cleared, and embedded in paraffin. Tissues were then stained with haematoxylin–eosin for histological evaluation. The villous height, crypt depth and the villous height to crypt depth ratio were determined. Measurements of villous height from the tip of the villous to the 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 each specimen.

Volatile Fatty Acid Analysis: The samples of digesta from the caecum were diluted in demonized distilled water (1:1) and centrifuged (TOMY model MX-301; TOMY Kogyo, Tokyo, Japan) in microfuge tubes at 17 800 g, 4 °C for 10 min, and 1 ml of supernatant were transferred to microfuge tubes. The concentrations of volatile fatty acids analyzed by gas chromatography (Shimadzu Model GC-2010 High-end; Shimadzu, Kyoto, Japan) and a flame ionization detector (GC-FID).

Statistical Analysis: Data were organized for mean, standard error, and relative value and for further

analysis. The data were analyzed statistically using analysis of variance. The different between the means of groups were separated by Duncan's New Multiple Range Test (Duncan, 1955). Statements of statistical significance were based on P<0.05. All statistical analyses were done in accordance with the method of Steel and Torrie (1980).

Results

Growth Performance: Effect of adding LMA in drinking water on growth performances are presented in Table 2. Growth performance of negative group was significantly poorer than those of the other groups. Growth rate, feed intake, water intake and FCR were clearly improved by DLM supplementation in diet. Adding LMA in drinking water at 0.025 or 0.05% significantly improved growth rate better than those of the Met sufficient diet group (0.0% LMA) (p <0.05). The pH of drinking water was significantly decreased parallel with the increasing of LMA in drinking water (p <0.01). Consequently, adding LMA at 0.1% in drinking water significantly depressed water consumption compare to diet without LMA in drinking water (p <0.05).

Table 2 Effect of adding DL-Methionine Hydroxy Analog Free Acid to drinking water on growth performance of broiler chickens at 42 Days of age

	Negative Control	DL-Met supplementing diet				SEM	*p*-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.100% in water		
Initial body weight (g)	41.87	42.33	41.33	41.87	41.87	0.68	0.99
Final body weight (g)	2455.41^{C}	2937.47^{B}	3029.09^{A}	3014.36^{A}	2977.16^{AB}	40.33	<0.001
ADG (g/d)	57.46^{C}	68.93^{B}	71.14^{A}	70.77^{A}	69.89^{AB}	0.96	<0.001
Feed intake (g/d)	107.50^{b}	118.00^{a}	120.68^{a}	115.27^{a}	118.86^{a}	1.29	0.005
Water intake (ml/d)	468.95^{c}	525.51^{a}	512.08^{ab}	507.25^{ab}	498.51^{b}	9.02	0.03
Water pH	7.54^{A}	7.54^{A}	5.43^{B}	3.62^{C}	3.20^{D}	0.37	<0.001
Dietary Met intake (mg/d)	316.34^{B}	601.91^{A}	614.28^{A}	587.63^{A}	605.46^{A}	0.03	<0.001
Watery LMA intake (mg/d)	0^{D}	0^{D}	128.51^{C}	254.24^{B}	498.32^{A}	0.02	<0.001
FCR	1.87^{A}	1.71^{B}	1.69^{B}	1.62^{B}	1.70^{B}	0.02	0.004
Mortality (%)	1.515	0.756	3.030	0.030	3.789	0.84	0.99

$^{a, b, c}$ Values within the same row with different superscripts are significantly different (p<0.05)
^{A,B,C} and ^{D} Values within the same row with different superscripts are significantly different (p<0.01)

Table 3 Effect of adding DL-Methionine Hydroxy Analog Free Acid to drinking water on carcass quality of broiler chickens at 42 days old of age

Carcass quality (% of body weight)	Negative Control	DL-Met supplementing diet				SEM	*p*-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.100% in water		
Carcass yield	90.81	92.46	92.53	91.81	92.53	0.24	0.14
Inner breast meat	3.13^{b}	3.72^{a}	3.74^{a}	3.79^{a}	3.77^{a}	0.06	<0.001
Outer breast meat	12.99^{b}	17.01^{a}	18.10^{a}	18.05^{a}	17.39^{a}	0.40	<0.001
Drumstick	9.93	9.68	9.07	9.03	9.26	0.12	0.08
Thigh	4.91^{A}	4.27^{B}	4.29^{B}	4.30^{B}	4.36^{B}	0.01	0.001
Wing	7.44^{b}	7.41^{b}	7.44^{b}	7.31^{b}	7.87^{a}	0.08	0.02
Abdominal fat	2.27^{A}	1.92^{B}	1.88^{B}	1.81^{B}	1.63^{B}	0.01	0.001

$^{a, b}$ Values within the same row with different superscripts are significantly different (p<0.05)
$^{A, B}$ Values within the same row with different superscripts are highly significant different (p<0.01)

Carcass Quality: Effects of adding LMA in drinking water on carcass quality are presented in Table 3. The carcass yield and drumstick were not affected by the treatments. The broiler received DL-Met supplementing diet had inner breast meat and outer breast meat heavier, while thigh and abdominal fat

lesser than those of the negative group ($p < 0.001$). Among the groups received DL-Met supplementing diet, both of inner and outer breast meat, thigh and abdominal fat were not differed by LMA levels in drinking water.

Acid-Base in Gastrointestinal Tract: The effect of LMA levels in drinking water on the pH of entire gastrointestinal tract present in Table 4. Adding LMA to drinking water did not significantly affect to the pH of entire GI tract ($p > 0.05$).

Bacteria Population and Volatile Fatty Acids in Ceacum: Population of *Lactobacillus* spp. and *E.coli* in ceacum of broiler chicks is present in Table 5. The number of *Lactobacillus* spp. and *E.coli* were not affected significantly by any treatment ($p >0.05$).

Effects of adding LMA in drinking water on volatile fatty acid in the ceacum are present in Table 6. Supplementing LMA in drinking water at 0.025% significantly increased the propionic acid and Total VFAs concentration of ceacal digesta ($p <0.05$).

Table 4 Effect of adding DL-Methionine Hydroxy Analog Free Acid to drinking water on pH of each portion of broiler chickens at 42 days old of age

	Negative Control	DL-Met supplementing diet				SEM	*p*-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.100% in water		
Crop	3.93	3.93	3.84	3.72	3.84	0.02	0.19
Proventiculus	2.61	3.21	2.55	2.11	2.53	0.10	0.18
Gizzard	2.17	2.78	2.59	2.88	2.13	0.06	0.34
Duodenum	5.14	4.74	4.80	4.74	4.69	0.09	0.85
Jejunum	4.90	4.89	4.18	4.84	4.81	0.11	0.99
Ileum	5.57	5.33	5.24	5.59	5.09	0.12	0.96
Ceacum	6.44	6.40	6.12	5.74	5.52	0.04	0.35
Colon	6.03	5.60	5.92	5.63	5.50	0.10	0.31

Table 5 Effect of adding DL-Methionine Hydroxy Analog Free Acid to drinking water on bacterial population (log CFU/g) in ceacum of chickens at 42 days old of age

	Negative Control	DL-Met supplementing diet				SEM	*p*-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.100% in water		
E. coli	8.83	8.99	8.57	8.67	8.53	0.18	0.92
Lactobacillus spp.	6.62	6.65	6.82	6.80	7.12	0.10	0.32

Table 6 Effect of adding Methionine Hydroxy Analog Free Acid to drinking water on volatile fatty acid of broiler chickens at 42 Days of age (mmol/l)

	Negative Control	DL-Met supplementing diet				SEM	*p*-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.100% in water		
Acetic Acid	2.92	4.71	4.21	4.81	6.33	0.48	0.230
Propionic Acid	13.35^{b}	11.74^{b}	57.80^{a}	16.96^{b}	21.68^{b}	5.37	0.028
Butyric Acid	2.43	1.69	7.86	2.38	2.44	0.79	0.108
Total Volatile Fatty Acid	18.71^{b}	18.65^{b}	68.83^{a}	23.26^{b}	27.74^{b}	6.18	0.041

$^{a, b, c}$ Values within the same row with different superscripts are significantly different ($p<0.05$)

Small Intestinal Morphology: The effect of adding LMA in drinking water on small intestine morphology is shown in Table 7. In duodenum segment, the negative group showed lower villous height, width, surface area and crypt depth than those of the other groups ($p <0.01$). Among the groups received LMA in drinking water, adding 0.05% LMA in drinking water have the highest villous surface area and villous width, while adding 0.025% and 0.1% LMA have villous surface area higher than those of 0% LMA supplementation. Adding 0.05-0.1% LMA showed the villous height and villous width/crypt depth ratio higher than those of 0% and 0.025% LMA in drinking water groups.

In jejunum segment, the negative group also has lowest villous height, surface area and villous width/crypt depth ratio. Among DL-Met supplementing diet groups, adding LMA 0.025% showed highest of villous height and surface area and crypt depth ($p <0.01$). The 0% LMA in drinking water

group has lower crypt depth, whilst higher villous width/crypt depth ratio compare to those of the others groups (p <0.01).

Discussion

In general, the production performance of chickens receiving low TSAA diet or amino acid imbalance diet is poor, and commercial recommendation of TSAA requirement is higher than that of NRC (1994) (Bunchasak *et al.*, 1996, 1997). In this study, poor growth performance of negative group (Met deficient group) may cause by low feed and water consumption. In agreement with general phenomena that feed consumption of growing chicks is directly related to body weight, and water requirement is related to feed consumption (Ward and McKague, 2007). To satisfy dietary TSAA consumption, DLM was supplemented in diet according to commercial recommendation that was higher than the recommendation of NRC (1994) for 0.1% and 0.05% during starter and grower period, respectively. Adding LMA in drinking water also caused significant stepwise increasing of daily TSAA intakes. Thus, it could be said that chickens received Met intake higher than commercial recommendation (additional Met source from watery LMA around 0.025-0.05%) could achieve maximum growth performance compare to the DLM supplementation alone.

Table 7 Effect of adding DL-Methionine Hydroxy Analog Free Acid to drinking water on small intestinal morphology of broiler chickens at 42 Days of age

	Negative Control	DL-Met supplementing diet				SEM	P-value
		LMA 0.0% in water	LMA 0.025% in water	LMA 0.050% in water	LMA 0.10% in water		
Duodenum							
Villous height (μm)	1437.71^{C}	1511.37^{BC}	1567.18^{B}	1729.73^{A}	1670.75^{A}	14.46	<0.01
Villous width (μm)	82.24^{D}	89.54^{C}	95.02^{AB}	96.92^{A}	90.97^{BC}	0.86	<0.01
Villous surface area (mm^2)	0.120^{D}	0.136^{C}	0.149^{B}	0.166^{A}	0.152^{B}	0.0002	<0.01
Crypt depth (μm)	208.60^{B}	235.02^{A}	234.80^{A}	235.66^{A}	222.53^{AB}	2.64	<0.01
Villous width/ Crypt depth	7.29A^{B}	6.70^{B}	6.80^{B}	7.66^{A}	7.76^{A}	0.10	<0.01
Jejunum							
Villous height (μm)	936.49^{C}	1107.73^{B}	1320.45^{A}	1151.91^{B}	1140.80^{B}	14.55	<0.01
Villous width (μm)	78.66^{AB}	81.50^{A}	76.19^{B}	81.94^{A}	78.64^{AB}	0.67	<0.05
Villous surface area (mm^2)	0.074^{D}	0.081^{CD}	0.100^{A}	0.096^{AB}	0.089^{BC}	0.0015	<0.01
Crypt depth (μm)	181.31^{CD}	168.28^{D}	220.58^{A}	208.73^{AB}	193.22^{BC}	2.95	<0.01
Villous width/ Crypt depth	5.59^{B}	6.83^{A}	6.14^{B}	5.75^{B}	6.15^{B}	0.10	<0.01

$^{a, b, c}$ Values within the same row with different superscripts are significantly different (p<0.05)
^{A,B,C} Values within the same row with different superscripts are significantly different (p<0.01)

Several researchers found the adversely effects of adding organic acid in drinking water on daily water intake (Cave, 1984; Avila et al., 2003; Moharrery and Mahzonieh, 2005) and Baker and Boebel (1980) reported that 50% diminish of water consumption in growing chicks given Met in drinking water. In this study, similarly, feed intake slightly decreased, particularly water intake was significantly diminished by increasing of LMA in drinking water due to strong acidification of the water, and excess Met intake always depress production performance. Therefore, ADG and FCR of 0.10% LMA in drinking water group was tended to be poor. However, LMA provided in the drinking water (0.025 - 0.05%) reduced pH of the water (from 5.43 to 3.62) did not negatively affect to feed intake, and high growth performance was maintained. This indicates that as long as low pH in drinking water caused by LMA supplementation is kept around 3.62-5.43, the supplementations would have benefit to the growth performance of broiler chickens.

The major portion of the protein synthesis in body represent by the breast meat yield that is sensitive

to amino acid status of the diet (Moran 1994; Café and Waldroup, 2006). Vieira et al. (2004) stated that TSSA supplementation strongly affected the production of breast meat (improved 10%). Moreover, Rakangtong and Bunchasak (2011) stated that the growth of breast meat is more sensitive to limiting amino acid (TSAA) in the diet than that of other edible meat component. Due to Met play functional role in protein synthesis of broiler chicken (Meirelles et al., 2003), it is general accepted that supplementing Met into diet increase breast meat, while abdominal fat is decreased (Mendoca and Jensen, 1989; Hickling et al., 1990; Gorman and Balnave, 1995; Albino et al., 1999; Wallis, 1999; Jianlin et al., 2004; Bunchasak and Keawarun, 2006). Since TSAA in diet was set at commercial requirement to achieve maximal protein synthesis, supplementing LMA in drinking water only slightly enhanced breast meat and reduced abdominal fat.

Maenz and Engele-Schaan (1996) found that dietary radiolabel remaining as unabsorbed form of ^{3}H-LMA in the broiler intestine was greater than that form of ^{3}H-L-Met-suplementing diets. This remanding may reduces pH of the GIT and show antimicrobial benefits (Dibner and Buttin, 2002). Unfortunately, adding LMA in drinking water did not significantly affect to the pH of entire GIT. Similarly, Avila et al. (2003), Moharrery and Mahzonieh (2005) and Chaveerach et al. (2004) stated that adding organic acid to drinking water did not reduce pH of gut digesta. It may because of an excellent acid-base homeostasis in the GIT and also the strong buffering capacity of dietary ingredients (corn–soybean based) (Dibner and Buttin, 2002; Gauthier, 2005). Moreover, dynamic situation of the GIT is dependent on many factors such as health, nutrients, microflora content as well as interrelation among these factors (Sarra et al., 1985). It is suggested that increasing acidity of drinking water by LMA supplementation is not directly related to the pH in GIT.

Organic acids have been studied as a tool to reduce unwanted bacteria (Griggs and Jacob, 2005), and formic acid in particular has been shown to be particularly effective against *Escherichia coli* (Ratcliff, 2000). The beneficial effects of organic acids on the productive traits of pigs have been demonstrated in many studies, but consistent data have not been obtained for poultry (Langhout, 2000). Most of the pathogens grow in a pH over 6 or slightly higher (Hai Meng, 2006; Dhawale, 2005), while beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens (Ferd, 1974). However, the lowering of pH in drinking water by LMA supplementation did not affect to gastrointestinal pH and bacteria population. Accordingly, Dahiya et al. (2007) could not found any effects of dietary Met source on intestinal microbial population in broiler chicken.

Based on the diversity of ceacal microbial ecosystem, the ceacal microflora utilize some components not digested by the host animal (Jozefiak et al. 2004) and transform into short-chain fatty acids (SCFAs) or VFAs and gases (Marounek et al., 1999; Jamroz et al., 2002). The quantity of VFAs produced in the large intestines depends on the amount and composition of the substrate and on the microflora present in the large intestines (van Beers-Schreurs et al., 1998). Ceacal propionic acid and total VFAs were increased by adding LMA in drinking water, particular in 0.025% LMA group. Since Met is glucogenic amino acid, which can be converted into succinate an intermediate in the Kreb's Cycle. High feed consumption of 0.025% LMA in drinking water group may increased the utilization of succinic acid by some anaerobic bacteria such as Bacteroides, Clostidia, Propionaibacteria and Veiilonella and resulting in propionate production (Józefiak et al, 2004). Moreover, increasing of total VFAs was agreed with Nisbet et al. (1994) who reported that a 100-fold increasing of competitive exclusion cultures was shown significantly increase VFAs in the ceca of 3-day-old chicks and this increase was mostly due to the increase in cecal propionic concentration.

High positive effect of adding LMA in drinking water on villous high and width and crypt dept were found in both duodenum and jejunum segments. Until LMA alter to L-Met, an acidifier property of LMA is similar to those of organic acids. There is an α-hydroxy mono-carboxylic acid that similar to lactic acid, therefore its biological activity resembles those of other organic acids (Dibner, 2003). Accordingly, Xia et al. (2004) and Pelicano et al. (2005) reported that higher villous in duodenum and jejunum in small intestine with most organic acidifiers supplementation in diet of chicks.

Met is a nutritionally essential amino acid required for many important metabolic functions: 1) protein synthesis; 2) transmethylation to form S-adenosylmethionine (SAM) that methylated compound to form such products as creatine, phosphatidylcholine and also participation of polyamine synthesis; and 3) transsulfuration to form L-Cys that can be encompassed into glutathione and taurine. As a glutathione and taurine precursor, L-Cys plays a functional role in intestinal epithelial antioxidant, and it may also regulate epithelial cell proliferation and differentiation via modulation of redox status (Huxtable, 1992; O'Flaherty et al., 1997; Lambert, 2004; Roig-Pérez et al., 2005; Shoveller et al., 2005). Recently, Yodseranee and Bunchasak (2012) reported that plasma taurine and uric acid concentration were significantly elevated by LMA supplementation. Therefore, adding LMA in drinking water may increase cell proliferation and differentiation of enterocyte processes by the enhancing of L-Cys, glutathione and taurine products.

Therefore, we suggest that adding LMA in drinking water may affect small intestinal morphology via three mechanisms: (i) LMA directly stimulates cell proliferation and/or cell number as amino acid 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 (Lambert, 2004; Roig- Pérez et al., 2005; Shoveller et al., 2005) and because of the tendency for low pH in the small intestines when LMA was added, this condition may be suitable to the growth of lactic acid bacteria population and then produce short chain fatty acids.

In conclusion, it could be implied that adding DL-LMA in drinking water can be used as methionine

source and an acidifier to achieve maximal growth performance of broiler chicks. Dose recommendation of DL-LMA in drinking water was 0.05 percentages.

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บทคัดย่อ

ผลของระดับ Liquid DL-Methionine Hydroxy Analog Free Acid ในน้ำดื่มต่อสมรรถภาพการผลิต และท่อทางเดินอาหารของไก่กระทงที่อายุ 42 วัน

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ศึกษาการเสริมเมทไธโอนีนเหลว (Liquid DL-methionine hydroxyl analog free acid: LMA) ในน้ำดื่ม ต่อสมรรถภาพการผลิตและทางเดินอาหารไก่กระทงอายุ 1-42 วัน โดยแบ่งไก่ออกเป็น 5 กลุ่ม ดังนี้ กลุ่มที่1 (negative control) ได้รับอาหารขาดกรดอะมิโนเมทไธโอนีน (DL-methionine: DLM) และไม่เสริม LMA ในน้ำดื่ม กลุ่มที่ 2 3 4 และ 5 ได้รับอาหารเสริม DLM ให้มีระดับกรดอะมิโนที่มีกำมะถันเป็นองค์ประกอบทั้งหมดตรงกับความต้องการ (total sulfur amino acids: TSAA) โดยแต่ละกลุ่มจะได้รับน้ำดื่มที่มีระดับ LMA 0% 0.025% 0.05% และ 0.1% ตามลำดับ การเสริม DLM ในอาหารปรับปรุงประสิทธิภาพการใช้อาหาร (FCR) และอัตราการเติบโตต่อวัน (ADG) นอกจากนั้นภายในกลุ่มที่ได้รับอาหารเสริม DLM ไก่ที่ได้รับการเสริม LMA ในน้ำดื่ม 0.025% และ 0.05% มีประสิทธิภาพการผลิตดีกว่ากลุ่มอื่นๆ อย่างมีนัยสำคัญยิ่งทางสถิติ ($p<0.01$) การได้รับอาหารที่มีเมทไธโอนีนไม่เพียงพอส่งผลให้ไก่กินอาหารและน้ำดื่มลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และการเสริม LMA ในน้ำดื่มทำให้ค่าความเป็นกรด-ด่างในน้ำดื่มลดลงอย่างมีนัยสำคัญยิ่งทางสถิติ ($p <0.001$) ส่งผลให้กลุ่มที่ได้รับน้ำดื่มเสริม LMA 0.1% มีปริมาณการกินน้ำน้อยกว่ากลุ่มที่ได้รับอาหารขาดกรดอะมิโนเมทไธโอนีน (0.0% LMA) อย่างไรก็ตามความเป็นกรดในน้ำดื่มไม่ส่งผลต่อค่าความเป็นกรด-ด่าง และจำนวน *E. coli* หรือ *Lactobasillus* spp. ในทางเดินอาหาร การเสริม LMA 0.05% ในน้ำดื่มทำให้ความยาว villi พื้นที่ผิว villi และความลึก crypth ในลำไส้เล็กส่วนต้น (duodenum) และส่วนกลาง (jejunum) เพิ่มขึ้นอย่างมีนัยสำคัญยิ่งทางสถิติ ($p <0.01$) ปริมาณกรด propionic และกรดไขมันระเหยได้ทั้งหมด (total volatile fatty acids: VFAs) เพิ่มขึ้นอย่างมีนัยสำคัญยิ่งทางสถิติ ($p <0.001$) จากผลการทดลองสรุปได้ว่าการเสริม LMA ในน้ำดื่ม0.05% สามารถเป็นทั้งแหล่งของกรดอะมิโนเมทไธโอนีนและสารให้ความเป็นกรด (Acidifier) เพื่อประสิทธิภาพ การเติบโตสูงสุดของไก่กระทงโดยการปรับปรุงลักษณะทางสัณฐานวิทยาในทางเดินอาหาร

คำสำคัญ: ไก่กระทง ทางเดินอาหาร เมทโธโอนีนเหลว ประสิทธิภาพการผลิต

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