Influence of Essential Oil Components on Growth Performance and the Functional Activity of the Pancreas and Small Intestine in Broiler Chickens*

I. S. Jang, Y. H. Ko, H. Y. Yang, J. S. Ha, J. Y. Kim, J. Y. Kim, S. Y. Kang, D. H. Yoo[1], D. S. Nam[1] D. H. Kim and C. Y. Lee**

Department of Animal Science & Technology, Graduate School, RAIRC, Jinju National University
Chilam-Dong 150, Jinju, Gyeongnam, 660-758, Korea

ABSTRACT : To investigate the efficacy of alternatives to antibiotics, the present study was conducted to compare the effects of antibiotic, lactic acid, a blend of commercial essential oils (EOs) and EOs in combination with lactic acid on growth performance and the functional activity of the gut in broiler chickens. A total of 168 broiler chickens were given the basal diet supplemented with 10 ppm colistin (T1), 0.1% lactic acid (T2), 25 ppm EOs (T3), 25 ppm EOs+0.1% lactic acid (T4), 50 ppm EOs (T5) or 50 ppm EOs+0.1% lactic acid (T6) in the period 3 to 35 days of age. As a result, the broiler chickens assigned to T4 group throughout the experimental period had apparently ($p<0.05$) greater body weight and total gain than these assigned to T1, T2, T3 and T5 groups. However, there was no difference in growth performance among the birds fed the diets supplemented with antibiotic (T1), lactic acid (T2) and EOs (T3 and T5) alone. The weights of digestive organs and the number of *lactobacilli* and *E. coli* in the lower ileum were not affected by dietary treatments. Total trypsin activity was significantly ($p<0.05$) greater in T4 than T1, T2, T3 and T5 groups. Total and specific pancreatic α-amylase activities were significantly ($p<0.05$) enhanced in the broiler chickens fed T4 diet compared with these fed T1, T2 and T3 diets. However, there were no differences in growth performance and digestive enzyme activities including pancreatic trypsin and α-amylase between T4 and T6 groups fed the diets supplemented with either low or high EOs levels in combination of lactic acid. In conclusion, a blend of commercial EOs combined with lactic acid showed significant increases in digestive enzyme activities of the pancreas and intestinal mucosa, leading to increase in growth performance. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 394-400)***

Key Words : Essential Oils, Lactic Acid, Trypsin, α-Amylase, Maltase, Broiler Chickens

INTRODUCTION

It has been widely recognized that the manipulations of gut functions and microbial habitat in pigs have a crucial role in promoting growth performance and feed efficiency (Collington et al., 1990). For the purpose of these accomplishments, a number of antimicrobial compounds, especially including antibiotics have been extensively used in the poultry and pig industries for several decades. It was reported that approximately up to 80% of domestic animals have been received drugs and synthetic chemicals for the purpose of either medication or growth promotion (Lee et al., 2001). The recent concerns about possible antibiotic residues and resistance infection have raised a great attention on the use of antibiotics in food animals. Thus, a great effort has been attempted to find alternatives to antibiotics as growth promoters in animal industry (Wenk, 2000). As one of alternatives, essential oils (EOs) generally recognized as safe admitted by Food and Drug Administration (FDA), which enhance nutrient digestibility and inhibit microbial growth in the gut, are of interest as feed additives to improve the efficiency of animal production under intensive management programs (William and Losa, 2001). It has been known that EOs extracted from herb and spices are composed of various mixtures of aromatic and volatile substances (Cowan, 1999). Thymol and carvacrol, two major components of EOs derived from thyme and oregano, have biological properties such as antimicrobial, antioxidant and antiseptic activities (Lawrence and Reynolds, 1984). There have been a few studies on the effects of EOs on growth performance and digestive functions in broiler chickens. The results obtained from several studies with chickens are either significant (Bassett, 2000; Kamel, 2001) or non-significant (Case et al., 1995; Botsoglou et al., 2002) in growth performance and digestive processes. A beneficial effect of EO-mediated improvement in animal production was associated with increases in antimicrobial and digestive activities (Lee, 2002), although there is still a lack of evidence of the underlying mechanisms by which dietary EOs affect growth performance. However, *in vivo* study, results in response to dietary EOs have shown to be affected by intrinsic and extrinsic factors including environment, diet and nutritional status.

Lactic acid has been also extensively utilized as a feed additive to improve feed hygiene and gut milieu (van de

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** Corresponding Author: In Surk Jang. Tel: +82-55-751-3236, Fax: , E-mail: isjang@jinju.ac.kr

[1] ALL THE BEST (Co, Ltd), Seongnae-dong 558-5, Kangdong-ku, Seoul, Korea.

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Table 1. Formula of the basal diets fed to broiler chickens

Item	Diets	
	Starter	Finisher
Ingredients, %		
Corn	38.16	44.13
Wheat	20.00	20.00
Wheat bran	5.00	4.00
Animal fat	2.20	3.00
Corn gluten	4.00	4.00
Soybean meal(44% CP)	23.00	16.50
Rapeseed meal	1.50	2.00
Fish meal	1.00	1.00
Meat meal	2.00	2.00
Salt	0.20	0.23
Calcium carbonate	0.40	0.20
Tricalcium phosphate	1.40	1.60
Lysine (liquid)	0.46	0.66
Methionine	0.13	0.12
Choline-HCl	-	0.01
Vitamin premix[1]	0.20	0.20
Mineral premix[2]	0.20	0.20
Maduramycin+nicarbazine	0.05	-
Salinomycin	-	0.05
Colistin sulfate (T1 treatment only)	0.10	0.10
Chemical composition, %		
Moisture	11.69	11.60
Crude protein	21.11	19.05
Crude fat	4.78	5.62
Crude fiber	3.63	3.57
Crude ash	4.86	4.68
Ca	0.82	0.81
P	0.64	0.66

[1] Contained per kg: vit. A, 5,500,000 IU; vit D3, 1,500,000 IU; vit E, 15,000 mg; vit K, 800 mg; thiamin, 1,000 mg; riboflavin, 4,000 mg; niacin, 25,000 mg; biotin, 30 mg; folic acid, 500 mg pantothenic acid, 5,000 mg, pyridoxine, 1,500 mg; vitamin B12, 15 mg.

[2] Contained per kg: Cu, 12,000 mg; Fe, 35,000 mg; Zn, 25,000 mg; Co, 150 mg; I, 500 mg; Se, 120 mg; Mn, 38,000 mg.

Broek, 2000). Thus, it is reasonable to postulate that the combination of these two feed additives would give positive effect on the performance of broiler chickens.

Therefore, to investigate possible efficacy of alternatives to antibiotics, the present study was conducted to compare the effects of antibiotic, lactic acid, a commercial EOs and EOs in combination with lactic acid on growth performance and digestive processes in broiler chickens.

MATERIALS AND METHODS

Animals and experimental design

A total of 168, one-day old male broiler chicks (ROSS) were purchased from Halim (Co.), Korea and kept in a wire cages in a room equipped with temperature (33-23°C) and on a light/dark cycle (light on 07:00-22:00). The experimental groups consisted of six dietary treatments; each treatment had fourteen replicate cages with 28 chicks. Immediately after a 2 days adjustment period, the broiler chicks were randomly assigned to the soy-corn-wheat basal diets (powdered form, Table 1) supplemented with 10 ppm colistin (T1), 0.1% lactic acid (T2), 25 ppm a blend of commercial essential oils (EOs, T3), 25 ppm EOs+0.1% lactic acid (T4), 50 ppm EOs (T5) and 50 ppm EOs+0.1% lactic acid (T6). A commercial blend of EOs was CRINA® Poultry contained 29% of active ingredients including thymol (Akzo Nobel, Crina S.A, Switzerland). The EOs and lactic acid (50% purity) were kindly provided by ALL THE BEST (Co. Ltd), Korea.

All birds were fed the respective starter (3 to 21 days) and finisher (22 to 35 days) diets *ad libitum* and had free acess to water for the entire period (day 3 to 35). Feed intake and BW were monitored on days 3, 21 and 35 after birth to determine growth performance and feed conversion ratio (FCR).

Tissue harvesting

At the end of 35 days feeding trial including adjustment period, eight broiler chickens weighing similar to average body weight per group were sacrificed by cervical dislocation to harvest the pancreas, small intestine and liver. Immediately after bleeding, the pancreas and liver were harvested and weighed. The small intestine was removed at the pylorus and at the ilio-cecal valve immediately after opening abdominal cavity. The harvested small intestine was perfused with 0.9% ice-cold saline and gently squeezed to remove remaining digesta. Sixty percent of the upper region was designated as the proximal intestine and the rest of the segment as the distal region. The length of each segment was rinsed in three successive baths containing mannitol buffer (5 mM $MgCl_2$, 150 mM mannitol, 10 mM Tris succinate, 5 mM K_2HPO_4 and 1 mM $MnCl_2$; pH 7.4). Immediately after washing, the intestinal segments were replaced in an aluminum pan on a bed of ice. The mucosal surface was removed by gentle scraping with a glass slide to obtain epithelial tissues. Residual fat and digesta were removed from the harvested mucosal tissues by resuspension in equal volumes of mannitol buffer followed by centrifugation at 10,000×g at 4°C for 12 min.

Microbial enumeration

The number of colony forming units (CFU) in intestinal digesta (1 g) harvested from the lower ileum was determined by 10 fold serial dilution using sterilized distilled H_2O. The *Lactobacillus* count was determined using MRS agar (Difco) after incubation an aerobic

Table 2. Growth performance, feed inake and feed conversion ratio (FCR) from 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, essential oils (EOs) and EOs in combination with lactic acid

Item	Treatment*					
	T1	T2	T3	T4	T5	T6
Initial BW	81.66±6.03	83.71±5.31	83.33±4.49	80.88±4.45	82.99±5.07	84.74±3.09
3-21 days						
BW	755.50±44.19	742.36±34.84	751.93±46.46	730.57±26.53	771.93±35.21	746.43±34.03
Gain	674.00±40.44	658.43±36.89	668.21±44.44	649.71±25.63	688.64±32.55	661.57±33.91
FI	985.64±73.73	958.07±55.77	999.64±59.63	962.29±65.57	1,006.50±58.90	938.71±64.22
FCR	1.47±0.10	1.46±0.09	1.50±0.10	1.48±0.11	1.46±0.07	1.42±0.09
22-35 days						
BW	1,532.36±75.39^{c}	1,574.71±72.68^{bc}	1,550.36±78.81^{bc}	1,707.79±69.71^{a}	1,595.43±76.73^{bc}	1,630.50±102.99^{ab}
Gain	776.86±83.45^{c}	833.07±85.28^{bc}	799.07±78.57^{bc}	977.43±56.66^{a}	824.14±75.40^{bc}	884.43±86.82^{b}
FI	1,662.86±151.70^{ab}	1,604.68±186.46^{b}	1,679.59±98.66^{ab}	1,801.36±127.77^{a}	1,639.61±110.08^{b}	1,685.89±102.10^{ab}
FCR	2.15±0.21^{a}	1.93±0.32^{abc}	2.10±0.21^{ab}	1.84±0.09^{c}	1.99±0.17^{abc}	1.92±0.14^{bc}
Over all						
Total gain	1,450.86±74.55^{c}	1,490.93±73.81^{bc}	1,467.07±78.74^{bc}	1,627.00±68.71^{a}	1,512.57±77.38^{bc}	1,547.07±103.34^{ab}
Total FI	2,648.50±156.54^{ab}	2,563.07±200.74^{b}	2,679.57±93.48^{ab}	2,763.86±171.80^{a}	2,646.29±108.96^{ab}	2,624.93±110.82^{ab}
Total FCR	1.83±0.08^{a}	1.72±0.18^{ab}	1.83±0.10^{a}	1.70±0.07^{b}	1.75±0.09^{ab}	1.70±0.07^{b}

* T1 (colistin, 10 ppm), T2 (lactic acid 0.1%), T3 (EOs, 25 ppm), T4 (EOs, 25 ppm+lactic acid, 0.1%), T5 (EOs, 50 ppm); T6 (EOs, 50 ppm+lactic acid, 0.1%). $^{a, b, c}$ Values with different superscripts are significantly ($P<0.05$) different between treatment groups. Mean±SD (pen=14).

chamber at 37°C for 48 h. The *E. Coli* were enumerated on MacConkey agar (Difco) after aerobic incubation at 37°C for 24 h.

Determination of enzyme activities

The whole pancreas was homogenized with 1:5 volumes of isolation buffer containing 0.5 M Tris and 0.154 M KCl (pH, 7.4) in a tissue grinder. The aliquots were stored at -70°C for later assay of enzyme activities. Activity of α-amylase was determined using starch potato as the substrate and measuring the amount of reducing sugars liberated with maltose as the standard at 450 nm (Bernfeld, 1955). One unit was defined as liberated 1.0 mg of maltose from starch per 3 min at pH 6.9 at 20°C. Trypsin activity was measured using benzoyl-L-arginine ethyl ester (BAEE) as the substrate at 253 nm according to the method of Geiger and Fritz (1986) after activation with 0.1 U/ml enterokinase. One unit of enzyme activity is defined as the amount of enzyme that hydrolyses 1 μmole of BAEE per min at pH 8.0 and at 25°C. The harvested mucosa was weighed and homogenized with 1:6 volumes of mannitol buffer in a tissue homogenizer. The aliquots were stored at -70°C for later assay of enzyme activities. To determine activities of intestinal enzymes, the homogenized tissue was diluted 2 times with 2% triton X-100 to separate enzymes from the membrane fraction. Alkaline phosphatase was assayed with a Sigma Diagnostic Assay Kit (Procedure No, 245) and an ELISA reader (V_{max} Molecular Device) to determine the continuous increase in absorbance at 405 nm. One unit of activity is defined as the amount of one μmole of p-nitrophenol per min under the assay conditions. Leucine aminopeptidase activity was determined by the slightly modified method of Rybina et al. (1997). The assay was performed with L-Leucine-p-nitroanilide as the substrate. The amount of liberated p-nitroaniline was measured using an ELISA reader at 405 nm. One unit is equal to the produced one μmole of p-nitroanilide per min under the assay conditions. Maltase and sucrase activities were determined by the modified procedure of Dahlgvist (1968). The end product, glucose was measured by an ELISA reader at 450 nm. The bicinchronic acid (BCA protein assay kit; Pierce) method was adapted to 96 well plate to determine protein concentration. Specific activity of each enzyme was expressed as the total activity per mg of protein.

Statistical analysis

Effect of diet was analyzed by Proc GLM (SAS Institute Inc., 1989). When the diet effect was significant at $p<0.05$, Tukey's test was applied to identify significant differences between groups. The level of probability for statistical difference was established at $p<0.05$.

RESULTS

Growth performance, FCR and mortality

Growth performance, feed intake and FCR in the broiler chickens fed the diets containing antibiotic, lactic acid, EOs and EOs combined with lactic acid are presented in Table 2. For the period of 3-21 days, BW, feed intake and FCR were shown to be similar among the six treatment groups.

Table 3. The weights of digestive organs from 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, essential oils (EOs) and EOs in combination with lactic acid

Treatment*	Liver	Pancreas	Proximal intestine	Distal intestine	Proximal mucosal tissues	Distal mucosal tissues
	g					
T1	56.10±18.87	3.39±0.53	16.90±1.93	12.61±1.34	10.64±2.22	4.97±1.10
T2	70.73±11.07	3.68±0.52	18.28±2.42	11.43±2.38	11.71±1.58	4.02±1.28
T3	73.08±13.67	3.42±0.44	18.77±3.35	`10.71±1.12	12.06±2.64	4.59±0.73
T4	55.16±7.12	4.13±0.75	18.23±2.16	12.61±1.54	12.73±1.39	5.42±1.20
T5	70.35±16.39	3.79±0.70	16.62±1.04	11.61±1.35	10.98±0.93	4.86±0.75
T6	73.94±13.12	3.84±0.53	18.20±2.33	11.90±1.97	12.63±2.10	5.35±0.73

* T1 (colistin, 10 ppm), T2 (lactic acid 0.1%), T3 (EOs, 25 ppm), T4 (EOs, 25 ppm+lactic acid, 0.1%), T5 (EOs, 50 ppm) and T6 (EOs, 50 ppm+lactic acid, 0.1%). Mean±SD (n=8).

Table 4. Microbial enumeration in ileal digesta from 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, essential oils (EOs) and EOs in combination with lactic acid

Treatment*	*Lactobacilli*	*E. coli*
	CFU, log/g	
T1	5.66±0.50	3.00±0.38
T2	5.94±0.28	2.84±0.64
T3	6.04±0.92	2.69±0.96
T4	5.61±0.92	2.78±0.92
T5	6.02±0.75	3.27±0.88
T6	6.04±0.72	2.39±1.10

* T1 (colistin, 10 ppm), T2 (lactic acid 0.1%), T3 (EOs, 25 ppm), T4 (EOs, 25 ppm+lactic acid, 0.1%), T5 (EOs, 50 ppm) and T6 (EOs, 50 ppm+lactic acid, 0.1%). Mean±SD (n=6-8).
CFU: Colony forming unit.

However, for the period 22-35 days, the broiler chickens assigned to T4 group resulted in significant ($p<0.05$) increases in BW and gain compared with those assigned to T1, T2, T3 and T5 groups. Also, BW and total gain on a cumulative basis (3-35 days) were remarkably ($p<0.05$) higher in T4 group compared with those in T1, T2, T3 and T5 groups. However, there were no differences in BW, total gain and FCR among the birds fed the diets supplemented with antibiotic (T1), lactic acid (T2) and EOs (T3 and T5) alone. BW, feed intake and FCR between T4 and T6 groups fed the diets supplemented with either low or high EOs levels in combination of lactic acid were not differed throughout the experimental period. There was only one bird dead in T6 group during the entire feeding period.

Organ weights

Table 3 presents changes in the weights of the liver, pancreas, intestine (proximal and distal regions) and wet mucosal tissues harvested from 35 day-old broiler chickens in response to dietary feed additives. The weights of the liver, small intestine and mucosal tissues from broiler chickens were not affected by dietary treatments. Pancreas weight tended to be numerically greater ($p<0.10$) by on a average 30% in the birds fed the diet supplemented with 25 ppm EOs+0.1% lactic acid (T4) compared with these fed the diets with antibiotics (T1), lactic acid (T2) and EOs (T3 and T5) alone.

Microbial enumeration

The effects of dietary feed additives on ileal microbial counts are shown in Table 4. The numbers of *lactobacilli* and *E. coli* in intestinal contents harvested from the lower ileum were not influenced by dietary supplementation of feed additives.

Digestive enzyme activities in the pancreas and small intestine

The activities of pancreatic and intestinal enzymes in 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, EOs alone and EOs combined with lactic acid are presented in Table 5 and 6. Total trypsin activity was significantly ($p<0.05$) greater in T4 than T1, T2, T3 and T5 groups, but specific activity of trypsin was not different among dietary treatments (Table 5). In pancreatic α-amylase, total and specific pancreatic amylase activities were markedly ($p<0.05$) greater in the broiler chickens fed T4 diet compared with those fed T1, T2 and T3 diets. There were no differences in pancreatic trypsin and α-amylase activities between T4 and T6 groups fed the diets supplemented with either low or high EOs levels in combination of lactic acid. In addition, both T5 and T6 groups showed a tendency to increase in total and specific amylase activities compared with T1, T2 and T3 groups without a statistical difference. However, no significant differences in pancreatic enzyme activities were found among the birds fed the diets supplemented with antibiotics (T1), lactic acid (T2) and the low (T3) and high (T5) levels of EOs alone. The specific activity of maltase in the proximal region was much greater ($p<0.05$) in the birds fed the diets T4 diet than these fed T2, T3 and T5 diets (Table 6). Proximal sucrase, alkaline phosphatase and leucine aminopeptidase activities were not affected by dietary feed additives.

Table 5. The activities of pancreatic trypsin and α-amylase from 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, essential oils (EOs) and EOs oils in combination with lactic acid

Treatment*	Total trypsin activity, U/total pancreas	Specific trypsin activity, U/mg protein	Total amylase activity, KU/total pancreas	Specific amylase activity, U/mg protein
T1	46.96±8.19^{b}	0.116±0.02	32.16±6.13^{bc}	79.12±9.35^{c}
T2	45.88±9.10^{b}	0.129±0.02	30.85±9.70^{c}	86.20±18.46^{bc}
T3	43.89±10.92^{b}	0.110±0.02	31.20±6.83^{c}	73.34±16.17^{c}
T4	68.24±14.80^{a}	0.135±0.03	57.99±16.58^{a}	112.47±22.40^{a}
T5	46.08±9.00^{b}	0.128±0.02	38.58±6.77^{bc}	107.57±14.14^{ab}
T6	54.26±7.61^{ab}	0.116±0.01	46.38±8.15^{ab}	99.32±12.84^{abc}

* T1 (colistin, 10 ppm), T2 (lactic acid 0.1%), T3 (EOs, 25 ppm), T4 (EOs, 25 ppm+lactic acid, 0.1%), T5 (EOs, 50 ppm) and T6 (EOs, 50 ppm+lactic acid, 0.1%). $^{a, b, c}$ Values with different superscripts are significantly ($p<0.05$) different between treatments. Mean±SD (n=8).

Table 6. The activities of disaccharidase, alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) in the proximal intestine from 35 day-old broiler chickens fed the diets containing antibiotic, lactic acid, essential oils (EOs) and EOs in combination with lactic acid

Treatment*	Total maltase activity, μmole/g mucosa	Specific maltase activity, μmole/mg protein	Total sucrase activity, μmole/g mucosa	Specific sucrase activity, μmole/mg protein	Total ALP activity U/g mucosa	Specific ALP activity U/mg protein	Total LAP activity U/g mucosa	Specific LAP activity U/mg protein
T1	10.60±2.80^{ab}	0.21±0.09^{ab}	2.01±0.45	0.036±0.005	5.19±3.48	0.091±0.042	433.04±54.67	7.94±1.41
T2	9.70±3.43^{b}	0.17±0.06^{b}	1.61±0.18	0.029±0.003	5.17±2.07	0.091±0.035	451.82±62.94	8.00±0.61
T3	8.50±3.96^{b}	0.15±0.07^{b}	1.64±0.24	0.03±0.004	3.37±1.17	0.059±0.015	413.34±82.45	7.45±1.51
T4	15.01±3.80^{a}	0.28±0.08^{a}	1.93±0.30	0.036±0.005	4.62±1.50	0.084±0.025	417.41±76.76	7.64±1.26
T5	10.15±2.19^{b}	0.18±0.05^{b}	1.74±0.26	0.031±0.009	5.58±1.96	0.097±0.040	391.85±65.51	6.95±2.01
T6	12.11±2.09^{ab}	0.21±0.04^{ab}	1.78±0.17	0.032±0.009	4.63±2.65	0.078±0.038	403.48±78.61	7.13±1.46

* T1 (colistin, 10 ppm), T2 (lactic acid 0.1%), T3 (EOs, 25 ppm), T4 (EOs, 25 ppm+lactic acid, 0.1%), T5 (EOs, 50 ppm) and T6 (EOs, 50 ppm+lactic acid, 0.1%). $^{a, b}$ Values with different superscripts are significantly ($p<0.05$) different between treatment groups. Mean±SD (n=8).

DISCUSSION

An EO extracted from herb and spices is a complicated mixture of various compounds, which consist of aromatic and volatile substances. Due to their biological properties such as antimicrobial, antioxidant and antiseptic activities, a commercial blend of EOs has been developed for use as alternatives to antibiotics in animal industry. Limited studies are available to assess the possible application of EOs as alternatives to antibiotics in broiler chicken production. The effects of EOs on growth performance in chickens are not consistent when they were fed the diets supplemented with 20-200 ppm of EOs. The positive results have been observed from several field studies (Langhout, 2000; Kamel, 2001), but the non-significant results have been also reported (Case et al., 1995; Botsoglou et al., 2002). It has been proposed that dietary EOs as growth stimulators could not give positive when the chickens are kept at optimal conditions such as highly digestible diets and clean conditions (Botsoglou et al., 2002). It was also well documented that dietary antibiotics did not promote growth rate when animals were raised in a clean environment (Coates et al., 1963). It is postulated that our study conducted under well-nourished and disinfected environments may do not show increased weight gain and FCR in broiler chickens fed the diet supplemented with antibiotics or a commercial blend of EOs (25 or 50 ppm) alone, although we did not test as to the effect of antibiotics or EOs in comparison with control group. However, the birds fed the diets containing EOs in combination with lactic acid showed a significant increase or a tendency to increase in growth performance compared with those fed the diets supplemented with antibiotic, lactic acid and EOs alone in our study. Presumably, there may have some synergistic effect of EOs in combination with lactic acid when they are added to the diet, although more detailed studies still need to be confirmed.

In order to understand the modes of action of alternatives to antibiotics, we should consider the effects of feed additives on the functional activities of digestive organs and gut microflora in animals. First, it is reasonable to investigate digestive enzymes of the pancreas and small intestine to reveal the effects of feed additives on intestinal functions, since major nutrient digestion processes are occurred in these sites. Several studies with chickens demonstrated that a blend of commercial EOs components stimulated the activities and secretion of digestive enzymes including amylase compared with control group (William

and Losa, 2001; Lee, 2002). It have been documented that spices and herbs from which EOs extracted, stimulated the secretion and activities of digestive enzymes in the intestine and pancreas in rats (Platel and Srinivasan, 1996 and 2000). However, similar to the results of Lee (2002), the diet fortified with EOs alone did not stimulated digestive enzyme activity as compared with that supplemented with antibiotic or lactic acid in our study.

Furthermore, lactic acid has been widespread for use as a dietary feed additive, mainly because of its positive effect on intestinal milieu and growth performance. Several studies have demonstrated that the positive effect of lactic acid on growth performance is due to antimicrobial activity, gut pH reduction, a useful energy source and pancreatic juice secretion in pigs (van de Broek, 2000). Especially, lactic acid has been known as a strong stimulator of pancreatic secretion (Ravindran and Kornegay 1993; Khan, 2003). Thaela et al. (1998) reported that the dietary inclusion of lactic acid stimulated the secretion of pancreatic enzymes in pigs after weaning.

Taken together, it could be postulated that the combination of EOs with lactic acid would synergistically trigger the secretion of pancreatic enzymes, which resulted in enhanced degestibilities of macronutrients.

The major mechanism associated with brush border membrane (BBM) from intestinal absorptive cells is the degradation and absorption of nutrients from the small intestine into the circulatory system (Jang et al., 2000). The BBM enzymes such as disaccharidase, alkaline phosphatase and leucine aminopeptidase are important constituents of the microvillous membrane in the intestinal absorptive cells (Ferraris et al., 1992). Presumably, a significantly increased BBM maltase activity in response to EOs in combination with lactic acid could be attributed to increase in the presence of amylose from intestinal digesta, which was produced by enhanced pancreatic α-amylase activity. These increased enzyme activities would have positive impact on starch digestion in the small intestine (Xu et al., 2002) especially at the later stage of growth when the birds consumed a greater amount of starch diet.

In addition, it should not ignore that most dietary feed additives have a profound impact on the gut microflora either directly or indirectly, although birds have little nutritional advantage from intestinal microflora compared with other species of animals. In our study, the dietary supplemented with EOs and lactic acid did not significantly affect ileal microbial populations. Antimicrobial activity has been recognized as the major beneficial effect of EOs on animal production, although the exact antimicrobial mechanism is not fully revealed. Many *in vitro* studies confirmed that EOs such as thymol, carvacrol, etc. have displayed antimicrobial activity against intestinal microbes such as *clostridium perfringer, salmonella typhimurium* and *E. coli* (Helander et al., 1998; Hammer et al., 1999). It has been reported that antimicrobial action of EOs is mediated by lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (Helander et al., 1998). On the other hand, *in vivo* study, it seems that the effects of EOs on gastrointestinal microflora are not constant, even though EOs have been generally recognized as antimicrobial agents. A study with chickens indicated that feeding the diet containing a commercial blend of EOs showed a significant decrease in colony forming units of *Clostridium perfringer* as compared with that containing 20 ppm zinc bacitracin (Kőhler, 1997). However, Evans et al. (2001) reported that chickens supplied the diet containing a commercial blend of EOs did not change in the number of *Clostridium perfringens* in the intestine. Therefore, it is suggested that the action of EOs or antibiotics on antimicrobial property may have more profound when the birds are given a less digestible diet and kept at a less clean environment. It is tentatively speculated from this study that the lack of responses of dietary antibiotic and EOs to ileal microbial counts is partially due to our experimental conditions undertaken well-nourished and clean conditions.

It is concluded that a commercial blend of EOs in combination with lactic acid showed a marked increase in digestive enzyme activities of the pancreas and intestinal mucosa from broiler chickens, leading to increase in a significant growth performance. More detailed studies are still needed to elucidate the effect of EOs alone and EOs in combination with lactic acid on the functional activities of digestive organs under various circumstances.

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