Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers

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ABSTRACT This study was conducted to evaluate the effects of the blend of organic acids (OAs) and medium-chain fatty acids (MCFAs) in broiler chickens. A total of 816 1-d-old male Ross 308 broiler chickens (35 ± 0.44 g) were randomly allocated into 1 of the following 6 dietary treatments (17 broilers per pen with 8 pens per treatment): dietary treatments consisted of corn-soybean meal-based basal diet and the basal diet supplemented with 0.02, 0.03, 0.04, 0.05, and 0.06% blend of OAs and MCFAs. The study lasted 5 wk during which growth performance was determined. In the current study, the inclusion of 0.02, 0.03, 0.04, 0.05, and 0.06% blend of OAs and MCFAs in the basal diet linearly increased ($P < 0.05$) body weight gain and improved feed conversion ratio ($P < 0.0001$) on day 7 to 14, day 14 to 35, as well as overall. Increasing inclusion of the blend of OAs and MCFAs levels in the diets also linearly increased ($P = 0.001$) the digestibility of dry matter on day 35. Broilers fed with different levels of the blend of OAs and MCFAs showed a linear increment ($P = 0.042$) in *Lactobacillus* concentration and decrease ($P = 0.002$) in *Escherichia coli* concentration. With regard to relative organ weight, a trend of linear reduction ($P = 0.052$) in bursa of Fabricius weight of broilers fed the blend of OAs and MCFAs was observed. There was a significant linear improvement ($P = 0.011$) in the IgG concentration associated with the inclusion of the blend of OAs and MCFAs levels in the diets. In conclusion, the blend of OAs and MCFAs supplementation positively influenced growth performance, nutrient digestibility, and excreta microflora in broiler chickens.

Key words: broiler, growth performance, microflora, nutrient digestibility, blend of organic acid and medium-chain fatty acid

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INTRODUCTION

Protein sources from poultry play an important role in human diet and hence they are of enormous economic value, and domesticated broiler chickens are the most common domestic animals in the world (Hou et al., 2016). Due to the demand for poultry meat and eggs, the use of antibiotics as growth promoters is still a common practice in developing countries largely (Khan et al., 2012; Shamlo et al., 2014). However, in consideration of the risk of bacterial resistance and antibiotic residues in animal products many countries including European Union have banned the use of antibiotics in animal feed in 2006 (Khan and Iqbal, 2016). A poultry industry challenge is to exploit the use of specific dietary supplements to boost the production and growth performance of poultry (Chand et al., 2014; Khan et al., 2014). Among a variety of candidates for the replacement of antibiotic growth promoters, organic acids (**OAs**) are promising alternatives (Mroz, 2005).

Organic acids are known to attribute to various factors, including (1) reducing the buffering capacity of diets, (2) controlling harmful microorganisms in digestive and respiratory organs by reducing pH levels in the stomach and gut, (3) promoting the availability of nutrients in the diet and their absorption and digestion, and (4) improving immune responses in poultry (Yesilbag and Colpan, 2006; Park et al., 2009; Abudabos et al., 2014), which can make a great contribution to the profitability in the poultry production and also can provide people with healthy and nutritious poultry products. However, an important limitation is that OAs are rapidly metabolized in the foregut (the crop to the gizzard; Khan and Iqbal, 2016). To overcome this limitation, matrix coating or encapsulation techniques to protect OAs for targeted delivery to different gut

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segments has gained considerable attention. It has been reported that dietary matrix coated OAs blend supplementation maintained the optimum pH in the intestinal tract and improve nutrient digestibility (Upadhaya et al., 2014a,b).

Medium-chain fatty acids (**MCFAs**) are another type of acids that could be considered as antibiotic replacers. MCFAs have strong antibacterial activity against Gram-positive cocci (Bergsson et al., 2001) and *Escherichia coli* (Skřivanová et al., 2006, 2009). Such positive changes (e.g., greater villus height) may result in improved performance of poultry. In addition, OAs could improve the antibacterial effects of MCFAs (Zentek et al., 2011). The combination of OAs and MCFAs has been reported to have beneficial effects on intestinal microecology in piglets (Zentek et al., 2013; Kuang et al., 2015) and nutrient digestibility in laying hens (Lee et al., 2015). However, there is still limited information on the influence of blend of OAs and MCFAs in poultry. Therefore, the objective of the present study was to assess the effect of the blends of OAs with MCFAs in matrix coating on growth performance, nutrient digestibility, blood profiles, excreta microbiota, and carcass quality in broiler chickens.

MATERIALS AND METHODS

Source of the blend of OAs and MCFAs

The blend of OAs and MCFAs used in the experiment was provided by a commercial company (Morningbio Co., Ltd., Cheonan, South Korea). The active ingredients were 17% fumaric acid, 13% citric acid, 10% malic acid, and 1.2% MCFAs (capric and caprylic acid, provided as 1:1 mixture product).

Experimental Design, Animals and Housing

In the present experiment, a total of 816 1-d-old Ross 308 male broiler chicks with an average initial body weight of 35 ± 0.44 g were used in a 5-wk feeding trial. The chicks were randomly allotted into 6 treatments with 8 pens per treatment and 17 birds per pen. Dietary treatments consisted of corn-soybean meal-based basal diet and the basal diet supplemented with 0.02, 0.03, 0.04, 0.05, and 0.06% blend of OAs and MCFAs. The composition of the basal diets is shown in Table 1. All diets were formulated to meet or exceed Aviagen (2007) recommendations. Blend of OAs and MCFAs was included in the diet by replacing the same amount of corn. Broiler chickens were housed in stainless steel pens (1.75 × 1.55 m^2). Room temperature was maintained at 33 ± 1°C for the first 3 d, and then gradually reduced by 3°C a week until reaching 24°C and maintained for the remainder of the experiment and the relative humidity was around 60%. Broiler chickens received diet and water ad libitum. Each pen had a pan feeder with a 35-cm diameter. Water was provided by evenly spaced nipple drinkers (5 nipples per pen) positioned along the side wall of the pen. Artificial light was provided 24 h per day by the use of fluorescent lights. The animal welfare committee of Dankook University approved the animal care protocol used for this study.

Table 1. Diet composition (as-fed basis).

Ingredient, g kg^{-1}	Starter (1 to 14 d)	Finisher (14 to 35 d)
Maize	556.7	632.1
Soybean meal (CP 48%)	282.5	246.1
Maize gluten meal (CP 60%)	65.0	35.0
Soybean oil	55.0	48.9
Tricalcium phosphate	24.6	22.9
Limestone	8.9	7.5
Salt	2.0	2.0
DL-methionine	0.7	0.7
L-lysine-HCl	0.6	0.8
Vitamin premix[1]	2.0	2.0
Trace mineral premix[2]	2.0	2.0
Calculated composition		
ME (MJ kg^{-1})	13.05	12.87
Crude protein	224.0	191.0
Lys	11.7	10.5
Ca	10.1	9.3
Available P	4.6	3.2
Met + Cys	9.8	10.0
Analyzed composition		
ME (MJ kg^{-1})	13.02	12.82
Crude protein	218.0	189.0
Lys	11.2	10.4
Ca	10.3	9.1
Available P	4.4	3.1
Met + Cys	9.8	9.8

[1]Provided per kg of premix: 15,000 IU vitamin A, 3,750 IU vitamin D3, 37.5 mg vitamin E, 2.55 mg vitamin K3, 3 mg vitamin B1, 7.5 mg vitamin B2, 4.5 mg vitamin B6, 24 μg vitamin B12, 51 mg niacin, 1.5 mg folic acid, 126 mg biotin, and 13.5 mg pantothenic acid.

[2]Provided per kg of diet: 37.5 mg Zn (as $ZnSO_4$), 137.5 mg of Mn (MnO_2), 37.5 mg of Fe (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 0.83 mg of I (as KI), 0.23 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$), and 1,408 mg of choline.

Sampling and Measurements

The broilers were weighed by pen and feed intake (**FI**) was recorded on day 1, 7, 14, and 35 to calculate body weight gain (**BWG**) and feed conversion ratio (**FCR**). As an indigestible marker (Fenton and Fenton, 1979), 0.2% chromium oxide (Cr_2O_3) (Duksan Pure Chemicals, Asan, South Korea) was added to the diets during the last week of the experiment and after feeding this for 4 d, fresh excreta samples were collected during the final 3 d of the experiment. All feed and excreta samples were stored immediately at −20°C until analysis. Before chemical analysis, the excreta samples were thawed and dried for 72 h at 60°C, after which they were finely ground to a size that could pass through a 1-mm screen. All feed and excreta samples were analyzed for DM (method 930.15, AOAC International, 2007) and crude protein (method 990.03, AOAC International, 2007). Chromium was analyzed using UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Nitrogen was

determined (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden). The gross energy was analyzed by measuring the heat of combustion using oxygen bomb calorimeter (Parr 6100 instrument Co., Moline, IL). The ATTD was calculated using the following formula: Digestibility (%) = $\{1 - [(Nf \times Cd)/(Nd \times Cf)]\} \times 100$, where Nf = nutrient concentration in excreta (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in excreta (% DM).

At the end of the experiment, 16 broilers were randomly obtained from each treatment (2 birds per pen) and blood samples were selected from the wing vein into a sterile syringe and stored at −4°C. Samples for serum analysis were then centrifuged at 3,000 × g for 15 min and serum was separated. The white blood cells (**WBC**), red blood cells (**RBC**), and lymphocyte counts in the whole blood were determined using an automatic blood analyzer (ADVIA 120, Bayer, NY). The immunoglobulin G (**IgG**) was analyzed using nephelometry (Behring, Germany).

After blood collection, the same broilers were weighed individually and killed by cervical dislocation. The stomach, breast meat, bursa of Fabricius, liver, spleen, and abdominal fat were removed by trained personnel and weighed. Organ size was expressed as a percentage of BW. The carcass quality was evaluated by measuring the lightness (**L***), redness (**a***), and yellowness (**b***) values that were determined using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Duplicate pH values for each sample were measured using a pH meter (Fisher Scientific, Pittsburgh, PA). The water holding capacity (**WHC**) was measured in accordance with the methods described by Kauffman et al. (1986). Briefly, a 0.3 g sample was pressed at 3,000 g for 3 min on a 125-mm-diameter piece of filter paper. The areas of the pressed sample and the expressed moisture were delineated and then determined using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water to meat area was then calculated, giving a measure of WHC (a smaller ratio indicates a higher WHC). Drip loss percentage was determined on day 1, 3, 5, and 7 by using the plastic bag method (Honikel, 1998). Cooking loss was determined using 5 g of breast meat, which was heat-treated in plastic bags separately in a water bath (100°C) for 5 min. Samples were cooled at room temperature. Cooking loss was calculated as (sample weight before cooking – sample weight after cooking)/sample weight before cooking × 100 (Cho et al., 2013).

On day 35, 1-g fresh excreta samples from each pen (8 replicated pens per treatment) was collected directly for the determination of *E. coli* and *Lactobacillus* counts according to the methods described by Nguyen et al. (2016). Excreta samples were diluted from 10^{-3} to 10^{-7} with 9 mL of 1% peptone broth (Becton, Dickinson, Franklin Lakes, NJ) and then mixed with a circulator (Seward Stomacher 400 circulator, West Sussex, UK). Tenfold serial dilutions of samples were plated onto agar plates in triplicates. The MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium III agar plates were used (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus,* respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately upon removal from the incubator.

Statistical Analysis

All data were analyzed as a completely randomized design using mixed procedures of SAS (SAS Institute 2004) to determine linear and quadratic effects. Polynomial regression was used to describe the shape of the response to increasing concentration of the blend of OAs and MCFAs in the diets, with the cage serving as the experimental unit. The initial BW was used as a covariate for the BWG and ADG. Before conducting statistical analysis of the microbial counts, the value was transformed logarithmically, and the initial values were used as a covariate for the blood profiles. Variability in the data was expressed as standard error. Probability level of less than 0.05 was considered as statistically significant whereas $0.05 \leq P < 0.10$ was considered as trend.

RESULTS

Growth Performance

The results of growth performance are summarized in Table 2. There was a linear improvement ($P < 0.05$) in BWG and FCR during day 7 to 14, day 14 to 35, and the whole experiment period with increasing levels of the blend of OAs and MCFAs in the diets. However, no influence of the blend of OAs and MCFAs supplementation was found in BWG, FI, and FCR from day 1 to 7 as well as FI during day 7 to 14, day 14 to 35, and the whole experiment period ($P > 0.05$).

Nutrient Digestibility

The data presented in Table 3 show the results of nutrient digestibility. Increasing inclusion of the blend of OAs and MCFAs levels in the diets linearly increased ($P = 0.001$) the digestibility of DM on day 35. No significant difference was observed in N and energy digestibility on day 35 with increasing the blend of OAs and MCFAs levels in the diets among 6 treatments ($P > 0.05$).

Excreta Microbial

Table 4 shows the results on excreta microbial assay. These results showed that there was a linear improvement in *Lactobacillus* concentration and decrease in

Table 2. Evaluation of the blend of OAs and MCFAs supplementation on growth performance in broilers.[1,2]

								P-value	
Items	T1	T2	T3	T4	T5	T6	SEM	Linear	Quadratic
Day 1 to 7									
BWG, g	87	86	86	84	85	85	3	0.529	0.542
FI, g	97	99	97	98	99	100	2	0.188	0.812
FCR	1.115	1.149	1.139	1.179	1.179	1.172	0.038	0.198	0.634
Day 7 to 14									
BWG, g	286	287	293	296	298	309	7	0.014	0.631
FI, g	416	413	416	418	419	423	9	0.452	0.744
FCR	1.456	1.441	1.422	1.413	1.408	1.371	0.015	<0.0001	0.679
Day 14 to 35									
BWG, g	1,316	1,325	1,347	1,357	1,393	1,384	20	0.002	0.871
FI, g	2,178	2,165	2,170	2,184	2,177	2,173	18	0.882	0.994
FCR	1.657	1.634	1.613	1.613	1.564	1.571	0.020	<0.0001	0.841
Overall									
BWG, g	1,689	1,698	1,726	1,737	1,776	1,779	21	<0.0001	0.934
FI, g	2,691	2,677	2,682	2,700	2,696	2,696	21	0.576	0.879
FCR	1.595	1.576	1.556	1.557	1.518	1.516	0.015	<0.0001	0.937

[1]T1, basal diet, T2, T3, T4, T5, and T6 were the basal diet supplemented with the blend of OAs and MCFAs at 0.02, 0.03, 0.04, 0.05, and 0.06%, respectively.
[2]Values of means represent 17 birds per 8 replicates pens (n = 136 birds) per treatment.
BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Table 3. Evaluation of the blend of OAs and MCFAs supplementation on nutrient digestibility in broilers.[1,2]

								P-value	
Items, %	T1	T2	T3	T4	T5	T6	SEM	Linear	Quadratic
Dry matter	70.12	71.67	71.93	72.27	72.50	73.57	0.69	0.001	0.692
Nitrogen	68.74	69.98	70.02	70.21	70.33	70.30	0.99	0.285	0.515
Energy	72.24	72.40	72.39	72.60	72.78	72.79	0.69	0.481	1.000

[1]T1, basal diet, T2, T3, T4, T5, and T6 were the basal diet supplemented with the blend of OAs and MCFAs at 0.02, 0.03, 0.04, 0.05, and 0.06%, respectively.
[2]Values of means represent 17 birds per 8 replicate pens pooled on a pen basis (n = 8) per treatment.

Table 4. Evaluation of the blend of OAs and MCFAs supplementation on excreta microbial in broilers.[1,2]

								P-value	
Items	T1	T2	T3	T4	T5	T6	SEM	Linear	Quadratic
Lactobacillus, $\log_{10}$cfu/g	7.12	7.29	7.31	7.45	7.44	7.47	3.53	0.002	0.314
E. Coli, $\log_{10}$cfu/g	6.33	6.27	6.25	6.19	6.20	6.18	2.49	0.042	0.518

[1]T1, basal diet, T2, T3, T4, T5, and T6 were the basal diet supplemented with the blend of OAs and MCFAs at 0.02, 0.03, 0.04, 0.05, and 0.06%, respectively.
[2]Values of means represent 1 bird per 8 replicate pens (n = 8 birds) per treatment.

E. coli concentration with increasing inclusion of the blend of OAs and MCFAs in the diets ($P < 0.05$).

Carcass Quality

The data presented in Table 5 show that carcass characteristics were not significantly influenced by treatment diets ($P > 0.05$). However, increasing inclusion of the blend of OAs and MCFAs led to a trend of increased relative weight of the bursa of Fabricius ($P = 0.052$).

Blood Profiles

There was a significant linear improvement ($P = 0.01$) in the IgG concentration (Table 6) associated with the inclusion of the blend of OAs and MCFAs levels in the diets. No significant difference was observed in lymphocyte percentage, WBC, and RBC concentrations on day 35 with increasing the blend of OAs and MCFAs levels in the diets among 6 treatments ($P > 0.05$).

DISCUSSION

Growth Performance

The feed industry and the poultry production sector still suffer from huge losses due to the contamination of food with pathogenic bacteria and their related impacts in the animal, such as lower body weight gain or even increased mortality. Poultry performance

Table 5. Evaluation of the blend of OAs and MCFAs supplementation on carcass quality and organ weight in broilers.[1,2]

								P-value	
Items	T1	T2	T3	T4	T5	T6	SEM	Linear	Quadratic
pH value	5.55	5.5	5.52	5.48	5.46	5.47	0.05	0.230	0.683
Breast muscle color									
Lightness (L*)	53.38	53.97	54.06	54.61	54.72	54.69	0.81	0.190	0.693
Redness (a*)	10.28	10.29	10.38	10.44	10.62	10.52	0.24	0.295	0.947
Yellowness (b*)	9.13	9.14	9.17	9.25	9.21	9.30	0.23	0.553	0.968
Cooking loss, %	34.65	34.30	34.3	33.84	33.30	33.38	0.81	0.170	0.999
Water holding capacity, %	51.32	52.74	53.00	53.55	53.46	53.51	1.24	0.208	0.477
Drip loss, %									
day 1	2.87	2.75	2.68	2.62	2.58	2.60	0.12	0.087	0.579
day 3	5.58	5.51	5.47	5.48	5.48	5.44	0.09	0.310	0.731
day 5	8.69	8.69	8.67	8.66	8.57	8.55	0.08	0.135	0.622
day 7	10.78	10.70	10.66	10.62	10.58	10.57	0.10	0.120	0.711
Relative organ weight, %									
Breast muscle	18.51	18.54	18.57	18.63	18.62	18.71	0.09	0.120	0.844
Liver	2.51	2.54	2.58	2.60	2.62	2.60	0.07	0.274	0.599
Bursa of Fabricius	0.12	0.13	0.14	0.14	0.15	0.15	0.02	0.052	0.930
Abdominal fat	3.42	3.54	3.58	3.64	3.66	3.62	0.16	0.325	0.590
Spleen	0.10	0.11	0.12	0.12	0.12	0.12	0.01	0.225	0.442
Gizzard	1.13	1.12	1.11	1.10	1.10	1.11	0.08	0.779	0.880

[1]T1, basal diet, T2, T3, T4, T5, and T6 were the basal diet supplemented with the blend of OAs and MCFAs at 0.02, 0.03, 0.04, 0.05, and 0.06%, respectively.
[2]Values of means represent 2 birds per 8 replicate pens (n = 16 birds) per treatment.

Table 6. Evaluation of the blend of OAs and MCFAs supplementation on blood profiles in broilers.[1,2]

								P-value	
Items	T1	T2	T3	T4	T5	T6	SEM	Linear	Quadratic
IgG, mg/dL	1.50	1.63	1.64	1.69	1.72	1.82	0.08	0.011	0.919
WBC, $10^3/\mu$L	12.61	13.45	13.55	13.52	13.83	13.80	1.47	0.573	0.800
RBC, 10^6/mm3	4.59	4.67	4.70	4.69	4.85	4.81	0.21	0.368	0.970
Lymphocyte, %	48.75	48.85	48.88	48.79	48.90	48.90	1.50	0.948	0.989

[1]T1, basal diet, T2, T3, T4, T5, and T6 were the basal diet supplemented with the blend of OAs and MCFAs at 0.02, 0.03, 0.04, 0.05, and 0.06%, respectively.
[2]Values of means represent 2 birds per 8 replicate pens (n = 16 birds) per treatment.
IgG, immunoglobulin G; WBC, white blood cells; RBC, red blood cells.

and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the host animal, including the load in the alimentary tract and in the environment (Islam et al., 2008). The blend of OAs and MCFAs used in the present study is protected by joint matrix coating technology on the base of lipid which allows the active components to reach the intestine in an intact form, be released slowly by reaction of lipase from the intestine thereby showing the beneficial effects to animals (De Lange et al., 2010; Upadhaya et al., 2016).

It has been previously reported that the blend of OAs and MCFAs supplementation in diets has beneficial effects on performance in pigs (Upadhaya et al., 2014a, 2016) as well as in laying hens (Lee et al., 2015). Based on the results of the present study, increasing inclusion of the blend of OAs and MCFAs levels in the diets linearly improved the BWG and FCR. In agreement with our findings, some researchers have shown positive effects with single or blends of dietary acidifiers. For example, Adil et al. (2010) and Sultan et al. (2014) reported that addition of the different levels of OA such as citric acid and lactic acid to the diet linearly improved growth performance and FCR. It is also reported that the supplementation of OAs to broiler chickens diet improved the BWG (Owens et al., 2008; Kaczmarek et al., 2016) and FCR (Boling et al., 2000; Sheikh et al., 2011). The enhancement in performance could be due to the antimicrobial activity of OAs, which helps in the reduction of pathogenic microbial load, resulted in reducing the metabolic demands of microbes and increasing the availability of dietary energy and nutrients to the host animals (Lee et al., 2015). In addition, Odle (1997) has shown that MCFAs could be a rapidly available energy source for young animals, due to their direct transport via portal blood to the liver. MCFAs also have an antibacterial function similar to short-chain fatty acids (Skrivanova et al., 2006). The improvement in FCR could possibly be due to better utilization of nutrients resulting in increased body weight gain in the birds fed the blend of OAs and MCFAs in the diets.

Nutrient Digestibility

Use of OAs in the diets is known to decrease pH levels in the stomach and gut, which could be conducive for the growth of favorable bacteria simultaneously hampering the growth of pathogenic bacteria which grow at a relatively higher pH (Abdel-Fattah et al., 2008). The beneficial microbiological and pH-decreasing abilities of OAs might have had resulted in the inhibition of intestinal bacteria leading to the reduced metabolic needs, thereby increasing the availability of nutrients to the host (Adil et al., 2010). This also had decreased the level of toxic bacterial metabolites as a result of lessened bacterial fermentation, causing an improvement in the protein and energy digestibility. In the current study we also found that the digestibility of DM linearly increased when birds were fed the diets with different levels of the blend of OAs and MCFAs supplementation. Similarly, Pirgozliev et al. (2008) reported that broilers fed OAs had higher metabolizability coefficients of DM values than those fed diets without OAs. Using the same product (the blend of OAs and MCFAs), increased DM digestibility in piglets was also reported in another study (Devi et al., 2016). However, the N and energy digestibility were not improved by the blend of OAs and MCFAs supplementation in this study, similar results were also acquired by previous researchers using the same additive in pigs (Upadhaya et al., 2014b, 2016) and in laying hens (Lee et al., 2015). On the contrary, other researchers have reported that broilers fed OAs had increased metabolizability coefficients of gross energy and N (Pirgozliev et al., 2008; Fascina et al., 2012) than those fed diets without OAs. The digestibility of DM was also not improved by supplementing the same product in laying hens (Lee et al., 2015). The variation in results could be due to the age of the animals, composition of diet, the amount and type of OAs and MCFAs supplemented (Lallès et al., 2009).

Excreta Microflora

The antimicrobial activity of acids changes microbial populations. The mode of action of OAs on bacteria in pigs and poultry involves the entry of these acids into the bacterial cell in an undissociated form, causing bacterial membrane disruption and inhibition of essential metabolic reactions. The stress on intracellular pH homeostasis causes the accumulation of toxic anions and the bacteria cannot tolerate large internal and external pH variations such as *Salmonella* and *E. coli* (Borsoi et al., 2011). In addition, MCFAs had a significant antimicrobial activity against *E. coli* (Marounek et al., 2003), which resulted in a beneficial microbial ecosystem. Our findings corroborate the above reports by showing a significant increase in *Lactobacillus* counts and decrease in the *E. coli* counts with the diets supplemented with the blend of OAs and MCFAs, which is in agreement with a previous study in laying hens (Lee et al., 2015). It is also reported that supplementing the diets with OA improved the counts of *Lactiobacilli* (Gheisari et al., 2007) and decreased the concentrations of *E. coli* in the small intestine (Sun et al., 2005; Gunal et al., 2006). As the microbial pathogenic population gets reduced, the metabolic need of the microbes is reduced and the availability of dietary energy and nutrients to the host animal is increased, leading to enhanced growth rate and enhanced feed efficiency (Upadhaya et al., 2016).

Carcass Quality and Blood Profiles

In this study, we found that supplementation of the blend of OAs and MCFAs led to a trend of increased development of the bursa of Fabricius. Similarly, other previous studies have reported that addition of OA significantly increased relative weight of the bursa of Fabricius in broilers (Mohamed et al., 2014; Sultan et al., 2014) and in quails (Saki et al., 2012). Measurement of immune organ weight is a common method for evaluation of immune status in chickens (Heckert et al., 2002). Immune organs constitute the immune system of the body together with lymphoid tissue and immune cells (Li and Verma, 2002). Generation, proliferation, differentiation, and maturation of immune cells usually take place in the thymus, spleen, and bursa (Brekelmans and Van Ewijk, 1990). Therefore, the immune organ index is an important indicator reflecting immune competence. A trend of increased development of the bursa of Fabricius was observed in the present study, which to some extent indicated that broiler in the group with the blend of OAs and MCFAs presented better immunity than in control group. It is shown that there is an important relationship between the weight of bursa of Fabricius and immune response, which indicated that antibody production in a breed with large bursa was higher than that of small bursa (Ammar, 2008). Therefore, we hypothesize that the increase in the IgG concentration in this study could be due to the increased development of the bursa of Fabricius.

CONCLUSION

Broiler fed the diets with the blend of OAs and MCFAs supplementation has improved the performance, FCR, digestibility of DM, *Lactobacillus* count, IgG concentration and decreased the *E. coli* count. In addition, dietary inclusion of the blend of OAs and MCFAs supplementation tended to increase the bursa of Fabricius. However, no significant difference was observed in other parameters of carcass quality and blood profiles on day 35, as well as FI throughout the experimental period among treatments.

REFERENCES

Abdel-Fattah, S. A., M. H. El-Sanhoury, N. M. El-Mednay, and F. Abdel-Azeem. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7:215–222.

Abudabos, A. M., S. I. Al-Mufarrej, A. H. Alyemni, H. M. Yehia, A. R. Garelnabi, and M. N. Alotybi. 2014. Effect of using organic acids to substitute antimicrobial growth promoters on broiler chickens' performance. J. Food Agric. Environ. 12:447–451.

Adil, S., T. Banday, G. A. Bhat, M. S. Mir, and M. Rehman. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 10:479–485.

Ammar, J. A. 2008. The Relationship between the Weight of Bursa of Fabricius and the Antibodies Production in Local and Commercial Broiler Chicks: A thesis for the Degree of Master of Science in Veterinary Pathology in University of Basrah.

AOAC International. 2007. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD, USA.

Aviagen, R. 2007. 308 Broiler: Nutrition Specification. Scotland, UK, 8.

Bergsson, G., J. Arnfinnsson, Ó. SteingrÍmsson, and H. Thormar. 2001. Killing of Gram-positive cocci by fatty acids and monoglycerides. APMIS. 109:670–678.

Boling, S. D., D. M. Webel, I. Mavromichalis, C. M. Parsons, and D. H. Baker. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. J. Anim. Sci. 78:682–689.

Borsoi, A., L. R. D, G. S. Diniz Santos, C. T. P. C Salle, H. L. S. Moraes, and V. P. Nascimento. 2011. Salmonella fecal excretion control in broiler chickens by organic acids and essential oils blend feed added. Rev. Bras. Cienc. Avic. 13:65–69.

Brekelmans, P., and W. Van Ewijk. 1990. Phenotypic characterization of murine thymic microenvironments. Semin. Immunol. 2:13–24.

Chand, N., S. Naz, Z. Shah, S. Khan, A. A. Shah, and R. U. Khan. 2014. Growth performance and immune status of broilers fed graded levels of albizia lebbeck seeds. Pak. J. Zool. 46:574–577.

Cho, J. H., Z. F. Zhang, and I. H. Kim. 2013. Effects of single or combined dietary supplementation of β-glucan and kefir on growth performance, blood characteristics and meat quality in broilers. Br. Poult. Sci. 54:216–221.

De Lange, C. F. M., J. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Sci. 134:124–134.

Devi, S. M., K. Y. Lee, and I. H. Kim. 2016. Analysis of the effect of dietary protected organic acid blend on lactating sows and their piglets. R. Bras. Zootec. 45:39–47.

Fascina, V. B., J. R. Sartori, E. Gonzales, F. B. D. Carvalho, I. M. G. P. D. Souza, G. D. V. Polycarpo, A. C. Stradiotti, and V. C. Pelícia. 2012. Phytogenic additives and organic acids in broiler chicken diets. R. Bras. Zootec. 41:2189–2197.

Fenton, T. W., and M. Fenton. 1979. An improved method for chromic oxide determination in feed and feces. Can. J. Anim. Sci. 59:631–634.

Gheisari, A. A., M. Heidari, R. K. Kermanshahi, M. Togani, and S. Saraeian. 2007. Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in broiler chickens. Pages 519–522 In Proceedings of the 16th European Symposium on Poultry Nutrition. Strasbourg, France.

Gunal, M., G. Yayli, O. Kaya, N. Karahan, and O. Sulak. 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci. 5:149–155.

Heckert, R. A., I. Estevez, E. Russek-Cohen, and R. Pettit-Riley. 2002. Effects of density and perch availability on the immune status of broilers. Poult. Sci. 81:451–457.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457.

Hou, Q., L. Y. Kwok, Y. Zheng, L. Wang, Z. Guo, J. Zhang, W. Huang, Y. Wang, L. Leng, H. Li, and H. Zhang. 2016. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Sci. Rep. 6:1–13.

Islam, M. Z., Z. H. Khandaker, S. D. Chowdhury, and K. M. S. Islam. 2008. Effect of citric acid and acetic acid on the performance of broilers. J. Bangladesh Agril. Univ. 6:315–320.

Kauffman, R. G., G. Eikelenboom, P. G. van der Wal, B. Engel, and M. Zaar. 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18:307–322.

Kaczmarek, S. A., A. Barri, M. Hejdysz, and A. Rutkowski. 2016. Effect of different doses of coated butyric acid on growth performance and energy utilization in broilers. Poult. Sci. 95:851–859.

Khan, R. U., Z. Nikousefat, V. Tufarelli, S. Naz, M. Javdani, and V. Laudadio. 2012. Garlic (Allium sativum) supplementation in poultry diets: effect on production and physiology. Worlds Poult. Sci. J. 68:417–424.

Khan, R. U., S. Naz, and K. Dhama. 2014. Chromium: pharmacological applications in heat-stressed poultry. Int. J. Pharmacol. 10:213–217.

Khan, S. H., and J. Iqbal. 2016. Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res. 44:359–369.

Kuang, Y., Y. Wang, Zhang Y., Song Y., X. Zhang, Y. Lin, L. Che, S. Xu, D. Wu, B. Xue, and Z. Fang. 2015. Effects of dietary combinations of organic acids and medium chain fatty acids as a replacement of zinc oxide on growth, digestibility and immunity of weaned pigs. Anim. Feed Sci. Technol. 208:145–157.

Lallès, J. P., P. Bosi, P. Janczyk, S. J. Koopmans, and D. Torrallardona. 2009. Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: a review. Animal 3:1625–1643.

Lee, S. I., H. S. Kim, and I. H. Kim. 2015. Microencapsulated organic acid blend with MCFAs can be used as analternative to antibiotics for laying hens. Turk. J. Vet. Anim. Sci. 39:520–527.

Li, Q., and I. M. Verma. 2002. NF-[kappa] B regulation in the immune system. Nat. Rev. Immunol. 2:725–734.

Marounek, M., E. Skřivanová, and V. Rada. 2003. Susceptibility of Escherichia coli to C2-C18 fatty acids. Folia Microbiol. 48:731–735.

Mohamed, M. A., E. F. El-Daly, N. A. El-Azeem, A. W. Youssef, and H. M. A. Hassan. 2014. Growth performance and histological changes in ileum and immune related organs of broilers fed organic acids or antibiotic growth promoter. Int. J. Poult. Sci. 13:602–610.

Mroz, Z. 2005. Organic acids as potential alternatives to antibiotic growth promoters for pigs. Adv. Pork Prod, 16:169–182.

Nguyen, D. H., M. Mohammadi Gheisar, S. D. Upadhaya, M. M. Hossain, and I. H. Kim. 2016. Effect of dietary extracted rice bran supplementation on production performance and excreta microflora in laying hens. Can. J. Anim. Sci. 97:372–377.

Odle, J. 1997. New insights into the utilization of medium-chain triglicerides by the neonate: observations from a piglet model. J. Nutr. 127: 1061–1067

Owens, B., L. C. M. A. Tucker, M. A. Collins, and K. J. McCracken. 2008. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. Br. Poult. Sci. 49:202–212.

Park, K.W., A. R. Rhee, J. S. Um, and I. K. Paik. 2009. Effect of dietary available phosphorus and organic acids on the performance and egg quality of laying hens. J. Appl. Anim. Res. 18:598–604.

Pirgozliev, V., T. C. Murphy, B. Owens, J. George, and M. E. E. McCann. 2008. Fumaric and sorbic acid as additives in broiler feed. Res. Vet. Sci. 84:387–394.

Saki, A. A., R. N. Harcini, E. Rahmatnejad, and J. Salary. 2012. Herbal additives and organic acids as antibiotic alternatives in broiler chickens diet for organic production. Afr. J. Biotechnol. 11:2139–2145.

SAS Institute. 2004. SAS User's Guide: Statistics. 9th ed. SAS Inst. Inc., Cary, NC.

Shamlo, R., J. Nasr, and F. Kheiri. 2014. Effects of various levels of pennyroyal (Mentha pulegium L.) on carcass characteristics and serum cholesterol in broiler. Res. Opin. Anim. Vet. Sci. 4:453–457.

Sheikh, A. D. I. L., T. Banday, G. A. Bhat, M. Salahuddin, M. Raquib, and S. Shanaz. 2011. Response of broiler chicken to dietary supplementation of organic acids. J. Cent. Eur. Agr. 12:489–508.

Skřivanová, E., M. Marounek, V. Benda, and P. Březina. 2012. Susceptibility of Escherichia coli, Salmonella sp. and Clostridium perfringens to organic acids and monolaurin. Vet. Med. (Praha) 51:81–88.

Skřivanová, E., Z. Molatová, V. Skřivanová, and M. Marounek. 2009. Inhibitory activity of rabbit milk and medium-chain fatty acids

against enteropathogenic Escherichia coli O128. Vet. Microbiol. 135:358–362.

Sultan, A., I. Ullah, S. Khan, R. U. Khan, and Z. Hassan. 2014. Impact of chlorine dioxide as water acidifying agent on the performance, ileal microflora and intestinal histology in quails. Archiv. Tierzucht. 31:1–9.

Sun, X., A. McElroy, K. E. Webb, Jr, A. E. Sefton, and C. Novak. 2005. Broiler performance and intestinal alterations when fed drug-free diets. Poult. Sci. 84:1294–1302.

Upadhaya, S. D., K. Y. Lee, and I. K. Kim. 2014a. Protected organic acid blends as an alternative to antibiotics in finishing pigs. Asian Australas. J. Anim. Sci 27:1600–1607.

Upadhaya, S. D., K. Y. Lee, and I. H. Kim. 2014b. Influence of protected organic acid blends and diets with different nutrient densities on growth performance, nutrient digestibility and faecal noxious gas emission in growing pigs. Vet. Med. (Praha) 59:491–497.

Upadhaya, S. D., K. Y. Lee, and I. H. Kim. 2016. Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. J. Appl. Anim. Res. 44:238–242.

Yesilbag, D., and I. Colpan. 2006. Effects of organic acid supplemented diets on growth performance, egg production and quality and on serum parameters in laying hens. Rev. Med. Vet. 157:280–284.

Zentek, J., S. Buchheit-Renko, F. Ferrara, W. Vahjen, A. G. Van Kessel, and R. Pieper. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim. Health. Res. Rev. 12:83–93.

Zentek, J., F. Ferrara, R. Pieper, L. Tedin, W. Meyer, and W. Vahjen. 2013. Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive tract of weaning piglets1. J. Anim. Sci. 91:3200–3210.