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Microencapsulated organic acid blend with MCFAs can be used as an alternative to antibiotics for laying hens

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Abstract: A total of 144 Hy-Line brown laying hens were used in a 10-week trial to evaluate the effects of a microencapsulated organic acid blend with medium chain fatty acids (MCFAs) on egg production, weight, quality, fecal microflora, and nutrient digestibility in the hens. The hens were divided into four groups and different dietary treatments were given to each group. The control group received no microencapsulated organic acid blend with MCFAs. The second group received 0.05%, the third group received 0.1%, and the fourth group received 0.2% of a microencapsulated organic acid blend with MCFAs. Laying hens fed on a microencapsulated organic acid blend with MCFAs showed an increase in the egg production rate (linear, $P > 0.001$), in the Haugh units (linear, $P = 0.010$), and in eggshell strength (linear, $P = 0.012$; quadratic, $P = 0.005$). Also, the same hens had higher calcium concentrations in the blood at 35 weeks (linear, $P = 0.042$). The dietary supplementation of a microencapsulated organic acid blend with MCFAs increased the *Lactobacillus* fecal content and decreased the *E.coli* fecal content in the hens. In conclusion, the microencapsulated organic acid blend with MCFAs had positive effects on egg production, egg strength, Haugh units, calcium concentration, and fecal *Lactobacillus* and *E. coli* contents in laying hens. Thus, we suggest that the microencapsulated organic acid blend with MCFAs could be used as an alternative to antibiotics in laying hens.

Key words: Alternative to antibiotics, laying hens, medium chain fatty acids, organic

1. Introduction

The use of antibiotics in feedstuffs has been limited, since the continuous use of antibiotics may contribute to a reservoir of drug-resistant bacteria and residue problems in animal products that may transfer resistance to pathogenic bacteria in both animals and humans, posing a potential health hazard to humans (1,2). An intensive amount of research has focused on the development of alternatives to antibiotics to maintain animal health and performance using different feed additives (3).

Organic acids are known to control harmful microorganisms in digestive and respiratory organs by reducing pH levels in the stomach and gut and improving immune responses (4,5). Organic acids are well accepted as an alternative to antibiotics as growth promoters by improving growth performance and nutrient digestibility in pigs (6,7), broilers (8), and laying hens (5,9). Youssef et al. (9) reported that dietary supplementation of organic acids improved egg production rate, egg mass, shell thickness, and yolk color in laying hens. In addition, it has been reported that the combination of an organic acid blend and inorganic acids such as medium chain fatty acids (MCFAs) has beneficial and synergistic effects on the performance of piglets by changing the acidity in the upper part of the digestive tract and causing structural changes of the small intestinal mucosa (10,11).

The microencapsulation technique has been used to deliver substances into specific sites of the gastrointestinal tract through the slow release of nutrients throughout the gastrointestinal tract, allowing for delayed drug absorption and protecting amino acids and proteins from rumen degradation (12). In our previous reports, the dietary supplementation of a microencapsulated organic acid blend with inorganic acids such as MCFAs or essential oil had positive effects on the growth performance and nutrient digestibility in growing (13) and finishing pigs (14,15), but not yet in layer hens. Thus, the objective of the present study was to evaluate the effects of a microencapsulated organic acid blend with MCFAs on egg production, weight, quality, fecal microflora, and nutrient digestibility in laying hens.

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2. Materials and methods

2.1. Source of microencapsulated organic acid blend with MCFAs

The microencapsulated organic acid blend with MCFAs used in the present study was provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). This microencapsulated organic acid blend consists of the active ingredients of 17% fumaric acid, 13% citric acid, 10% malic acid, and 1.2% MCFA, as well as capric and caprylic acid and a carrier.

2.2. Experimental birds and design

All animal-based procedures were in accordance with the Guidelines for the Care and Use of Experimental Animals of Dankook University. A total of 144 Hy-Line brown laying hens (25 weeks of age) were raised in a windowless and environmentally controlled room that was maintained at 23 °C for 10 weeks. They were randomly assigned to 4 treatments, with 6 replications of 6 hens in each treatment. Six layers were housed in a 114 × 50 × 40 cm wire cage, and were subjected to a 16 h light/8 h dark photoperiod. Layers were fed mash diets, formulated to supply their nutritional requirements (16) (Table 1). Feed and water were provided ad libitum. The dietary treatments were as follows: 1) the control group was on basal diet only; 2) the second group was on basal diet + 0.05% of a microencapsulated organic acid blend with MCFAs; 3) the third group was on basal diet + 0.1% of a microencapsulated organic acid blend with MCFAs; and 4) the fourth group was on basal diet + 0.2% of a microencapsulated organic acid blend with MCFAs.

2.3. Sample measurements

Eggs were collected and counted daily throughout the experimental period. The egg production was calculated using the total egg number divided by the number of hens per cage. In addition, a total of 30 eggs with the exception of soft and broken eggs were randomly collected at 1700 hours from each treatment on a weekly basis and were used to determine the egg quality. Egg weight was measured by an electronic scale. Eggshell strength was measured by using the eggshell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan). Eggshell thickness was measured at three locations (all cell, equator, and sharp end) of the egg by a dial pipe gauge (Ozaki MFG. Co., Ltd., Japan). Egg yolk color was evaluated by a yolk color fan (Roche, Switzerland). The Haugh units were measured according to the HU formula based on the height of albumen as determined using a micrometer with an egg multitester (Touhoku Rhythm Co. Ltd., Tokyo, Japan).

The selected birds were individually housed in metabolic cages to determine the digestibility of nutrients. Laying hens were fed their respective diets containing chromic oxide (Cr_2O_3 at the 0.20% level) for 4 days prior to the collection period. All excreta of the birds were collected daily for 3 days. All the fecal samples along with feed samples were then analyzed according to AOAC procedures (17).

Fecal samples were collected directly via massaging the rectum of each hen and then pooling and placing the samples on ice for transportation to the laboratory, where analyses were immediately carried out. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution)

Table 1. Basal diet composition (as-fed-basis).

Items	
Ingredients, %	
Corn	56.30
Soybean meal, 46% CP	22.72
Corn gluten meal, 60% CP	3.00
Dried distillers grains with solubles	5.00
Tallow	1.61
Limestone	9.36
Dicalcium phosphate, 18% CP	1.41
Salt	0.22
Sodium bicarbonate	0.10
DL-Methionine, 50%	0.02
Choline,50%	0.06
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Total	100
Calculated energy content, %	
ME, kcal/kg	2,800
Analyzed nutrient content, %	
Crude protein, %	18.00
Lysine, %	0.85
Methionine + cysteine, %	0.65
Calcium, %	3.87
Total phosphorus, %	0.61

[1]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; pantothenic acid, 29 mg; choline, 166 mg; and vitamin B12, 33 µg.
[2]Provided per kg of complete diet: Cu, 12 mg; Zn, 85 mg; Mn, 8 mg; I, 0.28 mg; and Se, 0.15 mg.

onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and Lactobacilli medium III agar plates (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 39 °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 after removal from the incubator.

Blood samples were randomly collected from 24 layers in each treatment at 30 and 35 weeks, using a sterilized syringe and K_3EDTA vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The blood samples were then centrifuged at 3000 rpm at 4 °C for 20 min within 1 h of collection, to separate the serum. The aliquots were stored at –4 °C for subsequent determination of the calcium and phosphorus concentrations using an automatic biochemistry analyzer (Hitachi 747, Hitachi, Tokyo, Japan).

2.4. Statistical analysis

Data were statistically analyzed by analysis of variance, using the general linear model procedure of the SAS program (SAS, 2002) for a completely randomized design. Mean values and standard errors of means are reported. An orthogonal polynomial contrast was conducted to measure the linear and quadratic effects of increasing the microencapsulated organic acid blend with MCFAs levels on all measurements. Differences among treatment means were determined using Duncan's multiple range tests. Statements of statistical significance were based on $P < 0.05$.

3. Results

3.1. Egg production and weight

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on egg production and weight are shown in Table 2. There was no difference in the egg production rate during the first half (25–30 weeks) among treatments ($P > 0.05$). During the second half (31–35 weeks), laying hens fed on a microencapsulated organic acid blend with MCFAs increased their egg production rate (linear, $P > 0.001$) and the 0.2% treatment had a higher egg production rate than the 0.05% and the 0% treatments. Also, laying hens fed with 0.05% of a microencapsulated organic acid blend with MCFAs had a higher egg production rate than those in the control group. There was no difference in egg weight among the treatments during the experimental period ($P > 0.05$).

3.2. Internal egg quality

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on yolk color and Haugh units are shown in Table 3. There was no difference in yolk color among treatments during the experimental period ($P > 0.05$). In terms of the Haugh units, laying hens fed on a microencapsulated organic acid blend with MCFAs showed an increase (linear, $P = 0.010$) and the 0.2% treatment had a higher score than the 0% treatment at 30 weeks ($P < 0.05$). At 35 weeks, laying hens fed on a microencapsulated organic acid blend with MCFAs increased in Haugh units (quadratic, $P = 0.043$).

3.3. External egg quality

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on eggshell thickness and eggshell strength are shown in Table 4. There was no difference in eggshell thickness

Table 2. The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on egg production weight in laying hens. Values with different subscripts are significantly different.

	Microencapsulated organic acid blend with MCFAs, %				SEM	P-value		
	0	0.05	0.1	0.2		Linear	Quadratic	Cubic
Egg production, %								
25 to 30 weeks	93.27	93.21	94.70	94.64	1.00	0.217	1.000	0.493
31 to 35 weeks	92.14^{c}	93.45^{b}	94.17^{ab}	94.88^{a}	0.46	<0.001***	0.517	0.772
Egg weight, g								
Initial (25 weeks)	55.63	56.58	57.06	57.88	0.94	0.072	0.943	0.837
31 weeks	58.89	59.69	59.84	60.39	1.18	0.371	0.914	0.835
Final (35 weeks)	61.62	62.29	62.26	63.14	0.79	0.164	0.284	0.616

***$P < 0.001$.

Table 3. The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on internal egg quality in laying hens. Values with different subscripts are significantly different.

	Microencapsulated organic acid blend with MCFAs, %				SEM	P-value		
	0	0.05	0.1	0.2		Linear	Quadratic	Cubic
Haugh Unit								
Initial (25 weeks)	86.80	87.16	87.80	88.18	0.89	0.232	0.991	0.989
30 weeks	84.29[b]	85.08[ab]	85.42[ab]	86.73[a]	0.64	0.010*	0.702	0.634
Final (35 weeks)	86.620	86.860	87.225	87.485	0.34	0.060	0.043	0.874
Yolk color								
Initial (25 weeks)	6.2	6.1	6.0	5.9	0.08	0.041*	1.000	0.865
30 weeks	5.80	6.05	6.00	6.10	0.15	0.212	0.622	0.503
Final (35 weeks)	5.40	5.40	5.40	5.55	0.11	0.508	0.375	0.767

*P < 0.05.

among the treatments during the experimental period (P > 0.05). Eggshell strength showed no difference among treatments at 30 weeks (P > 0.05). Laying hens fed on a microencapsulated organic acid blend with MCFAs showed increased eggshell strength (linear, P = 0.012; quadratic, P = 0.005) and the 0.2% treatment resulted in higher eggshell strength than the 0% treatment at 35 weeks (P < 0.05).

3.4. Concentration of calcium and phosphorus in blood

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on the concentration of calcium and phosphorus in the blood are shown in Table 5. There was no difference in phosphorus among treatments during the experimental period (P > 0.05). Calcium concentration showed no difference among the treatments at 25 and 30 weeks (P > 0.05). The hens fed on a microencapsulated organic acid blend with MCFAs showed increased calcium concentrations (linear, P = 0.042) at 35 weeks.

3.5. Nutrient digestibility

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on nutrient digestibility are shown in Table 6. There was no difference in digestibility of dry matter, nitrogen, and

Table 4. The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on external egg quality in laying hens. Values with different subscripts are significantly different.

	Microencapsulated organic acid blend with MCFAs, %				SEM	P-value		
	0	0.05	0.1	0.2		Linear	Quadratic	Cubic
Eggshell strength, kg/cm^2								
Initial (25 weeks)	3.40	3.39	3.39	3.50	0.07	0.414	0.451	0.777
30 weeks	3.69	3.71	3.75	3.77	0.05	0.300	0.990	0.925
Final (35 weeks)	3.99[b]	4.05[ab]	4.09[ab]	4.12[a]	0.04	0.012*	0.005**	0.099
Eggshell thickness, mm^{-2}								
Initial (25 weeks)	42.78	42.64	42.83	43.99	0.68	0.242	0.371	0.837
30 weeks	45.29	45.28	45.17	45.67	0.61	0.794	0.703	0.841
Final (35 weeks)	44.26	44.50	44.79	44.99	0.74	0.479	0.442	0.964

*P < 0.05, **P < 0.01.

Table 5. The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on calcium and phosphorus concentration in blood serum in laying hens.

	Protected organic acid blend, %				SEM	P-value		
	0	0.05	0.1	0.2		Linear	Quadratic	Cubic
Initial (25 weeks)								
Calcium	19.00	19.03	18.98	19.15	0.18	0.656	0.709	0.738
Phosphorus	6.60	6.73	6.55	6.65	0.13	0.970	0.933	0.397
30 weeks								
Calcium	19.00	18.83	18.78	19.23	0.17	0.416	0.084	0.623
Phosphorus	6.45	6.63	6.63	6.68	0.10	0.146	0.532	0.614
Final (35 weeks)								
Calcium	18.30	18.78	18.90	18.93	0.20	0.042*	0.459	0.770
Phosphorus	6.45	6.70	6.70	6.65	0.14	0.355	0.589	0.819

Table 6. The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on nutrient digestibility in laying hens.

	Microencapsulated organic acid blend with MCFAs, %				SEM	P-value		
	0	0.05	0.1	0.2		Linear	Quadratic	Cubic
30 weeks								
Dry matter	73.22	73.46	74.62	75.22	1.3	0.380	0.920	0.855
Nitrogen	60.79	62.15	63.13	64.45	2.4	0.377	0.995	0.957
Energy	77.11	77.44	78.60	79.85	1.2	0.264	0.848	0.896
35 weeks								
Dry matter	72.98	73.44	73.73	75.03	1.6	0.350	0.781	0.864
Nitrogen	60.34	62.34	62.88	63.99	2.5	0.244	0.837	0.834
Energy	77.15	77.69	78.72	78.68	1.3	0.341	0.825	0.787

energy among treatments during the experimental period ($P > 0.05$).

3.6. Contents of fecal Lactobacillus and *E. coli*

The effects of dietary supplementation of a microencapsulated organic acid blend with MCFAs on fecal microflora are shown in Figures 1A and 1B. Laying hens fed on a microencapsulated organic acid blend with MCFAs had an increased *Lactobacillus* fecal content at 30 and 35 weeks (linear, P = 0.002 and 0.004, respectively). Also, the same hens showed a decreased *E. coli* content at 30 and 35 weeks (linear, P = 0.001 and 0.008, respectively). Laying hens fed with 0.05%, 0.1%, and 0.2% of a microencapsulated organic acid blend with MCFAs had higher *Lactobacillus* content in feces than those fed on a diet with 0% of a microencapsulated organic acid blend with MCFAs. Laying hens fed with 0.1% and 0.2% of a microencapsulated organic acid blend with MCFAs had lower *E. coli* content in feces than those fed on a diet with 0% of a microencapsulated organic acid blend with MCFAs at 30 weeks. Furthermore, 0.05%, 0.1%, and 0.2% of a microencapsulated organic acid blend with MCFAs had lower *E. coli* content in feces than those fed on a diet with 0% of a microencapsulated organic acid blend with MCFAs at 35 weeks (Figure 1).

4. Discussion

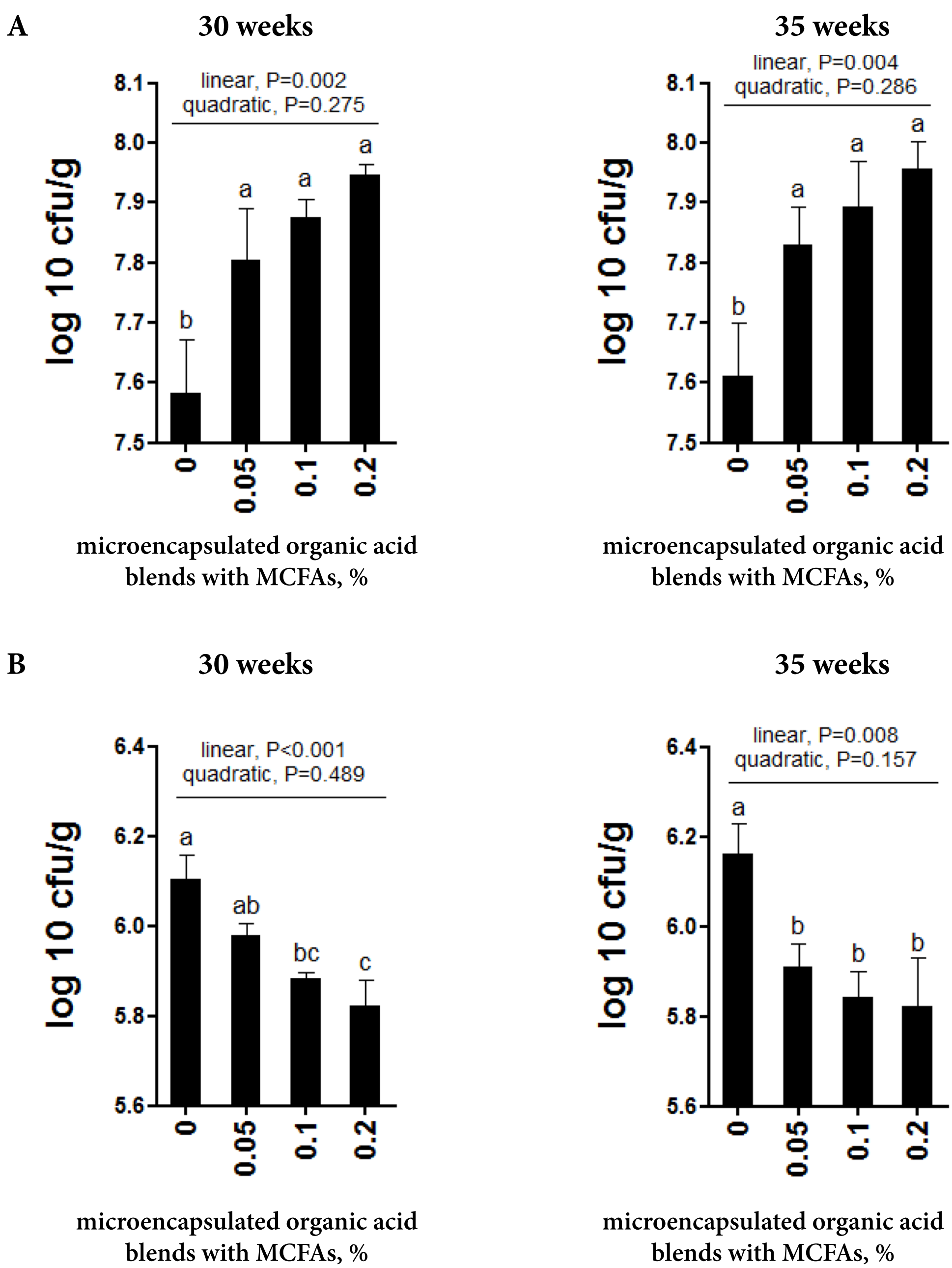

Figure 1. The effects of microencapsulated organic acid blends with MCFAs on *Lactobacillus* (A) and *E. coli* (B) contents at 30 and 35 weeks in laying hens. Fecal samples were collected directly via massaging the rectum and viable counts of bacteria were then conducted by plating serial 10-fold dilutions onto MacConkey and *Lactobacilli medium* III agar plates. An orthogonal polynomial contrast was conducted to measure the linear and quadratic effects of increasing the microencapsulated organic acid blend with MCFAs levels on all measurements. Differences among treatment means were determined using Duncan's multiple range tests. Statements of statistical significance were based on $P < 0.05$. Error bars indicate the standard error of quadruplicate analyses ($n = 4$).

Feed acidifiers such as organic acids including formic, propionic, acetic, sorbic, citric, fumaric, malonic, and short chain fatty acids are used as alternatives to antibiotics in animal diets (18). Organic acids improve gut health by reducing the pH level and buffering capacity of diets, and by promoting beneficial bacterial growth while inhibiting growth of pathogenic microbes such as *Salmonella* spp., *E. coli*, *Clostridium perfringens*, and *Campylobacter* (6,19). In addition to organic acids, MCFAs are also used as alternatives to antibiotics due to their similar effects as those of organic acids on antimicrobial activities outside the intestinal tract and on reducing pH (20). We have previously reported that dietary supplementation of a microencapsulated organic acid blend with MCFAs has positive effects on the growth performance and nutrient digestibility in growing (13) and finishing (14,15) pigs, but not yet in layer hens. In the present study, we used a microencapsulated organic acid blend with MCFAs to confirm the hypothesis that this dietary supplementation exerts synergistic effects on the performance of laying hens similarly to that of pigs. The results of the present study suggest that dietary supplementation of a microencapsulated organic acid blend with MCFAs had positive effects on laying hens' productivity including egg production and egg quality, and fecal microbiota content.

In the present study, laying hens fed on a microencapsulated organic acid blend with MCFAs showed an increased egg production rate. It is well accepted that dietary supplementation of an organic acid mixture significantly increases egg production in laying hens, as compared with hens that were not given such supplementation (5,9). In addition, our previous study showed that dietary supplementation of an organic acid such as phenyllactic acid linearly improved egg production in the laying hen (21). However, the study of dietary supplementation of MCFAs, SCFAs, and their combination had no significant effect on laying performance (22). Swiaatkiewicz et al. (18) reported that dietary supplementation of 0.50% VFA (0.20% formic, 0.15% propionic, and 0.15% acetic acid), 0.25% MCFA (0.125% caproic and 0.125% capric acid), and 0.30% SCFA + 0.20% VFA had no effect on laying rate, daily egg mass, egg weight, feed consumption, and feed conversion in the layer hens diet with different levels of calcium and phosphorus. Indeed, VFAs were included in the organic acids that increased egg production in layers. The reason for the difference is unknown; it may have occurred due to differences in organic acids used and in the concentrations of MCFAs. High concentrations of MCFAs had mainly antibacterial effects and lower concentrations of MCFAs exerted more selective effects in the upper digestive tract and led to a change in the spectrum of the intestinal microbiota (23,24).

Eggshell quality is an important criterion for consumption because eggshell strength and thickness are essential for protection against the penetration of pathogens into eggs. Eggshell quality is influenced by many factors such as genetics, environment, nutrition, and the health status of layer hens. Dietary supplementation of calcium, phosphorus, vitamin D3, and microelements such as Zn, Mn, and Cu could positively affect egg shell quality (25). Several studies demonstrated that a reduced intestinal pH by lactic and formic acid as organic acids had a beneficial effect on calcium and phosphorus, and mineral absorption and dietary utilization (26,27). In the present study, laying hens fed on a microencapsulated organic acid blend with MCFAs increased their calcium concentration in the blood serum. This may have occurred due to an increase in calcium absorption by the dietary supplemented organic acids. Also, the positive effect on calcium absorption may have enhanced the eggshell strength in laying hens fed on a microencapsulated organic acid blend with MCFAs.

Organic acids are used as alternatives to antibiotics in animal diets, since their main function is to improve gastrointestinal tract health by reducing pH, inhibiting the invasion and proliferation of pathogenic bacteria, and promoting beneficial bacterial growth such as *Lactobacillus* (6,19). In the present study, the dietary supplementation of a microencapsulated organic acid blend with MCFAs showed a significant increase in the *Lactobacillus* content and a significant decrease in the *E. coli* content during a 10-week trial, which is in agreement with previous reports in pigs (14,28). The microencapsulated organic acid blend with MCFAs used in the present study showed a promotion of the *Lactobacillus* population in the experimental period and an inhibition of the *E. coli* population of finishing pig feces in our previous study (14). It is well accepted that *Lactobacillus* bacteria produce acid through fermentation, resulting in improvement of gastrointestinal tract health by reducing the intestinal pH level (29). Thus, the inhibition of *E. coli* content was possible due to a low tolerance of *E. coli* to the low pH by the acid produced from *Lactobacillus.*

In conclusion, the microencapsulated organic acid blend with MCFAs showed positive effects on the growth performance and nutrient digestibility in growing and finishing pigs in our previous studies. Thus, we hypothesized that dietary supplementation of a microencapsulated organic acid blend with MCFAs can positively influence laying hen performance. To test this hypothesis, we investigated the effects of this dietary supplementation on egg production, weight, quality, fecal microflora, and nutrient digestibility in laying hens. From the results, we concluded that dietary supplementation of a microencapsulated organic acid blend with MCFAs had positive effects on egg production, egg strength, Haugh units, calcium concentration in blood, and fecal *Lactobacillus* and *E. coli* contents in laying hens. Thus, the microencapsulated organic acid blend with MCFAs could be used as an alternative to antibiotics for laying hens.

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