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Effects of feed supplementation with increasing levels of organic acids on growth performance, carcass traits, gut microbiota and pH, plasma metabolites, and immune response of broilers*

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The effects of organic acids included in broiler feed on growth performance, carcass traits, caecal microbiota, duodenal pH, plasma metabolites, and immune response were studied. A total of 210 one-day-old female chickens of the Cobb 500 strain were allocated to one of the following treatments (three replicates of 10 birds per treatment): control (basal diet with no added acids), and basal diet supplemented with 0.3 and 0.5% of either formic acid (F0.3 and F0.5 treatments), or propionic acid (P0.3 and P0.5 treatments), or a commercial mixture of both acids and their ammonium salts (S0.3 and S0.5 treatments). The F0.5, P0.5 and S0.5 treatments showed the best results in body weight gain, feed conversion rate, final body weight, and relative breast and drumstick weights. The S0.5 treatment had the highest relative liver weight and *Lactobacilli* counts, and the lowest *Escherichia coli* counts. The lowest duodenal pH values corresponded to the P0.3, P.05 and S0.5 treatments. Plasma calcium was higher in the supplemented treatments. The immune response to Newcastle disease was more sustained in the F0.5, S0.3 and S0.5 treatments. Supplemented treatments had a better response to infectious bronchitis, while the responses to SRBC were higher in the F0.5, P0.5 and S0.5 treatments. Supplementation of broiler feed with 0.5% of formic or propionic acids

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positively influenced the productivity traits, but a mixture of both acids with their ammonium salts was even more effective. Changes in the intestinal environment due to organic acid supplementation could cause increased nutrient availability for the animals and improved the immune response.

KEY WORDS: Formic acid / propionic acid / poultry / production / digestion/ immunity

Several substances have been investigated in recent years with the aim of finding alternatives to growth-promoting antimicrobials that are able to support productive performance and prevent the incidence of some diseases in poultry [Huyghebaert *et al.* 2011]. Among such substances, organic acids and their salts have received much attention because they have shown not only antimicrobial activity, but several additional effects that go beyond those of antibiotics and could improve productivity in poultry [Dibner and Buttin 2002].

The antimicrobial effects of organic acids have been traditionally explained by the ability of the undissociated form to pass through the cell membrane, dissociate in the more alkaline interior and acidify the cell cytoplasm. Therefore, a lower pH in the gut or a higher pKa of the acid will increase their antimicrobial efficacy [Van Immersel *et al.* 2006]. Some other claimed effects of supplementary organic acids include improved digestion and absorption of some nutrients due to a lower digesta pH and a higher solubility of feed ingredients [Brenes *et al.* 2003, Rafacz-Livingston *et al.* 2005, Ao *et al.* 2009]; modulation of the natural microbial population in the gut by reducing adhesion of pathogenic bacteria and favouring commensal bacteria, which in turn could improve intestinal histomorphology [Choct 2009] and modulate the immune response [Brisbin *et al.* 2008, Gabriel *et al.* 2006, Lan *et al.* 2005]; and direct trophic effects on the gastrointestinal mucosa, because they can act as rapidly absorbed energy sources for enterocyte cells [Sakata 1987].

In recent years, several authors have investigated the effects of broiler feed supplementation with formic and propionic acids and their salts, either alone [García *et al.* 2007, Hernández *et al.* 2006, Khosravi *et al.* 2012] or in combination [Gunal *et al.* 2006, Isabel and Santos 2009, Khodambashi *et al.* 2013], on growth performance and carcass traits, but the results are contradictory. Moreover, studies simultaneously comparing the effects of those acids at different inclusion levels in the feed are scarce. Besides, very few authors have reported data on plasma metabolites or immune response in broilers after supplementation with those substances.

The aim of the present work was to investigate the effects of feed supplementation with two levels of formic acid, propionic acid, and a commercial mixture of both acids and their ammonium salts on growth performance, carcass traits, caecal microbiota, duodenal pH, plasma metabolites and immune response of broiler chickens.

Material and methods

Animals, housing, diets and treatments

Use and care of birds and procedures in this study were approved by the Islamic Azad University Ethics Committee. A total of 210 one-day-old female chickens of the Cobb 500 strain (Cobb-Vantress Inc., Siloam Springs, AR) purchased from a commercial hatchery were used. The broiler chicks were placed in 1×1 m cages, which floor was covered with shredded paper. Each cage was equipped with a pan feeder and a manual drinker. The research facility was an open-sided poultry barn having thermostatically controlled curtains and equipped with thermostatically controlled gasoline rocket heaters, overhead sprinklers, wall-mounted fans in both ends of the barn, and fluorescent tubes in ceiling fixtures. Ambient temperature was set at 32°C at placement and then decreased gradually to reach 24°C from week 3 onwards. Lighting was constant at day 1. From day 2 to the end of the study, the light regime was 23L:1D. Feed (mash form) and water were provided *ad libitum* throughout the whole trial.

Table 1. Experimental diets fed to broiler chickens

Item	Starter 1-7 days	Grower 8-21 days	Finisher 22-42 days
Ingredients (%)			
maize	41.44	42.60	39.50
soybean meal	31.55	25.50	22.23
wheat	20.00	25.00	30.00
soybean oil	2.50	2.50	4.00
dicalcium phosphate	1.89	1.82	1.68
oyster shells	1.33	1.28	1.22
vitamin mixture[1]	0.25	0.25	0.25
mineral mixture[2]	0.25	0.25	0.25
dl-methionine	0.20	0.22	0.24
l-lysine hcl	0.10	0.19	0.23
salt	0.23	0.15	0.12
sodium bicarbonate	0.20	0.20	0.20
enzymes[3]	0.05	0.05	0.05
Calculated analysis[4]			
metabolizable energy (MJ/kg)	12.5	12.9	13.3
crude protein (%)	21.0	19.0	18.0
lysine (%)	1.20	1.10	1.05
methionine + cysteine (%)	0.46	0.44	0.43
tryptophan (%)	0.20	0.19	0.19
calcium (%)	1.00	0.96	0.90
available phosphorus (%)	0.50	0.48	0.45

[1]Supplied per kilogram of feed – Vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K_3: 2 mg; thiamine: 1.8 mg; riboflavin: 6.6 mg; calcium pantothenate: 10 mg; niacin: 30 mg; pyridoxine: 3 mg; folic acid: 1 mg; vitamin B12: 0.015 mg; biotin: 0.1 mg; choline: 25 mg.
[2]Supplied per kilogram of feed - Mn: 99.2 mg; Fe: 50 mg; Zn: 84.7 mg; Cu: 10 mg; I: 1 mg; Se: 0.2 mg.
[3]Kemzyme MAP Dry (Kemin Industries, Inc., U.S.A.).
[4]According to National Research Council (1994).

The experiment lasted 42 days. The feeding programme consisted of a starter diet until the chicks were 7 days old (starting period), followed by a grower diet up to 21 days of age, (growing period), and then a finisher diet until the end of the experiment (finishing period). All feeds were maize-soybean meal based and did not contain any antibiotic feed additives. The diets were formulated according to the Cobb 500 strain rearing catalogue recommendations (Tab. 1). Chicks were assigned into one of the following treatments: the control (basal diet without organic acids), and the same basal diet supplemented with 0.3 and 0.5% of either formic acid (F0.3 and F0.5 treatments), or propionic acid (P0.3 and P0.5 treatments), or Salgard (S0.3 and S0.5 treatments). Salgard (Optivite, Worksop, UK) contains a mixture of formic and propionic acids and their respective ammonium salts. Each treatment had three replicates, thus there was a total of 21 groups of 10 birds caged together.

Growth performance and carcass measurements

Body weight (BW) of the chicks and feed consumption were weekly recorded by cage, and body weight gain (BWG, g/period), feed intake (FI, g/period), and feed conversion ratio (FCR, feed to gain g/g) were determined. At the age of 42 days, after 4 hours of fasting to ensure complete evacuation of the gut, three chickens per treatment (one from each replicate) that had weights closest to the mean weight for the cage were selected and euthanized by cervical dislocation to determine carcass traits. Birds were fully plucked by the dry plucking method and the feet, head, and wingtips were removed. Broilers were eviscerated before determining their carcass weight. Weights of the breast muscles, drumsticks, wings, the liver and bile, and the small intestine were recorded.

Microbial enumeration and duodenal pH measurement

At 42 days of age, three chickens per treatment (one from each replicate) were selected as above and euthanized. From each euthanized bird, the caeca were quickly dissected and their contents were collected in sterilized sampling tubes. From those contents, 10-fold serial dilutions of 1 g sample were serially made in phosphate buffer solution (10^{-1}-10^{-6}). Subsequently, 100 µL were removed from 10^{-4}, 10^{-5}, and 10^{-6} dilutions and poured onto petri dishes containing the culture media. *Lactobacilli* were cultured in De Man, Rogosa and Sharpe agar and incubated at 37°C under anaerobic conditions for 72 h. *Escherichia coli* were cultured in eosin methylene blue agar and incubated at 37°C under aerobic conditions for 48 h. Bacterial colony forming units (CFU) in petri dishes were counted using a colony counter. The counts were reported as $\log_{10}$ CFU per 1 g of sample. To measure duodenal pH, the same number of animals was selected as above and euthanized. Their duodenal contents were gently expelled into containers and diluted with 10 ml deionized water; then, pH was measured with a pH meter.

Blood sampling and analysis and immune response study

T**o** measure plasma metabolites and minerals at 42 days of age, three chickens per treatment (one from each replicate) were selected as above to collect blood from their wing veins into EDTA tubes. After centrifuging blood samples (3000 g, for 10 min at room temperature), plasma was harvested and stored in Eppendorf tubes at -20°C until assayed. Biochemical analysis was conducted according to standard protocols using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran). Parameters measured included glucose, total protein, albumin, uric acid, triglycerides, cholesterol (total, HDL, LDL and VLDL), calcium and phosphorus.

Production of antibodies to different antigens was assessed during the experiment. First, the birds were vaccinated against infectious bronchitis (4^{th} and 17^{th} day of age), Newcastle disease (9^{th}, 20^{th} and 30^{th} day of age), influenza (1^{st} day of age) and Gumboro disease (14^{th} and 23^{rd} day of age). All vaccines were provided by Razi Co. (Tehran, Iran). Additionally, one bird per replicate was injected under the breast skin with 0.5 ml of a 10 % suspension in phosphate buffered saline of sheep red blood cells (SRBC) at 21^{st} and 35^{th} day of age. To determine the systemic antibody response, blood samples were collected from one chick per replicate from the wing vein at 21^{st}, 35^{th} and 42^{nd} days of age (Newcastle disease), at 42^{nd} day of age (infectious bronchitis and Gumboro disease), and at 28^{th} and 42^{nd} (SRBC). Blood samples were processed and analysed as described by Pourhossein *et al.* [2014]. Briefly, antibody responses to infectious bronchitis and Gumboro disease were measured by the enzyme-linked immunosorbent assay using commercially available kits. To determine the antibody response to Newcastle disease a hemagglutination inhibition assay was used. Total immunoglobulin (Ig) and immunoglobulin G (IgG) titers to SRBC were determined by the hemagglutination assay; then, immunoglobulin M (IgM) titers to SRBC were calculated as the difference between total Ig and IgG titers.

Statistical analysis

The GLM procedure of SAS 8.0 (SAS Institute Inc., Cary, NC) was used in the statistical analyses. The statistical analysis was based on following unitrait linear model: $y_{ij} = \mu + T_j + e_{ij}$, where yij is the dependent variable; μ is the overall mean; T_j is the effect of the treatment; and e_{ij} is the residual error. Treatment means were compared using the Duncan's multiple range test. Statistical significance was declared at $P<0.05$.

Results and discussion

Compared with the control, BWG was higher ($P<0.05$) in the F0.5, P0.3, P0.5, S0.3, and S0.5 treatments (Tab. 2). Except for P0.3, those treatments had better FCR ($P<0.05$) than the control. Regarding carcass traits, the F0.5, P0.5, S0.3 and S0.5 treatments had higher final BW ($P<0.05$) than the control (Tab. 2). Except for S0.3, those treatments had higher relative breast weights ($P<0.05$) and tended to have higher relative drumstick weights than the control. Clearly the best results were obtained

with the F0.5, P0.5 and S0.5 treatments. However, results presented in the literature are contradictory. Some authors have reported improved growth performance in broilers when fed diets supplemented with different levels of formic and propionic acids or their mixture [García *et al.* 2007, Khodambashi *et al.* 2013, Paul *et al.* 2007]. However, several other authors have failed to show any improvements related with those substances [Hernández *et al.* 2006, Isabel and Santos 2009, Khosravi *et al.* 2010]. Contrary to our results, the few authors that previously investigated the changes in carcass traits found no differences between treatments [Denli *et al.* 2003, García *et al.* 2007, Isabel and Santos 2009]. The highest relative weight of liver ($P<0.05$) was observed in the S0.5 treatment and it tended to be higher in all the acid supplemented treatments compared with the control. No significant changes ($P>0.05$) of small intestine relative weight were observed due to organic acid supplementation. Those results disagreed with the previous findings of Gunal *et al.* [2006] and Abdel-Fattah *et al*. [2008].

The observed positive results could be explained, at least in part, by a more favourable microbiota in the digestive tract and a more complete digestion of the feed. In the present work, *Lactobacilli* and *Escherichia coli* counts were higher

Table 2. Body weight gain (BWG), feed intake (FI) and feed conversion rate (FCR), carcass traits, organ weights, caecal microbiota, and duodenal pH of 6-week old broilers fed the control diet or the same diet supplemented with organic acids

Item	Control		F0.3		F0.5		P0.3		P0.5		S0.3		S0.5	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BWG (g/period)	2192^{d}	34.6	2087^{d}	36.1	2404^{abc}	37.3	2330^{c}	29.7	2341^{bc}	30.2	2341^{bc}	38.6	2497^{a}	42.6
FI (g/period)	5437	298.3	4983	269.2	4806	243.2	5193	280.4	4983	297.3	4933	298.9	4792	268.4
FCR (g/g)	2.48^{a}	0.14	2.38^{a}	0.10	1.99^{bc}	0.16	2.22^{ab}	0.23	2.12^{b}	0.08	2.10^{b}	0.14	1.91^{c}	0.16
Body weight (BW, g)	2167^{c}	69.3	2090^{c}	68.6	2403^{b}	43.6	2247^{bc}	57.9	2440^{a}	65.4	2358^{ab}	45.6	2440^{a}	41.9
Carcass weight (% BW)	63.85	1.98	63.84	2.96	68.80	2.64	65.80	3.00	68.72	1.91	64.36	0.89	68.69	1.56
Breast (% BW)	20.67^{b}	0.67	20.30^{b}	0.85	23.05^{a}	0.47	21.52^{ab}	0.51	23.06^{a}	0.67	21.84^{ab}	0.39	22.73^{a}	0.96
Drumsticks (% BW)	17.28^{bc}	0.47	16.91^{c}	0.71	19.49^{ab}	0.91	16.72^{c}	0.47	18.85^{ab}	0.57	17.39^{bc}	0.39	17.88^{abc}	0.97
Wings (% BW)	4.98^{a}	0.39	4.39^{ab}	0.11	5.24^{a}	0.64	4.08^{b}	0.31	5.13^{a}	0.15	4.66^{ab}	024	5.12^{a}	0.47
Organ weights														
liver and bile (% bw)	2.00^{b}	0.08	2.13^{ab}	0.19	2.30^{ab}	0.24	2.30^{ab}	0.06	2.24^{ab}	0.09	2.19^{ab}	0.14	2.42^{a}	0.16
small intestine (% bw)	4.27	0.29	4.26	0.26	4.93	0.22	4.74	0.44	4.65	0.36	3.92	0.28	4.51	0.14
Bacterial counts ($\log_{10}$ CFU/g digesta)														
Lactobacilli	7.96^{b}	0.32	8.80^{ab}	0.24	8.46^{ab}	0.29	8.73^{ab}	0.36	8.81^{ab}	0.24	8.27^{b}	0.19	9.29^{a}	0.30
Escherichia coli	8.12^{ab}	0.21	8.38^{ab}	0.45	8.69^{a}	0.24	8.14^{ab}	0.36	7.57^{bc}	0.28	7.49^{b}	0.20	6.99^{c}	0.38
Duodenal pH	5.96^{ab}	0.35	6.57^{a}	0.42	5.88^{ab}	0.40	5.56^{b}	0.14	5.42^{b}	0.20	6.15^{ab}	0.31	5.48^{b}	0.28

F0.3, and F0.5: diets supplemented with formic acid at 0.3 and 0.5%, respectively.
P0.3, and P0.5: diets supplemented with propionic acid at 0.3 and 0.5%, respectively.
S0.3, and S0.5: diets supplemented with a commercial mixture of formic and propionic acids and their ammonium salts at 0.3 and 0.5%, respectively.
[abcd] In rows means bearing different superscripts differ significantly at $P<0.05$.

(P<0.05) and lower (P<0.05) in the S0.5 treatment, respectively (Tab. 2). Moreover, *Lactobacilli* counts tended to be higher in the other supplemented treatments compared with the control, except for the S0.3 treatment that did not differ (P>0.05), while *Escherichia coli* counts tended to be lower in the P0.5 and S0.3 treatments compared with the control. The lowest duodenal pH values were observed in the P0.3, P0.5 and S0.5 treatments, although non-significant differences existed (P>0.05) between treatments (Tab. 2). Those modifications in the intestinal environment partially matched the above-mentioned improvements in growth performance and carcass traits. Changes in microbiota could cause a reduction in the host-microbe competition for nutrients and a better mucosa functionality, thus leaving more nutrients available to be absorbed and improving mucosa absorptive capacity. In addition, the reduced duodenal pH could contribute to a better solubilisation of feed ingredients and a more complete digestion, as observed by García *et al.* [2007] and Khodambashi *et al.* [2013]. Those effects could explain the higher calcium concentration in the plasma of the chickens fed the supplemented diets (Tab. 3). In this regard, Hernández *et al.* [2006] found no differences between the acid supplemented treatments and the control in the pH values measured at the jejunum, as well as

Table 3. Blood plasma constituents of 6-week old broilers fed the control diet or the same diet supplemented with organic acids

Item	Control		F0.3		F0.5		P0.3		P0.5		S0.3		S0.5	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Glucose (mg/dL)	75.36	13.24	88.94	14.69	101.82	26.32	82.12	16.78	71.07	14.38	86.53	19.65	71.30	17.64
Total protein (g/dL)	4.22	0.36	3.79	0.22	3.93	0.30	3.65	0.25	3.90	0.23	4.17	0.29	4.14	0.31
Albumin (g/dL)	2.11	0.26	2.29	0.30	2.51	0.29	2.25	0.32	2.39	0.24	2.37	0.23	2.25	0.21
Uric acid (mg/dL)	4.39	0.92	5.30	1.23	3.94	1.02	4.45	1.36	3.21	0.86	4.73	1.24	3.15	1.01
Triglycerides (mg/dL)	97.66	16.45	105.0	18.54	116.33	20.01	100.33	18.12	109.00	21.03	95.00	13.21	104.00	16.28
Cholesterol (mg/dL)														
Total	124.9	11.32	132.72	14.56	129.74	13.24	134.52	14.21	137.81	13.87	115.09	15.69	124.66	11.27
HDL	44.53	4.69	49.15	6.78	55.46	5.19	60.53	6.37	45.95	4.18	56.97	6.47	53.00	5.27
LDL	57.56	18.28	45.90	12.39	49.67	14.27	53.92	16.84	64.06	22.43	39.11	11.03	52.19	13.56
VLDL	16.20	3.68	21.00	6.41	24.60	7.52	20.06	6.32	27.80	4.23	19.00	3.92	19.46	4.01
Calcium (mg/dL)	8.13^{b}	0.68	10.84^{ab}	1.01	9.51^{ab}	0.98	10.36^{ab}	.87	8.95^{ab}	0.74	11.27^{a}	1.23	10.51^{ab}	0.98
Phosphorus (mg/dL)	8.6	0.24	8.93	0.36	8.82	0.32	8.55	0.23	8.64	0.19	9.0	0.34	8.90	0.36

F0.3, and F0.5: diets supplemented with formic acid at 0.3 and 0.5%, respectively.
P0.3, and P0.5: diets supplemented with propionic acid at 0.3 and 0.5%, respectively.
S0.3, and S0.5: diets supplemented with a commercial mixture of formic and propionic acids and their ammonium salts at 0.3 and 0.5%, respectively.
^{ab}In a rows, means bearing different superscripts differ significantly at P<0.05.

not in the calcium plasma levels. On the other hand, the fact that the S0.5 treatment showed more pronounced effects on intestinal microbiota and pH could be attributed to a more sustained action along the gastrointestinal tract due to the different pKa values of formic and propionic acids and their salts. Mixtures of organic acids with different pKa values are expected to have a broader spectrum of action throughout the gastrointestinal tract [Van Immerseel *et al.* 2006], in which increasing pH values are encountered when the feed moves towards its lower parts [Gabriel *et al.* 2006].

The lack of effects of organic acid supplementation on plasma metabolites (Table 3) is in agreement with the results reported by Hernández *et al.* [2006] and, except for LDL cholesterol, with Khosravi *et al.* [2008]. It is noteworthy that the suspected increase in nutrient digestion and absorption due to organic acid supplementation did not influence plasma metabolite levels in the present work. However, the fact that the relative liver weight was higher ($P<0.05$) in the S0.5 treatment and tended to be higher in the other supplemented treatments when compared with the control (Tab. 2) suggests an increased lipogenetic activity [Aletor *et al.* 2000], which could have been caused by a greater substrate availability.

The effects of dietary organic acid supplementation on the immune responses against vaccines and SRBC are shown in Table 4. There were no clear trends in the immune

Table 4. Immune response after vaccination or injection of sheep red blood cells (SRBC) in broilers fed the control diet or the same diet supplemented with organic acids

Item	Control		F0.3		F0.5		P0.3		P0.5		S0.3		S0.5	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Newcastle disease (log_2)														
21 d	4.33	0.82	4.00	0.70	4.66	0.76	3.66	0.69	2.66	0.58	4.00	0.42	4.66	0.80
35 d	4.00	0.46	4.33	0.52	4.00	0.69	4.33	0.38	4.66	0.40	4.66	0.54	5.00	0.69
42 d	2.33^{b}	0.39	2.33^{b}	0.42	3.00^{ab}	0.36	2.33^{b}	0.45	2.33^{b}	0.40	4.00^{a}	0.39	3.00^{ab}	0.31
Gumboro disease 42 d (log_{10})	6934^{a}	598.2	3743^{c}	670.1	4903^{abc}	425.3	5980^{ab}	658.3	4486^{bc}	728.8	3188^{b}	687.2	4028^{bc}	581.6
Infectious bronchitis 42 d (log_{10})	1722^{b}	498.3	2252^{ab}	505.6	2482^{ab}	671.2	2125^{ab}	482.1	3786^{a}	628.6	3064^{ab}	600.4	3314^{ab}	716.0
SRBC (log_2)														
Total Ig 28 d	2.61^{bc}	0.24	2.33^{c}	0.19	3.33^{a}	0.29	2.50^{bc}	0.32	3.66^{a}	0.24	2.50^{bc}	0.29	3.16^{ab}	0.24
Total Ig 42 d	3.00^{e}	0.29	3.16^{c}	0.42	5.00^{ab}	0.37	3.16^{c}	0.35	4.00^{bc}	0.42	3.50^{c}	0.31	5.16^{a}	0.47
IgG 28 d	1.16	0.27	1.16	0.32	1.83	0.34	1.33	0.16	1.66	0.28	1.50	0.19	1.83	0.32
IgG 42 d	1.66^{b}	0.39	1.83^{b}	0.16	2.33^{ab}	0.24	1.66^{b}	0.30	1.83^{b}	0.32	1.83^{b}	0.26	2.83^{a}	0.29
IgM 28 d	1.00^{e}	0.21	1.00^{e}	0.23	1.50^{b}	0.20	1.16^{de}	0.11	2.00^{a}	0.16	1.00^{e}	0.08	1.33^{bc}	0.16
IgM 42 d	1.33^{d}	0.21	1.33^{d}	0.28	2.50^{a}	0.30	1.50^{cd}	0.25	2.16^{abc}	0.21	1.66^{bcd}	0.23	2.33^{ab}	0.21

F0.3, and F0.5: diets supplemented with formic acid at 0.3 and 0.5%, respectively.
P0.3, and P0.5: diets supplemented with propionic acid at 0.3 and 0.5%, respectively.
S0.3, and S0.5: diets supplemented with a commercial mixture of formic and propionic acids and their ammonium salts at 0.3 and 0.5%, respectively.
[abcde]In a rows, means bearing different superscripts differ significantly at $P<0.05$.

response to the vaccines due to organic acid supplementation. The response to Newcastle disease was more sustained ($P<0.05$) in the F0.5, S0.3 and S0.5 treatments at 42 days of age. Supplemented treatments had no positive effects on Gumboro disease response compared with the control. The P0.5 treatment showed the highest ($P<0.05$) antibody response to infectious bronchitis and all supplemented treatments tended to have higher responses than the control. With regard to SRBC, the highest responses either at 28 or 42 days of age were in general observed in the F0.5, P0.5 and S0.5 treatments. Total Ig responses were higher ($P<0.05$) in the F0.5, P0.5 and S0.5 treatments at 28 days of age, and in the F0.5 and S0.5 treatments at 42 days of age. The highest ($P<0.05$) IgG responses at 42 days corresponded to the S0.5 treatment, whereas no significant differences ($P>0.05$) between treatments were observed at 28 days of age. The IgM responses at 28 and 42 days had the highest values ($P<0.05$) in the P0.5 and F0.5 treatments, respectively; also, they were higher ($P<0.05$) than in the control in the F0.5 treatment at 28 days, and in the P0.5 and S0.5 treatments at 42 days of age. Previous works reported contradictory results of organic acid supplementation in the immune response of broilers. Khosravi *et al.* [2010] found no differences in immune cell counts when supplementing broiler feed with 0.2% propionic acid. Other authors have reported a higher immune response after feed supplementation with 0.2% of a commercial mixture of formic and propionic acids and their ammonium salts [Khodambashi *et al.* 2013] or 0.2% citric acid [Rahmani and Speer 2005]. Improvements in the immune response of broilers due to feed supplementation with organic acids in the present work might be related to the observed increase of *Lactobacilli* counts in the gastrointestinal tract, since those bacteria have been reported to have beneficial effects on the host immune system [Xu *et al.* 2003].

In summary, supplementation of broiler feed with 0.5% of either formic acid or propionic acid, or a commercial mixture containing both acids and their ammonium salts clearly improved growth performance, some carcass traits, and titers of antibodies to infectious bronchitis and SRBC compared with no supplementation or 0.3% supplementationlevel. As a whole, our results indicate that the commercial mixture of organic acids buffered with their ammonium salts provides better results than the organic acids used separately. The observed effects were probably due to multiple complementary positive effects of the organic acids on the gastrointestinal tract, the most apparent being the reduction of duodenal pH and favourable changes in caecal microbiota, which in turn could increase nutrient availability for the animals and could improve the immune response.

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