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Does the Consumption of Acidified Drinking Water Affect Growth Performance and Lymphoid Organs of Broilers?

Hany M. S. Al-Mutairi [1], Elsayed O. S. Hussein [1], Abdul Rahman Jar El Nabi [1], Ayman A. Swelum [1,2], Mohamed E. Abd El-Hack [3,*], Ayman E. Taha [4] and Saud I. Al-Mufarrej [1,*]

1 Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460 Riyadh 11451, Saudi Arabia; halmutatiri@ksu.edu.sa (H.M.S.A.-M.); shessin@ksu.edu.sa (E.O.S.H.); a.nabi@arasco.com (A.R.J.E.N.); aswelum@ksu.edu.sa (A.A.S.)
2 Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt
3 Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
4 Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Egypt; Ayman.Taha@alexu.edu.eg
* Correspondence: dr.mohamed.e.abdalhaq@gmail.com (M.E.A.E.-H.); salmufarrej@ksu.edu.sa (S.I.A.-M.)

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Abstract: This study was conducted to compare the effects of single and synergistic organic acids (formic (F) and butyric (B) acids) on the performance and lymphoid organs of broiler chicks. In total, 450 one-day-old ISA JA57 chicks were assigned randomly to nine groups, each of five replicates (10 chicks/replicate): The control group, without added acids; groups 2 and 3, with 0.2% and 0.3% formic acid, respectively; groups 4 and 5, with 0.2% and 0.3% butyric acid, respectively; group 6, with 0.2% formic acid and 0.3% butyric acid; group 7, with 0.2% butyric acid and 0.3% formic acid; groups 8 and 9, with 0.2% butyric and formic acids and 0.3% butyric and formic acids, respectively. The control group received tap water, and other treatment groups received acidified drinking water, as previously described. The results indicated that treatment resulted in a significantly higher ($p < 0.05$) average live weight and weight gain at four weeks of age than in the control group. Nevertheless, group 7 showed significant decreases in the feed conversion ratio compared with the other treatments between four and five weeks of age. The carcass percentage was highest when B3, F3B2, and B2 were added to the drinking water, whereas control and F2B3 showed lower carcass percentage than the other treatments. At 42 days of age, the addition of organic acids to the drinking water of broilers had significant effects on the bursa of Fabricius and thymus percentages, but no effect on the spleen percentage. Water acidification by F and B alone and in combination did not affect poultry performance. However, it improved the lymphoid organ weight, indicating improved immunity and carcass percentage at 42 days of age.

Keywords: formic acid; butyric acid; poultry performance; lymphoid organs

1. Introduction

The development of livestock and animal health has not always led to sustainable increases in farmers' welfare or animal productivity due to the lack of understanding of livestock production systems. The multipurpose functions of livestock and the complex relationships between animal health, nutrition, breeding, and biotechnology require a systems approach to optimize the use of resources [1]. Therefore, multi-stakeholders directed their goals toward improving the environmental sustainability of livestock via better metrics and methods, such as acidification and fermentation of products, which provide better keeping quality [2].

In applied physiology, growth and nutrition are closely related and complementary subjects. The use of different strategies to explore this relationship can improve animal production [3,4]. The achievement of optimum broiler performance has led to the search for alternative growth promoters, especially owing to the ban on using antibiotics as growth promoters [5]. Thus, researchers have developed physiological additives, such as acidifiers, prebiotics, and probiotics, to enhance immunity and improve performance. These additives aid development of normal physiological functions in animals or ameliorate their deficiencies [6,7].

Organic acids are weak acids that enhance intestinal function. Correct usage of these compounds together with proper nutrition, management, and biosecurity measures confers several beneficial effects, such as enhancement of protein digestion [8], leading to improved feed conversion ratio (FCR), growth performance, and immunity [9], as well as enhancement of mineral absorption from the intestine [10,11]. Formic acid (CH_2O_2) is a derivative of formate and is considered the simplest form of carboxylic acid. It has a pleasant smell and is volatile. The free form is not used as a dietary supplement; however, its formats or salts, which are more comfortable to handle and less pungent, are widely used in broiler diets. Several reports were cleared of the positive effects of formic acid on body weight (BW) gain [12,13]. However, inconsistent effects were reported for dietary formats [14]. Butyric acid ($C_4H_8O_2$) is the most important source of energy to the epithelial cells; it required for normal epithelial cell development and has antibacterial activity in the gastrointestinal tract [15]. Many poultry researchers intensively studied butyric acid; diets supplemented with butyric acid improved feed efficiency and BW gain of broilers [16].

On the other hand, high levels of butyric acid inclusion may impair feed efficiency [17]. Recently, synergistic positives of organic acid effects on boiler growth performance were studied [18–21]. It is well known that lymphoid organs, such as the bursa, thymus, and spleen, are a part of the immune system [22], which responsible for the protection of the birds from microorganism invasion by producing immune cells (B and T cells). To date, limited reports have been published that evaluate the effects of organic acids on lymphoid immune organs, such as the thymus, bursa of Fabricius, and spleen in broiler chickens [23].

Therefore, the present study aimed to investigate the effects of water acidification using formic and butyric acids alone and in combination on growth performance of chickens through the determination of their impact on live body weight (LBW), feed intake (FI), feed conversion ratio (FCR), and carcass percentage. In addition, the estimation of the lymphoid organs of broiler chickens during the experimental period was conducted from 1 to 42 days of age.

2. Material and Methods

The experimental procedures and protocol used in the current study were approved by the Animal Use and Care Committee of the College of Food and Agricultural Sciences, King Saud University (KSU-0125-0125). In total, 450 one-day-old ISA JA57 chicks were obtained from a local hatchery and then assigned randomly to nine groups, each of five replicates (10 chicks/replicate, with five males and five females). Each pen was equipped with a feeder and an automatic drinker. The boilers were kept at 33 °C for the first week, and the temperature was reduced after that by 3 °C/week until it reached approximately 24 °C, when the broilers were four weeks of age. The light was provided continuously by using incandescent lamps. Chicks firstly received a commercial starter diet from 0–3 weeks of age, and then received a commercial grower diet from 3–6 weeks of age (Table 1).

Table 1. Ingredients and compositions of basal diets.

Ingredient	Periods (0-6) Weeks	
	Starter (0–3 Weeks)	**Finisher (3–6 Weeks)**
Soybean meal	27.00	22.80
Yellow corn	57.56	61.50
Corn gluten meal	8.80	6.0
Wheat bran	0.00	3.0
Limestone	0.70	0.62
Di calcium phosphate	2.30	2.09
DL-methionine	0.105	0.075
L-lysine	0.39	0.36
Choline chloride	0.05	0.05
Vitamin–mineral premix [1]	0.50	0.50
Salt	0.40	0.20
Threonine	0.17	0.17
Palm oil	2.20	2.80
	Nutrients	
Crude protein (%)	23.0	20.5
Metabolizable Energy (kcal/kg)	3000	3050
Available P (%)	0.48	0.44
Calcium (%)	0.96	0.88

[1] V–M premix; Vitamin–mineral premix contains in the following per kg: Vitamin A, 2,400,000 IU; vitamin D, 1,000,000 IU; vitamin E, 16,000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin, 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18,000 mg; selenium, 60 mg; zinc, 14000 mg.

2.1. Water Treatments and Experimental Design

Each of the five cages within a battery was provided with one of eight water treatments; treatments were started when the chicks were one day old. The treatments consisted of a control group without any supplements, while groups 2 and 3 received 0.2% and 0.3% formic acid (F2 and F3), respectively. Groups 4 and 5 received 0.2% and 0.3% butyric acids (B2 and B3). Group 6 (F2B3) received the addition of 0.2% and 0.3% of formic and butyric acids, respectively. Group 7 (B2F3) received the addition of 0.2% and 0.3% of butyric and formic acids, respectively. The treatments of groups 8 (F3B2) and 9 (F3B3) comprised the addition of 0.2% formic acid and 0.2% butyric acids, and 0.3% formic acid and 0.3% butyric and formic acid, respectively. The control group received tap water, and the other treatment groups received acidified drinking water with formic acid (F), butyric acid (B), and a mixture of F and B acids, respectively.

The 450 experimental birds, one-day-old ISA JA57 broiler chicks, were divided into groups and immunized against Newcastle disease (NDV) and infectious bronchitis (IBV) (Hb1 and Connecticut; LaSota and Massachusetts) at 1 and 18 days of age via drinking water, respectively. A vaccination against IBD (Bursine-2) was given at 14 days of age. Blood samples were collected at 1, 7, 14, 28, 35, and 42 days post-immunization of the primary and secondary doses. BW was determined at hatch and weekly up to 6 weeks of age by the cage, and then the average daily gain was calculated. The feed was weighed on days 7, 14, 21, 28, 35, and 42, and the average daily feed intake and feed conversion (gram feed: Gram gain) were determined weekly and for the total period (1–42 days). Mortality was recorded daily during the experiment. At 21 and 42 days of age, ten broilers/treatment were selected randomly as one male and one female and were euthanized by exsanguination. The live BW of each broiler was obtained before it was killed; subsequently, the spleen, thymus, bursa of Fabricius, and carcass were collected and weighed to determine the organ-to-BW ratio.

2.2. Relationship between Body Weights and Lymphoid Organs

The body weights (BW) of the lymphoid organs of the bursa of Fabricius, spleen, and thymus were measured at 21 and 42 days of age and expressed as a percentage related to BW. The body weight (BW) of the carcass was expressed as a percentage of BW measured at 42 days of age.

2.3. Statistical Analysis

One-way analysis of data (ANOVA) practices were used by using the general linear model (GLM) procedure of the SAS software. The mortality was lower and was not significant among treatments; therefore, this has not been reported.

3. Results

3.1. Growth Performance

The effects of the addition of organic acids on live BW of broiler chicks during the experimental period are shown in Table 2. The addition of organic acids to the broilers' drinking water significantly reduced the live body weight (LBW) compared with the control treatment at all ages; F3, F3B3, and F2B2 resulted in the best LBW, whereas B2 resulted in the lowest LBW. The data in Table 3 illustrate that the addition of different concentrations of organic acids to the drinking water of the birds significantly decreased body weight gain (BWG) compared with the control treatment at all time points analyzed; F2B2 and F3 were the best treatments, whereas F2 was the worst.

The results in Table 4 show that there were significant differences at all ages of chicks, except for in the fifth and sixth weeks of age. At one week of age, feed intake (FI) was decreased significantly in response to the F2, B2, B3, F2B2, F2B3, and F3B3 treatments compared with the control treatment; B2 treatment yielded similarly to the control. At two weeks of age, FI was decreased by the treatment with F2, B2, F2B3, and F3B3, whereas F3, B3, F2B2, and F3B2 treatments yielded similar results to the control. At three weeks of age, FI was decreased significantly compared with the control treatment only by the F3 treatment, and F2 and F3B2 yielded higher results than other treatments. At four weeks of age, FI was increased considerably by the F3B2 treatment compared with the other therapies, as well as by F2B3 and F3B3 compared with the control treatment. At five and six weeks of age, FI was not significantly different, except that FI in the F2B3 and B2 treatments was greater than in the control and F3, respectively. At six weeks of age, FI was greater when F2B3 and B2 were used compared with all treatments, whereas control and F3 treatment resulted in the lower values.

Table 2. The effect of organic acid treatments on weekly body weights of broilers.

Treatment	Body Weight (g), Weeks						
	0	1	2	3	4	5	6
Con	40.47	96.50 ^{a}	232.00 ^{a}	545.60 ^{a}	860.19 ^{ab}	1272.85 ^{a}	1861.09 ^{a}
F2	40.17	76.03 ^{e}	195.67 ^{cd}	468.81 ^{cd}	756.11 ^{c}	1118.56 ^{bc}	1593.20 ^{b}
F3	40.03	83.30 ^{bcd}	217.33 ^{ab}	506.09 ^{bc}	855.99 ^{ab}	1265.20 ^{a}	1816.54 ^{a}
B2	40.00	68.60 ^{f}	165.00 ^{e}	394.93 ^{e}	700.19 ^{d}	1043.87 ^{c}	1552.97 ^{b}
B3	40.00	81.27 ^{cde}	213.67 ^{abc}	502.14 ^{bc}	841.67 ^{ab}	1255.27 ^{a}	1792.52 ^{a}
F2B2	40.17	88.50 ^{b}	218.67 ^{ab}	510.23 ^{b}	888.81 ^{a}	1249.65 ^{a}	1806.59 ^{a}
F2B3	39.87	79.93 ^{de}	190.33 ^{d}	459.39 ^{d}	819.62 ^{b}	1192.35 ^{ab}	1731.09 ^{a}
F3B2	39.83	87.27 ^{b}	211.00 ^{bc}	508.78 ^{b}	854.78 ^{ab}	1244.54 ^{a}	1770.04 ^{a}
F3B3	39.87	86.30 ^{bc}	203.67 ^{bcd}	486.01 ^{bcd}	854.50 ^{ab}	1267.72 ^{a}	1808.90 ^{a}
SEM	0.08	1.55	4.00	8.54	11.98	16.49	22.78
P value	0.808	>0.000	>0.000	>0.000	>0.000	>0.000	0.001

- Carrying different superscripts within the same column shows significance at ($p < 0.05$).
- Con: Control; F2, 3: Formic acid 2%, 3%; B2, 3: Butyric acid 2%, 3%; SEM: Standard error mean.

Table 3. The effect of organic acid treatments on body weight gain of broilers.

Treatment	Bodyweight Gain (gm/bird/day), Weeks						
	0–1	1–2	2–3	3–4	4–5	5–6	0–6
Con	8.00 ^{a}	19.39 ^{a}	44.77 ^{a}	44.94	58.95 ^{a}	84.03	43.39
F2	5.13 ^{e}	17.08 ^{abc}	39.03 ^{b}	41.05	51.78 ^{bc}	67.81	36.78
F3	6.18 ^{bcd}	19.14 ^{a}	41.26 ^{ab}	49.98	58.46 ^{a}	78.76	42.50
B2	4.08 ^{f}	13.76 ^{d}	32.86 ^{c}	43.61	49.10 ^{c}	72.73	36.02
B3	5.90 ^{cd}	18.93 ^{ab}	41.19 ^{ab}	48.51	59.09 ^{a}	76.75	41.73
F2B2	6.91 ^{b}	18.57 ^{ab}	41.68 ^{ab}	54.08	51.55 ^{bc}	79.56	42.10
F2B3	5.72 ^{de}	15.79 ^{cd}	38.42 ^{b}	51.46	53.25 ^{abc}	76.96	40.27
F3B2	6.78 ^{b}	17.66 ^{abc}	42.56 ^{ab}	49.43	55.68 ^{ab}	75.07	41.22
F3B3	6.64 ^{bc}	16.73 ^{bc}	40.36 ^{b}	52.64	59.03 ^{a}	77.31	42.15
SEM	0.22	0.39	0.71	1.14	1.16	1.19	2.80
P value	>0.000	>0.000	>0.000	0.071	0.04	0.078	0.062

- Carrying different superscripts within the same column shows significance at ($p < 0.05$).
- Con: Control; F2, 3: Formic acid 2%, 3%; B2, 3: Butyric acid 2%, 3%; SEM: Standard error mean.

Table 4. The effect of organic acid treatments on feed intake of broilers.

Treatment	Feed Intake (gm/bird/day), Weeks						
	1	2	3	4	5	6	1–6
Con	9.60 ^{a}	24.33 ^{ab}	59.33 ^{bc}	80.73 ^{b}	103.32	118.31	65.94 ^{c}
F2	7.95 ^{cd}	23.75 ^{ab}	63.61 ^{a}	81.76 ^{b}	100.52	126.10	67.28 ^{bc}
F3	7.50 ^{d}	25.76 ^{ab}	57.62 ^{c}	82.54 ^{b}	106.28	119.43	66.52 ^{c}
B2	9.17 ^{ab}	20.69 ^{c}	59.10 ^{c}	83.85 ^{b}	102.49	137.43	68.79 ^{b}
B3	8.07 ^{bcd}	26.33 ^{a}	59.27 ^{bc}	81.32 ^{b}	108.25	125.71	68.16 ^{b}
F2B2	8.47 ^{abcd}	25.40 ^{ab}	59.49 ^{bc}	85.87 ^{b}	97.63	121.16	66.34 ^{c}
F2B3	8.63 ^{abcd}	23.16 ^{bc}	60.08 ^{abc}	93.69 ^{a}	95.42	140.97	70.33 ^{ab}
F3B2	8.60 ^{abcd}	24.78 ^{ab}	63.21 ^{a}	94.02 ^{a}	97.07	122.91	68.43 ^{b}
F3B3	8.88 ^{abc}	23.85 ^{ab}	62.97 ^{ab}	88.43 ^{ab}	107.83	136.62	71.43 ^{a}
SEM	0.15	0.39	0.51	1.15	1.28	2.22	1.95
P value	0.012	0.008	0.011	0.003	0.105	0.072	0.043

- Carrying different superscripts within the same column shows significance at ($p < 0.05$).
- Con: Control; F2, 3: Formic acid 2%, 3%; B2, 3: Butyric acid 2%, 3%; SEM: Standard error mean.

Concerning feed conversion ratio (FCR) (Table 5), there were non-significant ($p > 0.05$) differences between the control and different treated groups throughout the experimental period except at 1–2 and 4–5 weeks of age, where the control group showed the best conversion ratio (1.26 and 1.75, respectively).

Table 5. The effect of organic acid treatments on feed conversion ratios of broilers.

Treatment	Feed Conversion Ratio (gm/bird/day), Weeks						
	0–1	1–2	2–3	3–4	4–5	5–6	0–6
Con	1.20	1.26 ^{e}	1.32	1.80	1.75 ^{b}	1.41	1.46
F2	1.55	1.39 ^{cd}	1.63	1.99	1.94 ^{ab}	1.87	1.73
F3	1.22	1.35 ^{d}	1.40	1.67	1.82 ^{b}	1.53	1.50
B2	2.25	1.50 ^{a}	1.81	1.92	2.09 ^{a}	1.89	1.91
B3	1.38	1.39 ^{cd}	1.44	1.69	1.83 ^{b}	1.64	1.56
F2B2	1.23	1.37 ^{cd}	1.43	1.60	1.90 ^{ab}	1.52	1.51
F2B3	1.51	1.47 ^{ab}	1.56	1.85	1.79 ^{b}	1.83	1.67
F3B2	1.27	1.40 ^{bcd}	1.48	1.91	1.74 ^{b}	1.64	1.57
F3B3	1.35	1.43 ^{bc}	1.56	1.68	1.83 ^{b}	1.76	1.60
SEM	0.06	0.01	0.03	0.03	0.03	0.04	0.04
P value	0.324	0.015	0.063	0.056	0.036	0.087	0.07

- Carrying different superscripts within the same column shows significance at ($p < 0.05$).
- Con: Control; F2, 3: Formic acid 2%, 3%; B2, 3: Butyric acid 2%, 3%; SEM: Standard error mean.

3.2. Carcass Percentage

The results in Table 6 showed that there were significant differences between all treatments concerning carcass percentage. The carcass percentage was higher under B3, F3B2, and B2 treatments, whereas control and F2B3 showed lower carcass percentage than the other treatments.

Table 6. The effect of organic acid treatments on relative weights of carcasses and lymphoid organs of broilers.

Treatment	Carcass (%)	Lymphoid Organs as a Percentage of Live Weight (%)					
		At 21 Days			At 42 Days		
		Spleen	Bursa of Fabricius	Thymus	Spleen	Bursa of Fabricius	Thymus
Con	67.7 ^{c}	0.41 ^{abc}	1.44	2.07	1.27	3.37 ^{a}	9.83 ^{a}
F2	72.3 ^{b}	0.48 ^{ab}	1.27	2.23	1.20	3.11 ^{ab}	7.97 ^{b}
F3	72.3 ^{b}	0.28 ^{c}	1.04	1.88	1.02	2.43 ^{b}	5.67 ^{d}
B2	73.4 ^{b}	0.42 ^{ab}	1.61	2.50	1.31	3.72 ^{a}	5.30 ^{d}
B3	77.1 ^{a}	0.43 ^{ab}	1.41	2.25	1.18	3.91 ^{a}	5.31 ^{d}
F2B2	72.5 ^{b}	0.50 ^{a}	1.64	2.97	1.32	3.61 ^{a}	5.89 ^{cd}
F2B3	71.3 ^{b}	0.48 ^{ab}	1.61	2.64	1.37	4.00 ^{a}	6.34 ^{bcd}
F3B2	73.6 ^{b}	0.36 ^{bc}	1.45	2.73	1.55	4.05 ^{a}	6.82 ^{bcd}
F3B3	71.9 ^{b}	0.40 ^{abc}	1.56	2.69	1.44	3.85 ^{a}	7.46 ^{bc}
SEM	0.46	0.02	0.50	0.10	0.04	0.12	0.25
P value	>0.000	0.029	0.172	0.182	0.127	0.011	>0.000

- Carrying different superscripts within the same column shows significance at ($p < 0.05$).
- Con: Control; F2, 3: Formic acid 2%, 3%; B2, 3: Butyric acid 2%, 3%; SEM: Standard error mean.

3.3. Lymphoid Organ Percentages

At 21 days of age, the addition of organic acids to chicken feed had significant effects on the spleen and no effect on the percentages of bursa of Fabricius and thymus (Table 6). The differences were higher when F2B2, F2, and F2B3 were added, whereas F3 and F3B2 resulted in lower rates of change than control and other treatments. At 42 days of age, the addition of organic acids to chicken feed had significant effects on the bursa of Fabricius and thymus percentages, but no impact on the spleen. The percentage of bursa of Fabricius was the highest when F3B2 and F2B3 were added, compared to the control and other treatments. The portion of the thymus was greater in the control and F2 compared with other therapies.

4. Discussion

The growth-promoting action is not evident in our study, although the antimicrobial activity of formic and butyric acids is, and these results are strongly supported by [12,24]. The observed difference of organic acid in the present study compared with the results of the other studies may be associated with the different experimental diets and environmental conditions. Several researchers reported that when chicks were housed in a clean environment, organic acids did not affect their performance [12]. Vale et al. [25] reported that there was no impact on BWG and FCR when broiler chickens had supplements of an organic acid. Butyrate also had no effects on BW and BWG [24]. In contrast, Pinchasov and Elmalich [26] and Islam et al. [27] found reduced BW gain in broilers fed with acetic-acid-supplemented diets. Organic acid salts, mainly ammonium formate and calcium propionate, increased live weight and the weight gain of broiler chickens until 21 days of age. However, no significant difference was observed compared with the control at 42 days of age, although FCR was improved [14]. Esmaeilipour et al. [28] studied the performance of broilers fed 0, 20, or 40 g/kg citric acid for 24 days. At 40 g/kg, decreased feed intake and BW gain occurred, according to Centeno et al. [29]. In disagreement with our results in Table 4, Cave [30] found that FI was decreased by dietary supplementation of propionic acid. Collectively, the variations in the results of the present study could be attributed to the different concentrations of acids used, differences in specific acids used, or differences in feed ingredients or environmental conditions.

The results in Table 6 showed improvements in carcass percentages under B3, F3B2, and B2 treatments. However, the control group expressed better growth performance parameters than those of the treated groups; it had the lowest carcass percentage, and this may be explained by the greater weight of the digestive tract organs, including gut contents. Similarly to the results of the present study, Huda-Faujan et al. [31] reported an improvement in the dressing percentage of broilers when acetic acid was added to drinking water. Dehghani-Tafti and Jahanian [32] found that the dietary inclusion of organic acids (OA) (citric + butyric) increased the carcass yield ($p = 0.016$). Contrary to the results of the present study, Rehman et al. [33] revealed no significant effect of acetic acid (AA) supplementation on the dressing percentage of broilers.

For the impacts on the lymphoid organs, the results in Table 6 are partially in agreement with those of Ghazalah et al. [34], who found that the relative weights of primary lymphoid organs (spleen, bursa of Fabricius, and thymus) were significantly ($p < 0.01$) improved by supplementation of formic acid, especially for 0.5%, compared to the basal diet. These results indicated that the addition of acidifiers in the diet of the broiler chicks conferred better immune response and disease resistance. There was a lack of studies representing and illustrating the effects of organic acid on relative weights of organs such as the bursa of Fabricius, spleen, and thymus [35]. In this respect, Katanbaf et al. [36] reported that the increase in relative organ weight indicated beneficial immunological advances. Therefore, further studies are needed to analyze the effects of F or B acid alone or in combination in different doses on lymphoid organs.

Taken together, our findings indicate that water acidification by F and B alone and in combination did not affect poultry performance; however, acidification improved lymphoid organ weight at 42 days of age, indicating increased immunity and carcass percentage.

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