



Original Research Article

Dietary supplementation with acidifiers improves the growth performance, meat quality and intestinal health of broiler chickens



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ABSTRACT

This experiment was conducted to study the effects of dietary supplementation with acidifiers on the growth performance, meat quality, and intestinal health of broiler chickens. A total of 648 male Arbor Acres broiler chickens at 1 d old were randomly divided into 6 groups, and each group consisted of 6 replicates with 18 broilers per replicate. The dietary treatments were as follows: negative control (NC, the basal diet), NC + antibiotic (enramycin, 8 mg/kg, positive control [PC]), NC + phosphoric acid (PA, 0.1, 0.2, and 0.3 g/kg), and NC + lactic acid (LA, 0.3 g/kg). The feeding trial lasted for 42 d. The results showed that the feed-to-gain ratio of the NC + acidifier groups was lower than that of the NC and PC groups from 1 to 42 d ($P < 0.05$). Compared with the values in the NC group, the pH of breast muscle was significantly higher in the NC + PA (0.2 g/kg) and LA (0.3 g/kg) groups ($P < 0.05$), and the cooking loss was lower in the breast muscle of the NC + PA (0.1 g/kg) and LA (0.3 g/kg) groups ($P < 0.05$). In addition, the shear force of the breast muscle and thigh muscle and the pH value in the crop, gizzard and duodenum of the antibiotic and acidifier groups were significantly decreased ($P < 0.05$). Moreover, the trypsin, chymotrypsin, and lipase activities of the duodenum in the NC + PA (0.2 and 0.3 g/kg) groups, as well as the villus height-to-crypt depth (VH:CD) ratio of the duodenum in the NC + PA (0.1 g/kg) group was significantly greater ($P < 0.05$) compared with those in the NC group. Meanwhile, the number of total aerobic bacteria, *Escherichia coli* and *Salmonella* in the cecum of the NC + PA (0.1 g/kg) and LA (0.3 g/kg) groups were decreased ($P < 0.05$). Collectively, diet supplementation with acidifiers could improve the growth performance, meat quality, and intestinal health of broilers, in which the effects of PA (0.1 g/kg and 0.2 g/kg) are better than the other supplementations.

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1. Introduction

The advent of antibiotics and their use has had a profound impact on animal health and welfare (Goforth and Goforth, 2000).

Moreover, antibiotics promote the growth of livestock and poultry by at least 3 effects: modulating metabolism, improving nutrients efficiency, and preventing diseases. Afsharmanesh et al. (2013) reported that body weight and feed intake increased by the dietary inclusion of antibiotics in broiler chickens. The onion (*Allium cepa* L.), when used as an antibiotic growth promoter supplementing the broiler diet, could induce favorable effects on performance and ileum microflora composition (Goodarzi et al., 2014). Contrarily, the use of antibiotics in livestock and poultry farming has been debated, as side effects may occur with long-term usage, such as residues in meat and the development of microbial resistance (Muaz et al., 2018). Therefore, in response to the emergence of antibiotic resistance and the unreasonable use of antibiotics, several European countries have restricted or banned the use of antibiotics as growth promoters (Food, 2010; Lancet, 2013).

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Moreover, several alternatives to the use of antibiotics in poultry are under investigation (Inatomi and Otomaru, 2018; Salah et al., 2019).

Unfortunately, the ban on the use of antibiotics has caused slower growth of livestock and poultry, worsened feed efficiency and increased the incidence of disease, which in turn has increased the amount of antibiotic treatment, increased the treatment costs, and reduced the economic benefits (Castanon, 2007; Zhang et al., 2017). Our previous study found that the withdrawal of antibiotic growth promoters (AGP) from feed induces poor growth performance, but that the supplementation of AGP has adverse effects on the meat quality of broiler chickens (Hamid et al., 2019). Therefore, concerns over the increasing emergence of antibiotic resistance and the unreasonable use of antibiotics have prompted efforts to develop so-called alternatives to antibiotics (Cheng et al., 2014).

Among these alternatives, the organic acids (or simply acidifiers) play an important role in the gut health in animals, and also improve nutrient digestibility. Organic acids are natural components in several feeds, are produced during the metabolism of animals, and are often used in feed acidification. Performance and health-promoting impacts have been elucidated for a number of organic acids, such as fumaric acid, formic acid, lactic acid (LA), citric acid, and their salts (Yang et al., 2018). Among them, LA research has become one of the hotspots in contemporary animal husbandry. It has the characteristics of no pollution, no residue, rapid absorption in the body, participation in metabolism (Datta and Henry, 2010; Lemire et al., 2014) and the tricarboxylic acid cycle, and is an important energy carrier (Kim and Gadd, 2008).

In contrast, the inorganic acidifiers, particularly hydrochloric acid, sulphuric acid and phosphoric acid (PA) are under used, even though they are cheaper than organic acids. Phosphoric acid, the most widely used inorganic acidifier, has the dual functions of acidifying and providing a source of phosphorus to the body. It can release up to 3H^+ and slow down the release rate of H^+ to play a lasting and effective role (Andrys et al., 2003). Furthermore, it is more effective for young poultry that have an unmaturing digestive system (Andrys et al., 2003). Moreover, we found that acidified drinking water, which was acidified with a liquid acidifier (Lupromix NC, produced by BASF Co., Ltd., consisting of propionic acid, ammonium propionate, formic acid and ammonium formate as active ingredients), could improve growth performance, compensate for gastric acidity, and control pathogenic bacteria in broilers (Hamid et al., 2018).

Thus, acidifiers in livestock nutrition are a cost-effective performance-enhancing option, exerting their effects through the feed, intestine and metabolism of animals (Roth et al., 2017). However, organic acidifiers and inorganic acidifiers are different, and the price of organic acidifiers is higher. The organic acidifiers have a better flavor and strong bacteriostatic effects, but inorganic acidifiers have a high degree of dissociation, and the speed is fast, so they can quickly reduce the pH value of feed in the stomach. However, the sharp reduction rate of pH may inhibit gastric acid secretion, burn the esophagus and stomach, and then inhibit normal development of the gastric function (Xiao et al., 2016). Moreover, the role of adding acidifiers is mainly to enhance the effect of acidification. When the content of acidifier in the feed exceeds 3.0%, it will easily lead to the loss of vitamins in feed because of the high acidity. At these levels, it is also prone to dilution and agglomeration or moisture, which may result in difficulties when making a premix. With the above advantages and differences, some concerns still persist regarding a comparison of the effects of supplementing broiler diets with organic and inorganic acids, and whether supplementing these acids are better than supplementing AGP.

Thus, the objectives of the present study were to examine the effect of dietary supplementation of acidifiers on the performance, meat quality and intestinal health of broilers, and to provide data to support the application of acidifiers in broilers.

2. Materials and methods

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of China Agricultural University, and the experimental protocol was approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China).

2.1. Materials

Arbor Acre (AA) broilers were obtained from Beijing Huadu Suikou commodity generation and were conventionally healthy 1-d-old males.

Acidifiers (PA, purity $\geq 85\%$; LA, purity $\geq 99\%$) were purchased from Qingdao Haoli Special Feed Additive Co., Ltd. (Qingdao, China). The antibiotic was 8 mg/kg enramycin from Wuhan Xingding Biotechnology Co., Ltd. (Wuhan, China).

2.2. Experimental design and diets

A total of 648 AA broilers (mean body weight: 43.5 ± 0.1 g) were randomly allotted to 6 treatment groups, with 6 replicate pens per group, and each replicate contained 18 chicks. A negative control group (NC group) was fed a basic diet based on the nutrient requirements of broilers (National Research Council, 1994). The experiment was divided into 2 stages: feeding the early feed from 1 to 21 d, and feeding the late feed from 22 to 42 d. The ingredients and calculated chemical composition of the basal diet are shown in Table 1. Chemical composition of the ingredients was determined as proposed by AOAC: Official Methods of Analysis, 17th ed. (2006).

The antibiotic group was fed a basic diet + antibiotic (enramycin, 8 mg/kg, positive control [PC]), whereas the acidifier groups were fed a diet based on the NC group + PA (0.1, 0.2, and 0.3 g/kg) and LA (0.3 g/kg) from d 1 (the PA or LA was diluted by mixing with bentonite in a ratio of 1:1 before being added into the basic diet). Each replicate was assigned to one pen and the broilers were caged in an enclosed room with 3-layer galvanized iron wire cages, using exhaust fans for ventilation. The temperature of the room was maintained at approximately 35 °C during the first week, then decreased gradually to reach a constant temperature of 25 °C. The relative humidity was maintained at between 65% and 70%. All chicks were allowed food and water ad libitum throughout the experimental period. Artificial light was provided at 23-h lighting:1-h darkness with fluorescent lights.

2.3. Sampling procedure

At the end of this experiment (42 d), 6 broilers (1 bird per replicate) with the closest mean weights were selected from each treatment, deprived of feed for 12 h, and then euthanized. The breast and thigh muscles (on the right side of the carcass) were removed from each carcass, trimmed, weighed, and chilled on ice after carefully removing the skin, thigh bone, and subcutaneous fat. The samples of duodenum and cecal digesta were collected and homogenized with 4-mL ice-cold saline (0.9% NaCl) and stored immediately at -80 °C until use.

Table 1
Ingredients and chemical composition of the basal diet (as-fed basis, %).

Item	1 to 21 d	22 to 42 d
Ingredients		
Corn	57.67	59.80
Soybean meal	28.30	25.65
Extruded soybean	8.00	8.00
Soybean oil	1.90	3.00
Limestone	1.30	1.10
Dicalcium phosphate	1.70	1.60
Salt	0.30	0.30
Lysine-HCl, 98.5%	0.10	0.00
D,L-Methionine	0.25	0.12
Threonine	0.05	0.00
Vitamin premix ¹	0.03	0.03
50% Choline chloride	0.10	0.10
Micro-mineral premix ²	0.30	0.30
Nutrient level³		
Metabolizable energy, MJ/kg	12.56	12.98
Crude protein	20.51	19.28
Calcium	1.01	0.90
Total phosphorus	0.66	0.63
Non-phytic phosphorus	0.43	0.41
D,L-Methionine	0.56	0.42
Methionine + Cystine	0.91	0.76
Lysine	1.15	1.01
Tryptophan	0.24	0.22
Threonine	0.81	0.72

¹ Provided the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 20 mg; vitamin K, 3 mg; vitamin B₁, 2.50 mg; vitamin B₆, 0.40 mg; vitamin B₁₂, 0.02 mg; niacin, 25 mg; vitamin B₅, 8 mg; folic acid, 1.20 mg; choline chloride, 450 mg.

² Provided per kilogram of diet: Cu, 15 mg; Fe, 20 mg; Zn, 80 mg; Mn, 80 mg; I, 1.50 mg; Se, 0.30 mg.

³ The nutrient levels were calculated values.

2.4. Growth performance

Feed intake and body weight were recorded at 21 and 42 d of age. The average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F:G) were calculated per replicate from 1 to 21 d, 22 to 42 d, and 1 to 42 d.

2.5. Meat quality determinations

Meat color (*a** redness, *b** yellowness and *L** lightness), drip loss and pH were measured as previously described (Jin et al., 2018). Meat color was immediately measured on the medial side of the breast and thigh muscles by a colorimeter (CR410, MINOLTA, Japan) with a 50-mm aperture. Three color readings, each measured in triplicate and then averaged, were taken from different locations on each sample. The pH was immediately measured at approximately 45 min and 24 h after slaughter on the same section of the breast muscle and thigh muscle using a pH meter electrode (HI99161, Hanna, Villafranca Padovana, Italy). Drip loss was assayed as described by Jin et al. (2018). In brief, the muscle pieces (2 cm thick × 3 cm wide × 5 cm long) cut from the same location in the breast muscle and thigh muscle were measured as the initial weight, freely suspended in a zip-lock bag, stored at 4 °C for 24 h and then reweighed to record a final weight which was used to evaluate the drip loss.

$$\text{Drip loss(\%)} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100\%$$

The breast muscle and thigh muscle samples were dissected, weighed, placed into a zip-lock bag, and cooked in a water bath at 75 °C for 20 min. They were then cooled under tap water and

equilibrated at room temperature. Next, the muscle samples were weighed again for determination of cooking loss (%).

$$\text{Cooking loss(\%)} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100\%$$

Shear force was evaluated as described by Jin et al. (2018). In brief, the breast muscle and thigh muscle samples were cooked in a water bath at 70 °C for 30 min, and the muscle pieces (3 cm thick × 2 cm wide × 1 cm long) were measured by a digital muscle tenderness tester (C-LM3B, TENOVO, Beijing, China).

2.6. Gastrointestinal tract pH measurement

The pH was measured in the crop, gizzard, proventriculus, duodenum, jejunum, ileum, and cecal digesta of the gastrointestinal tract of the AA broiler chicks at 43 d of age, based on the method of Piel et al. (2005). The electrode was first calibrated with standard solutions of pH 4 and 7. Then, the gastrointestinal tract contents were collected and transferred into 3 mL of distilled water. Each sample was measured 3 times using a pH meter (HI99161, Hanna, Villafranca Padovana, Italy).

2.7. Determination of duodenal digestive enzyme activity

Duodenal digestive samples were weighed precisely (0.01 g) and then homogenized in ice-cold distilled water in a 1:9 (wt/vol) proportion. After centrifugation (2,500 × g, 10 min, 4 °C), the supernatants were separated and kept at 4 °C for analysis.

Trypsin, lipase, chymotrypsin, and amylase activities were analyzed according to Palamidi et al. (2016) with commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). One unit of amylase activity (U/mg protein) was defined as 1 mg of glucose liberated by hydrolyzing starch for 1 min at 40 °C. The protein concentration of the enzyme extracts was measured by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard protein.

2.8. Gut morphology

The intestines were washed with phosphate buffered saline, and then, the duodenum, jejunum and ileum were separated for the morphological analysis. Samples were first fixed with 4% paraformaldehyde and then stained with hematoxylin and eosin (H&E), as described by Kelly-Hooper et al. (2012). Sections of 6 μm were cut for histological analysis, and then, the slides were observed by optical microscopy. Finally, the villus height and crypt depth and their ratios were measured using the Image-Pro Plus software (American, Media Cybernetics).

2.9. Intestinal microflora analysis

Cecal contents were collected and homogenized with an anaerobic culture solution of 1:5 (wt:vol) mixture and then filtered with sterile gauze. The colony counts were determined by the plate count method, and the medium was a selective medium for each bacterial strain. The total aerobic bacteria were cultured in a nutrient agar medium at 37 °C for 18 to 24 h; *Escherichia coli* was cultured in MacConkey medium at 37 °C for 18 to 24 h; *Salmonella* was cultured in Hektoen enteric (HE) agar medium at 37 °C for 18 to 24 h; and *Lactobacillus* was anaerobically cultured at 37 °C for 48 h in MRS medium. Colony counts were performed to calculate the number of bacteria per gram of cecal contents.

2.10. Statistical analysis

The results were analyzed by a one-way analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC) software. Data are shown as the means and pooled SEM. Duncan's multiple-range tests were used to evaluate the differences between treatments, and those differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Growth performance

The effects of dietary acidifier supplementation on growth performance are shown in Table 2. From 1 to 21 d, the F:G ratio in the NC + PA (0.1 and 0.2 g/kg) groups showed a decreased trend compared with that in the NC group ($P = 0.05$). Further, the F:G ratio in the NC + PA (0.1 g/kg) group also showed a decreased trend compared with that of the PC group ($P = 0.05$); whereas from 1 to 42 d, the F:G ratio in the NC + PA (0.1 and 0.2 g/kg) groups was significantly lower than that in the NC group ($P < 0.05$). In addition, the F:G ratio in the NC + PA (0.1 g/kg) group was significantly lower than that of the PC group ($P < 0.05$). From 22 to 42 d, there was no significant difference in ADFI and ADG between different treatment groups ($P > 0.05$).

3.2. Breast muscle meat quality

The breast muscle meat quality results from the broilers fed an acidifier are presented in Table 3. The diets supplemented with an antibiotic or acidifier significantly decreased the cooking loss and shear force in comparison to those of the NC group ($P < 0.05$). Meanwhile, compared to that in the NC group, the pH at 24 h in the PC group, the NC + PA (0.2 g/kg) group and the NC + LA (0.3 g/kg) group was significantly increased ($P < 0.05$).

3.3. Thigh muscle meat quality

As shown in Table 4, the thigh muscle meat from diets supplemented with an antibiotic or acidifier had significantly lower shear force than that in the NC group ($P < 0.05$). Additionally, the pH at 24 h was significantly different between the treatment groups ($P < 0.05$); however, the pH was not significantly different at 45 min ($P > 0.05$). Compared to that in the NC group, the b^* value in the antibiotic and acidifier groups was significantly increased ($P < 0.05$).

Table 2

Effects of dietary acidifier supplementation on the growth performance of broilers.¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
ADFI, g/d								
1 to 21 d	54.2	54.4	53.5	53.5	53.2	53.1	0.40	0.94
22 to 42 d	145.0	148.4	151.0	147.21	143.7	147.9	1.30	0.68
1 to 42 d	102.1	101.4	102.2	100.4	98.5	100.5	0.76	0.74
ADG, g/d								
1 to 21 d	33.6	33.9	34.9	34.2	33.6	33.8	0.27	0.76
22 to 42 d	73.2	75.3	79.9	77.1	76.1	76.8	0.82	0.36
1 to 42 d	53.4	54.6	57.4	55.7	54.8	55.3	0.49	0.36
F:G, g/g								
1 to 21 d	1.62	1.60	1.53	1.56	1.58	1.57	0.01	0.05
22 to 42 d	2.05	1.97	1.89	1.91	1.89	1.93	0.02	0.08
1 to 42 d	1.91 ^a	1.86 ^{ab}	1.78 ^c	1.80 ^{bc}	1.79 ^{bc}	1.82 ^{bc}	0.01	<0.05

NC = negative control (basal diet); PA = phosphoric acid; LA = lactic acid; SEM = standard error of the mean; ADFI = average daily feed intake; ADG = average daily gain; F:G = feed-to-gain ratio.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).

¹ Each value represents the mean of 6 replicates ($n = 6$).

3.4. Digestive tract pH

The effect of dietary acidifier supplementation on the pH of the digestive tract is shown in Table 5. There was no significant difference in the pH of the proventriculus, jejunum, ileum and cecum among the different treatments ($P > 0.05$). However, compared to that in the NC and PC groups, the pH of the gizzard was significantly lower in the NC + PA (0.1, 0.2 and 0.3 g/kg) groups ($P < 0.05$). Additionally, the pH of the duodenum in the NC + PA (0.2 and 0.3 g/kg) groups as well as the LA (0.3 g/kg) group was significantly lower than that in the NC and PC groups ($P < 0.05$).

3.5. Duodenal digestive enzyme activity

The effect of dietary acidifier supplementation on the digestive enzyme activity of the duodenum is shown in Table 6. The results showed that the NC + PA (0.2 g/kg) group had the highest trypsin activity and that it was significantly higher than that in the NC and PC groups ($P < 0.01$). In addition, the activities of chymotrypsin and lipase in the NC + PA (0.2 and 0.3 g/kg) groups were higher than those in the PC group ($P < 0.05$), whereas these activities in the LA (0.3 g/kg) group were not significantly different compared to PC group.

3.6. Gut morphology

The results of the gut morphology are presented in Table 7. It was observed that compared to the NC group, the villus height-to-crypt depth (VH:CD) ratio of the duodenum in the NC + PA (0.1 g/kg) group was significantly increased ($P < 0.01$), reaching the level observed for the PC group, whereas in the LA (0.3 g/kg) group, VH:CD RATIO was not obviously different compared to that in the NC group. The villus height of the jejunum in the NC + PA (0.3 g/kg) group was significantly increased compared to that in the NC group ($P < 0.05$), and it reached the level observed for the PC group. Additionally, the crypt depth of the jejunum in the NC + PA (0.1 and 0.2 g/kg) group and LA (0.3 g/kg) group was not obviously different compared to that in the NC group.

3.7. Cecal microflora

The effects of acidifier supplementation on cecal microflora are shown in Table 8. The results showed that the total aerobic bacterial count in the NC + PA (0.1 g/kg) group was significantly lower than that in the other groups ($P < 0.05$). The counts of *E. coli* in the NC + PA (0.1 g/kg) group were significantly decreased compared to

Table 3
Effects of dietary acidifier supplementation on the breast muscle meat quality of broilers.¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Drip loss 24 h, %	1.52	1.60	1.85	1.42	1.56	1.71	0.05	0.19
Shear force, N	39.62 ^a	21.90 ^b	19.73 ^b	22.84 ^b	19.37 ^b	16.14 ^b	1.56	<0.01
Cooking loss, %	27.33 ^a	25.04 ^{ab}	23.80 ^{bc}	26.32 ^{ab}	24.57 ^{bc}	23.58 ^{bc}	0.40	<0.05
pH 45 min	6.27	6.47	6.40	6.43	6.47	6.42	0.02	0.08
pH 24 h	5.69 ^c	5.97 ^a	5.75 ^{bc}	5.99 ^a	5.80 ^{bc}	5.87 ^{ab}	0.03	<0.01
L*	52.62	54.11	51.71	53.42	51.40	51.30	0.33	0.06
a*	12.75 ^a	11.39 ^b	12.88 ^a	11.26 ^b	12.25 ^{ab}	12.35 ^{ab}	0.18	<0.05
b*	10.04 ^b	12.81 ^a	11.59 ^{ab}	13.60 ^a	11.80 ^{ab}	12.67 ^a	0.32	<0.05

NC = negative control (basal diet); PA = phosphoric acid; LA = lactic acid; a* = redness; b* = yellowness; L* = lightness.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).¹ Each value represents the mean of 6 replicates ($n = 6$).**Table 4**
Effects of dietary acidifier supplementation on the thigh muscle meat quality of broilers.¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Drip loss 24 h, %	1.47	1.89	1.59	1.48	1.29	1.55	0.06	0.15
Shear force, N	21.75 ^a	15.99 ^b	13.91 ^b	14.94 ^b	14.89 ^b	12.92 ^b	0.62	<0.0001
Cooking loss, %	20.73	21.96	19.90	19.86	21.72	19.13	0.45	0.43
pH 45 min	6.57	6.50	6.60	6.58	6.66	6.53	0.02	0.13
pH 24 h	6.35 ^{abc}	6.38 ^{ab}	6.34 ^{abc}	6.46 ^a	6.20 ^{bc}	6.18 ^c	0.03	<0.05
L*	56.24	56.51	56.01	57.51	56.15	55.77	0.28	0.56
a*	13.86	13.88	13.87	12.64	13.91	13.52	0.16	0.16
b*	10.82 ^b	13.79 ^a	12.59 ^a	13.49 ^a	13.85 ^a	14.44 ^a	0.36	<0.05

NC = negative control (basal diet); PA = phosphoric acid; LA = lactic acid.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).¹ Each value represents the mean of 6 replicates ($n = 6$).**Table 5**
Effects of dietary acidifier supplementation on the digestive tract pH of broiler chickens.¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Crop	5.81 ^a	5.80 ^a	5.58 ^{ab}	5.35 ^b	5.68 ^a	5.53 ^{ab}	0.05	<0.05
Gizzard	3.40 ^a	3.45 ^a	3.06 ^b	3.24 ^b	3.03 ^b	3.41 ^a	0.05	<0.05
Proventriculus	5.01	5.14	4.82	4.62	4.70	4.71	0.07	0.16
Duodenum	6.04 ^a	6.02 ^a	5.95 ^{ab}	5.84 ^b	5.82 ^b	5.83 ^b	0.02	<0.01
Jejunum	6.34	6.24	6.22	6.39	6.34	6.23	0.03	0.41
Ileum	7.59	7.42	7.64	7.32	7.39	7.50	0.08	0.88
Cecum	6.63	6.60	6.48	6.62	6.62	6.42	0.07	0.94

NC = negative control (basal diet); PA = phosphoric acid; LA = lactic acid.

^{a, b} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).¹ Each value represents the mean of 6 replicates ($n = 6$).**Table 6**
Effects of dietary acidifier supplementation on the duodenal digestive enzymes of broilers (U/mg prot).¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Trypsin	3,981.16 ^{bc}	3,307.94 ^c	2,692.83 ^c	8,252.86 ^a	7,008.07 ^{ab}	2,840.74 ^c	574.83	<0.01
Lipase	96.84 ^{ab}	64.31 ^b	64.64 ^b	160.87 ^a	149.77 ^a	67.56 ^b	11.53	<0.05
Chymotrypsin	6.94 ^{ab}	5.77 ^b	6.53 ^b	10.39 ^a	7.86 ^{ab}	4.48 ^b	0.54	<0.05
Amylase	5.97	9.11	6.13	7.57	7.23	2.73	1.12	0.72

NC = negative control (basal diet); PA = phosphoric acid; LA = lactic acid.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).¹ Each value represents the mean of 6 replicates ($n = 6$).

those in the NC and PC groups ($P < 0.05$), whereas the counts of *Salmonella* in the NC + PA (0.1 g/kg) and NC + PA (0.2 g/kg) groups were significantly decreased compared to those in the NC and PC groups ($P < 0.05$).

4. Discussion

The present study showed that adding acidifier to the diet improved the ADG and decreased F:G ratio of broilers, and that PA supplementation at 0.1 g/kg in particular, improved broiler

performance more than the antibiotic. Our results are similar to those of Hashemi et al. (2012), who reported that dietary supplementation with acidifiers had a positive impact on FCR, and Mourya (2011) who reported an increased ADG and FCR of broilers with a diet supplemented with acidifiers. This positive effect of the acidifier on the performance may be due to a decrease in the pH of the feed and digestive tract, direct antimicrobial action and reduced acidity of the muscle (Ghazalah et al., 2011; Luckstadt and Mellor, 2011). However, there was no significant difference on the performance between the PC group with antibiotic growth promoter

Table 7
Effects of dietary acidifier supplementation on the small intestine mucosal morphology of broilers.¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Duodenum								
Villus height, μm	416.42	445.55	444.43	440.83	426.18	433.28	3.82	0.18
Crypt depth, μm	122.42	126.12	124.62	127.02	127.60	129.72	1.14	0.57
VH:CD ratio	3.40 ^b	3.54 ^a	3.57 ^a	3.47 ^{ab}	3.34 ^b	3.34 ^b	0.02	<0.01
Jejunum								
Villus height, μm	349.53 ^c	381.35 ^{ab}	368.60 ^{abc}	356.63 ^{bc}	381.95 ^a	370.08 ^{abc}	3.60	<0.05
Crypt depth, μm	116.10 ^b	128.32 ^{ab}	116.82 ^b	117.13 ^b	130.50 ^a	124.23 ^{ab}	1.78	<0.05
VH:CD ratio	3.03	2.97	3.20	3.05	2.93	2.97	0.04	0.35
Ileum								
Villus height, μm	318.00	309.78	315.37	311.90	309.93	313.48	1.88	0.81
Crypt depth, μm	109.88	114.45	113.80	113.57	108.12	115.52	1.34	0.59
VH:CD ratio	2.90	2.70	2.78	2.75	2.88	2.74	0.03	0.19

NC = negative control (basal diet); PA = phosphoric acid; LA = Lactic acid; VH:CD ratio = villus height-to-crypt depth ratio.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).

¹ Each value represents the mean of 6 replicates ($n = 6$).

Table 8
Effects of dietary acidifier supplementation on the cecum microorganisms of broilers (\log_{10} CFU/g wet digesta).¹

Item	NC	NC + antibiotic (8 mg/kg)	NC + PA (0.1 g/kg)	NC + PA (0.2 g/kg)	NC + PA (0.3 g/kg)	NC + LA (0.3 g/kg)	SEM	P-value
Total aerobic bacteria	8.06 ^a	7.80 ^a	7.33 ^b	7.89 ^a	8.23 ^a	7.90 ^a	0.08	<0.05
<i>Escherichia coli</i>	7.64 ^{ab}	7.66 ^{ab}	6.88 ^c	6.99 ^{bc}	7.88 ^a	7.44 ^{abc}	0.11	<0.05
<i>Salmonella</i>	7.52 ^{ab}	7.53 ^{ab}	6.58 ^c	6.61 ^c	7.74 ^a	7.09 ^{bc}	0.11	<0.01
<i>Lactobacillus</i>	7.30	6.39	6.69	7.30	7.07	7.19	0.10	0.05

NC = negative control (basal diet); PA = phosphoric acid; LA = Lactic acid.

^{a, b, c} Within a row, values with different superscripts indicate a significant difference ($P < 0.05$).

¹ Each value represents the mean of 6 replicates ($n = 6$).

and the negative control group. That might have been caused by the supplementation level of antibiotic growth promoter not being enough. However, the reason will need to be further studied to be confirmed.

The results of this experiment showed that the addition of PA (0.2 g/kg) and LA (0.3 g/kg) increased the pH at 24 h of the breast muscle and thigh muscle, which is consistent with Lee et al. (2012), who reported that the pH of broiler thigh meat was increased by gallic and LA supplementation. The reason that the supplementation of acidifiers alleviated the decrease in muscle pH might be related to the increase of antioxidant activity in thigh meat from the birds fed the acidifiers. Previous study have indicated that antioxidant activity in meat can be affected by the acidifier supplementation (Wang et al., 2020). In addition, the supplementations of acidifiers might affect the gut microbiota and its metabolites, and alleviate the decrease in muscle pH.

Meat color is the most important factor affecting consumers buying livestock and poultry meat products. However, the present study showed that the addition of acidifiers had no meaningful effect on breast muscle and thigh muscle meat color. Shear force is an objective index for evaluating meat tenderness (Guo et al., 2010), and dietary acidifiers resulted in a decreased shear force of breast and thigh muscles in the present study. Thus, dietary supplementation with acidifiers might have the potential to improve meat tenderness in broilers. Unfortunately, no other studies using acidifiers have reported data on meat tenderness to compare with our results. The supplementation of acidifiers may improve meat tenderness via indirect mechanisms. The cause may be due to an improvement in the metabolism of nutrients, the slowing down of anaerobic digestion, and an improvement in the body's antioxidant capacity. In addition, some investigators have shown that energy has to be generated under anaerobic conditions after slaughter of the bird, which results in accumulation of lactic acid and affects the water holding capacity of meat due to protein breakdown. Meat quality is directly correlated with the metabolic state of the muscle

before slaughter. One previous study reported that dietary benzoic acid or amylase were effective in improving the antioxidant capacity, and nutrient digestion, and then improving the meat quality (Wang et al., 2020).

Both juvenile and adult animals have high GIT pH due to different factors. Juvenile animals have not developed the digestive tract system, and gastric acid secretion in the digestive tract is insufficient, while for adult animals, it is due to physiology, feed, environment and other factors. This often makes the gastrointestinal tract pH higher than the suitable range for enzyme activity and beneficial bacteria growth, and external feed must be replied on to improve the acid and alkali environment in the digestive tract. Lowering the pH of the diet can lower the pH of the digestive tract biology, thus providing a suitable environment (Kim et al., 2005). In the current study, dietary PA and LA reduced the pH of the duodenum, and dietary PA also reduced the pH of the crop and gizzard, providing a suitable environment for enzymes and microorganisms in the digestive tract of the animals. Incorporation of acidifiers in the diet helps to maintain the optimum pH in the stomach and duodenum for enzymatic actions and ensures proper protein digestion in the intestine. Moreover, pepsinogen, the inactive enzyme precursor of pepsin, has its active conversion catalyzed by a low pH environment (Luckstadt and Mellor, 2011). Our results are in agreement with those of He et al. (2013), who found that acidifier compounds could decrease the gastrointestinal pH. In contrast, the results of this study are not in agreement with those of other relevant studies, which reported that the gastrointestinal pH remained unaffected by acidifier supplementation (Giannenas and Papanephytous, 2014; Palamidi et al., 2016). This discrepancy may be a result of the difference in acidifier type and concentration, experimental animals, acidifier formulations and test sites, as well as diet type and composition, and other factors.

In the present study, the trypsin, chymotrypsin and lipase activities of the duodenum were increased when the diet was supplemented with PA (0.2 and 0.3 g/kg). Similar results have been

reported by Kong et al. (2003), who found that acidifiers in diets increased the activities of duodenal proteinase, amylase, and lipase. The increase in digestive enzyme activity may be due to the lowering of the pH of the crop, gizzard and duodenum and the increasing of pepsin activity and protein hydrolysate concentration, thereby stimulating digestive enzyme secretion after entering the duodenum. In addition, acidifiers stimulated the secretion of non-protease in the intestinal segment. It is speculated that the addition of acidifier stimulates the secretion of pepsin, and the chyme enters the intestine to further stimulate the decomposition and absorption of nutrients, thereby stimulating the development of the body's digestive system, which is manifested by the increased secretion of amylase and lipase and the increased capacity of intestinal digestion. Although as the concentration of the acidifier increases, the enzyme activity increases accordingly, until when a certain concentration is reached, the secretion of the acid and pepsinogen may be suppressed by the excess of acid in the stomach acid.

For young chicks, a longer villus increases the absorptive surface of the intestines, and shorter crypt depths indicate lower tissue turnover as well as a lower demand for tissue development (Kelly-Hooper et al., 2012). In this study, broilers fed diets supplemented with an antibiotic or acidifier increased the villus height in the jejunum. This result is in accordance with the study of Khatun et al. (2010), who stated that the length of the intestinal villi in broilers fed organic acid is longer than that in the feed control. The increase in villus height of the small intestine may be attributed to the role of the intestinal epithelium as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen (Khan, 2013). Additionally, the addition of an antibiotic as well as PA (0.1 g/kg) increased the VH:CD ratio of the duodenum, which was also demonstrated by Mohammadagheri et al. (2016). Consequently, there is a decrease in the villus height, increase in the cell turnover and decrease in the digestive and absorptive capacities (Pelicano et al., 2005).

Acidifiers in feed inhibit the growth of pathogenic bacteria (such as *E. coli* and *Salmonella*) by influencing the pH. The proliferation of most pH sensitive bacteria (*E. coli* and *Salmonella*) is minimized below pH 5 whereas acid-tolerant ones survive. The pathogenic bacteria diseases caused by the digestive tract bring harm to the poultry industry. The gastrointestinal tract is the front line of defense against the constant invasion of microbes (Markovi et al., 2009). The makeup of the gastrointestinal fauna of the bird is an important factor in improving poultry performance and flock health (Markovi et al., 2009). In the current experiment, birds fed diets containing PA (0.1 and 0.2 g/kg) and LA (0.3 g/kg) decreased the abundance of *E. coli* and *Salmonella*. This result is in close agreement with that of previous studies (Amaechi and Iheanetu, 2014; Hassan et al., 2010). The reason for improving the intestinal microflora is that the suitable growth environment of pathogenic bacteria has a neutral pH, whereas beneficial bacterium such as *Lactobacillus* are suitable for growth and reproduction in an acid environment. Additionally, once an acidifier enters the cell, where the pH is maintained near 7, the acid will dissociate and suppress bacterial cell enzymes (e.g., decarboxylases and catalases) and nutrient transport systems (Huyghebaert et al., 2011). However, the potential concern is the fact that *Lactobacillus* abundance was decreased in birds fed the antibiotic or acidifier diet. This finding was also made by Patrick et al. (2003). *Lactobacillus* is generally considered to be a beneficial bacterium that competes with pathogens in order to reduce their number in the gastrointestinal tract.

5. Conclusion

We conclude that the dietary supplementation of PA (0.1 g/kg), or PA (0.2 g/kg) could improve growth performance by increasing the F:G ratio, by improving the breast and thigh muscle meat quality by decreasing the cooking loss and shear force, and by increasing the pH and a^* value. In addition, intestinal health was improved with a dietary supplementation of PA (0.1 g/kg) by decreasing the abundance of aerobic bacteria, such as *E. coli* and *Salmonella*, and the PA (0.2 g/kg) group had the highest digestive enzyme activity. Overall, the supplementation of PA (0.1 g/kg) or PA (0.2 g/kg) had a better overall effect on broilers than the antibiotic supplementation or LA (0.3 g/kg) supplementation.

Author contributions

Chun-Qi Gao: Conceptualization, Validation, Writing – original draft, Writing – review & editing. **Hui-Qin Shi:** Methodology, Formal analysis, Writing – original draft. **Wen-Yan Xie:** Writing – original draft. **Li-Hong Zhao:** Supervision. **Jian-Yun Zhang:** Supervision. **Cheng Ji:** Supervision. **Qiu-Gang Ma:** Conceptualization, Validation, Writing – review & editing, Supervision, Project administration.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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