Feeding malic acid to chickens at slaughter age benefits poultry production and microbial safety in regard to *Campylobacter*

Fangzhe Ren
Yangzhou University
Wenbin Yang
Yangzhou University
Juanjuan Hu
Yangzhou University
Pinyu Huang
Yangzhou University
Xin-an Jiao
Yangzhou University
jinlin huang (jinlin@yzu.edu.cn)
Yangzhou University https://orcid.org/0000-0001-9447-3666

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Abstract

Background

Chicken meat has become popular for consumption worldwide. However, chicken flocks suffer the *Campylobacter* infection during the rearing period, which allows chicken meat products act as a vehicle for transmitting this pathogen through the food chain and bring great health and economic burden to the public. Malic acid is a dicarboxylic organic acid with antimicrobial activity, the application of malic acid during food animal rearing period also found could improve the performance of animals and the quality of their products. This study applied malic acid to chicken flocks and evaluated its potential benefits on the poultry production and microbial safety.

Results

In Experiment 1, AA broilers and partridge chicken were provided with the malic acid-supplemented drinking water for three weeks, although the *Campylobacter* carriages were found decreased by 0.91–0.98 log after the first week of use ($P < 0.05$). However, this effect was not consistent, significant decontamination could not be found in the second and the third week of application. Thus, in Experiment 2, the malic acid-supplemented drinking water was used for five days to flocks at slaughter age, the *Campylobacter* carriage was found decreased by 1.05–1.55 log ($P < 0.05$), suggesting an effective reduction effect exist. Malic acid has no adverse effects on chicken performance, including body weight, intestinal indices and microflora. However, the meat quality of AA broilers was found to be promoted, the moisture increased by 5.12% − 5.92% ($P < 0.05$), and the fat decreased by 1.60% ($P < 0.05$). In Experiment 3, the malic acid-supplemented drinking water was provided to AA broilers which were suffering from respiratory disease. The results showed that the mortality rate of malic acid treated group was consistently lower than that of the control group during the experimental period, and the total mortality was decreased from 52% in the control group, to 32% in malic acid treated group.

Conclusions

Our results suggest that feeding malic acid to flocks could decrease the contamination of *Campylobacter* while also benefit chicken farming, and is worthy of application to promote safe development of the poultry production and its products.

Background

Poultry production is closely related to human life, and chicken meat has become popular for consumption worldwide, due to its characteristics such as high protein, ample micronutrients, low fat content, and comparatively low price when compared to beef or pork(1, 2). However, chicken flocks suffer foodborne pathogen infections during the rearing period, which could then be transmitted through the

farm-to-fork chain and pose a serious threat to food safety and human health. This issue has been one of the major problems that has beset chicken meat production, while the contamination of *Campylobacter* is particularly serious(2–4).

The chicken is generally recognized as the natural host for *Campylobacter* in the intestine without developing symptoms(5). During the slaughter processing, the chicken intestinal tract may leak or rupture and contaminate the meat, which provide a potential source of cross-contamination in the food chain(2–4). It is estimated that the handling and consumption of chicken meat-related products may account for 20–30% of human infection cases, while 50–80% may be attributed to the chicken reservoir as a whole(6). Recent studies showed that the decontamination of *Campylobacter* in chicken flocks would significantly reduce the risk of human campylobacteriosis(4).

Due to the increasing antibiotic resistance in pathogens, the use of antibiotics is limited in chicken production(7, 8). Thus, finding alternative antimicrobial agents has recently become more and more important(3). Malic acid is a type of dicarboxylic organic acid which showed antimicrobial activity(9). Besides, malic acid is an intermediate in metabolic cycles of organisms for energy production, and could aid digestion and absorption by chelating various cations and enhancing the activities of some digestive enzymes(10). Malic acid has been permitted as GRAS (Generally Recognized As Safe), which does not has adverse effects for human intake or animal feeding when properly used. Attempts to provide malic acid to livestock were performed in previous studies, which showed its potential benefits in promoting the performance of animals and their products(10–12).

The bactericidal effect of malic acid on *Campylobacter* has been demonstrated in laboratory culture and on raw meat samples(13, 14). However, its antimicrobial efficacy during the chicken rearing period has never been investigated, and the potential effects of feeding malic acid to chickens on their performance and products still stay unclear. This study supplemented malic acid into the drinking water of the flocks that were found naturally *Campylobacter*-positive. The decontamination effect of malic acid-supplemented water and its influence on chicken performance were evaluated in AA broilers and partridge chicken. During the slaughter of AA broilers, the intestine and meat samples were collected, and the influence of this acidified drinking water on chicken products was analyzed. In another flock of AA broilers, which were suffering from respiratory disease. The malic acid-supplemented drinking water was provided to evaluate its potential role in helping the flocks resist disease. The obtained results provide a reference for the further practical application of malic acid to promote safe poultry production.

Results

The reduction effect of malic acid-supplemented drinking water on Campylobacter was not consistent for a long period of use

In Experiment 1, the malic acid-supplemented drinking water was provided to flocks continuously for three weeks. Before the experiment, the *Campylobacter* carriages in the control group and malic acid treated group were similar both in the partridge chickens and AA broilers ($p > 0.05$). After one week

treatment by acidified drinking water, the *Campylobacter* carriages were found to be significantly decreased by 0.98 log and 0.91 log in the partridge chickens and AA broilers, respectively, compared to the control group ($p < 0.05$). After two weeks of malic acid treatment, the average amounts of *Campylobacter* in the acid treated groups were slightly higher than the control group for both the partridge chickens and AA broilers, but no significant differences was observed ($p > 0.05$). Similar results were also observed in the third week, suggesting that the decontamination effect of the malic acid-supplemented water was not consistent, which is decreased with extended of the time of use (Fig. 1).

The use of malic acid-supplemented drinking water for 5 days before slaughter could reduce the contamination of Campylobacter in flocks

For Experiment 2, the acidified drinking water was given for five days to flocks at slaughter age. Before the experiment, similar carriages of *Campylobacter*, approximately 4 log cfu (colony forming units) /g were detected in all groups ($p > 0.05$). After five days treatment, for the partridge chicken, the *Campylobacter* carriage detected in the cloaca of the control group was 3.54 log cfu/g, which was similar to the data before the experiment. However, in the malic acid treated group, the *Campylobacter* carriage was significantly decreased to 1.98 log cfu/g, and a 1.55 log reduction was found when compared to the control group ($p < 0.05$) (Fig. 2a). In the AA broilers, the *Campylobacter* carriage in the cloaca of the control group was increased to 5.19 log cfu/g during the five days experimental period, while the carriage of the malic acid treated group was decreased to 4.14 log cfu/g and a 1.05 log reduction was found ($p < 0.05$) (Fig. 2b). In AA broilers caeca samples, the *Campylobacter* contamination load of the control group was 9.96 log cfu/g, while the corresponding colonization level in the malic acid treated group was only 8.40 log cfu/g with a 1.56 log reduction ($p < 0.05$) (Fig. 2c).

The treatment of malic acid-supplemented water does not influence the chicken performance, intestinal indices and microflora

In Experiment 2, the average weight and microflora of flocks in different groups were similar before the experiment ($p > 0.05$). For the partridge chickens, the average weight of the malic acid treated group was approximately 0.81 kg, which was slightly higher than that of the control group after treating by the malic acid-supplemented water for five days ($p > 0.05$). Similar results were also observed in AA broilers, where the average weights of the malic acid treated group and control group were approximately 1.52 kg and 1.50 kg, respectively ($p > 0.05$) (Table 1). For the intestinal microflora, in the cloaca of partridge chickens, they were slightly decreased to 11.66 log cfu/g in malic acid group after five days treatment, when compared to 11.72 log cfu/g in the control group($p > 0.05$). In AA broilers, the detected microflora in the malic acid treated group and control group were 11.79 and 11.51 log cfu/g, respectively ($p > 0.05$), suggesting malic acid does not affect the body weight and microflora of the flocks (Table 1).

Table 1
Body weight (g) and intestinal microflora (log cfu/g) of broilers and partridges receiving malic acid-supplemented drinking water at slaughter age

Parameter	Broiler		p-value	Partridge		p-value
	Control	Malic acid		Control	Malic acid	
Body weight[1]						
application of 0 d	1163.8 ± 103.8	1204.4 ± 97.6	0.39	675.2 ± 97.3	644.8 ± 53.7	0.39
application of 5 d	1504.1 ± 112.3	1523.8 ± 98.7	0.69	774.6 ± 80.4	813.3 ± 94.2	0.30
Microflora[1]						
application of 0 d	11.67 ± 0.74	11.66 ± 0.71	0.97	11.88 ± 1.10	11.85 ± 0.54	0.95
application of 5 d	11.51 ± 0.52	11.79 ± 0.49	0.22	11.72 ± 0.93	11.66 ± 0.82	0.89

[1] Values are given as means ± SD from 20 chickens per group.

The potential effect of malic acid-supplemented water on the intestines of chickens was evaluated in AA broilers. As shown in Table 2, after five days experimental period, the average weight of the intestine was approximately 25.1 g, the length of small intestine was approximately 112.6 cm, the caecum was approximately 11.0 cm, and the pH of the caecal content was approximately 7.26 in the control group. Meanwhile, in the malic acid treated group, the weight of the intestines was approximately 26.7 g, the length of small intestine was approximately 110.9 cm, the caecum was approximately 12.1 cm, and the pH of the caecal content was approximately 7.03. No significant differences observed between the treatment group and control group ($p > 0.05$).

Table 2
Intestinal weight, length and pH in broilers receiving malic acid-supplemented drinking water at slaughter age

Parameter	Broiler		p-value
	Control	Malic acid	
Intestine weight (g)[1]	25.1 ± 2.2	26.7 ± 3.0	0.16
Length (cm) of[1]			
Small intestine	112.6 ± 1.8	110.9 ± 1.9	0.07
Caecum	11.0 ± 1.1	12.1 ± 1.2	0.06
Intestinal pH[1]	7.26 ± 0.36	7.03 ± 0.40	0.17
[1] Values are given as means ± SD from 20 chickens per group.			

Drinking malic acid-supplemented water benefits the quality of chicken meat

In Experiment 2, the meat composition of AA broilers was evaluated by measuring the contents of moisture, crude protein, ash and fat. As shown in Table 3, there were no significant difference of the protein and ash contents in groups supplemented with and without malic acid ($p > 0.05$), suggesting that malic acid has no influence on the nutrition of chicken meat. Compared to the control group, the moisture content in the malic acid treated group was increased by 5.12% in the thigh meat portion, and 5.92% in breast meat portion, respectively ($P < 0.05$), indicating the juiciness of meat was improved. The fat content in the malic acid treated group was decreased by 1.60% in the thigh meat compared to the control group ($p < 0.05$), which contributes to the taste sensory of the meat.

Table 3
Chemical composition (%) of breast and thigh meats from broilers receiving malic acid-supplemented drinking water at slaughter age

Parameter	Breast meat		p-value	Thigh meat		p-value
	Control	Malic acid		Control	Malic acid	
Moisture[1]	63.25 ± 3.94	69.17 ± 2.30*	0.02	65.66 ± 2.50	70.78 ± 2.21*	0.01
Crude protein[1]	21.69 ± 1.72	20.70 ± 4.96	0.68	22.91 ± 6.34	21.15 ± 2.14	0.56
Crude ash[1]	1.15 ± 0.47	1.01 ± 0.09	0.48	1.18 ± 0.04	1.12 ± 0.20	0.48
Crude fat[1]	3.07 ± 2.30	3.36 ± 1.76	0.85	5.59 ± 0.93	3.99 ± 0.26*	0.01
[1] Values are given as means ± SD from 20 chickens per group.						
* Asterisks indicate significant difference ($P < 0.05$) was found in the malic acid treated group when compared to the control group						

Malic acid-supplemented drinking water decreased the mortality of the chicken flocks

In Experiment 3, the acidified water was provided to another chicken flock which suffers from respiratory disease, for three weeks of use. In the first week, none broilers were dead in both the control and malic acid treated group. During the second week, the mortality rate of control group was 36%, while it decreased to 24% in the malic acid treated group. On week 3, the mortality rates of the control group and malic acid treated group were 16% and 8%, respectively. In total, 13 of broilers were found dead in the control group, and the total mortality rate was 52%, while only 8 broilers were dead in the malic acid treated group with a total mortality rate of 32% (Table 4).

Table 4
Mortality of broilers receiving malic acid-supplemented drinking water for one, two and three weeks

Broiler	Mortality (n)[1]			
	1 W	2 W	3 W	Total
Control	0% (0)	36% (9)	16% (4)	52% (13)
Malic acid	0% (0)	24% (6)	8% (2)	32% (8)
W: week, n: number				
[1] Values are given as percentage and (number) of the dead broilers.				

Discussion

Campylobacter is one of the leading causes of food-borne gastroenteritis in humans worldwide(2–4), which accounts for approximately 96 million cases of human illness per year on a global scale(15). Poultry is the most common species associated with human *Campylobacter* illness, and most chicken flocks became *Campylobacter*-positive when reached slaughter age, making it an important reservoir for human infection. After *Campylobacter* infect humans, the clinical symptoms could include mild abdominal pain, headaches, fever, vomiting and sever watery and bloody diarrhea, and the infection can sometimes lead to serious sequelae such as Guillain-Barré syndrome, Miller Fisher syndrome and reactive arthritis(15), although most cases were self-limiting. Still, a number of patients required medication and hospitalization treatment, which brings great health and economic burden to the public(3, 4, 15). Therefore, the control of *Campylobacter* colonization in chickens at the farm level should be meaningful work.

Malic acid can be industrially produced at present and has the advantages of no pollution/residue, lack of toxicity and easy of application(16). In previous studies, it was found that malic acid could cause a 6 log reduction of *Campylobacter* in laboratory broth and a 4 log reduction in chicken juice after 24 h of exposure at 4 °C(13). The contamination of *Campylobacter* on chicken legs was also observed to decrease 1.18 log after treatment with malic acid solution at 4 °C for 8 days(14). These results foreshadow the promising application of malic acid in the poultry industries, but the antimicrobial effect of malic acid towards chicken feeding still stayed unclear. Malic acid is commonly recognized as a mild acid and has wide application in the food industry(16), which makes it possible to be applied in animal feeding. Our pre-experiment found that the effect of malic acid against the growth of *Campylobacter in vitro* was obvious (Supplementary Figure S1). Besides, an *in vivo* study showed that malic acid had a more stable effect to control the *Campylobacter* contamination when compared to other acids (Supplementary Figure S2). The minimum inhibitory concentration of malic acid against *Campylobacter* was also found to be lower than for other acids(17), suggesting that a more effective and economical potency may exist.

Organic acids exploit their antimicrobial activity in the undissociated form, which is closely related to the pH of the medium(18). Previous studies have indicated that the well bactericidal effect of organic acids on *Campylobacter* strains shows up at a pH of 4.0(19), and thus we decided to adjust the pH to 4.0 using malic acid in this study. There is no evidence of the vertical transmission of *Campylobacter* in chicken flocks, while the cross contamination from the environment seems to be an important infection source(4, 20). Malic acid could be supplemented into the feed or drinking water of the broilers. The dry conditions of the feed is lethal to *Campylobacter*, which is thus recognized as not being a potential source for contamination(21). Meanwhile, water is an important vehicle for spreading *Campylobacter* that is prominent in chicken flocks(4, 21, 22). Thus, malic acid was added into the drinking water in our study.

Although most studies reported the effectiveness of using organic acid to control the contamination of pathogenic bacteria during animal rearing(17, 22–24), some results showed its limited or variable effect

which could not be ignored(25–27). The bacteriostatic or bactericidal effect of the organic acid may depend on the manner of use (concentration, vehicle, and duration) and is also related to the status of the host. In our study, we found that supplementation of malic acid into the drinking water for the chicken flocks at slaughter age was effective in two chicken lines, the *Campylobacter* load in the cloaca was found to be decreased by 1.05–1.55 log cfu/g, while in the caeca was decreased by 1.56 log cfu/g. However, in a long period daily rearing for three weeks, the effect of malic acid was not consistent, with significant decontamination only found in the first week of application. Our present study suggests that application of acidified drinking water to the broilers at slaughter age could effective limited the *Campylobacter* infection load. Limitation of its use to the last week before slaughter also has other advantages, such as cost savings, thus making it more economical. In addition, *Campylobacter* infection of the broilers at slaughter age are epidemiologically more relevant to human infection, thus making the decontamination more meaningful. Nevertheless, the reason why a long period of application of acidified drinking water has a limited decontamination effect on *Campylobacter* still requires further investigation.

Malic acid is a flavoring agent, and it is also an intermediate in metabolic cycles, including the tricarboxylic acid (TCA) cycle and the glyoxylic acid cycle (GAC). Feeding diets with malic acid was found to increase the weight gain and feed consumption in Japanese quails(11). For dairy cows, the feed efficiency was improved, the milk yield was increased and the fat content in the milk was subtly changed(12). The growth and feed utilization were found to be improved in tilapia, but an excess supply would compromise the beneficial effect(10). Despite that inconsistent findings were reported(28), these results showed the potential benefits of feeding malic acid to animals when applied with suitable nutritional and managerial measures. In our study, the broiler body weight as well as intestinal indices, including length, weight, pH, and microflora were not influenced by the malic acid supplementation, indicating that the chicken performance was not affected at least. The bacteriostatic effect was found on *Campylobacter*, while not on the normal intestinal microflora, which may be attributed to the acid-intolerance characteristics of the pathogens when compared to the gut microflora (especially for the probiotics)(29). The propagation of the pathogens was inhibited, which will in turn promote the growth of microflora as it reduces microbial competition for nutrients(22, 30).

A previous study showed that feeding malic acid to cows could influence the composition of milk(12), and thus its potential effect on chicken meat was analyzed. Our results showed that the protein content of the chicken meat was not influenced by the malic acid treatment, suggesting that the nutrition was maintained. Compared to the control group, the moisture was increased by 5.12% and 5.92% in thigh and breast meat, respectively, while the fat content was decreased by 1.60% in thigh meat. This contributes to the tenderness, juiciness and taste sensory of the meat and promotes the acceptability and preference of the customers(31, 32). The changes in the chicken meat composition may be attributed to the influence of malic acid on the sense of chicken taste, and could affect the feed intake, nutrient digestion and conversion. Organic acids could exert beneficial effects on disease resistance, which was observed in tilapia(33, 34). In this study, the malic acid-supplemented drinking water was provided to one flock, which suffers from respiratory disease, and after three weeks of treatment with acidified water, the mortality was found to be decreased from 52–32%, which is also an additional benefit for chicken rearing.

This study showed that the malic acid-supplemented drinking water could benefit chicken production while also improving food safety. Our study only applied malic acid, and many improvements could be considered in the subsequent research, such as the use of malic acid in combination with prebiotics or bacteriophages(35) as well as with nutritional, managerial and biosecurity measures(30). Additionally, the bactericidal effect of malic acid was observed as not only restricted to *Campylobacter* but also on other food-borne pathogens(9), which foreshadows its promising application improving the safety of poultry production.

Conclusion

This study supplemented malic acid in drinking water of chicken flocks to evaluate its antimicrobial effect to the broiler chicken. The significant reduction of *Campylobacter* contamination loads was observed in the first week and decreased with extended of time supplementary. Thus, providing the acidified drinking water to flocks at slaughter age for five days showed to be an effective decontamination method. Malic acid has no adverse effect on chicken performance, including the body weight, intestinal indices, and microflora. Meanwhile, it could benefit the quality of chicken meat, by increasing the moisture and decreasing the fat content. In addition, malic acid was found to decrease the mortality of broiler suffering from disease. Our present results suggest that the application of malic acids to the flocks at slaughter age is a feasible and effective way to control *Campylobacter* contamination while also benefiting poultry production.

Methods

Animals and treatments

The AA broilers were provided by Jiangsu Jinghai Poultry Industry Group Co., Ltd, and raised in floor pens with dimensions of approximate 8 m^2 per group. The partridge chickens were provided by poultry farms in Nantong city, Jiangsu province, and raised in cages with dimensions of approximate 3.5 m × 1.75 m per group. For all chicken houses, the temperature was maintained around 21 - 28 ℃, and humidity was around 50 % - 60 %. The flocks were supplied with commercial feed with corn, wheat, soybean meal as the main ingredients, the detailed composition of the feed was listed in Supplementary Table S1. Normal water, or acidified water prepared by supplementation of L-malic acid to a final pH value of approximate 4.0, were providing to the flocks for drinking. Chicken had free access to water and commercial feed at all the time, the feed and drinking water were refreshed every day.

Experimental design

Three experiments were carried out in this study. In each experiment, chickens with less individual differences were randomly divided into two groups and reared in separate areas. The experimental group was provided the malic acid supplemented water, while the control group was given the non-

supplemented water. For experiment 1 and 2, the cloacal swabs were sampled for each flock before conducting the experiments to ensure *Campylobacter*-positive.

Experiment 1. Two flocks (2-week-old AA broilers and partridge chickens) were selected for evaluating the antimicrobial effect of the malic acid-supplemented drinking water on *Campylobacter* during rearing period. In each flock, the chickens were divided into two groups (20 chickens per group), the experimental group and control group were given the acidified water and non-supplemented water continuously for three weeks, respectively. The cloacal swab samples of the chickens were collected before the experiment and one, two and three weeks later after giving the specific water for counting the contamination load of *Campylobacter*.

Experiment 2. Another two flocks (5-week-old AA broilers and 10-week-old partridge chicken) were selected for evaluating the application of the acidified water to the flocks at slaughter age. In each flock, the experimental group and control group (20 chickens per group) were given the acidified water and non-supplemented water for five days. The body weights and cloacal swab samples of each chicken were recorded before conducting the experiment and five days later. The AA broilers were sent to slaughter house after the experimental period, the broilers were electrically stunned and killed by neck cutting, after defeathering process, the intestine and meat samples were collected and analyzed.

Experiment 3. For evaluate the effect of acidified water in helping the flocks to improve disease resistance, another chicken flock (AA broilers approximate 3-week-old) which were suffering from the respiratory disease was selected. 50 chickens were picked out from this sick flock, divided into two groups by giving the malic acid supplemented or normal water for three weeks, respectively. The mortality rates of the malic acid treated group and the control group was recorded after one, two and three week treatment.

Analysis of the chicken performance

The body weights of the chickens were recorded before and after giving the acidified water to analyze its influence on the chicken weight gain, the cloacal swabs were collected to analyze the intestinal microflora and *Campylobacter* carriage. During the slaughter of AA broilers (experiment 2), the intestines of the chicken were collected, the total weight of the intestine samples were recorded, then separated and ligated at the subsection to compare the length of the intestinal components. The caecal contents were squeezed into 10 mL tubes, diluted (1:8) with distilled water, vortexed, and read using a pH meter as described previously(23).

Enumeration of *Campylobacter* and microflora from cloacal swabs

The swabs were moistened in buffered peptone water (BPW) and weighed both before and after taking the fecal samples. The number of *Campylobacter* and microflora presented in the feces were than calculated as described previously with some modifications(26). The cloacal swab with sample was immersed in 1 mL of phosphate buffer saline (PBS) for 20 min and shaken several times. The swab was

then removed from the solution and appropriately diluted, with 100 µL of the diluent spread onto the CCDA agar containing rifampicin, polymyxin B, trimethoprim, cycloheximide, vancomycin, amphotericin B and cefoperazone and incubated at 42°C under a microaerophilic atmosphere for 36 h to count the *Campylobacter*. For enumeration of the intestinal microflora of the chicken, 100 µL of the appropriate diluent prepared above was spread on the plate count agar (PCA), the plate was incubated at 37°C for 24 h for colony count.

Determination of *Campylobacter* colonization in the caecum

Colonization assay was performed as described in a previous study(36). The caecum of the chicken was collected during the slaughter (experiment 2), and the lumenal contents were gently extruded. The tissue was weighed, homogenized, serially diluted and plated on selective CCDA agar to count the *Campylobacter* as described above.

Analysis of the proximate composition of chicken meat

The chicken meat samples were collected during slaughter (experiment 2), approximately 10 g of breast and thigh meat samples were collected and stored at −70°C. The samples were thawed overnight at 4°C, and ground when used for subsequent analysis. The moisture present in the samples was determined by drying meat samples at 105 °C until a constant weight was achieved. Crude proteins were determined according to the Kjeldahl method from the amount of ammonia ions neutralized by sodium hydroxide. Crude fat was determined by Soxhlet extraction procedure with petroleum ether. Crude fat was determined by burning the samples in the muffle furnace at 550 °C for 12 h. All procedures were performed according to the method described by the Association of Official Agricultural Chemists (AOAC) (37).

Statistical analysis

Statistical analyses were performed by GraphPad Prism software 8.0 (San Diego, CA, USA). Data were expressed as the mean ± standard deviation (SD). Normality was tested with the Kolmogorov-Smirnov test or with the D'Agostino-Pearson test to assure Gaussian distribution of values. Statistical significance between 2 groups was analyzed using unpaired t-test (Welch's correction was used if there were unequal variances) when normality test passed, otherwise using Mann-Whitney U test applying 95% confidence intervals, and $P < 0.05$ was considered statistically significant.

Abbreviations

AOAC: Association of Official Agricultural Chemists; BPW:buffered peptone water; cfu:colony forming units; GAC:glyoxylic acid cycle; GRAS:Generally Recognized As Safe; PBS:phosphate buffered saline; PCA:plate count agar; SD:standard deviation; TCA:tricarboxylic acid

Declarations

Availability of data and materials

The datasets used and analyzed in this study are available from the

Ethics approval and consent to participate

All experimental and animal management procedures were approved by the Animal Welfare and Ethics Committees of Yangzhou University and complied with the guidelines of the institutional administrative committee and ethics committee of laboratory animals (SYXK [Su] 2016–0020).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interest.

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Authors’ contributions

JH and XJ conceived and designed the experiments. FR, WY, JH and PH performed the experiments. FR, WY performed data analysis and interpretation of the results. FR, JH drafted the manuscript. All authors have read and approved the final manuscript.

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Figures

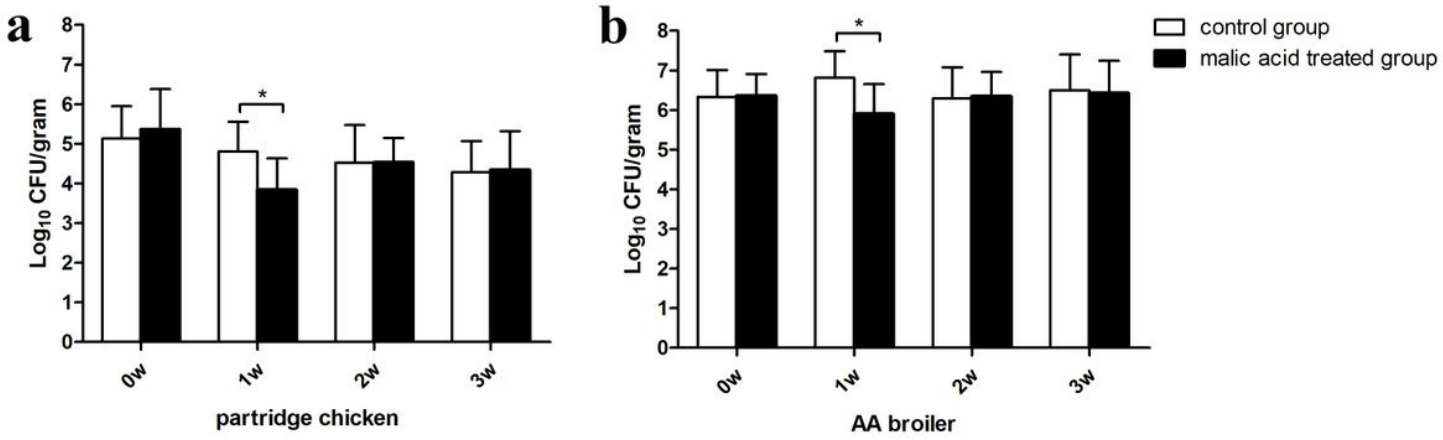

Figure 1

Campylobacter carriage in the cloaca of chickens after a long period of use of malic acid. Two-week-old partridge chickens (a) and AA broilers (b) were provided with the malic acid-supplemented drinking water for 3 weeks, and the cloacal swab samples were collected at designated time to determine the

Campylobacter carriage. Twenty chickens were assayed in each group, the data are presented as the mean ± SD, asterisks indicate significant differences (P < 0.05 *)

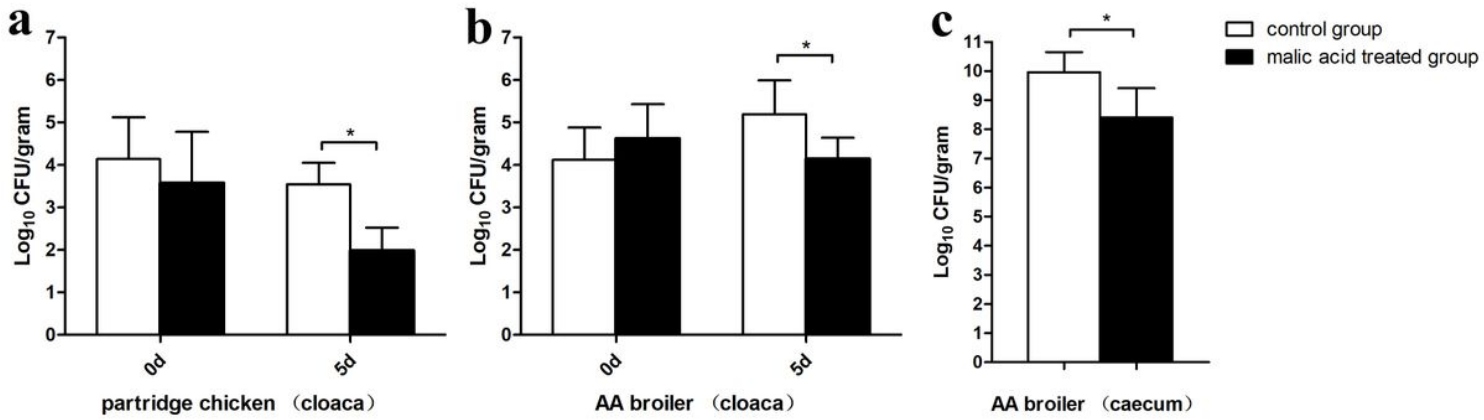

Figure 2

Campylobacter carriage in the chicken cloaca and caecum after the use of malic acid for five days. The partridge chickens (a) and AA broilers (b) at slaughter age were provided with the malic acid-supplemented drinking water, and the cloacal swab samples were collected before the experiment and 5 days later to determine the Campylobacter carriage. The caeca of AA broilers were collected during slaughter to determine the load of colonized Campylobacter (c). Twenty chickens were assayed in each group, the data are presented as the mean ± SD, asterisks indicate significant differences (P < 0.05 *)

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