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The effect of acidifier supplementation on egg production performance and intestinal histology of Japanese quail (*Coturnix japonica*)

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Abstract

Background: Acidifier are substances with antibacterial, antifungal, antimicrobial, performance and health benefits that are frequently employed in feed acidification, especially in poultry diet. Meanwhile, the most important factor for acidifier efficiency is the proportion of different acids in the final product.

Objectives: This study aimed to investigate the effect of dietary supplementation of a commercial acidifier on egg production and histology of the small intestine in laying Japanese quail.

Methods: One-hundred and sixty female quails at 15 weeks of age were divided into four groups and fed basal diet supplemented with different levels of acidifier (0, 1, 2 and 3 gr acidifier/kg of basal diet) for 8 weeks. Egg production, egg quality attributes and body weight (BW) were measured every 2 weeks. Histology of the small intestine and bacterial population of cecum as well as pH of crop, duodenum, jejunum, ileum and cecum contents were also investigated at the end of the experiment.

Results: Feed conversion ratio (FCR), yolk height, shell thickness, pH of the duodenum, jejunum, ileum, cecum; duodenum, villus width (VW), villus height (VH), crypt depth (CD); jejunum VH, VW and ileum VH to CD ratio (VCR) were linearly improved by the increasing levels of acidifier supplementation ($P < 0.05$). Duodenum VH increased in a linear and quadratic manner in response to increasing levels of acidifier. Egg weight, yolk diameter, jejunum CD, ileum CD, ileum VW, duodenum CD and jejunum VCR quadratically improved by grading levels of acidifier ($P < 0.01$). BW, albumen height, Haugh unit, ileum VH and ileum VCR were cubically enhanced ($P < 0.05$). Acidifier supplementation enhanced egg production, FCR, jejunum, ileum and cecum pH and VH, CD and VW of duodenum and jejunum, compared to the control group ($P < 0.05$); however, dietary acidifier did not affect egg mass, gizzard pH, ileum VH and bacterial count of the cecum ($P > 0.05$).

Conclusions: In conclusion, as calculated, the supplementation of 1 and 2.6 g acidifier per kg of diet was associated with beneficial effects on egg production and quality, gastrointestinal tract pH and histology of the small intestine in laying quails.

KEYWORDS
acidifier, egg production, intestinal histology, Japanese quail

1 | INTRODUCTION

High production and efficient feed conversion are the demand of the modern poultry industry, which can be achieved by using specific feed additives (Zampiga et al., 2021). Antibiotics as growth promoters have long been supplemented with poultry feed to stabilize the intestinal microbial flora, improve the general performance, and prevent specific intestinal pathogens (Khan & Iqbal, 2016). Some feed additives can affect the microbial population in the gastrointestinal tract (GIT), and there is tremendous interest in consuming them in the poultry industry (Ricke et al., 2020). During past decades, antibiotics have been widely used in the poultry industry to prevent disease and improve growth performance (Hedayati et al., 2013). Due to their residual effects and the risk of developing drug resistance in animal and human pathogenic bacteria, the use of antibiotics as growth promoters in animal feed diets has been highly regulated (Bonos et al., 2011). The small intestine is a crucial part of the digestive system due to its involvement in nutrient absorption (Lin et al., 1999). Hence, the healthy development of this digestive region is essential to quail health and performance (Wilkinson et al., 2016). It is well known that improving gut structural morphology leads to an increase in the digestive and absorptive function of the intestine due to expanding absorptive surface area (Choct, 2009; Yadav et al., 2022). In this respect, intestinal histology is closely related to intestinal absorptive function (Keating et al., 1995; Yamauchi, 2002).

The use of feed additives and supplements, such as acidifiers, has generally resulted in beneficial changes to gut morphology and growth performance of poultry species (Elhassan et al., 2019). Currently, many researchers are investigating different feed additives that may be used to alleviate the problems associated with the withdrawal of antibiotics from the feed. Several studies about dietary acidifiers used in chicken diets have been published previously (Bonos et al., 2011; Dizaji et al., 2012; Elhassan et al., 2019; Ibrahim et al., 2020; Gao et al., 2021). Most of the studies have shown that dietary supplementation of acidifiers positively affected growth performance (Ahmed et al., 2014; Dizaji et al., 2012; Eftekhari et al., 2015). The acidifiers make proteins and nutrients more digestible by reducing microbial competition for nutrients and resulting in better chicken growth and performance (Dibner & Buttin, 2002). An essential objective of dietary acidification is inhibiting intestinal bacteria from competing with the host for available nutrients and reducing possible toxic bacterial metabolites (Guo et al., 2022). Therefore, the objectives of the present study were to examine the effect of dietary supplementation of a newly developed acidifier on the production performance, intestinal histology and different parts of GIT pH in layer Japanese quail.

2 | MATERIALS AND METHODS

2.1 | Experimental design and bird management

One hundred and sixty laying Japanese quails at 15 weeks of age with an average body weight (BW) of 240± 0 gr were randomly allocated into four groups and four replicates (cage) with 10 birds per replicate. After 2 weeks of acclimation to the environmental and nutritional conditions, the birds were kept for 8 weeks. The quails were housed in 50 × 60 cm cages with an ambient temperature of 24 ± 2°C and a ventilation rate of 540 m^3/h. The quails were on a 16L:8D lighting schedule at an intensity of 10 lux (Rafieian-Naeini et al., 2021) and were fed a basal diet (30 g, every day) formulated to meet their nutritional requirements (NRC, 1994; Table 1) along with free access to nipple drinking system and freshwater. Treatments included dietary supplementation of a commercial acidifier (PROCID, Towhid Darou Pars Co.) at 0, 1, 2 and 3 gr acidifier/kg of diet (Tugnoli et al., 2020).

2.2 | Egg production, BW and egg quality indicators

Egg production, egg weight and feed conversion ratio (FCR) of each replicate and dead birds (if any) were recorded daily and presented on a weekly basis. Egg weight was measured by digital balance (model EK-1000H), and the FCR was calculated for each replicate (feed intake/egg mass × 100). Egg quality indicators, including yolk and albumen index, and shell thickness, were measured every 2 weeks. Five eggs per replicate were weighed, then broken into a flat surface and a micrometre (Ames company, model: s-6428) was used to determine the height of the yolk and albumen, and the yolk diameter was measured with a calliper from two perpendicular areas (Papaioannou et al., 2010; Rafieian-Naeini et al., 2022). The BW was recorded just at the beginning and end of the experiment. Shell thickness was measured with a micrometre from two equator points, and average numerical values were reported (Gaisford, 1965).

TABLE 1 Ingredients and chemical composition of the laying Japanese quail diet (as fed basis)

Ingredient	Amount (%)
Corn	54.25
Soybean meal, 44% CP	34.80
Dicalcium phosphate	1.45
$CaCO_3$	5.25
Common salt	0.20
$NaHCO_3$	0.17
Vegetable oil	3.23
DL-Met, 99%	0.15
Mineral premix[a]	0.25
Vitamin premix[b]	0.25
Total	100
Calculated nutrient content	
AME (kcal/kg)	2900
CP (%)	20
Calcium (%)	2.5
Available phosphorus (%)	0.35
Sodium (%)	0.15
Lysine (%)	1.59
Methionine (%)	0.45
Met + cys (%)	0.77
Threonine (%)	0.77

[a]Provides (per kg of diet): Choline (C5 H14 N O), 300 mg; iron (FeSO4.7H2O),50 mg; manganese (MnSO4.H2O), 120 mg; Zn (ZnO), 110 mg; copper(CuSO4.5H2O), 10 mg; selenium (Na2SeO3), 0 mg; iodine (KI), 2 mg.
[b]Provides (per kg of diet): vitamin A (retinyl acetate), 11,000 IU; vitamin D3 (cholecalciferol), 3500 IU; vitamin E (DL-α -tocopheryl acetate), 150 IU; vitamin K3(menadione), 5.0 mg; vitamin B1 (thiamin), 3.0 mg; vitamin B2 (riboflavin), 12 mg; vitamin B3 (niacin), 55 mg; vitamin B5 (Dpantothenic acid), 15 mg; vitamin B6(pyridoxine), 4 mg; vitamin B9 (folic acid), 2 mg; vitamin H2 (biotin), 0.25 mg; vitamin B12 (cobalamin), 0.03 mg.
Abbreviation: AME, apparent metabolisable energy.

2.3 | Necropsy, gastrointestinal sampling and pH measurement

At the end of the experiment, two birds per replicate were slaughtered, and small intestine samples from the duodenum, jejunum and ileum were collected for histology study (Schwartz & Bickford, 1986). In addition, samples of cecum contents were collected to measure the microbial population of the intestine and transferred to the microbiology lab immediately. A calibrated pH meter (Testo 206-pH2) measured the digesta pH of the crop, gizzard, duodenum, jejunum, ileum and cecum (Rune, 1968). Briefly, after calibrating the pH meter with pH 4 and 7 solutions, put it directly inside the content of the crop, gizzard, duodenum, jejunum, ileum and cecum (Panda et al., 2009).

2.4 | Histology analysis

About 2 cm of the duodenum, jejunum and ileum were dissected and washed with phosphate buffered saline (Siddiqi & Nou, 2021); then, samples were placed in 10% formalin (pH = 7) to stabilise the tissue for several days (De Souza et al., 2021). After locating samples in the baskets, they were transferred to a tissue processor (Didsabz company), dehydrated in ethyl alcohol, cleared in xylene and finally embedded in paraffin and then cut into 7-μm-thick sections using a microtome (Rotary microtome, Didsabz company, model DS4055) and then stained with hematoxylin–eosin dye (Fischer et al., 2008; Tosta et al., 2019; Rodig, 2021). The slides were mounted by Entellan new (Merck), and the samples were examined by a light microscope at the X40 magnification. Setting scale and measurements were conducted using image J 1.52a software (Lam et al., 2021). The villus height (VH) and crypt depth (CD) were measured in different parts, and the VH to CD ratio (VCR) was calculated.

2.5 | Intestinal microflora analysis

The cecum contents were collected instantly after slaughter, homogenised and filtered. The total number of aerobic bacteria, *Escherichia Coli* and *Lactobacillus* colonies were counted and reported. The anaerobic culture techniques used were similar to those described by Hungate (1969), and microscope cell counts were made on cecal samples following Meynell and Meynell (1970) method.

2.6 | Data analysis

Data were analysed by SAS version 9.4 (SAS Institute Inc.). Polynomial orthogonal analysis was used to evaluate the response to the increase in acidifier levels and orthogonal polynomial contrasts were used to test the linear, quadratic and cubic effects of acidifier supplementation on dependent variables. When the quadratic effect was significant, a second-order equation of $Y = a + bX + cX^2$ was fitted to the responses of the dependent variables (Y) to the increasing levels of acidifier (X) (Nasirikhah et al., 2019; Robbins et al., 2006). The optimum dosage of the acidifier (inflection point) was identified as the point at the first derivate of the curve function that was equal to zero. For traits that were affected in a cubical manner, third-order regressions of $Y = a + bX + cX^2 + dX^3$ were fitted on the responses of the dependent variables (Y) to graded levels of acidifier supplementation (X) (Nasirikhah et al., 2019; Robbins et al., 2006). To find the optimum acidifier dosage, a straight broken-line regression analysis was applied using the NLIN procedure of SAS. The egg production, as a binary distributed data, was analysed by GENMOD procedure using a logit odds ratio link function, and Tukey's multiple comparison tests were used to determine significant differences between experimental groups. Results were reported as least squares mean (*LSMEANS*) and standard error of the mean (*SEM*). Significance and tendency were declared at $P < 0.05$

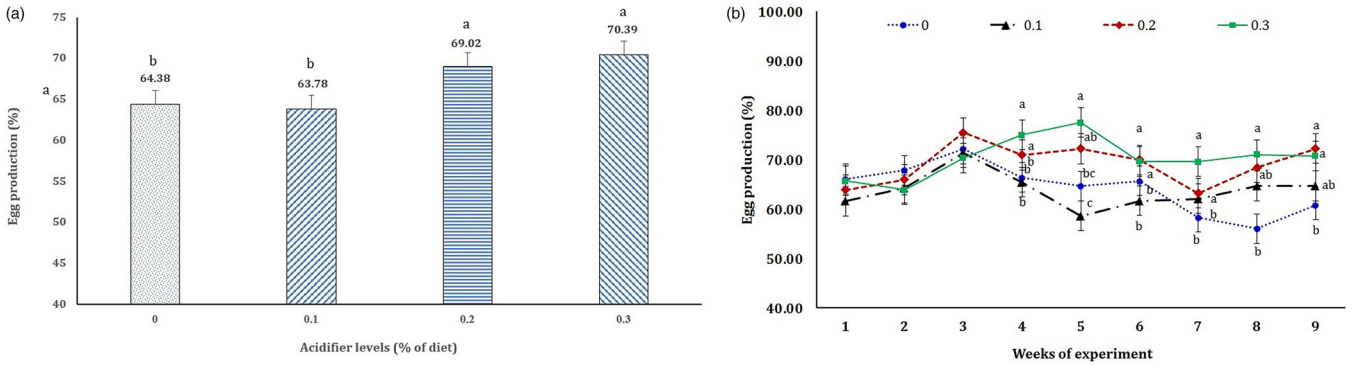

FIGURE 1 Egg production (a) and interactive effect of treatment and time on egg production (b) of quails fed by graded levels of acidifier. Values with different superscripts (a,b) are statistically different ($P < 0.05$).

TABLE 2 The effect of dietary acidifier supplementation on body weight (BW), productivity and egg quality

	Acidifier levels (gr/kg of diet)					*P*-value			
Item	0	1	2	3	SEM	ACDF vs. control	Linear	Quadratic	Cubic
BW (g)	266.04	286.36	266.86	266.14	5.14	0.25	0.42	0.06	< 0.05
Egg weight (g)	12.07	12.39	12.45	12.15	0.10	0.03	0.55	< 0.01	0.84
Egg mass (g/cage/day)	303.63	276.01	310.58	329.74	13.14	0.90	0.06	0.08	0.19
FCR (g FI/g egg)	4.22	3.99	3.47	3.75	0.18	0.03	< 0.05	0.18	0.20
Yolk height (mm)	11.71	11.94	11.97	11.94	0.07	< 0.01	< 0.05	0.07	0.68
Albumen height (mm)	4.35	4.35	4.61	4.33	0.07	0.35	0.50	0.08	< 0.05
Yolk diameter (mm)	24.55	25.03	25.30	24.81	0.12	< 0.01	0.06	< 0.01	0.32
Shell thickness (μm)	273.5	268.1	259.3	258.5	0.52	0.06	< 0.05	0.66	0.63
Haugh unit	88.19	84.89	89.38	88.08	0.99	0.51	0.35	0.31	< 0.01

Abbreviations: ACDF, acidifier; FCR, feed conversion ratio; SEM, standard error of the mean.

and $0.05 < P < 0.10$, respectively. To compare the control group and acidifier-fed groups, simple orthogonal analysis was used. Binomial data were analysed using logistic regression and GENMOD procedure.

3 | RESULTS

3.1 | Egg production and egg quality

The effect of acidifier supplementation on egg production is shown in Figure 1a. Supplementation of 2 and 3 g acidifier/kg diet increased produced eggs, compared to the control group ($P < 0.05$). The interactive effect of acidifier level and time showed that the first significant difference between treatments was obtained 4 weeks after acidifier feeding (Figure 1b); however, the egg production level remained relatively higher in the quails fed by 2 and 3 g acidifier/kg diet up to the end of the experiment. The effect of dietary acidifier supplementation on BW and egg quality attributes is presented in Table 2. Increasing levels of acidifier cubically affected BW ($P < 0.05$). The equation fitted for BW response (Y) to the graded level of acidifier (X) was $Y = 266.04 + 597.62X - 4920.50X^2 + 9765.41X^3$. The increasing levels of acidifier caused a quadratic response in egg weight ($P < 0.01$) with the equation of $Y = 12.07 + 5.02X - 15.79X^2$, in which X = acidifier level and Y = egg weight, suggesting 1.6 g acidifier/kg diet as optimum doses. Egg mass also tended to have a quadratic response to graded levels of acidifier response ($P = 0.08$), where the equation of $Y = 299.75 + 237.90X - 1169.31X^2$ was fitted for egg mass response to acidifier levels (X) and 1 g acidifier/kg diet calculated as the optimal dosage.

Results revealed a linear increase in feed conversation ratio (FCR) response to the increasing level of acidifier (X), resulting in a 5% and 17% reduction in 1 and 2 gr acidifier supplementation/kg of diet, respectively, compared to the control group ($P < 0.05$). Yolk height and shell thickness linearly ($P < 0.05$), albumen height ($P < 0.05$) and Haugh unit cubically ($P < 0.01$) and yolk diameter was quadratically improved by dietary supplementation of acidifier ($P < 0.01$). The equation fitted for yolk height, shell thickness, albumen height, Haugh unit and yolk diameter response to increasing levels of acidifier in diet were $Y = 11.72 + 2.66X - 6.43X^2$, $Y = 27.29 - 5.36X$, $Y = 4.35 - 4.02X + 53.31X^2 - 33.48X^3$, $Y = 88.19 - 117.27X + 1068.39X^2 - 2262.52X^3$ and $Y = 24.55 + 4.03X + 17.14X^2 - 92.35X^3$, respectively.

TABLE 3 The effect of dietary acidifier supplementation on pH of gizzard, different parts of small intestine and cecum

	Acidifier levels (gr/kg of diet)					*P*-value			
Item	0	1	2	3	SEM	ACDF vs. control	Linear	Quadratic	Cubic
Gizzard pH	3.98	3.86	3.70	3.67	0.15	0.20	0.13	0.78	0.82
Duodenum pH	6.04	6.00	5.91	5.88	0.04	0.06	0.01	0.93	0.66
Jejunum pH	6.32	6.22	6.00	6.01	0.06	< 0.01	< 0.01	0.37	0.25
Ileum pH	7.25	7.07	7.02	6.95	0.10	0.04	< 0.05	0.58	0.74
Cecum pH	6.06	5.63	5.310	5.37	0.17	< 0.01	< 0.01	0.17	0.73

Abbreviation: SEM, standard error of the mean.

TABLE 4 The total number of aerobic bacteria, colony count of *Escherichia coli*, and *Lactobacillus* (log CFU/g) in the quail cecum, fed with different levels of acidifier

	Acidifier level (gr/kg of diet)					*P*-value			
Item	0	0.1	0.2	0.3	SEM	ACDF vs. control	Linear	Quadratic	Cubic
Total aerobic	7.67	7.31	6.77	6.73	0.38	0.12	0.07	0.69	0.70
Lactobacillus	7.31	7.50	6.43	6.57	0.46	0.38	0.13	0.95	0.25
E. Coli	4.14	4.20	4.33	3.22	0.54	0.20	0.07	0.61	0.45

Abbreviation: SEM, standard error of the mean.

The optimal dosage improving these parameters ranged between 1 and 2.4 gr acidifier/kg of diet.

3.2 | GIT pH

The effect of dietary acidifier supplementation on gizzard pH, different sections of the small intestine and cecum are shown in Table 3. Acidifier supplementation decreased the pH of duodenum, jejunum, cecum ($P < 0.01$) and ileum ($P < 0.05$), compared to the control group; however, there were no differences among groups regarding gizzard pH. Duodenum, jejunum and ileum pH were linearly decreased ($P = 0.01$, $P < 0.01$ and $P < 0.05$, respectively) as acidifier dosage increased. The equations $Y = 6.04 - 0.55X$ and $Y = 6.045 - 0.55X$ explained the duodenum and jejunum pH (Y) response to graded acidifier (X) levels, respectively. The equation fitted for ileum pH (Y) response to the increasing level of acidifier was $Y = 7.22\text{-}0.96X$, in which X = acidifier level. The cecum pH was linearly ($P < 0.01$) affected by increasing levels of acidifier supplementation, where the equation $Y = 5.95 - 2.41X$ explained the cecum pH (Y) response to the increasing levels of acidifier (X).

3.3 | Microbial count

The total number of aerobic bacteria, *E. coli* and *Lactobacillus* in the cecum are represented in Table 4. The total number of aerobic bacteria and colony count of *E. coli* tended to have a quadratically decreasing response to graded levels of acidifier ($P = 0.07$), but *Lactobacillus* count was not affected by the acidifier supplementation.

3.4 | Small intestine histology

Results of adding different levels of acidifier supplementation on intestinal histology evaluation of VH, VW, CD and VH/CD ratio (VCR) of duodenum, jejunum and ileum are shown in Table 5. The dietary addition of an acidifier improved most histological parameters of the small intestine, compared to the control group ($P < 0.05$). Acidifier supplementation was associated with a cubic increase in the duodenum's VH ($P < 0.01$). The equation of $Y = 914.16 - 1918.77X + 24{,}394.18X^2 - 53{,}969.107X^3$ was fitted for duodenum VH (Y) response to increasing levels of acidifier (X), and the optimal dosage of 2.5 gr acidifier/kg of diet was calculated. The result showed a quadratic increase in duodenum CD ($P = 0.01$) under the fitted equation of $Y = 81.74651 + 148.57444X - 417.82188X^2$, which explains the CD (Y) response to increasing levels of acidifier (X) and 1.8 g of acidifier/kg of diet as optimum dosage. Increasing levels of acidifier supplementation improved duodenum VW and duodenum VCR in a linear manner ($Y = 58.25016 + 61.54600X$ and $Y = 10.94 + 7.0497X$, respectively; $P < 0.05$). The result indicated a cubical ($P < 0.01$), quadratic ($P < 0.01$) and linear ($P < 0.01$) increase in VH, CD and VW of jejunum in response to the graded level of acidifier supplementation, respectively. The equation $Y = 719.39044 - 2427.27079X + 21595X^2 - 42504X^3$ showed the jejunum VH (Y) response to increasing acidifier (X), where 2.6 g acidifier/kg of diet was calculated as the optimum dosage. Jejunum CD response (Y) to

TABLE 5 Effect of increasing acidifier levels on villi height (VH), villi width (VW), crypt depth (CD) and VH/CD (VCR) of duodenum, jejunum and ileum

	Acidifier level (gr/kg of diet)					*P*-value			
Item	0	1	2	3	SEM	ACDF vs. control	Linear	Quadratic	Cubic
Duodenum									
VH (μm)	914.15	912.25	1074.42	1076.84	20.83	< 0.01	< 0.01	0.91	< 0.01
CD (μm)	82.06	91.48	95.69	88.40	3.45	0.01	0.13	0.01	0.68
VW (μm)	59.36	62.80	70.45	77.31	1.14	< 0.01	< 0.01	0.32	0.33
VCR	11.39	10.89	12.53	13.17	0.42	0.09	< 0.01	0.17	0.09
Jejunum									
VH (μm)	719.39	650.10	757.69	787.12	13.26	0.42	<0.01	<0.01	< 0.01
CD (μm)	61.77	66.28	72.55	68.37	1.95	<0.01	<0.01	<0.05	0.17
VW (μm)	52.96	67.67	67.67	72.52	1.14	<0.01	<0.01	0.19	0.82
VCR	12.23	10.04	11.24	12.60	0.38	0.03	0.18	<0.01	0.07
Ileum									
VH (μm)	514.16	492.90	499.32	500.19	8.64	0.09	0.34	0.18	0.36
CD (μm)	51.20	60.50	61.21	61.19	1.61	< 0.01	< 0.01	< 0.01	0.27
VW (μm)	50.56	55.36	61.70	62.14	0.95	< 0.01	< 0.01	< 0.05	0.08
VCR	10.61	8.28	8.66	8.89	0.30	< 0.01	< 0.01	< 0.01	< 0.05

Abbreviation: SEM, standard error of the mean.

the graded level of acidifier (X) was fitted in a quadratic equation of $Y = 61.19531 + 92.67597X - 222.77057X^2$, suggesting 2.1 gr acidifier/kg of diet as the optimal dosage. The equation $Y = 53.64953 + 65.56065X$ explained that jejunum VW (Y) response to increasing acidifier (X) level. In addition, jejunum VCR showed a quadratic response to increasing acidifier levels ($P < 0.01$). The equations of $Y = 50.19 + 73.75X - 108.85X^2$ and $Y = 51.59 + 100.55X - 232.89X^2$ were fitted for the quadratic response of ileum VW ($P < 0.05$) and ileum CD ($P < 0.01$) to acidifier supplementation, respectively. The optimum dosage for these traits was calculated to be 3.4 and 2.1 g acidifier/kg diet. Cubical increase of ileum VCR was concluded from results with an optimum dosage of 2.7 g acidifier/kg and equation of $Y = 10.62 - 46.38X + 278.19X^2 - 475.93X^3$ in response to the increasing level of acidifier (X).

4 | DISCUSSION

The ban on antibiotics use in farm animals' feed, including poultry, has promoted a search for feed additives that control gut microbial status and improve production traits (Brzoska et al., 2013). Acidifiers, particularly in the poultry and swine sectors, are regarded as among such alternatives. It has been known for decades that acidifiers are excellent preservatives and nutritional additives for livestock feed (Partanen & Mroz, 1999; Spratt, 1987). Organic acids have a different mechanism of action depending on their pKa value; therefore, their effects will not be the same. Some researchers claim that the intestine's first part neutralizes acids by secreting bicarbonates (Pearlin et al., 2020). The current study results, as Fazilat et al.'s (2014) investigation, demonstrated positive effect of dietary supplemention of acidifier on Japanese quail's performance and gut health. In the present study, the pH of the duodenum, jejunum, cecum and ileum changed and decreased, but the gizzard pH did not. It may be due to the natural pH of gizzard in poultry, which is about 2.97–3.86 depending on age and feed type (Jiménez-Moreno et al., 2009). Therefore, because of the low pH of the gizzard, acidifiers and organic acids cannot reduce the pH of the gizzard, or if coat salts of acidifiers use, they pass through the gizzard and are released into the intestine. In contrast to our results, Hernandez et al. (2006) declare that adding different organic acids or their salts has not shown a significant effect on gut pH due to the strong buffering action of the digestive tract.

Better feed efficiency and producing more eggs in supplemented acidifier quails, compared to the control group, may be impressed by lower pH in the intestine and more feed digestion. The production of hydrochloric acid increases with age in the stomach, but incorporating acidifiers in their diet maintains the optimal pH for enzyme actions and proper protein digestion in the gut. Also, pepsinogen, the precursor to pepsin, is converted into an active form by an acidic environment and actively helps protein digestion (Lückstädt & Mellor, 2011). Ghazalah et al. (2011) enounced that the positive effect of 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. Earlier studies have shown that dietary benzoic acid or amylase can improve antioxidant capacity (Wang et al., 2020), and antioxidants can neutralize free radicals that significantly impact all aspects of quail egg quality (Rafieian-Naeini et al., 2022).

This study had greater values of yolk, albumen height and Hough unit, so we can postulate that more digestibility and antioxidant capacity in supplemented acidifier quails can improve those parameters (Yardibi & Hosturk, 2008). In our study, yolk height and shell thickness linearly, albumen height, and Haugh unit cubically and yolk diameter were quadratically improved by dietary supplementation of acidifier. Chen and Menon (2015) reported that acidifiers could prolong eggs' storage time by enhancing the Haugh unit. Our result regarding eggshell thickness was also in line with Chen and Menon (2015), which reported an increase in eggshell thickness due to the high utilisation ratio of calcium and phosphorus in Hyline Brown laying-supplemented acidifier hens. Also, Ziaie et al. (2011) stated that phosphorus (P) solubility and microbial phytase were more active through acidification, resulting in improved P absorption and mineral retention, and bone mineralisation was observed as a result of improved digestibility and availability of nutrients following organic acid supplementation and the development of desirable gut microflora.

In young chicks, a longer villus increases the absorbent surface of the intestines, while a shorter CD indicates a decreased tissue turnover as well as a reduced need for tissue growth (Emami et al., 2012). In this study, quails fed diets supplemented with an acidifier increased the VH in the duodenum and jejunum. According to Khatun et al. (2010), broilers fed with organic acid showed longer intestinal villi than those fed a control diet. Also, Khan (2013) stated that small intestinal VH rises due to the function of the intestinal epithelium as a natural barrier against pathogenic bacteria in the intestinal lumen as well as toxic substances. The present study showed no difference between treatments for total microbial aerobic, *Lactobacillus* and *E. Coli* count. Mroz et al. (2006) reported that under pH = 5, the proliferation of most pH-sensitive bacteria (*E. coli*, *Salmonella* and *Clostridium perfringens*) is minimized. Also, the order of the efficacy of acids against coliform bacteria is benzoic > fumaric > lactic > butyric > formic > propionic acid (Pearlin et al., 2020). Regarding our study acidifier components (acetic acid > formic acid > propionic acid > lactic acid), we can conclude that due to the use of a lower portion of strong acids against coliform bacteria and pH of the small intestine parts (< 5.31), no change in the number *E. Coli* can be justified. Contrasting the results of past research (decreasing *E. Coli* and increasing *Lactobacillus*) with our results might depend on the variation of acidifiers' ingredients (Getachew, 2016; Youssef et al., 2017).

5 | CONCLUSION

The result of this experiment showed that adding an acidifier (ranging between 1 and 2.6 g/kg diet) improved production performance and GIT pH. Irrespective of acidifier dosage, adding acidifier had a beneficial effect on the egg quality traits in the treatment group, compared control group. The findings indicate that the quail-fed acidifier had significantly better small intestine histology than the control. This positive effect of the acidifier on the performance may be due to a decrease in feed and digestive tract pH.

AUTHOR CONTRIBUTIONS

Visualisation, *investigation*, *writing—original draft*, *writing—review and editing*: Kimia Aliverdi-Nasab. *Conceptualisation*, *methodology*, *supervision*, *project administration*: Mahdi Zhandi. *Formal analysis*, *visualisation*, *writing—original draft*, *writing—review and editing*: Ali Reza Yousefi. *Resources*: Vahid Zahedi. *Writing—original draft*, *writing—review and editing*: Hamid Reza Rafieian-Naeini.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

All procedures in the present work were approved by the Animal Care and Welfare Committee of the Department of Animal Sciences, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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