Research Article

Effect of *In Ovo* Injection of Butyric Acid on Hatching, Physiological, and Productive Performance of Broilers

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Abstract | Butyric acid (BA) plays an important role in chicken embryonic development by stimulating digestive enzyme activity, promoting early intestinal growth and development, enhancing immune function, increasing the secretion of growth-related hormones and improving hatchability. In the present study, 540 fertilized eggs (17.5 days of incubation) were used to evaluate the effects of *in ovo* injection of BA on hatchability, physical characteristics, and growth performance of broiler chicks. The eggs were randomly allocated into six experimental groups: C1 (non-injected control), C2 (NaCl-injected control), C3 (0.3% BA), C4 (0.4% BA), C5 (0.5% BA), and C6 (0.6% BA). After hatching, chicks from each treatment group were reared under farm conditions. The results indicated that the C6 group showed a significant ($p \leq 0.05$) improvement in hatchability and mortality rate, along with a reduced proportion of pipped eggs. Additionally, all BA-treated groups exhibited a significant ($p \leq 0.05$) increase in chick hatch weight, as well as improvements in pecked chick mortality, incidence of abnormal chicks, chick length, and tonic immobility. The percentage of live pecked chicks was significantly ($p \leq 0.05$) higher in the C3 group, while leg length was significantly increased ($p \leq 0.05$) in the C4 group. Antioxidant status was significantly enhanced, with glutathione peroxidase (GSH-Px) activity being higher in C4 and superoxide dismutase (SOD) activity higher in C5 ($p \leq 0.05$). Furthermore, live body weight and weight gain were significantly increased ($p \leq 0.05$) in the C5 group, while feed conversion ratio was significantly improved ($p \leq 0.05$) in the C3, C4, and C5 groups. The study finding exhibited that *in ovo* injection of butyric acid, particularly at 0.5–0.6%, improved hatchability, chick quality, antioxidant status, and growth performance of broilers.

Keywords | *In ovo* injection, BA, Sustainable farming, Hatching traits, Broiler, Chicks

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INTRODUCTION

Modern fast-growing broiler strains are characterized by a high growth rate, and improper feeding of broiler breeder flocks can result in deficiencies in the nutritional content of eggs. As a consequence of genetic selection for rapid growth, the broiler rearing period has been shortened to approximately 35 days. When compared with the incubation period, the embryonic growth phase constitutes nearly 65% of the total rearing period, highlighting the critical importance of embryonic development. Therefore, considerable attention has been directed toward embryo growth and care to improve hatchability, post-hatch growth performance, and immune competence of chicks

(Alsudani *et al.*, 2025; Arkan *et al.*, 2025).

Any defect in hatchability components can reduce embryo survival, leading to early embryonic mortality. In some cases, embryos complete development but fail to hatch successfully due to insufficient nutrient availability, which weakens their ability to break the eggshell (Kadhim, 2025). Consequently, *in ovo* injection technology has been adopted as an effective method to supply essential nutrients directly to the developing embryo. This technique represents a sustainable approach to broiler production through the use of environmentally friendly compounds (Ibrahim and Al-Jebory, 2020; Ajafar *et al.*, 2024).

Organic acids are carbon-containing compounds with acidic properties that do not fully dissociate in water (Theron and Lues, 2007). They belong to the group of short-chain volatile fatty acids and are also referred to as weak acids containing one or more carboxyl groups; hence, they are known as carboxylic acids (Ricke, 2003). Their general chemical formula is RCOOH, where *R* represents an alkyl group or hydrogen (Hans, 2006). Butyric acid (BA), also known as butanoic acid ($CH_3CH_2CH_2COOH$), derives its name from the Greek word for butter due to its natural presence in butter, and its salts are referred to as butyrates. BA is produced through anaerobic fermentation in the colon and is characterized by a strong, unpleasant odor and pungent taste, detectable by humans at concentrations as low as 10 ppm (Lake, 2012).

Despite its odor, BA has several industrial and biological applications. Low-molecular-weight butyrate esters, such as methyl butyrate, possess pleasant aromas and are used in food and perfume industries. Additionally, BA has been reported to inhibit the proliferation of cancer cells in colon epithelial tissues, although its precise mechanism of action remains unclear (Vanhoutvin *et al.*, 2009). In animal nutrition, BA has gained attention as a feed additive due to its antimicrobial properties and ability to suppress harmful intestinal bacteria. Chaveerach *et al.* (2004) demonstrated that the inclusion of organic acids such as butyric, acetic, propionic, and formic acids in drinking water reduced intestinal Enterobacteriaceae and *Salmonella enteritidis* populations. Furthermore, BA serves as an important energy source for intestinal epithelial cells and plays a role in metabolic regulation and lymphoid tissue development in the digestive tract (Navd *et al.*, 2011).

The research has emphasized that embryonic growth and development reflect the management and nutritional status of broiler breeder flocks and represent the initial stage influencing subsequent growth performance and immune function of broilers (Salman *et al.*, 2024a). As this stage accounts for nearly two-thirds of the bird's productive lifespan, optimizing embryonic nutrition is crucial. Therefore, the present study aimed to investigate the effects of *in ovo* injection of butyric acid on hatchability traits, physiological and morphological characteristics of newly hatched chicks, and subsequent growth performance under farm conditions.

MATERIALS AND METHODS

The present study was conducted through two consecutive trials, one at the hatchery and the other at the broiler farm of Al-Anwar Poultry Company, during the period from October 1 to November 11, 2024. All laboratory analyses were performed at the Department of Animal Production, College of Agriculture, Al-Qasim Green University. All experimental procedures involving animals were carried out in accordance with the ethical standards and guidelines for the care and use of animals.

Experimental design and *in ovo* inoculatione

In the first trial, a total of 540 fertilized broiler eggs, at 17.5 days of incubation, were used to evaluate the effects of *in ovo* injection of butyric acid (BA). The BA used in this study was of English origin with a purity of 98–99%. Butyric acid solutions were prepared by dissolving the required amounts of BA in 100 mL of distilled water, and the concentrations were adjusted according to previously described protocols (Zaki and Al-Jebory, 2021; Khalil *et al.*, 2022; Al-Saeedi *et al.*, 2023).

Prior to injection, eggs were candled to identify fertilized eggs. The wide end of each egg was disinfected using an appropriate antiseptic, and a small hole was made using a sterile egg piercer. A sterile needle was then inserted vertically into the egg, and the prepared solution was injected following standard *in ovo* injection procedures. After injection, the puncture site was immediately sealed with paraffin wax to prevent contamination and moisture loss.

The fertilized eggs were randomly allocated into six experimental groups, each comprising 90 eggs, with three replicates per group (30 eggs per replicate), as follows:

- C1: Non-injected control
- C2: NaCl-injected control
- C3: 0.3% BA injection
- C4: 0.4% BA injection
- C5: 0.5% BA injection
- C6: 0.6% BA injection

The inclusion of two control groups was scientifically justified to allow comparison between natural incubation conditions (C1) and the effect of the injection process and diluent solution (C2), as NaCl may influence embryonic development due to its nutrient properties.

Hatchability and chick quality assessment

Following hatching, hatchability parameters and physical characteristics of chicks were evaluated according to the methodology described by Ajafar *et al*. (2024). Data related to hatchability, chick weight, chick length, and other physical traits were recorded immediately post-hatch.

Post-hatch rearing and productive performance

After hatching, chicks from each treatment group were transferred to the farm and reared under standard commercial conditions. Physiological traits were assessed at one week of age, following the procedures outlined by Al-Saeedi *et al*. (2024), Salman *et al*. (2024b), and Alsudani *et al*. (2025). Productive performance parameters, including body weight, weight gain, feed intake, and feed conversion ratio, were recorded throughout the rearing period up to 35 days of age, in accordance with Al-Jebory and Ibrahim (2021) and Al-Jebory *et al*. (2024).

Birds were fed starter, grower, and finisher diets formulated to meet the nutritional requirements of broilers as recommended in the Ross 308 Broiler Management Guide. The diets contained crude protein levels of 23.00%, 21.50%, and 20.08%, and metabolizable energy values of 2975, 3050, and 3100 kcal/kg, respectively.

Statistical analysis

All collected data were statistically analyzed using SAS Software (2012). Differences among treatment means were determined using Duncan's Multiple Range Test (1955), and statistical significance was declared at $p \leq 0.05$.

RESULTS

Hatching traits

The effects of the experimental treatments on hatchability traits are presented in Tables 1 and 2. A significant increase ($p \leq 0.05$) in hatchability percentage was observed in the C6 group compared with the C1 and C2 groups. In contrast, the hatch mortality rate was significantly higher ($p \leq 0.05$) in the C2 group than in the C4 and C6 groups. Hatch chick weight was significantly increased ($p \leq 0.01$) in the C3, C4, C5, and C6 groups compared with the C1 and C2 groups (Table 1).

Regarding the pipped egg ratio, a significantly higher value ($p \leq 0.05$) was recorded in the C2 group compared with the C4 and C6 groups. The live pecked chick percentage was significantly higher ($p \leq 0.05$) in the C3 and C5 groups than in the C1 and C2 groups. Meanwhile, the pecked chick mortality rate was significantly increased ($p \leq 0.05$) in the C3, C4, C5, and C6 groups compared with the C1 and C2 groups. Furthermore, the incidence of abnormal chicks was significantly higher ($p \leq 0.05$) in the C2 group than in the C3, C4, and C6 groups (Table 2).

Table 1: Effect of *in ovo* butyric acid (BA) injection on hatchability traits of broilers.

Treatments	Hatching ratio (%)	Hatch mortality ratio (%)	Hatch chick weight (g)
C1	86.00 ± 2.00^{bc}	14.00 ± 2.00^{ab}	38.73 ± 0.26^{b}
C2	84.00 ± 4.00^{c}	16.00 ± 4.00^{a}	38.32 ± 0.22^{b}
C3	91.00 ± 1.00^{abc}	9.00 ± 1.00^{abc}	40.94 ± 0.03^{a}
C4	93.00 ± 1.00^{ab}	7.00 ± 1.00^{bc}	39.99 ± 0.67^{a}
C5	90.00 ± 0.01^{abc}	10.00 ± 0.01^{abc}	40.18 ± 0.18^{a}
C6	94.00 ± 2.00^{a}	6.00 ± 2.00^{c}	40.77 ± 0.11^{a}
Significance	*	*	**

* $p \leq 0.05$; ** $p \leq 0.01$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Table 2: Effect of *in ovo* butyric acid (BA) injection on hatching quality traits of broilers.

Treatments	Pipped egg ratio (%)	Pecked chick live ratio (%)	Pecked chick mortality (%)	Abnormal chick ratio (%)
C1	14.00 ± 2.00^{ab}	0.50 ± 0.25^{c}	13.50 ± 1.50^{a}	4.00 ± 1.00^{ab}
C2	16.00 ± 4.00^{a}	0.50 ± 0.25^{c}	15.50 ± 4.50^{a}	5.00 ± 3.00^{a}
C3	9.00 ± 1.00^{abc}	5.50 ± 0.50^{a}	3.50 ± 1.50^{b}	0.00 ± 0.00^{b}
C4	7.00 ± 1.00^{bc}	3.50 ± 0.50^{abc}	3.50 ± 0.50^{b}	0.00 ± 0.00^{b}
C5	10.00 ± 0.01^{abc}	5.00 ± 1.00^{ab}	5.00 ± 1.00^{b}	0.50 ± 0.25^{ab}
C6	6.00 ± 2.00^{c}	2.00 ± 1.00^{bc}	4.00 ± 0.01^{b}	0.00 ± 0.00^{b}
Significance	*	*	**	*

* $p \leq 0.05$; ** $p \leq 0.01$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Chick physical traits

Table 3 shows the effects of the experimental treatments on chick physical traits. Chick length was significantly increased ($p \leq 0.01$) in the C3, C4, C5, and C6 groups compared with the C1 and C2 groups. No significant differences were observed among the experimental groups in wing length. Leg length was significantly greater ($p \leq 0.01$) in the C4 group compared with all other groups. In addition, tonic immobility was significantly improved ($p \leq 0.01$) in the C3, C4, C5, and C6 groups compared with the C1 and C2 groups.

Physiological traits

Tables 4 and 5 illustrates the effects of *in ovo* injection of butyric acid on selected physiological traits of broiler chicks. No significant differences were observed among the experimental groups in serum triglycerides, total cholesterol, high-density lipoprotein (HDL), or total protein concentrations (Table 4). Regarding antioxidant status and immune response (Table 5), glutathione peroxidase (GSH-Px) activity was significantly increased ($p \leq 0.01$) in the C3, C4, C5, and C6 groups compared with the C1 and C2 groups. Superoxide dismutase (SOD) activity showed a significant increase ($p \leq 0.01$) in the C5 group compared with the C1, C2, and C3 groups.

Table 3: Effect of *in ovo* butyric acid (BA) injection on physical traits of broiler chicks.

Treatments	Chick length (cm)	Wing length (cm)	Leg length (cm)	Tonic immobility (min)
C1	16.50±0.29^{d}	2.80±0.01	4.50±0.02^{b}	1.79±0.11^{a}
C2	16.10±0.05^{d}	3.10±0.15	4.57±0.27^{b}	1.77±0.18^{a}
C3	18.16±0.44^{bc}	3.60±0.49	4.16±0.03^{b}	0.65±0.04^{b}
C4	17.93±0.06^{c}	3.26±0.18	5.43±0.08^{a}	0.56±0.11^{b}
C5	19.10±0.37^{ab}	3.23±0.16	4.67±0.42^{b}	0.69±0.03^{b}
C6	19.30±0.55^{a}	3.56±0.16	5.90±0.02^{b}	0.46±0.12^{b}
Significance	**	N.S	**	**

N.S: not significant; ** $p \leq 0.01$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Table 4: Effect of *in ovo* butyric acid (BA) injection on physiological traits of broiler chicks.

Treatments	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	TP (g/L)
C1	101.77±1.40	69.13±4.30	9.93±2.04	5.24±1.00
C2	100.30±2.15	70.54±9.15	9.82±1.91	5.10±2.00
C3	97.89±4.65	71.32±5.31	10.01±5.31	4.98±1.48
C4	103.21±5.40	68.77±7.01	10.21±4.13	5.13±3.00
C5	98.78±3.61	72.94±12.45	9.74±5.08	5.27±4.21
C6	100.09±6.14	70.96±11.61	10.31±6.19	5.33±0.95
Significance	N.S	N.S	N.S	N.S

N.S: not significant ($p > 0.05$). TG = Triglycerides; TC = Total cholesterol; HDL= High-density lipoprotein; TP= Total protein.

Productive traits

Table 6 shows the impact of *in ovo* injection of BA on the live body weight of broilers. During the first week, C3, C4, C5, and C6 groups had significantly higher body weight ($p \leq 0.05$) than C1. In the second week, C3 and C4 were significantly higher ($p \leq 0.05$) compared to C1 and C2. In the third week, C3, C4, and C6 groups showed a significant increase ($p \leq 0.05$) over C2. In the fourth and fifth weeks, C3, C4, and C5 groups exhibited significantly higher body weights ($p \leq 0.05$) than the control groups.

The changes in weight gain (WG) are presented in Table 7. There was no significant difference between groups in the 1st and 5th weeks. In the 2nd week, the C5 group showed a significant increase ($p \leq 0.05$) compared to C1, C2, C3, and C6 groups. In the 3rd week, WG was significantly higher ($p \leq 0.05$) in the C3 group compared to C2 and C5. During the 4th week and for total weight gain, C3, C4, C5, and C6 groups exhibited a significant increase ($p \leq 0.05$) compared to C1 and C2 groups.

In feed intake, there was no significant difference between groups during the 1st, 2nd, and 3rd weeks. In the 4th week, C1 showed significantly higher feed intake ($p \leq 0.05$) compared to the other groups. In the 5th week, C5 had significantly higher feed intake ($p \leq 0.05$) than C1, C2, C3, and C4. The total feed consumption over the 5-week period was significantly higher ($p \leq 0.05$) in C6 compared to C1, C2, C3, and C4 (Table 8).

The impact of *in ovo* BA injection on feed conversion ratio (FCR) is presented in Table 9. In the 1st week, significant improvement ($p \leq 0.05$) was observed in C3 and C5 compared to the other groups. In the 2nd week, C5 showed a significant improvement ($p \leq 0.05$) over all other groups. No significant differences were observed among groups in the 3rd week. During the 4th week, C2, C3, C4, and C5 exhibited significant improvement ($p \leq 0.05$) compared to C1. In the 5th week and for total FCR, all BA-injected groups showed significant improvement ($p \leq 0.05$) compared to the non-injected control (C1).

Table 5: Effect of *in ovo* butyric acid (BA) injection on antioxidant and immune status of broiler chicks.

Treatments	GSH-Px (U/mL)	SOD (U/mL)	MDA (nmol/mL)	IgM (U/mL)	IgG (U/mL)
C1	191.66± 5.32^{c}	283.36 ± 10.23^{b}	20.51 ± 1.40	2.14 ± 0.58	6.41 ± 2.55
C2	194.47 ± 4.13^{c}	291.17 ± 5.89^{b}	20.33 ± 3.25	2.31 ± 0.25	6.21 ± 1.36
C3	270.89 ± 3.78^{ab}	293.14 ± 4.36^{b}	18.63 ± 2.69	1.98 ± 0.50	6.50 ± 2.00
C4	280.61 ± 4.14^{a}	325.13 ± 5.21^{ab}	19.57 ± 5.47	2.07 ± 0.61	6.33 ± 4.25
C5	253.15 ± 13.55^{b}	352.86 ± 9.11^{a}	19.69 ± 4.21	2.11 ± 0.27	6.15 ± 3.71
C6	259.30 ± 6.41^{b}	314.26 ± 7.35^{ab}	20.08 ± 1.57	2.10 ± 0.84	6.39 ± 2.97
Significance	**	**	N.S	N.S	N.S

N.S: not significant; ** $p \leq 0.01$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences. GSH-Px = Glutathione peroxidase; SOD = Superoxide dismutase; MDA = Malondialdehyde; IgM = Immunoglobulin M; IgG = Immunoglobulin G.

Table 6: Impact of *in ovo* injection of butyric acid (BA) on live body weight of broiler (g/bird).

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5
C1	160.17 ± 0.25^{c}	475.12 ± 1.00^{c}	872.65 ± 0.31^{b}	1409.28 ± 1.34^{d}	2109.23 ± 2.25^{d}
C299	162.25 ± 0.78^{bc}	477.18 ± 0.50^{c}	847.21 ± 1.56^{d}	1426.35 ± 0.78^{c}	2156.31 ± 2.00^{c}
C3	165.25 ± 0.18^{ab}	482.21 ± 0.75^{b}	889.21 ± 0.29^{ab}	1436.91 ± 1.79^{b}	2212.97 ± 1.59^{b}
C4	163.12 ± 0.41^{b}	488.98 ± 1.23^{a}	891.10 ± 1.00^{a}	1447.28 ± 0.21^{ab}	2235.26 ± 3.21^{b}
C5	160.78 ± 0.63^{b}	487.21 ± 2.00^{a}	867.17 ± 0.85^{c}	1451.63 ± 0.26^{a}	2248.82 ± 2.47^{a}
C6	166.21 ± 0.51^{a}	483.14 ± 1.63^{b}	886.27 ± 2.01^{b}	1446.08 ± 3.00^{ab}	2201.53 ± 2.56^{b}
Significance	*	*	*	*	*

* $p \leq 0.05$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Table 7: Impact of *in ovo* injection of butyric acid on weekly weight Gain (g) of broilers.

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Total
C1	121.44±0.77	314.95±1.25^{b}	397.53±2.82^{ab}	536.63±4.17^{c}	699.95±2.96	2070.50±2.45^{d}
C2	123.93±1.36	314.93±1.72^{b}	370.03±3.00^{b}	579.14±3.12^{ab}	729.96±1.58	2118.48±4.67^{c}
C3	124.31±2.01	316.96±0.16^{b}	407.00±4.51^{a}	547.70±1.50^{bc}	776.06±5.00	2172.03±5.31^{b}
C4	123.13±0.56	325.86±3.41^{ab}	402.12±0.55^{ab}	556.18±2.56^{b}	787.98±4.26	2165.27±3.12^{b}
C5	120.60±1.23	326.43±1.09^{a}	379.96±5.00^{b}	584.46±3.11^{a}	797.19±3.42	2208.64±5.09^{a}
C6	125.44±0.33	316.93±0.90^{b}	403.13±1.47^{ab}	559.81±1.14^{b}	755.45±6.00	2160.76±4.87^{b}
Significance	N.S	*	*	*	N.S	*

N.S: not significant; * $p \leq 0.05$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Table 8: Effect of *in ovo* injection of butyric acid on feed intake (g) of broilers.

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Total
C1	145.23±2.00	365.21±1.22	564.87±0.63	850.63±5.01^{a}	1106.31±0.06^{b}	3032.25±8.36^{b}
C2	144.92±2.50	374.96±1.00	546.21±2.14	837.21±4.62^{b}	1106.25±0.10^{b}	3009.55±5.23^{c}
C3	146.38±1.69	375.21±0.76	565.31±3.10	809.32±3.89^{c}	1059.23±3.15^{c}	2955.45±6.71^{c}
C4	145.68±3.25	387.39±2.00	574.63±1.72	810.87±6.21^{c}	1065.98±0.96^{c}	2984.55±9.85^{c}
C5	145.89±2.15	374.12±0.25	560.28±2.36	835.69±4.80^{b}	1134.52±11.02^{a}	3050.50±5.40^{ab}
C6	145.53±4.55	378.65±0.98	570.63±1.08	837.41±4.61^{b}	1124.69±1.47^{ab}	3056.91±14.67^{a}
Significance	N.S	N.S	N.S	*	*	*

N.S: not significant; * $p \leq 0.05$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

Table 9: Effect of *in ovo* injection of BA on feed conversion ratio (FCR) of broilers.

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Total
C1	1.195 ± 0.01^{a}	1.159 ± 0.03^{b}	1.420 ± 0.08	1.585 ± 0.01^{a}	1.580 ± 0.02^{a}	1.464 ± 0.03^{a}
C2	1.169 ± 0.01^{b}	1.190 ± 0.04^{ab}	1.476 ± 0.02	1.445 ± 0.02^{b}	1.515 ± 0.03^{ab}	1.420 ± 0.07^{b}
C3	1.117 ± 0.03^{c}	1.183 ± 0.02^{ab}	1.388 ± 0.04	1.397 ± 0.04^{c}	1.364 ± 0.06^{c}	1.360 ± 0.05^{c}
C4	1.183 ± 0.02^{b}	1.188 ± 0.05^{ab}	1.429 ± 0.01	1.457 ± 0.02^{b}	1.352 ± 0.04^{c}	1.378 ± 0.01^{c}
C5	1.209 ± 0.01^{a}	1.146 ± 0.02^{c}	1.474 ± 0.03	1.429 ± 0.01^{b}	1.423 ± 0.05^{b}	1.381 ± 0.06^{c}
C6	1.160 ± 0.02^{b}	1.194 ± 0.01^{a}	1.415 ± 0.04	1.495 ± 0.02^{b}	1.488 ± 0.08^{b}	1.414 ± 0.03^{b}
Significance	*	*	N.S	*	*	*

N.S: not significant; * $p \leq 0.05$. Values are expressed as mean ± standard error. Different superscript letters within the same column indicate significant differences.

DISCUSSION

Improvement in hatching characteristics and the physical traits of hatched chicks was observed in groups injected with BA, which supports the energy and growth of embryos. Injecting BA into the amniotic sac allows it to be absorbed through the digestive tract via the yolk diverticulum, leading to a decrease in the pH of the digestive tract. This

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increased acidity favors the growth of beneficial bacteria in the early intestinal tract of hatched chicks, while reducing harmful bacteria that thrive in more basic environments, thereby decreasing microbial competition. The enhanced microbial balance stimulates the secretion of digestive enzymes, improving the breakdown of proteins and energy sources, including fats and carbohydrates. Lactobacillus species, for example, promote the secretion of amylase, which aids in starch digestion (Jang *et al*., 2004).

The increase in the surface area of intestinal villi may also contribute, as this enhances yolk absorption and results in higher chick weight (Jahanian, 2011). Furthermore, the acidic environment created by BA stimulates the release of digestive hormones, including gastrin and cholecystokinin (CCK), which regulate protein digestion and absorption. These hormones influence gallbladder contractions, exocrine pancreatic secretions, and gastric acid production, thereby improving feeding behavior, nutrient utilization, and post-hatch growth performance (SAlAhi *et al*., 2015).

The development of intestinal epithelial tissue may also explain these effects (Born, 2002). Supplementation with organic BA increases villi length (Jahanian, 2011), expanding the surface area available for nutrient absorption (Ricke, 2003). Organic acids enhance nutrient digestibility, improving blood serum quality and increasing antioxidant enzyme activity, including SOD and GSH-Px. These effects result from increased acidity, stimulation of pancreatic enzyme secretion, and improved absorption of minerals such as calcium, phosphorus, magnesium, and zinc, all of which enhance the function of the antioxidant system.

Moreover, the presence of BA promotes the utilization of digested nutrients and supports the growth of beneficial gut bacteria, which in turn stimulate pancreatic and digestive enzyme activity for more efficient protein digestion (Ghazalah *et al*., 2011). Lowering intestinal pH increases the availability of nutrients for beneficial bacteria, reduces harmful bacteria, and minimizes protein degradation into ammonia by pathogenic microbes during fermentation (Adil *et al*., 2010; Al-Hussany and Kadhim, 2025).

CONCLUSION

The current study demonstrated that *in ovo* injection of BA enhanced embryo development, resulting in higher hatchability, increased chick weight, and improved physical traits. These improvements translated into better growth performance on the farm, highlighting the role of BA in supporting broiler growth. Among the treatments, the C6 group (0.6% BA) performed best in hatching, physiological, and productive traits.

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NOVELTY STATEMENT

This study is unique in that it is the first to use butyric acid to support the growth and development of chicken embryos in the egg.

AUTHOR'S CONTRIBUTION

Noor Ali Hussein and Hashim Hadi Al-Jebory performed the field and laboratory work and prepared the initial project, while Mohammed Khalil Ibrahim Al-Saiddi and Asha Fajer Al-Jebory contributed to the laboratory work, manuscript writing, and statistical analysis.

Generative AI and AI-assisted technology statement

Artificial intelligence tools were used to improve the language quality of the manuscript. The authors confirm that all scientific content, data analysis, interpretations, etc. are solely their own.

Conflict of interests

The authors have declared no conflict of interest.

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