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Application of butyric acid as a feed additive for improving quail performance and health

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ABSTRACT This study evaluated the effects of dietary butyric acid (BA) on the Japanese quail' performance, immunology, lipid profile, cecal microbiota, and antioxidant levels. 250 unsexed, one-week-old quail chicks were divided into 5 groups, each with fifty chicks (5 replicates of 10 chicks). The first group was given the basal diet (BD), while the 2nd to 5th groups were fed BD with 50, 100, 150, and 200 mg BA/kg, respectively. The results indicated that BA improved weight gain and FCR ($p < 0.05$) and decreased total FI. The 200 mg BA/kg of diet showed the lowest FI ($p < 0.05$) and the best FCR ($p > 0.05$). BA boosted immunity through increasing IgA, IgM, IgG, and Complement 3. Significantly lower alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), and lactate dehydrogenase (LDH) were observed at 150 and 200 mg BA/kg ($P < 0.05$) than the control group. The BA-supplemented quail showed lower total cholesterol (**TC**), triglyceride (**TG**), low-density lipoprotein (**LDL**), and very low-density lipoprotein (VLDL) than the control one. This effect was more pronounced for 100 and 200 mg of BA/kg. However, high low-density lipoprotein (**HDL**) did not differ from the control group ($p > 0.05$). BA at ≥100 mg/kg diet reduced malondialdehyde (**MDA**) and induced greater levels of superoxide dismutase (**SOD**), total antioxidant capacity (**TAC**), glutathione peroxidase (**GPX**), globulin, total protein, digestive enzymes than the control group ($P < 0.05$). BA decreased cecal *E. coli, Salmonella*, Enterococcus, and *Coliforms* and increased Lactic acid bacteria ($p < 0.05$) compared to non-supplemented group. Collectively, the inclusion of 100 mg BA/kg diet is ideal for Japanese quail production and health.

Key words: organic acid, acidifier, chicken, feed additive, antioxidant

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INTRODUCTION

The antibiotic alternatives should have the ability of increase growth performance through enhancing feed efficiency, nutrient absorption and utilization, and gut microbial regulation through increasing the proliferation of beneficial bacteria and decrease harmful ones (Deepa et al., 2018; Rama Rao et al., 2023). Dietary acidifiers as a feed additive can enhance the action of proteolytic enzymes, improve protein digestion (Kim et al., 2005), and inhibit the growth of harmful bacteria through lowering the intestinal pH (Kim et al., 2005; Hernández et al., 2006). Thus, favored acidifiers and potential substitutes for antibiotic growth promoters are organic acids, particularly short-chain fatty acids (**SCFA**) containing 1-6 carbon atoms like butyric, propionic, and acetic acids. Short-chain fatty acids are generated by bacterial fermentation of feed carbohydrates in the gut. Butyric acid is one of the most researched SCFA and is currently gaining more attention as a feed supplement for poultry, either in acidic or salty forms (sodium, potassium, and calcium); the latter can be digested by birds and turned into acid. According to Kwan and Ricke (2005), butyric acid (**BA**) has the strongest bactericidal activity against species that are acid-intolerant, like Salmonella spp. and *E. coli.* Furthermore, it has been shown that supplementing SCFAs with or without medium chain fatty acids (**MCFA**) can protect against *Clostridium perfringens* (Pereira et al., 2015; Song et al., 2017; Kumar et al., 2021; Abdel Aziz et al., 2024) and *Eimeria* spp. (Nguyen et al., 2018; Sadurní et al., 2022). Selective

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stimulation of beneficial gut bacteria is another aspect of BA activity (Deepa et al., 2018). Therefore, gut health and nutrient digestibility are improved, which in turn improves growth performance and immunity in birds (El-Saadony et al., 2022) that can be used as alternatives to growth-promoting antibiotics (Giacomini et al., 2022). Butyric acid is used as a primary energy source (**ATP**) for intestinal epithelial cells when adenine nucleoside triphosphate (ATP) is further synthesized (Xia et al., 2017;Gabbianelli et al., 2020) and is required for the development of leukocytes (Corrêa-Oliveira et al., 2016). In addition, it stimulates tolerogenic dendritic cells and generates anti-inflammatory cytokines; consequently it controls epithelial inflammation and tolerance to antigens (Akter et al., 2021). According to a number of studies (Bortoluzzi et al., 2017; Gao et al., 2022; Zhang et al., 2022), sodium butyrate (**SB**) is essential for regulating the animal intestinal microbiota, improving gut immunity, and enhancing production performance and meat quality. There is also evidence that SB has anti-inflammatory and antioxidant properties that help alleviate metabolic diseases (Zhang et al., 2011; Mattace Raso et al., 2013; Bortoluzzi et al., 2017; Lan et al., 2020a). Coated sodium butyrate (75 or 150 g/t) can be used as an alternative to the antibiotic growth promoters (**AGP**) in broiler chicken diet (Rama Rao et al., 2023). Despite the fact that butyric acid has many health benefits, consuming large amounts of saturated fat can have negative health effects (Akter et al., 2021). These effects include obesogenic changes and changes in serum biochemical parameters that can result in hypercholesterolemia, hypertriglyceridemia, and fatty livers (Koteish and Diehl, 2001), as well as other risk factors linked to metabolic complications (Koteish and Diehl, 2001). Likewise, higher butyric acid concentrations in mice resulted in degenerative hepatocellular alterations, as well as an increase in their lipid profile and blood glucose level (Akter et al., 2021), while lower concentrations did not exhibit any discernible changes. Therefore, the goal of the current study was to ascertain how different butyric acid supplementation levels for 35 d would affect the quail chicks' growth performance, gastrointestinal health, lipid profile, liver and kidney functions, antioxidant status, and immune system.

MATERIAL AND METHODS

Animals and Design

The experimental procedures were conducted in accordance with the guidelines set forth by the Local Experimental Animal Care Committee. The ethical approval code is (ZU-IACUC/2/F/313/2023). 250 unsexed one-week-old quail chicks were divided into 5 treatment groups, each with 50 chicks, at random. Each group had five replicates, each with ten chicks. The first group 1) was fed on basal diet (only); 2) basal diet included BA by 50 mg/kg; 3) basal diet included 100 mg of BA/kg; 4) basal diet plus 150 mg of BA/kg and 5) basal diet plus 200 mg of BA/kg.

Table 1. Ingredients and nutrient contents of basal diet of growing Japanese quail.

Items	(%)
Ingredient	
Yellow Corn 8.5%	51.80
Soybean meal 44%	36.70
Maize gluten meal 62 %	5.21
Soybean oil	2.90
Limestone	0.70
Di-calcium phosphate	1.65
Salt	0.30
Premix[1]	0.30
L-Lysine	0.13
DL-Methionine	0.11
Choline chloride	0.20
Total	100
Calculated composition[2] (%)	
Metabolizable energy (kcal/kg)	2998
Crude protein	24.0
Calcium	0.80
Nonphytate phosphorus	0.45
Lysine	1.30
Total sulphur amino acids	0.92

[1]Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D3, 5000 I.U; Vitamin E, 130.0 mg; Vitamin K3, 3.605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B12, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18.333 mg; Folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

Birds and Housing

The Japanese quail chicks were obtained from Agriculture Faculty, Zagazig University, Egypt and housed in open-sided ventilated housing. As per the NRC (1994), standard growth rations were supplied for 5 weeks. Water and food were given ad libitum. The composition of the chick diets is displayed in Table 1. The environmental, sanitary, and managerial circumstances were noted for all birds.

Growth and Carcass Properties

At 3, 5, and 6 wk of age, body weight (**BW**) was measured. The body weight gain (**BWG**) at intervals of 1−3, 3−5, and 1−5 wk of age was calculated. During the test periods, feed consumption (**FI**) and FCR were measured. Five birds were selected at random to assess the carcass. The weights of the liver, heart, gizzard, and carcass were recorded and reported as g/kg of carcass weighing.

Blood Chemistry

Five birds per group were slaughtered to collect blood samples in sterilized tubes. Following clotting, samples were centrifuged at 3,500 rpm (2,328.24 G) for 15 min, and serum was stored at -20°C until analysis. Protein differentiation, alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), creatinine, urea, uric acid, triglycerides, cholesterol, high-density lipoprotein (**HDL**), superoxide dismutase (**SOD**), total antioxidant capacity (**TAC**), malondialdehyde (**MDA**),

Table 2. Growth performance of quail chicks as affected by dietary treatments at 5 wk of age.

	Butyric acid level (mg/kg diet)							Contrasts	
Items	0	50	100	150	200	SEM[1]	*P* value[2]	Linear	Quadratic
Body weight (g)									
1 wk	30.06	30.15	30.07	30.05	30.04	0.562	0.9999	0.9436	0.9500
3 wk	102.96	107.05	116.83	107.67	110.80	1.033	<.0001	0.0007	0.0004
5 wk	201.31	210.42	226.49	220.34	215.42	2.292	0.0002	0.0004	0.0002
Body weight gain (g/day)									
1-3 wk	5.21	5.49	6.20	5.54	5.77	0.034	<.0001	<.0001	<.0001
3-5 wk	7.02	7.38	7.83	8.05	7.47	0.117	0.0033	0.0052	0.0024
1-5 wk	6.12	6.44	7.02	6.80	6.62	0.070	<.0001	0.0002	<.0001
Feed intake (g/day)									
1-3 wk	16.13	16.15	15.81	14.56	14.15	0.499	0.0755	0.0094	0.4045
3-5 wk	20.75	19.22	19.94	19.67	17.85	0.464	0.0299	0.0089	0.4378
1-5 wk	18.44	17.68	17.88	17.12	16.00	0.251	0.0025	0.0002	0.1762
Feed conversion ratio (g feed/ g gain)									
1-3 wk	3.101	2.941	2.552	2.630	2.450	0.092	0.0056	0.0006	0.3019
3-5 wk	2.950	2.603	2.551	2.451	2.391	0.066	0.0020	0.0002	0.0695
1-5 wk	3.022	2.751	2.550	2.523	2.420	0.066	0.0011	<.0001	0.0891

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

[1]Standard error means.

[2]Linear and quadratic effects.

glutathione peroxidase (**GPX**), immune globulins (**IgA, IgG**, and **IgM**), and complement were all measured using trade diagnostic tools from Biodiagnostic Co. (Giza, Egypt). Low-density lipoprotein (**LDL**) and cholesterol were computed using (Friedewald et al., 1972).

Digestive Enzymes

The methods described by Somogyi (1960) and Tietz and Fiereck (1966) were used to measure the activity of amylase, lipase, and protease enzymes.

Microbiology Traits

Fresh cecal contents were gathered, brought to the laboratory, and exposed to a CO2 stream contained in bottles. Total bacteria were counted at 30°C for 2 d on plate count agar for *E. coli, Salmonella,* and lactic acid bacteria, and then quantified after 1 day of incubation at 37°C on Eosin Methylene Blue agar plates and XLD agar plates (Alagawany et al., 2018).

Statistical Analysis

The gathered data were statistically analysed using SAS (version 9.2, 2008, SAS Institute, Cary, NC) software's One-way ANOVA with a generalized linear model according to the following model: $X_{ij} = \mu + T_j + e_{ijk}$, where X_i = value of any observation, μ = population mean, T_j = treatment effect, e_{ijk} = random error. The data was initially examined for normal distribution before applying the identity link function. Orthogonal polynomial contrasts (linear and quadratic) were used to test the significance of the different levels of dietary butyric acid. Statistical significance was declared at $P < 0.05$.

RESULTS

Growth Performance

From the third week of life, all BA-supplemented groups showed a considerably (linear and quadratic) higher body weight than the nonsupplemented group (Table 2). All BA doses significantly ((linear and quadratic) improved body weight, with the 100 mg/kg diet having a noticeably greater impact. In comparison to the control group, feed intake at all ages was linearly decreased by BA supplementation. When compared to the control group, FCR was linearly improved by BA supplementation at all studied ages (Table 2).

Carcass Properties

The carcass traits of Japanese quail fed the experimental diets are shown in Table 3. Heart % was linearly ($P = 0.0071$) increased with BA supplementation. Although liver percentage was linearly and quadratically ($P = 0.0283$ and 0.0022) higher in the groups supplemented with BA than in the control group, the effect of the 100 mg/kg diet was shown to be superior. Similarly, giblets % was linearly and quadratically ($P = 0.0256$ and 0.0159) increased in the BA-supplemented groups at 50 mg/kg diet ($P > 0.05$), 100, 150, and 200 mg/kg diet compared to the control birds.

Liver and Kidney Functions and Lipid Profile

Supplementing with BA resulted in a linear decrease in the values of all measured indices of liver function, including ALT, AST, and LDH. LDH was lowered by all BA dosages ($P = 0.0002$), but the substantial fall in ALT and AST was seen in the diet supplemented groups receiving 100, 150, and 200 mg BA/kg and 150 and 200 mg BA/kg, respectively ($P < 0.05$) (Table 4). BA supplementation beginning from 50 mg/kg diet

Table 3. Carcass traits of quail chicks as affected by dietary treatments at 5 weeks of age.

	Butyric acid level (mg/kg diet)							Contrasts	
Items	0	50	100	150	200	SEM[1]	*P* value[2]	Linear	Quadratic
Carcass %	79.92	80.49	80.98	81.01	80.91	1.229	0.9622	0.5344	0.7051
Liver %	2.21	2.52	2.74	2.44	2.51	0.065	0.0030	0.0283	0.0022
Gizzard %	2.10	2.27	2.29	2.49	2.15	0.122	0.3015	0.4354	0.1098
Heart %	1.00	1.02	1.09	1.14	1.20	0.047	0.0802	0.0071	0.8161
Giblets %	5.29	5.81	6.12	6.06	5.86	0.137	0.0385	0.0256	0.0159
Dressing %	85.21	86.3	87.09	87.07	86.77	1.184	0.7930	0.3341	0.4469

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[1]Standard error means
[2]Linear and quadratic effects

Table 4. Liver and kidney functions of quail chicks as affected by dietary treatments at 5 weeks of age.

	Butyric acid level (mg/kg diet)							Contrasts	
Items	0	50	100	150	200	SEM[1]	*P* value[2]	Linear	Quadratic
Liver and kidney functions[3]									
TP (g/dL)	2.31	2.37	3.00	3.08	3.31	0.119	0.0017	0.0001	0.7158
ALB (g/dL)	1.40	1.41	1.39	1.59	1.85	0.087	0.0182	0.0033	0.0534
GLOB (g/dL)	0.91	0.96	1.61	1.49	1.46	0.102	0.0017	0.0005	0.0379
A/G ratio	1.55	1.49	0.89	1.08	1.28	0.089	0.0030	0.0097	0.0042
ALT (IU/L)	12.54	9.72	7.00	6.13	8.04	0.957	0.0065	0.0022	0.0110
AST (IU/L)	184.71	159.55	171.25	151.90	102.12	9.100	0.0011	0.0002	0.0475
LDH	222.65	109.88	160.20	187.20	133.55	10.753	0.0002	0.0167	0.0456
Creatinine (mg/dL)	0.81	0.42	0.41	0.41	0.52	0.058	0.0027	0.0093	0.0010
Urea (mg/dL)	2.00	1.85	1.12	1.34	0.96	0.124	0.0005	<.0001	0.3199
Uric acid	10.06	8.28	7.26	8.26	7.98	0.360	0.0034	0.0043	0.0039

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).
[1]Standard error means
[2]Linear and quadratic effects
[3]TP: total protein; ALB: albumin; GLOB: globulin; A/G: albumin/ globulin ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

increased globulin and subsequently total protein compared to the control group ($P < 0.0001$). Table 4 shows that all doses resulted in a lower A/G ratio than the control group, with a significant effect seen for diets containing 100 and 150 mg/kg ($P < 0.05$). BA supplementation beginning from 150 mg/kg diet increased ALB compared to the other tested groups (linear, $P = 0.0033$).

All doses of BA supplementation significantly (linear and quadratic) reduced creatinine ($P = 0.0093$ and 0.0010) and uric acid ($P = 0.0043$ and 0.0039) when compared to the control group. Urea was lower in the groups supplemented with 100, 150, and 200 mg BA/kg than in the groups supplemented with 0 and 50 mg/kg (linear, $P < 0.0001$) (Table 4).

Table 5 displayed the quail's lipid profile after supplementation with BA. Compared to the control group, TC and LDL were significantly (linear and quadratic) lower in the 50, 100, 150, and 200 mg BA/kg groups ($P < 0.0001$ and $= 0.0077$). BA at 100 mg/kg diet showed the lowest TC and LDL values among BA-supplemented groups. On the other hand, the different diets produced no significant differences in the levels of TG, HDL, and VLDL.

Table 5. Lipid profile of quail chicks as affected by dietary treatments at 5 wk of age.

	Butyric acid level (mg/kg diet)							Contrasts	
Items	0	50	100	150	200	SEM[1]	*P* value[2]	Linear	Quadratic
Lipid profile[3]									
TC (mg/dL)	240.35	215.71	151.16	160.10	160.97	9.904	0.0002	<.0001	0.0077
TG (mg/dL)	166.13	134.75	148.60	151.65	142.25	4.564	0.0078	0.0601	0.0832
HDL (mg/dL)	56.72	55.00	59.13	63.36	63.27	5.014	0.7030	0.2126	0.8635
LDL (mg/dL)	150.41	133.76	62.31	66.41	69.26	4.609	<.0001	<.0001	<.0001
VLDL (mg/dL)	33.23	26.95	29.72	30.33	28.45	0.913	0.0077	0.0600	0.0831

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[1]Standard error means
[2]Linear and quadratic effects
[3]TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein

Table 6. Immunity and antioxidants of quail chicks as affected by dietary treatments at 5 wk of age.

Items	Butyric acid level (mg/kg diet)					SEM[1]	*P* value[2]	Contrasts	
	0	50	100	150	200			Linear	Quadratic
Anti-oxidants[3]									
SOD (U/mL)	0.18	0.23	0.27	0.25	0.28	0.009	0.0001	<.0001	0.0213
MDA (nmol/mL)	1.69	0.98	0.43	0.38	0.43	0.052	<.0001	<.0001	<.0001
TAC (ng/mL)	0.79	2.38	2.34	2.45	1.79	0.108	<.0001	0.0001	<.0001
GPX (mU/mL)	0.21	0.27	0.51	0.41	0.42	0.040	0.0073	0.0043	0.0299
Immunity[4]									
IgM (mg/dL)	0.47	0.60	1.21	1.19	0.97	0.081	0.0002	0.0001	0.0016
IgG (mg/dL)	0.81	0.90	1.44	1.31	1.86	0.087	<0.0001	<.0001	0.4832
IgA (mg/dL)	0.63	0.99	1.60	0.92	0.88	0.092	0.0004	0.1799	0.0001
Complement 3	120	133	135	146	126	5.023	0.0474	0.1806	0.0139

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).
[1]Standard error means
[2]Linear and quadratic effects
[3]SOD: superoxide dismutase, [3]MDA: malondialdehyde, [3]TAC: total antioxidant capacity, [3]GPX: glutathione peroxidase
[4]IgM, G and A: immunoglobulin M, G and A.

Antioxidant Activity and Immune Status

P values of the linear and quadratic models showed a significant modulation in SOD, MDA, TAC, and GPX (Table 6). Supplementing quail diet with BA resulted in a significant decrease in the oxidative stress as measured by the MDA level. Among BA doses, 150 mg/kg diet showed the lowest MDA level ($P < 0.0001$). When compared to non-supplemented quail, BA at 100 mg/kg diet and above statistically increased SOD, GPX, and TAC levels (Table 6). The higher effect of BA was seen by the 100 mg/kg diet across all antioxidant measures. Supplementing with BA was found to boost the quail's immunological status (Table 6). BA at 50-200 mg/kg diet linearly and quadratically increased IgM ($P < 0.0001$ and =0.0016) when compared to the control. In comparison to the control group, a linear ($P < 0.0001$) and quadratic ($P = 0.0001$) increase in IgG and IgA was observed with BA supplementation, respectively. The different diets did not affect ($p > 0.05$) quail complement 3.

Digestive Enzymes

Table 7 showed an increment of the levels of digestive enzymes by BA supplementation in quail. Amylase, lipase, and protease enzymes were linearly and quadratically increased by BA at all doses ($P < 0.05$). Of the administered doses of BA, 100 mg/kg diet of BA induced the greatest levels of amylase and protease ($P < 0.05$), while 150 mg/kg diet of BA induced the highest lipase level ($P < 0.05$).

Microbiology Traits

By increasing the dose of BA, the overall number of bacteria, yeast, and mold in the cecum was significantly reduced (Table 8). The total yeast and mold count at all doses were significantly lower than in non-supplemented quail ($P < 0.05$). The overall bacterial count in BA-supplemented quail linearly ($P = 0.0003$) decreased at all doses 50 to 200 mg/kg diet. As a result, at all doses, BA significantly (linear and quadratic) reduced the counts of *E. coli, Coliforms, Salmonella* spp., and *Enterococcus spp.* ($P < 0.05$). In contrast, supplementation with BA at 50, 100, and 150 mg/kg diet ($P < 0.05$) as well as 200 mg/kg diet linearly and quadratically ($P = 0.0289$ and <0.0001) increased the lactic acid bacterial count.

DISCUSSIONS

This study aimed to compare the effects of BA in different doses on the performance, oxidative status, lipid profile, immunity, and gut microbiota of Japanese quail for 5 weeks. Some studies found no statistically significant differences in growth performance in broiler chickens supplemented with different forms of butyrate under normal conditions (Sadurní et al., 2022; Bawish, et al.,

Table 7. Digestive enzymes of quail chicks as affected by dietary treatments at 5 wk of age.

Items	Butyric acid level (mg/kg diet)					SEM[1]	*P* value[2]	Contrasts	
	0	50	100	150	200			Linear	Quadratic
Amylase (U/L)	13.87	14.87	19.82	17.06	17.58	0.588	0.0003	0.0005	0.0032
Lipase (U/L)	8.63	10.79	10.65	13.85	10.77	0.607	0.0022	0.0037	0.0113
Protease (U/L)	0.77	1.47	1.72	1.43	1.28	0.046	<.0001	<.0001	<.0001

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[1]Standard error means
[2]Linear and quadratic effects

Table 8. Effects of treatments on caecal microbiota (Log CFU/g) (total bacterial count, lactobacilli count, *coliform, E. coli* and *Salmonella* spp.) in quail chicks.

Items	Butyric acid level (mg/kg diet)					SEM[1]	*P* value[2]	Contrasts	
	0	50	100	150	200			Linear	Quadratic
Total bacterial count	7.08	6.98	6.89	6.79	6.56	0.071	0.0043	0.0003	0.3694
Total yeasts and molds count	4.42	4.16	3.81	3.78	3.65	0.072	0.0001	<.0001	0.0582
E. coli	5.33	4.52	4.33	4.12	3.90	0.072	<.0001	<.0001	0.0022
Coliform	5.95	5.11	4.71	4.65	4.40	0.091	<.0001	<.0001	0.0013
Salmonella spp	3.63	3.17	2.52	2.63	2.94	0.079	<.0001	<.0001	<.0001
Lactic acid bacteria	6.20	6.42	6.76	6.71	6.31	0.061	0.0022	0.0289	<.0001
Enterococcus spp.	5.62	4.89	4.57	5.01	4.84	0.089	0.0001	0.0005	0.0003

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

[1]Standard error means

[2]Linear and quadratic effects

2023). According to Giacomini et al. (2022), butyric acid's evaluation as a performance-enhancing additive is strongly influenced by microbiological challenges. Nevertheless, under normal conditions, Japanese quail treated with varying doses of BA showed increased growth performance (weight gain and FCR). FCR improved linearly as the BA dose was increased up to 200 mg/kg of diet, especially at the late stage (3−5 wk) and over the entire trial period (1−5 wk). Our results are in line with Mohamed and Bahnas, (2009), Nuhocak et al. (2009), Raza et al. (2019), Deng et al. (2023) Abdel Aziz et al. (2024) in quails and chickens. Fouladi et al. (2018) reported a significantly improved FCR with no effect on feed intake in laying Japanese quail supplemented with BA by 1% of diet. Interestingly, the present feed intake was decreased in BA supplemented quails, yet their FCR increased significantly. Numerous possible causes could explain that. One of these is the central nervous system impact of glucagon-like peptide-1 (**GLP-1**), which decreases appetite, enhances satiety, and decreases chickens feed intake (Tachibana et al., 2007; Honda, 2016). The expression of these GLP-1 was upregulated in intestinal epithelial cells by SCFAs (acetate, propionate, and butyrate), as a consequence of changed microflora, through mitogen-activated protein kinase pathways (Zhang et al., 2019). From another point of view, compared to chickens given antibiotics, broilers treated with butyric acid gained greater weight in a meta-analyzed review (Giacomini et al., 2022) when they examined the association between weight gain and feed consumption. According to their interpretation, broilers receiving BA require fewer nutrients for body maintenance, which leaves more nutrients available for growth performance and less for metabolism. These reduced maintenance requirements could result from BA's positive effects on the health of the gastrointestinal system (Giacomini et al., 2022). A broiler's ability to grow was found to be closely linked to its small intestine's ability to digest and absorb nutrients (Gao et al., 2018; Li et al., 2019), which is linked to higher villus height (Upadhaya et al., 2020). The addition of butyrate to broiler chickens enhanced the gut development (relative weight and length) on d 21 (Lan et al., 2020a). In addition to the microstructural improvements including strong proliferation in intestinal crypts (Salmanzadeh, 2013; Abdel Aziz et al., 2024), increased goblet cell numbers (Sikandar et al., 2017; Sadurní et al., 2022), and increased intestinal villi development (Bortoluzzi et al., 2017; Abdel Aziz et al., 2024) might be responsible for the improved quail performance with BA supplementation. Khan et al. (2022) reported that organic acids promote intestinal cell growth and proliferation by upregulating the expression of GLP-2. GLP-2 is a 33-amino acid peptide hormone and secreted by gut endocrine cells. It regulates gastric motility, gastric acid secretion, intestinal hexose transport, and strengthens the gut epithelium's barrier function. It also increases the surface area of the mucosal epithelium by stimulating the proliferation of crypt cells and inhibiting enterocyte's apoptosis (Drucker, 2001).

Besides the modification of gut histology, butyrate increases the activity of digestive enzymes, which improves nutritional absorption and digestibility as well as the productivity of broilers (Le Gall et al., 2009; Adil et al., 2010). In our investigation, supplementing with BA consistently resulted in a considerable rise in digestive enzymes. Therefore, butyrate enhances weight gain and FCR by having a good effect on protein and mineral digestibility (Khan and Iqbal, 2016).

In the present investigation, supplementing with BA boosted total protein and globulin in the plasma. Likewise, albumin and total protein were increased in laying Japanese quail supplemented with BA by 1% of diet (Fouladi et al., 2018) and in broiler chickens supplemented with butyrate (250g/ton feed) (Abdel Aziz et al., 2024) or BA (3 or 4 g/kg diet) (Raza et al., 2019), compared to basal diet.

According to Martin-Gallausiaux et al. (2021), the gut microbiota is also associated with nutrient digestion and absorption, energy balance maintenance, and immune system development. In quail, cecal microflora was found to be significantly modulated by BA supplementation starting at 50 mg/kg of diet. Where, the numbers of *E. coli, Salmonella, Coliforms*, and Enterococcus dropped but the numbers of lactic acid bacteria rose. Consistently, intestinal *Salmonella* and *E. coli* counts in laying Japanese quail fed BA were significantly decreased (Fouladi et al., 2018). Giacomini et al. (2022) stated that butyric acid has an antibacterial impact because its non-dissociated form penetrates bacterial cell membranes and lowers intracellular pH, which inhibits bacterial growth. Moreover, the Salmonella bacterial invasion to the chicken cecal epithelial cells was decreased significantly by BA pretreatment (Van Immerseel et al., 2004). Thus, quail growth performance would be enhanced by preventing the growth of pathogenic microorganisms.

Elevated blood levels of AST, ALT, and LDH are suggestive of hepatocyte damage, along with increased generation of free radicals triggering oxidative stress (Arab et al., 2006). AST, ALT, and LDH were considerably lower in quails given BA supplementation in the current study. In line with Lan et al. (2020b) who found that butyrate treatment significantly reduced the levels of AST and ALT in heat-stressed birds. Additionally, the addition of 0.4% of BA stopped the elevated serum ALT and AST levels observed in the positive control group of Eimeria-challenged birds (Ali et al., 2014). According to Elnesr et al. (2019) butyrate had a negative impact on the values of AST and ALT in Japanese quails by day 42, and it had no effect on the birds at age 21. These hepatic biochemical results were in line with the increased liver size by BA supplementation that seems to have been interpreted as a positive parameter. These organic acids support liver functions by taking part in redox activities, improving vessel wall flexibility and decreasing permeability, and forming readily soluble compounds with cholesterol for simpler removal (Kvan et al., 2019).

The quails received BA showed lower blood levels of TC, TG, LDL, and VLDL in the current investigation. Consistently, adding different forms of BA to chickens or quail has been linked to lower levels of various lipid elements in the blood or liver (Taherpour et al., 2009; Deepa et al., 2017; Fouladi et al., 2018; Raza et al., 2019; Miao et al., 2023). Activation of GLP-1 has been demonstrated to improve hepatic steatosis, lower triglyceride (**TG**) content, and lower AST and ALT levels (Cuthbertson et al., 2012; Trevaskis et al., 2012). It has been demonstrated that butyrate is an agonist for G protein coupled receptors (Bultman, 2014). Sodium butyrate accelerated lipid metabolism, maybe by preventing hepatic lipogenesis and encouraging hepatic lipolysis and the increased oxidative stability may be the cause of the enhanced lipid metabolism (Miao et al., 2023). Different types of butyric acid increased SOD activity and decreased MDA levels, indicating reduced tissue or cell damage and enhanced capacity to scavenge free radicals (Deepa et al., 2018). Moreover, butyrate can increase the glutamate level which is the precursor for GSH synthesis involved in the antioxidant activity (Liu et al., 2021). It could account for the enhanced lipid profile, antioxidant activity, and liver function that we observed. Whereas the lipid peroxidation indicator, MDA level, was significantly decreased while a considerable increase in the antioxidant indices SOD, GPX, and TAC was observed due to BA supplementation to quails.

We also assessed BA's impact on quail chicks' immune systems. Serum IgA, IgM, and IgY are the primary markers of immunological response in chickens (Perez-Carbajalet al., 2010). Additionally, complement is primarily produced by the liver and macrophages to improve humoral and cell-mediated specific immunity (Sakai, 1992). During the process pathogenesis, an interaction occurs between bacteria and host cells, where, butyrate down regulates expression of invasion genes and decreases the virulence of bacteria (Van Immerseel et al., 2004). Additionally, butyrate produces mucin glycoproteins in the intestinal epithelium and increase the defence barrier of colon mucosa (Leonel and Alvarez-Leite, 2012). Butyrate increases blood globulin levels and lowers albumin to globulin ratio (Griminger, 1986). The significantly higher levels of IgM, IgG, and C3 in BA feed group compared to the control group demonstrated an immune-modulatory effect. The IgM and IgY results matched the findings of Abdel Aziz et al. (2024). Anabolic effect in broiler chickens is indicated by improved carcass yield when BA is added to the diet (Panda et al., 2009; Lakshmi and Sunder, 2015; Dehghani-Tafti and Jahanian, 2016). However, our investigation found no appreciable changes in carcass features, which is consistent with the findings of Raza et al. (2019) in broiler chickens.

In conclusion, BA supplementation to Japanese quail diets improved the growth performance (improved weight gain and FCR and decreased FI), modulated the cecal microbiota, and enhanced their IgM, IgG, and complement 3. By enhancing lipid metabolism and having hepatoprotective and antioxidant effects, supplementing with BA reduced oxidative stress.

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DISCLOSURES

The authors declare no conflicts of interest.

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