Use of fumaric acid as a feed additive in quail's nutrition: its effect on growth rate, carcass, nutrient digestibility, digestive enzymes, blood metabolites, and intestinal microbiota

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ABSTRACT To investigate the effects of dietary fumaric acid (**FUA**) on performance, carcasses, nutrient digestibility, blood metabolites, digestive enzymes, and cecal microbiota in Japanese quail chicks. Three hundred unsexed Japanese quail (1-wk-old) were randomly assigned to 5 groups. Supplementation of FUA in the diet of Japanese quail chicks exhibited a significant improvement in growth performance through the different experimental periods studied compared with those receiving unsupplemented one. The digestibility of crude protein (**CP**) and metabolizable energy (**ME**) were improved with 10 and 15 g/kg FUA, respectively. Apart from lipase enzyme, birds fed 5 and 15 g/kg FUA recorded higher activity of amylase. There were no significant changes among experimental groups on the relative weights of carcass, gizzard, heart, and dressing. Dietary supplementation of FUA at different levels ($P >$ 0.05) increased total protein (**TP**) and globulin (**GLB**) concentrations and A/G % compared with control group. A significant ($P < 0.01$) decrease in plasma low density lipoprotein (**LDL**) and total cholesterol (**TC**) levels and increase in high density lipoprotein (**HDL**) concentrations were observed in chicks fed with FUA containing diets. Immunoglobulin G **(IgG)** ($P = 0.0026$) and M **(IgM)** ($P = 0.0007$) levels were greater in groups treated with either 10 or 15 g FUA/kg diet. A significant increase in plasma Ca concentration was noticed in chicks received 15 g FUA/ kg compared with the other groups. Quail chicks received diets containing FUA at different levels exhibited reduced cecal count of *coliform, E. coli*, and *Salmonella* as compared with control group. In conclusion, supplementation of fumaric acid (especially 15 g/kg diet) in quail chick diets improved their growth, digestibility of nutrients, immune response, antioxidant status, digestive enzyme, and intestinal health.

Key words: fumaric acid, growth, blood, digestive enzyme, bacteriology

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INTRODUCTION

In the last 50 years, antimicrobial growth enhancers have been used in chicken feeds all over the world (Yegani and Korver, 2008; Salim et al., 2018). Because of antibiotic resistance and its consequences for animal and human health, the European Union has outlawed the use of antibiotic growth promoters in the chicken sector. To limit the use of antibiotic growth promoters in poultry diets, feed additives such as organic acids have recently been required. Because of their physiological and nutritional activities, as well as protection against enteric infections, these additives play an essential role in increasing productivity and health (Alagawany et al., 2018; Alagawany et al., 2021a,b). Phytogenic feed additives are promising natural alternatives to antibiotics (Abd El-Hack et al., 2020; Rehman et al., 2020; Reda et al., 2021).

Organic acids and their salts are allowed in chicken diets by the European Union since they are safe and boost performance (Ismail et al., 2020; Fikry et al., 2021; Pirzado et al., 2021). In broiler chicks fed a feed enriched with 1% fumaric acid, Pirgozliev et al. (2008) discovered an increase in metabolizable energy of the diets. Furthermore, dietary fumaric acid supplementation considerably increased broiler chick growth performance (Kamal and Ragaa 2014; Banday et al., 2015; Abd El-Haleem et al., 2018).

The organic acid fumaric-FUA ($C_4H_4O_4$) is primarily produced by the oxidation of succinate and then

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transformed to malic acid in the tricarboxlic cycle (Kim et al., 2015). Due to their physical and chemical qualities, short chain fatty acids such as acetic, butyric, propionic, and formic acid, as well as other carboxylic acids such as fumaric, tartaric, citric, and lactic acid, have been the most commonly utilized in chicken diets (Abdel Fattah et al., 2008; Elnesr et al., 2019, 2020). According to Banday et al. (2015), broiler chicks fed diets supplemented with FUA at various doses (0.5, 1.0, and 1.5 %) demonstrated a significantly ($P > 0.05$) linear improvement in body weight gain (**BWG**) when compared to control. Elnaggar and Abo El maaty (2017) discovered that dietary formic acid caused a considerable rise in serum TAC, GSH, GPX, and SOD in ducks (0.5 and 1%). Total coliform levels in the caecum and ileum of broilers treated with fumaric and ascorbic acids were significantly reduced, according to Pirgozliev et al. (2008). To our knowledge, there has been little research in quails on the effects of FUA on nutrient digestibility, digestive enzymes, and cecal microbiota. The purpose of this study was to see how different quantities of dietary fumaric acid supplementation affected the growth performance, carcass characteristics, nutrients digestibility, digestive enzymes, blood metabolites, and cecal microbiota in growing Japanese quail.

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

Items	(g/kg)
Ingredient	
Maize 8.5%	518.0
Soybean meal 44%	367.0
Maize gluten meal 62 %	52.1
Soybean oil	29.0
Limestone	7.0
Di-calcium phosphate	16.5
Salt	3.0
Premix[1]	3.0
L-Lysine	1.3
Dl-Methionine	1.1
Choline chloride	2.0
Total	1000
Calculated composition[2]	
Metabolizable energy (MJ/kg)	12.53
Crude protein (g/kg)	240.0
Calcium (g/kg)	8.0
Nonphytate phosphorus (g/kg)	4.5
Lysine (g/kg)	13.0
Total sulphur amino acids (g/kg)	9.2

[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.

[2]Calculated according to NRC (1994).

MATERIALS AND METHODS

The research was conducted at Zagazig University, Poultry Department, Faculty of Agriculture. Three hundred 1-wk-old Japanese quail chicks were divided into 5 groups of 60 chicks each, with 5 replicates of 12 chicks in each group. The average live body weight of the chicks in each group was virtually the same (**LBW**). Five levels of dietary fumaric acid (**FUA**) supplementation (0, 5, 10, 15, and 20 g FUA/ kg diet) were tested in a totally randomized manner. The first group was fed a standard diet with no supplements and acted as a control group. The basal diet was supplemented with 5, 10, 15, and 20 g FUA/ kg diet in the second, third, fourth, and fifth groups, respectively. The basal diet (Table 1) was created using NRC guidelines (1994).

Characteristics of Growth and Carcass

Weights of individual quail were recorded at wk 1, 3, and 5 in order to compute body weight (g) and gain (g) over the course of the experiment. Throughout the trial, consumption of feed (g) was also calculated and converted to g feed/g gain. Five birds at 35 d were chosen at random from each group and slain for carcass evaluation. A pH meter (Model 507; Crison Instruments S.A., Barcelona, Spain) was also used to measure the pH of the cecal content (Reda et al., 2020a,b).

Digestibility Trail and Metabolizable Energy

To determine the nutrient digestibility and the metabolizable energy (**ME**), at 5 wk of age, 5 birds from each group were chosen and kept in separate cages and provided appropriate experimental meals. The chicks were given three days to adapt before being fed and having their excrement collected every 24 h for 5 d. After collecting the excreta, all quail feathers were removed, and the samples were cleaned, weighed, and dried in ovens at 70°C for 36 h. Diet and fecal analyses (CF: curd fiber, CP: curd protein, NFE: nitrogen free extract and EE: ether extract) were performed in accordance with AOAC procedures (2006). According to Titus (1960), the ME was 4.2 Kcal per gram TDN (total digestible nutrients).

Blood Biochemistry

At 5 wk of age, 10 birds from each group were chosen at random, weighed, and slaughtered to collect blood samples in test tubes using EDTA. The test tubes were then gently shaken to combine the anticoagulants and blood. Isolated plasma was obtained by centrifuging whole blood at 3,000 × g for 20 min and then storing it at −20°C until analysis. Total protein (g/dL) was determined using Armstrong and Carr's (1964) by Biuret technique. Concentrations of albumin (g/dL) were calculated using a calorimetric method. The globulin concentrations (g/dL) were calculated by subtracting albumin concentrations from total protein concentrations. Allain et al. (1974) recommended measuring triglycerides and total cholesterol. Myers et al. (1994) proposed a method for estimating high-intensity lipoprotein levels (**HDL**). Friedewald et al. (1972) evaluated the levels of low-density lipoprotein (**LDL**). The hepatic enzymes and kidney indices (urea and creatinine) and very low-density lipoprotein (**VLDL**) were also determined. According to Reitman and Frankel (1957), plasma mineral (Ca and P) concentrations were determined. The activities of the amylase and lipase enzymes were determined according to Somogyi (1960) and Tietz and Fiereck (1966). According to Koracevic et al. (2001) total antioxidant capacity (**TAC**) was evaluated. To determine superoxide dismutase (**SOD**) and glutathione peroxidase (**GPX**), Nishikimi et al. (1972) employed the spectrophotometric technique. Malondialdehyde (**MDA**) was measured using the method published by Mihara and Uohiyama (1978). Also, we determined immunoglobulin (IgG and IgM) according to Bianchi et al. (1995).

Digestive Enzymes

At 5 wk of age, the activities of amylase and lipase were determined in the ileum of the quails (1 quail per replicate) according to the method of Najafi et al. (2005, 2006). The ileum part was dissected from the Meckel's diverticulum to 2 cm above the junction of ileum and cecum, and the contents of ileum were collected in screw-capped sterile specimen vials (Najafi et al., 2005, 2006).

Microbiology Characteristics

At 5 wk of age, 10 g of cecal contents of quails were collected and transferred to a 250 mL Erlenmeyer flask containing 90 mL of 0.1% peptone in NaCl solution (0.85%) and mixed thoroughly. The counts of total bacteria, lactobacillus, coliform, *Salmonella*, and *E. coli* were assessed using the methods of Xia et al. (2004) and Reda et al. (2020a,b).

Statistics

All data of performance, carcass, digestibility of nutrients, digestive enzymes, blood constituents, and bacteriology were analyzed with one way ANOVA (SAS, 2001) and the model is:

$Y_{ij} = + T_i + e_{ij}$, where Y_{ij} = observation, = overall mean, T_i = FUA effect, and e_{ij} = random error. We used Tukey's test to compare the means among the different groups ($P < 0.05$).

RESULTS AND DISCUSSION

Growth Performance

Table 2 summarizes the effects of dietary FUA supplementation on the growth performance indicators of Japanese quail chicks. The results showed that supplementing FUA in the feed of Japanese quail chicks increased their growth performance (LBW, BWG, and feed conversion ratio [**FCR**]) significantly ($P > 0.05$) during the various experimental periods compared to those receiving an unsupplemented diet.

However, according to the best growth performance (LBW, BWG, and FCR) compared to other lev (5, 10, and 20 g FUA/ Kg diet), 15 g FUA/ kg food supplementation appeared to be the ideal level. When compared to the control group, chicks fed a food enriched with FUA at various dosages showed considerably lower FI during all experimental periods.

Our findings are consistent with those of other studies that have found that FUA increases development performance in broiler chicks maintained under normal (thermoneutral) circumstances (Adil et al., 2010; Ding et al., 2020; He et al., 2020). The 1.5% FUA diet produced the best BWG and FCR values, according to Attia et al. (2018). According to Elnaggar and Abo El Maaty (2017), ducks fed a basal diet containing 0.5 or 1.0% formic acid had considerably higher LBW and BWG, as well as lower FI and best FCR, than ducks fed a control diet. According to Banday et al. (2015), broiler chicks fed diets supplemented with FUA at various doses (0.5, 1.0, and 1.5%) demonstrated a significantly ($P > 0.05$) linear improvement in BWG when compared to control. Hernández et al. (2006), on the other hand, found no significant influence of formic acid on broiler chick growth rate.

The intestinal protective effects of FUA, which enhance the pH in meals, gut microbiota, and digestive enzyme activities, could explain why FUA improves growth performance (Adil et al., 2010; Liu et al., 2017). FUA is also a byproduct of carbohydrate metabolism (the citric acid cycle), which is a major source of intercellular energy in the form of ATP.

Coefficients of Digestion and Digestive Enzymes

When compared to the control, all birds fed 10 to 20 g/kg FUA had improved (P 0.05) digestibility for NFE, EE, and CF (Table 3). When compared to the other treatment groups, 10 and 15 g/kg FUA improved the digestibility of CP and ME, respectively. A similar tendency was reported in Ndelekwute et al. (2019), who found that adding organic acids to CP, CF, and EE enhanced their digestibility. Table 3 shows the effect of dietary FUA supplementation on digestive enzyme activity in this study. Aside from lipase enzyme, birds fed 5 and 15 g/kg FUA had greater amylase activity. Organic acids supplementation may be responsible for the increase in FCR since acidic anions aid mineral absorption (Abdel-Latif et al., 2020; Alagawany et al., 2020; Pearlin et al., 2020). Broilers fed organic acid diets improved nutrient absorption by increasing the height of villus in the intestine (Xia et al., 2004). Denli et al. (2003) discovered that adding synthetic antibiotics and organic acid to the diet enhanced intestine weight and length by d 42. Broilers fed organic acid diets have greater levels of digestive enzymes such chymotrypsin and trypsin (Liu et al., 2017). The higher

Table 2. Growth performance of broiler chicks as affected by different levels of fumaric acid.

Items	Fumaric acid level (g/kg diet) 0	5	10	15	20	SEM	*P* value
Live body weight (g)							
w 1	30.39	30.44	30.52	30.51	30.48	0.078	0.7547
w 3	109.40[e]	112.72[d]	118.10[c]	123.44[a]	120.17[b]	0.633	<0.0001
w 5	190.07[d]	196.00[bc]	199.44[b]	208.59[a]	193.33[cd]	1.183	<0.0001
Body weight gain (g / day)							
1–3 wk	5.65[e]	5.88[d]	6.26[c]	6.64[a]	6.41[b]	0.045	<0.0001
3–5 wk	5.76[b]	5.95[b]	5.81[a]	6.08[a]	5.23[b]	0.104	0.0027
1–5 wk	5.70[d]	5.91[bc]	6.03[b]	6.36[a]	5.82[cd]	0.043	<0.0001
Feed intake (g / day)							
1–3 wk	14.72[a]	13.38[b]	12.62[c]	13.21[bc]	13.44[b]	0.188	0.0009
3–5 wk	25.71[a]	25.10[ab]	24.33[b]	23.13[c]	22.90[c]	0.300	0.0004
1–5 wk	20.22[a]	19.24[b]	18.48[c]	18.18[c]	18.17[c]	0.166	<0.0001
Feed conversion ratio (g feed/ g gain)							
1–3 wk	2.61[a]	2.28[b]	2.02[cd]	1.99[d]	2.10[c]	0.025	<0.0001
3–5 wk	4.47[a]	4.22[ab]	4.19[b]	3.81[c]	4.38[ab]	0.068	0.001
1–5 wk	3.55[a]	3.25[b]	3.06[c]	2.86[d]	3.12[c]	0.037	<0.0001

[abcd]Means within the same row with different common superscripts differ significantly.

Table 3. Apparent nutrient digestibility and digestive enzymes affected by different levels of fumaric acid.

Items	Fumaric acid level (g/kg diet) 0	5	10	15	20	SEM	*P* value
Crude protein	85.08[b]	85.63[b]	86.15[b]	88.07[a]	85.61[b]	0.525	0.0287
Ether extract	70.67[d]	72.85[c]	75.99[b]	82.00[a]	75.72[b]	0.557	<0.0001
Crude fiber	25.27[b]	24.95[b]	28.31[a]	26.35[b]	26.41[b]	0.510	0.0124
Nitrogen-free extract	77.91[b]	79.46[b]	81.93[a]	83.43[a]	82.18[a]	0.504	0.0001
Metabolizable energy	3021[b]	3121[b]	3379[a]	3044[b]	2981[b]	51.31	0.0060
Digestive enzymes (U/l)							
Amylase	13.43[c]	17.53[ab]	15.60[bc]	19.60[a]	15.30[bc]	0.613	0.0011
Lipase	9.33	11.00	10.50	12.00	9.00	0.902	0.2615

[abc]Means within the same row with different common superscripts differ significantly.

activity of digestive enzymes may have improved nutrient digestibility in the current investigation. Increased efficacy of digestive enzymes in birds may be an indicator for increasing nutrient digestibility and improving the productivity (Alagawany et al., 2021c).

Carcass Characteristics

The results of feeding different amounts of FUA on carcass features of 6-wkold Japanese quail chicks are summarized in Table 4. Dietary FUA supplementation was found to raise the relative weight of the liver substantially ($P > 0.05$). The diet containing 10 g FUA/kg resulted in the largest liver weight ($P > 0.05$). Due to the addition of FUA to the food, there were no significant differences in the relative weights of the carcass, gizzard, heart, and dressing of Japanese quail chicks between experimental groups. However, when chicks fed a diet containing 1.5 g FUA/kg were compared to control and other dietary treatment groups, numerical increases in the aforementioned parameters were detected. According to Banday et al. (2015), carcass characteristics of broiler chicks fed diets supplemented with FUA at various levels (0, 0.5, 1.5, and 1.5%) exhibited no significant differences ($P > 0.05$) between treatment groups. Broiler chicks fed FUA at 1.5 and 3% had no significant effect on the relative weights of dressing,

Table 4. Relative weights of carcass traits as affected by different level of fumaric acid.

Items	Fumaric acid level (g/kg diet) 0	5	10	15	20	SEM	*P* value
Carcass %	77.28	76.28	76.03	79.09	75.52	0.768	0.0952
Liver %	2.18[b]	2.67[ab]	3.17[a]	2.60[ab]	2.64[ab]	0.159	0.0366
Gizzard %	1.98	2.41	2.44	2.07	2.69	0.178	0.1407
Heart %	0.86	0.81	0.95	1.05	0.73	0.084	0.1800
Giblets %	5.02	5.89	6.56	5.72	6.06	0.296	0.0805
Dressing %	82.30	82.16	82.59	84.8	81.58	0.692	0.0725
Caecal content pH	6.92[a]	6.82[a]	6.33[bc]	6.23[c]	6.46[b]	0.055	<0.0001

[abc]Means within the same row with different common superscripts differ significantly.

giblets, and abdominal fat, according to Attia et al. (2018). In contrast, Elnaggar and Abd EL-Maaty (2017) found that supplementing formic acid at 0.5 and 1.0% in the diet of broiler chicks increased the relative weights of total edible pasts, belly fat, and dressing when compared to the control group. The percentages of liver, heart, and spleen of broiler chicks were not significantly altered by dietary FUA (0, 1.25, 2.50, 3.75, 5.0, and 7.5%). supplementation, according to Islam et al. (2008).

As the level of FUA was increased from 5 to 10 or 1.5 g/ kg diet, a substantial ($P > 0$) decrease in the pH values of cecal contents was detected, as shown in Table 4. Banday et al. (2015), on the other hand, reported that the pH of proventriculus, crop, and gizzard in broiler chicks fed diets enriched with FUA at 0.5, 1.0, and 1.5% decreased insignificantly.

Blood Metabolites

Proteins and Their Fractions Table 5 shows the plasma total protein (**TP**), globulin (**GLB**), albumin (**ALB**), and A/G percent as a function of dietary FUA supplementation. The results of this investigation showed that dietary supplementation with FUA at various levels increased plasma TP and GLB concentrations and A/G percent significantly ($P > 0.05$). Furthermore, when compared to control and other dietary treatment groups, Japanese quail chicks fed a diet supplemented with 15 g FUA/ Kg had significantly greater plasma TP and GLB concentrations and A/G percent. However, dietary supplementation of FUA had no significant effect on TP and its fractions in the experimental group (Table 5).

The results of plasma TP, GLB, and A/G percent agree with those of Ghazala et al. (2011), who found that serum content of TP and GLB increased significantly with 0.5% FUA supplementation in broiler chick diet as compared to the control group, indicating that the immune response improved with FUA supplementation, which could indicate that broilers fed diets supplemented with a FUA supplement improved their immune response. Supplemental FUA may improve immunological response, according to these findings (Kamal and Ragaa, 2014).

The improvement in immune indices linked to dietary acidity could be attributable to an inhibitory effect on pathogens in the GI tract (Rahmani and Speer, 2005). Ducks fed a meal enriched with formic acid at 0.5 and 1% had considerably greater serum TP and GLB concentrations than control groups, according to Elnaggar and abo El-Maaty (2017).

Functions of the Liver and Kidneys Table 5 shows the results of the liver and renal functions obtained in this investigation. It's worth noting that Japanese quail chicks given a diet feed supplement containing FUA at various doses had significantly lower plasma activity of AST and urea levels than controls ($P > 0.05$). Furthermore, chicks fed the basal diet supplemented with 15 g FUA/Kg had the lowest AST activity and were level compared to the control and other dietary treatment groups ($P > 0.05$).

Table 5. Blood chemistry as affected by different levels of fumaric acid.

Items[1]	Fumaric acid level (g/kg diet) 0	5	10	15	20	SEM	*P* value
Liver and kidney functions							
TP (g/dL)	2.83[b]	3.10[a]	2.74[b]	3.17[a]	2.77[b]	0.061	0.0064
ALB (g/dL)	1.63	1.70	1.50	1.66	1.61	0.049	0.2103
G (g/dL)	1.20[c]	1.40[b]	1.24[c]	1.51[a]	1.16[c]	0.020	<0.0001
AG (%)	1.35[a]	1.21[b]	1.21[b]	1.10[c]	1.39[a]	0.028	0.0006
AST (IU/L)	203.53[a]	201.65[a]	161.65[c]	150.55[c]	182.00[b]	5.028	0.0001
ALT (IU/L)	14.32	14.42	12.05	13.37	14.18	0.755	0.2501
Creatinine (mg/dL)	0.54	0.55	0.50	0.57	0.55	0.038	0.8002
Urea (mg/dL)	1.26[a]	1.13[ab]	0.84[bc]	0.70[c]	0.86[abc]	0.086	0.0448
Lipid profile							
TC (mg/dL)	214.79[a]	212.73[a]	191.80[b]	197.12[b]	206.25[a]	2.612	0.0004
TG (mg/dL)	153.86	152.87	144.43	139.49	144.79	3.192	0.0576
HDL (mg/dL)	48.66[c]	52.62[b]	53.71[ab]	56.98[a]	51.92[bc]	1.111	0.0071
LDL (mg/dL)	135.36[a]	129.55[ab]	109.20[c]	112.24[c]	125.38[b]	2.715	0.0002
VLDL (mg/dL)	30.77	30.57	28.89	27.90	28.96	0.638	0.0576
Antioxidant parameters							
GPX (ng/ml)	0.22[c]	0.29[b]	0.32[a]	0.34[a]	0.32[ab]	0.010	<0.0001
TAC (ng/mL)	0.16[c]	0.22[b]	0.21[b]	0.30[a]	0.26[a]	0.012	0.0003
SOD (U/mL)	0.14[b]	0.15[b]	0.23[a]	0.27[a]	0.24[a]	0.016	0.0011
MDA (nmol/mL)	0.46[a]	0.38[b]	0.26[c]	0.21[c]	0.34[b]	0.023	0.0002
Immunology							
IgG (mg/dL)	0.82[c]	0.90[bc]	1.03[b]	1.23[a]	0.80[c]	0.054	0.0026
IgM (mg/dL)	0.56[b]	0.53[b]	1.06[a]	0.94[a]	0.69[b]	0.064	0.0007
Minerals							
Ca (mg/dL)	8.78[b]	8.61[b]	8.07[b]	10.22[a]	8.02[b]	0.242	0.0055
P (mg/dL)	5.10	5.37	4.72	4.41	4.99	0.200	0.1452

[abc]Means within the same row with different common superscripts differ significantly.

[1]Abbreviations: Alb, albumin; AG, albumin/ globulin ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Ca, calcium; G, globulin; GPX, glutathione peroxidase; HDL, high density lipoprotein; IgG and M, immunoglobulin G and M; LDL, low density lipoprotein; MDA, malondialdehyde; P, phosphorus; SOD, superoxide dismutase; TC, total cholesterol; TAC, total antioxidant capacity; TG, triglycerides; TP, total protein; VLDL, very low density lipoprotein.

Because uric acid is the main and result of protein metabolism, the lower plasma urea levels of groups treated with FUA at various dosages could indicate a higher use of protein and amino acid digestibility. The current findings are consistent with those of Elnaggar and Abo El Maaty (2017), who found that ducks fed a diet supplemented with 0.5 or 1% formic acid, had considerably lower serum urea levels and AST and ALT activity.

Lipid Constituents Table 5 shows that there were no significant variations in plasma triglycerides (**TG**) and VLDL concentrations between the different experimental groups and the control group. In chicks fed FUA-containing diets, however, there was a substantial ($P < 0.01$) drop in plasma total cholesterol (**TC**) and LDL, as well as a significant rise in HDL. Furthermore, chicks fed diets enriched with 10 or 15 g FUA/kg meal exhibited decreased TC and LDL concentrations and considerably higher plasma HDL concentrations ($P < 0.01$).

The ability of FUA supplementation in the diet to reduce microbial intracellular pH may explain the considerable drop in plasma TC and LDL (Abd El Halim et al., 2018). The findings were in line with those of Kamal and Ragaa (2014), who discovered a significant drop in blood cholesterol and total lipids as a result of consuming 3% fumaric acid in the diet. According to Elnugar and Abo El Maaty (2017), the level of TC and LDL in blood serum was dramatically reduced, while HDL was significantly elevated by either 0.5 or 1.5% FUA.

Immune Indices and Antioxidant Status

Table 5 indicates the influence of dietary FUA on Japanese quail chicks' immunological response (IgG and IgM) and antioxidant indices (GPX, SOD, TAC, and MDA). Table 5 shows that when comparing groups treated with 10 or 15 g FUA/kg diet to control groups, IgG ($P = 0.0026$) and IgM ($P = 0.0007$) levels were higher in groups treated with 10 or 15 g FUA/kg diet. The FUA level had no effect on IgM and IgG levels.

Dietary FUA improved immunological response in Japanese quail chicks, which could be linked to FUA's effect on enhanced amino acid and mineral availability (He et al., 2020). Furthermore, Ghazala et al. (2011) found that broiler chicks fed a diet supplemented with 0.5% formic, 0.5% fumaric, 0.75 % acetic, and 1.0 to 2.0 % citric acid had larger immune organs and higher levels of serum globulin, and that the improvement in immunity could be attributed to the organic acids' inhibitory effect on gut pathogens.

Emami et al. (2017) found that include FUA in broiler meals can increase humoral and cellular immunity in *E. coli* K88-infected chicks. Elnaggar and Abo El-Maaty (2017) discovered that eating 0.5 or 1.0% formic acid raised the serum concentration of IgM and IgG in ducklings.

In terms of antioxidant stats, Table 5 shows that there were significant ($P < 0.05$) variations between experimental groups in TAC, SOD, GPX, and MDA. TAC, SOD, and GPX levels were considerably ($P < 0.0$) greater in FUA supplemented groups than in control groups, but plasma MDA levels were significantly ($P = 0.0002$) lower in FUA supplemented groups than in control groups. Formic acid lowered serum hydrogen peroxide levels and enhanced TAC in hens challenged orally with pathogenic bacteria, according to Abudabos and Al-Mufarrej (2014).

Elnaggar and Abo El Maaty (2017) discovered that dietary formic acid caused a considerable rise in serum TAC, GSH, GPX, and SOD in ducks (0.5 and 1%). He et al. (2020) found that 10 g/kg dietary FUA supplementation boosted GPX activity and lowered total carbonyl in the bursa and thymus of broiler chicks, indicating that FUA improved the oxidative state of certain immune organs.

Minerals in Plasma

Supplementation of FUA in the diet of Japanese quails considerably influenced plasma Ca concentrations, as seen in Table 5. It was discovered that chicks fed a diet containing 15 g FUA/kg had a significantly higher plasma Ca content ($P = 0.0055$) than those fed an unsupplemented diet and other dietary treatment groups. Our findings support those of Kamal and Ragaa (2014), who found that acid acidification of % fumaric acid, resulted in a considerable increase in serum Ca content. In broiler chicks, Ghazala et al. (2011) discovered that dietary 0.5% fumaric acid dramatically elevated blood serum Ca and P. When turkey meals were supplemented with 1.5% FUA, Pinwu and Chen (2016) reported no significant variations in serum p and Ca levels.

Bacteriology

Table 6 shows the effects of dietary FUA on cecum bacterial counts (total bacterial count, *lactobacillus,*

Table 6. Bacteriology as affected by different levels of fumaric acid.

	Fumaric acid level (g/kg diet)						
Items	0	5	10	15	20	SEM	*P* value
Caecal bacterial count (Log CFU/g)							
Total bacterial count	8.51[b]	8.54[b]	8.45[b]	8.67[a]	8.55[b]	0.023	0.0169
Lactobacillus	7.41[d]	7.64[c]	7.94[b]	8.89[a]	7.45[d]	0.047	<0.0001
Coliform	5.12[a]	4.33[b]	3.90[c]	4.41[b]	4.28[b]	0.052	<0.0001
E. coli	4.29[a]	3.38[b]	3.46[b]	3.05[c]	3.27[bc]	0.071	<0.0001
Salmonella	4.52[a]	3.12[b]	3.26[b]	3.20[b]	3.13[b]	0.073	<0.0001

[abcd]Means within the same row with different common superscripts differ significantly.

salmonella, coliform, and *E. coli*). Japanese quail chicks fed diets containing FUA at various dosages had a considerably lower ($P = 0.0001$) cecal count of coliform, *E. coli,* and *Salmnella*. *Lactobacillus* bacteria count, on the other hand, was dramatically increased ($P = 0.0001$) in the cecum of chicks fed diets containing FUA at various doses as compared to control, with no significant difference between them. When compared to control and other dietary treatment groups, addition of 15 g FUA/kg feed significantly enhanced ($P = 0.0001$) cecal of total bacteria count in Japanese quail chicks. When compared to the control, other FUA levels had no effect on the overall bacteria count in the cecum.

Total coliform levels in the caecum and ileum of broilers treated with fumaric and ascorbic acids were significantly reduced, according to Pirgozliev et al. (2008). Our findings corroborate those of Attia et al. (2018), who found a substantial reduction in total bacteria and Enterobacteriaceae counts in fumaric and citric acid-treated groups. Elnaggar and Abo El Maaty (2017) discovered that supplementing duck meals with either 0.5 or 1% fumaric acid reduced overall bacteria count, *salmonella*, and *E. coli*.

CONCLUSIONS

It was concluded that including fumaric acid (particularly 15 g/kg diet) in the diets of growing Japanese quails enhanced their growth, immunological response, and overall health. In addition, quail chicks fed diets containing FUA at various dosages had a much lower ($P = 0.0001$) cecal count of coliform, *E. coli,* and *Salmonella* than the normal group.

DISCLOSURES

The authors have no conflicts of interest to report.

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