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# The effects of formic acid or herbal mixture on growth performance, carcass quality, blood chemistry, and gut microbial load in broiler chickens Formic Acid & Herbal Mixture in Broiler Diets

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ABSTRACT

The purpose of this research was to assess the effects of formic acid (**FORM**) and an herbal mixture (**HM**) as antibiotic alternatives in broiler meals on growth efficiency, carcass criteria, blood parameters, and intestinal bacterial populations. A total of 360 one-day-old Ross 308 broiler chicks were assigned to six trial treatments using a totally randomized method. Each group consisted of 6 replicates, with 10 unsexed chicks per replicate. The groups were arranged as follows: NC (negative control, basal diet); **PC** (positive control, basal diet + 0.5 g Polymyxin E antibiotic/kg diet); Form2, Form4, HM3, and HM6 (basal diet supplemented with 2 or 4 ml formic acid, or 3 or 6 g herbal mixture/kg diet, respectively). The findings showed no substantial variations in "body weight (**BW**) or body weight gain (**BWG**)" among the formic acid and herbal mixture groups relative to the NC and PC groups. The HM 3 and 6 g/kg groups exhibited higher "feed intake (**FI**) and improved feed conversion ratio (**FCR**)" relative to the other treatments. Carcass characteristics were not substantially impacted by formic acid or herbal mixture supplementation, except for spleen weight, which was highest in the NC group. Significant changes in blood parameters were observed, with notable variations in **ALT** and **AST** activity and "urea, creatinine, total protein, globulin, and albumin" levels in the formic acid and herbal mixture groups. The supplementation of formic acid and herbal mixture also increased antioxidant levels (**GPx, GST**, and **GSH**) compared to the **NC** group while reducing **MDA** levels relative to the NC and PC groups. Additionally, formic acid supplementation significantly reduced harmful bacterial populations in cecal samples. Conversely, Lactobacilli counts greater than those in the NC and PC groups, although no significant variances in *Clostridium* populations were observed. In conclusion, supplementing broiler feed with formic acid and herbal mixtures positively influences blood parameters, antioxidant status, and intestinal microbiota.

## Introduction

Due to a significant global surge in demand for meat, eggs, and dairy products from animals and birds fed on natural resources (Bist et al., 2024; Kamal et al., 2023a), nutritional supplements have become essential in the poultry industry, where feed charges constitute 60–70 % of the overall cost of production (Abd El-Hack et al., 2024a; Mohamed et al., 2024). Livestock and poultry commonly rely on organic feed additives, which often include synthetic growth promoters and antibacterial agents to boost growth performance and carcass yield (El-Abasy et al., 2025). As a result, researchers are increasingly investigating alternatives to antimicrobial growth promoters (AGPs) to improve birds'

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health, quality, and productivity (Yang et al., 2024). Consequently, herbs and phytogenic substances are being used as functional nutritional elements to bolster bird immunity and productivity (El-Hack et al., 2024b; El-Ratel et al., 2024).

Overcrowding in large poultry farms, coupled with the utilization of antimicrobial agents, has led to the creation of resistant microorganisms, which presents a significant public health threat. As a result, farmers are exploring dietary acids as a potential solution (Gržinić et al., 2023; Salam et al., 2023). Organic acids (OA), including fatty acids, are commonly incorporated into poultry meals as acidifiers, though not all are utilized as feed additives (Abd El-Ghany, 2024; Dai et al., 2021). The blending of OA into poultry feed reduces the pH in the gastrointestinal tract, enhancing nutrient utilization and thus enhancing growth efficiency, feed efficiency, and disease resistance (Waghmare et al., 2025).

The introduction of OA in bird diets not only improves growth performance and immunity but also reduces pathogenic colonization. Furthermore, they possess bacteriostatic properties, acting as effective anti-*Salmonella* agents (Naeem and Bourassa, 2024). Additionally, feed acidifiers contribute to enhanced feed efficacy, growth, and overall well-being in broilers by reducing gut microbiota, minimizing nutrient competition, and preventing mold growth, all while preserving the nutritional value of the feed (Dibner and Buttin, 2002; Pearlin et al., 2020).

The antibacterial properties of essential oils derived from spices and herbs are particularly noteworthy, as these oils may serve as nutritional supplements, potentially replacing antibiotics (Ivanova et al., 2024). A multitude of investigations have demonstrated the beneficial effects of herbal plants on broilers, highlighting their antibacterial properties, hypocholesterolemic impacts, growth-promoting properties, and availability (Vlaicu et al., 2021). Herbs possess a range of beneficial effects, including anti-coccidial, antioxidant, and antifungal properties, thanks to their secondary metabolites, such as phenols, oils, and saponins, which make them popular feed additives (Alem, 2024).

The novelty of this study lies in its investigation of formic acid and herbal mixtures as alternatives to traditional antibiotics and growth stimulants in broiler diets. While individual studies have examined the influences of organic acids and herbal additives on poultry health and efficiency, this research uniquely compares the influence of these two supplements, specifically addressing these additions' influence on growth efficiency, carcass criteria, blood parameters, and intestinal microbiota. Moreover, the study explores the potential of formic acid and the herbal mixture to replace antibiotics, a growing concern in the poultry industry because of the emergence of microbes resistant to antibiotics and the associated public health risks.

The hypothesis of this study is that supplementing broiler diets with formic acid and the herbal mixture will improve growth performance, enhance carcass quality, better blood parameters, and promote healthier intestinal microbiota, while serving as effective substitutes for antibiotics and growth stimulants. It is further hypothesized that these alternatives will reduce pathogenic bacterial populations, improve antioxidant levels, and support overall poultry well-being without the negative effects associated with conventional antibiotic use.

## Materials and methods

### *Study site and ethical approval*

The investigation was executed at the Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee and the Department of Poultry Ethics Committee at Zagazig University. The authorizations guaranteed that all study protocols adhered to ethical norms for the handling and utilization of animals in scientific investigations.

### *Herbal mixture (plant materials)*

The herbal mixture consisted of "*Thymus vulgaris* (TV), *Pimpinella anisum* (PA), *Capsicum annuum* (CA), *Mentha spicata* (MS), *Salvia rosmarinus* (SR), *Allium sativum* (AS), and *Nigella sativa* (NS)." These plant materials were obtained from local botanical suppliers in Zagazig City, Egypt.

### *Chemical characterization*

Folin and Ciocalteu's phenol reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ethanol, gallic acid, quercetin, aluminium chloride, and methanol were sourced from Merck (Merck KGaA, Darmstadt, Germany).

### *Specimen preparation*

Each of the plant materials (10 g each) was mixed with 200 mL of 70 % methanol and stirred for 3 h. The mixture was then filtered utilizing Whatman No. 2 filter paper. Methanol was evaporated under a vacuum using a BuCHI water bath (B-480 evaporator) at 45 °C, and the remaining extract was lyophilized using a freeze dryer (Thermoelectron Corporation-Heto Power Dry LL 300 Freeze Dryer). The resulting powder was kept at -20°C until further analysis (Abd El-Hack et al., 2018; Ashour et al., 2020).

### *Total phenolic compounds (TPCs) estimation*

The TPCs of the methanolic extracts (1000 mg/mL) were detected utilizing the Folin–Ciocalteu method. Gallic acid was utilized as a standard to create a calibration curve [$y = 0.001x + 0.0563$ ($R^2 = 0.9792$)], where y represents absorbance, and x denotes concentration (mg/mL). One milliliter of every specimen or normal gallic acid solution was combined with 3 mL of diluted Folin–Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate solution and incubated in the darkness at 25°C for 30 Min. Absorbance was determined at 760 nm utilizing a spectrophotometer (JENWAY 6405 UV/Vis, UK).

### *Total flavonoids (TFs) evaluation*

The TFs in the extracts (1000 mg/mL) were determined following the method described by Ordonez et al. (2006). Quercetin was utilized as a standard for calibration [$y = 0.0012x + 0.008$ ($R^2 = 0.944$)], where y represents absorbance and x is the concentration of quercetin (mg/mL). One milliliter of quercetin solution or extract was mixed with 1 mL of a 20 g/L $AlCl_3$ ethanol solution. The absorbance was quantified at 420 nm with a spectrophotometer.

### *Antioxidant activity estimation*

The antioxidant status of the methanolic extracts was assessed utilizing the DPPH assay (Ramadan and Asker, 2008). One mL of every extract was mixed with 3 mL of a methanolic DPPH solution. After a 30 min incubation, the absorbance was detected at 520 nm. The extract concentration that scavenged 50 % of the DPPH radicals (SC50) was calculated as delineated by Abdel-Hamid et al., 2020.

### *Design, birds, and diets*

A total of 360 Ross 308 broiler chicks with one-day-olds with similar initial body weights were randomly assigned to six groups in a completely randomized design. Every group comprised 6 replicates, with 10 unsexed chicks/replicates. The trial lasted for 32 days post-hatch. Basal meals were formulated to meet the nutritional requirements as per NRC (1994). The chicks were fed pelleted diets from day 1 to day 32, as detailed in Table 1. The trial diets were divided into

**Table 1**
Composition and calculated analysis of the basal diets.

| Items | Starter (1 – 21 days) | Finisher (22 – 32 days) |
|---|---|---|
| Ingredients (%) | | |
| Yellow corn | 54.03 | 58.70 |
| Soybean meal (44% crude protein) | 34.50 | 29.78 |
| Corn germ (60% crude protein) | 5.50 | 5.50 |
| Limestone | 1.08 | 0.95 |
| Dicalcium phosphate | 2.00 | 1.75 |
| Premix[1] | 0.30 | 0.30 |
| Salt | 0.30 | 0.30 |
| L-lysine | 0.29 | 0.24 |
| DL-methionine | 0.20 | 0.18 |
| Soybean oil | 1.80 | 2.30 |
| Calculated analysis[2] | | |
| CP, % | 23.12 | 21.10 |
| ME, Kcal/kg | 3001 | 3180 |
| Calcium, % | 0.99 | 0.89 |
| Available phosphorus, % | 0.51 | 0.46 |
| Potassium, % | 0.54 | 0.52 |

[1] Minerals and vitamins premix manufactured by Multi Vita Animal Nutrition® (Tenth of Ramadan City, Sharkia Governorate, Egypt) provides vitamin A 12000 IU, vitamin D3 2500 IU, vitamin E 20 mg, vitamin K3 2 mg, vitamin B1 2 mg, vitamin B2 5 mg, vitamin B6 2 mg, vitamin B12 0.05 ug, niacin 30 mg, biotin 0.05 ug, folic acid 1 mg, pantothenic acid 10 mg, manganese 60 mg, zinc 50 mg, iron 40 mg, copper 10 mg, iodine 0.6 mg, selenium 0.3 mg per 1 kg diet. DL-methionine (manufactured by Evonik Industries, Essen, Germany) contains 99 % methionine. Lysine = lysine hydrochloride (Evonik Industries) and contains 70 % Lysine. CP, crude protein; ME, metabolizable energy.

[2] Calculated according to NRC (1994).

two phases: starter (days 1–21) and finisher (days 22–32). Birds were raised under control of environmental, managerial, and health conditions. The groups were as follows: NC (basal diet); PC (basal diet + 0.5 g Polymyxin E antibiotic/kg feed); Form2, Form4, HM3, and HM6 (basal diet supplemented with 2 or 4 ml of formic acid and 3 or 6 g of herbal mixture/kg feed, respectively). Formic acid and a mixed herbal powder were incorporated into the feed ingredients at specified concentrations in the mixer prior to the pelleting process. The mixture was then pelleted at a temperature of 80°C for 30 seconds, with a moisture content of approximately 17% relative humidity. After pelleting, the feed was cooled, and the pellets were subsequently crushed to a diameter of less than 1 mm for the starter phase (7 to 21 days of age). Gradually, over the course of 3 days, the feed transitioned to the finisher feed, which was then provided to the birds until the end of the experiment at 32 days of age. The birds were housed in traditional cages (100 × 100 × 50 cm³) with *ad libitum* nourishment and clean water. The illumination regimen comprised 23 h of light and 1 h of darkness.

### *Growth efficiency and carcass traits*

Birds were assessed separately on a weekly basis to determine live body weight (**LBW**) and BWG. Average daily FI and FCR were calculated. At the end of the trial (32 days), 6 birds from every treatment were randomly allocated for carcass analysis. Birds were slaughtered, and the carcass weight, along with other edible components, was evaluated. The performance index (PI) was computed utilizing the subsequent formula: PI = the final BW kg divided by the FCR, multiplied by 100 (North, 1981).

### *Blood indicators*

Blood specimens were obtained from six birds for each treatment after an overnight fast at 32 days of age. The blood was drawn into non-heparinized tubes and centrifuged at 5000 rpm for 15 min at 4°C to acquire serum, which was kept at -20°C for additional examination. The serum was analyzed for "alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), total protein (**TP**), albumin, urea, and creatinine" using commercially available kits. Antioxidant parameters, including "malondialdehyde (**MDA**), glutathione peroxidase (**GPx**), glutathione-S-transferase (**GST**), and reduced glutathione (**GSH**)," were measured as aforementioned mentioned (Abd El-Hack et al., 2017).

### *Microbiological assessment*

Cecal specimens have been acquired from six birds per treatment at 32 days of age. The specimens were homogenized, and 10 g of the cecum was mixed with 90 mL of sterile peptone water and incubated for 30 min. Serial dilutions were made up to $10^{-7}$. Total bacterial count (TBC), Lactobacilli, coliforms, *E. coli*, and *Salmonella spp*. Microbes were plated to a miniature size using the methods described by Sieuwerts et al. (2008). The bacterial counts were expressed as log CFU/mL (Sheiha et al., 2020).

### *Statistical analysis*

Data were analyzed using one-way ANOVA with a completely randomized design through the GLM procedures in SPSS (v 27). Mean differences were evaluated utilizing Duncan's post-hoc test. Statistical significance was ascertained at $P \leq 0.05$ unless otherwise stated. Orthogonal contrasts were utilized to assess linear or quadratic impacts of dose levels on specific parameters. Graphs were generated using GraphPad Prism 8.0. The statistical approach that has been used is as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where $Y_{ij}$ represents the value that was noticed of the relevant treatment, μ denotes the recorded mean for the relevant treatment, $T_i$ signifies the treatment impact, and $e_{ij}$ indicates the error associated with the individual observation.

## Results

### *Growth efficiency*

Table 2 illustrates the effects of formic acid and herbal mixture on BW, BWG, FI, FCR, and performance index (**PI**) in broiler chicks. The ANOVA analysis indicated that the treatments with formic acid and herbal mixture had no substantial impact ($P > 0.05$) on BW and BWG at 32 days. The PC group exhibited the highest BW and BWG, followed by the herbal mixture at 6 g/kg. However, the birds in the groups that received the herbal mixture at 3 and 6 g/kg showed substantial improvements in FI and FCR at both the 22–32 day and 1-32-day periods, compared to the other groups. Additionally, the herbal mixture treatments (3 and 6 g/kg) exhibited a higher PI compared to the other treatments.

### *Carcass criteria*

Table 3 shows that most carcass characteristics were not substantially influenced ($P > 0.05$) by the addition of formic acid and herbal mixture, except for spleen weight. The spleen weight in the NC group was considerably higher relative to the other treatments.

### *Blood biochemical indicators*

Biochemical indicators of liver and kidney function, such as the values of urea, TP, creatinine, albumin, globulin, and the activities of ALT and AST, provide valuable insights into avian health and nutrition. Table 4 summarizes the effects of formic acid and herbal mixture supplementation on blood biochemical parameters in broilers. Birds fed diets containing herbal mixture had significantly higher ($P \leq 0.05$)

**Table 2**
Effect of dietary formic acid and herbal mixture on broiler growth performance.

| Traits | Age (days) | Treatments | | | | | | SIM | P value[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NC | PC | Form 2 | Form 4 | HM 3 | HM 6 | | T | L | Q |
| BW (g/ bird) | 1 | 45.31 | 45.68 | 45.80 | 45.60 | 45.49 | 45.61 | 0.21 | 0.723 | 0.945 | 0.715 |
| | 21 | 971.02 | 957.44 | 951.50 | 977.57 | 939.00 | 691.00 | 7.22 | 0.756 | 0.630 | 0.791 |
| | 32 | $1939.46^{ab}$ | $2025.33^{a}$ | $1886.72^{b}$ | $1988.94^{ab}$ | 1878.39 | $1994.22^{ab}$ | 17.64 | 0.042 | 0.834 | 0.417 |
| BWG (g/ bird/ day) | 1-21 | 44.08 | 43.43 | 43.13 | 44.42 | 42.55 | 43.59 | 0.34 | 0.735 | 0.629 | 0.798 |
| | 22-32 | $87.95^{bc}$ | $97.19^{a}$ | $85.01^{c}$ | $91.94^{abc}$ | $85.40^{c}$ | $93.93^{ab}$ | 1.34 | 0.017 | 0.931 | 0.393 |
| | 1-32 | $59.26^{abc}$ | $61.86^{a}$ | $57.52^{bc}$ | $60.76^{ab}$ | $57.27^{c}$ | $60.89^{ab}$ | 0.55 | 0.042 | 0.837 | 0.423 |
| FI (g/ day) | 1-21 | 57.78 | 60.86 | 59.05 | 59.85 | 57.83 | 57.80 | 0.42 | 0.149 | 0.308 | 0.072 |
| | 22-32 | $149.73^{a}$ | $148.17^{a}$ | $148.01^{a}$ | $150.44^{a}$ | $116.26^{b}$ | $116.81^{b}$ | 4.10 | 0.000 | 0.000 | 0.019 |
| | 1-32 | $103.75^{a}$ | $104.46^{a}$ | $103.53^{a}$ | $105.14^{a}$ | $87.05^{b}$ | $87.31^{b}$ | 2.13 | 0.000 | 0.000 | 0.010 |
| FCR (g feed/ g gain) | 1-21 | 1.32 | 1.42 | 1.37 | 1.35 | 1.36 | 1.33 | 0.01 | 0.544 | 0.870 | 0.189 |
| | 22-32 | $1.71^{ab}$ | $1.55^{bc}$ | $1.74^{a}$ | $1.63^{ab}$ | $1.36^{cd}$ | $1.24^{d}$ | 0.04 | 0.000 | 0.000 | 0.007 |
| | 1-32 | $1.7^{a}$ | $1.69^{a}$ | $1.80^{a}$ | $1.73^{a}$ | $1.52^{b}$ | $1.43^{b}$ | 0.03 | 0.001 | 0.000 | 0.005 |
| PI | 32 | $110.67^{bc}$ | $119.99^{b}$ | $105.09^{c}$ | $114.94^{bc}$ | $123.89^{b}$ | $139.48^{a}$ | 3.12 | 0.004 | 0.001 | 0.013 |

NC: negative control (basal diet); PC: positive control (basal diet + 0.5g Polymyxin E antibiotic/kg diet); Form, formic acid; HM, herbal mixture; PI, performance index. SEM, Standard error of means
[a,b,c,d] Means within a row by different superscripts are significantly different ($P \le 0.05$) or ($P \le 0.001$).
BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio
[1] T, overall effects of treatments; L, linear effects of increasing treatment levels of broiler; Q, quadratic effects of increasing treatment levels of broiler.

**Table 3**
Carcass traits as affected by formic acid and herbal mixture in broiler rations.

| Traits | Treatments | | | | | | SEM | P value[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NC | PC | Form 2 | Form 4 | HM 3 | HM 6 | | T | L | Q |
| LBW | 2191.95 | 2287.00 | 2283.00 | 2047.00 | 2092.00 | 2229.00 | 55.85 | 0.810 | 0.621 | 0.772 |
| Carcass weight | 1585.00 | 1654.00 | 1663.00 | 1514.00 | 1533.00 | 1631.00 | 40.60 | 0.890 | 0.763 | 0.855 |
| Breast meat | 972.75 | 1020.00 | 1057.00 | 966.00 | 967.00 | 1013.00 | 24.81 | 0.909 | 0.932 | 0.816 |
| Drum meat | 612.00 | 633.00 | 604.00 | 545.00 | 552.00 | 621.00 | 18.28 | 0.697 | 0.534 | 0. 404 |
| Liver | 49.05 | 48.00 | 57.00 | 48.50 | 49.00 | 49.50 | 1.90 | 0.556 | 0.394 | 0.454 |
| Heart | 11.10 | 10.10 | 8.50 | 9.00 | 10.00 | 10.00 | 0.37 | 0.536 | 0.582 | 0.111 |
| Spleen | $3.10^{a}$ | $2.00^{b}$ | $2.50^{ab}$ | $2.00^{b}$ | $2.00^{b}$ | $2.00^{b}$ | 0.12 | 0.088 | 0.028 | 0.238 |
| Gizzard | 26.08 | 24.50 | 27.00 | 24.500 | 24.00 | 25.00 | 0.57 | 0.671 | 0.560 | 0.943 |

NC: negative control (basal diet); PC: positive control (basal diet + 0.5g Polymyxin E antibiotic/kg diet); Form, formic acid; HM, herbal mixture; LBW, live body weight; SEM, Standard error of means;
[a,b] Means within a row followed by different superscripts are significantly different ($P \le 0.05$).
[1] T, overall effects of treatments; L, linear effects of increasing treatment levels of broiler; Q, quadratic effects of increasing treatment levels of broiler.

**Table 4**
Effect of diet supplementation by formic acid and herbal mixture on serum parameters of broiler.

| Traits | Treatments | | | | | | SEM | P value[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NC | PC | Form 2 | Form 4 | HM 3 | HM 6 | | T | L | Q |
| ALT (IU/L) | $53.67^{a}$ | $51.56^{a}$ | $27.00^{b}$ | $19.66^{c}$ | $10.28^{d}$ | $9.80^{d}$ | 4.36 | 0.000 | 0.000 | 0.000 |
| AST (IU/L) | $106.33^{b}$ | $118.06^{a}$ | $44.66^{d}$ | $55.33^{c}$ | $119.50^{a}$ | $115.50^{a}$ | 7.57 | 0.000 | 0.005 | 0.000 |
| Urea (mg/dL) | $12.13^{a}$ | $12.00^{a}$ | $8.00^{b}$ | $5.66^{c}$ | $9.32^{b}$ | $9.06^{b}$ | 0.58 | 0.000 | 0.000 | 0.000 |
| Creatinine (mg/dL) | $0.52^{a}$ | $0.47^{b}$ | $0.35^{d}$ | $0.41^{c}$ | $0.55^{a}$ | $0.42^{c}$ | 0.01 | 0.000 | 0.016 | 0.000 |
| TP (mg/dL) | $4.30^{b}$ | $4.11^{c}$ | $2.63^{e}$ | $3.26^{d}$ | $6.38^{a}$ | $6.25^{a}$ | 0.34 | 0.000 | 0.000 | 0.000 |
| Albumin (mg/dL) | $2.40^{b}$ | $2.30^{b}$ | $1.30^{d}$ | $1.60^{c}$ | $3.32^{a}$ | $3.35^{a}$ | 0.18 | 0.000 | 0.000 | 0.000 |
| Globulin (mg/dL) | $1.93^{b}$ | $1.82^{b}$ | $1.33^{c}$ | $1.66^{b}$ | $3.05^{a}$ | $2.90^{a}$ | 0.15 | 0.000 | 0.000 | 0.000 |
| A/G ratio | $1.26^{ab}$ | $1.39^{a}$ | $0.98^{b}$ | $0.98^{b}$ | $1.08^{ab}$ | $1.15^{ab}$ | 0.04 | 0.115 | 0.144 | 0.043 |

NC: negative control (basal diet); PC: positive control (basal diet + 0.5g Polymyxin E antibiotic/kg diet); Form, formic acid; HM, herbal mixture; AST: aspartate transaminase; ALT: alanine transaminase; TP, total protein; SEM, Standard error of means
[a,b,c,d,e] Means within a row followed by different superscripts are significantly different ($P \le 0.05$) or ($P \le 0.001$).
[1] T, overall effects of treatments; L, linear effects of increasing treatment levels of broiler; Q, quadratic effects of increasing treatment levels of broiler.

levels of TP, albumin, and globulin compared to the other groups. Moreover, birds receiving formic acid supplementation displayed significantly lower ($P \le 0.05$) levels of AST, ALT, creatinine, and uric acid compared to other groups, indicating that the supplements influenced liver and kidney function, protein metabolism, and overall health.

### *Antioxidant indices*

The influence of formic acid and herbal mixture addition on the antioxidant levels of chickens was evaluated by measuring enzyme activities of GPx, GST, and GSH, as well as MDA concentrations, a marker of oxidative stress. Table 5 shows that all antioxidant criteria were considerably impacted ($P \le 0.05$) by the formic acid and herbal mixture treatments relative to the NC and PC groups. The data show that formic acid and herbal mixture supplementation alleviated GPx, GST, GSH, and MDA levels ($P \le 0.05$). The highest concentrations of GPx, GST, and GSH were observed in the birds that received FORM 2 $cm^3$ and HM 6 g, while the lowest values for MDA were recorded in the same groups.

**Table 5**
Effect of diet supplementation with formic acid and herbal mixture on blood antioxidant indicators of broiler.

| Traits | Treatments | | | | | | SEM | *P value*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NC | PC | Form 2 | Form 4 | HM 3 | HM 6 | | T | L | Q |
| GPx (nmol/L) | 152.51[b] | 169.46[a] | 160.00[ab] | 157.00[ab] | 156.19[ab] | 160.52[ab] | 2.00 | 0.226 | 0.944 | 0.504 |
| GST (nmol/L) | 4.68[bc] | 5.57[ab] | 6.39[a] | 4.09[c] | 6.59[a] | 6.36[a] | 0.26 | 0.003 | 0.014 | 0.859 |
| GSH (nmol/L) | 62.01[b] | 73.52[a] | 74.00[a] | 62.50[b] | 71.45[a] | 71.96[a] | 1.39 | 0.002 | 0.077 | 0.266 |
| MDA (nmol/L) | 6.15[a] | 4.61[b] | 3.80[b] | 4.50[b] | 3.76[b] | 3.79[b] | 0.23 | 0.008 | 0.001 | 0.049 |

NC: negative control (basal diet); PC: positive control (basal diet + 0.5g Polymyxin E antibiotic/kg diet); Form, formic acid; HM, herbal mixture; GPx: glutathione peroxidase; GST: glutathione-S-transferase; GSH: reduced glutathione; MDA: malondialdehyde; SEM, Standard error of means
[a,b,c] Means within a row followed by different superscripts are significantly different ($P \leq 0.05$).
[1] T, overall effects of treatments; L, linear effects of increasing treatment levels of broiler; Q, quadratic effects of increasing treatment levels of broiler.

*Enumeration of microorganisms in cecal samples*

Fig. 1 illustrates the microbial populations in cecal samples, including TBC, lactobacilli, coliforms, *E. coli, Salmonella*, and *Clostridium*. The results show that formic acid treatments significantly reduced all microbial communities in the ceca of the broilers. However, lactobacilli counts were higher in the formic acid treatments relative to the NC and PC groups. No substantial variations were found in Clostridium counts across all treatments.

## Discussion

The poultry industry is increasingly exploring alternatives to AGPs, such as organic acids and herbal mixtures, to enhance the health and productivity of broilers. This trend is driven by regulatory changes and growing concerns over antimicrobial resistance (Ashour et al., 2025a, b). The results of our study provide insights into how formic acid and herbal mixture, as feed additives, affect various performance and health criteria in broiler chickens.

In terms of growth efficiency, our study showed no substantial effects on BW and BWG across all trial groups, with the PC group exhibiting the highest values. However, the groups added with herbal mixtures at 3 and 6 g/kg demonstrated improved FI and FCR compared to other treatments. This finding matches the outcomes of Ali et al. (2024), who indicated that there was no significant impact of formic acid supplementation on growth performance. While the absence of a significant effect on BW and BWG may seem inconclusive, it is important to note that growth efficacy in chickens can be influenced by several factors, such as dietary acid concentrations, the form of acid (single vs. mixed), the age of the birds, and ecological circumstances (Adewole et al., 2021). Organic acids, including formic acid, are known to reduce gut pH, improving nutrient absorption and microbial control in the gut (Huang et al., 2024), which may subsequently result in improved feed efficiency, as observed in the improved FI and FCR in our study.

Abo-Samaha et al. (2022) demonstrated that the use of an herbal mixture in poultry feeds improved productivity. Jagadeeswaran and Selvasubramanian (2014) noted that broiler chicks receiving a basal diet augmented with an herbal mixture exhibited improved body weight at 42 days. Toson et al. (2023) demonstrated that incorporating an herbal mixture into broiler chicks' feeds improved growth efficiency and physiological condition. Rashnou et al. (2023) discovered that the incorporation of purslane and an herbal mixture into quail diets did not affect FI or FCR. Moreover, the advantageous impacts of herbal mixtures may be ascribed to the bioactive compounds they contain, like thymol and carvacrol, which possess antimicrobial, antioxidant, and immune-modulating properties (Ragaa et al., 2016). These compounds may enhance nutrient digestion and absorption, thus improving feed efficiency and FCR without necessarily increasing BW or BWG.

While most carcass traits were unaffected by formic acid and herbal mixtures, there were substantial discrepancies in spleen weight, with the NC group showing the highest values. This finding suggests that dietary treatments may influence immune function, as the spleen plays a crucial function in immunological responses in avians (Beug et al., 1996). The noted reduction in spleen size in the treatment groups may reflect improved overall health and immune function, potentially due to the antimicrobial properties of formic acid and herbal mixtures. Consequently, Rashid et al. (2020) found that phytogenic nutrients and organic acids improved carcass yield and meat texture in broilers, suggesting that these additives may also promote better immune responses and healthier growth in broilers.

Biochemical indicators of liver and kidney function, along with blood chemistry, offer valuable insight into the overall health and physiological state of broilers (Adeyeye et al., 2020; Kamal et al., 2023b). In our research, the inclusion of formic acid and herbal mixtures resulted in significant differences in blood serum concentrations, along with the activities of liver enzymes (ALT and AST) and kidney function indicators (urea and creatinine). Elevated values of AST and ALT are commonly related to liver damage or dysfunction (Gu et al., 2021), while reduced levels are often indicative of improved liver health (Sang et al., 2023). The decline in AST and ALT levels noted in our study suggests that the formic acid and herbal mixture treatments may enhance liver function, likely due to their antioxidant and anti-inflammatory effects. These outcomes correspond to the findings of Ali et al. (2024), who observed improvements in immune indicators following formic acid supplementation. Moreover, our results demonstrate a reduction in creatinine levels, indicating enhanced kidney function. This is particularly relevant as creatinine is a metabolic byproduct of muscle metabolism, and lower levels of creatinine are often associated with improved renal health (Oke et al., 2017).

The augmentation in TP, globulin, and albumin levels in broilers fed formic acid and herbal mixtures suggests enhanced nutritional status and immune response. Herbal mixtures have been shown to have antioxidant and immune-modulating effects, which may contribute to improved serum protein levels (Ibrahim et al., 2024; Vlaicu et al., 2021). The enhancement of immune function could be one of the underlying mechanisms by which these additives improve growth performance and health in broilers.

In terms of oxidative stress, our study demonstrated that formic acid and herbal mixture supplementation significantly increased the efficiency of antioxidant enzymes involving GPx, GST, and GSH while reducing MDA levels. MDA is a byproduct of lipid peroxidation and serves as a marker of oxidative damage (Halliwell and Gutteridge, 2015). The observed reduction in MDA suggests that formic acid and herbal mixtures help mitigate oxidative stress, which is known to impair growth, immunological function, and general health in poultry (Hernández et al., 2012). The increased antioxidant enzyme activity may be ascribed to the bioactive substances in herbal mixtures, such as carvacrol and thymol, that have demonstrated the ability to boost the antioxidant status in poultry (Hashemipour et al., 2013). Additionally, formic acid has been reported to improve oxidative stress markers by modulating gut microbiota and enhancing nutrient absorption (Ali et al., 2024). Therefore, the addition of formic acid and herbal mixtures may provide a synergistic effect in improving oxidative balance in broiler chickens.

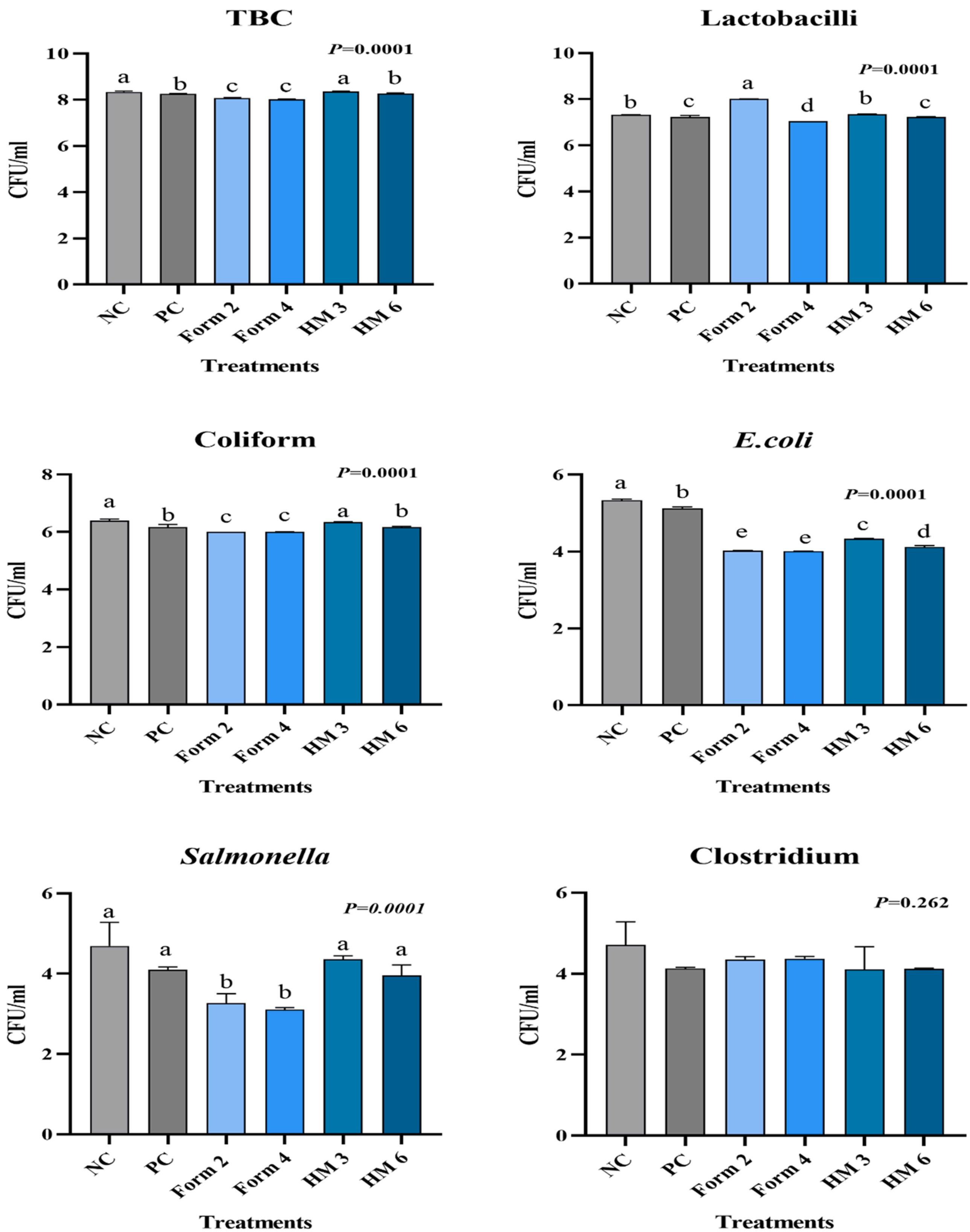


**Fig. 1.** Effect of formic acid and herbal mixture on cecal microbiota (TBC, lactobacilli, *E. coli*, coliform, *Salmonella*, and clostridium) represented by (Log CFU/ml) in broiler during feeding.
NC, negative control; PC, positive control; TBC, total bacterial count; *E. coli, Escherichia coli*.

Microbiological analysis of cecal samples revealed that formic acid significantly reduced bacterial populations, including *E. coli* and coliforms, while fostering the proliferation of beneficial lactobacilli. This finding supports the well-established role of organic acids, particularly formic acid, in modulating gut microbiota by decreasing the pH of the digestive system, which inhibits the growth of pathogenic bacteria while fostering beneficial microbes (Attia, 2018). The reduction in pathogenic microbes, like *E. coli*, and the promotion of lactobacilli are crucial for maintaining gut health, improving nutrient absorption, and enhancing overall performance (Ricke et al., 2020). Our results accord with that of Saki et al. (2011), who showed that organic acids effectively reduced *Salmonella spp*. and *Enterobacteriaceae* in chicks. Furthermore, Elnaggar and El-Kelawy (2024) highlighted the ability of organic acids to improve antioxidant conditions in poultry by modulating gut microbiota, suggesting that this mechanism could explain the improvements in both antioxidant levels and gut health observed in our study.

Taken together, the outcomes of this study provide strong evidence for the beneficial effects of formic acid and herbal mixtures as dietary supplements in broiler chicken diets. These additives not only enhance feed efficiency and antioxidant status but also improve blood biochemical indicators, support better immune function, and modulate gut microbiota. The beneficial impacts on growth efficiency and overall health observed in our study underscore the possibility of formic acid and herbal mixtures as viable substitutes for AGPs in poultry production systems. Additional investigation is required to examine the long-term impacts of these additives and their possible synergistic associations with other dietary elements.

## Conclusion

In conclusion, the supplementation of formic acid and herbal mixtures in broiler diets demonstrated positive effects on several health and performance parameters, though no significant impact was noticed on BW and BWG. Notably, the herbal mixture groups found improved FI and FCR, indicating enhanced feed efficiency. While carcass characteristics were generally unaffected, the treatments led to increased blood protein levels, improved liver enzyme activities, and elevated antioxidant enzyme levels, alongside a reduction in oxidative stress markers. Additionally, the supplementation contributed to healthier gut microbiota by decreasing harmful bacterial populations and promoting beneficial lactobacilli. The results suggest that including 4.0 ml of formic acid/kg and 6 g of herbal mixture/kg in broiler diets can enhance overall health, blood parameters, and gut microbiota, thereby promoting the well-being and productivity of broilers. These findings highlight the potential of formic acid and herbal mixtures as effective, sustainable replacements for traditional feed additives in poultry nutrition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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