Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
31 (6): 791-800, 2025
DOI: 10.9775/kvfd.2025.35119

Research Article

A Study on the Effect of Rutin in the Antibiotics Enhancement Controlling *E. coli* Infection in Broiler Chicken

Randa M. KHALLAF [1], Sahar S. ABD EL-HAMIED [2], Azza Z. REFAIE [3], Omnia A. ELEWASY [3], Safaa M. ELMESALAMY [4]

[1] Biochemistry, Toxicology and Nutritional Deficiency Diseases Dept., Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Dokki, Giza, EGYPT
[2] Pathology and Clinical Pathology Deptartment, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, EGYPT
[3] Bacteriology Deptartment, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, EGYPT
[4] Biochemistry, Toxicology and Nutritional Deficiency Diseases Deptartment, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, EGYPT

(*) **Corresponding author:**
Safaa M. Elmesalamy
Phone: +20 1062268081
E-mail: drsafaamelmesalamy@gmail.com

How to cite this article?

Khallaf RM, Abd El-Hamied SS, Refaie AZ, Elewasy OA, Elmesalamy SM: A study on the effect of rutin in the antibiotics enhancement controlling *E. coli* infection in broiler chicken. *Kafkas Univ Vet Fak Derg,* 31 (6): 791-800, 2025. DOI: 10.9775/kvfd.2025.35119

Article ID: KVFD-2025-35119
Received: : 14.10.2025
Accepted: 19.12.2025
Published Online: 20.12.2025

Abstract

This study aimed to identify synergistic antibacterial combinations containing rutin in both *in vivo* and *in vitro* settings. *Escherichia coli*, a major cause of poultry mortality, was isolated from broiler chickens and tested for antibiotic resistance using various methods, and the results showed that *E. coli* was resistant to all antibiotics, with only colistin showing a zone of inhibition. In the in vivo study, 50 one-day-old broilers were divided into five groups: control (non-infected, untreated), *E. coli* challenged (infected at 2 weeks old, untreated), rutin-treated (fed rutin at 1000 mg/kg diet without infection), *E. coli* + colistin (infected at 2 weeks old and treated with colistin at 1 g/5 L drinking water for 5 days), and rutin + colistin (fed rutin from day 1, infected at 2 weeks old, then treated with colistin). E. coli infection increased liver enzyme activity [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], globulin levels, oxidative stress markers [malondialdehyde (MDA)], and tumor necrosis factor-alpha (TNF-α), while lowering total protein, albumin, and antioxidant enzyme activity. Treatment with both rutin and colistin resulted in decreased serum ALT, AST, MDA, and TNF-α, while increasing total protein, albumin, and antioxidant enzymes. In conclusion, rutin administration provided excellent protection against the adverse consequences of E. coli infection through its antibacterial, hepatoprotective, antioxidant, and anti-inflammatory properties, and the combination with colistin showed the greatest improvement, highlighting a synergistic effect.

Keywords: Antioxidant, Drug-resistant, *E. coli*, Poultry, Resistant gene, Rutin

Introduction

Avian pathogenic *Escherichia coli* are a tiny, rod-shaped bacterium that is 0.5 mm in diameter and 1-2 mm long. This gram-negative bacterium was found within the fecal flora and can lead to both systemic and local illnesses, causing significant threat to poultry businesses globally which may lead to substantial economic losses [1]. Though *E. coli* can cause extremely serious illnesses it also has a significant role in the autochthonous microbiota of humans and animals. Due to its impact on animal and human health, it is vital to require a full conclusion effective management of the disease and its fundamental needs, providing information about the epidemiological and microbiological circumstances associated with it. The possibility of pathogenic and/or resistant *E.coli* moving from animals to people through the food chain, direct contact, or contact with animal waste is quite concerning. Additionally, *E. coli* is a substantial source of resistance genes that could be the cause of human and animal treatment failures [2]. Colistin is a narrow-spectrum bactericidal antibiotic that is efficient against Gram-negative bacteria which has been applied intensively in the veterinary field primarily to avoid or control gastrointestinal infections in animals that provide food [3]. Antibiotic-resistant genes have emerged as a result of the widespread use of antibiotics, even though they have been effective in treating infectious diseases. Because of its potential to develop virulence traits, *E. coli* is becoming increasingly widespread worldwide, which worries both

people and veterinarians. These characteristics enable it to evade host defenses and withstand antibiotics [4].

A growing number of resistance genes, some acquired by horizontal gene transmission, have been discovered in *E. coli* strains in recent years. Antimicrobial resistance in *E. coli* should be taken very seriously as a public health concern since it is regarded as one of the main problems impacting both humans and animals globally [5]. As a result, the development of novel and unusual drugs to replace antibiotics has been forced by the reduced effectiveness of antibiotics brought on by resistance [6].

Due to the growing demand for food safety, natural-origin compounds like phytochemicals and plant polyphenols found in plant extracts, such as flavonoids, which have growth-promoting potential, antioxidants, and immunomodulation qualities became the new trend for usage as growth advocates and meat value enhancers and antibiotic substitutes because they are generally controlled and do not leave any harmful residue in animal products [7].

Rutin is valuable in biomedical applications because it is regarded as a safe chemical which has a number of therapeutic uses, including immune-stimulating, anti-inflammatory, anti-oxidant and anti-cancer properties. Furthermore, rutin has a significant level of reactive oxygen species scavenging ability [8]. Antibiotics are more effective against tested bacteria when combined with rutin [9].

The purpose of this study was to evaluate the protective and therapeutic potential of rutin, a natural flavonoid, against *(E. coli)* infection in broiler chickens, and to determine whether its combination with colistin produces a synergistic antibacterial effect. Specifically, the study focused on assessing both *in vitro* antibacterial activity and *in vivo* outcomes, including liver enzyme activity [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], serum protein profiles, oxidative stress markers [malondialdehyde (MDA)], and inflammatory cytokine tumor necrosis factor-alpha (TNF-α). By comparing five experimental groups (control, *E. coli* challenged, rutin alone, *E. coli* + colistin, and rutin + colistin), the study aimed to clarify the extent to which rutin supplementation can mitigate the physiological and biochemical consequences of *E. coli* infection and enhance the efficacy of colistin treatment.

Material and Methods

Ethical Approval

This protocol was approved by the Animal Health Research Institute (AHRI) authorized the experimental procedure in accordance with the Agriculture Research Center (ARC) and IACUC committee in Egypt (ARC, AHRI, IACUC, 150/24).

E. coli Isolation, Culture, and Purification

Specimens from the liver, intestine, kidney, and lung were cultured on MacConkey agar, then E.M.B agar (eosin methylene blue) in an aseptic environment. The plates were tested for the distinctive *E. coli* colonies after 24 h/37°C [10].

Serological Identification of *E. Coli*

According to Kok et al[.11] the putative isolate was identified serologically using Denka Seiken Co. (Japan) as quick diagnostic *E. coli* antisera sets for the identification of the Enteropathogenic kinds, and the diagnostic sera were used to achieve slide agglutination assays.

Susceptibility Test of *E. Coli* to Antibiotics

The disc diffusion method was used to analyze the *E. Coli* strain's sensitivity pattern to twenty-two antibiotics (Oxoid, UK) in accordance with the Clinical and Laboratory Standards Institute's [12], as Doxycycline (Do, 30 µg), Erythromycin (E, 15 µg), Ciprofloxacin (CIP ,5 µg), Ampicillin (AMP, 10 µg), Streptomycin (S, 100 µg), Chloramphenicol (C, 30 µg), Amoixcillin (AX, 10 µg), Neomycin (N, 30 µg), Gentamycin (Cn, 10 µg), Colistin (CT, 10 µg), Kanamycin (K, 30 µg), Nalidixic acid (NA, 30 µg), Ampicillin/sulbactam (SAM, 20 µg), Amoxicillin/clavulanic acid (Amc, 30 µg), Sulfamethaxazole/trimethoprim (SXT, 25 µg), Levofloxacin (LEV, 5 µg), Cefotaxim (CTX, 30 µg), Tetracyclin (TE, 30 µg), Oxacillin (OX, 30 µg), Rifampin (RF, 30 µg), Fusidic acid (FA, 10 µg), Spectinomycin (SPT, 100 µg). The plates were inoculated with an *E. Coli* suspension that was adjusted to the 0.5 McFarland standard (1.5×10^8 CFU/mL), and they were cultured for 18 to 24 h at 37°C. The inhibitory zone was then measured.

The *in vitro* Antibacterial Activity of Rutin

Using the agar well diffusion technique, a well is cut using a sterilized borer (6 mm in diameter), 50 µL of rutin is dissolved in DMSO (25 g/mL), and the bacterial suspension equal to 0.5 McFarland standard ($1.5x10^8$ CFU/mL) is distributed on Mueller Hinton agar plate. The well is then incubated for 18 to 24 h at 37°C [13].

The minimal inhibitory concentration (MIC) is calculated as follows: Two-fold dilutions of rutin with concentrations of (512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL) were dispensed in tubes with a minimum capacity of 2 mL to perform a broth macro-dilution test. A microbial inoculum suspension that has been adjusted to 0.5 McFarland scale ($1.5x10^8$ CFU/mL) is then added to each tube. The infected tubes are thoroughly mixed and then incubated for 18 to 24 h at 37°C [12].

PCR Assay

Molecular identification of E. coli: Genomic DNA was extracted to serve as a template [14]. The housekeeping

gene for *E. coli* alkaline phosphatase, phoA, exists in all *E. coli* utilized to identify particularity in polymerase chain reaction (PCR) to identify maintained strains *(Table 1)*. The QIA quick PCR product extraction kit was used (Qiagen, Valencia). The sequence reaction was obtained using the PerkinElmer Bigdye Terminator V3.1 cycle sequencing kit, and purification was carried out using a Centrisep spin section. DNA sequences were obtained by A BLAST® investigation (Basic search technique for local alignment) [15] was initially conducted to generate a character sequence to accessions in GenBank using the genetic analyzer Biosystems3130 (HITACHI, Japan). The phylogenetic tree was constructed using the lasergenednastar12.1 MegAlign module [16] and analyses of Phylogenetic were prepared utilizing highest probability, maximum parsimony and neighbor-joining in MEGA6 [17].

Molecular Detection of Resistance and Virulence Genes: Real-Time PCR was used to detect genes encoding resistance to gentamicin *(aac*(3)-Ia), sulphonamides *(sul*1), trimethoprim *(dfr*A), tetracycline *(tet*A, *tet*B), colistin *(mcr*1), erythromycin *(ere*A), and aminoglycosides *(aadA*1). In addition, virulence genes including Shiga toxin *(stx*1, *stx*2) and intimin *(eae*A) were identified. Primers were provided by Metabion (Germany). DNA was extracted using the QIAamp DNA Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions with minor adaptations *(Table 1)*.

Table 1. *Target genes, primer sequences and amplicon sizes*

Target Gene	Primers Sequences	Product Size (Bp)
phoA	F: CGATTCTGGAAATGGC AAAAG R: CGTGATCAGCGGTGACTATGAC	720
TetA(A)	F: GGTTCACTCGAACGAC GTCA R: CTGTCCGACAAGTTGCATGA	570
TetB	F: CCTTATCATGCCAGTCT TGC R: ACTGCCGTTTTTTCGCC	773
EreA	F: GCCGGTGCTCATGAAC TTGAG R: CGACTCTATTCGATCAGAGGC	420
Aada1	F: TATCAGAGGTAGTTGG CGTCAT R: GTTCCATAGCGTTAAGGTTTCATT	484
Stx1	F: ACACTGGATGATCTCA GTGG R: CTGAATCCCCCTCCATTATG	614
Stx2	F: CCATGACAACGGACAG CAGTT R: CCTGTCAACTGAGCAGCACTTTG	779
EaeA	F: ATGCTTAGTGCTGGTTTAGG R: GCCTTCATCATTTCGCTTTC	248
Sul1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433
DfrA	F: TGGTAGCTATATCGAAGAATG R: GAGT TATGTTAGAGGCGAAGTCTTGGGTA	425
Mcr1	F: CGGTCAGTCCGTTTGTTC R: CTTGGTCGGTCTGTAGGG	308
aac(3)-Ia	F: TTGATCTTTTCGGTCGTGAGT R: TAAGCCGCGAGAGCGCCAACA	150

In vivo Antibacterial Activity

E coli Strain: The experimentally employed strain of *E. coli,* PP291565, was obtained from diseased birds. Each 2-week-old chicken in the infected groups received an injection of 1 mL of saline containing 10^8 *E. coli* colony forming units (CFU/mL) [18].

Rutin: Rutin is manufactured by Changsha huir Biological-tech Co., Itd China. Product name: Rutin NFI I, appearance: light yellow needle crystal powder. Part used: Whole flower bud. Extract Solvent: Ethanol & Water. Batch number: 23061514. Rutin was given to the baseline diet of chicken at a level of 1000 mg/kg from day one until the completion of the experiment [19].

Colistin: Pharma Swede, Egypt, is the manufacturer of Colistin Sulphate®. The powder dissolves in water. 5.000.000 IU of colistin sulfate are present in one gram. Administration and Dosage: Drinking water is used to administer the product orally. Poultry dosage: 1 g/5 L of drinking water for three to five days.

Experimental Design

Fifty Cobb broiler chicks (one-day old) were purchased from a commercial poultry company. Chicks were sectioned and housed in separate floor pens, each unit containing 10 chicks, under standard hygienic conditions. All chicks were vaccinated against Newcastle disease at 8 days of age and Gumboro disease at 13 days of age.

Animal Housing Conditions

Housing units: Each group of 10 chicks was housed in a separate floor pen (dimensions: 1.0 x 1.0 m).

Flooring: Pens were bedded with clean, dry wood shavings at a depth of ~5 cm, replaced weekly.

Ventilation: Natural ventilation supplemented with fans to maintain air circulation.

Temperature: Brooding temperature maintained at 32-34°C during the first week, gradually reduced by 2-3°C per week until reaching 24°C.

Humidity: Relative humidity maintained between 55-65%.

Lighting: Continuous light (23 h light:1 h dark) during the first week, then adjusted to 20 h light:4 h dark.

Stocking density: 10 chicks/m^2, in line with commercial recommendations.

Biosecurity: Pens disinfected before chick placement; footbaths and restricted access maintained throughout the experiment.

Water supply: Fresh, clean drinking water provided *ad libitum* via nipple drinkers.

Feed supply: Corn-soybean balanced ration provided *ad libitum* in trough feeders.

Group Classification

Group 1 (Negative control): Basal diet only, no challenge.

Group 2 (Positive control): Basal diet, challenged with *E. coli* at 2 weeks old.

Group 3 (Rutin group): Basal diet supplemented with rutin (1000 mg/kg feed) from day one, challenged with *E. coli.*

Group 4 (Colistin group): Basal diet, challenged with *E. coli* at 2 weeks old, then treated with colistin (1 g/5 L drinking water) for 5 days.

Group 5 (Rutin + Colistin group): Basal diet supplemented with rutin from day one, challenged with *E. coli* at 2 weeks old, then treated with colistin.

At the end of the experiment, *E. coli* was re-isolated and bacteriologically examined in all groups by obtaining samples from the colon, lung, liver, and spleen of slaughtered chickens.

Sampling

On day 21 of the experiment, blood samples were obtained from each chicken's wing vein. 3 mL of blood was drawn without an anticoagulant. The serum was isolated and stored at -20°C for biochemical analysis. Chicks were slaughtered by neck dislocation, and liver tissue samples were taken to test antioxidant levels.

Biochemical Analysis

Serum total protein determination was carried out using Spectrum &CO kit (Diuret kit) (CAT. No. 310 001). While albumin was determined using Spectrum & CO kit (CAT. No. 211 001). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were measured using spectrum kit CAT. NO. 260 001 and 265 001, respectively. While globulin was computed following [20], Malonaldehyde (MDA), reduced glutathione (GSH), and catalase (CAT) levels in liver tissue were determined with the Bio-diagnostic kit CAT.NO MD 25 29, GR 25 11, and CA 25 17, respectively. Serum tumor necrotic factor-α (TNF-α) was tested using commercially available ELISA kits, following manufacturer's instructions (R and D Systems; Minnesota; Minneapolis, USA).

Statistical Analysis

The One-Way Analysis of Variance (ANOVA) test was performed to statistically analyze the data. The Duncan test was performed after presenting the data as mean ± standard error (SE) with SPSS 14.0 (2006). The statistical significance level was $P<0.05$.

Results

E. Coli Identification

The microscopic examination exhibited gram-negative, non-sporulated, straight rods. On MacConkey *E. coli*, ferments lactose, and appears red/pink colony. While on EMB agar, colonies produced distinctive pink colonies with a metallic sheen. The serotyping of isolated *E. coli*'s discovered good results for O91:H21 latex agglutination was considered likely *E. coli* EHEC.

Antibiotic Susceptibility Test

Twenty-two antibiotic discs were applied to the *E. coli* strain, and the results showed that the *E. coli* isolate was resistant to all of the tested antibiotics, with only a 13 mm inhibition zone for colistin.

The *In vitro* Antimicrobial Activity of Rutin

By Agar Well Diffusion, Rutin showed antibacterial activity against *E. coli* PP291565 with a growth inhibition zone valued 15 mm *(Fig. 1)*. Rutin had a minimum inhibitory concentration of 128 µg/mL against the *E. coli* strain.

E. Coli Molecular Identification and Virulence and Resistance Gene Detection

The appearance of characteristic bands at 720 bp demonstrated that the 16S rRNA genes of the *E. coli* DNA samples were amplified successfully *(Fig. 2)*. The sequences, which were submitted to the Gene Bank with accession number PP291565, showed similarities to *E. coli* when compared to published sequences using the Local Alignment Search Tool. The cluster analysis and

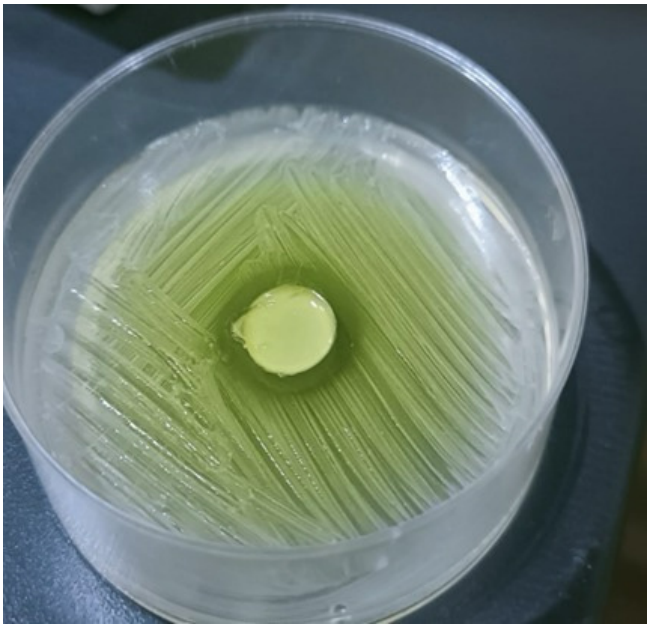

Fig 1. Antibacterial efficacy of Rutin against *E. coli* strain by agar well diffusion assay

dendrograms produced by the Local Alignment Search Tool application *(Fig. 3)*.

The existence of resistance and virulence genes is summarized in *Fig. 4, Fig. 5*, and *Fig. 6. Tet*(A) 570 bp, *Tet* (B) 773 bp, *ere*A (420 bp), *Aada*1 (484 bp), *dfr*A (425 bp) and *Mcr*1 (308 bp), *Stx*1 (614 bp), *eae*A (248 bp) and *Sul*1 (433 bp). while *aac* (3)-Ia and *stx*2 genes not detected. The isolate was classified as an enterohaemorrhagic *E. coli* (EHEC) because it possessed both the *stx* and *eae* genes.

Results of Experimental Infection

Re-isolation of *E. coli* at the end of the experimental period was negative in both the non- infected and non-treated group (control negative) (G1) and the infected group treated with Rutin and colistin together (G5). While in the infected and non-treated group (control positive) (G2), *E. coli* was isolated from all sacrificed birds. Also, it was isolated from 40% of the birds in the infected group

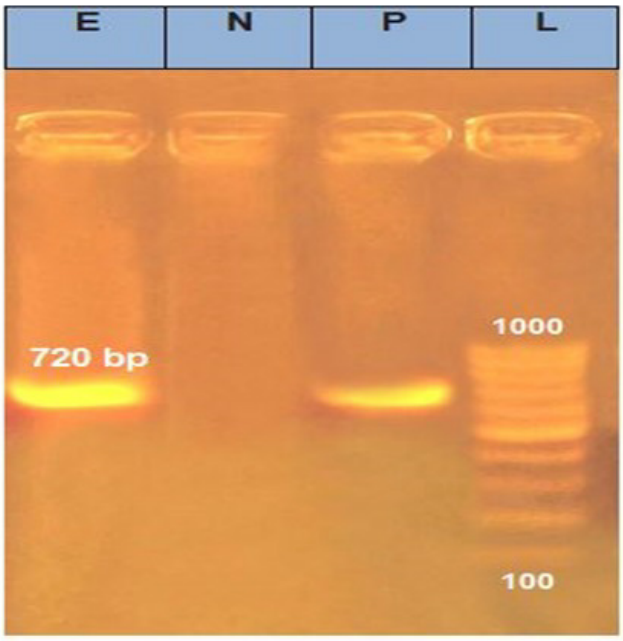

Fig 2. PCR results of the amplified 16 S rRNA gene of *E. coli*

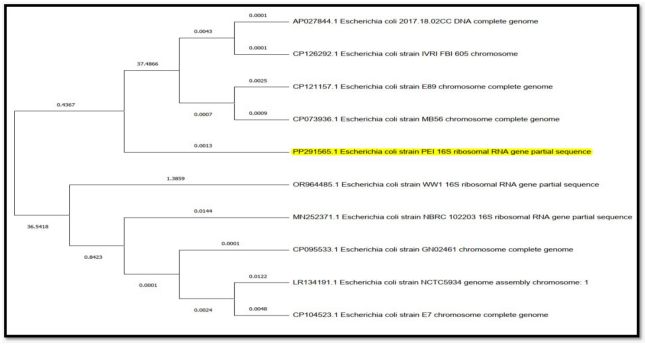

Fig 3. *Escherichia coli*'s 16S rDNA sequence similarity phylogenetic tree

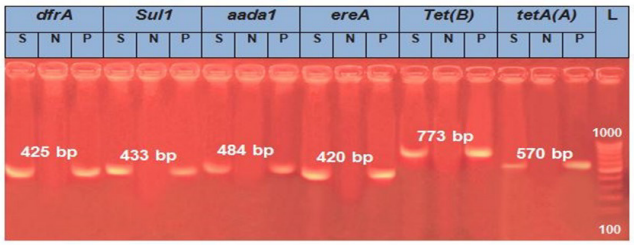

Fig 4. Gel electrophoresis of *dfrA, Sul1, aada1, ereA , Tet* (A) and *te t* (B) genes in *E. coli*

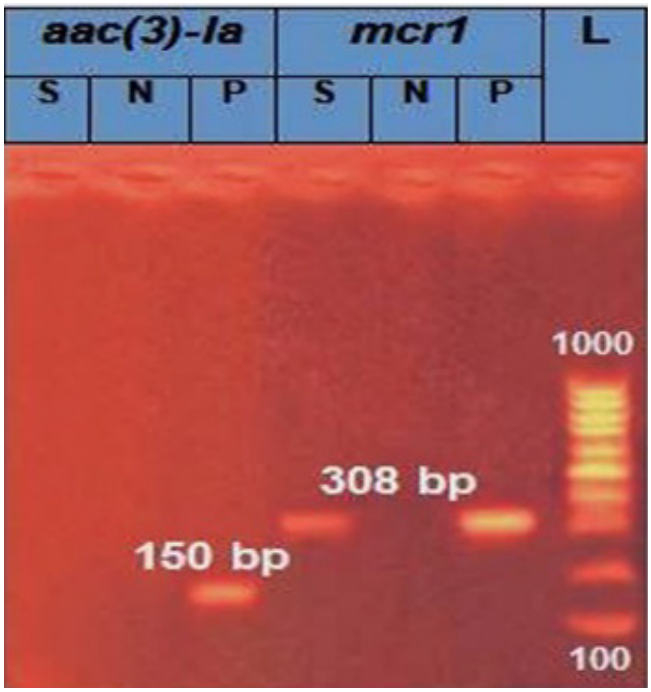

Fig 5. Gel electrophoresis of *mcr1* gene

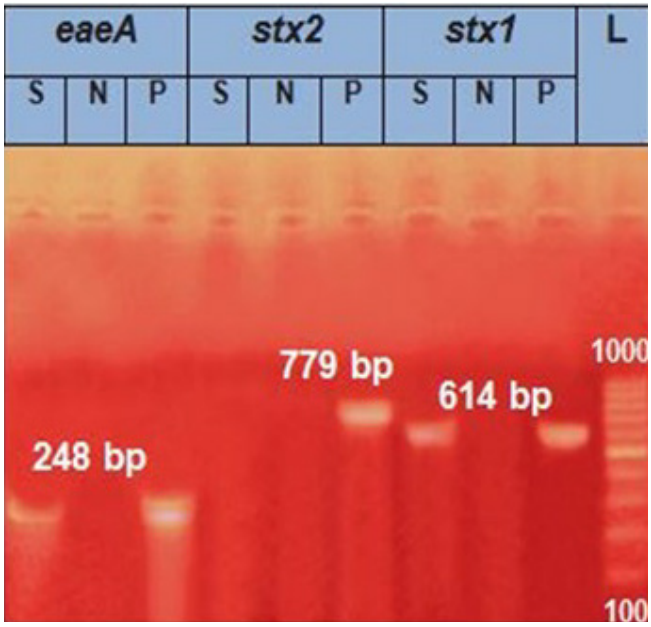

Fig 6. Gel electrophoresis of *Stx1* and *eaeA* gene

treated with Rutin only (G3). Finally in the infected group treated with colistin (G4) *E. coli* was re-isolated from 20% of the birds.

The *In vivo* Antimicrobial Activity of Rutin

The effects of rutin, colistin, and their combination on the liver functions, antioxidant enzymes, and inflammatory markers in broilers experimentally infected with E. coli were shown in *Table 2* and *Table 3.* Regarding total protein (TP) it significantly declined (P<0.05) in G2 (3.66±0.09), and G4 (3.91±0.09) in contrast to G1 (4.50±0.10), and G5 (4.37±0.11) treated with both rutin and colistin. Albumin level was significantly reduced (P<0.05) in G2 (0.81±0.03) compared to G1 (1.94±0.02), and to other groups as G3 (1.68±0.05), G4 (1.55±0.05), and G5 (1.89±0.05) *(Table 2).* Treatment with the combination of rutin and colistin in G5 revealed a non-significant change in albumin level in comparison to G1. Contrary, globulin levels were significantly increased in G2 (2.85±0.04) compared with G1 (2.56±0.02) and other groups as G3 (2.37±0.03), G4 (2.35±0.04) andG5 (2.48±0.05). The liver function parameters, as presented in *Table 2,* showed that AST activity of G2 had significantly (P<0.05) elevated activity reaching (92.03±2.09) compared to G1 group (66.70±1.19). Rutin and colistin treatment caused significant improvement in the AST activity in comparison to the infected control group reaching (77.33±1.20) and (79.03±2.07) in G3 and G4 respectively. Likely, G5 the infected and treated group with a combination of rutin and colistin had normal AST activities (68.33±2.03) compared to G1. Moreover, G1 had ALT activity of (16.17±1.01), while G2 had significantly (P<0.05) higher ALT activity of (29.84±1.00). Such values within G3 and G4 were significantly reduced to (21.03±0.58) and (22.63 ±0.69). However, G5 did not have essentially different ALT activity reaching the normal levels of (18.02±0.58).

Regarding the antioxidant status, the obtained findings in *Table 3* showed that CAT activity of (G1) was (102.40±2.75), while G2 had significantly (P<0.05) lower CAT activity of (56.17±2.17). Such values in G3 and G4 were significantly increased reaching (88.33±2.04) and (79.03±1.79) compared to G5. G5 did not have significantly different CAT activity than G1 reaching (96.69±1.35).

Regarding MDA concentration, G2 had significantly (P<0.05) elevated levels reaching (12.60±1.06) compared to G1 (4.35±0.18). Rutin caused significant improvement in the MDA concentration reaching levels of (5.43±0.19) in G3. Likely, G4 and G5 the infected and treated groups with colistin alone or in combination with rutin had relatively reduced MDA concentrations of (6.59±0.02) and (5.13±0.16), respectively in comparison to the infected non-treated group.

Referred to GSH activity, it was shown that after treatment, G5 had the highest activity (28.06±0.24), followed by G3 (26.69±0.27), G4 (24.65±0.50), while the infected non-treated group had the least GSH activityG2 (16.06±1.04) compared with G1(29.45±0.57)

Regarding the inflammatory marker, TNF-α, it was much upregulated (P<0.05) in G2 (385.03±6.14) compared to G1 (261.66±2.95), and to other groups as G3 (292.66±3.18), G4 (293.33±2.40), and G5 (279.21±1.42) *(Table 3).*

Table 2. *The impact of colistin, rutin, and their combination on the liver function of broilers subjected to experimental E. coli infection*

Parameters	Groups				
	G1	G2	G3	G4	G5
Total protein (g/dL)	4.50±0.10 ^{a}	3.66± 0.09 ^{d}	4.04±0.10 ^{bc}	3.91±0.09 ^{c}	4.37±0.11 ^{ab}
Albumin (g/dL)	1.94±0.02 ^{a}	0.81±0.03 ^{d}	1.68±0.05 ^{b}	1.55±0.05 ^{b}	1.89±0.05 ^{a}
Globulin (g/dL)	2.56±0.0 2 ^{b}	2.85±0.04 ^{a}	2.37±0.03 ^{c}	2.35±0.04 ^{c}	2.48±0.05 ^{bc}
AST (U/L)	66.7±1.19 ^{c}	92.03±2.09 ^{a}	77.33±1.20 ^{b}	79.03±2.07 ^{b}	68.33±2.03 ^{c}
ALT (U/L)	16.17±1.01 ^{c}	29.84±1.0 ^{a}	21.03±0.58 ^{b}	22.63±0.69 ^{b}	18.03±0.58 ^{c}

Table 3. *The impact of colistin, rutin, and their combination on inflammatory and antioxidant indicators in broilers experimentally infected with E. coli*

Parameters	Groups				
	G1	G2	G3	G4	G5
CAT (U/g liver tissue)	102.40±2.75 ^{a}	56.17±2.17 ^{d}	88.33±2.04 ^{b}	79.03±1.79 ^{c}	96.70±1.35 ^{a}
MDA (nmol/g liver tissue)	4.35±0.18 ^{d}	12.60±1.06 ^{a}	5.43±0.19 ^{c}	6.59±0.02 ^{b}	5.13±0.16 ^{c}
GSH (nmol/g liver tissue)	29.45±0.57 ^{a}	16.06±1.04 ^{d}	26.69±0.27 ^{b}	24.65±0.50 ^{c}	28.06±0.24 ^{a}
TNF (Pg/mL)	261.67±2.95 ^{d}	385.03±6.14 ^{a}	292.67±3.18 ^{b}	293.33±2.40 ^{b}	279.22±1.42 ^{c}

Data are expressed as mean ± SE
a,b,c,d *Superscript: Mean significance difference among groups on P<0.05*

Discussion

The necessity to generate a novel antibacterial drug in the period of antibiotic resistance has spurred researchers worldwide to revert to natural medicine techniques to explore the potential antibacterial mechanisms of action of compounds originating from plants, e.g. polyphenols, particularly rutin [21].

In this work, *E .coli* isolated from the broiler was investigated for the presence of virulence and antibiotic resistance genes, this is a public health concern due to the frequent interactions between people and companion animals.

Multiple tetracycline determinants were existent in *E. coli* PP291565 isolated strain in agreement with Van et al.[22] studies that stated the presence of *tet*A and *tet*B genes.

Resistance to streptomycin and gentamicin is mostly dependent on the *aa*d (A1) and *aac3* genes respectively which was investigated by Szczepanowski et al.[23]. In our study we detected only *aa*d (A1) gene while *aac3* was negative by PCR assay.

Our results agrees with Shahrani et al.[24] and other studies who detected the presence of *stx* gene encodings shiga toxin which reflects a major virulent component in the pathogenicity of the EHEC pathotypes. Likewise our study revealed that *E. coli* isolate was containing genes that code Shiga toxin *(stx1)* and intimin production *(eae),* which are essential virulence determinants in *E. coli* connected with human infection but he also detected *sxt2* gene which was not detected in our study.

Poirel et al.[25] stated that the mcr-1 gene, which encodes colistin resistance, has predominantly been discovered in environmental and animal samples since its discovery worldwide, as well as in *E. coli* isolated from broiler samples.

Due to the existence of poultry products that contain antibiotic residues, and because of growing user demand for products free from antibiotic residues, have accelerated the look for options that might exchange antibiotics that do not reduce production or product value.

Our result revealed that Rutin is one of the antibiotic alternates for *E. coli* with MIC value of 128 µg/mL that agrees with Miklasińska-Majdanik et al.[26] who reported that RH demonstrated variable effects against *E. coli*.

Several studies have revealed that natural-origin compounds have activity against bacteria alone and in combination with specific antibiotics [7]. In our study, we have concentrated on the antibacterial impact of Colistin and Rutin against *E. coli* which could have useful uses in the era of resistance to antibiotics. Also that agrees with Wang et al.[27] who stated that rutin has antibacterial activity against both Gram-positive and Gram-negative bacterial strains.

Serum globulin concentration, AST, and ALT activity were significantly higher in the *E. coli*-infected non-treated group as *E. coli* infection has a characteristic symptom that damages the liver in chicken is perihepatitis [28]. Similar results were observed previously by Ghandour et al [29]. The elevated globulin levels may result from the birds' immune response to *E. coli* infection. The findings aligned with the previous findings [30]. Elevated serum AST signifies cellular damage to cardiac myocytes and hepatocytes, while increased serum ALT is predominantly due to hepatic diseases. These findings are consistent with the studies conducted by Abd-Allah et al.[31]. That Increase in hepatic enzyme activity may be associated with modified hepatocyte membrane permeability induced by the microorganism; consequently, the cell membrane's functional integrity is compromised, leading to the efflux of these enzymes into the bloodstream [30]. Bacterial toxins impact liver cells, especially those next to the central vein, resulting in an increase in ALT activity. These cells get the least amount of nutrients from the blood and are very vulnerable to hepatotoxins and inflammatory chemicals, which directly affect how permeable the membranes of liver cells are [32].

According to the current study, dietary rutin supplementation significantly affects serum AIT levels. This suggests that rutin has hepato-protective qualities, which may be related to the production of cytokines, which have been shown to provide hepato-protection in a variety of liver injury models [33].

Biochemical assays showed a significant decrease in albumin which is the carrier to anions, cations, fatty acids, and hormones. Changing the rate at which they are made affects the amounts of these compounds and the physiological responses that birds need to stay alive [34]. Declined result of total protein concentration, in agreement with Vikash et al.[35], who posted that this hypoproteinemia may be associated with renal pathology, leading to protein depletion, hepatic impairment resulting in impaired plasma protein synthesis, or congestive heart failure. Hyperglobulinemia in *E. coli* infection has been documented by Abd El-Ghany et al.[36], associated with liver cirrhosis, hepatitis, and Kupffer cell expansion.

The *E. coli*-infected non-treated group's CAT and GSH activity was significantly lower than that of the control group, according to the data regarding antioxidant status. Indicators of oxidative stress and inflammation, malondialdehyde (MDA) levels were significantly greater ($P<0.05$) in the infected non-treated group than in the negative control group. The findings are consistent with those of El-Kilany et al.[37] who found that broiler SOD and GPX levels were lowered as a result of reactive oxygen species build up and oxidant/antioxidant imbalance brought on by an *E. coli* infection. Furthermore,

hens infected with *E.coli* had considerably decreased antioxidant enzyme levels and higher MDA [29]. These alterations may result from the bacterial LPS (endotoxin) it produces damages multiple organs, including the liver, by boosting lipid peroxidation and generating reactive oxygen intermediates [38].

Natural antioxidants, such as flavonoids, taking a great concern for preserving health and preventing disease. Flavonoids have been shown to be superior antioxidants that can enhance T-SOD and CAT activity while lowering MDA levels, which is consistent with our current findings [8]. As a powerful antioxidant flavonoid, rutin exhibits its antioxidant properties by chelating iron to eliminate free radicals, hence preventing lipid peroxidation and xanthine oxidase activity [39].

According to Tufarelli et al.[40], rutin supplementation significantly increased GSH and CAT activities while decreasing malonaldehyde levels in the current study. This suggests that rutin has the ability to transfer electrons and free radicals in addition to its capacity to activate antioxidant enzymes and lessen oxidative stress Rutin's chemical makeup may directly scavenge ROS, according to the first reports of its antioxidant qualities *in vitro* and *in vivo* [41]. Second, it increases GSH production, and it is believed that enhanced expression of different antioxidant enzymes, including SOD and CAT, promotes cellular oxidative defense mechanisms [42].

Tumor necrosis factor TNF-α is an inflammatory cytokine, which can cause hepatocytes to generate a number of acute-phase proteins during the acute phase response. Broilers infected with *E. coli* most likely had higher levels of cytokines and inflammatory markers, including TNFα. The idea that infection-induced tissue damage requires increased production of inflammatory cytokines is further supported by the current study's findings regarding elevated tissue levels of the pro-inflammatory cytokines TNF-α following *E. coli* tumor necrosis factor (TNF) [43]. TNFα was considerably ($P<0.05$) higher in the experimentally infected group than in the treated and negative control groups [29].

Numerous studies have been conducted to comprehend the pharmacological processes and efficacy of flavonoids, including rutin, since they have been identified as naturally occurring anti-inflammatory compounds a great deal of research has been done to understand their pharmacological mechanisms and effectiveness [44]. In line with our findings, rutin has potent anti-inflammatory properties by lowering levels of pro-inflammatory molecules such TNF-a, and NF-κB production [45]. Our results collectively imply that rutin may enhance immune response and reduce inflammation in broilers by blocking the NF-κB signaling pathway.

The effectiveness of colistin is impacted by the widespread use of antibiotics to treat germs that are resistant to many drugs, especially Enterobacteriaceae. Additionally, as was primarily shown in a number of *E. coli* strains, it was contributing to the development of colistin-resistant bacteria [46]. On the other hand, the limited gastrointestinal absorption of colistin, even in contaminated animals, indicates that oral colistin administration is increasing colistin resistance by placing selection pressure on the intestinal flora of animals due to antibiotics [47]. Numerous researches have demonstrated that using certain flavonoids reduces antibiotic resistance and reveals vulnerability in a synergistic manner, providing drug-resistant bacteria with a significant therapeutic strategy [48].

Food safety demands that plant extracts and their phytochemicals (such as flavonoids) to become the new trend for use as growth promoters and meat quality improvers. In the current study, rutin significantly improved antioxidant status activity more than colistin therapy, bringing it back to normal levels in group 5 treated with a combination of rutin and colistin. It is suggested that resuming the antibacterial activities of antibiotics when flavonoids are present may be caused by flavonoids' ability to improve the efficiency of the antibiotic medicine [49].

From this study we conclude that the most serious issue in this research is that the demonstrated *E. coli* PP291565 (EHEC) isolate not only was resistant to 95% of the antibiotics tested but also its molecular screening clarified that it carries multiple resistance and virulence genes, which makes it very virulent, difficult to be treated. But rutin demonstrated a considerable growth inhibition zone and MIC value against *E. coli in vitro,* and a synergistic spectacular impact with colistin *in vivo.*

In conclusion, rutin administration provided excellent protection against the adverse consequences of *E. coli* infection through its anti-bacterial, hepato-protective, antioxidant, and anti-inflammatory properties. With the colistin treatment combination, it shows the greatest improvement. So that the synergistic effect of rutin with colistin suggests that rutin can convert antimicrobial resistance pathways into susceptible ones, restoring the drug's efficacy which raised hopes that it could be used as a future treatment for highly resistant and virulent *E. coli.*

Declarations

Availability of Data and Materials: The data sets analyzed during the current study are available from the corresponding author S. M. Elmesalamy on reasonable request.

Acknowledgements: The authors declare that they have no acknowledgements.

Funding Support: The authors declare that this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Ethical Approval: This protocol was approved by the Animal Health Research Institute (AHRI) authorized the experimental procedure in accordance with the Agriculture Research Center (ARC) and IACUC committee in Egypt (ARC, AHRI, IACUC, 150/24).

Competing Interests: The authors declare that they have no competing interests.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, including tables and figures, was not written or created by generative artificial intelligence (AI) or AI-assisted technologies. Such technologies were only used to improve the readability and language of the manuscript.

Author Contributions: R. M. Khallaf, S. S. Abd El-Hamied, S. M. Elmesalamy were participate in research design and conceptualization and experimental design, methedology, project administration, literature review, A. Z. Refaie, O. A. Elewasy conceptualization, methodology, validation, writing original draft preparation, literature review, investigation, data curation, visualization, and funding acquisition. All authors critically reviewed and approved the final version of the manuscript.

References

1. **Miglani S:** APEC report. *Aust Endod J,* 49 (3): 466-467, 2023. DOI: 10.1111/aej.12818
2. **Karch H, Tarr PI, Bielaszewska M:** Enterohaemorrhagic *Escherichia coli* in human medicine. *Int J Med Microbiol,* 295 (6-7): 405-418, 2005. DOI: 10.1016/j.ijmm.2005.06.009
3. **Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S:** Antimicrobial resistance in *Escherichia coli. Microbiology Spectrum,* 6:10.1128/microbiolspec.arba-0026-2017, 2018. DOI: 10.1128/microbiolspec.ARBA-0026-2017
4. **Biran D, Ron EZ:** Extraintestinal pathogenic *Escherichia coli. Curr Top Microbiol Immunol,* 416, 149-161, 2018. DOI: 10.1007/82_2018_108
5. **Puvača N, De Llanos FR:** Antimicrobial resistance in *Escherichia coli* strains isolated from humans and pet animals. *Antibiotics (Basel)*, 10 (1):69, 2021. DOI: 10.3390/antibiotics10010069
6. **Xiao P, Shen Z, Wang D, Pan Y, Li Y, Gong J, Wang L, Wang D, Tang BZ:** Precise molecular engineering of type I Photosensitizers with near-infrared aggregation-induced emission for image-guided photodynamic killing of multidrug-resistant bacteria *Adv Sci,* 9:e2104079, 2022. DOI: 10.1002/advs.202104079
7. **Long SF, He TF, Wu D, Yang M, Piao XS:** Forsythia suspensa extract enhances performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function and gut morphology in broilers. *Poult Sci,* 99 (9): 4217-4226, 2020. DOI: 10.1016/j.psj.2020.05.011
8. **Negahdari R, Bohlouli S, Sharifi S, Maleki DS, Rahbar SY, Khezri K, Jafari S, Ahmadian E, Gorbani JN, Raeesi S:** Therapeutic benefits of rutin and its nanoformulations. *Phytother Res,* 35, 1719-1738, 2021. DOI: 10.1002/ptr.6904
9. **Amin MU, Khurram M, Khattak B, Khan J:** Antibiotic additive and synergistic action of rutin, morin and quercetin against methicillin-resistant *Staphylococcus aureus. BMC Complement Altern Med,*15:59, 2015. DOI: 10.1186/s12906-015-0580-0
10. **Sarba EJ, Kelbesa KA, Bayu MD, Gebremedhin EZ, Borena BM, Teshale A:** Identification and antimicrobial susceptibility profile of *Escherichia coli* isolated from backyard chicken in and around Ambo, Central Ethiopia. *BMC Vet Res,*15:85, 2019**.** DOI: 10.1186/s12917-019-1830-z
11. **El-Ashram S, Hashad ME, Abdel-Alim GA, Abdelhamid T, Deif HN:** Seroprevalence of mycoplasmosis in broiler, layer, and native chickens in Giza, Egypt. *PLoS ONE,* 16 (7):e0254220, 2021. DOI: 10.1371/journal.pone.0254220
12. **Clinical and Laboratory Standards Institute:** Performance Standards for Antimicrobial Susceptibility Testing. 26th ed., Wayne**,** PA: CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2017.
13. **Ginovyan M, Petrosyan M, Trchounian A:** Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Complement Altern Med,* 17:50, 2017. DOI: 10.1186/s12906-017-1573-y
14. **Hu Q, Tu J, Han X, Zhu Y, Ding C, Yu S:** Development of multiplex PCR assay for rapid detection of *Riemerella anatipestifer, Escherichia coli,* and *Salmonella enterica* simultaneously from ducks. *J Microbiol Methods,* 87, 64-69, 2011. DOI: 10.1016/j.mimet.2011.07.007
15. **Altschul SF, Gish W, Miller W, Myers EW, Lipmanl DJ:** Basic Local Alignment Search *Tool. J Mol Biol,* 215, 403-410, 1990. DOI: 10.1016/S0022-2836(05)80360-2
16. **Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG:** Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol,* 7:539, 2011. DOI: 10.1038/msb.2011.75
17. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S:** MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol,* 30:2725–2729, 2013. DOI: 10.1093/molbev/mst197
18. **Almremdhy HAA, Neamha GAK, Al-Hilli ZB, Awadh RJ:** Pathological, hematological, and biochemical alteration in broiler chickens infected with mycotoxin in Babylon province. *Open Vet J,* 14 (11): 2848-2859, 2024. DOI: 10.5455/OVJ.2024.v14.i11.13
19. **Fardos AMH, Elshimaa MR, Asmaa TYK, Asmaa WZ, Tukur HA, Saadeldin I:** Growth performance, antioxidant capacity, lipid-related transcript expression and the economics of broiler chickens fed different levels of rutin. *Animals,* 9 (1):7, 2019. DOI: 10.3390/ani9010007
20. **Tóthová C, Sesztáková E, Bielik B, Nagy O:** Changes of total protein and protein fractions in broiler chickens during the fattening period. *Vet World,* 12 (4): 598-604, 2019. DOI: 10.14202/vetworld.2019.598-604
21. **Rodríguez-Valdovinos KY, Salgado-Garciglia R, Vázquez-Sánchez A, Bernal DÁ, Oregel-Zamudio E, Ceja-Torres LF, Medina-Medrano JR:** Quantitative analysis of rutin by HPTLC and *in vitro* antioxidant and antibacterial activities of phenolic-rich extracts from Verbesina sphaerocephala. *Plants,* 10:475, 2021. DOI: 10.3390/plants10030475
22. **Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ:** Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *Int J Food Microbiol,* 124, 217-223, 2008. DOI: 10.1016/j.ijfoodmicro.2008.03.029
23. **Szczepanowski R, Linke B, Krahn I, Gartemann KH, Gützkow T, Eichler W, Pühler A, Schlüter A:** Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. *Microbiology,* 155 (7): 2306-2319, 2009. DOI: 10.1099/mic.0.028233-0
24. **Shahrani M, Dehkordi FS, Momtaz H:** Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biol Res,* 47 (1):28, 2014. DOI: 10.1186/0717-6287-47-28
25. **Poirel L, Jayol A, Nordmann P:** Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev,* 30, 557-596, 2017. DOI: 10.1128/CMR.00064-16
26. **Miklasińska-Majdanik M, Kępa M, Wąsik TJ, Zapletal-Pudełko K, Klim M, Wojtyczka RD:** The direction of the antibacterial effect of rutin hydrate and amikacin. *Antibiotics,* 12:1469, 2023. DOI: 10.3390/antibiotics12091469
27. **Wang Z, Ding Z, Li Z, Ding Y, Jiang F, Liu J:** Antioxidant and antibacterial study of 10 flavonoids revealed rutin as a potential

antibiofilm agent in *Klebsiella pneumoniae* strains isolated from hospitalized patients. *Microb Pathog,* 159:105121, 2021. DOI: 10.1016/j.micpath.2021.105121

28. **Hu J, Afayibo DJA, Zhang B, Zhu H, Yao L, Guo W, Wang X, Wang Z, Wang D, Peng H, Tian M, Qi J, Wang S:** Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic *Escherichia coli* (APEC). *Front Microbiol,* 13:1049391, 2022. DOI: 10.3389/fmicb.2022.1049391
29. **Ghandour M, Shams GA, Hassan HM, Baz HA, SaadEldin WF:** Effects of ceftriaxone on body performance and immunological status of broilers experimentally challenged with *E. coli. Pak J Zool,* 55, 1-10, 2023. DOI: 10.17582/journal.pjz/20230206200202
30. **Ghahramani Z, Mosleh N, Shomali T, Nazifi S, Khodakaram-Tafti A:** A study on selected responses and immune structures of broiler chickens with experimental colibacillosis with or without florfenicol administration. *BMC Vet Res,* 20:371, 2024. DOI: 10.1186/s12917-024-04232-3
31. **Abd-Allah OA, Kilany OE, Elhosiny ME:** Biochemical and immunological studies on the effect of *Echinacea purpura* in broilers. *Glob Anim Sci J,* 6 (1): 1-10, 2018. DOI: 10.21608/jappmu.2020.102707
32. **Salasia SIO, Hariono B:** Patologi Klinik Veteriner. Penerbit Samudra Biru, Yogyakarta, 2014.
33. **Zhang Y, Li H, Wang J, Chen X, Liu Y, Zhao Y:** Rutin attenuates liver injury by modulating oxidative stress and inflammatory cytokine production in experimental models. *Biomed Pharmacother,* 170:116123, 2024. DOI: 10.1016/j.biopha.2024.116123
34. **da Rosa G, Alba DF, Silva AD, Gris A, Mendes RE, Mostardeiro VB, Lopes TF, Schetinger MRC, Stefani LM, Lopes MT, Boiago MM, da Silva AS:** Impact of *Escherichia coli* infection in broiler breeder chicks: The effect of oxidative stress on weight gain. *Microb Pathog,* 139:103861, 2019. DOI: 10.1016/j.micpath.2019.103861
35. **Sharma V, Jakhar KK, Nehra V, Kumar S:** Biochemical studies in experimentally *Escherichia coli* infected broiler chicken supplemented with neem *(Azadirachta indica)* leaf extract. *Vet World,* 8 (11): 1340-1345, 2015. DOI: 10.14202/vetworld.2015.1340-1345
36. **Abd El-Ghany WA, Ismail M:** Tackling experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. *Iran J Vet Res,* 15 (2): 110-115, 2014. DOI: 10.22099/ijvr.2014.2341
37. **Wang X, Fang C, He J, Dai Q, Zhu J, Luo YZ:** Effects of *Escherichia coli* challenge on growth performance, immunity, and antioxidant capacity in broilers. *Poult Sci,* 98 (5): 2199-2206, 2019. DOI: 10.3382/ps/pey569
38. **Kono H, Asakawa M, Fujii H, Maki A, Amemiya H, Yamamoto M:** Edaravone, a novel free radical scavenger, prevents liver injury and mortality in rats administered endotoxin. *J Pharmacol Exp Ther,* 307, 74-82, 2013. DOI: 10.1124/jpet.103.053595
39. **Al-Sayed E, Abdel-Daim MM, El-Sheikh A, Khalifa S:** Protective role of rutin against chemically induced hepatotoxicity: Involvement of antioxidant defense, iron chelation, and cytokine modulation. *Environ Toxicol Pharmacol,* 99, 104-112, 2023. DOI: 10.1016/j.etap.2023.104112
40. **Tufarelli V, Rizzo A, Lacalandra GM, Guaricci AC, Laudadio V, Valentini L:** Effects of supplementation with a high-polyphenols extra-virgin olive oil on kinetic sperm features and seminal plasma oxidative status in healthy dogs. *Reprod Domest Anim,* 53, 582-587, 2018. DOI: 10.1111/rda.13145
41. **Hanasaki Y, Ogawa S, Fukui S:** The correlation between active oxygen scavenging and antioxidative effects of flavonoids. *Free Radic Biol Med,* 16 (6): 845-850, 1994. DOI: 10.1016/0891-5849(94)90202-X
42. **Al-Enazi MM:** Protective effects of combined therapy of rutin with silymarin on experimentally induced diabetic neuropathy in rats. *Pharmacol Pharm,* 5 (9): 876-889, 2014. DOI: 10.4236/pp.2014.59098
43. **HH, Nielsen JP, Heegaard PM:** Application of acute phase protein measurements in veterinary Petersen clinical chemistry. *Vet Res,* 35 (2): 163-187, 2004. DOI: 10.1051/vetres:2004002
44. **Rakshit S, Shukla P, Verma A, Nirala SK, Bhadauria M:** Protective role of rutin against combined exposure to lipopolysaccharide and D-galactosamine-induced dysfunctions in liver, kidney, and brain: hematological, biochemical, and histological evidences. *J Food Biochem,* 45:e13605, 2021. DOI: 10.1111/jfbc.13605
45. **Su S, Li X, Li S, Ming P, Huang Y, Dong Y, Ding H, Feng S, Li J, Wang X, Li Y, Wu J:** Rutin protects against lipopolysaccharide-induced mastitis by inhibiting activation of the NF-κB signaling pathway and attenuating endoplasmic reticulum stress. *Inflammopharmacol,* 27 (1): 77-88, 2019. DOI: 10.1007/s10787-018-0521-x
46. **Mead A, Richez P, Azzariti S, Pelligand L:** Pharmacokinetics of colistin in the gastrointestinal tract of poultry following dosing via drinking water and its bactericidal impact on enteric *Escherichia coli. Front Vet Sci,* 8:634, 2021. DOI: 10.3389/fvets.2021.698135
47. **Kumar H, Chen BH, Kuca K, Nepovimova E, Kaushal A, Nagraik R, Kumar D:** Understanding of colistin usage in food animals and available detection techniques: A review. *Animals,* 10 (10):1892, 2020. DOI: 10.3390/ani10101892
48. **Miklasińska-Majdanik M, Kępa M, Wojtyczka RD, Idzik D, Wąsik TJ:** Phenolic compounds diminish antibiotic resistance of Staphylococcus aureus clinical strains. *Int J Environ Res Public Health,* 15 (10):2321, 2018. DOI: 10.3390/ijerph15102321
49. **Vipin C, Saptami K, Fida F, Mujeeburahiman M, Rao S, Athmika ABA, Rekha PD:** Potential synergistic activity of quercetin with antibiotics against multidrug-resistant clinical strains of *Pseudomonas aeruginosa. PLoS One,* 15 (11):e0241304, 2020. DOI: 10.1371/journal.pone.0241304