Pak. j. life soc. Sci. (2025), 23(1): 7214-7224 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences

www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2025-23.1.00561

RESEARCH ARTICLE

Impact of Pathogenic Bacterial Challenges on Growth Performance, Gut Morphology, Serum Biochemistry, and Meat Quality in Broiler Chickens

Nareen A. Shekhu[1,2], Jassim Mohammed Abdo[3]

[1]Department of Animal Production
[2] Department of Food Sciences and Technology, College of Agricultural Engineering Sciences, University of Duhok, Iraq
[3] Department of Biomedical Sciences, College of Medicine, University of Zakho, Iraq

ARTICLE INFO

Received: Jan 10, 2025

Accepted: Feb 23, 2025

Keywords

Broiler chickens
Escherichia coli Salmonella spp Staphylococcus aureus
Gut health
Meat quality

***Corresponding Author:**

nareen.shekhu@uod.ac

ABSTRACT

This study aimed to evaluate the effects of Escherichia coli, Salmonella spp., and Staphylococcus aureus infections on broiler growth performance, gut morphology, serum biochemical markers, and meat quality. A total of 480 one-day-old Ross 308 broiler chicks were randomly assigned to four treatment groups: a control group and three groups challenged with E. coli, Salmonella spp., and S. aureus. Birds challenged with pathogenic bacteria exhibited significantly lower ($P < 0.05$) body weight gain and feeding conversion efficiency compared to the control group. The intestinal morphology of Salmonella- and Staphylococcus-challenged birds showed a marked reduction in villous height and crypt depth, indicating impaired nutrient absorption. However, E. coli-infected birds significantly increased villous tip width, possibly as an adaptive response. Serum analysis revealed a significant suppression ($P < 0.0001$) of Newcastle Disease Virus (NDV) antibodies in all challenged groups, suggesting compromised immune function. GPT levels were significantly elevated in infected birds, indicating potential liver stress, whereas GOT, total protein, albumin, and globulin levels remained unaffected. Meat quality assessments showed significant variations ($P < 0.0001$) in lightness (L), redness (a), and yellowness (b) values among treatments, with E. coli-challenged birds exhibiting the lowest pH. The results indicate that pathogenic bacterial infections negatively impact broiler health, growth performance, and meat quality while also posing significant food safety risks.

INTRODUCTION

Poultry meat and eggs serve as crucial dietary protein sources and income in many middle- and low-income countries worldwide. Poultry product's cost-effective and profitable protein production presents significant challenges, particularly due to the widespread use of antibiotics in the poultry industry (Doski *et al.* 2016; M'Sadeq *et al.*). The consumption of poultry meat, eggs, and exposure to poultry waste can contribute to the transmission of multidrug-resistant bacteria such as Escherichia coli, Salmonella spp., and Staphylococcus aureus, which are frequently associated with foodborne illnesses. These bacteria, often found in poultry-derived proteins and by-products, have zoonotic potential, posing a serious threat to public health(El-Ghany 2021; Rafiq *et al.* 2024).

The extensive use of antibiotics in poultry farming exerts selection pressure, leading to antimicrobial resistance (AMR) among commensal bacteria such as *E. coli*, Salmonella spp., and Staphylococcus aureus (M'Sadeq *et al.* 2015; Salman *et al.* 2024). These microorganisms, which are naturally present in the human microbiota, can harbor resistance genes or plasmids capable of spreading between humans and animals, thereby exacerbating drug resistance issues (Djordjevic *et al.* 2013) . *E. coli* and

Staphylococcus are commensals in the gastrointestinal tract of both humans and animals but can become pathogenic under specific conditions. In poultry, pathogenic *E. coli* strains are responsible for various diseases, including colibacillosis, meningitis, diarrhoea, and septicaemia, leading to significant economic losses (Hasan and M'Sadeq 2020). While most *E. coli* strains are harmless, approximately 15% are pathogenic, categorized into enterohemorrhagic, enterotoxigenic, enteropathogenic, enteroaggregative, and enter invasive groups based on their mechanisms of disease induction (Smith and Fratamico 2017). Additionally, *E. coli* is classified into commensal, extraintestinal pathogenic, and extraintestinal pathogenic strains based on virulence factors, including toxin production, haemolysis, siderophores, proteases, and adhesins, which play crucial roles in disease development (Sarowska *et al.* 2019).

Staphylococcus aureus, another significant pathogen, can cause infections in both humans and animals under favorable conditions (Haag *et al.* 2019). Some strains produce enterotoxins that lead to food poisoning, with antimicrobial resistance primarily resulting from mutations in penicillin-binding protein (PBP2a) encoded by the Meca genes. S. aureus possesses numerous virulence factors, including extracellular toxins, enterotoxins, and surface proteins such as protein A, β-hemolysin, and staphylococcal enterotoxins (SEs) (Omwenga 2022). These virulence factors contribute to the bacterium's ability to colonize various environments and cause a wide range of clinical manifestations. Globally, S. aureus and its enterotoxins are the third most common cause of foodborne illnesses.

Salmonellosis remains one of the most severe bacterial diseases affecting the poultry industry, leading to significant economic losses due to mortality and reduced productivity (Tariq *et al.* 2022). Nearly all known Salmonella serovars have the potential to infect both animals and humans. The most common serovars of Salmonella enterica responsible for human salmonellosis are Enteritidis and Typhimurium (Andino and Hanning 2015). Annually, Salmonella infections impact approximately 20 million individuals and animals, resulting in an estimated 150,000 deaths and decreased animal productivity. Typhoid fever, caused by specific Salmonella strains, is particularly prevalent in South-Central and Southeast Asia (Hossain *et al.* 2021). Poultry meat is one of the leading sources of Salmonella-related foodborne illnesses, with the pathogen being responsible for an estimated 155,500 deaths worldwide each year(Rafiq *et al.* 2024). Thus, this research has consistently demonstrated the presence of various foodborne bacteria in different parts of poultry meat, highlighting the importance of stringent food safety measures.

MATERIALS AND METHODS:

The research was performed in the animal facility of the Department of Animal Production at the College of Agricultural Engineering Sciences, University of Duhok, situated in the Kurdistan Region, Iraq. The experiment received approval from the Animal Ethics Committee of the College of Agricultural Engineering Sciences, Department of Animal Production (Approval No: AEC08072022).

Animal husbandry:

A total of 480 one-day-old Ross 308 chicks were randomly assigned to four treatment groups, each consisting of four replicates. Each replicate contained 15 chicks. The birds were vaccinated against the Newcastle Disease Virus (NDV) on the first day. The study design incorporates different challenge conditions. The treatments included a control group, along with groups challenged with Salmonella, Staphylococcus, and *E. coli*. Housing was divided into two rooms based on the challenge designation: four negative control pens were placed in a non-challenged room, while 12 challenged pens were assigned to a separate room within the same environmentally controlled facility.

The birds were provided with a control diet, which included starter, grower, and finisher feed formulations. The temperature and lighting schedules followed the guidelines outlined by Aviagen (2018). Each pen was equipped with an individual feeder and nipple drinkers to ensure standardized feeding and hydration conditions. Feed and water were supplied ad libitum throughout the experimental period. The dietary phases included a starter diet from day 0 to day 10, a grower diet

from day 10 to day 24, and a finisher diet from day 24 to day 35. The birds were monitored for growth performance, health status, and response to bacterial challenges throughout the study.

Bacterial Challenge:

This procedure was conducted following the methodology described by M'sadeq (2019) and Hassan (2024). Escherichia coli, Salmonella spp., and Staphylococcus spp. were individually incubated overnight at 37˚C in 1000 mL of sterile nutrient broth. On day 14, birds in the challenged groups were inoculated with 1.5 mL of bacterial suspension (3.8×10^8 CFU/mL) for each pathogen, while the non-challenged birds received 1.5 mL of distilled water.

Collection and isolation of pathogenic microorganisms for challenging chicks:

Some infected chicken samples were collected for the detection and isolation of salmonella, E. coli & Staphylococcus aureus to get bacterial stocks which were required for the challenging process. According to Harrigan (1998), conventional methods for the detection and isolation of pathogenic microorganisms were conducted. isolates were sub-cultured onto the selective culture media to obtain pure bacterial colonies. Then a typical colony for each required microorganism was selected and enriched in the Nutrient broth at 37oC for around 24-48 hrs, then glycerol stocks were established and stored in a freezer at - 21 °C for DNA extraction. Regarding to molecular method, DNA Extraction for bacterial stocks was done by using Addprep Bacterial Genomic DNA Extraction Kit (add bio /Korea), while considering and applying the manufacturer's recommended protocol for gram-negative and gram-positive bacteria. Nanodrop and agarose gel electrophoresis were used to check the quality and quantity of extracted DNA and then the samples were saved at -20°C. Additionally, the extracted DNA samples were confirmed by conducting conventional PCR assays using the universal primers targeting the partial region of 16*S* rRNA (1200 bp) Forward-(5'-GACCTCGGTTTAGTTCACAGA-3') and Reverse-(5'-CACACGCTGACGCTGACCA-3') which was previously published by Schippa et al. (2010) . The thermocycling program for PCR was Initial denaturation at 95 °C for 6 minutes followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 56 °C 60 seconds, extension at 72oC 60 seconds, and Final extension at 72 °C for 5 minutes. Then, the PCR products were sequenced by Sanger sequencing method in both forward and reverse directions (Macrogen, South of Korea). The sequences were cleaned and confirmed for targeted bacteria in Blast, NCBI. Then the genetic sequence data for salmonella, E. coli & Staphylococcus aureus were submitted to NCBI GenBank.

Sample Collection:

By day 24, two chickens from each pen were randomly selected, weighed, and humanely slaughtered via cervical dislocation. A 1 cm segment of the jejunum was collected from each pen for morphometric analysis. The tissue samples were carefully dissected, rinsed with phosphate-buffered saline (PBS, pH 7.4), and preserved in 10% buffered formaldehyde for 24 hours.

Histological Analysis:

Tissue samples were dehydrated, cleared, and embedded in paraffin wax for histological examination, following the method of M'Sadeq (2023). Serial longitudinal sections (7 µm) were mounted on Superfrost® slides (Thermo Scientific, Rockville, MD, USA) and stained with haematoxylin and eosin. Villus height and crypt depth were quantified using the Dino-eye application with images captured via a colour video camera (Dino-eye 20). From each replicate, measurements were taken for ten villi, ten crypts, apical width, basal width, and muscle width.

Serum Biochemical Analysis:

Blood samples were collected from birds at 24 days of age. Two birds per pen were randomly selected and euthanized, after which blood was drawn from the jugular vein, centrifuged at 3000 rpm for 15 minutes, and the serum was separated and stored at -20°C. Serum samples were analyzed for glutamic pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), total protein, albumin, globulin, and Newcastle disease virus (NDV) using an automatic analyzer (TOKYO BOEKI MEDICAL SYSTEM) and commercial kits (Prestige 24i LQ CHOL and Glucose (COD-PAP).

Bacterial Identification Using Conventional Cultural Methods:

A 10 g sample was homogenized with 90 mL of Buffered Peptone Water and incubated at 37˚C for 24 hours for enrichment. For Salmonella isolation, 0.1 mL of the pre-enriched culture was transferred to 10 mL of Rappaport-Vassiliadis medium (Oxoid, UK) and incubated for secondary enrichment at 37˚C for 24 hours. The secondary culture was then plated onto Xylose Lysine Deoxycholate (XLD) agar (Liofilchem, Italy) and incubated at 37˚C for another 24 hours. For *E. coli* isolation, a loopful of the pre-enriched culture was streaked onto Eosin Methylene Blue Levine (E.M.B Levine) agar plates (Liofilchem, Italy) and incubated at 37˚C for 24±6 hours. For Staphylococcus aureus identification, a loopful of the pre-enriched culture was streaked onto Mannitol Salt Agar plates (Liofilchem, Italy) and incubated at 37˚C for 24 hours.

Statistical Analysis:

The SAS statistical software (PROC GLM) was used to assess the significance of main effects (SAS, 2013). Duncan's multiple range test was applied to identify differences among treatment means.

RESULTS:

1- Broiler performance:

The performance data are summarized in Tables 1, 2, and 3 . On day 10, no significant differences were observed in feed intake, body weight gain, and feed conversion ratio (FCR) among the treatment groups. However, by day 24, birds were challenged with Escherichia coli, Salmonella spp., and Staphylococcus spp. exhibited a significant reduction in feed intake and body weight gain, alongside a notable increase in FCR compared to the non-challenged group.

By day 35, all challenged broiler chickens demonstrated a significant decline in body weight gain, further emphasizing the detrimental impact of bacterial infections on growth performance. Additionally, a marked increase in FCR was recorded in the challenged groups, indicating poorer feed efficiency. However, feed intake did not show significant variations among the treatment groups at this stage. These findings suggest that bacterial challenges negatively affect growth performance parameters, primarily by impairing feed utilization efficiency and reducing weight gain in broiler chickens.

Table 1: Effect of different treatments on broiler performance at day 10

Treatment means	Feed Intake g/bird	Weight gain g/bird	FCR
Control	342a	306	1.115
Salmonella	313a	280	1.109
Staphylococcus	312a	306	1.018
E.coli	315a	308	1.022
Pooled SEM	7.603	5.205	0.015
P > F	0.471	0.163	0.12

Table 2: Effect of different treatments on broiler performance at day 24

Treatment means	Feed Intake g/bird	Weight gain g/bird	FCR
Control	1609a	1344a	1.198b
Salmonella	1712a	1146b	1.494a
Staphylococcus	1728a	1160b	1.49a
E.coli	1717a	1143b	1.502a
Pooled SEM	22.821	21.183	0.033
P > F	0.224	0.0001	0.0001

[a, b,] means in rows with different superscripts are significantly different (P< 0.05).

Table 3: Effect of different treatments on broiler performance at day 35

Treatment means		**Feed Intake g/bird**	**Weight gain g/bird**	**FCR**
Control		2834a	1890a	1.500
Salmonella		2729a	1793b	1.520
Staphylococcus		2637a	1698b	1.553
E.coli		2689a	1777b	1.513
Pooled SEM		32.714	21.356	0.011
P > F		0.179	0.005	0.400

[a, b] means in rows with different superscripts are significantly different (P< 0.05).

2- Organs percentage:

On the 24th day of the study, the relative proportions of various organs to live body weight were evaluated in birds that were provided with different treatment diets (Table 4). The analysis revealed no significant differences in the percentages of the liver, heart, gizzard, and bursa among the birds consuming the various experimental diets. This indicates that the dietary treatments did not have a notable impact on the development or relative weight of these organs by day 24.

Table 4: Effect of different treatments on broiler organ percentage from live body weight of birds in different experimental treatments

Treatment means	**Liver g/kg**	**Heart g/kg**	**Gizzard g/kg**	**Bursa g/kg**
Control	2.33	0.534	3.57	0.237
Salmonella	2.21	0.515	2.98	0.201
Staphylococcus	2.46	0.494	3.22	0.184
E.coli	2.32	0.452	3.04	0.237
Pooled SEM	2.55	0.486	3.36	0.176
P > F				

3-Bacteria isolation

The required pathogenic microorganisms *salmonella, E. coli* & *Staphylococcus aureus* were isolated from infected chicken samples and confirmed by the conventional method, Aasa consequence; pure glycerol stocks were gained. furthermore, The DNA extracted by the recommended protocol for gram-negative and gram-positive bacteria. Moreover, the molecular assay was applied for extracted DNA samples and confirmed by Conventional PCR protocol using universal 16S rRNA primers (Figure 1). Additionally, the sequencing results revealed that these samples belong to Salmonella enterica strain SNJ10, Escherichia coli strain DNJ23 and Staphylococcus sp. strain STAN01and the GenBank accession numbers for these isolates were PV122192, PV122079, and OR342420) Respectively.

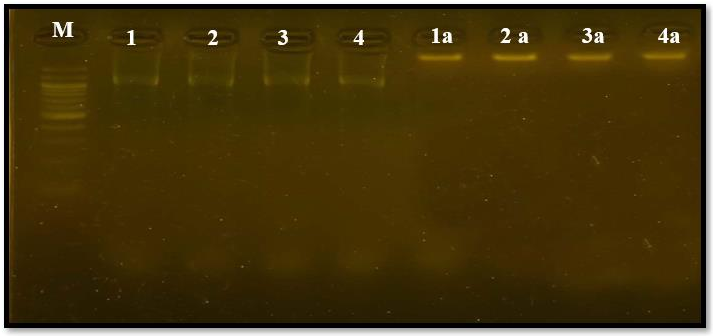

Figure 1. Agarose gel electrophoresis images of the extracted genome and the PCR reaction products of the bacteria samples for partial 16S rRNA region (1200 bp), where M is the DNA ladder and samples (1,2,3,4) represent the PCR reaction products and samples (1a,2a,3a,4a) are the genomic products from the bacteria under study.

4- Gut morphology:

Treatment means	GPT	GOT	Protein	Albumin	Globuline	NDV
Control	2.9b	221	2.7	1.2	1.54	2870a
Salmonella	3.95ab	200	3.1	1.27	1.65	2161b
Staphylococcus	4a	219	2.77	1.27	1.5	2335b
E.coli	5.75a	231	2.7	1.`12	1.57	2103b
Pooled SEM	1.35	3459	0.305	0.113	0.193	383.46
P > F	0.004	0.68	0.16	0.18	0.76	0.0006

The morphological characteristics and structural integrity of jejunal tissue samples were thoroughly examined following bacterial challenge, with the corresponding findings summarized in Table 5. By the 24th day of the experiment, the impact of bacterial infection on intestinal morphology became distinctly apparent. Birds challenged with Salmonella and Staphylococcus exhibited a significant reduction in villous height, crypt depth, and the villous-to-crypt ratio, indicating compromised intestinal architecture and potential impairment in nutrient absorption.

Conversely, birds exposed to Escherichia coli (*E. coli*) displayed a notable increase in villous tip width, suggesting a distinct structural adaptation or response to the bacterial presence. Additionally, birds challenged with Salmonella demonstrated a significant increase in villous surface area compared to the unchallenged control group, which may reflect a compensatory mechanism in response to intestinal stress or damage. These findings highlight the varying effects of different bacterial pathogens on jejunal morphology, emphasizing their potential implications for gut health and overall nutrient assimilation in poultry.

Table 5: Effect of different treatments on jejunal muscle thickness, villus height, and crypt depth at day 24

Treatment means	Villi (µm)	Crypt (µm)	Muscle (µm)	Tip (µm)	Basal (µm)	Villicrypt	Area (mm^2)
Control	1251c	159c	184a	151ab	180a	8.053a	207670b
Salmonella	1477a	209a	199a	146b	176a	7.287b	239419a
Staphylococcus	1388b	179b	200a	152ab	178a	7.935ab	229516ab
E.coli	1278c	18b	206a	164a	174a	7.191b	216882ab
Pooled SEM	14.341	3.532	3.646	2.834	2.939	0.127	4016.36
P > F	0.0001	0.0001	0.2614	0.103	0.918	0.0337	0.0411

$^{a, b, c}$ means in rows with different superscripts are significantly different ($P< 0.05$).

5- Serum biochemicals:

The effects of different dietary treatments, including both challenged and unchallenged conditions, on serum biochemical markers in broiler chickens at 24 days of age are presented in Table 6. The results revealed a significant elevation ($P < 0.0001$) in Newcastle Disease Virus (NDV) antibody titers in the unchallenged control group compared to all challenged birds. This suggests that bacterial infection may have suppressed the immune response, potentially compromising the birds' ability to mount an effective defense against viral infections.

Furthermore, serum levels of glutamate pyruvate transaminase (GPT) were significantly increased in response to bacterial challenge, indicating potential liver stress or tissue damage in infected birds. In contrast, no significant differences were observed among the treatment groups for glutamate oxaloacetate transaminase (GOT), total protein, albumin, or globulin levels. These findings suggest that while bacterial infection influences certain biochemical markers related to liver function and immune response, other serum parameters remained unaffected, highlighting the specific physiological responses induced by the bacterial challenge.

Table 6: Effect of different treatments on broiler serum biochemical parameters at day 24

Treatment means	GPT	GOT	Protein	Albumin	Globuline	NDV
Control	2.9b	221	2.7	1.2	1.54	2870a
Salmonella	3.95ab	200	3.1	1.27	1.65	2161b
Staphylococcus	4a	219	2.77	1.27	1.5	2335b
E.coli	5.75a	231	2.7	1.`12	1.57	2103b
Pooled SEM	1.35	3459	0.305	0.113	0.193	383.46
P > F	0.004	0.68	0.16	0.18	0.76	0.0006

[a, b] means in rows with different superscripts are significantly different (P< 0.05).

GOT = serum glutamic oxaloacetic transaminase, and GPT = serum glutamic pyruvic transaminase, NDV = Newcastle disease virus.

6- Bacteria identification:

Figure 2 illustrates the identification of bacterial contamination in broiler chicken carcasses, specifically Salmonella, E. coli, and Staphylococcus aureus. The study findings indicate that E. coli was detected in 66.67% of carcasses, while 33.33% tested negative. Salmonella was present in 20.83% of samples, with the remaining 79.17% testing negative. Additionally, all birds challenged with Staphylococcus aureus (100%) tested positive for this bacterium.

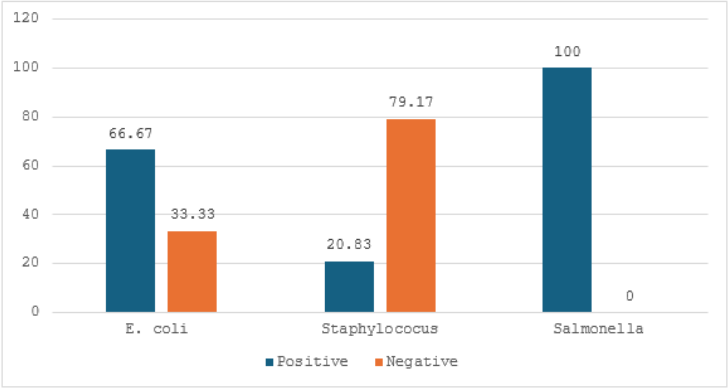

Figure 2. The Percentage of Positive and Negative Bacterial Presence in Broiler Chicken Carcasses.

7- Meat Colour and pH at Day 24:

The effects of different bacterial challenges on meat colour parameters (L*, a*, b*) and pH at day 24 are summarized in Table 7. Significant variations (P < 0.0001) were observed among treatments for lightness (L*), redness (a*), yellowness (b*), and pH values. Birds challenged with Salmonella and Staphylococcus exhibited significantly higher (P < 0.0001) lightness (L*) values (54.64 and 54.89, respectively) compared to the control (51.509) and *E. coli* (52.70) groups. Redness (a*) was significantly affected by bacterial challenge, with Staphylococcus and *E. coli*-challenged birds showing the highest values (3.73 and 3.82, respectively), while Salmonella-infected birds had the lowest (2.1). The yellowness (b*) value was significantly higher in Staphylococcus-challenged birds (2.46) compared to the other groups, whereas Salmonella-infected birds had the lowest value (1.84).

Regarding pH, although slight variations were noted, birds challenged with *E. coli* exhibited a significantly lower (P < 0.0001) pH (5.75) compared to the control (5.83), Salmonella (5.84), and Staphylococcus (5.82) groups.

Table 7: Effect of different treatments on broiler colour and pH parameters at day 24

Treatment means		L	A	B	pH
Control		51.509c	2.90b	2.12ab	5.83a
Salmonella		54.64a	2.1c	1.84b	5.84a
Staphylococcus		54.89b	3.73a	2.46a	5.82a
E.coli		52.70c	3.82a	2.08ab	5.75b
Pooled SEM		0.297	0.079	0.067	0.006
P > F		0.0001	0.0001	0.011	0.0001

[a, b, c] means in rows with different superscripts are significantly different (P< 0.05).

DISCUSSIONS:

The application of feed additives to the intestinal microbiota of chickens is well acknowledged for its considerable metabolic potential. It affects the host's health and nutrition (Beski *et al.* 2021; Khishtan *et al.* 2024; N Sadeeq *et al.* 2024). Elevated levels of some pathogenic bacteria, such as *E. coli*, Salmonella and staphylococcus may adversely impact broiler chickens' body weight, feed intake, feed conversion ratio, nutrient absorption, and gut health, which serves as an indicator of digestion and intestinal integrity (M'sadeq 2019).

The presence of pathogenic bacteria such as Escherichia coli, Salmonella spp., and Staphylococcus aureus in poultry not only compromises broiler health and productivity but also raises significant concerns regarding food safety and public health (Nakhaee and Hafez 2025). The findings of this study align with previous research demonstrating the detrimental impact of bacterial infections on growth performance, gut morphology, biochemical markers, and meat quality in broilers. Moreover, the emergence of multidrug-resistant (MDR) strains of these pathogens in poultry production presents an additional challenge, as it increases the risk of antimicrobial resistance (AMR) transmission through the food chain (de Mesquita Souza Saraiva *et al.* 2022).

Bacterial infections significantly reduced feed intake, body weight gain, and feed conversion efficiency in broilers, findings that are consistent with previous studies indicating that *E. coli*, Salmonella, and Staphylococcus infections impair nutrient absorption and overall growth performance (M'Sadeq *et al.* 2016). These reductions may be attributed to intestinal inflammation, microbial dysbiosis, and competition for nutrients, which ultimately compromise gut integrity and digestion(Sadeeq *et al.* 2024). The structural damage observed in the jejunal morphology, particularly in Salmonella- and Staphylococcus-challenged birds, further supports the notion that these pathogens negatively impact nutrient absorption. Reduced villous height and crypt depth, which are critical indicators of intestinal health, suggest impaired absorptive capacity, leading to poor feed efficiency and lower body weight gains.

Interestingly, birds challenged with *E. coli* exhibited an increase in villous tip width, possibly as an adaptive response to microbial stress or tissue remodeling following infection (Hussein *et al.* 2021). Additionally, Salmonella-challenged birds showed a significant increase in villous surface area compared to the control group, which may indicate a compensatory mechanism in response to intestinal damage. These findings underscore the complex interactions between bacterial infections and gut morphology, which in turn affect overall poultry productivity.

The suppression of Newcastle Disease Virus (NDV) antibody titters in all challenged birds suggests that bacterial infections may weaken the immune system, making broilers more vulnerable to viral infections. This immunosuppressive effect is particularly concerning in commercial poultry farming, where disease outbreaks can lead to significant economic losses (Hoerr 2010). Elevated levels of glutamate pyruvate transaminase (GPT) in challenged birds further indicate potential liver stress or systemic inflammation, which could be attributed to bacterial toxins or metabolic disruptions (Deepthi *et al.* 2017). However, the absence of significant differences in glutamate oxaloacetate transaminase (GOT), total protein, albumin, and globulin suggests that bacterial infections did not cause severe disruptions in systemic protein metabolism. These results agree with previous research that has highlighted selective biochemical alterations in response to bacterial infections, primarily

affecting liver function and immune responses without significantly altering overall protein synthesis (M'Sadeq, 2019).

The contamination of poultry meat with *E. coli*, Salmonella, and Staphylococcus aureus is a well-documented concern in food safety research. Studies have shown that Salmonella can be transmitted through infected poultry feces, leading to contamination of eggshells and subsequent bacterial growth inside eggs (Fearnley *et al.* 2011). The presence of Salmonella in more than 25% of poultry meat is considered unsafe for human consumption, highlighting the importance of stringent hygiene measures in poultry production (Alam *et al.* 2019).

Similarly, *E. coli* contamination in poultry meat poses a serious health risk, as these bacteria are often used as indicators of microbiological safety in foodborne pathogen screening. Previous studies have reported *E. coli* prevalence rates ranging from 41.1% to 63.5% in chicken meat, with even higher detection rates in layer birds (Alam *et al.* 2019; Jakaria *et al.* 2017). The presence of *E. coli* in poultry meat is largely attributed to fecal contamination during slaughter and processing, emphasizing the need for improved biosecurity measures and sanitary handling practices in the poultry industry.

The growing threat of antibiotic-resistant S. aureus also presents a significant challenge, as multidrug-resistant strains such as Methicillin-Resistant Staphylococcus aureus (MRSA) have been linked to increased mortality rates and treatment failures in infected individuals (Lika 2019). Studies have reported S. aureus isolation rates as high as 33.3% in poultry meat, with variations depending on hygiene conditions, slaughterhouse practices, and storage environments (Al-Humam *et al.* 2021). The emergence of MRSA in poultry products further exacerbates public health concerns, as these strains are more pathogenic and resistant to conventional antibiotic treatments.

The widespread use of antibiotics in poultry farming has led to the emergence of multidrug-resistant bacterial strains, which pose a serious threat to both animal and human health. The resistance of bacterial pathogens to antibiotics can occur due to genetic inheritance and horizontal gene transfer, particularly in environments where antibiotic use is frequent (Goh et al. 2024). Studies have shown that bacterial isolates with a multiple antibiotic resistance (MAR) index above 0.2 originate from high-risk sources, such as farm animals frequently exposed to antibiotics (Rafiq *et al.* 2024).

Furthermore, the unhygienic handling of poultry during slaughter and processing increases the risk of cross-contamination. Poor sanitation practices, unclean equipment, and contaminated water have been identified as major factors contributing to bacterial contamination in chicken meat (Wardhana *et al.* 2021). The formation of bacterial biofilms in slaughterhouses, particularly under humid conditions, provides a protective environment for pathogens such as Salmonella, making them more resistant to sanitizers and increasing the risk of foodborne infections (Tan *et al.* 2022).

The significant differences in meat color parameters observed in this study suggest that bacterial infections influence muscle composition and oxidative stability. The increased lightness (L value) in Salmonella- and Staphylococcus-challenged birds may be attributed to muscle degradation or oxidative stress induced by bacterial toxins. The higher redness (a value) in Staphylococcus- and *E. coli*-challenged birds could be linked to alterations in muscle pigment composition or stress-induced changes in oxygenation.

The lower pH in *E. coli*-challenged birds suggests increased glycolytic activity or lactic acid accumulation postmortem, which may affect meat texture and shelf life. These findings agree with previous research indicating that bacterial infections can influence meat quality by altering muscle metabolism and oxidative stability (M'Sadeq, 2019).

CONCLUSION:

This study highlights the significant impact of *E. coli*, Salmonella, and Staphylococcus aureus infections on broiler performance, gut morphology, biochemical markers, and meat quality. The findings emphasize the urgent need for improved biosecurity measures, proper hygiene practices, and alternative antimicrobial strategies to mitigate bacterial infections in poultry production. Given the rising threat of antimicrobial resistance, future research should focus on evaluating natural feed

additives, probiotics, and vaccine strategies to enhance gut health and improve poultry productivity while minimizing the use of antibiotics.

REFERENCES:

Al-Humam N A, Reda L, Mohamed R E, El-Ghareeb W R, Darwish W S, and Ibrahim N A. 2021. Prevalence and antibiogram of Listeria monocytogenes in retailed buffalo raw meat and mince with a protection trial using nisin, and gingerol. *Buffalo Bulletin* 40(4): 47-57.

Alam M-U, Rahman M, Islam M A, Asaduzzaman M, Sarker S, Rousham E, and Unicomb L. 2019. Human exposure to antimicrobial resistance from poultry production: assessing hygiene and waste-disposal practices in Bangladesh. *International journal of hygiene and environmental health* **222**(8): 1068-1076.

Andino A, and Hanning I. 2015. Salmonella enterica: survival, colonization, and virulence differences among serovars. *The Scientific World Journal* **215**(1): 520179.

Beski S, M'Sadeq S, Hussein S, and Mahmod A. 2021. Performance and physiological responses of broiler chickens to dietary combination of yeast and medical plants. *Iraqi Journal of Agricultural Sciences* **52**(5): 1117-1125.

de Mesquita Souza Saraiva M, Lim K, do Monte D F M, Givisiez P E N, Alves L B R, de Freitas Neto O C, Kariuki S, Júnior A B, de Oliveira C J B, and Gebreyes W A. 2022. Antimicrobial resistance in the globalized food chain: A One Health perspective applied to the poultry industry. *Brazilian Journal of Microbiology*: 1-22.

Deepthi B, Somashekaraiah R, Poornachandra Rao K, Deepa N, Dharanesha N, Girish K, and Sreenivasa M. 2017. Lactobacillus plantarum MYS6 ameliorates fumonisin B1-induced hepatorenal damage in broilers. *Frontiers in microbiology* **8**: 2317.

Djordjevic S P, Stokes H W, and Chowdhury P R. 2013. Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. *Frontiers in microbiology* **4**: 86.

Doski j m m, M'Sadeq s a, and Beski S s. 2016. Reality and the productivity study for broiler projects in dohuk governorate. *Volume 19 december*: 203.

El-Ghany W A A. 2021. Staphylococcus aureus in poultry, with special emphasis on methicillin-resistant strain infection: a comprehensive review from one health perspective.

Fearnley E, Raupach J, Lagala F, and Cameron S. 2011. Salmonella in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *International Journal of Food Microbiology* **146**(3): 219-227.

Goh Y-X, Anupoju S M B, Nguyen A, Zhang H, Ponder M, Krometis L-A, Pruden A, and Liao J. 2024. Evidence of horizontal gene transfer and environmental selection impacting antibiotic resistance evolution in soil-dwelling Listeria. *Nature communications* **15**(1): 10034.

Haag A F, Fitzgerald J R, and Penadés J R. 2019. Staphylococcus aureus in Animals. *Microbiology spectrum* **7**(3): 10.1128/microbiolspec. gpp1123-0060-2019.

Harrigan, W. F. 1998. Laboratory methods in food microbiology (Vol. 308). Academic Press.

Hasan H, and M'Sadeq S. 2020. Effect of peppermint supplementation as powder or extract on broiler performance, serum biochemical content and gut health under e coli challenge. *The Iraqi Journal of Agricultural Science* **51**(1): 299-310.

Hassan, S.N.2024. Response of Broiler Chickens to Marjoram (Origanum majorana) Medical Plant Challenged with E. coli .Pak. j. life soc. Sci. **22** (2): 12943-12954

Hoerr F J. 2010. Clinical aspects of immunosuppression in poultry. *Avian diseases* **54**(1): 2-15.

Hossain M J, Attia Y, Ballah F M, Islam M S, Sobur M A, Islam M A, Ievy S, Rahman A, Nishiyama A, and Islam M S. 2021. Zoonotic significance and antimicrobial resistance in Salmonella in poultry in Bangladesh for the period of 2011–2021. *Zoonoticdis* **1**(1): 3-24.

Hussein M, Khattak F, Vervelde L, Athanasiadou S, and Houdijk J. (2021). *Effect of amino acid supplementation to reduced crude protein diets on growth performance, organ weights and nutrient digestibility of broiler chickens exposed to sub-clinical enteric health challenges.* Paper presented at the 2021 Poultry Science Association.

Jakaria J, Khasanah H, Priyanto R, Baihaqi M, and Ulum M. 2017. Prediction of meat quality in Bali cattle using ultrasound imaging.

Khishtan A, M'Sadeq S, Beski S, and Omar S A. 2024. Effect of adding chlorogenic acid on broiler performance, some blood biochemical parameters and gut health. *Iraqi Journal of Agricultural Sciences* **55**(2): 652-658.

Lika L. 2019. The European regional order in the Western Balkans facing the disrupting role of middle and great emerging powers.

M'Sadeq S A, Wu S, Swick R A, and Choct M. 2015. Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide. *Animal Nutrition* **1**(1): 1-11.

M'sadeq S. 2019. Effect of dietary supplementation of miaclost on performance and gut morphology in broiler chickens challenged with escherichia coli. *Iraqi Journal Of Agricultural Sciences* **2**(50).

M'Sadeq S A. 2023. Ameliorative effect of yeast cell walls on broiler chickens' performance and gut health under coccidiosis challenge. *Czech J. Anim. Sci* **68**: 346-355.

M'Sadeq S A, Omer F A, Abdullah H M, Mohammed N I, Mizzouri N S, Russell M A, Dickson A J, and Hirst P M. 2024. Assessment of Livestock Production Resources and Challenges Faced By Farmers in Nineveh Plains.

M'Sadeq S A, Wu S-B, Choct M, and Swick R A. 2016. Heat production and energy efficiency of broilers infected with necrotic enteritis. *Avian diseases* **60**(1): 50-55.

Nakhaee P, and Hafez H M. 2025. Public Health Impact of Staphylococcal and Clostridial Infections of Poultry: A Comprehensive Review. *Journal of Poultry Sciences and Avian Diseases* **3**(1): 13-25.

Omwenga I M. (2022). *Pathotyping and antimicrobial resistance-characterization of Staphylococcus aureus in milk for human consumption in Marsabit and Isiolo Counties, Kenya.* University of Nairobi.

Rafiq K, Sani A A, Hossain M T, Hossain M T, Hadiuzzaman M, and Bhuiyan M A S. 2024. Assessment of the presence of multidrug-resistant Escherichia coli, Salmonella and Staphylococcus in chicken meat, eggs and faeces in Mymensingh division of Bangladesh. *Heliyon* **10**(17).

Sadeeq, M. Sadeq S A, and Beski S M. 2024. Effect of nettle on productive performance of broilers and its biochemical, histological, immunological, and antioxidant characteristics. *Mesopotamia Journal of Agriculture* **52**(1): 106.100-121.100.

Sadeeq N, M'Sadiq S, and Beski S. 2024. Effects of nettle content and delivery route on performance, nutrient digestibility, intestinal morphology, immune response, and antioxidant capacity of heat-stressed broiler chickens. *South African Journal of Animal Science* **54**(2): 197-207.

Salman S M, M'Sadeq S a, and Can A. 2024. Effect of in-ovo injection of lavender oil and dietary supplementation of lavender powder on growth, intestine histomorphology, and serum parameters in broilers. *The Indian Journal of Animal Sciences* **94**(12): 1084-1089.

Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, and Choroszy-Krol I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. *Gut pathogens* **11**: 1-16.

Schippa, S., Iebba, V., Barbato, M., Di Nardo, G., Totino, V., Checchi, M. P., ... & Conte, M. P. 2010. A distinctive microbial signature'in celiac pediatric patients. BMC microbiology, 10, 1-10.

Smith J, and Fratamico P. (2017). Escherichia coli as a Pathogen *Foodborne diseases* (pp. 189-208): Elsevier.

Tan S J, Nordin S, Esah E M, and Mahror N. 2022. Salmonella spp. in chicken: prevalence, antimicrobial resistance, and detection methods. *Microbiology Research* **13**(4): 691-705.

Tariq S, Samad A, Hamza M, Ahmer A, Muazzam A, Ahmad S, and Amhabj A M A. 2022. Salmonella in poultry; an overview. *International Journal of Multidisciplinary Sciences and Arts* **1**(1): 80-84.

Wardhana D K, Haskito A E P, Purnama M T E, Safitri D A, and Annisa S. 2021. Detection of microbial contamination in chicken meat from local markets in Surabaya, East Java, Indonesia. *Veterinary world* 14(12): 3138.