



Effects of Ceftriaxone on Body Performance, and Immunological Status of Broilers Experimentally Challenged with *E. coli*

Marwa Ghandour^{1*}, Gamal A. Shams², Heba M. Hassan¹, Heba A. Baz³ and Walaa Fathy Saad Eldin³

¹Animal Health Research Institute, Agriculture Research Center, Cairo, Egypt

²Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt

³Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

The present study was conducted to evaluate the therapeutic effects of ceftriaxone on colibacillosis-induced infection in broilers. Besides, the effects of both colibacillosis infection and ceftriaxone treatment on the body performance and immune parameters of broilers were evaluated. A total of 200 one-day-old broiler chicks (100 healthy, and 100 experimentally challenged with *E. coli*) were used in this investigation. Broilers were divided into four equal groups (n= 50 /each). Group (G1): healthy broilers were kept as a control group. G2: healthy broilers that received a therapeutic dose of ceftriaxone. G3: broilers were experimentally infected with *E. coli* with no treatment. G4: broilers were experimentally infected with *E. coli* and received a therapeutic dose of ceftriaxone. The obtained results revealed that challenged broilers with *E. coli* showed significant decreases in body weight, weight gain, phagocytic percentage, phagocytic index, total protein, albumin, β , γ globulin, total globulin, Superoxide dismutase (SOD), and Glutathione peroxidase (Gpx). Besides, insignificant decreases in lymphocytes, basophils, eosinophils, and monocytes coupled with increases in food conversion ratio (FCR), WBCs, heterophils, nitric oxide, and lysosome, A/G ratio, malondialdehyde (MDA) were recorded in comparison to the control broilers. Infected broilers treated with ceftriaxone showed significant improvement in body weight, weight gain, total protein, albumin, γ globulin, and total globulin. Moreover, other parameters such as WBCs, α , and β globulin, MDA, FCR, heterophils, nitric oxide, lysosome, phagocytic percentage, and phagocytic index, A/G ratio, Tumor necrosis factor α (TNF- α), Interleukin 10 (IL-10), Gpx, and SOD were significantly improved compared with the challenged birds with no treatment. Such results demonstrated the colibacillosis-induced adverse effects on broilers and the use of ceftriaxone as an effective therapeutic candidate.

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Authors' Contribution

MG, GAS and HMH designed the research proposal. MG, HAB and WFS performed the experimental part, funded the study, and wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

Key words

Colibacillosis, Ceftriaxone, Body performance, Broilers, Immunity

INTRODUCTION

With all of the essential amino acids needed for growth, along with unsaturated fatty acids and low cholesterol levels, chicken meat offers a good source of animal protein with a high nutritional value (Darwish *et al.*, 2018).

Avian pathogenic colibacillosis is a disease that is

caused by *Escherichia coli* (*E. coli*) and causes acute, deadly, or subacute infections (Chansiripornchai and Sasipreeyajan, 2002). *E. coli* is one of the common bacteria found in the lower intestines of birds and mammals (Rosario *et al.*, 2004). *E. coli*, a gram-negative, rod-shaped, non-spore-forming bacterium that is also a facultative anaerobe and a member of the Enterobacteriaceae family, is responsible for significant economic losses and mortalities in the chicken industry (Abd El-Tawab *et al.*, 2015). Colibacillosis causes severe symptoms in different poultry species, such as disease symptoms, and lesions include septicemia, arthritis, omphalitis, panophthalmitis, salpingitis, peritonitis, perihepatitis, pericarditis, granuloma, and severe mortalities (Riva *et al.*, 2000).

Chemotherapy is one of the most rapidly advanced branches of applied pharmacology. New drugs are continually being introduced to cure infection with the least possible side effects on the host (Amer *et al.*, 2005).

* Corresponding author: marwaamir554@gmail.com
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A powerful antibacterial cephalosporin with a broad spectrum of activity against both gram +ve and gram -ve bacteria, ceftriaxone is resistant to beta-lactamases. Based on 7-amino-cephalosporic acid, which is equivalent to 6-penic-illanic acid in penicillins, cephalosporins are a class of antibiotics obtained from *Cephalosporium* species (Mohammed, 2018). Similar to other β -lactam antimicrobials, they prevent the production of bacterial cell walls by interfering with penicillin binding proteins (PBPs), which are made up of a six-membered dihydrothiazine ring and a β -lactam ring and are necessary for their antibacterial activity (Prescott, 2013).

The current study aimed to elucidate the effects of *E. coli* infection on body performance and immune status as well as the therapeutic effects of ceftriaxone in the treatment and the control of colibacillosis in broiler chickens.

MATERIALS AND METHODS

Materials

Ceftriaxone (Ceftriaxone^R) was supplied as 250 mg, 500 mg, and 1000 mg of ceftriaxone sodium by SmithKline Beecham for Novartis Pharma Company, Egypt.

E. coli O78 strains were kindly provided by the Animal Health Research Institute, EL-Dokki, Cairo, Egypt.

Experimental phase

For this investigation, 200 one day old cobb broiler chicks purchased from El-Dakahlea Poultry Company in Egypt were kept in clean cages, fed a balanced diet devoid of any medications, and given unlimited access to water and food. Broilers were divided into four groups, each of 50: G1: healthy broilers, and kept as a control group. G2: healthy broilers received ceftriaxone at 50 mg/kg bwt for 5 successive days at the 19th day of age (Pardeep *et al.*, 2011). G3: this group was challenged with *E. coli* O78 without receiving treatment. G4: received ceftriaxone at 50 mg/kg bwt for 5 successive days at the 19th day of age, after two days of experimental infection. Chicks of groups 3 and 4 were experimentally infected with *E. coli* O78 via intranasal administration of 0.3 mL of the bacterial culture containing 3×10^7 (Nakamura *et al.*, 1992). All birds were weighed at the start of the experiment and on the 1st-day post-treatment, the amount of feed consumed was calculated. Body weight gain and feed conversion rate were recorded. Clinical signs, morbidity, and mortality rates were also recorded for *E. coli*-challenged groups. Blood and tissue samples were taken for biochemical and histological analyses.

Blood sampling and analysis

Three blood samples were taken from five different birds from each group on the first post-treatment day; the first sample was taken in a heparin-containing tube to assess the phagocytic percentage and phagocytic index (Lucy and Larry, 1982). To estimate the total and differential leukocytic count, the second sample was collected in an EDTA-containing tube (Jain, 2000). To measure total protein (Doumas *et al.*, 1981), albumin (Bauer, 1982), and protein fraction using cellulose acetate electrophoresis, the third sample was obtained and centrifuged to produce a clear serum (Henry *et al.*, 1974). In addition, lysozyme activity (Schultz, 1987), and nitric oxide (Rajarman *et al.*, 1998) were also evaluated. Antioxidant enzymes superoxide dismutase (SOD), and glutathione peroxidase (Gpx) and malondialdehyde (MDA) were additionally evaluated according to the methods reported before (Draperi and Hadly, 1990; Nishikimi *et al.*, 1972).

Tissue sampling and residue analysis

Five birds were slaughtered on the 1st, 3rd, 5th, 7th, and 9th days post administration of ceftriaxone, tissue specimens were collected from liver, kidney, and breast muscles for estimation of ceftriaxone residues by HPLC (Diven *et al.*, 1981). The method validation was reported before (Abd El-Aziz *et al.*, 2021). Liver samples were also collected for RNA extraction to examine changes in the expression of interleukin 10 (IL10) and tumor necrosis factor α (TNF- α).

Expression of TNF- α and IL10

Using the QIAamp RNeasy Mini kit from Qiagen, GmbH, Germany, and following the manufacturer's instructions, total RNA was extracted from the liver samples. A 25- μ L reaction including 10 μ L of the 2x HERA SYBR[®] Green RT-qPCR Master Mix (Willowfort, UK), 1 μ L of RT Enzyme Mix (20X), 5 μ L of DDW, and 3 μ L of RNA template was used to test the gene expression of IL10, TNF- α , and β -actin as a housekeeping gene. The primers were provided by Metabion, Germany (Table I). An initial real-time PCR system was used to carry out the reaction. Step One Software was used to calculate CT values and amplification curves. The CT of each sample was compared to that of the positive control group according to the " $\Delta\Delta$ Ct" method (Yuan *et al.*, 2006).

Immunohistochemical analysis

Sections from the liver, lung, and intestine were prepared on positively charged coated slides and subjected to immunohistochemical analysis using an antibody that was specifically designed to target CD4 as a marker for T helper cells by a specialized kit bought from Sigma-Aldrich, Germany, and stained with peroxidase dye (Kim *et al.*, 2016).

Table I. Primers sequences, target genes, amplicon sizes, and cycling conditions for qRT-PCR.

Target genes	Primers sequences 5' → 3'	Reverse transcrip- tion	Primary denatura- tion	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	
<i>β-actin</i>	CAACACAGTGCTGTCTGGTGG ATCGTACTCCTGCTTGCTGAT	50°C 30 min.	94°C 15 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	Abdul-Careem <i>et al.</i> (2008)
<i>TNF-α</i>	CCCCTACCCGTGTTCCACAA ACTGCGGAGGGTTTCATTCC						Strong <i>et al.</i> (2015)
<i>IL-10</i>	CATGCTGCTGGGCCTGAA CGTCTCCTTGATCTGCTTGATG						Rothwell <i>et al.</i> (2004)

Table II. Effect of *E. coli* challenge and ceftriaxone treatment on body performance in broilers.

Groups	IBW	1 st -day post treatment				7 th -day post treatment			14 th -day post treatment		
		BW	WG	FCR		BW	WG	FCR	BW	WG	FCR
G1	758.0±0.45 ^b	1250.2±0.37 ^b	492.4±0.24 ^b	1.27±0.08 ^d		1620.0±0.45 ^b	370.0±0.45 ^d	2.16±0.09 ^b	1580±0.45 ^c	230.0±0.45 ^b	4.79±0.02 ^{bc}
G2	760.0±0.45 ^a	1279.6±0.24 ^a	520.0±0.45 ^a	1.30±0.0 ^c		1799.6±0.24 ^a	519.6±0.24 ^a	1.56±0.06 ^d	2000±0.45 ^a	200.0±0.45 ^c	5.77±0.02 ^a
G3	749.6±0.24 ^d	949.6±0.24 ^d	199.60±0.24 ^d	3.29±0.06 ^a		1349.6±0.24 ^d	399.6±0.24 ^b	1.77±0.02 ^c	1550±0.45 ^d	200.0±0.45 ^c	5.08±0.26 ^b
G4	755.0±0.45 ^c	1193.4±6.86 ^c	445.0±0.45 ^c	1.57±0.09 ^b		1588.0±0.45 ^c	388.0±0.45 ^c	2.32±0.01 ^a	1850±0.45 ^b	262.0±0.45 ^a	4.58±0.01 ^c

IBW, initial body weight; BW, Final body weight; WG, Weight gain; FC, Feed consumption; FCR, Feed conversion rate. Values represent mean ± SE. Values carrying different superscript letters within the same column are significantly different at $p < 0.05$. G₁, Control; G₂, Ceftriaxone-treated; G₃, infected with *E. coli*; G₄, *E. coli* infected treated with ceftriaxone.

Statistical analysis

Using the computerized SPSS program version 16, the collected data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD test (Tambane and Dunlop, 2000).

RESULTS AND DISCUSSION

In the veterinary field, antibiotics are used in the treatment of bacterial infections and used as feed additives to enhance feed conversion. However, many of these antibiotics can suppress the immune system even at therapeutic levels via their interference with the protein or immunoglobulin synthesis (Shalaby, 1989).

Challenge of broiler chicks with *E. coli* O78 could lead to induction of colibacillosis. The affected birds showed general clinical signs of sickness including reduction in appetite, diarrhea, dehydration, and weakness. At postmortem inspection of the dead birds, enlargements in the liver with fibrinous perihepatitis, pericarditis, and air sacculitis were observed. The morbidity and mortality rates were 60%, and 20%, respectively. Use of ceftriaxone as a treatment in the second day after the appearance of the symptoms could reduce morbidity and mortality rates

to 10%, and 4%, respectively. Similar clinical signs and postmortem lesions were reported before (Lutful-Kabir, 2010).

The recorded results of the current study revealed that healthy broilers that received ceftriaxone at the recommended doses for 5 successive days showed an increase in body weight with a significant increase ($p < 0.05$) in weight gain with an improved FCR when compared with the control group. Broiler chicks infected with *E. coli* showed a significant decrease in body weight and weight gain besides an increase in FCR in comparison to control broilers. Meanwhile, infected broilers treated with ceftriaxone showed a significant decrease in body weight and weight gain besides an increase in FCR but lesser than the infected non-treated broilers (Table II). The reduction in body performance of infected birds might be due to damage in gut epithelium impairing food absorption by pathogenic bacteria (Abd El-Aziz, 2002). The same author reported that antibacterials if given in very small amounts, produce an increase in the growth rate in growing chicks and increase body weight gain with improved FCR via their antibacterial effects. Besides, El-Kadeem (2009) stated that another cephalosporin, ceftiofur, could induce a significant increase in body weight, weight gain, and feed

consumption with a decrease in FCR. In addition, [Elena *et al.* \(2015\)](#) stated that ceftriaxone up to 2 g/kg/day is safe and improves body performance. [Ashraf *et al.* \(2019\)](#) found that infected broilers with *E. coli* showed a significant decrease in weight gain and an increase in FCR. Infected broilers showed a reduction in growth performance and an increase in FCR ([El-Tahawy *et al.*, 2022](#)). Likely, [Mithin *et al.* \(2022\)](#) mentioned that ceftriaxone is very beneficial in the control of *E. coli* infection and improves weight gain and body performance.

Healthy broilers that received ceftriaxone at the tested doses showed substantial changes in their lymphocyte and monocyte counts as well as a significant drop in their WBC and heterophil counts. WBCs and heterophils significantly increased in *E. coli*-infected broilers, but lymphocytes, basophils, eosinophils, and monocytes significantly decreased. Ceftriaxone treatment of *E. coli*-infected broilers resulted in a large increase in lymphocytes and monocytes while significantly decreasing WBCs and heterophils ([Table III](#)). Likely, infected broilers with *E. coli* showed an increase in WBCs and heterophils ([Khalid *et al.*, 2019](#)). In addition, broilers infected with *E. coli* showed a considerable rise in leukocytes and heterophils, according to [Ashraf *et al.* \(2019\)](#). WBC levels increased in broilers with *E. coli* infection ([El-Tahawy *et al.*, 2022](#)).

[Haq *et al.* \(2015\)](#) observed that cefpodoxime-treated pigeons with *E. coli* infections saw a similar shift in their leukogram. [Mohammed \(2018\)](#) also claimed that the leukogram improved after taking cephalosporin, which is useful in treating colibacillosis.

Healthy broilers that received ceftriaxone in the examined doses revealed a significant decrease in nitric oxide and lysosome activity. However, infected broilers with *E. coli* revealed a significant increase in nitric oxide and lysosome in comparison to control broilers but the infected group with *E. coli* and treated with ceftriaxone revealed a significant increase in nitric oxide, lysosome as compared to control broilers but lesser than the infected group without treatment ([Table IV](#)). In agreement with the obtained results, [Paulsen *et al.* \(2003\)](#) recorded that *E. coli* infection increased serum lysozyme activity. The same change in nitric oxide and lysozyme activity was observed by [Mohammed \(2018\)](#) in boilers infected with *E. coli*. Similar results were recorded by [Ashraf *et al.* \(2019\)](#) who reported that infected broilers with *E. coli* displayed a significant increase in nitric oxide and lysozyme activity. Moreover, infected broilers treated by cefquinome showed a significant decrease in nitric oxide and lysozymes in comparison to infected non-treated broilers ([El-Tahawy *et al.*, 2022](#)).

Table III. Effect of *E. coli* challenge and ceftriaxone treatment on leukogram in broilers.

		1 st day				7 th day			
		G1	G2	G3	G4	G1	G2	G3	G4
Total WBCs		10.80±1.09 ^c	9.96±1.04 ^d	12.84±1.14 ^a	11.70±1.19 ^b	10.78±0.78 ^a	10.09±0.74 ^a	12.17±0.44 ^a	11.06±0.38 ^b
Differen- tial count	Lymphocytes	4.63±0.13 ^a	4.00±0.19 ^b	3.84±0.43 ^b	4.07±0.75 ^b	4.90±0.61 ^a	4.19±0.39 ^b	3.98±0.36 ^b	4.23±0.51 ^b
	Heterophils	3.73±0.38 ^c	3.35±0.52 ^c	5.92±0.74 ^a	4.29±0.59 ^b	3.79±0.83 ^{bc}	3.61±0.42 ^c	5.19±0.25 ^a	4.03±0.27 ^b
	Monocytes	1.75±0.66 ^b	2.05±0.46 ^a	1.46±0.42 ^c	1.71±0.37 ^b	1.76±0.11 ^b	1.99±0.05 ^a	1.48±0.29 ^c	1.74±0.24 ^b

Values represent mean ± SE. Values carrying different superscript letters within the same row on the same day are significantly different at $p < 0.05$. For details of groups (G), see [Table II](#).

Table IV. Effect of *E. coli* challenge and ceftriaxone treatment on nitric oxide, lysosome, and phagocytic activities in broilers .

Groups	Nitric oxide			Lysosome			Phagocytosis on the 7 th day	
	1 st day	2 nd day	3 rd day	1 st day	2 nd day	3 rd day	%	Index
G1	90.80 ± 0.37 ^a	91.20 ± 0.58 ^b	88.80 ± 0.37 ^b	6.57 ± 0.12 ^c	6.88 ± 0.05 ^c	6.73 ± 0.08 ^b	74.20±0.58 ^b	3.48±0.11 ^b
G2	79.00 ± 0.84 ^c	79.60 ± 0.51 ^c	76.80 ± 0.73 ^d	4.36 ± 0.09 ^d	5.02 ± 0.12 ^d	5.57 ± 0.15 ^c	76.80±0.58 ^a	3.96±0.07 ^a
G3	98.60 ± 0.51 ^a	97.40 ± 1.69 ^a	95.60 ± 1.12 ^a	10.68 ± 0.10 ^a	9.01 ± 0.22 ^a	8.52 ± 0.13 ^a	56.80±0.8 ^d	1.88±0.11 ^d
G4	86.40 ± 1.08 ^b	82.60 ± 1.69 ^c	84.20 ± 1.16 ^c	7.86 ± 0.20 ^b	7.59 ± 0.12 ^b	6.63 ± 0.34 ^b	64.40±0.87 ^c	2.70±0.07 ^c

Values represent mean ± SE. Values carrying different superscript letters within the same column are significantly different at $p < 0.05$. For details of groups (G), see [Table II](#).

Table V. Effect of *E. coli* challenge and ceftriaxone treatment on protein picture in broilers.

Groups	T protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)				A/G ratio
			α	β	γ	Total	
7 th day							
G1	5.67 ± 0.11 ^a	2.27 ±0.04 ^{ab}	1.01 ± 0.11 ^a	1.00 ± 0.06 ^a	0.96 ± 0.01 ^a	3.02 ± 0.22 ^a	0.60 ± 0.04 ^b
G2	3.87 ± 0.19 ^c	1.68 ± 0.18 ^c	0.48 ± 0.07 ^b	0.97 ± 0.01 ^a	0.89 ±0.02 ^a	2.19 ± 0.2 ^{bc}	0.97 ± 0.07 ^a
G3	3.38 ± 0.19 ^d	1.86 ±0.12 ^{bc}	0.92 ± 0.05 ^a	0.86 ± 0.11 ^a	0.31 ± 0.03 ^c	1.83 ± 0.16 ^c	1.04 ± 0.09 ^a
G4	4.82 ± 0.04 ^b	2.63 ± 0.16 ^a	0.98 ± 0.11 ^a	1.00 ± 0.06 ^a	0.54 ± 0.03 ^b	2.38 ± 0.08 ^b	1.05 ± 0.05 ^a
14 th day							
G1	5.26 ± 0.16 ^a	2.48 ± 0.04 ^a	0.93 ± 0.27 ^a	0.63 ± 0.18 ^a	0.88 ± 0.16 ^a	2.83 ± 0.17 ^a	0.89 ± 0.06 ^a
G2	4.22 ± 0.08 ^b	1.73 ± 0.08 ^b	0.83 ± 0.07 ^a	0.73 ± 0.05 ^a	0.72 ± 0.14 ^a	2.42 ±0.09 ^{ab}	0.60 ± 0.05 ^a
G3	3.61 ± 0.16 ^c	1.73 ± 0.15 ^b	0.65 ± 0.1 ^a	0.76 ± 0.05 ^a	0.49 ± 0.03 ^b	1.92 ± 0.13 ^b	0.91 ± 0.08 ^a
G4	5.11 ± 0.17 ^a	1.86 ± 0.1 ^b	0.94 ± 0.27 ^a	0.64 ± 0.17 ^a	0.66 ±0.06 ^{ab}	1.99 ± 0.24 ^b	0.91 ± 0.16 ^a

Values represent mean $\pm$ SE. Values carrying different superscript letters within the same column on the same day are significantly different at $p < 0.05$. For details of groups (G), see Table II. A, Albumin; G, Globulin.

The treated group with ceftriaxone showed an increase in phagocytic percentage and index. Infected broilers with *E. coli* revealed a significant decrease in Phagocytic percentage and index in comparison to the control broilers; while the infected broilers treated with ceftriaxone showed a significant increase in phagocytic percentage and index in comparison to infected nontreated broilers (Table IV). The above-mentioned results were supported by Ashraf *et al.* (2019) who stated that broilers infected with *E. coli* revealed a significant decrease in phagocytic percentage and index. Besides, Mohamed and Younis (2018) stated that colibacillosis induced a significant decrease in phagocytosis. Interestingly, ceftriaxone was reported to improve phagocytosis (Peter and Micheal, 2021).

The study was extended to investigate the effects of either the challenge with *E. coli* or the treatment with ceftriaxone on the protein picture in the examined broilers. The obtained results demonstrated that birds exposed to ceftriaxone without bacterial infection showed a significant decrease in total protein, albumin, α , β , γ globulin, and total globulin besides a significant increase in A/G ratio as compared to control birds. Such reduction in the total protein and albumin levels might indicate ceftriaxone-induced hepatotoxicity (Galvão *et al.*, 2014). Likely, ceftriaxone induced liver damage and reduced total protein and albumin in a previous report (Ahmed, 2015). Infected broilers with *E. coli* revealed a significant decrease in total protein, albumin, β , γ globulin, and total globulin besides a significant increase in the A/G ratio. Infected broilers with *E. coli* and treated with ceftriaxone revealed a significant decrease in total protein, albumin, α , γ globulin, and total globulin beside a non-significant decrease of α and β globulin coupled with a significant increase in A/G ratio as compared with control broilers

(Table V). Similarly, broilers infected with *E. coli* showed a significant reduction in serum total protein and albumin (El-Keredy *et al.*, 2019). Besides, Ashraf *et al.* (2019) stated that infected broilers with *E. coli* and treated with cephradine showed improvement in the protein picture.

The current investigation additionally investigated the changes in the gene expression of some hepatic inflammatory cytokines including TNF- α and IL-10 (Table VI). Broilers that received ceftriaxone without infection revealed an insignificant decrease in TNF- α and IL-10 as compared with control birds. Infected broilers with *E. coli* revealed a significant increase in TNF- α and IL-10 in comparison to control broilers. Ceftriaxone treatment of the challenged birds significantly reduced TNF- α and IL-10 gene expression compared with the challenged group without treatment. The obtained results agree with Rao *et al.* (2014) who stated that ceftriaxone induced a significant decrease in plasma levels of IL-10 and TNF- α . In addition, Abd-El Rhman *et al.* (2018) stated that chickens infected with *E. coli* showed an elevation in the liver IL-10. Moreover, Elnagar *et al.* (2021) stated that *E. coli* displayed a significant increase in the level of ileal IL-10. The protective effects of ceftriaxone agree with Arhoumah (2018) who reported that cefepime at a dose of 45 mg/kg bwt significantly ameliorated the alterations in TNF- α and IL-10.

We further explored the effects of *E. coli* and/or ceftriaxone treatment on the antioxidant status in the challenged birds. The obtained results revealed that ceftriaxone caused a significant decrease in serum Gpx, and SOD, and an increase in MDA when compared to control broilers. Similarly, infected birds with *E. coli* showed a significant decrease in serum Gpx and SOD with an increase in MDA. However, broilers infected with *E. coli* and treated with ceftriaxone showed a significant increase

Table VI. Effect of *E. coli* challenge and ceftriaxone treatment on SOD, Gpx, and MDA in broilers.

		G1	G2	G3	G4
SOD	1 st day	97.80 ± 8.21 ^a	72.20 ± 3.37 ^{bc}	63.60 ± 3.46 ^c	82.0 ± 35 ^b
	7 th day	90.80 ± 1.56 ^a	89.20 ± 1.96 ^a	87.0 ± 1.67 ^b	90.0 ± 0.89 ^a
	14 th day	92.80 ± 1.66 ^a	90.60 ± 2.98 ^{ab}	83.0 ± 2.59 ^b	87.80 ± 3.43 ^{ab}
Gpx	1 st day	112.40 ± 3.97 ^a	100.40 ± 1.14 ^b	88.0 ± 1.58 ^d	94.60 ± 1.14 ^c
	7 th day	112.20 ± 2.59 ^a	101.80 ± 1.92 ^b	87.80 ± 1.79 ^d	96.0 ± 1.87 ^c
	14 th day	112.0 ± 2.65 ^a	100.60 ± 8.26 ^b	98.40 ± 6.80 ^b	106.20 ± 4.60 ^{ab}
MDA	1 st day	5.99 ± 0.32 ^d	8.15 ± 0.29 ^c	10.49 ± 0.35 ^a	9.41 ± 0.36 ^b
	7 th day	5.84 ± 0.31 ^d	9.33 ± 0.42 ^b	10.70 ± 0.04 ^a	7.79 ± 0.13 ^c
	14 th day	5.94 ± 0.24 ^d	5.99 ± 0.67 ^b	10.91 ± 0.16 ^a	5.58 ± 0.21 ^b

Values represent mean ± SE. Values carrying different superscript letters within the same row are significantly different at $p < 0.05$. For details of groups (G), see Table II. SOD, superoxide dismutase; Gpx, glutathione peroxidase; MDA, malondialdehyde.

in serum Gpx and SOD besides a decrease in MDA (Table VII). These results coincided with that obtained by Khaled *et al.* (2014) who stated that ceftriaxone induced an increase in serum MDA coupled with a significant decrease in SOD and Gpx. These findings were supported by Dayana and Manasa (2020) who confirmed that ceftriaxone induced lipid peroxidation and altered the activities of antioxidant enzymes. Besides, *E. coli* infection induced the accumulation of reactive oxygen species, and oxidant/antioxidant imbalance which led to decreased levels of SOD and GPX (El-Kilany *et al.*, 2018). In addition, Ashraf *et al.* (2019) recorded that infected broiler with *E. coli* treated with another cephalosporin (cephradine) showed improvement in Gpx, SOD, and MDA.

Table VII. Effect of *E. coli* challenge and ceftriaxone treatment on TNF- α , and IL-10 in broilers' livers.

		G1	G2	G3	G4
TNF- α	1 st	0.97±0.02 ^c	0.90±0.01 ^c	3.66±0.08 ^a	1.34±0.12 ^b
	3 rd	0.98±0.02 ^b	0.86±0.02 ^{bc}	2.61±0.1 ^a	0.72±0.01 ^c
IL-10	1 st	0.98±0.01 ^c	0.88±0.03 ^c	3.84±0.23 ^a	1.65±0.1 ^b
	3 rd	0.99±0.01 ^b	0.72±0.01 ^c	2.65±0.15 ^a	0.33±0.08 ^d

Values represent mean ± SE. Values carrying different superscript letters within the same row are significantly different at $p < 0.05$. For details of groups (G), see Table II. TNF- α , tumor necrosis factor α ; IL-10, interleukin 10.

Peroxidase-stained lung and intestinal tissues showed negative expression of CD4, a type 1 transmembrane protein that reflects the immune status of the birds. While peroxidase-stained tissues of *E. coli*-infected broilers showed a strong expression for CD4. While tissues of broilers challenged with *E. coli* and received ceftriaxone treatment showed moderate expression of CD4 compared with the infected group without treatment (Fig. 1). Likely, co-challenge of broiler chicken with colibacillosis and

viral respiratory infections caused a drastic induction of CD4 (Weerts *et al.*, 2021).

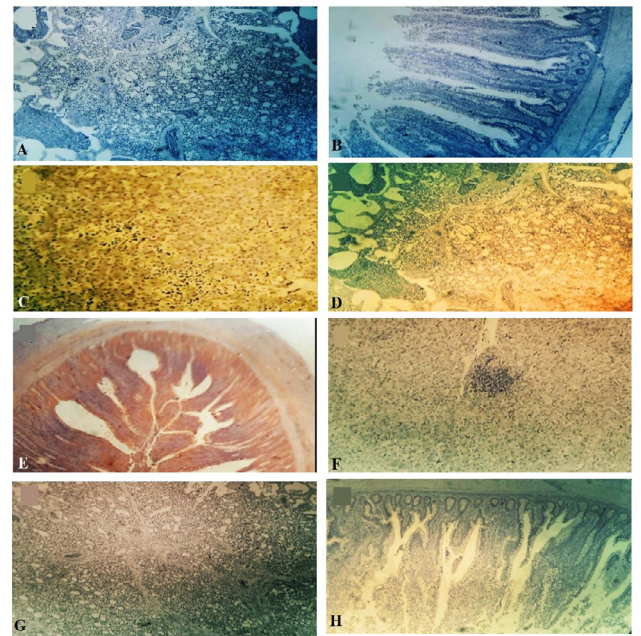


Fig. 1. Peroxidase stained slides of A) lung of control broilers showing negative expression of CD4 (X200), B) intestine of control broilers showing negative expression of CD4 (X200), C) liver of *E. coli*-infected broilers showing strong expression of CD4 (X200), D) lung of *E. coli*-infected broilers showing severe expression of CD4 (X400), E) intestine of *E. coli*-infected broilers showing strong expression of CD4 (X400), F) liver of *E. coli*-infected broilers and treated with ceftriaxone showing moderate expression of CD4 (X200), G) lung of *E. coli*-infected broilers and treated with ceftriaxone showing weak expression of CD4 (X200), H) intestine of *E. coli*-infected broilers and treated with ceftriaxone showing weak expression of CD4 (X400), at the first-day post-treatment.

Table VIII. Ceftriaxone residues in the tissues of the healthy and infected broilers.

	G2			G4		Kidney
	Muscle	Liver	Kidney	Muscle	Liver	
1 st day	1564.50 ± 44.72 ^a	3719.70 ± 89.44 ^a	4341.30 ± 89.44 ^a	945.37 ± 54.77 ^a	2340.200 ± 112.25 ^a	2655.50 ± 136.93 ^a
3 rd day	175.75 ± 2.24 ^b	415.13 ± 6.71 ^b	641.35 ± 17.89 ^b	172.70 ± 8.94 ^b	257.15 ± 22.36 ^b	290.770 ± 8.94 ^b
5 th day	57.39 ± 3.13 ^c	141.12 ± 8.94 ^c	149.30 ± 14 ^c	ND	82.77 ± 3.74 ^c	113.040 ± 0.75 ^c
7 th day	ND	ND	ND	ND	ND	ND
9 th day	ND	ND	ND	ND	ND	ND

ND, Not detected. Values represent mean ± SE of ceftriaxone residues in different tissues of broilers of G2 and G4. Values carrying different superscript letters within the same column are significantly different at $p < 0.05$.

In order to estimate ceftriaxone residues in the different tissues of the treated broilers, an HPLC approach was employed. The obtained results showed that healthy or diseased broilers that received ceftriaxone showed ceftriaxone residue detected in muscles, livers, and kidneys from the 1st to 5th-day post-injection and disappeared on the 7th-day after treatment. The highest residual level was detected in the liver, followed by the kidney, then muscles, respectively particularly in the healthy birds (Table VIII). Likely, Li *et al.* (1995) detected ceftriaxone in muscles, livers, and kidneys. In addition, El-Sayed *et al.* (2018) stated that cefotaxime was detected in liver tissues (120 h), and kidneys (96 h) of chickens post-last administrations. The same residue was detected in the plasma, liver, kidney, heart, lungs, and muscle tissue of treated birds (Mithin *et al.*, 2022).

CONCLUSION

In conclusion, colibacillosis could induce several adverse effects related to FCR, immune response, inflammatory cytokines, and antioxidant balance in broilers. Ceftriaxone treatment of diseased birds could ameliorate such adverse effects. Therefore, ceftriaxone can act as an ideal candidate for the treatment of colibacillosis in broilers.

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IRB approval

All the procedures were approved by the Institutional

Review Board (IBR number, ZU-IACUC/2/F/52/2022).

Ethical statement

All experiments using animals were conducted according to Zagazig University guidelines. This study received an ethical approval number ZU-IACUC/2/F/52/2022.

Statement of conflict of interest

The authors have declared no conflict of interest.

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