

## Haematological And Biochemical Studies On Danofloxacin And Ceftiofur Sodium In Healthy And Experimentally Infected Broiler Chickens With E. coli

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### ABSTRACT

Oral administration of danofloxacin in therapeutic dose (5mg /kg b. wt in drinking water) or subcutaneous administration of ceftiofur sodium (2mg/kg b.wt s/c) for three successive days in healthy chickens displayed a significant increase in body weight, body weight gain, leucocytic count, AST, ALT and ALP activities, globulin, uric acid, creatinine,  $Ca^{2+}$  and  $Mg^{2+}$  levels. Also a significant decrease in RBCs, haemoglobin concentration and packed cell volume percent, total protein, albumin and phosphorus levels were recorded.

Chickens experimentally infected with E.coli O<sub>78</sub> produced a significant decrease in body weight, body weight gain, RBCs count, haemoglobin concentration and packed cell volume percent, total protein, albumin and phosphorus levels. Moreover, a significant increase in total leucocytic count, AST, ALT and ALP activities, globulin, uric acid, creatinine,  $Ca^{2+}$  and  $Mg^{2+}$  levels were recorded in E.coli infected chickens.

Medication of the E.coli infected chickens with therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/kg b.wt s/c) improved haematological picture and serum biochemical constituents toward control level.

Sensitivity of E.coli O<sub>78</sub> used in this study to antimicrobials revealed that it was highly sensitive to danofloxacin and ceftiofur sodium whereas other antimicrobials showed variable sensitivity.

### INTRODUCTION

Antimicrobials agents are widely used in veterinary medicine to overcome bacterial infection in both poultry and animal farms. Danofloxacin has broad-spectrum bactericidal activity against Gram +ve and Gram-ve bacteria. It is effective against many chloramphenicol resistant organisms like E.coli, S. typhi as it is not affected by chloramphenicol acetyl transferase (1). Ceftiofur sodium is cephalosporin antibiotic which has worldwide approvals for respiratory diseases in chicks and turkey poults (2).

E.coli infection is considered as one of the most serious problems affecting poultry (3). It causes high losses among infected broilers, reduction in body weight and increase in therapy costs. E.coli infection includes various forms of infection such as

colibacillosis characterized by septicemia, pericarditis, airsacculitis, perihepatitis and enteritis (4).

Hence, the present study was conducted to evaluate some pharmacological studies of danofloxacin or ceftiofur sodium on colibacillosis in experimentally infected chickens with E.coli. Throwing light on possible side effects of danofloxacin or ceftiofur sodium that could be reflected on body weight and body weight gain, haematological as well as liver and kidney dysfunctions and its effect on some electrolytes such as  $Ca^{2+}$ ,  $ph^{2+}$  and  $Mg^{2+}$ .

### MATERIALS AND METHODS

#### Drugs:

Danofloxacin (Advocin)<sup>®</sup> was obtained from Pfizer Inc. New York USA. Drug is a cream-

colored, free-flowing powder packed in 75 and 150 gm laminated foil packs. The recommended therapeutic dose is 5 mg / kg b.wt for 3 successive days in drinking water (5).

**Ceftiofur sodium** (EXCENEL)<sup>®</sup> was obtained from GlaxoSmithKline – Pennsylvania – USA. Drug is in the form of sterile powder (one or four gram vials). The recommended therapeutic dose is 2mg/kg b.wt s/c for 3 successive days (6).

#### Chickens

One hundred and twenty (120) apparently healthy, one-day old unsexed Hubbard broiler chicks were obtained from El-Kahera farm 10<sup>th</sup> of Ramadan city. They were reared in environmentally conditioned separated units. They were fed on a balanced commercial ration free from medication from Cairo poultry company. The ration were free

from medication. Water was provided *ad-libitum*.

#### E.coli strain

Serotype O<sub>78</sub> was kindly supplied from Animal health research Institute, El-Dokki, Cairo.

#### Vaccines:

**Hitchener B<sub>1</sub> strain:** was obtained from vaccine Nobilis and manufactured by intervet international B.V. Boxmeer Holland.

**Gumboro vaccine:** was obtained from Gumboro vaccine Nobilis and manufactured by intervet international B. V. Boxmeer Holland.

#### Experimental design

On the 15<sup>th</sup> day of age, chicks were divided into 6 groups each, of 20 chicks in environmentally separated units as shown in Table 1.

Table 1. Experimental design applied for studying the effect of therapeutic dose of danofloxacin or ceftiofur sodium (n= 20).

Group	Dose
1-Non-infected non treated (Healthy control)	_____
2-Non-infected treated with danofloxacin	5mg /kg b. wt. in drinking water for 3 successive days
3-Non-infected treated with ceftiofur sodium	2mg/ kg b. wt. s/c for 3 successive days
4-Infected non treated (Infected control)	_____
5-Infected treated with danofloxacin	5mg /kg b. wt. in drinking water for 3 successive days
6-Infected treated with ceftiofur sodium	2mg/ kg b. wt. s/c for 3 successive days

#### Vaccinations

All chicks were vaccinated by Hitchener B<sub>1</sub> vaccine at one week old and Gumboro vaccine at 14<sup>th</sup> day.

#### Experimental infection

Experimental infection by E.coli was performed intrathoracically at the left posterior thoracic sac on the 15<sup>th</sup> day with 0.5 ml broth containing 10<sup>6</sup> colony forming unit (C.F.U).

#### Treatment

Treatment with danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/kg b.wt s/c) for 3 successive days started 48 hours post infection.

#### Blood sampling

First blood sample was mixed with EDTA as anticoagulant for hematological studies. Second blood sample was taken without anticoagulant, left to clot, centrifuged

at 3000 r.p.m for 20 minute and sera were separated for estimation of liver and kidney functions as well as concentration of some electrolytes ( $\text{Ca}^{2+}$ ,  $\text{ph}^{2+}$  and  $\text{Mg}^{2+}$ )

#### Body weight and body weight gain

The effect of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/kg b.wt s/c) for 3 successive days on body weight and body weight gain, of healthy and experimentally infected chickens with E.coli measured all over the experimental period.

#### Haematological studies

Blood sample with anticoagulant were examined for RBCs count and WBCs count (7), haemoglobin concentration (8) and packed cell volume % (9).

#### Biochemical analysis

Blood sample were analyzed for transaminases, AST and ALT (10), alkaline phosphatase (11), total protein (12), albumin (13), uric acid (14), creatinine (15), inorganic calcium (16), inorganic phosphorus (17) and magnesium (18).

#### Statistical analysis

Student "t" test was carried out (19).

### RESULTS

#### Sensitivity test

Danofloxactn or ceftiofur sodium has potent inhibitory effect on the tested organisms than other tested antimicrobial agents.

#### Clinical signs

Two days post inoculation of E.coli, all infected chickens (infected non-treated) showed clinical symptoms such as: diarrhea, depression, respiratory symptoms including sneezing, gasping, mouth breathing, rales, nasal discharge and cough.

Infected birds and treated with therapeutic doses of danofloxactin or ceftiofur sodium showed milder degree of clinical symptoms than that of infected non-treated chickens.

#### Mortality rate

Mortality rate was high (25%) in group infected with E. coli which decreased to 5%

after treatment with danofloxacin or ceftiofur sodium.

#### Lesion scores

Post- mortem examinations of both dead and sacrificed chicks of all groups were recorded. All chickens of the group non-infected non-treated and non infected treated with danofloxacin or ceftiofur sodium revealed no lesions scores in different organs. The main pathological lesions in chickens experimentally infected with E.coli were air sacculitis (45%), Pericarditis (33%), perihepatitis (25%), ascitis (25%), and enteritis (30%).

Medication of E.coli infected chickens with danofloxactn or ceftiofur sodium reduced gross lesions of E.coli infection, air sacculitis (15% and 20%), percarditis (10% and 15%), perihepatitis (5% and 5%), ascitis (5% and 5%) and entritis (10% and 15%) respectively.

#### Body weight and body weight gain

Chickens non-infected treated with danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for 3 successive days produced a significant increase in body weight and body weight gain on 1<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post treatment.

Experimental infection of chickens with E.coli evoked a significant decrease in both body weight and body weight gain on 1<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post infection.

Administration of danofloxacin or ceftiofur sodium for 3 successive days to chickens experimentally infected with E.coli induced a significant increase in body weight and body weight gain when compared with infected non-treated group on 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post treatment (Table 2).

#### Haematological results

In chickens non-infected treated with danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for 3 successive days displayed a significant increase in WBCs count with a significant decrease in RBCs count, haemoglobin

concentration and packed cell volume percent on 1<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post treatment.

Chickens experimentally infected of with E.coli and non medicated evoked a significant decrease in RBCs count, haemoglobin concentration and packed cell volume percent with a significant increase in leucocytic count on 1<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post infection compared with control group all over experimental period.

Medication of E.coli infected chickens with danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for 3 successive days produced a significant increase in RBCs count, haemoglobin concentration and packed cell volume percent with a significant decrease in leucocytic count (Tables 3 and 4).

#### Enzyme activities

Groups non-infected and treated with danofloxacin or ceftiofur sodium displayed a significant increase in AST, ALT and ALP activities all over experimental period. The groups infected of with E.coli and non medicated evoked a significant increase in AST, ALT and ALP activities all over experimental period. Chickens experimentally

infected with E.coli and medicated with danofloxacin or ceftiofur sodium displayed a significant decrease in AST, ALT and ALP activities on 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post treatment when compared with infected non treated group (Table 5).

#### Serum biochemical and electrolytes

Groups non-infected and treated with danofloxacin or ceftiofur sodium revealed a significant decrease in total protein, albumin and Ca<sup>2+</sup> levels with a significant increase in globulin, uric acid, creatinine, phosphorus and Mg<sup>2+</sup> levels post treatment all over experimental period.

Birds experimentally infected of with E.coli and non treated evoked a significant decrease in total protein, albumin, Ca<sup>2+</sup> levels with a significant increase in globulin, uric acid, creatinine, Mg<sup>2+</sup> and phosphorus levels all over experimental period.

Medication of E.coli infected chickens with danofloxacin or ceftiofur sodium displayed a significant decrease in uric acid, creatinine, Mg<sup>2+</sup> and phosphorus levels with a significant increase in total protein, albumin and calcium levels all over experimental period (Tables 6- 8).



Table 2. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on body weight and body weight gain of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5)

Groups	Body weight at beginning of experiment (gm)	Days post - treatment					
		1 <sup>st</sup> day		7 <sup>th</sup> day		14 <sup>th</sup> day	
		Body weight (gm)	Body gain (gm)	Body weight (gm)	Body gain (gm)	Body weight (gm)	Body gain (gm)
1-Non-infected non- treated (healthy control)	286.7 $\pm$ 4.95	383.6 $\pm$ 4.92	97.85 $\pm$ 1.2	743.6 $\pm$ 4.70	533.6 $\pm$ 3.84	1190.3 $\pm$ 5.26	477.2 $\pm$ 3.74
2-Non-infected treated with danofloxacin	291.1 $\pm$ 4.73	* 422.8 $\pm$ 5.66	** 131.7 $\pm$ 26	* 824.1 $\pm$ 5.34	* 401.9 $\pm$ 4.52	*** 1389.5 $\pm$ 6.83	* 500.6 $\pm$ 4.53
3- Non-infected treated with ceftiofur sodium	276.5 $\pm$ 3.15	* 417.8 $\pm$ 4.63	*** 141.3 $\pm$ 2.2	** 808.8 $\pm$ 6.43	* 390.1 $\pm$ 3.75	** 1266.6 $\pm$ 6.96	*** 457.6 $\pm$ 5.56
4-Infected non-treated (infected control)	280.2 $\pm$ 3.84	** 339.2 $\pm$ 5.25	** 58.8 $\pm$ 1.76	** 617.5 $\pm$ 5.81	** 277.4 $\pm$ 3.38	*** 850.8 $\pm$ 7.21	*** 343.3 $\pm$ 4.28
5- Infected treated with danofloxacin	282.2 $\pm$ 3.84	+ 370.2 $\pm$ 6.18	++ 87.9 $\pm$ 2.45	++ 716.9 $\pm$ 6.65	++ 346.8 $\pm$ 3.27	+++ 1126.3 $\pm$ 6.83	++ 409.7 $\pm$ 4.48
6-Infected treated with ceftiofur sodium	295.9 $\pm$ 4.35	+ 372.8 $\pm$ 4.28	+ 76.4 $\pm$ 2.47	+ 705.6 $\pm$ 7.61	++ 333.2 $\pm$ 3.56	+++ 1079.2 $\pm$ 6.93	++ 391.6 $\pm$ 4.68

. or + P < 0.05    .. or ++ p < 0.01    ... or +++ p < 0.001

. Compared with non-infected non-treated group

+ Compared with infected treated group

Fig 3. The effect of therapeutic dose of danofloxacin(5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on body weight gain of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5)

Groups	Days post - treatment								
	1 <sup>st</sup> day			7 <sup>th</sup> day			14 <sup>th</sup> day		
	RBCs count (10 <sup>6</sup> /mm <sup>3</sup> )	Hb (gm/dl)	PCV %	RBCs count (10 <sup>6</sup> /mm <sup>3</sup> )	Hb (gm/dl)	PCV %	RBCs count (10 <sup>6</sup> /mm <sup>3</sup> )	Hb (gm/dl)	PCV %
1-Non-infected non-treated (healthy control)	2.87 $\pm$ 0.057	11.29 $\pm$ 0.046	33.2 $\pm$ 0.25	2.95 $\pm$ 0.063	11.26 $\pm$ 0.038	34.51 $\pm$ 0.41	3.11 $\pm$ 0.074	10.71 $\pm$ 0.039	33.42 $\pm$ 0.41
2-Non-infected treated with danofloxacin	* 2.45 $\pm$ 0.076	* 10.82 $\pm$ 0.057	* 31.5 $\pm$ 0.29	* 2.56 $\pm$ 0.072	* 10.36 $\pm$ 0.027	* 32.6 $\pm$ 0.35	* 2.89 $\pm$ 0.063	* 10.11 $\pm$ 0.035	* 32.56 $\pm$ 0.37
3- Non-infected treated with ceftiofur sodium	** 2.38 $\pm$ 0.054	** 10.21 $\pm$ 0.049	* 31.2 $\pm$ 0.34	** 2.49 $\pm$ 0.058	* 10.98 $\pm$ 0.065	* 31.9 $\pm$ 0.41	* 2.62 $\pm$ 0.075	* 10.29 $\pm$ 0.037	* 31.45 $\pm$ 0.26
4-Infected non-treated (infected control)	** 2.15 $\pm$ 0.062	*** 8.79 $\pm$ 0.063	*** 25.9 $\pm$ 34	*** 2.24 $\pm$ 0.064	*** 8.94 $\pm$ 0.056	* 28.15 $\pm$ 0.35	* 2.46 $\pm$ 0.054	** 9.32 $\pm$ 0.025	** 29.17 $\pm$ 0.39
5- Infected treated with danofloxacin	+ 2.26 $\pm$ 0.035	++ 9.36 $\pm$ 0.059	+ 27.8 $\pm$ 0.27	+ 2.36 $\pm$ 0.048	+ 9.49 $\pm$ 0.034	++ 29.95 $\pm$ 0.95	+ 2.68 $\pm$ 0.047	+ 9.61 $\pm$ 0.053	+ 29.97 $\pm$ 0.25
6-Infected treated with ceftiofur sodium	+ 2.37 $\pm$ 0.049	+++ 9.86 $\pm$ 0.036	++ 28.3 $\pm$ 0.38	++ 2.45 $\pm$ 0.049	+ 9.88 $\pm$ 0.045	++ 29.88 $\pm$ 0.26	+ 2.75 $\pm$ 0.053	+ 9.94 $\pm$ 0.056	+ 29.94 $\pm$ 0.27

\* or + P < 0.05

\*\* or ++ P < 0.001

\*\*\* or +++ P < 0.001

\* Compared with non-infected non-treated group

+ Compared with infected treated group

Table 4. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on leucocytic count ( $10^3 /\text{mm}^3$ ) of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5)

Groups	Leucocytic count ( $10^3 /\text{mm}^3$ )		
	Days post - treatment		
	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day
1-Non-infected non-treated (Healthy control)	22.04 $\pm$ 0.79	21.95 $\pm$ 0.52	22.25 $\pm$ 0.38
2-Non-infected treated with danofloxactn	25.87 $\pm$ 0.54*	23.51 $\pm$ 0.46*	23.46 $\pm$ 0.54
3-Non-infected treated with ceftiofur sodium	26.41 $\pm$ 0.72*	24.16 $\pm$ 0.43*	24.05 $\pm$ 0.65
4-Infected non treated (Infected control )	28.56 $\pm$ 0.64***	27.81 $\pm$ 0.58***	25.37 $\pm$ 0.46*
5-Infected treated with danofloxactn	27.61 $\pm$ 0.76+	25.64 $\pm$ 0.34+	24.69 $\pm$ 0.87
6-Infected treated with ceftiofur sodium	25.84 $\pm$ 0.49+	25.28 $\pm$ 0.44+	24.58 $\pm$ 0.94

\* or + P < 0.01

\*\* or ++ P < 0.001

\*\*\* or +++ P < 0.0001

\* Compared with non-infected non-treated group

+ Compared with infected treated group

Table 5. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on Liver enzymes of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5)

Groups	Days post - treatment								
	1 <sup>st</sup> day			7 <sup>th</sup> day			14 <sup>th</sup> day		
	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
1-Non-infected non- treated (healthy control)	23.7 $\pm$ 1.36	12.97 $\pm$ 0.16	133.5 $\pm$ 2.9	25.69 $\pm$ 1.8	11.36 $\pm$ 0.15	142.5 $\pm$ 3.1	27.88 $\pm$ 1.65	11.88 $\pm$ 0.25	151.8 $\pm$ 3.9
2-Non-infected treated with danofloxacin	** 30.5 $\pm$ 1.84	** 15.69 $\pm$ 0.18	** 156.3 $\pm$ 3.1	* 28.75 $\pm$ 1.46	** 14.58 $\pm$ 0.26	* 153.2 $\pm$ 2.8			
3- Non-infected treated with ceftiofur sodium	** 33.6 $\pm$ 1.57	** 14.85 $\pm$ 0.14	*** 164.9 $\pm$ 3.8	* 27.92 $\pm$ 1.53	* 13.98 $\pm$ 0.22	** 156.8 $\pm$ 2.9			
4-Infected non-treated (infected control)	*** 57.8 $\pm$ 2.05	*** 17.93 $\pm$ 0.19	*** 198.7 $\pm$ 4.9	*** 48.93 $\pm$ 2.16	*** 15.27 $\pm$ 0.29	*** 176.3 $\pm$ 3.9	** 33.96 $\pm$ 2.75	** 14.62 $\pm$ 0.21	*** 168.6 $\pm$ 3.7
5- Infected treated with danofloxacin	+++ 34.7 $\pm$ 1.86	++ 13.67 $\pm$ 0.21	+++ 164.6 $\pm$ 3.6	+++ 32.64 $\pm$ 1.95	++ 13.86 $\pm$ 0.23	++ 154.1 $\pm$ 3.5	++ 30.15 $\pm$ 2.64		
6-Infected treated with ceftiofur sodium	+++ 35.9 $\pm$ 1.85	++ 14.34 $\pm$ 0.18	+++ 168.9 $\pm$ 3.6	+++ 33.25 $\pm$ 2.09	+ 14.11 $\pm$ 0.19	++ 151.6 $\pm$ 3.7	++ 30.29 $\pm$ 1.81		

\* or + P &lt; 0.01

.. or ++ P &lt; 0.001

... or +++ P &lt; 0.0001

\* Compared with non-infected non-treated group

+ Compared with infected treated group



Table 6. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on total protein, albumin and globulin levels of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5).

Groups	Days post - treatment								
	1 <sup>st</sup> day			7 <sup>th</sup> day			14 <sup>th</sup> day		
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1-Non-infected non-treated (healthy control)	3.66 $\pm$ 0.03	1.98 $\pm$ 0.09	1.68 $\pm$ 0.08	3.71 $\pm$ 0.11	1.99 $\pm$ 0.11	1.72 $\pm$ 0.09	3.75 $\pm$ 0.12	1.96 $\pm$ 0.08	1.79 $\pm$ 0.09
2-Non-infected treated with danofloxacin	* 3.32 $\pm$ 0.08	** 1.42 $\pm$ 0.04	* 1.9 $\pm$ 0.09	* 3.36 $\pm$ 0.09	** 1.47 $\pm$ 0.05	* 1.89 $\pm$ 0.07	* 3.47 $\pm$ 0.09	** 1.51 $\pm$ 0.08	* 1.96 $\pm$ 0.09
3- Non-infected treated with ceftiofur sodium	* 3.34 $\pm$ 0.09	** 1.58 $\pm$ 0.08	* 1.76 $\pm$ 0.08	* 3.42 $\pm$ 0.06	* 1.55 $\pm$ 0.09	* 1.87 $\pm$ 0.08	* 3.54 $\pm$ 0.12	* 1.58 $\pm$ 0.11	* 1.96 $\pm$ 0.09
4-Infected non-treated (infected control)	*** 3.15 $\pm$ 0.06	*** 1.32 $\pm$ 0.08	** 1.83 $\pm$ 0.09	*** 3.22 $\pm$ 0.09	** 1.48 $\pm$ 0.08		** 3.39 $\pm$ 0.07	** 1.49 $\pm$ 0.08	* 1.90 $\pm$ 0.08
5- Infected treated with danofloxacin	+ 3.45 $\pm$ 0.07	+ 1.54 $\pm$ 0.07		+ 3.49 $\pm$ 0.08	+ 1.63 $\pm$ 0.07	+ 1.86 $\pm$ 0.05	+ 3.56 $\pm$ 0.09	+ 1.65 $\pm$ 0.09	
6-Infected treated with ceftiofur sodium	+ 3.43 $\pm$ 0.08	+ 1.48 $\pm$ 0.07		+ 3.51 $\pm$ 0.08	+ 1.69 $\pm$ 0.09	+ 1.82 $\pm$ 0.04	+ 3.54 $\pm$ 0.08		

\* or + P < 0.01

\*\* or ++ P < 0.001

\*\*\* or +++ P < 0.0001

\* Compared with non-infected non-treated group

+ Compared with infected treated group

Table 7. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on uric acid and creatinine levels of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5).

Groups	Days post – treatment					
	1 <sup>st</sup> day		7 <sup>th</sup> day		14 <sup>th</sup> day	
	Uric acid (mg/l)	Creatinine (mg/l)	Uric acid (mg/l)	Creatinine (mg/l)	Uric acid (mg/l)	Creatinine (mg/l)
1-Non-infected non- treated (healthy control)	4.160.09	1.45 $\pm$ 0.11	4.37 $\pm$ 0.15	1.36 $\pm$ 0.12	4.35 $\pm$ 0.14	1.26 $\pm$ 0.11
2-Non-infected treated with danofloxacin	* 4.58 $\pm$ 0.07	* 1.92 $\pm$ 0.08	** 5.29 $\pm$ 0.11	** 1.86 $\pm$ 0.09	4.91 $\pm$ 0.18	* 1.62 $\pm$ 0.14
3- Non-infected treated with ceftiofur sodium	** 4.75 $\pm$ 0.11	*** 2.05 $\pm$ 0.09	** 4.97 $\pm$ 0.11	* 1.78 $\pm$ 0.12	4.89 $\pm$ 0.16	* 1.68 $\pm$ 0.12
4-Infected non-treated (infected control)	*** 6.29 $\pm$ 0.15	*** 2.48 $\pm$ 0.12	*** 5.96 $\pm$ 0.12	*** 2.44 $\pm$ 0.14	** 5.37 $\pm$ 0.12	** 2.08 $\pm$ 0.13
5- Infected treated with danofloxacin	+ 5.56 $\pm$ 0.16	++ 1.88 $\pm$ 0.14	+ 5.33 $\pm$ 0.14	+ 1.98 $\pm$ 0.13	5.07 $\pm$ 0.11	+ 1.81 $\pm$ 0.12
6-Infected treated with ceftiofur sodium	+ 5.69 $\pm$ 0.12	++ 1.76 $\pm$ 0.15	+ 5.26 $\pm$ 0.15	+ 1.86 $\pm$ 0.16	+ 5.01 $\pm$ 0.12	+ 1.77 $\pm$ 0.16

\* or + P < 0.01

\*\* or ++ P < 0.001

... or +++ P < 0.0001

\* Compared with non-infected non-treated group

+ Compared with infected treated group

Table 8. The effect of therapeutic dose of danofloxacin (5mg /kg b. wt in drinking water) or ceftiofur sodium (2mg/ kg b. wt s/c) for three successive days on some electrolytes ( $\text{Ca}^{2+}$ ,  $\text{ph}^{2+}$  and  $\text{Mg}^{2+}$ ) of healthy and experimentally infected chickens with E.coli (Mean  $\pm$  S.E) (n = 5).

Groups	Days post - treatment								
	1 <sup>st</sup> day			7 <sup>th</sup> day			14 <sup>th</sup> day		
	$\text{Ca}^{2+}$ (Mg/l)	$\text{Ph}^{2+}$ (Mg/l)	$\text{Mg}^{2+}$ ( $\mu$ /l)	$\text{Ca}^{2+}$ (Mg/l)	$\text{Ph}^{2+}$ (Mg/l)	$\text{Mg}^{2+}$ ( $\mu$ /l)	$\text{Ca}^{2+}$ (Mg/l)	$\text{Ph}^{2+}$ (Mg/l)	$\text{Mg}^{2+}$ ( $\mu$ /l)
1-Non-infected non- treated (healthy control)	10.87 $\pm$ 0.07	4.36 $\pm$ 0.08	1.38 $\pm$ 0.04	11.14 $\pm$ 0.05	5.28 $\pm$ 0.09	1.64 $\pm$ 0.04	11.08 $\pm$ 0.08	5.25 $\pm$ 0.06	2.03 $\pm$ 0.04
2-Non-infected treated with danofloxacin	** 10.24 $\pm$ 0.05	** 5.08 $\pm$ 0.07	* 1.75 $\pm$ 0.06	* 10.58 $\pm$ 0.06	** 5.87 $\pm$ 0.06	* 2.09 $\pm$ 0.05	* 10.96 $\pm$ 0.07	5.61 $\pm$ 0.08	2.17 $\pm$ 0.05
3- Non-infected treated with ceftiofur sodium	* 10.47 $\pm$ 0.04	** 4.84 $\pm$ 0.09	** 1.85 $\pm$ 0.08	* 10.61 $\pm$ 0.09	* 5.74 $\pm$ 0.08	** 2.12 $\pm$ 0.07	10.81 $\pm$ 0.06	5.54 $\pm$ 0.07	2.24 $\pm$ 0.07
4-Infected non-treated (infected control)	** 10.22 $\pm$ 0.05	** 4.87 $\pm$ 0.06	*** 1.94 $\pm$ 0.07	** 10.44 $\pm$ 0.07	** 5.95 $\pm$ 0.07	* 2.23 $\pm$ 0.06	* 10.56 $\pm$ 0.08	** 5.67 $\pm$ 0.06	2.25 $\pm$ 0.08
5-Infected treated with danofloxacin	10.66 $\pm$ 0.05	++ 4.51 $\pm$ 0.07	+ 1.54 $\pm$ 0.06	+ 10.69 $\pm$ 0.08	++ 5.01 $\pm$ 0.08	+ 1.91 $\pm$ 0.05	+ 10.85 $\pm$ 0.05	++ 5.09 $\pm$ 0.07	2.09 $\pm$ 0.07
6-Infected treated with ceftiofur sodium	10.59 $\pm$ 0.06	+ 4.49 $\pm$ 0.06	+ 1.48 $\pm$ 0.05	+ 10.71 $\pm$ 0.07	+ 4.89 $\pm$ 0.05	+ 1.89 $\pm$ 0.04	+ 10.44 $\pm$ 0.07	++ 5.12 $\pm$ 0.06	2.08 $\pm$ 0.09

\* or +, P < 0.01

\*\* or ++, P < 0.001

... or ..., P < 0.0001

\* Compared with non-infected non-treated group

+ Compared with infected treated group

## DISCUSSION

Danofloxacin is a third generation fluoroquinolones antibacterial agent, it is broad spectrum antibacterial drugs highly effective against Gram+ve and Gram-ve bacteria. Danofloxacin is introduced in veterinary medicine to overcome acute bovine disease and avian colibacillosis (20). Ceftiofur is a third generation cephalosporin that has broad spectrum antibacterial agent and is effective in control of Gram+ve and Gram-ve bacterial pathogens of veterinary importance (2).

E.coli is considered one of the most serious problems responsible for economic losses in poultry industry (21). Colibacillosis is one of the most causes of morbidity and mortality in poultry farms (22).

Experimental infection of chickens with E.coli (Infected non-treated) displaying clinical symptoms such as loss of appetite, diarrhea, depression, and respiratory manifestation such as sneezing, gasping, mouth breathing, rales, nasal discharge, and cough. Moreover E.coli infection elicited gross pathological lesions such as air sacculitis, Pericarditis, perihepatitis, ascitis, and enteritis. Our finding coordinates with previous studies (23,24) which reported that E.coli infection was associated with air sacculitis, hepatitis, salpingitis and respiratory manifestation. While medication of E.coli infected chickens with danofloxacin or ceftiofur sodium showed milder degree of clinical symptoms than infected non-treated group.

Experimentally infected chickens with E.coli infection produced high mortality rate (25%) which reduced to 5% by treatment with therapeutic dose of danofloxacin or ceftiofur sodium. Nearly similar results were observed (24,25) where mortality rate due E.coli Infection was up to 20%-30%. Treatment of E.coli infected chickens with danofloxacin or ceftiofur sodium resulted in disappearance of clinical symptoms and reduced mortality rate. Moreover, treatment with danofloxacin or ceftiofur sodium was accompanied by absence or reduction of gross pathological lesions.

These results added further support for the usefulness of danofloxacin or ceftiofur sodium in treating E.coli infection and reduction of mortality rate. Treatment of chickens infected with E.coli by danofloxacin improved health status and decrease mortality rate(26).

The use of danofloxacin or ceftiofur sodium to non infected chickens resulted in a significant increase in body weight and body weight gain. This may be attributed to the antimicrobial effect of the drug which consequently improved metabolic activity of birds and food consumption ratio.

In the present experiment, E.coli infection lead to significant decrease in body weight and body weight gain as well as food consumption were observed post infection which may be due to the deleterious effect of microorganisms that invade the host and retard the metabolic activity through disturbance of general health condition (27). Similar results were also recorded where experimental infection of chickens with E.coli resulted in a significant decrease in body weight and body weight gain as well as food consumption ratio (24,26).

Danofloxacin or ceftiofur sodium administration in healthy chickens produced a significant increase in leucocytic count with a significant decrease RBCs count, haemoglobin concentration and packed cell volume percent. Administration of pefloxacin produced a significant increase in RBCs count, haemoglobin concentration and packed cell volume % in chickens (24). Moreover, cefamandole in laboratory animals decreased haemoglobin concentration and blood cell count (28).

The haematological investigation post infection with E.coli in chickens revealed a significant decrease in RBCs count, Hb and PCV% with a significant increase in WBCs count. The previous authors by several studies (24,29, 30) reported that respiratory bacterial infection in ostriches resulted in a significant decrease RBCs count, Hb and PCV%. Moreover, total leucocytic count,



lymphocytes, and monocytes were elevated whereas neutrophil count was decreased.

Medication of *E. coli* infected chickens with danofloxacin or ceftiofur sodium improved blood picture which reverted to their control values. Needless to say, our data closely reinforced by those obtained previously (24) and showed that treatment of infected birds with pefloxacin reverted blood picture to control values.

In the present investigation it has been shown that administration of danofloxacin or ceftiofur sodium in healthy chickens produced a significant increase in serum AST, ALT and ALP activities. A significant increase in AST, ALT, and ALP activities was cited post treatment with pefloxacin. The elevated enzyme activities (AST, ALT and ALP) in *E. coli* infected chickens in the present study may probably due to some pathological changes induced by infection. The infection produced alteration in cellular permeability due to changes in cell membrane which allows the escape of these enzymes into serum in abnormal high level (31). The investigated enzymes are mostly of hepatic origin and so their level in the serum was indicative to extent of liver damage (32).

Similar results were also reported (29) and showed that respiratory bacterial infection in *Ostriches* elicited in a significant increase in AST, ALT, and ALP activities. *E. coli* infection in chickens resulted in a significant increase in AST, ALT, and ALP activities (24).

Danofloxacin or ceftiofur sodium administration to healthy chickens produced a significant increase in total protein, globulin, uric, phosphorus and magnesium levels with a significant decrease in albumin and calcium levels. Our finding confirm (26) previous study which showed that administration of danofloxacin to healthy chickens produced a significant increase in total protein level.

Experimental infection of chickens with *E. coli* resulted in a significant increase in total protein, globulin, uric acid, phosphorus and magnesium levels with a significant decrease in albumin and calcium levels. These results

are in agreement with that carried out on broiler infected with *E. coli* (33).

These observed results could be due to some pathological changes in the liver and kidney as a result of experimental infection with *E. coli* in chickens. The decrease in albumin level may be attributed to its small size and osmotic sensitivity to fluid movements. Moreover, the decrease in albumin level met with post bacterial infection might be due to amino acid utilization as defense against pathogens and renal damage produced by bacteria (34). Further explanation of hypoalbuminemia is important feature of liver disease (35). On the same ground the decrease in serum albumin level may be due to the destructive effect of bacteria and its toxins on the liver cells which is the main source of albumin and protein synthesis in the body.

*E. coli* infection in chickens evoked a significant decrease in albumin level (24,33). Hyperalbuminemia recorded in chickens indicating the immune defense mechanism against infection enhanced synthesis of immunoglobulin (36). The elevated uric acid and creatinine level in *E. coli* infected chickens in this study was in accordance with previous study (37). The observed decrease in serum calcium concentration in *E. coli* infected chickens throughout experiment period may be attributed to hypoproteinemia because the bound calcium level decreased (38). *E. coli* infection induced in a significant decrease in calcium level in infected broiler chickens (39).

The detected hyperphosphatemia and hypermagnesemia in *E. coli* infected chickens in the present study may be attributed to renal insufficiency because the kidney is the main regulator of phosphorus and magnesium concentration. Our results supported by previous study (40) which reported that avian renal disease is often associated with hyperuricemia and hyperphosphatemia.

Medication of *E. coli* infected chickens with danofloxacin or ceftiofur sodium improved total protein, albumin, globulin, uric acid, creatinine, calcium and phosphorus levels of treated chickens. This shift toward control

level in the previous parameters may be attributed to improved state of the liver in the treated groups as synthesis of albumin, the largest individual protein fraction in avian plasma takes place in the liver or inhibit its renal excretion by improving state of the kidney. The improvement in calcium, phosphorus and magnesium levels in E. coli infected chickens medicated with danofloxacin or ceftiofur sodium may be attributed to absence of clinical symptoms and pathological lesion in kidney.

Hence, it could be concluded from the present study that medication of E. coli infected chickens with therapeutic dose of danofloxacin or ceftiofur sodium is highly effective in treatment of infection which hinder progress of symptoms, lesions and reduce mortality rate as well as improved haematological and biochemical parameters. Moreover, our study demonstrated that danofloxacin or ceftiofur sodium greatly improved healthy condition of E. coli infected chickens and corrected the adverse effects inflicted by infection.

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### الملخص العربي

## دراسة تأثير الدانوفلوكساسين والسفتيفور صوديوم على صورة الدم والمكونات البيوكيميائية في الدجاج السليم والمصاب بالميكروب العصى

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لقد أجريت هذه الدراسة بهدف تقييم الدانوفلوكساسين والسفتيفور صوديوم في الدجاج السليم والمصاب بالميكروب العصى. أستخدم في هذه الدراسة ١٢٠ كتكوت قسمت الى ستة مجموعات متساوية، كل منها ٢٠ كتكوت. المجموعة الأولى مجموعة ضابطة غير معده وغير معالجه. المجموعة الثانية مجموعة غير معده ومعالجه بالجرعة العلاجية من الدانوفلوكساسين ٥مجم/كجم من وزن الجسم الحى فى الماء لمدة ثلاثة أيام. المجموعة الثالثة مجموعة معده ومعالجه بتأجرعة العلاجية من السفتيفور صوديوم ٢مجم/كجم من وزن الجسم الحى بالحقن تحت الجلد لمدة ثلاثة أيام. المجموعة الرابعة مجموعة معده بالميكروب العصى وغير معالجه. المجموعة الخامسة مجموعة معده بالميكروب العصى ومعالجه بالجرعة العلاجية من الدانوفلوكساسين ٥مجم/كجم من وزن الجسم الحى فى الماء لمدة ثلاثة أيام. المجموعة السادسة مجموعة معده بالميكروب العصى ومعالجه بالجرعة العلاجية من السفتيفور صوديوم ٢مجم/كجم من وزن الجسم الحى بالحقن تحت الجلد لمدة ثلاثة أيام.

ولقد تبين من هذا البحث أن استخدام الدانوفلوكساسين أو السفتيفور صوديوم بالجرعة العلاجية فى الكتاكيت الغير معده أحدث زيادة معنوية فى وزن الجسم، كرات الدم البيضاء، إنزيم إسبرتيت أمينو ترانس فيريز وإنزيم إسبرتيت الألانين ترانس فيريز، وخميرة الفوسفاتيز القاعدى والجلوبيولين وحامض اليوريك والكرياتنين ونسبه الكالسيوم والماغنسيوم وأيضا حدث نقص معنوى فى كرات الدم الحمراء ونسبة الهيموجلوبين وحجم كرات الدم المرصوصة والبروتين الكلى ومستوى الالبومين والفسفور فى الكتاكيت المعالجه والغير معده.

ولقد أظهرت نتائج هذه الدراسة أن الكتاكيت المصابة بالميكروب العصى والتي لم تعالج بها نقص معنوى فى وزن الجسم وكذلك عدد كرات الدم الحمراء ونسبة الهيموجلوبين وحجم كرات الدم المرصوصة والبروتين الكلى والإلبومين ومعدل الفسفور ولقد وجد أن هناك زيادة معنوية فى كرات الدم البيضاء وأنزيم الاسبيرتيت أمينو ترانس فيريز، إنزيم الالانين أمينو ترانس فيريز، خميرة الفوسفاتيز القاعدى، والجلوبيولين، وحامض اليوريك، والكرياتنين والكالسيوم والماغنسيوم.

ولقد تبين أن إعطاء الدانوفلوكساسين (مجم/كجم من وزن الجسم الحى فى ماء الشرب) أو السفتيفور صوديوم ٢مجم/كجم من وزن الجسم الحى بالحقن تحت الجلد بالجرعة العلاجية للكتاكيت المصابة بالميكروب العصى أن هناك تحسن فى صورة الدم ومكونات المصل حيث عادت للمستوى الطبيعى للمجموعة الضابطة.

ونستخلص من هذه الدراسة أن الدانوفلوكساسين أو السفتيفور صوديوم فى الدجاج المصاب بالميكروب العصى له تأثير جيد وفعال فى علاج الإصابة بالميكروب العصى وله أثر ملحوظ فى تحسن الآثار الجانبية الناتجة عن الإصابة.