EFFICACY OF OFLOXACIN AGAINST INDUCED COLIBACTERIOSIS IN BROILER CHICKS

F. A. Abdel-Alim, M. S. Amer*, O. E. Ramadan,**

T. A. Mohamed, and Nagah O. Edres,

Departments of Pharmacology, Fac. Vet. Medicine Zagazig and Mansoura*

*Universities and **Animal Health Research Institute - Zagazig

ABSTRACT

The antibacterial activity of ofloxacin, a novel fluoroquinolone, was determined in-vitro against some pathogenic bacterial strains compared with enrofloxacin Ofloxacin and enrofloxacin had nearly similar antibacterial activities against the tested strains.Moreover, the effect of the orally administered ofloxacin (50 mg & 100 mg/L) for 5 days on performance (live body weight, gain weight, feed consumption, feed efficiency and mortality rate) and some serum constituents of 100 Lohman broiler chicks (either non infected or experimentally infected with E.coli) was studied. Ofloxacin at therapeutic dose (50 mg/L) induced a significant increase of live body weight and gain weight in non infected chicks.On the otherhand, it evoked a significant decrease in feed consumption of infected treated chicks.Mortality rates were markedly decreased in all treated groups.Furthermore, ofloxacin licited a significant increase in serum activities of alkaline phosphatase, AST, ALT, creatinine and uric acid of treated groups.

INTRODUCTION

Bacterial diseases of poultry results in major economic losses in poultry industry, so the control of these diseases is of critical importance.Resistance of microorganisms to existing antimicrobial agents is wide spread and of great concern to veterinarians (**Fllall et al., 1988 and Minta et al.,1990**).

In-vitro antimicrobial susceptibility testing of avian pathogens can provide valuable guidance to veterinarians in selecting appropriate chemotherapeutic agents (**Woolcock and Mutimer,1983**).

During the last decade or so, fluoroquinolones have been established as therapeutic options for a wide range of infections. They exert bactericidal effect by inhibiting bacterial DNA gyrase

enzyme (**Drlica and Zhao,1997**). They are of low toxicity and have a long elimination half-life (**Vancutsem et al.,1990**). They are highly active against a wide range of Gram-negative and Gram-positive bacteria, including those resistant to β-lactam antibiotics and sulfonamides (**Scheer,1987 and Sato et al.,1982**).

Ofloxacin is a synthetic antibacterial agent of the fluoroquinolone class that was developed for veterinary use.It is a new pyridonecarboxylic acid derivative of nalidixic acid, chemically known as 9 fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piprazinyl)-7 oxo-7H-Pyrido- [1,2,3-de] -1,4 - benzoxazine-6-carboxilic acid. It differs from other quinolones in having a tricyclic structure (**Chu et al.,1986**).

The objectives of this study were planned to determine the in-vitro antibacterial activity of ofloxacin against some bacterial pathogens, as well as its effect on broiler performance (live body weight, gain weight, feed consumption, feed efficiency and mortality rate). Moreover, changes in some serum biochemical parameters were also studied.

MATERIAL AND METHODS

Antimicrobial agents :

Ofloxacin (Ofloxacin - 20) 20% solution, Samu Chemical Ind. Co., LTD, Deungchon - Dong Kangseo-Ku, Seoul, Korea.

Enrofloxacin (Spectrama Vet) was obtained as a 10% pharmaceutical preparation from the Amoun Company, Egypt.

Test organisms :

A strain of *E.coli* (K 78) was used for experimental infection of broiler chicks. Strains of *Escherichia coli*, *Salmonella gallinarum*, *Pseudomonas aeruginosa*, *Staphyloccus aureus*, *Bacillus subtilis* and *Bacillus cereus* were obtained from Animal Health Research Institute, Dokki, Cairo, for in-vitro antibacterial activity of ofloxacin.

Susceptibility test :

Minimal inhibitory concentration values of ofloxacin were determined against certain bacterial pathogens compared with enrofloxacin using the standard tube microdilution with an inoculum of 5×10^5 CFU/ml according to **Cohen et al., (1985)**.

Experimental Chicks :

A total of 100 apparently healthy unsexed, one day old broiler chicks (Lohman strain) of nearly the same weight were used in this study. The chicks were kept in wire floored battery brooders

provided with thermostatic control unit. They were fed on a balanced comercial ration free from any medicaments, and water was provided ad-libitum with a 24 hour light throughout the experimental period. They were divided randomly into 5 main groups of 20 chicks each. Then each group was divided into 2 subgroups. Chicks of 3rd, 4th and 5th groups were experimentally infected by subcutaneous injection of 3 x 10^5 CFU/ml of *E.coli* (K78) suspended in saline.

The experiment lasted for 3 weeks and the chicks of the 1st subgroups were individually weighed weekly.Body weight gain, feed consumption, feed efficiency and mortality rates were also recorded for the same subgroups.

Experimental design :

Ofloxacin was given in drinking water in therapeutic (50 mg/L) or double therapeutic (100 mg/L) doses for 5 successive days as follows:

Group I : non infected & non treated control.

Group II : non infected & treated with therapeutic dose.

Group III : infected & non treated.

Group IV : infected & treated with therapeutic dose.

Group V : infected & treated with double therapeutic dose.

Sampling & analysis :

At 1st & 2nd week post treatment, 5 chicks from each 2nd subgroups were slaughtered and blood samples were collected into clean and dry centrifuge tubes for serum analysis. The serum samples were separated by centrifugation at 3000 r.p.m for 15 minutes, and kept frozen at 20°C until analysed. Serum samples were analysed for total protein (**King and Wooton,1982**) AST & ALT (**Reitman and Frankel, 1957**), creatinine (**Young et al, 1975**), uric acid (**Caraway, 1963**) and alkaline phosphatase (**John, 1982**).

The obtained data were statistically analyzed using Student's 't' test (**Snedecor and Cochran, 1980**).

RESULTS AND DISCUSSION

Fluoroquinolones are a new class of antibacterial agents, having tremendous potential for use in Veterinary Medicine because of their broad-spectrum activity and good absorption.They are of low toxicity and have a long elimination half life (**Vancutsem et al, 1990**).

The in-vitro activity of ofloxacin against some bacterial strains is shown in table (1). The obtained results revealed that, the tested strains were highly sensitive to ofloxacin as well as enrofloxacin. These findings were supported by **Khodary and Ahlam (1997)** who found that dano-

floxacin, enrofloxacin and norfloxacin are highly effective against *E.coli*, *Salmonella* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus aureus* and *Streptococcus* sp. Moreover, **Abd El-Galil and El-Naenaeey (1993) and Watts et al (1993)** reported that enrofloxacin was more active against *E.coli* and *Salmonella* species. Furthermore, the MICs of fluoroquinolones against the most pathogenic bacteria were two to four folds less than the average serum concentration (0.5 - 1.9 µg/ml) of these drugs when used orally at therapeutic doses in broiler chicks and turkeys (**Scheer, 1987**).

The effect of ofloxacin on body weight gain, feed consumption, feed efficiency and mortality rate for broiler chicks up to 3 weeks were illustrated in tables (2,3& 4).The weekly means of body weight per bird in grams for broiler chicks (Table 2) had shown a significant decrease ($P< 0.01$) up to the 3rd week in infected non treated group compared with the control group. However, a significant increase ($P< 0.05$) was recorded in non infected treated group with therapeutic dose (50 mg/L). As shown in Table (3), the weight gain was significantly increased ($P<0.01$) in non infected treated group, meanwhile, the weight gain and feed consumption were significantly decreased in infected non treated group. Moreover, infected group treated with ofloxacin at 50 mg / L showed a significant decrease in feed consumption on 1st and 2nd week. The increase in body gain of treated chicks might be attributed to the effect of the drug on the subclinical infections, specially of the intestine, promoting an increase absorption of nutrients and consequently improvement of general health condition (**Alexander, 1985**). The obtained result was confirmed by the previously recorded by **El-Azzawy et al. (1997)**, who stated that, therapeutic doses of danofloxacin resulted in a significant increase of body gain in treated chicks. A similar effect on body gain of birds was recorded by **Kempf et al . (1992) and Tanner et al. (1993)**.

Table (4) shows that, no recorded mortalities in all treated groups. This might be attributed to the broad-spectrum activity of the drug against many pathogens. This suggestion confirmed the findings reported by **Shceer (1987) and Bauditz (1990)** who found that fluoroquinolones were active against a wide range of Gram negative, a number of Gram positive bacteria and mycoplasma at low concentrations. Moreover, in- vitro studies revealed a good intrinsic activity of the new quinolones against a wide spectrum of infectious agents (**Cohen et al.,1985**) .

The obtained results regarding the effect of ofloxacin on some serum constituents of treated chicks were summarized in Table (5). It was noticed that ofloxacin induced a significant increase in the activities of serum alkaline phosphatase ($P<0.05$), AST & ALT ($P<0.01$), creatinine and uric acid ($P<0.05$) in both infected and non infected chicks at 2nd week after treatment. Our findings seem conceivable to be attributed to disturbance in the liver and kidney functions resulting from the use of the drug. These results coincides with those previously recorded by **Halkin (1988)** who stated that, enrofloxacin caused hepatic dysfunction. In addition the serum level of alkaline

phosphatase may increase as a result of liver injury and obstructive jaundice **(Harper et al., 1977).** This finding is reinforced with those reported by **Davoren** and **Mainstone (1993)** who found that norfloxacin induced hepatitis.Moreover, **Shimada and Hori (1992)** reported that enrofloxacin evoked renal damage.Furthermore, our results are consistent with those obtained by **Mervat et al. (1997)** who reported that, oral administration of ofloxacin to rats for one week induced a significant increase in sGOT, alkaline phosphatase and creatinine. In addition, **Fraser et al. (1991)** noticed that ALT and AST may be elevated after administration of enrofloxacin in animals.

In conclusion, it was evident that ofloxacin has an efficient antibacterial activity for the control of colibacillosis in chickens.

Table 1 : Minimum inhibitory concentration (ug/ml) of ofloxacin against some pathogenic microorganisms in comparison with enrofloxacin.

Drugs	*E. coli*	*Salmonella gallinarum*	*Pseudomo. auruginosa*	*Staphyloc. aureus*	*Bacillus subtilis*	*Bacillus cereus*
Ofloxacin	0.03	0.03	1.00	0.12	0.25	0.06
Enrofloxacin	0.03	0.06	1.00	0.12	0.25	0.12

Table 2 : Effect of ofloxacin (50 & 100 mg/L) on weekly means of body weight (gm) of broiler chicks. (X ± S.E) n = 10.

Group	1st day (zero time)	1st week	2nd week	3 rd week
G1 (control) (non infe/non treates)	43.8 ± 0.7	109.5 ± 4.18	252.5 ± 9.87	426.5 ± 10.79
G2 (non inf/treated 50mg)	42.6 ± 0.93	106.3 ± 4.51	262.0 ± 9.3	457.0 ± 12.45*
G3 (infec./non treated)	42.3 ± 0.65	105.0 ± 2.24	1981 ± 14.45**	359 ± 17.89**
G4 (infect./treated 50mg)	44.2 ± 1.51	110.0 ± 4.88	228.0 ± 10.63	407 ± 10.44
G5 (infe/treated 100mg)	41.8 ± 1.55	110.0 ± 7.36	242.0 ± 10.74	422.0 ± 12.37

* P < 0.05 ** P < 0.01

Table 3 : Weekly means of gain weight (gm), feed consumption (gm) per bird and feed efficiency ratio of treated or non treated broiler chicks in response to ofloxacin (50 & 100 gm/L). (X ± S.E) n = 10.

Time	Parameter	Group 1 (control)	Group 2 non infect./ treat. 50mg	Group 3 Infect./ non treated	Group 4 infected/ treat. 50mg	Group 5 Infected/ treat 100mg
1st week	Gain weight	65.2±1.48	63.9±2.58	62.7±1.69	65.8±1.37	68.1±2.81
	Feed consumption	135.2±1.12	131.3±6.28	117.4±7.3*	122.6±4.9*	130.6±0.91
	Feed efficiency	2.07±0.75	2.04±0.09	1.87±0.42	1.86±0.36	1.92±0.32
2nd week	Gain weight	143.0±5.69	155.5±4.79	93±6.21**	138.0±5.75	132.0±4.38
	Feed consumption	291.5±3.72	284.39±4.5	197±9.8**	270.1±5.4*	279.3±6.2
	Feed efficiency	2.67±1.61	1.83±0.09	2.12±1.57	1.96±0.93	2.12±1.42
3rd week	Gain weight	174.0±0.59	195.0±6.8*	155±5.89*	179±0.19	180.0±1.69
	Feed consumption	465.2±7.05	452.5±8.1	375±10.6**	450.2±6.3	458.7±5.6
	Feed efficiency	2.67±1.61	2.32±1.19	2.09± 0.15	2.55±0.23	2.55±0.23

* P < 0.01 ** P < 0.005

Table 4 : Effect of ofloxacin (50 mg & 100 mg/L) on live body weight, body weight gain, feed consumption, feed efficiency and mortality rate of brolier chicks up to 3 weeks of age (X ± S.E)

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Initial No of chicks	10	10	10	10	10
Mortality No.	--	--	2	--	--
Mortality %	0	0	20%	0	0
Initial live body wt. (gm) (1 day old)	43.8±0.7	42.6±0.93	42.3±0.65	44.2±1.51	41.86±1.55
Final live body wt. (gm) (3 weeks old)	426.5±10.39	457.0±12.49	353.0±15.66	407.0±10.64	422.0±12.37
Live body wt. gain (gm/chick/period)	382.2	414.4	310.7	362.8	380.14
% of control	100	108.4	81.3	94.9	99.5
Total feed consumption (gm/chicks/period)	891.9	868.19	679.54	842.93	868.6
% of control	100	97.42	76.19	94.5	97.5
Feed efficiency (gm feed conc./gm gain)	2.33	2.1	2.19	2.32	2.28
% of control	100	90.13	90.94	99.57	97.85

Table 5 : Weekly means of gain weight (gm), feed consumption (gm) per bird and feed efficiency ratio of treated or non treated broiler chicks in response to ofloxacin (50 & 100 gm/L). (X ± S.E) n = 10.

Time	Time post treatment	Alk.phosh. (mg/dl)	ALT (U/L)	AST (U/L)	T. protein (gm/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
G 1 Control	1 week	340.4±16.57	63.8±2.67	85.2±4.8	3..9±0.29	1.34±0.07	8.54±0.45
	2 weeks	365.3±18.55	65.8±3.82	92.4±4.7	3.67±0.18	1.32±0.08	8.2±0.52
G 2 (non infec/ treated)	1 week	389.6±10.34*	69.0±4.03	117.1±6.77**	3.22±0.32	1.64±0.15	9.62±0.24*
	2 weeks	437.3±15.80*	83.6±4.5**	121.4±5.34**	3.62±0.14	1.89±0.2*	9.28±0.5
G 3 (infec./non treated)	1 week	368.8±11.91	69.8±3.26	95.8±4.65	4.04±0.45	1.26±0.08	7.92±1.17
	2 weeks	359.2±10.69	73.7±5.96	96.1±5.30	3..97±0.41	1.58±0.06	8.13±0.38
G 4 (infe/treat. 50 mg)	1 week	356.1±7.34	80.8±5.53**	125.2±8.9**	4.06±0.33	1.64±0.21	9.9±0.39*
	2 weeks	425.6±8.12**	85.2±4.36**	126.6±8.7**	4.20±0.69	1.5±0.07	10.5±0.83*
G 5 (infe/treat 100 mg)	1 week	376.2±9.17	84.8±6.62**	127.0±6.1**	4.13±0.34	2.13±0.32*	10.8±0.58*
	2 weeks	451.6±14.9**	85.8±5.8**	131.5±8.5**	4.23±0.47	1.85±0.21*	10.8±0.85*

* P < 0.05 ** P < 0.01

REFERENCES

Abd El-Galil,Y. and El-Naenaeey, Y. E. (1993) : Laboratory and field trial to evaluate the antibacterial action of enrofloxacin.Zag. Vet. J., 21 (3) : 558-563.

Alexander, F. (1985) : An Introduction to Veterinary Pharmacology.4th Ed. Longman (London and New York).

Bauditz, R. (1990) : Enrofloxacin, clinical evaluation in several animal species. Veterinary Pharmacology, Toxicology and Therapy in Food Producing. Animals.21-26.

Caraway, W. T. (1963) : Standard Methods of Clinical Chemistry. Edited by Seligsol, D., Acadimic Press, New York and London, 4 : 239.

Chu, D. T.; Fernandes, P. B. ; Claiborne, A. K.; Gracey, E. H. and Pernet, A. G. (1986) : Synthesis and structure activity relation ship of new arylfluoronaphthy- ridine antibacterial agents. J. Med.Chem., 29 : 2363-9.

Cohen, M. A.; Griffin, T. J.; Bein, P. A.; Heifetz, C. L. and Domagola, J. M. (1985) : In vitro activity of CI-934, a quinolone carboxylic acid active against Gram-positive and negative bacteria.Antimicro. Agents Chemother. 28, 766-772.

Davoren, P. and Mainstone, K. (1993) : Med.J.Aust., 150 (20), 423-426.

Drlica, K. and Zhao, X. (1997) : DNA gyrase, topoisomerase IV and the 4-quinolones. Microbiol. Mol. Biol. Rev., 61: 377-392.

El-Azzawy, M. H.; Hamdy, I. R.; Khodary, R. M. and Amina, A. Nawwar (1997) : Studies on danofloxacin in chicks vaccinated with Newcastle disease virus vaccine. Alex. J. Vet. Science. 13 (2) : 17-30.

Fllali, E. J.; Bell, G.; El-Houadfi, M. B.; Huqqino, J. and Cook, K. A. (1988) : Antibiotic resistance of Escherichia coli strains isolated from chickens with coli- septicemia in Moracco. Comp. Immunol. Microbiol. Infec. Dis., 11:121-124.

Fraser, M.; Bergerson, A.; Mays, A. and Susan, E. (1991) : Chemotherapeutics. In the Merck Veterinary Manual. 7th Ed., Rahway, USA. P. 121.

Halkin, H. (1988) : Rev. Infec. Dis., 10 : 258-261.

Harper, H. A., Rodwell, V. W. and Mayes, P. A. (1977) : Review of Physiological Chemistry. 16th Ed., P.76.

John, D. B. (1982) : Clinical Lab. method for determination of alkaline phosphatase. 9th Ed., 580-581.

Kempf., I.; Gesbert, F.; Guittet, M.; Ben Nejean, G. and Cooper, A. C. (1992) : Efficacy of danofloxacin in the therapy of experimental mycoplasmosis in chicks.Res.Vet.Sci., 53 (2) : 257-259.

Khodary, R. M. and Ahlam E. Abd-Allateif (1997) : In vitro susceptibility of some avian pathogens to fluoroquinolone compounds compared with the commonly used antimicrobial agents. Alex. J. Vet. Science. 13 (2) : 41-48.

King, E. G. and Wooton, N. P. (1982) : Microanalysis of Medical Biochemistry. 6th Ed., Churchill Livingstone London.

Mervat, E. A.; Fatma,R. A. and Rawia,S. A. (1997) : Effects of norfloxacin (Noroxin) and ofloxacin (Taravid) on liver and kidney functions and some other parameters of adult male rats.Zag. J. Pharm. Sci., 6 (1), 7-11.

Minta, Z.; Bagazak, P. and Karezewski, W. (1990) : The evaluation of the effectiveness of Baytril in the treament of Salmonellosis in chicken broilers. Medycyna Vrterynaria. 46 (9) : 325-328.

Reitman, S. and Frankel, S. (1957) : Colorimetric determination of glutamic oxalacetic and glutamic pyruvic transaminase. Am.J.Clin.Path., 28, 56.

Sato, K; Matsuura, Y.; Osada, Y.; Ogawa, H. and Mitsuhashi, M. (1982) : Antimicrob. Agents Chemother., 22 : 548.

Scheer, M. (1987) : Concentration of active ingredient in the serum and in tissues after oral and parentral administration of Baytril.Vet.Med.Rev.,2:104-118.

Shimada, J. and Hori, S. (1992) : Prog. Drug Res., 38:135-143.

Snedecor, G. W. and Cochran,E. G. (1980) : Statistical Methods.The Iowa State Uni. Press, Ames., Iowa, USA.

Tanner, A. C.; Davidson, J. N; Odor, E. M.; Quories, C. L.; Lautz, C. A. and Migaki, T. I. (1993) : Efficacy of danofloxacin against induced colisepticemia in broiler chickens. Proc. 19th World s Poultry Congress, Amestrdam.19-24.

Vancutsem, P. M.; Babish, J. G. and Schwark, W. S. (1990) : The fluoroquinolones antibacterials: structure antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicology. Cornell Veterinarian, 80 : 173-186.

Watts, J. L.; Salmon, S. A.; Yancey, R. J. and Kounrev, Z. V. (1993) : Minimum inhibitory concentration of bacteria isolated from septicemia and airsaccullitis in ducks. J. Vet. Diagnostic Invistigation. 5 (4) : 625-628.

Woolcock, J. B. and Mutimer, M. D. (1983) : Antibiotic susceptibility testing : caecl caecos duents.Vet.Rec.,113:125-128.

Young, D.; Pestaner, L. and Giberman, V. (1975) : Clin.Chem.,21:10.

الملخص العربى

فعالية الأوفلوكساسين ضد الكولى باسيللوزيس المكتسب فى كتاكيت اللحم

المشتركون فى البحث

عبدالعليم فؤاد و مجدى عامر*

أسامه رمضان** و طه عبدالفتاح ولحاج إدريس

أقسام الفارماكولوجيا - كلية الطب البيطرى - جامعتى الزقازيق والمنصورة *

معهد بحوث صحة الحيوان - الزقازيق **

تم إجراء هذه الدراسة لمعرفة فعالية الأوفلوكساسين معملياً ضد بعض سلالات البكتريا المسببة للأمراض مقارنة بالانروفلوكساسين، وكذلك تأثير إعطاء العقار بالفم للكتاكيت على كل من وزن الجسم الحى، معدل الزيادة فى وزن الجسم، كمية الغذاء المستهلك، الكفاءة الغذائية والحيوية، بالإضافة إلى تأثيرة على بعض مكونات مصل الدم.

أجريت التجربة على عدد ١٠٠ كتكوت عمر يوم من سلالة لوهمان، وقد قسمت إلى ٥ خمس مجموعات رئيسية (تحتوى على ٢٠ كتكوت لكل). ثم قسمت كل مجموعة إلى مجموعتين (بواقع ١٠ كتاكيت لدراسة كفاءة الأداء، و ١٠ للتغيرات بمصل الدم). تمت عدوى لكل من المجموعات الثالثة والرابعة والخامسة معملياً بميكروبات الاشريشيا كولاى.

تم إعطاء الدواء لمدة ٥ أيام متتالية فى ماء الشرب بالجرعة العلاجية (٥٠ مجم ١ كجم) وضعف العلاجية (١٠٠ مجم ١ كجم) لبعض المجاميع كالنحو التالى :

١- المجموعة الأولى : كمجموعة ضابطة (دون عدوى - دون علاج).

٢- المجموعة الثانية : أعطيت الجرعة العلاجية (دون عدوى).

٣- المجموعة الثالثة : مصابة بالميكروب (دون علاج).

٤- المجموعة الرابعة : مصابة وأعطيت الجرعة العلاجية.

٥- المجموعة الخامسة : مصابة وأعطيت ضعف الجرعة العلاجية.

وقد أظهرت النتائج إن عقار الأونلوكساسين قد أحدث :-

١- تأثير قوى ومشابه لتأثير الأنروفوكساسين على فصائل البكتريا المرضية المستخدمة معملياً.

٢- زيادة معنوية فى وزن الجسم الحى ومعدل الزيادة فى الجسم للمجموعة الثانية (معالجة - دون عدوى).

٣- زيادة معنوية فى مستويات كل من الفوسفاتيز القاعدى، إنزيم الالانين أمينوترانس فيريز، إنزيم الاسبرتات أمينوترانس فيريز، الكرياتينين وحمض البوريك فى مصل المجاميع المعالجة.