EFFICACY OF PHOSPHOMYCIN IN THE CONTROL OF *E. COLI* INFECTION IN BROILERS

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ABSTRACT

Ca fosfomycin is active against wide range of bacteria e.g. E. coli, Staphylococci and Streptococci. The present work was carried out to evaluate the antibacterial effect of Ca fosfomycin in healthy and experimentally infected broilers with E. coli. The effect of therapeutic dose of ca fosfomycin (40 mg/kg B.Wt) in drinking water for 3-5 successive days on healthy and experimentally infected broilers, with E. coli was determined. The minimal inhibitory concentration (MIC) of fosfomycin against tested E. coli was 0.5 ug/ml. In vivo studies were carried out on 200 broiler chickens. They were divided into 4 equal groups: Group I served as healthy control (non infected, non treated), the other 3 groups were infected with 0.25ml of broth containing $10^{3.3}$ C.F.U E. coli at age of 20 days. Group 2 remained infected, non treated. Group 3 and 4 were treated orally with Ca fosfomycin 40 mg/kg b.wt. for 3 and 5 successive days respectively. Treatment starts twenty four hours post infection. At the age of 2 and 3 weeks post-infection, the obtained results concerning clinical signs, mortality rate, re-isolation of E. coli and the body weight gain showed that Ca fosfomycin (40 mg/kg) for 3 and 5 successive days was highly effective agent in the control of experimental infection.

Moreover, serum AST, ALT, uric acid, blood urea nitrogen and leucocytic count were significantly increased in chicken infected with E. coli while serum total protein and albumin were significantly decreased. These parameters were improved toward to the normal levels in chicks infected with E. coli and treated with fosfomycin for 3 and 5 successive days.

INTRODUCTION

Antibiotics are widely used in veterinary medicine to overcome mainly bacterial infection in poultry farms. Fosfomycin is one of broad spectrum bactericidal antibiotic, which is useful in treatment of *E. coli* infection in poultry farms.

E. coli infections in chicken's farms are considered as one of the most serious problem, affecting poultry industry **(Curtis et al., 1970)**.

Ca fosfomycin is the newest generation in fosfonic acid derivatives (potassium fosfomycin and disodium fosfomycin) developed totally for poultry use and medication by Bedson-SA over 15 years of continuous research and development.

Fosfomycin is a broad spectrum antibiotic produced by Streptomysis jradiae strain **(Christensen et al., 1969)**. It's a derivative of fosfonic acid, unrelated to other antibiotic groups, its molecule is very small containing only two carbon elements in its structure, this allow its greater permeability in area of abscesses and poor vascularization **(Ishizawa and Cole, 1987)**.

The bactericidal effect of fosfomycin is due to inhibition of the first step in peptidoglycan synthesis among processes of ell wall biosynthesis in bacteria, incorporating it self into the organism by an active transport system. It's active against both Gram-positive and Gram-negative organisms **(Perea et al., 1977)**.

This study was planned to study the activity of Ca fosfomycin against isolates of *E. coli* spp. in vitro and vivo. Also to investigate the optimum effective dose and duration of therapy for control spp. of *E. coli* as well as its effect on body weight gain and some liver and kidney functions.

MATERIAL AND METHODS

Material:

(1) Drug:

Ca fosfomycin (Adwia Co.).

(2) Microorganism: pathogenic E. coli serotype O_{78}, obtained from Hamada Lab.

Media:

MacConky's agar, Nutrient agar, MacConky's broth (Oxoid) and Nutrient broth **(Cruickshank et al., 1971)**.

(3) Experimental chicks:

Two hundred apparently healthy one day old, baby chicks (Cup breed), were used in the present study. They were obtained from a poultry farm in Sharkia Governorate.

Methods:

Antibacterial effects:

(A) Antibacterial activity in vitro:

(1) Determination of minimum inhibitory concentration (MIC) using broth dilution method for fosfomycin according to **Cruickshanke et al., (1975).**

(2) Sensitivity test of E. coli to fosfomycin in comparison with other antimicrobial agents was studied in vitro using disc diffusion method according to **Backer et al., (1980).**

(B) Antibacterial activity in vivo:

Experimental evaluation of fosfomycin:

Two hundred and twenty days old broiler chicks were used. Five chicks were randomly sacrificed and bacteriologically examined to confirm that free from *E. coli*. Chickens were divided into 4 equal groups, each of five. Group 1 chicks (non infected non treated) where 2nd group was infected with *E. coli* and remained non treated (positive control). The 3rd and 4th groups were infected with *E. coli* and treated with fosfomycin at a dose level of 40 mg/k b.wt. orally for 3 and 5 successive days respectively. Infection was done at the 20 days of age with *E. coli* 0.78 strain by intramuscular injection of 0.75 ml of $10^{3.3}$ C.F.U **(Badr, 2003).**

Treatment started 24 hrs post infection, the period of experiment lasted to the age of 6 weeks. All chicks were weighted; mortality rate, clinical symptoms and PM lesions were recorded for all groups at the end of the experiment. Blood samples were obtained and sera were collected.

(C) Haematological studies:

Blood samples were collected from each chicken at the 3rd, 7th and 21 days post treatment in all groups. Blood samples were colleted in 2 tubes; one without anticoagulant for separation of serum. The collected serum samples were used for estimation of AST, ALT **(Reitman and Frankel, 1957)**, total proteins **(Weichselbaum, 1946)**, albumin **(Doumas et al., 1981)** and uric acid & blood urea **(Trinder, 1969).**

The other tube with EDITA as anticoagulant for erythrocytic and total leukocytic counts **(Natt and Herrick, 1952)** and packed cell volume was determined using the micro haematocrit method **(Cohen, 1967).**

Statistical analysis:

Statistical analysis was carried out according to **Snedecor and Cochran, (1982).**

RESULTS

Results of antibiogram study revealed that fosfomycin, pefloxacin, kanamycin and ciprofloxacin were the most effective antimicrobials against E. coli (Table 1). The MIC of fosfomycin against *E. coli* was 0.5 ug/ml.

Experimentally infected chicks with *E. coli* O_{78} showed clinical signs manifested by depression, loss of appetite, dropping of wings, diarrhea and respiratory symptoms. Post mortem examination of dead infected chicks as in Table (2). Oral administration of Ca phosphomycin at a dose of 40 mg/kg b.wt. for 3 and 5 successive days induced a significant ($P > 0.05$) increase of body weight gain in comparison to infected non-treated group (Table 3).

The effect on erythrocytic count, PCV, haemoglobin concentration and leuckocytic count was shown in Table (4). Alteration of serum biochemical values in all groups was detected. Infected chicks with *E. coli* denoted significant increase in activities of AST, ALT, uric acid and urea but decreased significantly compared with control group.

These parameters were revealed to nearly normal level in infected treated chicks with fosfomycin (Tables 5 and 6).

DISCUSSION

Avian E. coli infection is a problem of economic concern to all phases of the poultry industry **(Culnak et al., 1991)**.

Antibiogram of E. coli O_{78} to ca fosfomycin and other commonly used antimicrobials was performed. The results obtained indicated that *E. coli* was highly sensitive to ca fosfomycin. These results are in similar to previously cited results **(Goto et al., 1981)**, they reported that *E. coli* showed sensitivity to ca fosfomycin. Also, other numbers of quinolone groups are still effective in inhibiting *E. coli* infection **(Fernandez et al., 1998)**.

In addition, the MIC of ca fosfomycin was determined as 0.5 ug/ml as reported by **Marchese et al., (2003)**.

The clinical signs observed on the infected and non-treated chicks were depression, loss of appetite respiratory manifestations and loss of body weight. Similar symptoms, were previously recorded **(Awaad, 1972)**. The treatment of infected chicks with Ca fosfomycin at dose 40 mg/kg b.wt for 3 and 5 successive days reduced clinical signs and decreased mortality rate from 50% to 15% and 5% respectively. Numerous reports have been indicated the effectiveness of Ca fosfomycin in the treatment of *E. coli* infection **(Laurance et al., 1997)**.

The obtained results revealed that administration of Ca fosfomycin at a dose level of 40 mg/ kg b.wt for 3 and 5 successive days to infected chicks as well as healthy non infected chicks (control group) showed no significant variation in body weight gain. On contrary infected non treated chicks had loss body weight gain when compared with control and infected treated groups.

The decrease in the body weight post infection may be attributed to the deleterious effect of microorganism which invade the host and retarded its metabolic activity **(Anani, 1993)**. On other hand the body gain of chicks infected with *E. coli* was increased after treatment with Ca fosfomycin in compared to infected non-treated **(Mekllar and Verma, 1998)**. The improvement of body weight gain in infected and treated chicks might be attributed to bactericidal effect of the drug on the infection and consequently improved general health condition **(Ferandez et al., 1998)**. These results provide a farther reason for efficacy of Ca fosfomycin in control *E. coli* infection. In present investigation, it has been shown that Ca fosfomycin administration in therapeutic dose in infected chickens results in non-significant changes in erythrocytic count and packed all volume, where as leucocytic count was significantly increased. Our results coincide with that obtained by **Mckellar and Varma (1996)**.

Biochemical analysis of serum from chicks infectived with *E. coli* showed that serum AST, ALT were significantly ($P > 0.05$) increased. These parameters were improved towards the normal levels as result of treatment of infected chickens with Ca fosfomycin.

The increase is serum AST and ALT after infection indicating a hepatocellular damage **(Doxey, 1971)**. Treatment with Ca fosfomycin at level of 40 mg b.wt for 3 and 5 successive days displayed non-significant changes in serum AST, ALT as well as total proteins and albumin. Recent investigation recorded similar results **(Ali and Youssef, 2003)**. Our results agree with those obtained by **Ali et al., (2003)**.

Uric acid and blood urea levels were significantly increased in infected non-treated chicks with *E. coli*, this finding was agreed with the results obtained by **Mckellar and Varma (1996)**. Our results revealed that there were non significant changes in uric acid and blood urea levels in infected and treated group. These results coincide with **Halliwell (1981)** who reported that Ca fosfomycine had no significant nephrotoxicity in rats.

Finally it could be concluded that the treatment of *E. coli* infection in broiler chicks with Ca fosfomycin at dose of 40m/kg b.wt for 5 successive days has superior activity and efficiency than its administration for 3 successive days.

Table (1): Sensitivity test for some types of antibiotics against the used *E. coli* organism.

Antimicrobial	*Disc concentration (µg)*	*Zone of inhibition (mm)*
Fosfomycin	15	17
Erythromycin	30	18
Oxytetrayclin	30	24
Gentanycin	10	16
Ciprofloxacin	5	12
Colistin	25	15

Table (2): Effect of Ca. Fosfomycin (40 mg/kg) on the mortality rate (%) and lesion scores of non-infected and *E. coli* experimentally infected chickens (Mean ± SE) (n= 5).

Groups	Mortality %	Air saculitis %	Pericarditis	Perihepatitis	Ascitis	Enteritis	Reisolation
Non-infected, non-treated	0	0	0	0	0	0	0%
Infected, non-treated	15	70	50	75	40	35	100%
Infected, treated wit Ca fosfomycin (40 mg/kg) for 3 successive days.	5	20	15	5	3	8	10%
Infected, treated wit Ca fosfomycin (40 mg/kg) for 5 successive days.	2	12	8	3	0	2	5%

Table (3): The effect of Ca. fosfomycin (40 mg/kg) in drinking water for 3-5 successive days on the body weight (gm) in healthy and experimentally infected chickens with *E. coli* (Mean ± SE) (n=5).

Groups	Body weight (gm) time post infection			
	3rd day	7th day	14th day	21st day
Non-infected, non-treated	740 ± 40	1128 ± 80	1680 ± 210*	2030 ± 305*
Infected, non-treated	662 ± 54	920 ± 511	1398 ± 1452***	1680 ± 442***
Infected, treated with Ca fosfomycin (40 mg/kg) for 3 successive days.	721 ± 25	994 ± 224	1582 ± 219*	1822 ± 155*
Infected, treated with Ca fosfomycin (40 mg/kg) for 5 successive days.	718 ± 32	1062 ± 32	1570 ± 374*	1922 ± 301**

*P > 0. 05 **P > 0.01 ***P > 0.001

Table (4): Effect of Ca fosfomycin (40 mg/kg) in drinking water for 3-5 successive days on erythrocytic counts (10^6 UL), packed cell volume (%) and leukocytic counts (10^3 UL) in healthy and experimentally infected broilers with *E. coli* (mean ± SE) (n=5).

Groups	Red blood corpuscles (10^6 UL) time post infection (days)			Packed cell volume (P.C.V.) (%) time post infection (days)			Leukocytic count (10^3 UL) time post infection (days)		
	3rd day	7th day	21st day	3rd days	7th days	21st days	3rd days	7th days	21st days
Non-infected, non-treated	2.98 ± 0.08	321 ± 0.12	3.12 ± 0.14	32.61 ± 0.08	32.15 ± 1.2	34 ± 0.83	14.5** ± 1.5	15.8*** ± 0.88	15.8*** ± 1.2
Infected, non-treated	2.87 ± 0.05	2.5*** ± 0.07	2.06*** ± 0.06	24.22*** ± 0.1	23.8** ± 1.2	26.4** ± 0.55	17.4 ± 1.1	19.4 ± 0.55	20.6 ± 1.2
Infected, treated with Ca fosfomycin for 3 successive days	2.79 ± 0.04	2.85** ± 0.07	2.83* ± 0.14	32.1 ± 0.41	30.12 ± 12	32.4 ± 0.9	13.5** ± 0.22	17.5* ± 0.43	15.5*** ± 1.3
Infected, treated with Ca fosfomycin for 5 successive days	2.85 ± 0.08	2.92* ± 0.06	2.99* ± 0.07	32.2 ± 0.12	32.2 ± 0.2	33.2 ± 0.68	13.8** ± 0.18	16.8** ± 0.44	15.8*** ± 0.54

**P > 0.05 **P > 0.01 ***P > 0.001

Table (5): Effect of Ca fosfomycin (40 mg/kg) in drinking water for 3-5 successive days on total serum proteins and albumin (gm/dl) of non-infected and *E. coli* experimentally infected chickens (mean ± SE) (n=5).

Groups	Total serum protein (gm/dl) Time post infection (days)			Albumin (gm/dl) Tim post infection (days)		
	3rd day	7th day	21th day	3rd days	7th days	21th days
Non-infected, non-treated	3.1 ± 0.42	3.7 ± 0.44	4.2 ± 0.22	1.71 ± 0.18	2.12 ± 0.12	2.17 ± 0.08
Infected, non-treated	2.7* ± 0.34	2.5* ± 0.11	2.9* ± 0.18	1.2* ± 0.11	1.8* ± 0.12	1.6* ± 0.03
Infected, treated with Ca fosfomycin for 3 successive days	4. ± 0.12	4.2 ± 0.12	3.8 ± 0.23	1.7 ± 0.24	1.9 ± 0.11	2.1 ± 0.03
Infected, treated with Ca fosfomycin for 5 successive days	4.2 ± 0.17	4.1 ± 0.34	4.3 ± 0.24	1.6 ± 0.26	1.87 ± 0.03	2.2 ± 0.02

*P > 0.05

Table (6): Effect of Ca-fosfomycin (40 mg/kg) in drinking water for 3-5 successive days on blood urea, uric acid, alanine amino transferase (AST & ALT) and alkaline phosphatase in healthy and experimentally infected broilers with *E. coli* (Mean ± SE), (n=5)

Groups	Asparate amino Transferase "AST" (UL) Time post treatment			Alkaline phosphatase "AP" (UL) Time post infection			Blood urea nitrogen (mg/dl) Time post infection			Uric acid (mg/dl) Time post infection		
	3rd day	7th day	21st day	3rd days	7th days	21st days	3rd days	7th days	21st days	3rd days	7th days	21st days
Non-infected, non-treated	11.54 ± 2.36	11.22 ± 1.8	11.98 ± 2.41	30.4 ± 1.8	27.6 ± 1.2	29.4 ± 2.3	18.2 ± 1.5	14.78 ± 1.1	11.3 ± 0.55	4.68 ± 0.21	4.8 ± 0.32	4.6 ± 0.21
Infected, non-treated	25.61 ± 2.7	28.6** ± 3.1	35.2*** ± 2.8	37.4** ± 2.1	34.6* ± 1.7	35.8* ± 2.3	29.2 ± 012	24.2*** ± 1.65	12.1 ± 0.12	5.9* ± 0.81	7.4*** ± 0.27	5.2 ± 1.1
Infected, treated with Ca fosfomycin for 3 successive days	24.81 ± 1.67	29.17 ± 2.1	34.88 ± 2.8	31.6 ± 1.88	29.55 ± 1.42	30.66 ± 2.23	27.2 ± 1.16	19.46** ± 1.8	11.87 ± 1.64	5.1 ± 0.22	5.5* ± 0.25	5.2 ± 0.91
Infected, treated with Ca fosfomycin for successive 5 days	25.31 ± 2.1	30.81 ± 2.7	35.87	30.91 ± 1.96	31.4*** ± 1.4	29.78 ± 2.41	26.99 ± 2.41	18.82 ± 1.8	10.77 ± 0.55	5.1 ± 0.66	5.4* ± 0.17	4.98 ± 1.2

* P > 0.05 ** P > 0.01 *** P > 0.001

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الملخص العربى

كفاءة الفوسفومايسن فى مقاومة الإشيريشيا كولاى فى بدارى التسمين

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عقار الكالسيوم فوسفين له إستعمال واسع ضد البكتريا ومنها ميكروب الإيشريشيا كولاى والإستافيلوكوكس (ميكروب العنقود الذهبى والاستربتوكوكاى (الميكروب المكور).

إستهدفت هذه الدراسة إستخدام هذا العقار الفوسفوميسين بالجرعة العلاجية كعلاج للعدوى المعملية بميكروب الأيشريشياكولاى فى مياه الشرب لمدة من ٣ إلى ٥ أيام.

وبعمل إختبار الحساسية لميكروب الإيشريشياكولاى لعقار الفوسفومايسين وبعض مضادات الميكروبات شائعة الاستخدام إتضح كفاءته العالية وكذلك تم تحديد الحد الأدنى القاتل وجد أنه ٥ر٠ ميكروجرام لكل مللى.

وبالإضافة لما سبق فقد تم تقييم فاعلية الدباج البلدى المصاب معملياً بميكروب الإيشيرشياكولاى O_{78} وذلك بتقسيم عدد ٢٠٠ كتكوت تسمين أى أربعة مجاميع.

(١) المجموعة الأولى : مجموعة ضابطة سليمة وغير معالجة.

(٢) المجموعة الثانية : كتاكيت مصابة وغير معالجة.

(٣) المجموعة الثالثة : كتاكيت مصابة ومعالجة بعقار الفوسفومايسن ٠٤مجم/ك وزن حى لمدة ٣ أيام.

(٤) المجموعة الرابعة : كتاكيت مصابة ومعالجة بعقار الفوسفومايسن ٠٤مجم/ك وزن حى لمدة خمسة أيام.

واستناداً للأعراض ونسبة النفوق وإعادة عزل الميكروب من الكتاكيت المصابة وغير المعالجة ومقارنتها بالكتاكيت السليمة الغير معالجة بالإضافة إلى الوزن المكتسب يمكن الحكم بكفاءة عقار الفوسفومايسين علاوة على ذلك فقد لوحظ إرتفاع معنوى فى الإنزيمات الكبد والكلى فى مصل الكتاكيت المصابة وغير المعالجة بينما تحسن معدل القيم فى الكتاكيت المصابة والتى تم علاجها بالعقار ٣ أو ٥ أيام.

وخلاصة القول أن هذه النتائج أثبتت كفاءة عقار الفوسفومايسين على العدوى المعملية بميكروب الإيشريشياكولاى بالجرعة ٠٤مجم/وزن حى وأن يستمر العلاج ٥ أيام وبهذه الطريقة يمكن التحكم وعلاج ميكروب الإيشريشيا كولاى فى الدواجن.