

SOME PHARMACOLOGICAL STUDIES ON DRUG INTERACTIONS

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ABSTRACT

In recent years great extensions were achieved in poultry industry in Egypt to increase its production, this trend is unfortunately hampered by recurrent episodes of diseases like coccidiosis and E. coli infection, worldwide bacterial infections affecting all ages of birds, are of continuing economic concern to poultry production due to major losses includes high morbidity and mortality rates, loss of body weight and other bird's performance. Control of both coccidiosis and colibacillosis is very important in poultry farms, which depend mainly on the use of certain anticoccidial drug as diclazuril and antimicrobials as fosfomycin.

This study was directed to test the interaction between fosfomycin and diclazuril in case of infection of E.coli.

In the present work one hundred and twenty, one-day old mixed sex avian 48 broiler chicks were used from a commercial hatchery. Chicks were divided into 8 equal groups each of 15 chicks. All groups were taken same treat and were fed on ordinary ration free from any drugs all over the experiment (42 days).

Chickens were infected with pathogenic field strains of E. coli (strain

078) through intratracheal route in dose 0.2 ml.

The groups were treated with fosfomycin in drinking water after the clinical signs had appeared 3rd day post of *E.coli* infection for 5 successive days from 28th day to 32nd day old age using recommended therapeutic dose 160 mg / kg B.wt. daily in drinking water or feed for 3-5 days , the groups were treated with (diclazuril) in drinking water for 3 successive days from 30th day to 32nd days old age used in the recommended therapeutic dose from the producer 1 ml Diclosol liquid /4 liters of drinking water (2.5 ppm). The results showed improvement in infected chickens condition represented in disappearing of clinical signs and decreasing mortality rate and using fosfomycin and diclazuril together did not effect on general activity of chickens.

INTRODUCTION

Antibiotics have been widely used in the livestock and poultry industries since their discovery more than 50 years ago. They represent an extremely important tool in the efficient production of animal products such as milk, meat and eggs; *Cromwell (1999)*.

Significant improvements in the performance of commercially reared poultry have been made during the last half of the twentieth century ; *Chapman et al., 2003*.

Fosfomycin has attracted great interest, since it was a completely new molecule, because of its broad spectrum antibacterial activity and excellent tolerability; *Forsgren and walder (1983)*.

Coccidiosis, coccidian, coxy or cocci is a disease of poultry caused by a protozoan type parasite. This parasite of the genus *Eimeria* lives and multiplies in the intestinal tract and causes tissue damage. This damage can interfere with food digestion and nutrient absorption as well as

causing dehydration and blood loss. The tissue damage can also expose the bird to bacterial infections, like Clostridium and Salmonella. Diseases that suppress the bird's immune system may act with coccidiosis to produce a more severe problem. For example, Marek's disease may interfere with the development of coccidiosis immunity and infectious Bursal disease may exacerbate the coccidian infection (*Julie, 1999*).

In modern broiler management, preventive measures are taken to control economically important diseases such as coccidiosis and bacterial enteritis, which reduce feed utilization and live performance characteristics.

MATERIALS AND METHODS

I. Materials:

1. Drugs:

a. Ca Fosfomycin (Adwifos)®:

It is a broad spectrum antibiotic produced by streptomyces jradiae strain 1,2. it is a phosphonic acid derivative unrelated to other antibiotic.

It presents in form of 250 gm powder Ca fosfomycin 25% produced by ADWIA Company.

Used in the recommended therapeutic dose from the producer company at a level of 160 mg / kg B.wt. daily in drinking water or feed for 3-5 days.

b. Diclazuril (Diclosol)®:

Synthetic anticoccidial drug belongs to benzeneactetonitrile derivative developed and described by pharma Swede-Egypt; it is available in the form of vials containing 100 ml. It is used in the

recommended therapeutic dose from the producer 1 ml Diclosol liquid /4 liters of drinking water (2.5 ppm).

2. Experimental chicks:

A total of one hundred and twenty (120), one day old chicks obtained from faculty of agriculture Tanta University, with an average body weight of 40-50 gm. They were housed in a well isolated floor pens under complete hygienic conditions, chicks were reared in a complete block design provided with wood shaving litter, plastic feeders and waters. The temperature was adjusted according to the age (the first week of age temperature 32-33°C and decreased 2°C per week, until it reached 22°C and fixed till the end of 42 day of age) according to *Yeo and Kim, (1997)*. The light was provided for 24 hours throughout the experimental period.

3. Experimental Ration: from Abd El-Salam Hagazy company

4. Vaccines:

All chickens were vaccinated against Newcastle and Gumboro diseases according to *Giambrone and Ronald, (1986)*. The vaccines used in this experiment were:

5. Materials used in bacteriological studies:

- a. Pathogenic Escherichia coli (E. coli stain) serotype O:78 was kindly obtained from Department of Bacteriology, Animal Health research Institute Dokki, Giza, Egypt.

1- Experimental design:

One hundred and twenty mixed sex one-day old chicks were classified into eight equal groups, 15 chicks each and kept separately. The chicks were provided with wood shaving litter, plastic feeders and water troughs. The birds were fed on a starter ration for first two weeks,
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on a grower ration for next two weeks and then on a finisher ration till the end of experiment. The chicks were supplied with 24 hours of light daily. The breeding period started with a temperature of 33°C over first week, and this temperature was then decreased by 2° C each week until it reached 22°C and fixed till the end of experiment according to *Yeo and Kim (1997)*.

All groups were kept under the same conditions and received the same procedures of management

Number	Group	Infection	Drug	Dose
1	A) infected treated with Ca Fosfomycin	Pathogenic Escherichia coli (E. coli stain) serotype O:78	Ca Fosfomycin	40 mg/kg B.W for 5 successive days
2	B) non infected treated with Ca Fosfomycin	None	Ca Fosfomycin	40 mg/kg B.W for 5 successive days
3	C) non infected treated with Ca Fosfomycin and Diclazuril	None	- Ca Fosfomycin - Diclazuril	- Ca Fosfomycin: 40 mg/kg B.W for 5 successive days - Diclazuril :
4	D) non infected treated with Diclazuril	None	Diclazuril	
5	E : Infected treated with Diclazuril	Pathogenic Escherichia coli (E. coli stain) serotype O:78	Diclazuril	
6	F : Infected treated with Ca Fosfomycin and Diclazuril	Pathogenic Escherichia coli (E. coli stain) serotype O:78	- Ca Fosfomycin - Diclazuril	- Ca Fosfomycin: 40 mg/kg B.W for 5 successive days - Diclazuril :
7	G : Infected non treated (Control +ve)	Pathogenic Escherichia coli (E. coli stain) serotype O:78	None	_____
8	I) Non infected non treated (Control -ve)	None	None	_____

2- Exposure to the infection:

Experimental infection by E.coli Serotype O:78 was performed on 23 days with dose 1 ml concentration of 1×10^8 cfu/ ml inoculation per os (*perminetal 2006*) via inoculation of 0.2 ml of E.coli strain serotype O78 containing (1×10^8 CFU/ml) intratracheally by using an automatic micropipette according to *Khairy et al., (2012)*.

- Treatment with Ca Fosfomycin started 48 hours post infection.

3- Blood sampling:

Blood sampling were taken at end of 7th , 14th and 21st day post-treatment in all groups.

4- Sampling for histopathological examination: specimens from liver, kidney and heart were collected from all groups on the day 49th and fixed in 10% neutral formalin.

5- Evaluation of growth performance parameters:

6- Hematological parameters:

7- Biochemical studies:

- a) Determination of serum Aspartate aminotransaminase (AST) AST - (Colorimetric method)
- b) Determination of serum alanine aminotransaminase (ALT): ALT – (colorimetric method)
- c) Determination of total serum protein: Total protein – Biuret Reagent (coloremtric method)
- d) Determination of serum uric acid Quantitative determination of uric acid
- e) Determination of Albumin – BCG

Tissue specimens from kidney, liver and heart were taken immediately from chickens after sacrificed at the end of the experiment.

Statistical analysis:

Data are represented as mean \pm SE (standard error). One-way analysis of variance (ANOVA) was used to compare the means of values of all groups at a significance level pf $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

Statistical analysis was performed using the method of *Patrie and Watson, (1999) and computerized using SPSS 11(2001)*.

RESULTS

1- Clinical signs:

Infected non treated chickens and infected chicken treated with Diclazuril only showed various degrees of clinical manifestations vary from mild to severe, depending on the defense ability of each bird. Such as anorexia, depression, loss of appetite, nasal discharge, loss of weight, foul smell greenish diarrhea at the 3rd day post E.coli serotype O78 inoculation

2- Lesion scoring and mortality rate:

Infected chickens non treated or treated with Diclazuril only produced gross pathological lesions such as air sacculities, thoracic and abdominal air sacs were opaque , pericarditis as heart was enlarged with fibrinous pericarditis, trachea revealed tenacious exudate with linear hemorrhages, lungs revealed dark red congested appearance in some cases and liver was enlarged with focal congestion.

3- Performance:

In the present study, it has been shown that a slight increase in feed intake in all groups received fosfomycin, in the same time groups received fosfomycin and not infected showed a significant increase in final body weight and infected group received fosfomycin showed a slight increase in final body weight and body weight gain the same. As well as the feed conversion ratio was decreased and feed efficiency was slightly increase.

Regarding group treated with Diclazuril show decreasing in body weight comparable to groups treated with fosfomycin or fosfomycin and diclazuril together and slightly near to control group, Groups treated with fosfomycin and Diclazuril together did not show any significant change only non-infected group show more improvement in performance than infected one.

4- Hematological examination:

Infected groups with E. coli revealed drop in PCV%, Hb concentration and RBCs count and after treated with fosfomycin show slightly increase post treatment. This finding may be attributed to the pathogenesis and virulence properties of these particular invasive strains. Findings might be attributed also to enterohemolysin, which consider as cell damaging protein toxin produced by pathogenic E. coli that causes changes in cell membrane permeability and formation of surface lesions leading to RBCs destruction.

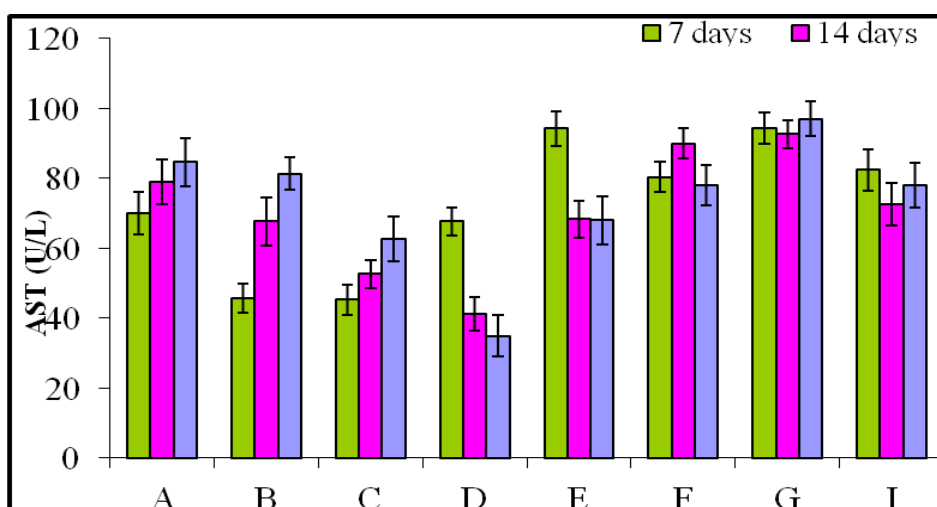
Groups treated with Diclazuril show slightly decrease in PCV%, Hb concentration and RBCs count. Our observation is disagree with what reported by *El-Sayed (2002)* and *Hassan, (2002)* who demonstrated that when given diclazuril to infected chicks with coccidian showed a significant increase in RBCs count, hemoglobin concentration, PCV% and a significant decrease in total leucocytic count when compared to positive control group and reported that diclazuril treated groups showed non-significant changes in the hemogram or leucogram when compared to non-infected non treated during the experimental period.

Non-infected group treated with both fosfomycin and diclazuril showed slightly decreasing in PCV%, Hb concentration and RBCs count when compared to non-infective non-treated group. On the other hand we observed that infected group treated with fosfomycin and diclazuril

showed decreasing in PCV%, Hb concentration and RBCs count compared to infective non treated group and infected group treated with fosfomycin and diclazuril.

5- Biochemical examination:

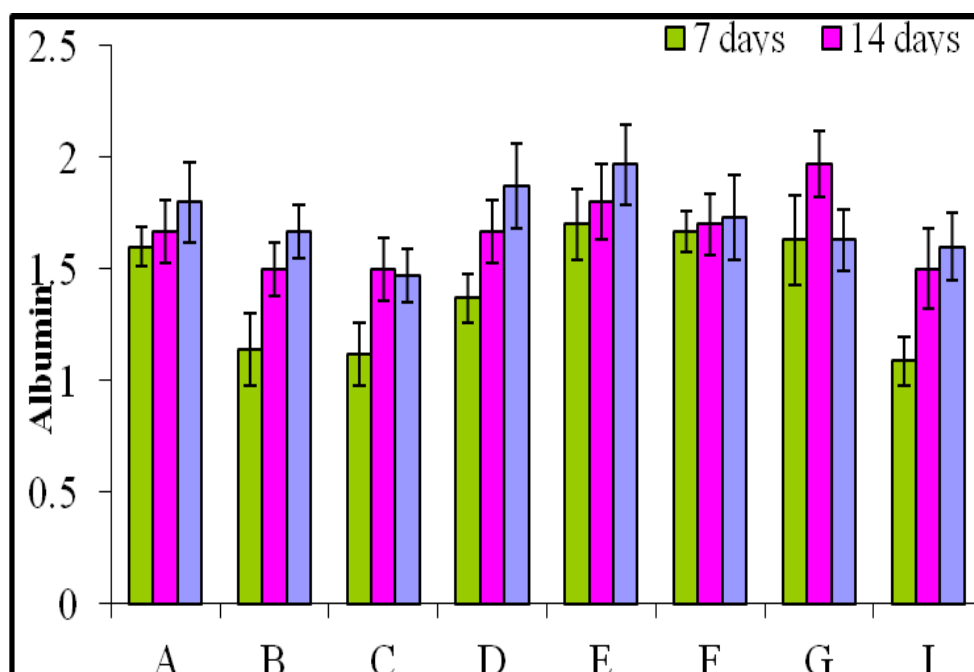
AST: showed increase in AST level in infected chickens of *E. coli* (O78) and about to normal when treated with fosfomycin but in chickens treated with diclazuril showed decrease in AST level



ALT: showed increase in ALT level in infected chickens of *E. coli* (O78) when compared with non-infected chickens group and observed sharp decrease in ALT level in groups treated with fosfomycin and insignificant decrease in groups treated with Diclazuril.

In total protein showed that infected chicken with *E. coli* (O78) slightly decrease in total protein and when it treated with fosfomycin increase about normal, there is no significant change in total protein in case of treating with diclazuril.

Albumin: only observed the difference between infected chickens with E. coli (O78) and non-infected chickens as showed elevation in albumin level in infected chickens when compared with non-infected one.



Uric acid: infected chickens with E. coli (O78) showed increase in uric acid level when compared with non-infected one, treated chickens with fosfomycin resulted with slightly decrease in uric acid level and no significant changes happened to groups treated with Diclazuril.

DISCUSSION

The purpose of this study was to determine drug interaction between two drugs may be used together as antibiotic (Fosfomycin) and anticoccidal drug (Diclazuril), this study was conducted on 120 chicken

divided to 8 groups and monitor the effect of both drugs treatment and their interaction on body performance, hematological and biochemical parameters also pathological studies and post mortem investigations on E.coli infected chicken were performed.

1- Clinical signs:

Infected non treated chickens and infected chicken treated with Diclazuril only showed anorexia, depression, loss of appetite, nasal discharge, loss of weight, foul smell greenish diarrhea at the 3rd day post E.coli serotype O78 inoculation. Greenish diarrhea which also reported by *Hafez, (2008) and Younes, (2012)* also recorded diarrhea and respiratory symptoms including nasal discharge, gasping, rales and cough in experimentally E. coli infected broiler chickens at 23rd day of age. *Jacy et al., (2010)* found that E.coli strains colonized and provoked cytotoxic effects in the ileal and colonic mucosa as total of partial villi destruction, epithelium detachment and rearrangement of mucosa structure, which could explain the perpetuation of the diarrhea. Similar signs also reported by *Ameh et al., (2011)*.

2- Lesion scoring and mortality rate:

Infected chickens non treated or treated with Diclazuril only produced gross pathological lesions such as air sacculities, thoracic and abdominal air sacs were opaque , pericarditis as heart was enlarged with fibrinous pericarditis, trachea revealed tenacious exudate with linear hemorrhages, lungs revealed dark red congested appearance in some cases and liver was enlarged with focal congestion. *Hughes and Heritage, (2001) and Melha et al., (2003)* stated that E. coli is capable of causing problems at almost any site of the body when enter chickens by

the respiratory tract and produce extra intestinal disease, the most common form of colibacillosis is an intitial respiratory infection (airsacculitis) which is fecquently followed by a generalized infection (perihepatitis, pericarditis and septicemia). Similar gross lesions have been reported by *Ameh et al., (2011)*.

3- Performance:

In the present study, it has been shown that a slight increase in feed intake in all groups received fosfomycin, in the same time groups received fosfomycin and not infected showed a significant increase in final body weight and infected group received fosfomycin showed a slight increase in final body weight and body weight gain the same. As well as the feed conversion ratio was decreased and feed efficiency was slightly increase. Similar results were observed by *Fuller (1995)* found that the use of probiotics had beneficial effects on the growth rate, feed conversion efficiency and resistance to diseases. Also, *Abd El-Aziz, (2002)* recorded that antimicrobials produced an increase in growth rate, reduced mortality in growing chicks and increase body weight gain with improved FCR through inhibiting pathogenic organisms which damage the gut epithelium impairing food absorption, inhibiting nonpathogenic organisms which complete for growth factors in the gut and inhibiting organisms producing substances affecting the growth.

Regarding group treated with Diclazuril show decreasing in body weight comparable to groups treated with fosfomycin or fosfomycin and diclazuril together and slightly near to control group, these findings agree with that recorded by *El- Sayed (2002)* who demonstrated that there is no significant change occurs by given diclazuril to non-infected chicks. Groups treated with fosfomycin and Diclazuril together did not show any

significant change only non-infected group show more improvement in performance than infected one.

4- Hematological Examination:

Infected groups with *E. coli* revealed drop in PCV%, Hb concentration and RBCs count and after treated with fosfomycin show slightly increase post treatment. This finding may be attributed to the pathogenesis and virulence properties of these particular invasive strains. Findings might be attributed also to enterohemolysin, which consider as cell damaging protein toxin produced by pathogenic *E. coli* that causes changes in cell membrane permeability and formation of surface lesions leading to RBCs destruction as reported by *Dagmar et al., (2002)*.

Pervious assumption is supported by *Hamouda (2008) and Zaki et al., (2012)* who stated that respiratory infection caused by *E. coli* provoked a significant decrease in RBCs count, Hb concentration, PCV% whereas the leucocytic count and lymphocytes were significantly increased. This indicates that *E. coli* causes deleterious effect on the hematopoietic system.

Groups treated with Diclazuril show slightly decrease in PCV%, Hb concentration and RBCs count. Our observation is disagree with what reported by *El-Sayed (2002)* and *Hassan, (2002)* who demonstrated that when given diclazuril to infected chicks with coccidian showed a significant increase in RBCs count, hemoglobin concentration, PCV% and a significant decrease in total leucocytic count when compared to positive control group and reported that diclazuril treated groups showed non-significant changes in the hemogram or leucogram when compared to non-infected non treated during the experimental period.

Non-infected group treated with both fosfomycin and diclazuril showed slightly decreasing in PCV%, Hb concentration and RBCs count when compared to non-infective non-treated group. On the other hand we observed that infected group treated with fosfomycin and diclazuril showed decreasing in PCV%, Hb concentration and RBCs count compared to infective non treated group and infected group treated with fosfomycin and diclazuril.

REFERENCES

- **CROMWELL G., T. STAHLY and T. MONEGUE (1999):** Effect of level of sulfamethazine in finishing feed for swine on sulfa residues in pork. J. Anim. Sci. 51(1), 79.
- **Chapman, H. D. (2003).** Origins of coccidiosis research in the fowl - the first fifty years. Avian Dis. 47: 1-20.
- **Julie D. Helm (1999):** DVM, Clemson University, Livestock Poultry Health Programs, POB 102406, Columbia, SC 29224, (803) 788-2260 jhelm@clemson.edu, www.clemson.edu/LPH , February 17.
- **JULIAN, R.J., M. WHITE and P. GAZDZINSKI, (1999):** Efficacy of Vaccination to Control Turkey Coccidiosis. Proceedings of the 2nd International Symposium on Turkey diseases. Berlin 24th–27th March 1999 (ed. HAFEZ, H.M. and MAZAHERI). ISBN 3-903511-75-4. pp. 159-164.
- **A. Forsgren, M. Walder (1983):** Antimicrobial activity of fosfomycin in vitro. J Antimicrob Chemoth, 11, pp. 467–471.
- **Patrie and Watson (1999):** Variables of hepatic function, body condition score, milk fat-protein concentration and sampling period as a repeated measure.
- **Petrie, Paul Watson. Wiley, Jun 9, 1999 - Medical - 241 pages ... and other animal science students approach with foreboding**

- ***Dagmar Zweytick, Karl (2002)***: by the Commission of the European Communities grant QLK2-CT-01001, “Antimicrobial.
- ***HAFEZ, A.S (2008)***: pharmacodynamics studies on drug interzction of Ca phosphomycin and/or gallipro and entramycin in broiler. Ph. D Pharmacology Dep., Fac. Vet. Med., Kafer El-Sheikh University.
- ***Ameh, J.A., J.Y. Adamu, and T,L. Ikpa, (2011)***: Experimental infection of chicks with Avian Enteropathogenic Escherichia coli (APEC) Serotype O78:K8. African Scientist, 12(1): 27-32.

بعض الدراسات الفارماكولوجية علي التداخلات الدوائية

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في السنوات الحديثة توسعت صناعة الدواجن في مصر لزيادة الانتاج، ولكن للأسف تهاجم بالامراض مثل الاصابة بالكوكسيديا و الايكولاي، تصاب الدواجن بانحاء العالم بالاصابة البكتيرية في كل الاعمار، الذي يسبب خسارة اقتصادية نتيجة ما يسببه من زيادة نسبة الاصابة والنفوق ونقص معدلات النمو في الدجاج. تعتبر الوقاية من الكوكسيديا و الايكولاي مهمة في مزارع الدواجن باستخدام مضاد الكوكسيديا مثل (دايكلزوريل) و مضاد بكتريا مثل (فوسفومايسين).

استهدف هذا البحث دراسة التداخل الدوائي بين فوسفومايسن و الدايكلزوريل في حالة الاصابة بالايكولاي. تمت التجربة علي 120 دجاجة من عمر يوم قسمت الي 8 مجموعات كل مجموعة 15 دجاجة ومدة التجربة 42 يوم، تمت الاصابة بالايكولاي عن طريق الحقن داخل القصبه الهوائية بجرعة 0.2 ملي عند عمر 23 يوم تم مراقبة وتسجيل الاعراض تم ادخال الفوسفومايسين بعد العدوي بالايكولاي بثلاثة ايام لمدة 5 ايام متتالية من اليوم 28 الي اليوم 32 من العمر، و المجموعات التي تم معاملتها بالدايكلزوريل في ماء الشرب لمدة 3 ايام متتالية من اليوم 30 الي اليوم 32 من العمر و اظهرت النتائج تحسن في حالة استخدام عقار الفوسفومايسن المتمثل في اختفاء الاعراض المرضية، نحسن حالة الاكياس الهوائية، تقليل الناقل و في حالة استخدام العقارين مجتمعين (الفوسفومايسين و الدايكلزوريل) تبين ان العلاج بالدوائيين معا لا يؤثر علي النشاط العلاجي ولم يظهر اي تأثير سلبي علي الحالة العامة للطيور.