BIOCHEMICAL STUDIES ON ENROFLOXACIN AND SOME FEED ADDITIVES COMMONLY USED IN BROILER CHICKS.

Suez Canal University, Faculty of Veterinary Medicine, Pharmacology Department.

ABSTRACT

The present study aimed to evaluate the comparative effect of probiotics and some antibiotics on the chicken gut microflora as well as their effect on some biochemical parameters in broiler chicks. The experiment was carried out on 600 one day old chicks divided into six groups each contain 100 chicks. The chicks were reared in separated blocks, provided with wood shaving litter, plastic feeders and water troughs. The birds were fed on a starter ration for the first three weeks and then on a grower ration for the next three weeks and the water was provided continuously. The serum AST, ALT, uric acid, total protein and albumin were measured. The results showed elevation in serum AST, ALT, uric acid levels of enrofloxacin treated birds, also revealed hypoproteinaemia and hypoalbuminaemia in the same treated group which indicated pathological changes in liver and kidney of this group. These changes occurred in less severity in the first weeks of treatment in enramycin treated birds and soon returned to the normal levels. Meanwhile other treated groups showed normal biochemical results all over the experiment compared to the control.

INTRODUCTION

The conditions of poultry production have changed over the years. Breeding and husbandry have changed in respect to faster growing and more productive birds, hatching in more or less hygienic environment, more birds per square meter, use of antimicrobials as a result of intensive production etc. These changes lead to stress conditions in the birds which may result in less natural resistance of the birds against infection with pathogens. Under such circumstances, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents were used to suppress or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency (Jausselin, et al., 1960; Morch, et al., 1982; Radostitis, et al., 1994). The production of food stuff of animal origin connected with the application of feed additives and veterinary drugs is
necessary for increasing production of meat and eggs *(Joint FAO/WHO, 1994)*. Healthy birds with a stable gastrointestinal microflora are thought to be maintained by using antibiotics in preventive dosages but these low dosages have negative effects on gastrointestinal microflora. To decrease the use of antibiotics, whose usage may result in bacterial resistance and in the formation of residues in organs and in tissues of treated birds, the use of probiotic can considered *(Mujeeb, 2000)*. Probiotics are group of microorganism strains that are robust enough to survive the harsh physico-chemical conditions present in the gastrointestinal tract (this condition include gastric acid, bile secretions and competition with the resident microflora). *Fooks and Gibson (2002)*. Our study is used to investigate the effects of probiotic, enramycin, enrofloxacin and their combinations on some biochemical parameters.

**MATERIAL & METHODS**

**I) Drugs:**

a) **Probiotic bacteria** *(Galibro)®:* It is a powder product (freeze dried bacteria) administered through feeding ration. 200 gm/ton of broiler's ration, Composed of *Bacillus Subtilus* 4×10¹², carrier Whey permeate and sodium alumonium silicate, produced by Biochem International, Germany.

b) **Enramycin hydrochloride**®: It is a basic polypeptide antibiotic developed by Schering- plough, Animal Health, America, and used as 250 gm/ton of broiler's ration. Its molecular formula is: Enramycin A *(C₁₀⁷H₁₃₈N₂₆O₃₁CL₂)* and enramycin B *(C₁₀₈H₁₄₀N₂₆O₃₁CL₂)*.

c) **Enrofloxacin** *(Spectrama®- Vet):* Enrofloxacin is a quinolone carboxylic acid derivative with antimicrobial action. It was obtained from Amoun Pharmaceutical Company, Egypt, in the form of 10% solution. Its Chemical name is Cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-[(4-ethyl)-1-piperaziny1]-3-quinolinecarboxylic acid, hydrochloride. Its Molecular formula: *C₁₉H₂₂FN₃O₃*.

II) **Experimental design:**

600 one day old chicks were classified randomly into six groups (each of 100 chicks) and kept separately using wooden chick partitions. The chicks were reared in a complete block design, provided with wood shaving litter, plastic feeders and water troughs. The birds were fed on a starter ration for the first three weeks and then on a grower ration for the next three weeks and the water was provided *ad libitum*.

The chicks were supplied with 24 hours of light daily. The brooding period started with a temperature of
32°C over the first week, and this temperature was then decreased by 2°C each week until it reached 22°C and fixed till the end (42 day of age) according to Yeo and Kim (1997).

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

The chicks were divided into six groups each of one hundred. The first group served as control received basal diet free from any additives or treatments, The second group received basal diet supplemented with probiotic (Galibro®) (200 gm/ton ration) all over the experiment, the third group received basal diet supplemented with enramycin at a dose of 250 gm/ton feed all over the experiment, the forth group received basal diet supplemented with enrofloxacin at a dose of (2 ml/ liter water) for three days and water free for seven days in an alternative manner till the end of experiment, the fifth group received basal diet supplemented with probiotic mixed with enramycin (200 gm/ton ration, 250 gm/ton feed), respectively together all over the experiment, the six group received basal diet supplemented with enrofloxacin (2 ml/ liter) in the water for three days and probiotic (200gm/ton) in the ration for the next seven days in an alternative manner till the end of experiment.

IV) Biochemical studies:

Blood samples from each control and treated groups were taken weekly by slaughtering the chicks and collected in dry clean centrifuge tubes without anticoagulant for separation of serum (to obtain clear serum the blood samples were left to clot at room temperature then centrifuged for about 20 minutes at 3500 r.p.m). The sera were labeled and stored in deep freezer at -20°C until used for biochemical analysis.

The collected sera were used to investigate the effect of probiotic, enramycin, enrofloxacin and their combinations on some hepatic and renal function tests such as serum aspartate aminotransferas (AST) and serum alanine aminotransferas (ALT) (Reitman and Frankel, 1957) total serum proteins (Henry, 1964), serum albumin (Doumas, 1971) and serum uric acid (Henry, et al.,1974). All previous tests were determined by colorimetric method using ready made kits of Diamond diagnostics®.

V) Statistical analysis:

The Statistical analysis was performed using the least square means described by Likelihood program of SAS (1996). The significances of group differences were carried out according to Duncan’s (1955). The obtained results were expressed as Mean± S.E.
RESULTS & DISCUSSION

The results showed that probiotic treated group of birds showed a decrease in serum AST and ALT in the 1\textsuperscript{st} week only then returned to the normal level along the rest of the experiment as compared with the control group; this might be due to the stress of transporting birds and changing their environment.

All treated groups of birds showed non significant changes in serum AST and ALT compared to the control group. Versus of enrofloxacin treated group of birds showed elevation in serum AST and ALT in the 3\textsuperscript{rd} and 4\textsuperscript{th} week of the experiment and then returned to the normal levels compared to the control group.

\textit{(Doxy, 1971)} mentioned that the level of sAST and sALT is increased following liver damage, accordingly this finding might be attributed to damage of hepatic cells by the direct effect of enrofloxacin resulting in escape of these enzymes into the plasma.

It is well documented that the increase of sAST and sALT activity is mainly recorded with liver damage and myocardial infarction leading to alteration in cellular permeability due to change in normal cell membrane. This allows the escape of this enzyme into serum in abnormal high levels \textit{(Charles, et al., 1959; Ross, et al., 1976; Joan and Pannal, 1981)}. These results also supported by \textit{Khodary and El-Sayed (1997) and Shawky et al. (1998)} who mentioned that, treatment with fluoroquinolones (enrofloxacin) showed an increase of AST.

Concerning serum ALT, many studies recorded that this enzyme doesn't increase in all cases of hepatic diseases in birds and therefore is not useful as diagnostic indicator of liver disease in these species \textit{(Harrison and Harrison, 1986)}.

Uric acid is the primary catabolic product of protein, non protein nitrogen and purines in birds. Birds are uricotelic and produce uric acid not urea as the major nitrogenous end product of metabolism; therefore blood urea nitrogen is not diagnostic test of renal affection in birds \textit{(Harrison and Harrison, 1986)}.

The avian kidney excretes urea entirely by filtration \textit{(Shannon, 1938 and Osbaldiston, 1968)}.

Therefore, the elevation in uric acid values was expected with impaired renal function, massive tissue destruction and gout or starvation \textit{(Halliwell, 1981)}.

In the present study birds, treated with probiotic and birds treated with enramycin showed non significant changes in serum uric acid all over the experiment compared to the control group, similarly probiotic plus enramycin and probiotic plus enrofl oxacin showed non significant changes in serum uric acid all over the exp-
eriment except the 2\textsuperscript{nd} week they showed an increase in serum uric acid which soon returned to the normal levels during the rest of the experiment compared to the control group. Versus of enrofloxacin treated group which showed a significant increase in serum uric acid all over the experiment compared to the control group, which may be attributed to impaired renal function caused by direct effect of enrofloxacin on the kidney.

These results coordinated with those reported by Khodary and El-Sayed (1997). They reported that a fluorquinolone enrofloxacin induced significant increase in uric acid also the same authors mentioned that these changes regained to their normality one week post treatment.

Birds treated with probiotic showed normal serum level of protein all over the experiment except for the second week, it showed mild hypoproteinamia which soon returned to the normal levels compared to the control group, also showed normal level of serum albumin all over the experiment.

These results were agreed with Ghoin and Sapcota (1998) they mentioned that probiotic increased growth of broiler chicks but serum protein and cholesterol not affected.

Enramycin treated birds showed decreased level of serum protein and albumin in the 2\textsuperscript{nd} and 3\textsuperscript{rd} weeks but showed normal levels of them all over the experiment compared to the control group. Meanwhile birds treated with enrofloxacin showed decreased level of serum protein and albumin from the 2\textsuperscript{nd} week to the end of the experiment. Other treated groups showed normal levels of both serum protein and albumin.

These results could be presumably attributed to the pathological changes in the liver and kidney caused by administration of enrofloxacin or sometimes enramycin to the normal chicks.

The occurrence of this hypoproteinenaemia may be attributed to damage of endothelium of glomerular tuft or most likely associated with inhibition of protein synthesis in the liver (Tung, et al., 1975).

The decreased in albumin might be possibly attributed to its small size and osmotic sensitivity to fluid movements as in case of renal diseases as glomerulonephritis, nephrosis and nephritic syndrome (Hoe and Harvey, 1961). Further, the liver is the sole site of albumin synthesis and hypoalbuminaemia is an important feature of liver disease (Kaneko, 1980).

The present results were supported with Ramadan (1996), who mentioned that the oral administration of fluoroquinolones to healthy chicks at first 3 successive days of age produced a significant decrease in total serum protein levels at the end of the experiment and these changes regained their normality one week post
treatment. Moreover, the decreased total serum protein levels in the present data might be explained by renal damage provoked by the drug.

CONCLUSION

Although enrofloxacin is effective in treating many serious infections in broiler chicks but as seen in the present study the drug has bad effects on serum biochemical parameters compared to enramycin or probiotic So the present study recommends elimination of enrofloxacin from poultry farms and using its recommended alternatives, or using it at the same time with addition of probiotics to the diet of the chicks and avoids long time administration.

![Graph](image1.png)

**Fig. (1)**: Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg /liter drinking water) on serum level AST of broilers.

![Graph](image2.png)

**Fig. (2)**: Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg /liter drinking water) on serum level of ALT of broilers.
Fig (3): Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg/liter drinking water) on serum level of uric acid of broilers.

Fig. (4): Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg/liter drinking water) on serum level of protein of broilers.
Fig. (5) : Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg/liter drinking water) on serum level of albumin of broilers.

Fig. (6): Effects of probiotic (200 g/ton ration), enramycin (250 g/ton ration) and enrofloxacin (200 mg/liter drinking water) on serum level of glucose of broilers.
REFERENCE

الملخص العربي

دراسات بيوكيمائية على الانروفلوكساسين و بعض الاضافات العلفية في بداري التسمين

أ/د/ مصطفى فايز محمد حسن, أ/د/ حاتم عبد الخالق جماز, أ/د/ وليد حلمي خليل, طب/ راشة أحمد بخيت
قسم الأدوية البيطرية، كلية الطب البيطرى، جامعة قناة السويس

استهدفت هذه الدراسة المقارنة بين تأثير البروبيوتيك البكتيرى و بعض المضادات الحيوية على بعض معدلات الدم الكيميائية الخاصة بوظائف الكبد والكلى وقد تم ترتيب 100 كتكتي عمر يوم واحد لهذا الغرض.

قامت الكتاكيت إلى ست مجموعات كل منها يحتوى على 100 كتكتي تم تمت معاملتها واحدة من حيث المناخ (درجة الحرارة و الرطوبة والإضاءة). تم ذبح خمسة طيور عشوائية من كل مجموعة أسبوعيا و تجمعت عينات الدم و فصل السيرم بالطرد المركزي لعمل تحليل لبعض معدلات الدم الكيميائية الخاصة بوظائف الكبد والكلى.

أظهرت الفحوصات الكيميائية زيادة نشاط الإنزيمات الخاصة بوظائف الكبد و نقص في بروتينات الدم مع حدوث زيادة في حمض النيكولا الذي يعتبر المؤشر الحساس لخلل وظائف الكلى، وذلك في المجموعة المعاملة بالانروفلوكساسين منفردا مما يشير إلى تأثيره السببي على وظائف الكبد.

كما أن المجموعات التي تم خلط البروبيوتيك فيها مع المضادات الحيوية لم تظهر أي نتائج سلبية في وظائف الكبد أو الكلى.