Scientific Journal of October 6 University
ISSN (Print): 2314-8640
ISSN (Electronic): 2356-8119

Available online at: http:// sjou.journals.ekb.eg
Original Article

Citation: Kadry MS and Hazem MS (2014). Biopharmacodynamical Effect of Clindamycin and Spectinomycin Combination in Experimentally *E.Coli* Infected Broilers. Sci.J. of Oct. 6 Univ.2(2), 166-174.

Biopharmacodynamical Effect of Clindamycin and Spectinomycin Combination in Experimentally E.Coli Infected Broilers

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Received: 17-03-2014/ Revised: 03-04-2014 / Accepted: 06-04-2014

Abstract
Antimicrobials are co-administered frequently worldwide in medical as well as veterinary practices. Often, the therapeutic efficiency of two antimicrobial drugs may induce adverse effects. In this study, the possibility of a clindamycin/spectinomycin interaction effect and the efficacy of the combination were investigated in 23 day-old chickens experimentally infected with *E. coli*. The challenged chickens were orally treated with *Clindamycin* (11mg/ kg body weight) or spectinomycin (51.1 mg/L) for 5 successive days with a combination of both drugs at therapeutic and half-therapeutic doses. Two blood samples were collected from five chickens of each group on the 4th day during the treatment course and on the 2nd, 7th, 14th and 21st days post-treatment. The results revealed that co-administration of clindamycin/spectinomycin reduced the severity of clinical signs and altered the biochemical function parameters related to liver, kidney and of oxidative stress. The effect of both drugs was short-lived, and most of the parameters returned to normal within 2 weeks after administration of them. It is concluded that, the therapeutic doses co- administration of clindamycin/spectinomycin reduced the severity of *E. coli* infection clinical signs and showed significant alterations in some liver and kidney function parameters as well as oxidative stress. This effect is short-lived and most of the parameters went back to normal within 2 weeks post drug(s) administration.

Key Words: Biochemical, Clindamycin, Bpectinomycin, Broilers, Escherichia coli

Introduction

Avian colibacillosis continues a problem in poultry production and remains one of the major serious endemic and economic threats affecting the poultry industry worldwide. It causes mortality and destruction of the flocks [(1)]. Avian pathogenic *Escherichia coli* (APEC) causes airsacculitis, polyserositis, septicaemia and diseases, primarily extraintestinal, in chickens, turkeys and other avian species. APEC is found in the intestinal microflora of healthy birds, and most of the diseases associated with these bacteria are secondary to environmental factors and predisposing factors in the hosts [(2)].

The *in vitro* sensitivity of these pathogens to antibiotics, intervention to address these infections may be warranted. During and after therapeutic intervention, the flock should be carefully evaluated as to the success of treatment. These evaluations can be performed by attending veterinarian or by service personnel under the veterinarian's direction. Accurate records should be maintained on all treatment outcomes and included in the farm history records for future reference [(3)].

Regardless of the actual contribution that antibiotic use in animal production has on the incidence of antibiotic-resistant infections in humans, serious consideration is being given to establishing regulations that would severely restrict the use of antibiotics in animal production [(1)]. If antibiotic use is restricted in poultry production, it would be anticipated that colibacillosis would become an even greater problem. Therefore, there is a real need to find a new approach to antibiotic use for the prevention and treatment of colibacillosis in poultry production.

One of these approaches is the co-administration of different antimicrobials. This may, however, result in unpredictable therapeutic results, potentially reducing the therapeutic efficacy or increasing the toxicity of the administered drugs [(4)].

There are some very efficacious antimicrobials, clindamycin and spectinomycin, are frequently

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used as prophylactic medications, as well as therapeutically[5].

Clindamycin inhibits protein synthesis in susceptible bacteria by binding to the 50S subunits of the bacterial ribosomes and preventing peptide bond formation. The lincosamides are usually considered bacteriostatic; however, when clindamycin is present at sufficient concentrations, it may be bactericidal towards sensitive organisms. It has a spectrum of activity that includes *Mycoplasma* species, *Staphylococcus* species, and *Streptococcus* species (except *Streptococcus faecalis*), as well as anaerobic organisms such as *Clostridium perfringens*. The administration of clindamycin to rats with peritonitis due to haemolytic *E. coli* reduce mortality. Subinhibitory concentrations of clindamycin can inhibit growth and haemolysin production by *E. coli* and reduce mortality in an animal model of haemolytic *E. coli* peritonitis. Oral absorption of clindamycin is high and unaffected by food. The drug is widely distributed into most tissues, including respiratory tissue, soft tissue, bones, and joints [6].

Spectinomycin is active against a variety of aerobic gram-negative and gram-positive organisms as well as *Mycoplasma* species. Spectinomycin is clinically used primarily for its activity against gram-negative organisms, but some gram-positive organisms may also be susceptible to it. Spectinomycin binds to the 30S ribosomal subunit of the microorganism and inhibits protein synthesis by preventing elongation of the polypeptide chain at the translocation step. It is slightly absorbed from the gastrointestinal tract. Twenty-four hours following oral administration, it is found at decreasing concentrations in kidney, liver, lung, muscle, and fat [7].

Unfortunately, there is little published information concerning the use of clindamycin and spectinomycin to treat *E. coli* or other infections in poultry. These data are required to determine dosage schedules for clinical use in birds. Therefore, this study has been designed to investigate the efficacy of clindamycin and spectinomycin combination usage *E. coli*-infected chickens and the effects of this combination on some biochemical parameters indicative of the general health of the chickens.

Material and Methods

This study was carried out in strict accordance with the recommendations in the guide for the care and use of laboratory animals of the national Institutes of Health (NIH). The protocol was approved by the local committee on the ethics of animal experiments of the University of Damanhur. All efforts were made to minimize suffering of two hundred and fifty clinically healthy, unvaccinated one-day old unsexed Hubbard broiler chicks were used. The chickens were obtained from a commercial hatchery. They were placed in the animal housing at the Faculty of Veterinary Medicine, Damanhour University. The birds were monitored for two weeks for any apparent clinical signs of disease prior to drug administration. The temperature was maintained at 25±2°C, and humidity was maintained at 45–65%. The chickens had free access to water and food without additives, such as antibiotics and growth promoters.

Chemotherapeutic agents

Clindamed® (Clindamycin 40%) was obtained from Arabcomed Co. Ltd. Egypt. Each 47 grams was equivalent to 40 gm. of the Cindamycin base. The recommended dose is 5-11 mg/ kg body weight for five successive days in the drinking water [8].

Moraspectam® (Spectinomycin 50%) obtained also from Arabcomed Co. Ltd. Egypt. Each 77 grams was equivalent to 50 gm. of the Spectinomycin base. The recommended dose is 51.1 mg/L for five successive days in the drinking water [5].

E. coli challenge

E. coli strain O78 was obtained from the Animal Health Research Institute, Dokki, Giza, Egypt. The broiler chickens of the treated groups were orally infected at 23 days of age with a 1 ml inoculum containing 10^9 CFU (colony forming units) as described by Chansiripornchai and Sasipreeyajan [9].

Efficacy of clindamycin and spectinomycin against E. coli

Prior to use in the birds, the sensitivity of the specific *E. coli* strain to clindamycin and spectinomycin was tested using the disc diffusion method. Clindamycin and spectinomycin discs (Oxoid, UK) were used. The diameters of the inhibition zones were interpreted by referring to the tables [10].

Experimental design

On the 23rd day of age, the chicks were classified into 10 groups of 25 chicks each as follows:

Group 1. These birds served as a control group (non-infected – non-treated).

Group 2. Non-infected birds were treated orally with clindamycin at a dose level of 11mg/kg for 12 hours, once daily, for five successive days.

Group 3. Non-infected birds were treated orally with spectinomycin at a dose level of 51.1mg/L for 12 hours, once daily, for five successive days.

Group 4. Non-infected birds were treated orally with a combination of clindamycin and spectinomycin at half-doses of each drug for 12 hours, once daily, for five successive days.

Group 5. Non-infected birds were treated orally with a combination of clindamycin and spectinomycin at the full doses for 12 hours, once daily, for five successive days.

Group 6. Birds were infected experimentally with *E. coli*.

Group 7. Infected birds were treated orally with clindamycin at a dose level of 11mg/kg after the

start of symptoms, available for 12 hours, once daily, for five successive days.

Group 8. Infected birds were treated orally with spectinomycin at a dose level of 51.1 mg/L after the start of symptoms, available for 12 hours, once daily, for five successive days.

Group 9. Infected birds were treated orally with a combination of clindamycin and spectinomycin at half doses, after the start of symptoms, available for 12 hours per each, once daily for five successive days.

Group 10. Infected birds were treated orally with a combination of clindamycin and spectinomycin at the full doses after the start of symptoms, available for 12 hours, once daily, for five successive days.

The clinical signs of *E. coli* infection in broilers were diarrhoea, lack of appetite and ruffled feathers. Before starting the treatment, three infected birds were slaughtered and examined post-mortem for lesions and for bacterial isolation from liver and heart. At necropsy, the bird's liver, air sac and heart were aseptically excised, and swabs from liver and heart were incubated in beef infusion broth and then plated on MacConkey agar at 37°C for 24 hours. The serogroup of *E. coli* was confirmed by agglutination reaction with *E. coli* O78 antiserum.

Sampling

Blood samples were collected from the wing veins of chickens of all groups on the 4th day of treatment and on the 2nd, 7th, 14th and 21st days post-treatment for biochemical studies. The sera were collected by centrifugation at 3000 rpm for 15 min. and kept frozen at -20ºC until used for biochemical analysis. Serum biochemical parameters were analysed by commercially available kit methods.

Statistical analysis

The recorded data were presented as the mean ± SE. The significant differences were calculated based on two-way test of ANOVA, and $p<0.05$ was considered as significant between the groups. All statistical analyses were carried out using SigmaStat for Windows, version 2.0, Jandel Corp., San Rafael, CA, U.S.A.

Results and Discussion

The present study showed that non-infected chickens treated with clindamycin displayed significant increases in serum ALT and ALP activities on the 4th day of treatment. Non-infected chickens treated with spectinomycin showed a significant increase in serum ALT activities on the 4th day of treatment. Non-infected chickens treated with a half-dose of the clindamycin-spectinomycin combination revealed non-significant changes in serum ALT and ALP activities on the 4th day of treatment and on the 2nd day post-treatment. However, non-infected chickens treated with a full dose of the clindamycin-spectinomycin combination showed a significant increase in serum ALT on the 4th day of treatment, Tables 1 and 2. The previous investigations reported that many drugs induce changes in hepatic and renal functions[11]. The increased ALT and ALP activities in the clindamycin and/or spectinomycin-treated chickens might be attributed to alteration of membrane permeability or damage of the hepatic cells by direct effects of the drugs, resulting in escape of these enzymes to the plasma[12]. Our results parallel those obtained by Smith and Reynard[13], Plumb[14] and Gilman[15], who reported that both drugs at therapeutic doses caused minor elevations in these hepatic enzymes. Interestingly, chickens experimentally infected with *E. coli* but not treated with the antibiotics displayed non-significant changes in serum ALT on the 4th day of treatment and on the 14th and 21st days post treatment. These results contrast with previous studies of Zaki, et al.,[16] who reported a significant increase in ALT in *E. coli*-infected chickens. The discrepancy might be due to differences in the ages of the chickens at infection or the dose and route of the *E. coli* infection.

Chickens experimentally infected with *E. coli* and treated with clindamycin showed no changes in serum ALT either on the 4th day of treatment or the 2nd, 14th and 21st days post-treatment. These results agree with Elmore, et al.[17], who showed that clindamycin is associated with hepatotoxic activity and with abnormalities in liver enzymes. Clindamycin caused a significant increase in ALP in the *E. coli*-infected chickens on the 4th day of treatment, and this is consistent with the results obtained by Plumb[14]. The chickens experimentally infected with *E. coli* and treated with spectinomycin showed no significant changes in serum ALT either on the 4th day of treatment or the 2nd and 14th days post-treatment, which conflicts with the results of Smith and Reynard[13], who reported that spectinomycin is associated with minor abnormalities in alkaline phosphatase, serum ALT and creatinine clearance. Regarding the effect of spectinomycin on ALP in chickens experimentally infected with *E. coli*, the results revealed non-significant changes in ALP on the 4th day of treatment and on the 2nd, 14th and 21st days post-treatment. These results do not agree with Plumb[14], who found that spectinomycin increases ALP at single and multiple doses. The current study also included an evaluation of the effect of a combination of half-doses of clindamycin and spectinomycin. This treatment did not result in any significant changes in ALT activity on the 4th day of treatment or on the 2nd and 14th days post-treatment. This result contrasts with the results of Ali [18], who found that the drug combination significantly decreased ALT in the experimentally infected chickens. The differences may be attributed to the age of the chick at infection and the dose of *E. coli*. The current study showed that

non-infected chickens treated with clindamycin displayed a significant decrease in serum total proteins on the 2nd, 7th and 21st days post-treatment, Table 3. In contrast, Ali[18] and Mohamed[19] found non-significant changes in the total serum protein of chickens treated with lincomycin and clindamycin. These differences may be attributed to the antibiotic doses, age of birds or the method of oral administration. Spectinomycin caused a significant decrease in total protein on the 7th and 21st days post-treatment in non-infected chickens. Non-infected chickens treated with the half and full doses of the clindamycin-spectinomycin combination displayed a significant decrease in total protein on the 2nd day post-treatment. These results could reflect some pathological changes in the liver and kidney induced by these drugs. In contrast, Ali [18] and Mohamed [19] reported that the clindamycin-spectinomycin combination produced no changes in total protein in non-infected chickens. This discrepancy might be related to the antibiotic doses and method of oral administration. The chickens experimentally infected with *E. coli* but not treated with antibiotics exhibited a significant decrease in total protein on the 2nd, 7th, 14th and 21st days post-treatment compared with the non-infected, non-treated chickens. The results obtained by El-Kadeem[20] revealed a significant decrease in total protein in *E. coli*-infected chickens, thus supporting the results of the present study. These results could be due to pathological changes in the liver and kidney as a result of experimental infection with *E. coli* in chickens [21]. The amino acid utilisation as a defence against the pathogens and renal damage produced by bacteria could also explain the hypoproteinaemia observed in the infected chickens[22]. Experimentally infected chickens treated with the clindamycin-spectinomycin combination at half or full doses showed significantly increased levels of total protein on the 14th and 21st or 7th and 21st days post-treatment, respectively. This shift toward the control values of total protein might be attributed to an improved liver status in the treated groups because the synthesis of albumin, the largest individual fraction in avian plasma, takes place in the liver. Alternatively, the treatment might inhibit renal excretion of albumin by improving the status of the kidneys. With respect to the effect of clindamycin and or spectinomycin on serum creatinine and uric acid, the present study revealed that the drugs induced a significant increase in creatinine level on the 2nd day post-treatment. This effect of the drugs, alone or in combination, was observed in the non-infected chickens, Table 4 and 5. These results are consistent with those obtained by Ali[18] and Mohamed[19]. The experimentally infected chickens that were not treated with the antibiotics demonstrated a significant increase in serum creatinine on the 2nd day post-treatment compared with non-infected, non-treated chickens. The previous results [18, 20] support these findings. Clindamycin administration in chickens experimentally infected with *E. coli* lead to a significant increase in creatinine on the 7th day post-treatment. This result also agrees with the study by Ali[18], who found that clindamycin significantly increased serum uric acid in infected chickens on the 1st day post-treatment. On the other hand, spectinomycin caused non-significant changes in the creatinine levels in the infected birds [18]. The clindamycin-spectinomycin combinations at both half and full doses significantly increased creatinine on the 4th day of treatment in the infected chickens. The study also evaluated the effect of experimental infection with *E. coli* on total antioxidant capacity (TAC) and lipid peroxidation (MDA, a biomarker of oxidative stress), Table 6 and 7. The results revealed that there were significant decreases in TAC and significant increases in MDA compared with the non-infected group, and this reflects the increased oxidative stress induced by infection. MDA, an important product of lipid peroxidation, is produced as a result of the peroxidation of fatty acids containing three or more double bonds. The MDA product can crosslink membrane elements and affect the ion exchange across the cell membranes, which results in changes in ion permeability and enzyme activity. In this study, the MDA level was found to be significantly higher in the *E. coli*-infected chickens than in the non-infected birds, suggesting that the presence of the *E. coli* caused oxidative stress in the hosts. The decreased TAC in the infected chickens occurred as a result of consumption of whole body antioxidants due to an increase in the level of oxygen radicals, such as MDA[23]. *E. coli* secretes various products, including lipopoly-saccharide and Haemolysin (HlyA). The latter is a pore-forming toxin. Numerous effects on different cellular populations have been attributed to sublytic concentrations of this toxin, including secretion of ROS and nitric oxide [24]. The enhanced generation of ROS may be responsible for the tissue injury due to the septic shock and endotoxaemia induced by *E. coli*[25]. *E. coli* infection increases the carcinogenicity of nitrosamine precursors and enhances oxidative and nitrosative stresses by increasing the levels of nitric acid, hydrogen peroxide and malondialdehyde[26]. In contrast, clindamycin and spectinomycin either alone or in combination ameliorate this adverse effect, as reflected by the significant increases in TAC and significant decreases in MDA compared with the infected, non-treated group. In an experimental model of maternofoetal *E. coli* infection in rabbits, the infection induced an inflammatory response in the foetal lung, causing NO-derived oxidative stress and programmed cell death. Early antibiotic therapy can limit this inflammatory response and decrease the infection-induced oxidative stress and cell death [27]. The authors stated that the antibiotics

achieve these effects by reducing bacterial growth and subsequent inoculum size, and by limiting the inflammatory response, especially cytokine production. Indeed, gentamycin has been shown to inhibit superoxide generation in activated human neutrophils in vitro[28]. The present data contrast with the results of Talla and Veerareddy[29] who found that ciprofloxacin and levofloxacin, widely used today for the treatment of bacterial infections, induce more reactive oxygen species in Indian patients. The discrepancy might be attributed to the species studied or the differences in the antibiotics or their doses used.

Conclusion

From the presented data, it is concluded that oral administration of both clindamycin and / or spectinomycin (therapeutic doses) for 5 successive days therapy has a prime efficacy in the control of the *E. coli* infection in poultry. Furthermore, paradoxical effects of both clindamycin and spectinomycin combinations showed alterations in some liver and kidney biochemical function parameters as well as oxidative stress. Most of the parameters went back to normal within 2 weeks post drug(s) administration, based on some biochemical measures.

Conflict of Interest statement: The author declares no conflicts of interest.

Table 1. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum alanine aminotransferase activity (ALT) of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum ALT (U/L)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	11.04±0.55c	12.70±0.87a	13.90±0.13a	11.50±0.67a	8.60±0.77a
Non-infected, treated with clindamycin	14.80±0.47b	9.80±0.33ab	11.80±0.54a	8.90±0.85b	8.80±0.86a
Non-infected, treated with spectinomycin	15.50±0.07b	10.30±0.17a	11.70±0.67a	11.60±0.45a	7.20±0.05ab
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	11.80±0.95c	11.80±0.74a	10.70±0.37ab	12.30±0.71a	6.20±0.57b
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	18.90±0.15a	8.60±0.67b	12.20±0.76a	9.50±0.67ab	6.80±0.63b
Infected, non-treated	11.23±0.85c	8.16±0.13b	11.80±0.96a	8.80±0.72b	7.10±0.81ab
Infected, treated with clindamycin	13.74±0.16bc	8.20±0.91b	11.20±0.17a	9.80±0.34ab	7.30±0.67ab
Infected, treated with spectinomycin	12.88±0.24c	8.70±0.96b	9.60±0.89b	10.90±0.19ab	8.10±0.49a
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	11.15±0.81c	8.33±0.66b	8.90±0.78b	9.70±0.82b	9.70±1.09a
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	12.67±0.19c	7.87±0.77b	8.80±0.83b	11.50±0.22a	8.10±0.91a

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
+ Compared with infected control group (infected, non treated).

Table 2. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum alkaline phosphatase activity (ALP) of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum ALP (U/L)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	17.73±0.15c	16.20±0.27b	16.50±0.93b	13.70±0.87b	17.60±0.66a
Non-infected, treated with clindamycin	20.60±0.97b	16.40±0.63b	15.80±0.77cd	14.10±0.09b	13.80±0.61b
Non-infected, treated with spectinomycin	17.09±0.76c	16.70±0.23b	15.70±0.31cd	13.90±0.24b	13.30±0.59b
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	15.40±0.31c	14.30±0.58c	14.20±0.69c	16.80±0.69a	17.80±0.61a
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	16.30±0.47c	15.40±0.00cd	14.70±0.32c	16.50±0.30a	15.50±0.31b
Infected, non-treated	17.83±0.33c	21.26±0.57a	24.10±0.16a	13.40±0.88b	13.70±0.25b
Infected, treated with clindamycin	28.57±0.47a	24.15±0.11a	26.40±0.54a	12.70±0.75b	17.10±0.07a
Infected, treated with spectinomycin	19.68±0.38b	18.60±0.36b	14.30±0.28c	14.10±0.59b	12.90±0.06b
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	13.15±0.01d	16.83±0.72b	13.90±0.62c	16.30±0.14a	15.60±0.46b
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	15.27±0.89c	15.97±0.61cd	19.60±0.36b	16.50±0.92a	13.80±0.28b

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
+ Compared with infected control group (infected, non treated).

Table 3. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum total protein of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum total protein (g/dl)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	6.57±0.19a	5.30±0.08a	6.09±0.13a	5.30±0.07a	5.20±0.06a
Non-infected, treated with clindamycin	5.87±0.16b	4.90±0.13ab	5.60±0.17b	4.60±0.09ab	4.80±0.11ab
Non-infected, treated with spectinomycin	5.85±0.11b	4.97±0.12ab	5.87±0.11ab	4.40±0.14b	4.70±0.17b
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	5.82±0.16b	4.67±0.18b	5.85±0.19ab	4.30±0.11b	4.80±0.11ab
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	6.41±0.11a	4.61±0.10b	5.70±0.12b	4.30±0.10b	5.00±0.18a
Infected, non-treated	5.97±0.15b	4.76±0.17b	5.50±0.16b	4.10±0.11b	4.70±0.04b
Infected, treated with clindamycin	6.07±0.12ab	4.85±0.11ab	5.40±0.14b	5.10±0.15a	5.50±0.07a
Infected, treated with spectinomycin	5.86±0.15b	5.00±0.10a	5.50±0.18b	5.10±0.17a	5.60±0.16a
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	6.19±0.11ab	4.93±0.12ab	5.60±0.12b	4.80±0.04ab	4.80±0.16b
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	6.55±0.19a	4.37±0.11b	6.20±0.13a	4.20±0.16b	5.30±0.11a

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
+ Compared with infected control group (infected, non treated).

Table 4. The effect of oral administration of clindamycin and /or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum creatinine of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum creatinine (mg/dl)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	0.68±0.01c	0.45±0.01d	0.64±0.01a	0.60±0.01bc	0.65±0.01a
Non-infected, treated with clindamycin	0.64±0.01d	0.48±0.006d	0.59±0.01ab	0.58±0.02bc	0.55±0.01b
Non-infected, treated with spectinomycin	0.65±0.01cd	0.51±0.02c	0.56±0.01b	0.65±0.01b	0.56±0.01b
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	0.68±0.01c	0.51±0.01c	0.59±0.01ab	0.78±0.01a	0.55±0.002b
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	0.66±0.01cd	0.51±0.004c	0.60±0.02ab	0.52±0.005c	0.55±0.01b
Infected, non-treated	0.67±0.03c	0.52±0.01c	0.58±0.01b	0.66±0.01b	0.51±0.01c
Infected, treated with clindamycin	0.84±0.01a	0.67±0.01a	0.65±0.01a	0.59±0.01bc	0.53±0.02c
Infected, treated with spectinomycin	0.63±0.01d	0.55±0.02b	0.56±0.03b	0.71±0.01a	0.55±0.006b
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	0.75±0.01b	0.49±0.003d	0.52±0.02b	0.58±0.02bc	0.52±0.008c
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	0.79±0.02b	0.56±0.01b	0.57±0.01b	0.52±0.01c	0.57±0.004b

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
+ Compared with infected control group (infected, non treated).

Table 5. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum uric acid of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum uric acid (mg/dl)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	10.38±0.01ab	9.15±0.01b	8.54±0.01b	8.66±0.01a	5.65±0.01b
Non-infected, treated with clindamycin	b.24±0.01b	7.38±0.01c	7.49±0.01c	7.78±0.02b	4.55±0.01b
Non-infected, treated with spectinomycin	11.85±0.01a	10.61±0.03a	8.56±0.01b	6.55±0.01b	4.56±0.01b
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	9.68±0.01b	9.71±0.01b	9.49±0.01a	7.88±0.01b	6.55±0.01a
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	8.86±0.01b	8.61±0.01b	8.65±0.01b	5.62±0.01c	5.55±0.01b
Infected, non-treated	9.67±0.01b	9.82±0.01b	9.55±0.01a	6.46±0.01b	6.51±0.01a
Infected, treated with clindamycin	11.84±0.01a	9.47±0.02b	8.75±0.01b	9.69±0.01a	7.53±0.02a
Infected, treated with spectinomycin	9.83±0.01b	9.85±0.01b	8.46±0.01b	9.61±0.01a	6.55±0.01a
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	11.45±0.01a	10.19±0.01a	8.72±0.02b	9.68±0.01a	6.52±0.01a
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	12.89±0.01a	11.66±0.01a	9.67±0.01a	8.57±0.01a	7.57±0.01a

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
+ Compared with infected control group (infected, non treated).

Table 6. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum (TAC) of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum TAC (mM/L)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	0.545±0.003b	0.567±0.01b	1.540±0.008ab	0.779±0.006b	0.539±0.004b
Non-infected, treated with clindamycin	0.636±0.007ab	0.589±0.10b	1.573±0.001ab	0.813±0.004ab	0.547±0.003b
Non-infected, treated with spectinomycin	0.743±0.001a	0.766±0.12a	1.811±0.004a	1.060±0.009a	0.789±0.007a
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	0.676±0.001ab	0.787±0.14a	1.845±0.001a	0.991±0.002a	0.764±0.005a
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses)	0.824±0.007a	0.833±0.16a	1.876±0.009a	1.084±0.005a	0.878±0.003a
Infected, non-treated	0.314±0.008c	0.281±0.003c	0.767±0.002b	0.471±0.007c	0.380±0.005c
Infected, treated with clindamycin	0.360±0.002c	0.369±0.005c	0.798±0.006b	0.665±0.008b	0.471±0.007b
Infected, treated with spectinomycin	0.477±0.003b	0.518±0.008b	0.901±0.007b	0.878±0.003ab	0.760±0.008a
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	0.486±0.006b	0.531±0.003b	1.198±0.003b	0.802±0.006ab	0.687±0.006ab
Infected, treated with clindamycin and spectinomycin (therapeutic doses)	0.525±0.004b	0.543±0.006b	0.987±0.008b	0.868±0.009ab	0.794±0.004a

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
\+ Compared with infected control group (infected, non treated).

Table 7. The effect of oral administration of clindamycin and / or spectinomycin (therapeutic and half therapeutic doses) for 5 successive days on serum (MDA) of non infected and experimentally infected chickens with *E. coli*. (n=5)

Parameters / Groups	Serum MDA (nmol /ml)				
	During treatment	Post- treatment			
	4th day	2nd day	7th day	14th day	21st day
Non-infected, non-treated	32.16 ± 2.77c	37.22 ± 3.30bc	42.56 ± 2.17c	46.27 ± 3.15c	39.83 ± 4.03c
Non-infected, treated with clindamycin	30.78 ± 3.68c	33.15 ± 4.08bc	43.32 ± 3.30c	41.18 ± 4.90c	40.02 ± 5.87c
Non-infected, treated with spectinomycin	26.11 ± 2.32d	25.58 ± 3.41c	40.69 ± 2.16c	44.83 ± 4.21c	39.42 ± 2.62c
Non-infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	29.71 ± 3.81c	28.19 ± 2.13c	43.58 ± 4.39c	41.92 ± 3.38c	38.17 ± 3.18c
Non-infected, treated with clindamycin and spectinomycin (therapeutic doses) combination	24.65 ± 2.93d	25.62± 3.72c	40.06± 4.84c	42.28± 5.24c	40.34± 2.29c
Infected, non-treated	53.16 ± 5.87a	59.74 ± 6.43a	71.08 ± 5.17a	76.62 ± 3.67a	64.92 ± 4.16a
Infected, treated with clindamycin	48.80± 4.95a	52.91± 6.18a	68.78± 4.72a	72.31± 5.49a	63.57± 3.46a
Infected, treated with spectinomycin	46.31 ± 3.55b	47.29 ± 4.31b	55.27 ± 3.29b	66.17 ± 5.71b	52.03 ± 3.93b
Infected, treated with clindamycin and spectinomycin (half therapeutic doses) combination	41.57 ± 2.79b	51.18 ± 3.58a	56.02± 4.13b	68.09± 2.83b	57.43± 4.62b
Infected, treated with clindamycin and spectinomycin (therapeutic doses) combination	42.17 ± 3.63b	48.30 ± 4.19b	52.13 ± 3.82b	68.79 ± 4.71b	50.28 ± 2.41b

The means with different superscripts in the same column indicate significantly different, ($p<0.05$).
* Compared with control group (non infected, non treated).
\+ Compared with infected control group (infected, non treated).

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