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Original Research Article

Comparative studies on the efficacy of lincomycin and bacitracin for the control of necrotic enteritis in broiler chickens

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ABSTRACT

Background: The present study was conducted to evaluate the efficacy of lincomycin and/or bacitracin for control of experimentally-induced Clostridium perfringens (CP) infection in broiler chickens.
Methods: A total of 100 one-day-old Cobb-mixed chicks were divided into five groups (A, B, C, D and E, each of 20 bird). At the 15th day of age, all birds (except group A) were inoculated orally with CP broth culture (109 CFU/mL). Two days later, drugs were orally administered once daily for five consecutive days as follow; Group A and B were left untreated. Group C, D, and E were treated with lincomycin (0.5 g/l), bacitracin (100 mg/l), lincomycin and bacitracin, respectively. The efficacy of used drugs was estimated based on clinical symptoms, body weight, weight gain, feed conversion rate. Hematobiochemical changes were also determined.
Results: Necrotic enteritis in broiler chickens induced a significant decrease in body weight, weight gain, erythrocytic count, hemoglobin content, PCV %, serum proteins, catalase, and superoxide dismutase. Additionally, a marked decrease in serum lipids was obtained. Furthermore, a significant increase in feed conversion rate, leukocytic count, phagocytic activity, phagocytic index, serum total globulin, γ globulin and malondialdehyde coupled with a marked increase in β and α globulins were determined. Medication of infected broilers with lincomycin and/or bacitracin improved clinical signs and reduced mortality rate to 8, 6 and 2%, respectively, as well as restored the performance and hematobiochemical alterations.
Conclusions: a combination of lincomycin and bacitracin was of considerable value for the control of necrotic enteritis in broiler chickens.

Keywords: Bacitracin, Broiler chickens, Clostridium perfringens, Lincomycin, Necrotic enteritis

INTRODUCTION

In Prevention of diseases is a major concern in the poultry industry, due to resulting decreased growth and increased mortality.[1] Poultry diseases cause severe economic losses to the poultry industry by increasing mortality, reducing weight gain and feed conversion ratio. One of the most important worldwide diseases affecting broiler industry is necrotic enteritis caused by Clostridium perfringens (CP) infection, leading to major economic losses in the poultry industry and severely affecting the performance of birds.[2,3] Clostridium perfringens (types A and C) found in the intestinal tract of healthy birds can induce NE in broilers flocks.[4] It is also an enteric bacterial pathogen in humans, poultry, other farm animals and a widespread anaerobic, Gram-positive, spore-forming, non-motile rod.[5] Lincomycin, belonging to a group of lincosamides, has been in use for the past 40 years. Lincomycin comes from

actinomycete Streptomyces lincolenensis.[6] It is a broad-spectrum antibiotic indicated in treating serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci. It aids in preventing chronic respiratory disease associated with Mycoplasma and coliform infections in chickens.[7] Bacitracin is an antibiotic produced by Bacillus licheniformis bacteria in 1945.[8] Bacitracin is a widely used metallopeptide antibiotic produced by Bacillus subtilis and Bacillus licheniformis with a potent bactericidal activity directed primarily against Gram-positive organisms.[9] Bacitracin is a bactericidal peptide antibiotic which inhibits cell wall synthesis and has additional effects on bacterial membranes.[10]

To our knowledge, there are little data about lincomycin and bacitracin for controlling CL infection in broiler chickens. Therefore, the present study was carried out to evaluate the efficacy of lincomycin and bacitracin either alone or in combination against experimentally-induced NE in broiler chickens with regard to the hematobiochemical alterations.

METHODS

Drugs

Lincomycin hydrochloride and bacitracin methylene disalicylate were obtained from ATCO pharma for pharmaceutical industries, Cairo, Egypt with the commercial name of ATO LINC 400® and BACITOP®, respectively. Each gram powder of ATO LINC 400® includes 453.6 mg lincomycin Hcl (eq. to 400 mg lincomycin base) and that of BACITOP® contains 62 mg bacitracin methylene disalicylate (eq. to 44.06 mg bacitracin base). It is administered orally at a dose of 100 mg/l drinking water for 5-7 days for treatment of CP infection in broiler chickens.

Experimental chickens

A total of 100, one-day-old Hubbard mixed breed broiler chicks were bought from a local poultry farm and used in this trial. Birds were kept in wire floor batteries under strict hygienic measures. All chicks were vaccinated with Newcastle vaccines (HitchnerB1 at 7 days and Lasota at 18 days, Intervet International BV Company, Boxmeer, Holland) and Gumboro vaccine (Rhone-Merieau Company, France) at 14 days. The birds were maintained at a suitable temperature and humidity according to their ages. The chickens had free access to water and feed. The feed was free from antibacterial and anticoccidial drugs. The experiments were carried out in accordance with the guidelines set by the Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt.

Experimental infection

Clostridium perfringens type A was kindly obtained from the Department of Microbiology, Animal Health Research Institute. Dokki, Giza, Egypt. At 15th day of age, chickens in group B, C, D, and E were administered an oral inoculation of 2 ml freshly prepared, 24 h incubated thioglycolate broth culture of CP type C (1.5×109) organisms/ml).[2]

Experimental design

At the 17th-day of age (two days of inoculation) where the clinical symptoms of NE were established and reported, drugs were orally (in drinking water) administered once daily for five consecutive days. chickens were divided into 5 equal groups (20 each). Group A (control) was kept non-infected and non-treated; group B one was infected with CP and not treated. Group C was infected and treated with lincomycin at 0.5 g/l; group D infected and treated with 100 mg of bacitracin per liter drinking water and group E was infected with CP and treated with lincomycin and bacitracin at the above-mentioned doses.

Blood sampling

Twenty four h after last administration of drugs, two blood samples from each bird were collected, one sample with EDTA as an anticoagulant for estimation of hematological parameters (erythrocytic count, packed cell volume, hemoglobin content), total leucocytic count, phagocytic activity and phagocytic index. Another sample was used for the estimation of serum total protein, protein fractions using cellulose acetate electrophoresis test, total lipid, cholesterol, triglyceride, superoxide dismutase (SOD), catalase and malondialdehyde (MDA).[11-20]

Statistical analysis

Data were expressed as mean ± standard error and were statistically analyzed using according to Petrie and Watson.[21] Comparison of the mean values was performed and differences were considered statistically significant when $P < 0.05$.

RESULTS

In the present study, broilers infected with CP showed clinical signs as depression, ruffled feathers, decreased appetite, diarrhea, dehydration, weight loss, a decrease in weight gain, increased feed conversion rate (Table 1) and the mortality rate was 34% in the non-treated group (B). The present study revealed that broilers infected with CP showed significant decreases in the total erythrocytic count, hemoglobin content, Packed Cell Volume % associated with significant increase leukocytic count, phagocytic activity and phagocytic index all over the experimental period post infection (Table 2). Additionally, the alone treatment of infected broilers by bacitracin or lincomycin in drinking water for 5 successive days showed significant decreases in the erythrocytic count, hemoglobin content, Packed Cell Volume % associated with significant increase in leukocyte, phagocytic activity % and phagocytic index at 1st-day post-treatment (Table

2). However, the combination of bacitracin and lincomycin displayed a non-significant increase in phagocytic activity % and phagocytic index. Necrotic enteritis in broiler chickens induced a significant decrease in serum total protein, albumin, A/G ratio (Table 3), catalase and SOD (Table 4). Additionally, non-significant decrease in total lipid, cholesterol, triglyceride levels was recorded (Table 4). Furthermore, a significant increase in feed conversion rate, leukocytic count, phagocytic activity, phagocytic index, serum total globulin, γ globulin and MDA coupled with a non-significant increase in □ globulin and α globulin were determined. Medication of infected broilers with lincomycin and bacitracin either alone or in combination showed improved clinical signs as they were healthy and viable as well as decreased mortality rate up to 8, 6 and 2% in group C, D, and E, respectively.

DISCUSSION

Although several clinical cases of NE have been reported in poultry industry, including broilers raised on floor, cage-reared layers, commercial layers either raised in cages or in floor pens,[22,23] there is a significant lack of data in literature about the clinical efficacy of antimicrobials and their combinations for control of NE in broiler chickens. The current study was conducted to check the efficacy of lincomycin and bacitracin against experimentally induced NE in broiler chickens.

Table 1: Effect of lincomycin (0.5 g/l) and bacitracin (100 mg/l) in drinking water for 5 successive days on body performance in chickens infected with CP at 1st, and 10th-day post-treatment. Values are Mean±SE (n= 5).

Group	15th day of age			1st-day post-treatment			10th-day post-treatment		
	Bodyweight (g)	Weight gain (g)	FCR	Bodyweight (g)	Weight gain (g)	FCR	Bodyweight (g)	Weight gain (g)	FCR
Control	499.6±9.36	461.1±13.3	1.25	1284.5±14.6	784.9±19.43	1.25	2079.2±16.3	794.7±13.9	1.65
Infected non-treated	504.3±5.89	465.8±12.8	1.25	1089.4±27.7***	585.1±15.8***	1.55	1874.5±23.6***	697.9±14.4***	1.83
Infected lincomycin-treated	498.8±17.8	460.3±12.6	1.25	1132.3±24.7**	633.5±20.9**	1.49	1912.5±24.5**	780.2±9.10	1.66
Infected bacitracin-treated	500.7±18.9	462.0±13.1	1.25	1139.3±21.8**	638.6±18.8**	1.49	1920.5±24.8**	781.1±8.20	1.66
Infected lincomycin- and bacitracin-treated	502.7±15.9	464.4±13.1	1.25	1144.2±22.9**	641.5±20.9**	1.48	1936.8±28.9**	792.6±9.37	1.64

FCR: Feed conversion rate, * *Significant at P <0.01 * ** Significant at P <0.001

Table 2: Effect of lincomycin (0.5 g/l) and bacitracin (100 mg/l) in drinking water for 5 successive days on erythrogram, leukocytic count, phagocytosis and phagocytic index in chickens infected with CP at 1st-day post-treatment. Values are Mean±SE (n= 5).

Group	RBCs (10^6/µl)	Hb (gm/dl)	PCV (%)	Total WBCs ($\times10^3$/µl)	Phagocytosis	Phagocytic index
Control	3.75±0.33	10.74±0.93	31.46±0. 84	10.86±0.33	62.67±0.54	4.74±0.38
infected non-treated	2.08±0.24**	7.75±0.54**	26.98±0.58**	12.96±0.39**	65.21±0.29 **	5.79±0.18*
Infected lincomycin-treated	2.66±0.25*	8.60±0.20*	28.17±0.76*	11.90±0.14*	64.03±0.12 *	5.63±0.11*
Infected bacitracin-treated	2.69±0.13*	8.54±0.14*	28.31±0.73*	11.92±0.18*	64.15±0.21 *	5.64±0.12*
Infected-lincomycin and bacitracin treated	3.59±0.19	9.96±0.68	30.20±0.38	10.91±0.52	62.71±0.33	4.75±0.23

*Significant at P <0.05 ** Significant at P <0.01

Table 3: Effects of lincomycin (0.5 g/l) and bacitracin (100 mg/l) in drinking water for 5 successive days on serum levels of total protein, albumin and globulin fraction in chickens infected with CP at 1st-day post-treatment. Values are Mean±SE (n= 5).

Group	T. P	Alb	Globulin (gm/dl)				
			α	β	γ	Total	A/G ratio
Control	5.41±0.21	3.20±0.21	0.64±0.10	0.69±0.18	0.88±0.16	2.21±0.21	1.45±0.12
Infected non-treated	4.55±0.10**	2.18±0.18**	0.68±0.17	0.70±0.13	0.99±0.23	2. 37±0.17	1.08±0.11
Infected lincomycin-treated	4.69±0.17*	2.44±0.14*	0.66±0.18	0.71±0.18	0.89±0.22	2.25±0.13	1.08±0.11
Infected bacitracin-treated	4.75±0.21*	2.55±0.18*	0.65±0.18	0.70±0.16	0.89±0.24	2.22±0.11	1.16±0.13
Infected lincomycin- and bacitracin- treated	5.28±0.18	3.08±0.22	0.63±0.16	0.70±0.18	0.88±0.21	2.21±0.16	1.40±0.16

*Significant at P <0.05 ** Significant at P <0.01

Table 4: Effects of lincomycin (0.5 g/l) and bacitracin (100 mg/l) in drinking water for 5 successive days on lipid profile and levels of catalase, SOD and MDA in chickens infected with CP at 1st-day post-treatment. Values are Mean±SE (n= 5).

Group	lipid profile (mg/dl)			Antioxidants enzyme (U/mL)		MDA mmol/ml
	T. lipid	cholesterol	Triglyceride	Cat	SOD	
Control	226.3±1.87	93.6±1.94	114.1±1.82	37.1±1.42	256.31±1.49	22.24±0.39
Infected non-treated	224.9±2.94	92.0 ±1.90	113.9±1.93	43.4±1.42**	250.12±1.15**	31.41±1.15**
Infected lincomycin-treated	226.0±2.85	93.4 ±1.69	114.0±1.87	37.02±1.09*	252.09±1.01*	37.76±1.20
Infected bacitracin-treated	225.7±2.93	93.4 ±1.67	113.9±1.55	37.4±1.03*	252.03±1.16*	37.36±1.37
Infected lincomycin- and bacitracin- treated	226.0±2.55	93.6 ±1.45	113.9±1.72	37.6±1.34	253.33±1.89	37.88±1.53

*Significant at P <0.05 ** Significant at P <0.01

The experimental birds were divided into five equal groups, each of 20 bird. After 24-36 h of CP infection, the chickens of infected groups (B, C, D, and E) developed clinical symptoms of NE. The recorded clinical symptoms are similar to those recorded previuosly.[2,3] Reduction in weight gain in broiler chickens infected with CP was recorded before.[24] Typical clinical signs and reduction of body weight due to NE were also recorded in broiler chickens suffering from NE.[2,3] Our results are reinforced by results reported that bacitracin abolished the development of NE and reduced the mortality rate and suppressed the bacterial growth and feed efficiency associated with NE in broilers.[25] In the same way, the treatment of broilers infected with CP using lincomycin showed improved body performance.[26] Moreover, CP isolated from chicken layer flocks in Germany was sensitive to lincomycin.[27] Furthermore, the reduction of mortality rate beside the disappearance of clinical signs and improved body gain in broiler chickens treated with lincomycin were reported.[28]

The phagocytosis recorded in the present study after CP inoculation was also recorded previously and same changes in blood picture of the chickens in the current study were reported previously,[3,29] and stated that broilers suffering from NE showed a significant decrease in the total erythrocytic count, hemoglobin content, Packed Cell Volume % associated with significant increase leukocytic count, phagocytic activity and phagocytic index all over the experimental period post infection. Similarly, in ducklings suffering from NE, the same alterations in blood picture were reported previously.[30] Furthermore, in turkey poults, CP showed a decrease in the total erythrocytic count, hemoglobin content and PCV%.[31] Treatment of infected CP-infected broilers chickens with lincomycin has completely stopped the development of NE and improved the erythrocytic count, hemoglobin content, packed cell volume % and leukocytic count.[28] Also, zinc bacitracin improved hematological parameters, phagocytosis% and phagocytic index in broilers suffered from NE.[32] Also, in turkey, zinc bacitracin showed good efficacy against clostridial infection and improved the hematological values.[33] Moreover, in rabbits, zinc bacitracin induced improvements in erythrogram and leukogram in rabbits after CP infection.[34]

The reduction of plasma protein in infected chickens suffering from NE may be due to liver damage caused by

clostridial toxins, thus impaired the synthesis of albumin.[35] Previously, in broiler chickens, NE induced a significant reduction in protein and albumin coupled with an increase in total globulin and globulin fractions, α, □ and γ globulin.[36]

In the present study, treatment of infected chickens with bacitracin and/or lincomycin restored the plasma protein alterations. Same results were recorded by Engberg et al., and Hofacre et al. They found that zinc bacitracin significantly reduced the number of CP in broiler chickens and improved the total protein, albumin and globulin.[37] Such finding was compatible with those reported previously where CP isolated from broilers is sensitive to bacitracin and improved protein picture.[38]

Infected broilers with CP showed non-significant decrease in total lipid, cholesterol and triglyceride levels throughout the experimental period after infection, while treatment of infected broilers by bacitracin and/or lincomycin restored the lipid profile compared with control broilers. Similar results were recorded previously in broilers[39] and in turkeys[40] suffering from NE.

The results of this study revealed that broiler chickens infected with CL showed a significant reduction in catalase and SOD together with a significant increase in MDA level. Treatment infected boilers by bacitracin and/or lincomycin induced improvements in catalase, SOD activities and serum MDA levels. Infected broilers with CP resulted in weakening the antioxidant defense mechanism as evidenced by decreases in SOD and catalase associated with an elevation in MDA.[39]

It could be concluded that the combination of lincomycin and bacitracin at the tested doses was of considerable value for the control of NE in broiler chickens than using them separately.

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