

Studies on the Compatibility between Lincomycin and Kitassamycin in the Control of Necrotic Enteritis in Broiler Chickens

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Abstract

A total of 75 one-day old broiler chickens were used in this study for investigate compatibility between Lincomycin and Kitassamycin for treating *clostridium perfringens* infection and its effects in hematobiochemical parameters. At day 15 of age broilers were divided into 5 equal groups. Chicks in groups (2, 3, 4 and 5) were infected with *clostridium perfringens* type C, 1st group non-infected, non-treated (-ve control), 2nd group-infected non-treated(+ve control), 3rdgroup infected lincomycin treated for 5 days, 4th group infected kitassamycin treated for 5 days and 5th group infected treated with both drugs together by same dose and period. At 1st day post treatment 2 blood sample was taken from all birds for hematobiochemical study. Infected broilers showed clinical signs as loss of appetite ruffled feathers, depression, loss of appetite, diarrhea, dehydration, polydipsia, emaciation, significant reduction in body weight gain total protein, albumin, catalase and superoxide dismutase beside significant increase in mortality rate, feed conversion rate, leukocytic count, heterophile, lymphocyte, monocyte, eosinophile, basophile, phagocytosis and index, liver enzyme (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) uric acid, creatinine, malanodialdehyde coupled with insignificant increase in total globulin, alpha, beta and gamma globulin beside insignificant decrease in total lipids, triglycerides and cholesterol. Necrotic enteritis induced many pathological lesion in liver, kidney and intestine Treatment infected broilers using lincomycin and kitassamycin lead to improved in hematobiochemical parameter and reduction in *clostridium perfringens* counts in faeces and bacterial reiolation but infected broilers treated with both drugs together showed superior improvement in hematobiochemical parameter. It could be concluded that Combination of lincomycin and kitassamycin is effective in treatment necrotic enteritis than each alone.

Keyword: *Clostridium perfringens*; Lincomycin; Kitassamycin; Broilers; Pathology

Introduction

Poultry diseases cause severe losses to poultry industry by increasing mortality reducing weight gain [12] *clostridium perfringens* is the most important clostridial pathogen of poultry [24]. It is a fatal disease and its mortality rates about 30% [56]. Its produced serious disease in chicks named necrotic enteritis [54]. The disease occurred in broilers 2-6 weeks of age and is characterized by sudden onset of diarrhea and mucosal necrosis [38] Antibiotics are widely used in veterinary practice to overcome bacterial infections in poultry. In many parts of the world, food-producing animals are given antibiotics daily to make them grow faster and prevent diseases [55].

Among well developed antibiotics that seem promising in veterinary use are lincomycin and Kitassamycin [51]. Antibiotics are used as therapeutic agents for treatment of infectious diseases in humans and livestock [31].

Lincomycin is a member of aminooglycoside antibiotic that inhibits protein synthesis by binding to 50s ribos-omal subunit and has specific activity aganist closterdial infection [25 & 44]. It is used in treatment of bacterial enteric infections [4]. Macrolide antibiotics are a group of antibiotics widely used to treat and prevent diseases and promote growth in food-producing animals [47]. Macrolides has antimicrobial activity by inhibiting protein synthesis. Macrolides are bacteriostatic active against Gr+ve and Gr-ve bacteria [22]. Kitassamycin is a member of macrolide antibiotic, has high degree of efficacy in control disease as it a wide tissue distribution and high intracellular concentration [2].

This study was planned to investigate the effect of Lincomycin and Kitasamycin for treating *clostridium perfringens* infection in broilers with regarding to its effects in some biochemical parameters in broilers as well as pathological changes.

Materials and Methods

Drugs

1. *kitasamycin tartarate* water soluble powder (100gm/packag) obtained from ADWIA Comp. for Pharmaceuticals SAE administered orally at dose 1gm/liter drinking water.
2. *Lincomycin hydrochloride* (atolinc 40%)^R produced from Atico company administ-ered orally at a dose of 0.5 gm/liter drinking water.

Closterdium infection

At 15th, 17th and 19th day of age chicks in group (2, 3, 4 and 5) were administered three oral inocula-tion of 2 ml freshly prepared 24 hrs incubated thioglycolate broth culture of *clostridium perfringens* type C (1.5×10^9) organisms/ml [13].

Birds and Experimental design

A total of 75, one-day old Hubbard broiler chicks were used in this trial. Chicks were floor reared in separate units under hygienic measures. All chicks were vaccinated with Newcastle vaccines and Gumboro vaccine at 14 day. Broilers feed ration obtained from El-Kahera Poultry Comp. At day 15 of age broilers were divided into 5 groups (15 each) 1st group non-infected, non-treated (-ve control). 2nd group infected with *clostridium perfringens* non-treated (+ve control), 3rd group infected with *clostridium perfringens* treated with 1 gm/liter lincomycin, 4th group infected with *clostridium perfringens* treated with 10 mg/Kgm Bwt kitasamycin in drinking water, 5th group infected with *clostridium perfringens* treated with lincomycin and kitasamycin together by same dose and period. Treatment started in all groups at 21thday of age for 5 successive days.

Body weight all broilers were weighted individually at start of experiment and at 1st day post treatment for calculation weight gain and feed conversion rate.

Blood samples At 1st day post treatment 5 broilers from each groups were sacrificed and two blood samples from each bird were taken.

1st sample was taken in tub contain EDTA, used for determination total and differential leucocytic count according [30], phagocytosis% and phagocytic index according [46 & 58].

2nd sample was tacking in centrifuge tube for obtain clear serum for estimation total protein [17], serum protein fractions were performed using cellulose acetate electrophoresis test [26]. laspartate aminotransferase and alanine aminotransferase were Determined according [45], alkaline phosphatase [33]. Serum uric acid [23], creatinine [29], total lipid [37], Cholesterol [10], Triglyceride [57],

super oxide dismutase [43], Catalase [50] and malanodialdehyde [42].

Counting of *Clostridium perferenges*

Post chicks sacrificed 1gram faeces from each chicks was placed in sterile tube for *clostridium perfringens* counting pre treatment and at 1st day post treatment [39].

Re-isolation of *clostridium perferenges*

Loopfulls from intestine were inoculated asepetically into cooked meat broth then incubated anaerobically at 37°C for 48h. Then a loopful was inculated on agar plates cont-ain 10% sheep blood and 10% gentamycin and this plates were incubated anaerobically at 37°C for 48h. Colonies were identified morphologically and biochemically [16].

Histopathological studies

Post chicks sacrificed sample from Liver, kidney and intestine were taken, fixed in 10% formaline solution. Collected samples were dehydrated and embedded in paraffin wax, then sectioned to 4 micron thickness, stained by H&E and examined microscopically [14].

Statistical analysis

Obtained data was analyzed by using computerized SPSS program version 16 according to [53].

Results and Discussion

Broilers suffering from necrotic enteritis showed clinical sign as loss of appetite, diarrhea, depression, ruffled feather, polydipsia, dehydration, emaciation, increase mortality rate beside reduction in body weight gain, food intake and increase in feed conversion rate. Beside increase *clostridium perfringens* counts in faeces at pre-treatment and increase reisolation of *clostridium perfringens* in infected non treatment (Table 1 and 2). Our findings coordinates with [4] reported that broiler suffering from necrotic enteritis showed reduction in body weight gain, loss of appetite, diarrhea, depression, ruffled feather, polydipsia, dehydration. These clinical signs and reduction in weight gain were observed [7] in broiler chickens suffering from necrotic enteritis. Same clinical signs were observed in broilers suffering from necrotic enteritis [19 & 9].

Parameter	Mortality rate (10 bird/group)		<i>clostridium perfringens</i> counts/gm Faeces (5 bird/group)		<i>clostridium perfringens</i> Re-isolation (5 bird/ group)	
	No	%	Pre treatment	1 st day post treatment	No	%
control	00	00	00	00	00	00
Inf. non treated	3	30	8.38±1.47a	8.38±1.47a	5	100
Inf. Linco. treated	1	10	9.97±1.46a	3.27±0.89b	2	40
Inf. Kitasa. treated	1	10	8.68±1.55a	2.61±1.03b	2	40
Inf. kitasa & linco	00	00	9.51±1.48a	0.71±0.08b	00	00

Means with different superscripts of the same raw indicate significant difference at P < 0.05

Table 1: The effect of lincomycin and kitasamycin on Mortality rate, *clostridium perfringens* reisolation and counts/gm faeces (Log 10).(5 bird/group).

Parameters Group	initial weight (15 day age)	Body weight	Weight gain	FC	F.C.R
475.20	367.89±2.79a	818.84±3.98a	450.95±1.93a	control	1.30
454.10	339.74±2.98b	795.62±2.78b	455.88±2.93a	Inf. non treated	1.34
475.08	359.90±2.87b	816.64±2.83a	453.74±1.61a	Inf. Linco. treated	1.32
473.58	357.54±2.89b	815.52±3.98a	457.98±1.72a	Inf. Kitasa. treated	1.32
480.97	369.01±3.97b	820.48±3.84a	451.47±1.69a	Inf. kitasa & linco	1.30

Means with different superscripts of the same raw indicate significant difference at P < 0.05

Table 2: The effect of lincomycin and kitasamycin on body performance in chickens infected with *clostridium perfringens* (n= 5).

Its clear evident from table (3) revealed that broilers suffering from necrotic enteritis showed significant increase in leukocyte, heterophil, lymphocyte eosinophils, basophils, monocyte, phagocytic activity and phagocytic index. Our data fit with those recorded [18] stated that changes in leukogram in broilers suffering from necrotic enteritis may be due to bacterial toxin. Same change in phagocytosis% and index was recorded by [48] in chickens suffering from necrotic enteritis. Similar result was observed [36] in broilers. Broilers suffering from necrotic enteritis revealed significant increase in leukocytic, phagocytosis% and index [35].

Parameter		Control	Inf non treated	Inf. linco treated	Inf. kitasa treated	Inf. Lin & kit treated	
leukocytic count(X10 ³ /	Total WBCs	10.98±0.38b	13.73±0.58a	12.23±0.89b	12.21±0.74b	12.10±0.81b	
	Deferential count	heterophils	2.44±0.13b	2.97±0.14a	2.62±0.30b	2.59±0.25b	2.61±0.37b
		lymphocyte	3.50±0.18b	4.24±0.20a	3.83±0.21b	3.80±0.30b	3.76±0.43b
		monocyte	1.54±0.17b	2.13±0.14a	1.92±0.29b	1.93±0.26b	1.89±0.31b
		eosinophils	1.77±0.15b	2.33±0.17a	1.98±0.27b	1.97±0.29b	1.94±0.44b
		Basophils	1.73±0.11b	2.09±0.10a	1.91±0.25b	1.92±0.31b	1.89±0.27b
phagocytosis%		63.21±1.83b	68.55±1.34a	65.21±1.73b	65.62±1.55b	64.43±1.55b	
Phagocytic index		4.47±0.68b	6.62±0.41a	5.24±0.61b	5.40±0.44b	4.87±0.64b	

Means with different superscripts of the same cullum indicate significant difference at P < 0.05

Table 3: effect of lincomycin and kitasamycin on leukogram, phagocytic activity % and index in chickens infected with *clostridium perfringens* (n= 5).

In the present investigation, it has been shown that broilers suffering from necrotic enteritis showed significant reduction in total protein and albumin associated with insignificant increase in total, a, b and g globulin and insignificant decrease in A/G ratio (table 4). Broilers suffering from necrotic enteritis showed decrease in total protein, albumin and globulin in due to anorexia and male absorption of nutrients from inflamed intestine or due to inability of liver to protein synthesis [34]. Our observed data are fit with [40 & 6] reported that necrotic enteritis induced significant reduction in total protein and albumin. Broilers suffering from necrotic enteritis show-ed decrease in total protein and albumin associated with insignificant increase in gamma globulin and insignificant decrease in alpha, beta globulin (35). Broilers offering from necrotic enteritis showed significant reduction in total protein, albumin and globulin [5].

Parameter	control	Inf non treated	Inf. Linco treated	Inf. kitasa treated	Inf. linco & kitasa treated	
T.Protein	6.99±0.22a	5.19±0.21b	6.09±0.26b	6.01±0.18b	6.70±0.32a	
Albumin	3.70±0.28a	2.15±0.21b	2.69±0.20b	2.70±0.19b	3.38±0.35a	
Globulin	a	0.70±0.08a	0.66±0.09a	0.75±0.06a	0.75±0.09a	0.75±0.07a
	b	0.79±0.07a	0.78±0.08a	0.86±0.07a	0.82±0.06a	0.85±0.09a
	g	1.80±0.17a	1.80±0.16a	1.89±0.14a	1.83±0.15a	1.82±0.17a
	total	3.29±0.62a	3.04±0.54a	3.33±0.62a	3.31±0.52a	3.32±0.48a
A/G ratio	1.12±0.22a	0.79±0.18a	0.81±0.19a	0.85±0.20a	0.86±0.23a	

Means with different superscripts of the same cullum indicate significant difference at P < 0.05

Table 4: The effect of lincomycin and kitasamycin on serum protein picture in chickens infected with *clostridium perfringens* (n= 5).

In present investigation, it has been shown that infected broilers with *clostridium perfringens* showed significant increase in liver enzymes (apartate aminotranferae, alanine aminotranferae and alkaline phosphatae) uric acid, creatinine beside insignificant decrease in total lipids, triglycerides and cholesterol (table 5). Our results were supported by the results reported [1] reported that broilers suffering from necrotic enteritis showed increase in liver enzymes, uric acid and creatinine beside insignificant decrease total lipids, triglycerides and cholesterol. Same observation was recored [49] observed that necrotic enteritis induced significant increase in liver enzymes, uric acid, creatinine. Necrotic enteritis induced decrease in serum total lipids, triglycerides and cholesterol [28]. Liver enzymes uric acid and creatinin were increased in broiler chickens suffering from necrotic enteritis [35].

Parameter	Control	Inf non treated	Inf. Linco treated	Inf. kitasa treated	Inf. linco & kitasa treated	
Liver enzymes (U/L)	AST	42.17±1.25b	47.32±1.32a	44.63±1.22b	44.64±1.21b	42.83±1.44b
	ALT	64.72±0.78b	67.22±0.55a	64.18±0.57b	65.32±0.71b	64.12±0.66b
	ALP	56.78±0.89b	61.33±0.96a	58.21±0.82b	58.55±0.77b	57.62±0.59b
Kidney function	Uric acid	4.63±0.38b	5.92±0.28a	4.93±0.34b	4.88±0.42b	5.08±0.40b
	Creatinine	1.38±0.16b	2.14±0.15b	1.59±0.16b	1.55±0.13b	1.46±0.16b
lipid profile (mg/dl)	T. lipid	214.14±2.99a	211.17±1.89a	212.48±1.88a	210.43±1.81a	212.39±1.93a
	cholesterol	86.38±1.84a	82.34 ±1.28a	84.55±1.27a	84.71±1.66a	857.0±1.88a
	triglyceride	123.17±1.95a	119.18±1.78a	120.88±1.69a	121.12±1.56a	121.88±1.89a

Means with different superscripts of the same cullum indicate significant difference at P < 0.05

Table 5: The effect of lincomycin and kitasamycin on serum Liver enzymes, Kidney function and lipid profil in chickens infected with *clostridium perfringens* (n= 5).

Infected broilers with *clostridium perfringens* showed significant reduction in catalase and super oxide dismutase beside significant increase malanodialdhyde (table 6). Increase in malanodialdhyde and reduction in catalase, super oxide dismutase in broilers suffering from necrotic enteritis may be due to increase oxidative stress iduced by *clostridium perfringens* bacteria [32]. Our finding confirmed by those obtained [8] found that necrotic enteritis induced reduction in catalase, super oxide dismutase and increase in malanodialdhyde in broilers. Same changes in antioxidant enzymes were reported [52] in broilers suffering from necrotic enteritis.

Parameter		Control	Inf non treated	Inf. Linco treated	Inf. kitasa treated	Inf. linco & kitasa treated
MDA (mmol/ml)		19.80±1.51b	24.61±1.24a	22.16±1.81b	21.99±1.72b	21.18±1.83b
antioxidant enzymes	CAT(U/mL)	42.08±1.89a	35.03±1.93b	40.21±1.73a	40.74±1.65a	41.32±1.77a
	SOD (U/mL)	190.62±1.29a	184.05±1.44b	187.21±1.81a	41.32±1.77a	188.50±1.89a

Means with different superscripts of the same cullum indicate significant difference at P < 0.05

Table 6: The effect of lincomycin and kitassamycin on serum lipid profile, serum malanodialdhyde and antioxidant enzymes in chickens infected with *clostridium perfringens* (n= 5).

Concerning the histopathological changes, the liver of broilers suffering from necrotic enteritis show congestion of hepatic blood vessels (Fig 1), fibrosis in portal area and hyperplastic bile duct epithelium (Fig 2). Necrotic epithelial lining villi and desquamation of intestine ((Fig 3), necrotic epithelial lining villi, desquamation and presence of heterophilic and lymphocytic infiltration in the lamina propria and submucosa (Fig 4). Kidney showed subscapular aggregation of lympho-cytes, renal papillae show-ed perivascular edema (Fig 5), shrinkage of moderate number of glomeruli and degenera-tive changes mainly hydropic degeneration in renal tubular epithelium (Fig 6). Liver of infected broilers lincomycin and kitassamycin treated together showed normal structures with hydropic degeneration in hepatocytes (Fig 7) normal structures with focal villous basal cells proliferation (Fig 8), Same lesion was observed [15 & 11] in broilers suffering and duckling suffering from necrotic enteritis. Our findings are agreed with [4] mentioned that broilers suffering from necrotic enteritis showed congestion of hepatic blood vessels and hydropic degeneration in renal tubular epithelium.

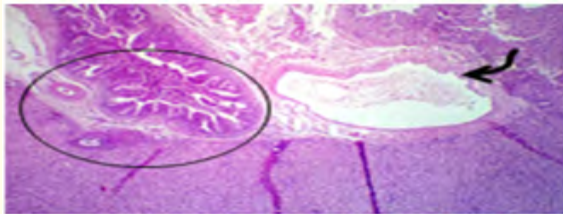


Figure 1: liver of infected broilers by *cl perfringens* showing sever congestion of hepatic blood vessels (curved arrow).

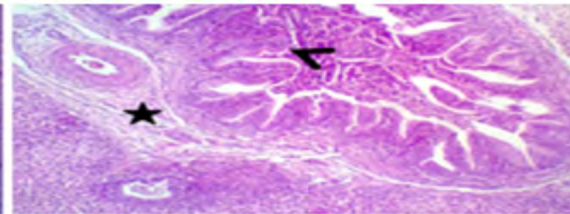


Figure 2: liver of infected broilers by *cl perfringens* showing portal area showing fibrosis (star) and hyperplastic bile duct epithelium (arrow head).

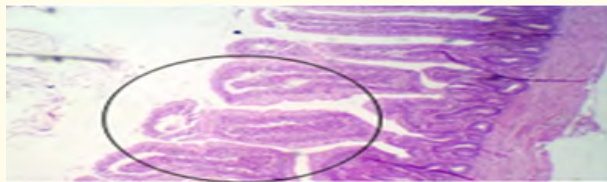


Figure 3: Intestine of infected broilers by *cl perfringens* showing necrotic epithelial lining villi and desquamation (open arrow) H&E X 100.

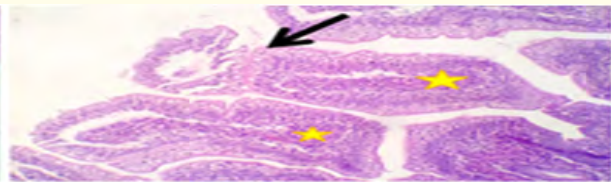


Figure 4: Intestine of infected broilers by *cl perfringens* showing necrotic epithelial lining villi and desquamation (arrow) and presence of heterophilic and lymphocytic infiltration in the lamina propria and submucosa (yellow stars) H&E X100.

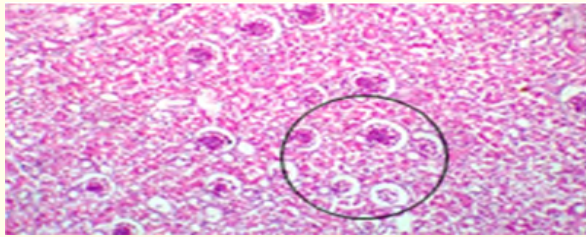


Figure 5: Kidney of infected broilers by *cl perfringens* showing subcapsular aggregation of lymphocytes (star). the renal papillae showing perivascular edema (open arrow).

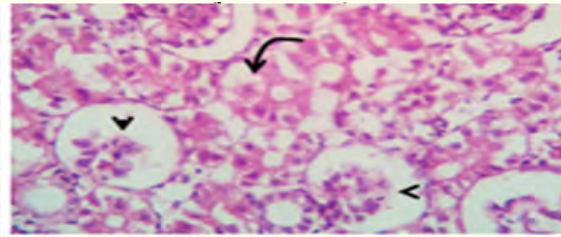


Figure 6: Kidney of infected broilers by *cl perfringens* showing shrinkage of moderate number of the glomeruli (arrow heads) and degenerative changes mainly hydropic degeneration in renal tubular.

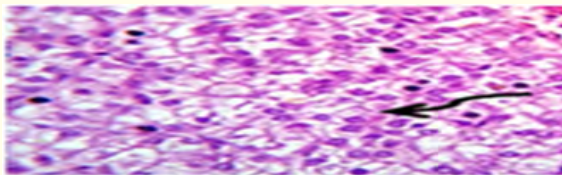


Figure 7: Liver of infected broilers by *cl perfringens* treated with lincomycin and kitassamycin together showing normal structures with mild hydropic degeneration (curved arrow) in some hepatocytes. H&E X 100.

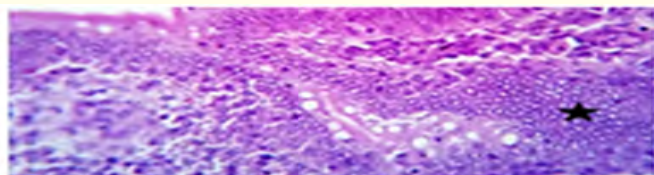


Figure 8: Intestine of infected broilers by *cl perfringens* treated with lincomycin and kitassamycin together showing normal structures, focal villous basal cells proliferation (star) H&E X 100.

Treatment infected broilers by lincomycin or kitassamycin either alone or together were effective and resulted in disappearance of clinical signs and improved body performance, leukogram, phagocytosis %, phagocytic index toward to nearly normal beside improved in pathological lesion (table 1-6)... Disappearance of clinical signs and improvement body performance leukogram, phagocytosis %, phagocytic index post treatment by lincomycin and kitassamycin might be due to reduction reisolation and suppress *clostridium perferenges* invade host [2]. Kitassamycin play an a role in controls *clostridium perfringens* infection in broilers and improved clinical signs and improve body performance [41]. These results agreed with those stated [3] mentioned that *Cl. Perfringens* sensitive to lincomycin. In addition [6] found that kitassamycin is effective against *clostridium perfringens* infection leading to disappear clinical signs and improved hemato-biochemical parameters. lincomycin is effective in treatment *clostridium perferenges* infection in chickens leading to improved clinical signs and body performance [4 & 21]. lincomycin decreased incidence of necrotic enteritis in broilers so improved leukogram, phagocytosis %, phagocytic index, protein picture, lipid profile, liver enzymes, kidney function, catalase, super oxide dismutase and malanodialdehyde return to normal levels [20].

It could be concluded that necrotic enteritis in broilers induce some adverse effect on hematobiochemical parameters and this adverse effects were returned to the nearly normal levels post treatment with lincomycin or kitassamycin either alone or together but combination of both drug have superior effect in *clostridium perfringens*. So, its good using combination of lincomycin and kitassamycin in treatment *clostridium perfringens*.

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