

Study on Effect of Anticlostridial Antibiotics Medication and Acidifier Copper Sulfate Mixture in Improvement of Performance against Clostridial Infection in Broiler Chickens

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Abstract: This study was conducted to evaluate different regimen usually used in field for treatment of necrotic enteritis (NE) as bacitracin methylene disalicylate (BMD[®]), amoxycillin (Bioamoxi[®]) and acidifier copper sulphate mixture (Sanifeed NC[®]). Two hundred broiler chicken were divided into eight groups; 25 each. Performance parameters were studied including average weekly body weight, feed conversion rate (FCR), mortalities, postmortem (P.M.) lesions together with liver and intestinal tissue samples for histopathological examination. Results revealed that all control negative chicks and groups received bacitracin methylene disalicylate (bacitracin M.D.), amoxycillin and acidifier copper sulphate mixture from one day old or after challenge shows no mortalities and clinical signs, while those control positive group shows typical clinical signs and mortalities of 70% and 30%; respectively, clinical signs including depression, tendency to huddle and watery dropping. P.M. findings in dead birds were severe inflammation in different parts of small intestine. Regarding average weekly body weight and FCR, it was found that control positive group was the most affected group challenged with *Clostridium perfringens* (*C. perfringens*) as average weekly body weight and FCR were 1015 gm and 2.2; respectively by the end of week 4 of age. In case of treatment therapy groups, they showed improved body weight and FCR that were better in groups received treatment therapy start from one day old chicks than those treated post challenge comparatively. Moreover, treated post challenged groups had lower body weight and FCR when compared with control negative group. The group received bacitracin M.D. was the best followed by acidifier copper sulphate mixture then those received amoxycilline in terms of average weekly body weight and FCR. Regarding histopathological changes, it was found that chicken groups treated from one day old are more better than those treated after challenge, on the other hand control positive group are severely affected as intestine of control positive group showed severe necrosis in the mucosa with inflammatory cells infiltration, liver showed severe congestion of the portal veins and vacuolar degeneration of the hepatocytes by 2nd day post infection, at 4th day post infection liver of control positive groups showed area of coagulative necrosis infiltrated with inflammatory cells together with congestion of the portal veins and vacuolar degeneration of the hepatocytes. It could be concluded that bacitracin M.D. still standard effective medication against NE and it is preferable to use acidifier as safe alternative for antibiotic medication with long treatment period as it act as growth promotor either by enhancing digestibility or competitive inhibition of colonization of pathogenic bacteria.

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1. Introduction

Necrotic Enteritis (NE) is one of great economic important poultry disease that affect poultry industries (Cooper and Songer, 2009) as it causes low growth rate and affect feed conversion together with variable mortalities (Lovland and Kaldhusdal, 2001). NE is enterotoxaemic disease in chicken caused by alpha toxin-producing bacterium *C. perfringens* in which were toxogenic strains which were isolated from both diseased and healthy chickens (Timbermont et al., 2009). The disease risk factors include concurrent coccidial infection or feed poultry flock in ration contains cereal grains high in nonstarch polysaccharides (Saif et al., 2003; Jia et al., 2009 and Palliyeguru et al., 2010), moreover coccidial vaccine

concedered as co factor in induction of the disease (Pedersen et al., 2008). The disease can cause both clinical and subclinical disease in poultry (Engström et al., 2003; Saif et al., 2003 and Merati, 2010). The disease cause severe economic losses due to high mortality rates and poor feed efficiency (Skinner et al., 2010 and Cooper et al., 2013). Control of the disease could be applied by vaccination against the pathogen and the use of probiotic and prebiotic products together with balancing the composition of the feed (Van Immerseel et al., 2004). There are many antimicrobale medication including Amoxycillin are still effective against *C. perfringens* treating the disease and found that it decrease and control NE lesions (Lankriet et al., 2010). Recently many studies

support the use of antibiotic alternative such as mixture of propionic acid, formaldehyde and terpenes or organic acids and concluded their efficiency in control the disease when used for five days treatment course (Casagrande *et al.*, 2013).

From the above mentioned data this study applied for updating effect of different anticlostridial medication together with effect of copper sulfate acidifier commercial mixture under field condition.

2. Material and Methods

Experimental birds:

A total of 200, one day old Hubbard broiler chickens were fed commercial ration and reared under strict hygienic measures simulating all farm conditions and management.

C. perfringens pathogenic strain:

C. perfringens strain for challenge both control positive and treated chicks throughout the experiments, were kindly supplied by Central Laboratories for Evaluation of Biological products "CLEVB". *C. perfringens* culture in cooked meat media broth (CMB) was given per os (intracrop) at 18, 19 and 20 days of age (1:1 w.v.) with dose of 10^7 CFU/ml and given twice daily according to *pedersen et al.* (2008).

Cooked meat medium (FLUKA):

It was used to support the growth of Clostridia organisms as a suitable medium for enrichment, purification and preservation, procedures according to *Smith and Holdeman* (1968).

Antibacterial agents and acidifier copper sulphate commercial mixture:

1- BMD[®] soluble 50%: bacitracin methylene disalicylate (bacitracin M.D.) Reg. No. 3177/2008 – Batch No. SE 13002 – Exp. Date 1/2016 – Company Pfizer-USA.

Dosage: 1-2 grams BMD soluble 50% / 10 liters of drinking water.

2- Bioamoxi[®]: Amoxicillin antibiotic powder for drinkable solution sachet of 100 gm. Batch No. 0111718 – Exp. Date 11/2014 – company biove laboratoires – France.

Dosage: 40 mg of Bioamoxi per kg of body wt / day

3- Sanifeed NC[®]: Acidifier mixtures together with copper sulphate. Batch No. 11/012 – Exp. Date 16/12/2015 – Company IQF – Espania.

Dosage: 0.5-2.0 ml /liter of drinking water.

Coccivac D vaccine:

Live coccidiosis vaccine was produced by Schering plough and used as stress complicating condition to produce clinical NE at 10 days of age by intracrop method.

Feed conversion rate (FCR):

It was calculated by total weight/g of food consumption / birds of specific group during a given period over total weight gain /g of the same group birds during a given period (including weight gain of birds which died during the given period) according to *Sainsbury* (1984).

Experimental design:

Total numbers of 200 broiler chickens were divided into eight groups 25 per each. Groups 1, 2 and 3 received bacitracin M.D. (BMD[®]), amoxycillin (Bioamoxi[®]) and mixture of acidifier with copper sulphate (Sanifeed NC[®]); respectively; from the day one of age till the end of the experiment. While groups 4, 5 and 6 received bacitracin M.D. (BMD[®]), amoxycillin (Bioamoxi[®]), and mixture of acidifier with copper sulphate (Sanifeed NC[®]); respectively post infection with *C. perfringens* field isolate and starting from 21st day of life for five successive days. Chicken groups 7 and 8 were kept as control negative (receive no medication) and control positive groups; respectively. At 10th day of age groups 1, 2, 3, 4, 5, 6 and 8 were given 1 ml of coccidial vaccine by intracrop route and at 18, 19 and 20 days of age groups 1, 2, 3, 4, 5, 6 and 8 were orally (intracrop route) challenged with *C. perfringens* culture containing 10^7 CFU/ml. twice daily.

3. Results and Discussion

All control negative chicks and groups received bacitracin M.D., amoxycillin and acidifier copper sulphate mixture from one day old or after challenge shows no mortalities and clinical signs. After experimental infection with *C. perfringens* pathogenic bacteria, control positive (those received pathogenic bacteria together with anticoccidial vaccine) shows typical clinical signs and mortalities of 70% and 30%; respectively. Clinical signs including depression, tendency to huddle and watery dropping, post-mortem finding in dead birds there was severe inflammation in different parts of small intestine, these results were parallel with *Wilson et al.* (2005).

Regarding average weekly body weight and FCR, it was found that group challenged with *C. perfringens* (control positive group) was the most affected as average weekly body weight and FCR were 1015 gm and 2.2; respectively by the end of week 4 of age. These results were parallel with those results found by *Sathishkumar et al.* (2013). While in case of all treatment therapy groups, they showed improved body weight and FCR that were better in groups received treatment therapy start from one day old chicks than those treated post challenge comparatively (tables 1 and 2). Better early access dietary supplementations were studied by *Ao et al.* (2012) as they studied early supplementation of either antibiotic, acidifier, or mannanoligosaccharides

against challenge with *C. perfringens* in broiler chickens and concluded that all of them improve performance parameter and could be used to prevent NE in the broiler industry. Moreover those treated post challenge group had lower body weight and FCR when compared with control negative groups this may be due to use of coccidial vaccine and challenge with *C. perfringens* bacteria prior treatment which affect villi and cause intestinal damage special when presence of stress factor (coccidial vaccine), this affect digestion and absorption of nutrient before treatment of causative agent (*C. perfringens*). These results were parallel with those of *Bahram et al. (2012)* who stated that *C. perfringens* with critical stress factors including coccidial vaccines may cause intestinal damage. Same results were found by *Alaeldein and Hany (2013)* who noticed that challenge with *C. perfringens* infection affect growth parameters including FCR and body weight. Group received

bacitracin M.D. was the best followed by acidifier copper sulphate mixture then those received amoxycillin (in terms of average weekly body weight and FCR). These results explained by *Knarreborg et al. (2002)* who explained improvement in FCR and body weight caused by bacitracin M.D. were due to it causes altering the composition and activities of microflora while *Aarestrup (2000)* and *Singer and Hofacre (2006)* were explained this improvement not only to improve microflora activities but also create a selective pressure in favor of resistant bacteria. Moreover, *Manoj et al. (2008)* stated that bacitracin M.D. supplementation have an positive impact on growth performance and carcass characteristics due to it control enteric pathogens and improve digestion. Also, *Lankriet et al. (2010)* compared the effect of different antibacterial agents includes amoxycillin on NE and concluded that they cause reduction in clinical symptoms.

Table (1): Average weekly body weight (gms) for all test groups.

| Age/ Week | Continuous treatment groups | | | Post challenge treatment groups | | | Control negative (gp. 7) | Control positive (gp. 8) |
|--------------------------------|-----------------------------|---------------------|---|---------------------------------|---------------------|---|--------------------------|--------------------------|
| | Bacitracin M.D. (gp. 1) | Amoxycillin (gp. 2) | Acidifier copper sulphate mixture (gp. 3) | Bacitracin M.D. (gp. 4) | Amoxycillin (gp. 5) | Acidifier copper sulphate mixture (gp. 6) | | |
| 1 | 188 | 160 | 177 | 155 | 156 | 156 | 156 | 156 |
| 2 | 425 | 395 | 401 | 390 | 387 | 388 | 390 | 380 |
| 3 | 855 | 795 | 815 | 784 | 778 | 780 | 785 | 590 |
| 4 (one week post challenge) | 1351 | 1265 | 1285 | 1255 | 1230 | 1239 | 1215 | 1015 |

Table (2): FCR for all test groups

| Age/ Week | Continuous treatment | | | Post challenge treatment | | | Control negative (gp. 7) | Control positive (gp. 8) |
|--------------------------------|-------------------------|---------------------|---|--------------------------|---------------------|---|--------------------------|--------------------------|
| | Bacitracin M.D. (gp. 1) | Amoxycillin (gp. 2) | Acidifier copper sulphate mixture (gp. 3) | Bacitracin M.D. (gp. 4) | Amoxycillin (gp. 5) | Acidifier copper sulphate mixture (gp. 6) | | |
| 1 | 1.23 | 1.25 | 1.24 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| 2 | 1.38 | 1.4 | 1.38 | 1.39 | 1.38 | 1.38 | 1.4 | 1.45 |
| 3 | 1.65 | 1.67 | 1.65 | 1.69 | 1.71 | 1.70 | 1.69 | 1.77 |
| 4 (one week post challenge) | 1.91 | 1.93 | 1.93 | 1.93 | 1.95 | 1.94 | 1.95 | 2.2 |

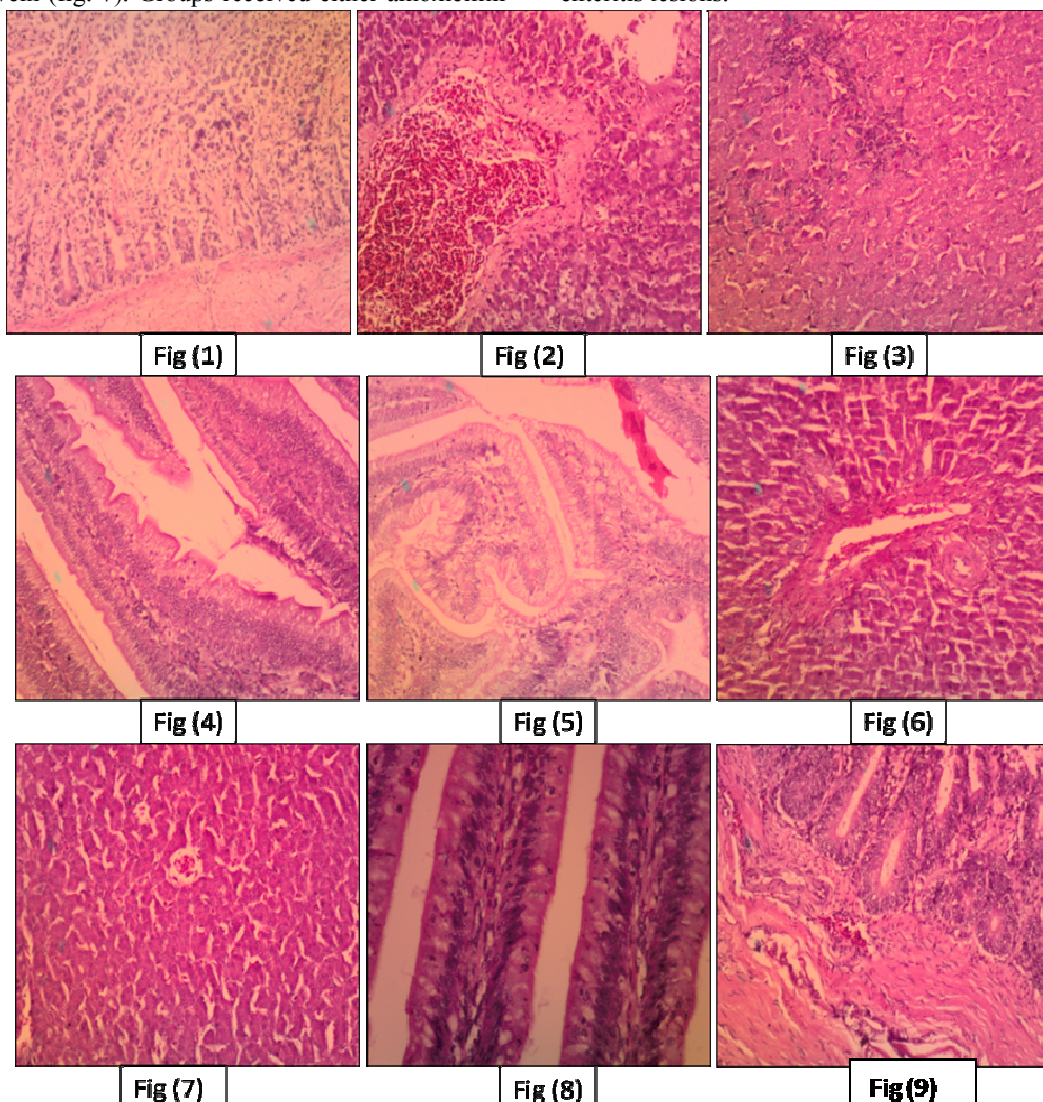
Regarding acidifier copper sulphate mixture results revealed it has positive impact in improvement of body weight and FCR this was matched with results found by *Mountzouris et al. (2010)* and *Taheri et al. (2010)* and was parallel with results found by *Eman et al. (2012)* on capability of organic acid in reduce colonization of pathogenic microorganism and improving digestibility and body weight, also the presence of copper sulphate combination has a synergistic effect in terms of improves body weight

and feed conversion rate as it was found by *Kim et al. (2011)* who concluded that it improves body weight and has antimicrobial effect.

Regarding histopathological changes, intestine of control positive group showed severe necrosis in the mucosa with inflammatory cells infiltration (fig. 1), liver showed severe congestion of the portal veins and vacuolar degeneration of the hepatocytes by 2nd day post infection (fig. 2) and by the 4th day post infection liver of control positive groups showed area of

coagulative necrosis infiltrated with inflammatory cells together with congestion of the portal veins and vacuolar degeneration of the hepatocytes (fig. 3), this results was matched by *Anthony et al. (2006)* who studied the histopathological changes caused by *C. perfringens*. Chicken groups treated from one day old are more better than those treated after challenge only, as intestine of chicken groups treated from one day old with bacitracin M.D. showed slight inflammatory cells infiltration of the mucosa after challenge with *C. perfringens* field isolates (fig. 4) while intestine of groups treated with bacitracin M.D. after challenge showed mild inflammatory cells infiltration of the intestinal mucosa (fig. 5). Liver of bacitracin M.D. treated groups from one day old were apparently normal and continue after challenge (fig. 6) while those infected then treated showed slight congestion of the portal vein (fig. 7). Groups received either amoxicillin

or acidifier copper sulphate mixture showed nearly similar histopathological changes as their intestine after challenge in groups received treatment start from one day old showed slight inflammatory cells infiltration of the mucosa (fig. 8) while intestine of those groups treated after challenge showed congestion of the blood vessels in the submucosa and inflammatory cells infiltration of the mucosa (fig. 9). While liver in chicken groups treated with amoxicillin or acidifier copper sulphate mixture from one day old and after experimental infection showed congestion of the portal vein (fig. 10). While those treated after challenge showed severe hydropic degeneration of the hepatocytes (fig. 11). This results was matched with *Lankriet et al. (2010)* who studied the effect of antimicrobial agents used in treatment of necrotic enteritis and found that it decrease and control necrotic enteritis lesions.



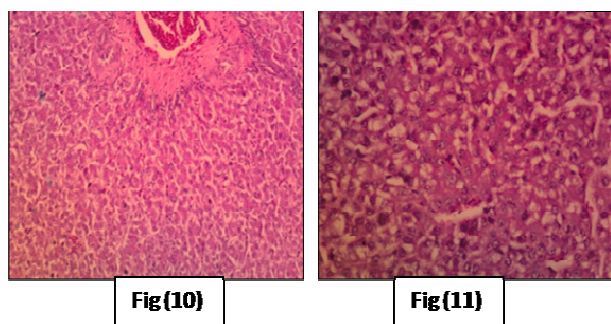


Fig. (1- 11): the histopathological changes caused by *C. perfringens* in intestine and liver of chicken in presence of treatment either continuous therapy start from one day old or post challenge (H&E x 200).

It could be concluded that bacitracin M.D. still standard effective medication against NE and it is preferable to use acidifier as safe alternative for antibiotic medication with long treatment period as it act as growth promotor either by enhancing digestibility or competitive inhibition of colonization of pathogenic bacteria.

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