

Effect of zinc bacitracin and oligosaccharids on chickens vaccinated with chicken necrotic enteritis vaccine

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SUMMARY

Chicken necrotic enteritis vaccine was used to vaccinate chicks in a mutual manner with zinc bacitracin or oligosaccharides (MOS). The study included four groups of chicks (50 birds/group). The first group was vaccinated with chicken necrotic enteritis vaccine and zinc bacitracin. The second group was vaccinated with chicken necrotic enteritis vaccine and oligosaccharides while the third group was vaccinated with chicken necrotic enteritis vaccine only. Fourth group was kept as unvaccinated control. The obtained results suggest that the use of (MOS) with necrotic enteritis vaccine represent a practical preventive method against necrotic enteritis in broiler chicken and improve production performance.

INTRODUCTION

The cost of necrotic enteritis to the poultry industry globally is nearly \$2 billion (Anonymous, 2000). Both clinical and subclinical necrotic enteritis is common in all poultry growing areas of the world (Van der Sluis, 2000).

The disease was first described by Parish (1961). The main cause of necrotic enteritis is *Clostridium perfringens* (Hofacre et al., 1986 and Prukner et al., 1995). *Clostridium perfringens* type A and to a lesser extent type C have been reported to be the causative agent (Kaldhusdal and Lovland, 2000 and Van Immerseel et al., 2004). In Egypt alpha toxin of *Clostridium perfringens* was detected in the intestinal filtrate of died chicken (Hussien et al., 2007 and Salama et al., 2008).

Current efforts to control *C. perfringens* rely upon sanitary measures and placing antibiotics in the poultry feed. However, antibiotics are costly and subject to increasing concerns related to the promotion of bacterial resistance. The European Union prohibited the use of the antibacterial feed additives that have most successfully controlled the incidence of both clinical and subclinical necrotic enteritis.

Therefore, new methods for prevention of necrotic enteritis must be investigated such as using vaccination. The necrotic enteritis vaccine was produced in Egypt (Hussien et al., 2007). The vaccine was prepared from locally isolated toxigenic strain of *Clostridium perfringens* type A. Supplementation with salinomycin and zinc bacitracin, alone or in combination, resulted in significantly lower counts of *C. perfringens* (Engberg, et al., 2000). The performance-enhancing effect of Zinc bacitracin has been observed in broiler (Rosen, 1980, Foster and Stevenson, 1983 and Choi and Ryu, 1987). Zinc bacitracin (ZnB) is largely unabsorbed from the intestine at the dietary concentration usually used. The main site of antibiotic activity is within the gastrointestinal tract, where ZnB acts to modify the intestinal flora as well as the gut wall structure (Boorman, 1987 and Bernsten, 1994). Mannan oligosaccharide (MOS) is derived from the cell wall of *Saccharomyces cerevisiae*. The benefits

of MOS are based on specific properties that include modification of the intestinal flora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system in the intestinal lumen. The quantum of *Clostridium* species in the cecal contents of 6 week old turkey poult was significantly reduced by addition of MOS in the diet at a level of 1 g/kg. In a replicate pen trial, total counts of *Clostridium* spp. were significantly reduced from 4.2 to 2.8 log CFU/g cecal contents (Finucane et al., 1999).

Inclusion of MOS at 1 g/kg diet enhanced both IgG and IgA serum anti-body levels in turkey poult (Savage et al., 1996).

The aim of this work is to study the effect of MOS and ZnB on the immune response of chicken vaccinated with chicken necrotic enteritis vaccine.

MATERIAL AND METHODS

1. Chickens:

Two hundred, day-old Hubbard broiler chicks were obtained from a commercial hatchery. The chicks were reared on litter floor. The chicken were given broiler ration ad libitum, also water was given ad libitum. The used chicks were vaccinated against Newcastle disease using Hitchner B1 and LaSota vaccines

at 8 and 21 days of age, respectively, and against infectious bursal disease at 14 days of age. All the vaccines were given through eye drop method. The chicks were divided into four groups each of fifty birds. First group was injected with necrotic enteritis vaccine, the second group was injected with necrotic enteritis vaccine and take bio-MOS in ration, the third group was injected with necrotic enteritis vaccine and take zinc bacitracin in ration, the fourth group was not vaccinated necrotic enteritis vaccine and take ration only without additives (control group).

2. The necrotic enteritis vaccine:

Aluminum hydroxide gel vaccine was supplied from veterinary sera and vaccines Research Institute (Abbasia, Cairo) and injected 0.5 ml subcutaneous at 14 days old chick as recommended by manufacture.

3. Mannan oligosaccharides (MOS)

A fraction of the *Saccharomyces* yeast outer cell wall, mannan oligosaccharides (MOS), were used as a feed additive for poultry (Bio-Mos®, Alltech Inc.). Bio-Mos® levels of adding for broiler chicken feeds were: 0.2% from zero to 7 days, 0.1% from 7 to 21 days and 0.05% from 21 to 42 days.

4. Zinc bacitracin (ZnB) 15% (Albac ® Alpharma):

It was used as a feed additive for poultry at (0.35 kg/ton feed - 52.5 ppm) as recommended of manufacture.

During the experiment period, the data about daily mortality, feed conversion rate and body weight gain were reported in the vaccinated and unvaccinated chickens.

Pooled serum samples were collected from each group at different interval as follow: two, three, four and five weeks after first dose. Pooled sera of each group were tested for determining alpha antitoxin titre of *Clostridium perfringens* type A using hemolytic activity test according to Norris and Ribbons (1971).

5-Biological reagent:

- Standard research antisera of *Clostridium perfringens* type A and *Clostridium perfringens* type A toxin were obtained from welcome Research Laboratories, England.
- Sheep red blood cells.

6. Alpha Anti-Toxin Unit:

It was determined by mouse serum neutralization test (SNT) according to European Pharmacopoeia (2001).

RESULTS AND DISCUSSION

Both clinical and subclinical necrotic enteritis (NE) is common in all poultry growing areas of the world (Van der Sluis, 2000). The causative agent of necrotic enteritis is *Clostridium perfringens*. The incidence of *Clostridium perfringens* associated (NE) in poultry has increased in countries that stopped using antibiotic growth promoters (Van Immerseel, 2004). Vaccination could be a helpful tool in preventing necrotic enteritis in poultry. It is known that flocks with high titers of antibodies against alpha toxin had lower mortality rate than flocks with low titers (Heier et al., 2001). In order to enhance the immunogenic effects of vaccine, Mannan oligosaccharide (MOS) is used. In the same time, (MOS) have beneficial effect till antibody production. The benefits of MOS are based on specific properties that include modification of the intestinal flora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system in the intestinal lumen (Loddi et al., 2002 and Ferket et al., 2002)

The results obtained in table (1) and table (2) showed that the anti-toxin titers were higher in group with (MOS) supplement than those without (MOS) supplement, and those

with zinc bacitracin supplement till 5th week post vaccination. These results agreed with Ellis et al. (1991), Heier et al. (2001) and Lovland et al. (2004), whom showed that the vaccination of broiler chickens with NE vaccine resulted in a strong specific antibodies against alpha toxin of *Clostridium perfringens* type A. Also, Savage et al., (1996) found that inclusion of MOS in diet enhanced both IgG and IgA serum anti-body levels.

Concerning broiler performance; the results obtained in table (3) showed that the use of (MOS) with NE vaccine decreased the mortality rate, decreased feed conversion ratio and increased body weight gain of chicken during the production period, which agreed with Kaldhusdal et al. (2001) and Hofacre et al. (2003) who stated that the damage of the intestinal mucosa caused by *Clostridium perfringens* type A in subclinical form of necrotic enteritis leads to decreased digestion and absorption leading to reduction in body weight gain and increased feed conversion ratio.

In conclusion, the obtained results suggest that the use of (MOS) with necrotic enteritis vaccine represent a practical preventive method against necrotic enteritis in broiler chicken and improve production performance.

Table (1): Immune response of chicken vaccinated with necrotic enteritis vaccine using haemolytic test

Vaccination	Antibody Titre (IU)			
	Weeks Post Vaccination			
	2	3	4	5
Necrotic enteritis vaccine + ZnB	2	3	2	2
Necrotic enteritis vaccine + Bio-MOS	3	3	3	3
Necrotic enteritis vaccine	2	3	2	2
Unvaccinated control	0	0	0	0

Table (2): Immune response of chicken vaccinated with necrotic enteritis vaccine using SNT in mice

Vaccination	Antibody Titre (IU)			
	Weeks Post Vaccination			
	2	3	4	5
Necrotic enteritis vaccine + ZnB	2.5	3.5	3.0	3.0
Necrotic enteritis vaccine + Bio-MOS	3.0	4.0	4.0	4.0
Necrotic enteritis vaccine	2.5	3.5	3.0	3.0
Unvaccinated control	0	0	0	0

Table (3): Effect of necrotic enteritis vaccine, zinc bacitracine and Bio-mos on broiler performance

Chicks group	Mean body weight	Mean feed conversion rate	Mortality %
Necrotic enteritis vaccine + ZnB	1.77 Kg	1.98	4%
Necrotic enteritis vaccine + Bio-MOS	1.82 Kg	1.87	4%
Necrotic enteritis vaccine	1.73 Kg	1.99	4%
Unvaccinated control	1.6 Kg	2.3	6%

REFERNCES

- Anonymous (2000): World growth continues. Poultry International 39, No.1:8-14.
- Bernsten, J.O. (1994): The use of Zinc bacitracin. World Poult. 10 (11): 41.
- Boorman, K.N. (1987): Mode of action of gut-active (antibiotic) performance promoters. Pages D12-20 in: Proceedings of the Sixth European Symposium on Poultry Nutrition. H. Vogt, ed. World's Poultry Science Association, Germany.
- Choi, J. H. and K. S. Ryu (1987): Responses of broilers to dietary Zinc bacitracin at two different planes of nutrition. Br. Poult. Sci. 28:113-118.
- Engberg, R.M.; Hedemann, M.S., Leser, T.D. and Jensen B.B. (2000): Effect of Zinc Bacitracin and Salinomycin on Intestinal Microflora and Performance of Broilers. Poultry Science 79:1311-1319.
- Ellis, T.M.; Gregory, A.R. and Logue, G.D. (1991): Evaluation of a toxoid for protection of rabbits against enterotoxaemia experimentally induced by trypsin-activated supernatant of Clostridium spiriforme.
- European Pharmacopoeia (2001): 4th Ed. Council Europe, Strasbourg Codex, France, p. 2297-2298.
- Ferket, P.R., Park, C.W. s and Grimes, J.L. (2002): Benefits of dietary antibiotic and mannan-oligosaccharide supplementation for poultry. In: Proc. Multi-State Poult. Feeding and Nutr. Conf., Indianapolis, IN. May 14-16.
- Finucane, M.C., Dawson, K.A., Spring, P. and K.E. Newman. (1999): Effects of mannan oligosaccharide on composition of the gut microflora of turkey poults. Poultry Sci. 78 (Suppl. 1):77.
- Foster, W. H. and M. H. Stevenson (1983): The interaction of food additives and protein

- content in broiler diets. *Br. Poult. Sci.* 24:455-462
- Heier, B.T.; Lovland, A.; Soleim, K.B.; Kaldhusdal, M. and Jarp, J. (2001): A field study of naturally occurring specific antibodies against *Clostridium perfringens* alpha toxin in Norwegian broiler flocks. *Avian Dis.* 45:724-732.
- Hofacre, C.L.; Beacom, T.; Collett, S. and Mathis, G. (2003): Using competitive exclusion mannan-oligosaccharide and other intestinal products to control necrotic enteritis. *Journal of Applied Poultry Research*, 12: 60-64.
- Hofacre, C.L. French, J.D., Page, R.K. and Fletcher, O.J (1986): Subcutaneous clostridial infection in broilers. *Avian Dis.*, 30(3): 620-622.
- Hussien, A.Z., Fathia, Shafie; El-Boraay, I.M.; Diab, R.A. and El-Harby, H.A. (2007): Necrotic enteritis in chicken and trials for vaccine preparation from the toxins of isolated *Clostridium perfringens*. *Benha Vet. Med. J.*, 18(1): 19-28.
- Kaldhusdal, M. and A.L. Lovland. (2000). The economical impact of *Clostridium perfringens* is greater than anticipated. *World Poultry* 16, No. 8:50-51.
- Kaldhusdal, M.; Schneitz, C.; Hofshagen, M. and Skjerve, E. (2001): Reduced incidence of *Clostridium perfringens* associated lesions and improved performance in broilers chickens treated with normal intestinal bacteria from adult fowl. *Avian disease.* 45: 149-156.
- Loddi, M.M.; Nakaghi L.S.O; Edens, F.; Tucci, F.M.; Hannas, M.I.; Moraes, V.M.B. and Ariki, J. (2002): Mannan-oligosaccharide and organic acids on intestinal morphology integrity of broilers evaluated by scanning electron microscopy. In: *Proc. 11th*
- European Poult. Sci. Conf., Bremen, Germany. Sept. 6-10. p. 121.
- Lovland, A.; Kaldhusdal, M.; Redhead, K.; Skjerve, E. and Liihaug, A. (2004): Maternal vaccination against subclinical necrotic enteritis in broilers. *Avian Pathol.* , 33(1): 83-92.
- Norris, J.R. and D.W. Ribbons (1971): *Methods in microbiology*, Vol.5A. Academic Press London and New York.
- Parish, W.E. (1961): Necrotic enteritis in the fowl. *J. Comp. Path.* 74:377- 393.
- Prukner, R.E, Milakovic, N.I.; Ivesa, P.S. and Grigis, N. (1995): *Clostridium chauvoei* in hens. *Avian Pathol.*, 24(1): 201-206.
- Rosen, G.D. (1980): Multifactorial models for antibacterials in broiler nutrition. Pages 302-309 in: *Proceedings European Poultry Conference. World's Poultry Science Association, Hamburg, Germany*
- Salama, S.S.; Afaf A. Khedr and Makharita M.A.M. (2008): Using Of polymerase chain Reaction (PCR) for differentiation of local field isolates of *Clostridium perfringens* from chickens. *SCVMJ*, XIII (2) 643-652.
- Savage, T.F., Cotter, P.F. and E.I. Zakrzewska. 1996. The effect of feeding a mannan-oligosaccharide on immunoglobulin plasma IgA and bile IgA of Wrolstad MW male turkeys. *Poultry Sci.* 75(Suppl. 1): 143.
- Van der Sluis, W. (2000): Clostridial enteritis is an often underestimated problem. *World Poultry* 16, No. 7:42-43.
- Van Immerseel, I., Buck, J., Pasmans, F., Huygheboert, G., Haesbrouck, F. and Ducatelle, R. (2004): *Clostridium perfringens* in poultry: an emerging threat for animal and public health. *Avian Pathol.*, 33(6): 537-549.

تأثير استخدام الزنك باستراسين والاوليجوسكاريد علي الدجاج المحصن بلقاح الالتهاب المعوي التنكزي للدجاج

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المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية العباسية. القاهرة

تم خلال هذا العمل استخدام لقاح التهاب التنكزي المعوي فى صورة تبادلية مع زنك باسيتراسين واوليجوسكاريد فى اربع مجموعات من كذاكيت (٣٠ طائر/مجموعة) تم تحصين الاولى بلقاح التهاب التنكزي المعوي و زنك باسيتراسين الثانية بلقاح التهاب التنكزي المعوي واوليجوسكاريد والثالثة بلقاح التهاب التنكزي المعوي فى حين تم ترك المجموعة الرابعة دون تحصين كضابط للتجربة. وقد اوضحت نتائج هذه التجربة أنه باستخدام أوليجوسكاريد يرفع من الكفاءة المناعية للقاح التهاب التنكزي المعوي.