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Effects of Probiotic Bacteria On Chicken Salmonellosis

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ABSTRACT

In the present study100 samples collected from diarrheic and apparently healthy birds revealed a total of 3 bacterial isolates serologically typed as S. Typhimurium. Two hundred day old Ross chicks were divided into 8 groups (25 birds/pen): Gp 1 which contains the control negative, those of Gp 2 were administered L.acidophilus only, Gp 3 contains chicks that were administered S.cerevisiae, Gp 4 contains chicks that were administered E.faecium. Gp 5 contains chicks that were administered L.acidophilus plus S.cerevisiae, Gp 6 contains chicks that were administered L.acidophilus plus E.faecium, Gp7 contains chicks that were administered L.acidophilus, E.faecium and S.cerevisiae. While was keeping Gp 8 which contains the positive control.Oral challenge was performed using 0.2 ml suspension of 10^6 CFU of S.Typhimurium from 19 hrs old nutrient broth culture on the third day of age. Results indicated that administration of L. acidophilus group plus E.faecium alone or with S.cerevisiae reduced the mortality rate to 8%.
Our study pointed out that the usage of probiotics is of value in controlling of Salmonellae in chickens also usage of probiotic mixture from 2 or 3 is better. The application of L. acidophilus and E. faecium orally with or without S. cerevisiae in the feed reduced the mortality rate and shedding (table2) of Salmonella in chickens so it can replace antibiotic therapy in control of avian salmonellosis.

Key words :(S. Typhimurium , S.cerevisiae , challenge, Probiotic).

INTRODUCTION

Salmonellae are among the major bacterial pathogens of poultry in the whole world and most Salmonella infection in humans result from the ingestion of contaminated poultry (Carli et al., 2001).There is no doubt that Salmonella species are among the most important causative agents which infect poultry populations and cause great losses and constituted hazard to public health (EL-Sayed, 1997).

Poultry producers are challenged to improve production while using fewer antibiotics due to increased restriction on antimicrobial usage due to internal regulations as in Europe, because of export market restrictions, and because of consumer or customer preferences in local markets. For these reasons, there is continued research on sustainable alternatives to antibiotic for animal production such as Probiotics or Direct feed microbials consisting of live or dead organisms and spores (Patterson and Burkholder, 2003).

Probiotics are "a mono-or defined mixed-culture of live microorganisms which, applied to animal or man, beneficially affect the host by improving the properties of the indigenous gastrointestinal microbiota, but restricted to products that (a) contain live microorganisms (e.g., as freeze-dried cells or in fresh or fermented product), (b) improve the health and well-being of animals or man (including growth promotion of animals), and (c) can have their effect on all host mucosal surfaces, including the mouth and gastrointestinal tract (e.g., applied in food, pill, or capsule form), the upper respiratory tract (e.g., applied as an aerosol), or in the urogenital tract (local application).(Havenaar and Huisin't Veld1992).

The use of probiotics in poultry has been investigated since Rantala and Nurmi (1973) who observed that exposure of young chicks to bacteria from the gut of mature birds conferred protection from infection. Selected beneficial bacteria such as lactic acid bacteria have been proposed as probiotics for the prevention of various enteric diseases and the improvement of overall health for many years (Tellez etal., 2006).

So the aims of the present study were detection the effects of different probiotics on avian

Salmonellosis as new methods for control of

MATERIALS AND METHODS

- **Sample**

A total of 100 samples were collected fromdiarrheic and apparently healthy chicks. These were 45 cloacal swabs, 20 drag swabs and 35 from different internal organs.

- **Isolation and identification of Salmonella**

Samples were placed in 10 ml ofbuffered peptone water (Oxoid, Basingstoke, UK) as pre-enrichment media, and incubated at 37 °C for 18 h. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1:10 in Rapport-Vasiliadis broth and incubated at 41.5 °C for 24 h. A loopful of broth was streaked on plates of XLD agar, MacConkey agar, and Salmonella-Shigella agar

Commercial probiotics:

Biosol®: produced by Biochem company, Germany. Based on containing stabilized probiotic strain Enterococcus faecium with a concentration of 10^{12} CFU / g and given with a dose of 200g / 5000 bird (as the manufacturer instructions) in the drinking water.

Megayeast®:

produced by View Trade company, Egypt.Based on containing stabilized probiotic strain S. cerevisiae with a concentration of 5000 viable cell / g and given with a dose of 1kg / ton feed (according to the manufacturer instructions) via mixing with the feed.

Prepared bacteria: L.acidophilus

- **Effects of Probiotic Bacteria On Chickens Salmonellosis**

Cloacal swabs

Ice box

It was used for transporting of the samples to the laboratory as soon as possible.They were divided into 8 groups (25 birds/pen) and treated as following: Gp 1 contains the control negative, Gp 2 was administered L.acidophilus,Gp 3 was administered S.cerevisiae, Gp 4 was given E.faeciumonly.Gp5wasadministeredL.acidophilus plus S.cerevisiae, Gp 6 was given L.acidophilus plus E.faecium, Gp7 contains given mixture ofL.acidophilus,E.faecium andS.cerevisiae. Gp 8 was kept as non treated to be positive control.Oral challenge was performedusing 0.2 aviansalmonellosis.

(Oxoid, Basingstoke, UK)according toSoomroet al., (2010).The temperature and the period of incubation were standardized at 37 °C for 24 h. The isolated colonies were identified on the basis of morphology, cultural characters and their biochemical profile according to Edwards and Ewing (1972). Colonies with biochemistry profile of Salmonella were submitted to serological tests by using polyvalent serum against O and H Salmonella antigens(Salmonella diagnostic antiserain Central Laboratories of Ministry of Health. The colonies that agglutinated during the period of 1 to 2 min were considered as positive for Salmonella, and were preserved in nutrient agar at 4 °C. **(Kauffmann and Das Kauffmann 2001)**

Two hundred, one-day-old, male Ross chicks were obtained from a commercial hatchery. They were salmonella free and transported in a closed vehicle and the temperature was kept at 35°C along the trip. They were vaccinated against Newcastle Disease (ND) and Infectious Bronchitis (IB) upon arrival. They were vaccinated against Infectious Bursal Disease (IBD) at day 14 and against ND and IB at day 17. The experiment was carried out for 24 days. The housing was layered with tibin as bedding and with a stocking density of 10 chicks/m^2. They were provided with a commercial starter broiler chicken ration. The ration contained crude protein not less than 21.64%, crude fat not less than 2.7%, crude fibers not more than 2.7% and metabolizing energy not less than 2950 Kcal/kg ration. No antibiotics were added to the ration. Feed and water were provided ad libitum in each pen.

Sterile cotton swabs containing normal saline were used for monitoring shedding of S.Typhymurium.

ml suspension of 10^6 CFU S.Typhimuriumfrom 19 hours old nutrient broth culture on the third day|(Rahimi et al., 2007) . Collection of samples for shedding of S.Typhimurium (Teresa et al., 1997) Ten chicks from each group were monitored and cloacal swabs were collected by gentle rotatory movement into the cloaca of the living chicks at the third day post infection, then every 3 days until the twenty one day post infection, and then transported in ice box as soon as possible to the Central Laboratory of Quality control on Poultry production.

RESULTS

Out of 100 samples collected from diarrheic and apparently healthy birds, a total of 3 bacterial isolates then serologically typed as S. Typhimurium.Was detected all challenged groups with 10^6 cells orally of challenging organism showed signs of dullness and had diarrhea between days two and seven. Allseen clinical symptoms disappeared after the7th day. During this experiment no clinical symptoms were of infection with S. Typhimurium were observed and the mortality rate for these chickens was not higher than that of the control positive group. At 3,7,15 and 21 days post infection stage10 chicks were selected from all groups. The control –vechickens were free of Salmonella throughout the experiment.The mortality rates of chickens supplemented with probiotics and challenged with S.Typhimurium are shown in table (1) and fig (1). Nine birds out of 25 died as the result of S.Typhimurium challenge (positive control) with mortality rate 36%. On the other hand, supplementation of S. cerevisiae alone reduced the number of dead birds to 4 with mortality rate 16% while administration of L. acidophilus group, E. faecium or S. cerevisiae plus L. acidophilus group reduced the number of dead birds to three with mortality rate 12%. Mean; while, as regards to administration of L. acidophilus group plus E. faecium alone or with S. cerevisiae reduced the number of dead birds to two with mortalityrate8%.

Table (1): Mortality rate in chicken groups given probiotics :

	Gp 1	Gp 2	Gp3	Gp4	Gp5	Gp 6	Gp 7	Gp8
Total No. of birds	25	25	25	25	25	25	25	25
No. of died	0	3	4	3	3	2	2	9
mortalities%	0	12	16	12	12	8	8	36

Table(2): Proportion of chickens shedding S. Typhimurium during the experimentation period (21):

Number of birds		10	10	10	10
Days post infection		3	7	15	21
Group(2)	Number of positive	3	2	0	0
	%	30	20	0	0
Group(3)	Number of positive	5	3	3	0
	%	50	30	30	0
Group(4)	Number of positive	2	1	0	0
	%	20	10	0	0
Group(5)	Number of positive	3	2	1	0
	%	30	20	10	0
Group(6)	Number of positive	3	2	2	0
	%	30	20	20	0
Group(7)	Number of positive	2	2	0	0
	%	20	20	0	0
Group(8)	Number of positive	7	5	8	4
	%	70	50	80	40

DISCUSSION

Poultry is one of the main reservoirs of Salmonella(Mohamed et al., 1999). Probiotic organisms, like those of the genera Lactobacillus, Pediococcus, Bifidobacterium, and others, consist of live microorganisms that exert a beneficial effect on the host by enhancing immune response, nutrient absorption, and control of pathogens (Bielkeet al., 2003).Introduction of such probiotics is believed to prevent or attenuate the growth of clinical enteric pathogens in poultry, resulting in enhanced growth and performance of the host bird. This phenomenon has prompted a widespread interest in the poultry industry of probiotic usage. It is considered an alternative to the prophylactic use of antibiotics for the prevention of disease within poultry flocks (Salminenaand Von Wright., 1998).

The present study revealed that out of 100 samples collected from diarrheic and apparently healthy birds revealed a total of 3 bacterial isolates serologically typed as S. Typhimurium. Various theories have been proposed as to the mechanisms by which probiotics protect their host against invading enteropathogens. Some of these include: competition for limiting nutrients, competition for attachment sites on the intestinal mucosa (Baleviet al., 2001) and production of short-chain volatile fatty acids (Humphrey et al., 1991).In the present study all birds which received orally $10^6$of S. Typhimurium were dull and had diarrhea between days two and seven. During this experiment clinical symptoms of Salmonellosis in poultry is worldwide problem both for poultry and as a vehicle for human disease (Sharp, 1991).

infection with S. Typhimurium were observed and the mortality rate for these chickens was lowerthan(8-16%) that of the positive control group (36%). At every sampling stage, chickens were selected from all groups. The control chickens were free of Salmonella throughout the experiment.anduseage of probiotics results indicated that administration of L. acidophilus group plus E.faeciumalone or with S.cerevisiaereduced the mortality rate and shedding of Salmonella .The administration of antimicrobial agents in chickenscreates selection pressure that favors the survival ofantibiotic resistant pathogens. Resistance of Salmonella to commonly used antimicrobials is increasing both in the Veterinary and public health sectors and has emerged as a global problem , this is lead to search for new way for control of avian salmonellosis as probiotics (Mollaet al. ,2003).

CONCLUSION

Our study pointed out that the usage of probiotics is of value in controlling of Salmonellae in chickens also usage of probiotic mixture from 2 or 3 is better.

The application of L. acidophilus and E. faecium orally with or without S. cerevisiae in the feed reduced the mortality rate and shedding of Salmonella in chickens so it can replace antibiotic therapy in control of avian salmonellosis.

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الملخص العربي

تم فحص 100 عينة تم جمعها من طيور سليمة ظاهرياً وأخرى مريضة و تم عزل ثلاث عترات بكتيرية من ميكروب السالمونيلا. وبالفحص السيرولوجي وجد أنها تتبع ميكروب السالمونيلا تيفيميوريم. تم عمل هذه الدراسة على 200 كتكوت روص عمر يوم والتي قسمت الى 8 مجموعات كل مجموعة تحتوي على 25 كتكوتا: المجموعة الأولى: لم تتعرض لأية معاملات. المجموعة الثانية: تم إعطاؤها مجموعة اللاكتوباسيليس أسيدوفيليس. المجموعة الثالثة: تم إعطاؤها سكارومايسيس سيرفيسي. المجموعة الرابعة: تم إعطاؤها إنتيروكوكس فايشيم. المجموعة الخامسة: تم إعطاؤها مجموعة اللاكتوباسيليس أسيدوفيليس و سكارومايسيس سيرفيسي. المجموعة السادسة: تم إعطاؤها مجموعة اللاكتوباسيليس أسيدوفيليس و إنتيروكوكس فايشيم. المجموعة السابعة: تم إعطاؤها مجموعة اللاكتوباسيليس أسيدوفيليس و إنتيروكوكس فايشيم و سكارومايسيس سيرفيسي. المجموعة الثامنة: تم إعطاؤها ميكروب السالمونيلا تيفيميوريم فقط .تم عمل عدوى للكتاكيت عند اليوم الثالث ب 0.2 مل من محلول يحتوي على 10^6 من ميكروب السالمونيلا تيفيميوريم. وبقياس معدل النفوق وجد أن معاملة الكتاكيت بمجموعة اللاكتوباسيليس أسيدوفيليس و إنتيروكوكس فايشيم وحدهما أومع سكارومايسيس سيرفيسي قد قللت نسبة النفوق الي 8%.

الكلمات الدالة (سالمونيلاتيفيميوريم - سكارومايسيس سيرفيسي– التحدي – البروبيوتك)