Advances in Environmental Biology, 5(7): 1857-1860, 2011
ISSN 1995-0756

ORIGINAL ARTICLE

Comparison of Different Methods of Probiotic Prescription against *Salmonella* Infection in Hatchery Broiler Chickens

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Navid Hosseini-Mansoub, Tohid Vahdatpour, Mohammad Arjomandi and Sina Vahdatpour: Comparison of Different Methods of Probiotic Prescription against *Salmonella* Infection in Hatchery Broiler Chickens

ABSTRACT

An experiment was conducted to evaluate the effects of various methods of probiotic administration in hatchery on prevention of *Salmonella Enteritidis* (SE) in broiler chickens. A total of 150 one-day-old chicks (Ross 308) were assigned to five experimental groups (30 birds per group) including control and four treatment with different hatchery probiotic administration methods comprised of *in ovo* injection, oral *gavage*, spray and *vent lip* application. All chickens were challenged by 8 Log CFU SE using oral *gavage* one day after administration of probiotics. At 1 and 7 days post-challenge (PC) 15 birds per experimental group were sampled for SE recovery. Administration of probiotics reduced the number of colonized chicks, compared with control group. Decline was non significant 1 day after PC ($P>0.05$), but after PC in day 7 was significant ($P<0.05$). The most number of infected chicks was observed in control group, and the lowest was observed in *vent lip* method.

Key words: probiotic, *Salmonella*, broiler chicken, *vent lip* application, oral *gavage*, spray

Introduction

Various kinds of Antibiotics have been used in poultry industry in order to treat of infectious diseases [1]. In some countries, the usage of antibiotics have been forbidden because of some problems caused by lavish usage of antibiotics e.g., bacterial resistance [2].

Probiotics prescription is a good alternative for antibiotics. Probiotics are microbial supplements which can prevent host body from infection by several ways: microbial balance of intestine, synthesis of B group vitamins, immune system stimulating, competition with other microorganisms, digestive enzymes producing and increasing the level of low density lipoproteins (LDL) [2,3,4,5]. There are a few ways to prescription of probiotics. The most common way is adding probiotics to food and drinking water, spraying and oral *gavage* are other ways. Edens *et al.* [6] reported that perishing percentage decreased and body weight optimized by *in ovo* injection in *Lactobacillus* infected hens. *Vent lip* application prevents microbial entrance from posterior digestive system [7]. Corrier *et al.* [7] used *vent lip* method for first time and reported *Salmonella* colonies in cecum were decreased by this method.

Because this infection is highly contagious, it can be transmitted directly and can decrease body weight and the production rate also it is infectious and can caused elevated mortality to 90 percent [8]. Other studies have reported increased body weights in poultry fed with *Lactobacillus* supplemented diets in both the starter and grower periods [9,10,11].

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In order to find the best method of prescription of probiotics, all ways of probiotic administration in hatchery need to study and compare. Therefore, present study investigated effects of various methods of probiotic prescription to prevention of *Salmonella Enteritidis* (SE) infection in broiler hens.

Materials and methods

A total of 150 one-day-old broiler chickens (Ross 308) were divided in 5 groups, each group inclusions 30 chicks. Business protexin were used as probiotics (protexin probiotics). This product contains 7 species of intestinal bacteria and 2 species of fungi. First group were used as control group and not recieved probiotics. Second group used for *in ovo* injection. Eggs were chosen from 18-day embryonated eggs. 100 µl of probiotics solution at rate of 7×10^7 CFU were injected to air space of embryonated eggs by acetic-alcohol swap. Injection points have been coated by paraffin oil.

Third group was inoculated with 25 µl of probiotics solution at rate of 2.8×10^8 CFU by *vent lip* application. 7×10^7 CFU/100 µl was inoculated to crop for forth group and the spray method was used for group 5. Each 1-day chicken has gotten 7×10^7 CFU/ 250 µl probiotic by spraying in chick's boxes (90×60×40cm).

After one day, chickens were challenged by 10^8 CFU *Salmonella Enteritidis* (RITCC 1695) which they have been received by oral *gavage*. One day and 7 days after bacterial inoculation, 15 chickens were used for sampling of SE for each of one and 7 days. Samples have been obtained from 1 *gr* of cecum in 9 *ml* peptone water medium. After one day incubation, 0.1 *ml* of medium has been added to 10 *ml* rappaport-vassiliadis and incubated for one day in order to selective differentiation of SE. XLD medium were used for final culture. SE have been determined by black color of colonies and for confirmation, TSI test was used.

All data from the presented study were analyzed by SAS [12] software by K square and also SPSS package (18^{th} version, Tukey HSD & Bonferroni).

Results and discussion

The results of current study are showed in Table 1 and Figure 1. According to analyzed data, there is no significant difference among groups in one day after probiotics prescription, but the most infected chicks was detected in the control group and the lowest value observed when *vent lip* application was used.

There were significant difference between control group and all of the other experimental groups, 7 days after challenge by SE. There was significant difference between results of 1 day and 7 days after challenge in each group. Significant difference between *in-ovo* injection and *vent lip* application was detected.

Discussion:

In the control of Salmonella infection in poultry there is a need to prevent intestinal colonization of the organism in chicken.

Mead [13] reported that 4 ways of competition can be theorized between probiotics and intestinal pathogens: competition for specific places in intestine, producing of baceriocin and volatile fatty acids which have negative effects on some of pathogens like *Salmonella* and competition of nutrients. Digestive system is completely sterile in hatch time but microflora can be placed during time [13].

Hassanzadeh *et al*. [14] in the study related to *in ovo* injection, immune complex vaccine of infectious bursal disease (IBD) were applied *in ovo* to embryonated eggs and subcutaneously to newly-hatched chickens in the hatchery, while the other group of chickens received a conventional IBD vaccine at days 12, 17 and 22 of age. They reported that immune complex vaccine similar to that of conventional vaccine is able to provoke active immunity of birds and seem to protect chickens sufficiently from the IBD. Therefore immune complex vaccine applied either *in ovo* or subcutaneously is able to induce humoral antibody and IBD protection.

The current vaccination programs failed to protect chicks sufficiently. Vaccination failures were mainly due to the inability of the intermediate vaccines to protect the birds before they became susceptible to challenge with virulent field virus. However, when progency are vaccinated at an early age with a mild or highly attenuated live vaccine, high levels of maternal antibody may interfere with the development of active immunity [15]. Because of maternal antibody interference associated with lack of uniform antibody titers in progency, repeated vaccinations are needed until maternal antibody wanes [16].

The best results in the presented study have been obtained when chickens got probiotics directly from digestive duct (*vent lip* administration and oral *gavage*). The least infected chickens were in *vent lip* application group; in this method, microorganisms get directly to digestive duct and there is no need for passing through upper parts of digestive system (crop, gizzard and pre gastric).

Higgins [17] compared *vent lip* application with drinking water method and reported that *vent lip* application were more efficient even when lower dose of probiotics were prescribed than drinking water method.

Table 1: Proportion of infected chickens to total chickens in various kinds of probiotics prescription

Perscription methods	1-day after challenge	7-days after challenge
Control	13/15	12/14
In ovo injection	8/15	7/15
Oral *gavage*	9/15	2/15
Spray	8/15	3/15
Vent lip method	6/15	1/15

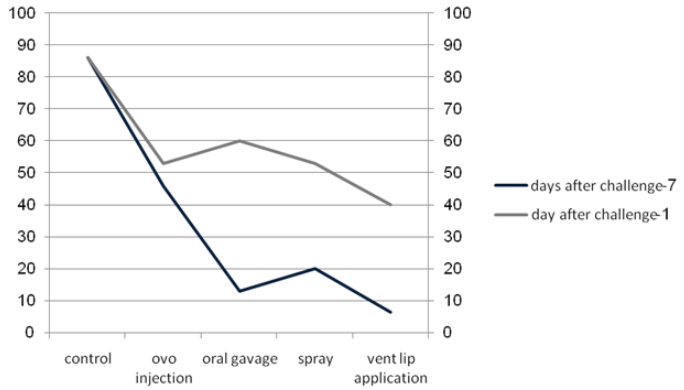

Fig. 1: Percentage of infected chicks in various kinds of probiotics prescription

Microorganisms should pass through upper parts of digestive system and tolerate low PH and digestive enzymes in oral *gavage* method, so there is less chance for surviving [18,19]. *In ovo* injection had not good efficiency even though chickens got probiotics sooner than other groups. In this method most of chickens do not get the whole dose of microorganisms.

Edens *et al.* [6] compared oral *gavage* method with *in ovo* injection and reported that the oral *gavage* had more effect on prevention of *Salmonella* growth.

In general, there is significant difference when we used probiotics and compared findings with control group results. *Vent lip* application is the most efficient method but it is not economical in high number of chickens by present method of administration. More studies should be done in order to find new techniques for administrating of this useful method.

However, spray method is not efficient as *vent lip* application and oral *gavage* but there were no significant difference between them. From the results of the present study, it is concluded that spray method is an easy and economical way for prescription of probiotics. Nowadays this method can be more efficacious.

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