

The effect of Aquablend Avian probiotic ® including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* on systemic antibody response against Newcastle and Influenza disease vaccine in broiler chickens

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Abstract

Background: Finding alternatives to antibiotics for poultry production is very important because there are increasing concerns about antibiotic resistance. So, researchers have been directed to the research back to natural antimicrobial products. Some researchers stated that probiotics can stimulate the immune system and play an important role in shaping the immune system.

Objectives: The aim of this study was to examine the effect of a commercial probiotic mixture (Aquablend Avian®) supplementation to the drinking water of broiler chickens on the immune response against Newcastle and influenza diseases vaccines.

Materials and Methods: In this study, 180 one-day-old broiler chickens were purchased and divided randomly into 3 groups (n = 60 for each group). Chickens in groups A and B received 300 mg of the probiotic in drinking water for first 3 days and first 7 days, respectively. Chickens in group C were kept as a control group and did not receive probiotic. All groups were vaccinated with live Newcastle vaccine (B1 strain) intraocularly on 8th day, and AI-ND killed vaccine (subtype H9N2) subcutaneously at the back of the neck on 8th day. Two mL of blood samples were collected before vaccination as well as on days 14, 28 and 35 postimmunization. Ten chickens of each group were bled randomly and an antibody titer against Newcastle disease vaccine and AI-ND killed vaccine (subtype H9N2) was determined by the hemagglutination-inhibition test.

Results: The results of the present study showed that oral administration of the probiotic for 7 days significantly increased the specific antibody response to Newcastle vaccine compared to the control group (0.75 - 1.6 log, based on log₂), while the probiotic administration had no significant effect on antibody productions against avian influenza vaccine as compared to the control group.

Conclusions: Oral administration of Aquablend Avian® probiotic strains including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* for 7 days can enhance the systemic antibody response to Newcastle vaccine in broiler chickens.

Keywords: Avian Influenza Virus, Probiotic, Immune Response, Newcastle Disease Virus, Broiler Chicken

1. Background

The successful poultry production is based on feeding, breeding, marketing, management and the well-developed immune status of the birds. Immunity means the power of resistance, against the pathogenic microorganisms. Such power of defense has two main sources, natural and induced. Schat and Myers (1991) stated that the natural immunity in the birds is concentrated in egg yolk, which directly discharges its antibodies into the intestine and assures protection against infections in the young bird. Efforts have been made to compensate the natural immune status of the broilers through the artificial means, like vaccinating or feeding antibiotics (1). Nowadays, using antibiotics at subtherapeutic levels has caused concerns about antibiotic residues in the animal productions which lead to the development of drug-resistant bac-

teria in animals and human. Thus, at the beginning of 2006, in the European Union, medical and public concerns focused on the complete omitting of the antibiotics from animal feed (2-4). Therefore, the substances that could replace antibiotic growth promoters (AGP) in the feed are important in poultry industry (5). Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut (6).

Probiotics are live microorganisms which, when used in adequate amounts, improve the intestinal microbial balance (7). The mode of action of probiotics in poultry includes: (i) maintaining normal micro flora of intestine; (ii) decreasing bacterial enzyme activity and ammonia production and increasing digestive enzyme activity; (iii) improving digestion and feed intake; and (iv) stimulating the immune system and neutralizing enterotox-

ins. Lactobacilli used as probiotics are nonpathogenic Gram-positive bacteria that live in animal intestine (8). In the chicken, as well as the ability to limit food-borne pathogens and to improve production parameters, administration of various members of the *Lactobacillus* species could stimulate multiple aspects of the immune response. These activities include improving systemic antibody response, increasing the number of intestinal epithelial lymphocytes (IELs) expressing CD3, CD4, CD8, and T cell receptor (TCR), modulation of chicken chemokine and cytokine gene expression, and improving the function of T cells (8). Despite the interest in the administration of probiotics in commercial poultry production, to date there is little information about the mechanisms of stimulation of chicken immune response by probiotic bacteria (8).

Due to the beneficial effects of probiotics, the present study was conducted to study the effect of Aquablend Avian probiotic strains including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* on the immune response of commercial broiler chicks using the influenza and Newcastle disease (ND) titer as an indicator.

2. Objectives

The aim of this study was to examine the effect of a commercial probiotic (Aquablend Avian®) supplementation to the drinking water of broiler chicks on the immune response against Newcastle and influenza diseases vaccines.

3. Materials and Methods

3.1. Chickens and Housing

One hundred and eighty one-day-old (mean of weight was about 50 g) broiler chickens (Ross 308) were purchased from Sahraye Jonob's Broiler Breeder Company. These newly hatched commercial broiler chicks were obtained from Pirmoradi Co. (Khozestan, Iran). The birds were maintained in floor pens. The chicks were provided with free access to water and broiler starter rations.

3.2. Vaccine

Hitchner B1 vaccine was bought from Razi vaccine and serum research institute.

AI-ND killed vaccine (subtype H9N2) was bought from Razi vaccine and serum research institute.

3.3. Commercial Probiotic Supplements

An Aquablend Avian® probiotic was bought from USA AGRANCO Co. This probiotic had different strains including *Lactobacillus*, *Streptococcus* and *Bifidobacterium*.

3.4. Experimental Design

The chickens were divided into three equal groups (A, B and C, n = 60 for each group). Chickens in the group A and B received 300 mg of the probiotic in drinking water for first 3 days and first 7 days, respectively. Chickens in the group C were kept as a control group and did not receive probiotic. All groups were vaccinated with live Newcastle (B1 strain) by ocular route on 8th days and AI-ND killed vaccine (subtype H9N2) subcutaneously in the dorsal of the neck on 8th day (Table 1).

Table 1. Experimental Design of This Study

Group	Vaccination	Probiotic
A	+	300 mg / 60 birds for 3 days
B	+	300 mg / 60 birds for 7 days
C	+	-

3.5. Blood Collection and Serological Tests

On the day of immunization, blood samples were collected as well as on days 14, 28 and 35 postimmunization. Ten chickens of each group were bled randomly, and sera were collected and submitted for serologic tests to assess antibody levels. Blood samples were drained from the brachial vein and sera were separated, identified and frozen at -20°C until the serological tests were performed. Serum samples were analyzed by the hemagglutination inhibition (HI) test to detect antibodies against AIV and NDV according to Alexander et al. (9).

3.6. Microplate Hemagglutination Inhibition Assay

To determine the antibody level of the sera samples collected from the chicks of different groups, beta procedure of the micro-plate HI test was done in U-bottomed 96-well microtiter plates with 1% chicken erythrocytes. The test was performed using 4HA unit AIV and ND virus.

3.7. Statistical Analysis

The titers obtained by HI were submitted to analysis of variance using the SPSS software version 18.0. To determine the significant differences in HI titers of each group after vaccination, one-way ANOVA and LSD test were used. The mean values were compared at a significance level of 5%.

4. Results

The results of this study, as shown in Table 2, indicated that probiotic had a significant effect on antibody response against Newcastle disease vaccines as compared to the control groups ($P < 0.05$). Fourteen days postvaccination, treatment B had higher antibody titers against Newcastle disease vaccines ($P < 0.05$) and there was a significant difference between the group B and C. Twenty-eight days postvaccination, treatment B had higher antibody titers against Newcastle disease vaccines ($P < 0.05$) and there was a significant difference between the group B and A, as well as between the group B and C.

Thirty-five days post vaccination, treatment B had higher antibody titers against Newcastle disease vaccines ($P < 0.05$) and there was a significant difference between the group B and C. This comparison showed that group B exhibited the highest mean HI antibody titer against ND compared to the groups A and C. The results of the present study showed that oral administration this probiotic for 7 days significantly increased the specific antibody response to Newcastle vaccine compared to the control group.

The results of this study, as shown in Table 3, indicated that probiotic had no significant effect on antibody productions against avian influenza vaccine as compared to the control groups and also the probiotic administration did not affect specific antibody synthesis to AI vaccine. In fact, the best antibody response among the groups following vaccination was in the group 2; however, there was no significant difference among the groups.

The results of the present study showed that receiving of the probiotic for 7 days significantly increased the specific antibody response to Newcastle vaccine compared to the control group (0.75 - 1.6log, based on \log_2), while the probiotic administration had no significant effect on antibody productions against avian influenza vaccine as compared to the control groups.

5. Discussion

The results of the present study showed that receiving of Aquablend Avian[®] probiotic strains including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* for 7 days significantly increased the specific antibody response to Newcastle vaccine compared to the control group. Previous work with chickens has indicated that probiotics improve the systemic antibody response against soluble antigens, such as SRBC and trinitrophenyl (TNP)-keyhole limpet hemocyanin (KLH) and KLH alone, which are classified as thymus-dependent immunogens (10, 11). Koenen et al. showed that administration of fermented liquid feed supplemented with various lactobacilli in chickens enhanced

IgM and IgG responses to TNP (11). In another study, the using of probiotics containing *Lactobacillus casei* and *Lactobacillus acidophilus* improved the serum IgA response to KLH, while the treatment did not affect the IgG response to this antigen (10). Hajati et al. reported that MOS has the capacity to bind pathogenic organisms such as Salmonella and Escherichia coli, and can stimulate the immunity system (12). Cross showed that some probiotics could stimulate a protective immune response against microbial pathogens in animals (13). Rhee et al. showed that using some commensal gut microflora as probiotic can increase the antibody-mediated immune response (14). Haghighi et al. (2005) indicated that birds that received probiotic had significantly more serum antibody than the birds that did not receive probiotics (15). The results of the present study showed that receiving of this probiotic had no significant effect on antibody productions against avian influenza vaccine as compared to the control groups. In fact, some reports showed that combining probiotics with immunization may not increase specific antibodies and could even result in the decrease of the antibody response in the gut contents in or serum (16-18). May be some parameters are involved in determining the efficacy of probiotics in the stimulation of the immune response; so, the immunostimulating effects of probiotics may not be generalized. For example, Huang and coworkers indicated that probiotics containing *Lactobacillus casei* and *Lactobacillus acidophilus* improved the serum IgA response against KLH, but that the treatment did not affect the IgG response against this antigen (10). Some studies showed that the genetic background of birds plays an important role in the mediation of immunostimulatory activities of probiotics, for example broiler chickens and egg layer treated with probiotics responded differently against TNP, and layer chickens had a significantly higher antibody response than broiler chickens (11). So, the immunostimulatory activities of probiotics in improving the antibody response are highly dependent on the genetic background of the host, antigen, immunization regimen, type and number of species of bacteria present in probiotics.

Abd El-Samee et al. (2012) reported that supplementing diets of growing Japanese quails reared during summer in Egypt with 20 or 40mg Bioplex zinc/kg alone or in combination with 1.0g/kg prebiotic (mannan oligosaccharides) had no significant effect on the productive performance, but improved their immune response (19). Sadeghi et al. (2013) reported that prebiotic supplementation improved the immune responses and health of the chicks infected with pathogens (20).

Table 2. The Effect of Probiotic on Hemagglutination Inhibition Antibody Titer Against Newcastle Disease Virus in Broiler Chicks^a

Days Post Vaccination Groups	0	14	28	35
A	6.2 ± 0.44 [*]	4.25 ± 0.86	4.9 ± 0.31 ^B	5.6 ± 0.36
B	6.2 ± 0.83	4.75 ± 0.73 ^{*C}	6 ± 0.86 ^{AC}	6.1 ± 0.65 ^C
C	5.7 ± 0.89	4 ± 0.44 ^B	4.4 ± 0.64 ^B	5.1 ± 0.85 ^B

^aThe data in this study are expressed as Mean ± SD. *C, A significant difference with group C.

Table 3. The Effect of Probiotic on Hemagglutination Inhibition Antibody Titer Against Avian Influenza Disease Virus^a

Days Post Vaccination Groups	0	14	28	35
A	6 ± 0.58	2.54 ± 0.52	3.5 ± 0.99	3.01 ± 0.2
B	6 ± 0.23	2.63 ± 0.5	3.6 ± 0.9	3.33 ± 0.53
C	6 ± 0.22	2.36 ± 0.5	3.3 ± 1.16	2.77 ± 0.42

^aThe data in this study are expressed as Mean ± SD.

5.1. Conclusions

The results of the present study show that administration of this probiotic for 7 days can significantly increase the specific antibody response to Newcastle vaccine compared to the control group. However, the probiotic administration had no significant effect on antibody productions against avian influenza vaccine as compared to the control groups. This comparison shows that group B exhibited the highest mean HI antibody titer against ND compare to the groups A and C.

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