Journal of Zoonotic Diseases
2023, 7 (1): 237-243
doi: 10.22034/jzd.2023.15971
https://jzd.tabrizu.ac.ir/article_15971.html

Original Article

Effect of probiotic *Pediococcus Lolii* bacteria on antibody level of laying out specific pathogen-free chickens

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(Received 8 January 2023, Accepted 14 February 2023)

Abstract
In this study, the effect of probiotic supplementation of *Pediococcus Lolii* bacterial strain NGRI 0510Q (T) on the immune response to Newcastle disease virus and avian influenza virus vaccines was investigated. Sixty-four chicks from specific pathogen-free (SPF) eggs were divided into four groups. Indeed, groups 1, 2, 3, and 4 were treated with normal ration without probiotic; normal ration with probiotic as 1 g/L of water; normal ration without probiotic with 0.2 mL dual oil vaccine during the breeding period, and normal ration with probiotic with 0.2 mL dual oil vaccine during the breeding period, respectively. Blood sampling were performed eight times weekly (3^{rd} to 10^{th} weeks) and obtained serum samples were evaluated by HI test to determine the antibody levels. Seventh post-vaccination was significant at $p < 0.05$, whereas this effect was not significant for the H9N2 strain. In the present study, the effect of *Lactobacilli* probiotic consumption on the humoral immune system of laying hens was investigated. Results showed positive effects of this probiotic on its immunogenicity along with vaccination with injectable oil vaccines against Newcastle disease strain V4 and influenza strain H9N2.
Keywords: Probiotic; *Pediococcus Lolii*; Antibody level; Newcastle disease; Influenza; Vaccine

Introduction

The use of probiotic products has spread rapidly over recent years, improving the performance of meat chicks, laying eggs, and producing free products from any remains. Recent evidence suggests that the use of microbial probiotics can play an important role in the future of the poultry industry. Indeed, probiotics are organisms that provide benefits to host animals by improving the microbial balance of digestion (Fuller, 1992). Previous studies have shown that eating with probiotics may improve growth performance (Apata, 2008), improve nutrient uptake (Apata, 2008), improve immune response and increase mucus function (Mountzouris et al., 2009), increase the immune response against antigens (Patterson and Burkholder, 2003), production of digestive enzymes (Saarela et al., 2000); improvement of non - digestible nutrients, lactic

acid production and volatile fatty acids and competition with other enzymes to bind bacteria to the digestive system. The beneficial effects of probiotics in improper health conditions and during the occurrence of stress are sensible.

For the evaluation of the poultry safety system, several criteria are used, the foremost of which can be called immunoglobulin and the weight of the lymph nodes (Yang et al., 2012). Therefore, it can be concluded that the addition of probiotics affects the safety of the chicks to the effect that these effects are observable in increasing the antibodies of the observable antibodies. The measurement of the antibody levels against the antigen SRBC and the Newcastle vaccine is the evaluation criteria of the safety system. The headline shown in feed - fed chicks is higher than in the control group (Khaksefidi and Ghoorchi, 2006).

The positive effects of prebiotics and probiotics on the poultry safety system have already been reported by different researchers (Ricke, 2018, Yang et al., 2012, Shehata et al., 2022). Recently, Yang et al. (2012) reported that in fed chicks with probiotic *Clostridium botulinum*, the concentrations of IgA and IgM were higher compared to the control group. Some bacteria in the probiotics, especially *Lactobacilli*, can improve the functioning of the immune system and macrophage activity (Yang et al., 2009). Alavi et al. (2012) studied the effects of prebiotics and probiotics seen on meat chicks, that the use of these additives caused significant safety improvement. Roller et al. (2004) reported an improved immune system performance by employing inulin and lactobacilli. In the present study, the effect of probiotic supplementation of *Pediococcus Lolii* bacterial strain NGRI 0510Q (T) on the immune response to both Newcastle and avian influenza vaccines was investigated.

Materials and methods

This research was carried out at the research and development unit of Razi Vaccine and Serum Research Institute, Marand, which is located in the northwest Iran, in collaboration with the quality control unit. In this study, lactic acid bacteria *Pediococcus lolii* (strain NGRI 0510Q (T)) was used as a probiotic. The main objectives of this study are: A. Investigating the effect of probiotics (*Paducococcus Lolii*) on the response of the humoral immune system created against the Newcastle disease V4 vaccine. B. Evaluation of the effect of probiotics (*Pediococcus Lolii*) on the humoral immune response induced against the H9N2 influenza vaccine.

Molecular identification of the bacteria

Isolation and purification of bacteria were performed in the Biotechnology Laboratory of Tabriz Agricultural Research Training Center. For DNA extraction, 20 mL of tissue buffer was poured in epindroph, and some bacterial colony was then centrifuged for 10 min at 95 ° C, then added up to 180 µL water and maintained in the fridge till next use.

Experimental design for the chickens breeding period

This study was done on 64 SPF-laying chickens with respective steps of preparation of chickens breeding hall, hatching, vaccination, etc., in standard conditions. Chickens were randomly categorized into four groups (n=16). Table 1 shows rations for each group prepared according to company recommendations.

Table 1. Experimental groups in the presented study.

Group	Ration type
1	Regular ration without probiotic
2 (Positive control)	Ration with probiotic as 1 g/L of water
3	Regular ration without probiotic with 0.2 mL dual oil vaccine during the breeding period
4	Ration with probiotic with 0.2 mL dual oil vaccine during the breeding period

Chickens of groups 3 and 4 were vaccinated with injection on the skin of the back of the neck at the age of 21 days using the oil vaccine of Newcastle and influenza produced by the Marand branch of the Razi organization. Blood sampling was done on days 21, 28, 35, 42, 49, 56, 63, and 70 from the start of the breeding period.

Hemagglutination inhibition (HI) and statistical analyses

Test tubes containing blood samples were placed on an inclined plane for 2 hours to separate their serum. Antibody titers against Newcastle and influenza viruses was measured by HA and HI test according to Sun et al. (2005). A one-way ANOVA was used for statistical analysis of the data using SPSS software (version 22, Inc., Chicago, IL, USA) and a $P < 0.05$ was considered significant.

Table 2- Serum antibody titer (mean ± SD) of chickens against Newcastle disease virus by HI test at different times of sampling.

Groups	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	5.9 ± 2.8	6.6 ±2.3	6.8 ± 2.2	6.9 ± 2.2	7.1 ±1.4	6.7 ±1.4	6.1 ± 1.5	6.0 ±2.3
4	7.3 ±1.4	8.1 ± 1.6	8.5 ± 0.7	8.5 ± 1.6	8.1 ± 2.1	8.2 ± 1.5	8.1 ±1.6	8.1 ± 1.6
p-value	*0.01	*0.04	0.003*	0.38	0.29	0.76	0.63	0.36

*p<0.05 was considered significant.

Results

Results of micro titration of blood samples within 3rd to 10th week after vaccination against influenza and Newcastle diseases by HT test are presented as mean ± SD and showed in Table 2 and Table 3, and Figures 1 and 2 (supplementary data), respectively. Increased antibody titers against the Newcastle disease virus in the third to fifth weeks after injection into vaccinated probiotic chickens have shown positive effects on the activity of the humoral immune system of the chickens being tested, as well as high levels of serum immunoglobulins after weeks. The next step is to stabilize the immune system in these chicks if the unvaccinated probiotic birds are vaccinated after the fifth week onwards, with a decrease in serum antibody titers. Regarding the antibody titer against the H9N2 strain of influenza virus, the results showed that the probiotic has not been able to have a significant effect on the antibody titer against the virus, but in the third to fifth weeks, it has a slightly higher ability to stimulate the immune system than other groups.

Table 3- Serum antibody titer (mean ± SD) of chickens against Influenza disease virus by HI test at different times of sampling.

Groups	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	6.4±1.0	6.5 ±0.7	7.1 ± 1.0	6.9 ± 0.9	6.2 ±1.4	6.4 ±0.6	5.8 ± 0.6	6.0 ±0.8
4	7.0 ±0.7	6.8 ± 0.7	7.9 ± 0.6	7.3 ± 0.8	6.5 ± 1.0	6.4 ± 1.0	6.0 ±0.8	6.2 ± 0.9
p-value	0.12	0.35	0.069	0.59	0.06	0.85	0.87	0.54

*p<0.05 was considered significant.

Discussion

The overall high grade of the antibody can be attributed to the high age of laying hens. At the herd level, healthier animals show a more limited response, making the results appear less pronounced at the herd level and seem ineffective.

Therefore, it is difficult to determine the usefulness of using probiotics in poultry farms that produce life under normal conditions. The results of working with probiotics are sometimes very varied due to the fact that the methods and conditions under which probiotics are processed are not the same, and sometimes even probiotic microorganisms may be inactive. The negative results reported so far can also be attributed to the poor viability of probiotic microorganisms. Other factors that contribute to the diversity of the results include the period (phase) of animal growth, the type of probiotic used, and the health conditions of the animal farm. Given the variations in the results of experiments with probiotics, it would not be surprising that the use of probiotics does not always produce the desired results. However, it should be noted that significant results from probiotic experiments have shown that probiotics can be used as an effective oral supplement if used correctly with probiotics in combination with management conditions and proper feeding methods.

The positive effects of probiotics on the performance of meat - fed chickens with food containing lower levels of nutrient intake are reported by Angel et al. (2005). The researchers found that reducing the amount of nutrients (protein, lysine, calcium, calcium, non - phytate) would have a negative effect on performance, but adding by maintaining and improving the efficiency of using nutrients helps the meat chicks to overcome the lack of nutrients. The reported use of probiotics is associated with the creation of some changes in the body, the immune system, food consumption, probiotic nutrient uptake, morphology, and reduction of pathogenic factors (Sun et al., 2005, Chichlowski et al., 2007). The beneficial effects of probiotics may be related to changes caused in intestinal microorganisms and body metabolism (Angel et al., 2005). Previous research has shown that the use of probiotics on meat chicks will affect the function, the ability to digest the nutrients, and the composition of the blind bowel.

As mentioned before, the use of probiotics has been associated with different results in feeding meat chicks. The instability observed in the results of the research can depend on different reasons, and many factors can affect the response of the chicks to these additives. The environment, the way of managing, nutrition, type and amount of additive, and bird characteristics (age, species, production stage and use), as an example, through water or food, can affect the response of meat chicks to additives (Yang et al., 2009).

The typical processes in raising new chicks, like the sudden change of rations, rations, transportation, operations carried out at the incubation site, and the high fertility of the bird at the time of breeding cause stress (Spreeuwenberg et al., 2001, St-Pierre et al., 2003, Humphrey, 2006). This can undermine the safety of the immune system, thus providing the ground for pathogenic bacteria that can be propagated within the digestive tract and harm host health as well as food products (Gaggia et al., 2010, Vilà et al., 2010).

Zulkifli et al. (2000) reported that adding probiotics to the diet of broilers under heat stress significantly increased the antibody titer. The findings of Huang et al. (2005) showed that feeding broilers with fructooligosaccharides could improve the integrity of the intestinal mucosal layer and thus be effective in improving the immune response. Kabir et al. (2004), in another experiment, by adding probiotics containing several strains of bacteria to the drinking water of chickens showed that antibody production against SRBC increased significantly compared to the control group, as well as the weight of stock and spleen increased significantly. Koenen et al. (2004) reported that feeding broiler chickens with the probiotic *Lactobacillus casei* increased the alienation activity of intestinal cells. *Lactobacillus*-based probiotics have been reported to improve the function of the immune system against certain river pathogens by activating and increasing cell-mediated immunity (Dalloul et al., 2003). The use of probiotics increases macrophage activity and enhances immune function (Panda et al., 2005).

For example, in a recent study, Kim et al. (2011) showed that adding prebiotics to the diet of broilers did not affect plasma immunoglobulin, immunoglobulin A, and G concentrations. The findings of Houshmand et al. (2011) also showed that the addition of prebiotics did not have a significant effect on immunity.

The exact mechanisms of action of probiotics to increase the function of the immune system remain largely unknown. Experimental results have shown that the effect of probiotics on improving poultry immunity has not been the same (Yang et al., 2009). Probiotics are associated with the activation of innate immunity by acting on foreign cells (Higgins et al., 2007). Nutrition with probiotics has been reported to increase the proliferative and functional activity of antibody-producing B cells (Panda et al., 2008). Special regulatory effects of probiotics on the immune system to strains or bacterial species depend on the type of the probiotics (Hooge, 2004). In addition, Zulkifli et al. (2000) showed that the effect of probiotics on antibody titers could be affected by the age and direction of broilers. Murry et al. (2006) reported that the digestive tract of birds receiving lactobacilli-containing probiotic supplements had higher lactobacilli than the control group. Mountzouris et al. (2010) reported that the use of probiotics had a significant effect on the natural flora of the appendix, increasing the population of lactobacilli and bifidobacteria.

Ng et al. (2009) showed in their study that probiotics might stimulate the natural flora of the intestine in various ways, including mucosal cellular immune responses, facilitate antibody production, improve the integrity of epithelial cell barriers, and reduce epithelial cell mortality. Zhang et al. (2011) reported that adding probiotics containing *Clostridium butyrium* to the diet of chickens increased the population of lactic acid bacteria in the appendix. The researchers said that a diet containing probiotics increased the production of acetic acid, butyric acid, valeric acid, and all short-chain fatty acids in the chicks' intestines. This condition reduces the acidity of the contents of the appendix, and inhibits the growth of pathogenic bacteria and stimulates the growth of beneficial bacteria.

Conclusions

The results of this study suggest that since the development of Newcastle disease can affect the respiratory, gastrointestinal, and nervous systems of poultry, this is necessary for the occurrence of secondary poultry diseases, including microbial and viral diseases. Hence, the use of probiotic compounds such as *Pediococcus Lulii* bacteria studied in this study aims to increase cellular and humoral levels of the poultry immune system, it can prevent many economic losses, along with other preventive factors, including vaccination.

Acknowledgement

The authors want to acknowledge Razi Vaccine and Serum Research Institute for its financial support for doing this study.

Ethical approval

The used data is extracted from the approved project of the Razi Vaccine and Serum Research Institute with research code 3-35-1851-114-961101.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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