IJVR
ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 24 No. 2 Ser. No. 83 2023

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Effects of *Escherichia coli* strain Nissle 1917 on immune responses of Japanese quails (*Coturnix japonica*) to Newcastle disease vaccines

Abbasi Kali, R.[1]; Rajabi, Z.[2*]; Nofouzi, K.[3] and Khordadmehr, M.[3]

[1]Graduated from Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran; [3]Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[*]***Correspondence:*** *Z. Rajabi, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. E-mail: rajabi@tabrizu.ac.ir*

10.22099/IJVR.2023.44852.6612

(Received 19 Sept 2022; revised version 28 Jan 2023; accepted 12 Mar 2023)

Abstract

Background: The development of proper immune responses to Newcastle disease (ND) vaccines is important in controlling the disease. *Escherichia coli* strain Nissle 1917 (EcN) is involved in regulating the immune system. **Aims:** The current study evaluated the effects of EcN on immune responses to ND live vaccines in Japanese quails. **Methods:** A total of 150 one-day-old quails were divided into three equal groups. Groups A and B received 10^7 and 10^6 CFU/ml/day of EcN, respectively, sprayed on their diets, while group C received 1 ml/day of PBS. All birds were vaccinated with B1 and Lasota vaccines at 10 and 20 days of age, respectively. Serum samples were collected in order to assay the levels of IgA and certain cytokines, including IL4, IFN-α, and IFN-γ, as well as antibody titers to NDV by HI and ELISA methods. **Results:** No significant difference ($P>0.05$) was observed in serum IgA and IFN-α levels among the groups. However, concentrations of IFN-γ and IL-4 in 42-day-old chicks in group A were significantly ($P<0.05$) higher than in both other groups. After 15 days of the second vaccination, the mean HI titer following NDV was significantly higher in group A than group C. Groups B and C showed significantly lower HI titer than group A after 22 days of the second vaccination. Mean ELISA titer to NDV was significantly ($P<0.05$) higher in group A than in groups B and C after 22 days of the second vaccination. **Conclusion:** It seems that the spraying of 10^7 CFU/ml/day of EcN on quail diets enhances the immune response to NDV vaccines by increasing serum levels of IFN-γ and IL-4.

Key words: *E. coli*, Immunity, Probiotic, Quail, Vaccine

Introduction

Newcastle disease (ND) is a viral disease caused by virulent strains of avian avulavirus 1. The disease is associated with adverse effects on economy of both poultry industry and backyard poultry around the world. Vaccination is one of the main methods of preventing ND. Development of the immune responses to ND vaccines will help to better control the disease (Suarez *et al*., 2020).

Gut microbiota have a crucial role in the development of innate and adaptive immune responses (Pan and Yu, 2014). In this regard, it is believed that there are extensive interactions between the intestinal microbiota community and the innate immune response and, indirectly, the adaptive immune response (Pan and Yu, 2014). Manipulation of the gut microbiota community in the early life of chicken affects the expression of some cytokine genes, such as IL-4 and IFN-γ (Corthay, 2006), which may subsequently enhance antibody and cell-mediated immune responses (Pan and Yu, 2014).

Probiotics regulate the immune response by modifying the composition of the intestinal microflora and, subsequently, through microbial metabolites, regulation of gene expression and signaling pathways in the host cells, especially if administered through food in the early days of age (Galdeano *et al*., 2019). Indeed, probiotic bacteria adhere to intestinal epithelial cells through toll-like receptors (TLR) and trigger signals that stimulate local and systemic immune responses (Galdeano *et al*., 2019). Growing evidence demonstrated that the administration of five species of *Lactobacillus* could increase the antibody titers against avian influenza virus subtype H9N2 (Yitbarek *et al*., 2019).

Escherichia coli strain Nissle 1917 (EcN) is a common probiotic isolated from human feces by Alfred Nissle (Sonnenborn, 2016). Previous studies in mammals have shown that the probiotic EcN is involved in

modulating the immune system by enhancing immune responses (Hu *et al*., 2020; Ukena *et al*., 2005). Moreover, EcN regulates the expression of proinflammatory genes and proteins in human and mouse intestinal epithelial cells (Ukena *et al*., 2005). In poultry, a study showed that EcN enhanced the maturation of the gastrointestinal tract of young turkeys (Moyle *et al*., 2012). Meanwhile, in an *in-vitro* study, EcN was able to inhibit many of the pathological effects of *Clostridium perfringens* (Jiang *et al*., 2014), and modified EcN could also reduce the amount of *Salmonella enterica* in turkey intestines by expressing and secreting antimicrobial peptides (Forkus *et al*., 2017). Another study indicated that oral administration of EcN can improve the weight gain and immune responses of poultry in challenges with *Campylobacter jejuni* (Helmy *et al*., 2022). To date, the effect of EcN on the immune systems and immune responses to commercial vaccines in poultry has not been studied. Therefore, the current study was conducted to evaluate the effects of *Escherichia coli* strain Nissle 1917 on immune responses to Newcastle disease vaccines in Japanese quails (*Coturnix japonica*).

Materials and Methods

Culture of *E. coli* strain Nissle 1917

EcN procured from the Pharma-Zentrale Company (Herdecke, Germany) was cultured on a nutrient agar medium and incubated at 37°C for 24 h. After overnight incubation with shaking, probiotic EcN cells were centrifuged for 15 min at 2500 g, then suspended in PBS, and finally adjusted to different concentrations (0, 10^6, and 10^7 CFU/ml). The process was repeated weekly. Bacterial suspensions were kept refrigerated during the week. After batch preparation, the viability of probiotic cells in each group was determined to be over 96% by culturing on Luria-Bertani broth. The probiotic bacteria were also amplified from the main stocks frozen at -80°C to prevent genetic instability.

Experimental design

One hundred and fifty one-day-old Japanese quail chicks from a breeder flock were randomly divided into three groups of 50 per cage. To prevent the transmission of EcN bacteria among groups, the quails were raised in three separate rooms with the same conditions. Groups A and B, respectively, received 1 ml/day of EcN bacteria using concentrations of 10^7 and 10^6 CFU/ml sprayed on their diet, from one day of age to the end of the study (Al-Khalaifa *et al*., 2019). The bacterial suspensions were sprayed on one-third of the daily feed of the groups every morning. Then, the food was provided to the birds in a feeder that had enough space for simultaneous access to the food. Group C, as a control group, received 1 ml of PBS instead of the bacteria as described above. The quails in all three groups were vaccinated with two commercial ND live vaccines, B1 (HIPRAVIAR® B1), which contains NDV B1 strain $\geq 10^{6.5}$ EID_{50}/dose, and LaSota (CEVAC® NEW L), which contains NDV La Sota strain $\geq 10^{5.5}$ EID_{50}/dose, by the eye-drop administration, at 10 and 20 days of age, respectively. Blood samples were taken from quails at 7, 14, 21, 28, 35, and 42 days of age for serological evaluations. At seven days of age, blood samples were taken from five chicks in each group. In the following weeks, 20 quails in each group were bled using a brachial vein. Serum samples were used to determine the amount of IgA and some cytokines, including IFN-α, IFN-γ (on days 7 and 42), and IL4 (only on day 42), by ELISA method, and antibody titers to Newcastle disease virus (NDV) using HI and ELISA methods for all sampling days (Table 1).

Determination of antibody titers to NDV by ELISA

Antibody titers of IgA against NDV were assayed using ELISA kits (IDDEX, USA) according to the manufacturer's instructions.

Determination of serum IgA, IFN-α, IFN-γ, and IL-4 levels by ELISA

Interleukin-4 (IL-4), IFN-α, and IFN-γ concentrations were determined using respectively, Chicken IL-4, chicken IFN-α, and chicken IFN-γ ELISA Kits (Cusabio, USA) as described in manufacturer's instructions. Also, an indirect ELISA was performed to quantify IgA using the commercial chicken IgA ELISA quantification set (Cusabio, USA) according to the manufacturer's instructions.

Determination of antibody titers to NDV by HI

HI test was performed based on the World Organization for Animal Health (OIE) Terrestrial Manual (Chapter 3.3.14), (Afonso *et al*., 2018).

Statistical analysis

The results were expressed as means ± standard error of the mean (SEM), and all data were statistically analyzed by one-way analysis of variance using SPSS version 22.0 software for Windows (SPSS Inc., Chicago, IL). Differences between the groups were tested by the Tukey's multiple comparison test, and differences were considered significant at $P<0.05$.

Table 1: Experimental design of the study

Experimental groups	Quails/group	Bacteria received (1 ml/day)	Vaccination schedule to NDV/day-old		Blood sampling/day-old
		CFU/ml	B1	Lasota	
A	50	10^7	10	20	7, 14, 21, 28, 35, 42
B	50	10^6	10	20	7, 14, 21, 28, 35, 42
C	50	0	10	20	7, 14, 21, 28, 35, 42

Results

Serum IgA concentration

At 7 days of age, IgA levels were slightly higher in the serum of birds in group B in comparison with the other groups (P>0.05). At 42 days of age, the blood of birds in group A had the highest mean IgA level. But, there was no remarkable difference when compared with the other groups (Table 2).

Table 2: Serum IgA concentrations (ng/ml) (mean±SEM) of the three groups of Japanese quails

Group	Serum IgA concentration	
	Age (day)	
	7	42
A	2105±250.5	7273±1444
B	2199±260.2	5881±576.1
C	1957±351.9	5540±406.5

Group A received 1 ml/day of 10^7 CFU/ml of *E. coli* strain Nissle 1917 bacteria, group B received 1 ml/day of 10^6 CFU/ml of *E. coli* strain Nissle 1917 bacteria daily, and group C as a control group received 1 ml of PBS. No significant difference was observed among the groups

IFN-α and IFN-γ levels in serum

At 7 days of age, the concentration of IFN-α was higher in group B compared to the other two groups, but there was no significant difference among the groups (P>0.05). At 42 days of age, the concentration of IFN-α followed the order of A>B>C. However, there was no considerable difference among the groups (P>0.05) (Table 3).

There was no significant statistical difference in IFN-γ concentration among the different groups studied at 7 days of age (P>0.05). But, at 42 days of age, there was a notable difference between group A and group B, and also group A and group C, with group A having the highest concentration (P<0.05) (Table 3).

IL-4 levels in serum

At 42 days of age, chicks in group A had the highest concentration of IL-4, and there was a significant difference between group A and group B, and also between group A and group C (P<0.05) (Table 3).

Antibody titers to NDV by HI

After vaccination, the mean antibody titers against the hemagglutinin antigen of NDV increased in all groups, especially in the group that received the high dose of bacteria. Up to 28 days of age, there was no statistically significant difference among the groups. But, at 35 days of age, a significant difference was observed between group A (the group that received the high dose of bacteria), and group C (the control group) (P=0.033). At 42 days of age, there was a considerable difference between group A and group B, and also between group A and group C (P<0.05), while the mean antibody titers were statistically similar in the control group and the group that received the low dose of bacteria (Table 4).

Antibody titers to NDV by ELISA

Although antibodies against all NDV proteins were higher in the treatment groups, particularly the group that received the higher dose of bacteria than in the control group, from 7 to 35 days of age, there was no statistically significant difference among them. At 42 days of age, however, there was a notable difference between group A and group B, and also between group A and group C (P<0.05), (Table 5).

Table 3: Serum IFN-α, IFN-γ, and IL-4 concentrations (pg/ml) (mean±SEM) of the three groups of Japanese quails

Group	IFN-α concentration		IFN-γ concentration		IL-4 concentration
	Age (day)				
	7	42	7	42	42
A	19.34±2.88^a	38.66±2.15^a	41.55±7.72^a	259.23±50.91^b	214.87±27.6^b
B	20.63±3.03^a	37.74±1.00^a	35.40±8.43^a	123.59±26.55^a	117.19±7.89^a
C	19.60±2.79^a	34.97±1.77^a	29.39±6.96^a	117.36±21.04^a	66.54±4.53^a

Group A received 1 ml/day of 10^7 CFU/ml of *E. coli* strain Nissle 1917 bacteria, group B received 1 ml/day of 10^6 CFU/ml of *E. coli* strain Nissle 1917 bacteria daily, and group C, as a control group, received 1 ml of PBS. $^{a, b}$ Different superscripts within each column show significant differences among groups (P<0.05)

Table 4: HI titers (log_2) to Newcastle disease virus (NDV) (mean±SEM) of the three groups of Japanese quails

Group	HI titers to NDV (days-old)					
	7	14	21	28	35	42
A	1.77±0.22^a	2.66±0.33^a	4.33±0.29^a	5.88±0.51^a	6.89±0.20^b	7.44±0.30^b
B	1.55±0.24^a	2.44±0.29^a	4.00±0.37^a	5.55±0.44^a	5.88±0.35^{ab}	6.11±0.35^a
C	1.77±0.22^a	2.33±0.29^a	3.66±0.24^a	4.88±0.39^a	5.44±0.50^a	6.11±0.35^a

Group A received 1 ml/day of 10^7 CFU/ml of *E. coli* strain Nissle 1917 bacteria, group B received 1 ml/day of 10^6 CFU/ml of *E. coli* strain Nissle 1917 bacteria daily, and group C, as a control group, received 1 ml of PBS. $^{a, b}$ Different superscripts within each column show significant differences among groups (P<0.05)

Table 5: Serum antibody titer assayed by ELISA test (mean±SEM) in the three groups of Japanese quails

Group	Antibody titers to all NDV proteins (days-old)					
	7	14	21	28	35	42
A	769±165[a]	1501±224[a]	1888±215[a]	2289±95[a]	2442±190[a]	3073±85[b]
B	584±157[a]	1317±201[a]	1778±167[a]	1808±179[a]	2177±137[a]	2199±166[a]
C	406±136[a]	997±208[a]	1318±178[a]	1784±225[a]	1859±251[a]	1913±193[a]

Group A received 1 ml/day of 10^7 CFU/ml of *E. coli* strain Nissle 1917 bacteria, group B received 1 ml/day of 10^6 CFU/ml of *E. coli* strain Nissle 1917 bacteria daily, and group C, as a control group, received 1 ml of PBS. Different superscripts within each column show significant differences among groups ($P<0.05$)

Discussion

In this study, we investigated the effects of probiotic EcN on immune responses to live NDV vaccines in Japanese quails. Interestingly, the present results demonstrate that adding EcN probiotics to the quails' feed improves humoral response to the live NDV vaccines, strains B1 and LaSota. This improvement was associated with an increase in the HI and ELISA titers to NDV. It seems that oral administration of probiotic EcN can contribute in immune regulation. In line with this observation, alterations of some cytokines, including, IFN-γ, and IL-4, confirmed the present hypothesis.

Numerous studies have indicated that there is a relationship between the administration of probiotics and the regulation of IFN-γ, and IL-4 levels. It is assumed that the binding of bacterial products to TLR receptors at the surface of innate immune system cells, including natural killer and mast cells, activates them and leads to the production of IFN-γ and IL-4 (Corthay, 2006; Zhao *et al*., 2021). Michael *et al*. (2021) revealed that EcN enhances innate and adaptive immune responses in a ciprofloxacin-treated defined-microbiota piglet model of human rotavirus infection. It is assumed that the fatty acid part of lipopolysaccharide (LPS) of EcN, which is present in lipid A, binds to the CD14/TLR4/MD-2 receptor on human blood monocytes; This complex leads to extensive pro-inflammatory reactions, like the expression of genes associated with cytokine activity and the presence of antigens (Huang *et al*., 2006). Hu *et al*. (2020) showed that EcN-outer membrane vesicles (OMVs), such as LPS, peptidoglycan, and DNA activate the expression of T helper cell type 2 (Th2)-polarizing cytokines (IL-4) in RAW 264.7 macrophages. The results of the present study determine that if proper amounts of EcN (10^7 CFU/ml) are added daily to quail's diet, it can increase the production of IFN-γ and IL-4 cytokines at 42 days of age. In general, the present findings show that at 42 days of age, the levels of IFN-γ and IL-4 were higher in quails that received higher number of bacteria. Importantly, these results indicate that the amount of administered bacteria is effective in the production and secretion of cytokines. Yan and Polk (2011) reported that the amount of probiotics is important for their beneficial effects.

IFN-γ and IL-4, act as bridges between innate and acquired immunity (Corthay, 2006). These cytokines help dendritic cells to produce IL-12, which is necessary for the differentiation of naive T cells. Previous reports have shown that the production of IFN-γ and IL-4 is critical for the differentiation of naive T cells into Th1 and Th2 cells, respectively (Yan and Polk, 2011); Th1 and Th2 cells lead to a cell-mediated and humoral immune response, respectively. IL-4 also enhances humoral immune responses by switching B cells to IgG1 (Luzina *et al*., 2012). In addition, it has been previously shown that the lipopeptides and LPS of EcN-conditioned medium bind to TLR2 and TLR4, respectively, which leads to increased nuclear factor kappa B (NF-κB) activity (Grabig *et al*., 2006), which in turn, controls the processes of differentiation and maturation of B cells (Barnabei *et al*., 2021). Both cellular and humoral immunity play important roles in the development of immunity against NDV (Suarez *et al*., 2020). In the current study, although the effect of EcN on cellular immune system response against the ND vaccines was not studied, group A, which received the high dose of probiotic EcN bacteria, showed the highest level of IFN-γ at 42 days of age, which may enhance cell-mediated immunity (Corthay, 2006) by Th1 response to the live NDV vaccines. The results also indicate that the group that received the highest dose of EcN produced the highest titer of neutralizing antibody (HI) to NDV. Moreover, ELISA titer to NDV antigens showed a significant increase in group A, which may be related to the administration of the probiotic EcN. Therefore, it can be concluded that adding EcN probiotic to the feed in sufficient quantities, will significantly increase the antibody titers to NDV antigens possibly by the mechanisms mentioned above.

Type 1 interferons of birds, including IFN-α, are similar in activity, structure, and evolution to their mammalian counterparts (Santhakumar *et al*., 2017). In this regard, a previous study showed that oral administration of EcN reduces the IFN-α level (Manzhalii *et al*., 2016). Another study focusing on the effect of EcN on the prevention of human rotavirus in pigs showed that this probiotic induces the production of IFN-α (Vlasova *et al*., 2016). However, in the current study, EcN administration did not increase IFN-α level. In mouse dendritic cells and human peripheral blood mononuclear cells, bacterial transfer RNA (tRNA) induces IFN type I; however, Jöckel *et al*. (2012) showed that EcN tRNA could not induce IFN type I, because tRNA of EcN is non-immunostimulatory. The increase in IFN-α levels at 42 days of age in the three groups of the present study appears to be due to vaccination with the B1 and Lasota vaccines. There are studies that show IFN-α is produced and secreted in poultry mainly against viruses that cause Marek disease, infectious bursal

disease, infectious bronchitis, and avian influenza disease (Santhakumar *et al*., 2017). Another study reports that NDV infection induces the production of IFN-α and velogenic strains increase interferon levels more rapidly than lentogenic strains (Liu *et al*., 2012). Consistently, Liu *et al*. (2012) reported that the LaSota strain, which is a lentogenic strain, gradually increases the level of IFN-α mRNA expression in the peripheral blood of chickens.

There is a hypothesis that mammalian IgA is homologous with avian IgA (Carlander *et al*., 1999). Human IgA has both inflammatory and anti-inflammatory activities. Serum IgA binds to Fcαreceptor I (FcαRI) expressed by myeloid cells and induces pro-inflammatory responses, such as the release of cytokines and chemokines (Hansen *et al*., 2019). But, the most important role of serum IgA is anti-inflammatory activity (Monteiro, 2014). Additionally, IgA has a passive protective role through immune exclusion (Breedveld and Van Egmond, 2019); the IgA FcαRI may stimulate inhibitory signals to a set of active receptors and may reduce IgG-FcR-mediated signaling (Monteiro, 2014). Various reports exist on the role of probiotics in increasing the IgA concentration in the blood (Karamzadeh-Dehaghani *et al*., 2021; Lin *et al*., 2021; Takeuchi and Ohno, 2022). For instance, in humans, the probiotic *Lactobacillus johnsonii* La1 has been shown to increase serum IgA by 10% (Wold, 2001); Although, another report has stated that other probiotic strains or yogurt products have no appreciable effect on serum IgA (Wold, 2001). In the current study, EcN was not able to significantly increase IgA, which is consistent with the previous report. Although, in group A, which received higher number of EcN bacteria than group B, the IgA concentration increased slightly at the age of 42 days.

In conclusion, it seems that spraying 10^7 CFU/ml/day of *E. coli* strain Nissle 1917 bacteria on quail diets, from the first day of rearing to 42 days of age, in particular, increases serum levels of IFN-γ and IL-4, which may lead to enhancement of the immune response to the NDV live vaccines.

Acknowledgement

The authors would like to thank the Research Council of the University of Tabriz for funding this research.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Afonso, CL; Miller, PJ; Grund, C; Koch, G; Peeters, B; Selleck, PW and Srinivas, GB (2018). Newcastle disease. *Manual of diagnostic tests and vaccines for terrestrial animals*. (8th Edn.), OIE: Paris, France. PP: 964-983.

Al-Khalaifa, H; Al-Nasser, A; Al-Surayee, T; Al-Kandari, S; Al-Enzi, N; Al-Sharrah, T; Ragheb, G; Al-Qalaf, S and Mohammed, A (2019). Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poult. Sci. J., 98: 4465-4479.

Barnabei, L; Laplantine, E; Mbongo, W; Rieux-Laucat, F and Weil, R (2021). NF-κB: at the borders of autoimmunity and inflammation. Front. Immunol., 12: 1-27. https://doi.org/10.3389/fimmu.2017.00913.

Breedveld, A and Van Egmond, M (2019). IgA and FcαRI: pathological roles and therapeutic opportunities. Front. Immunol., 10: 553-572.

Carlander, D; Stalberg, J and Larson, A (1999). Chicken antibodies, a clinical chemistry perspective. Upsala. J. Med. Sci., 104: 179-190.

Corthay, A (2006). A three-cell model for activation of naive T helper cells. Scand. J. Immunol., 64: 93-96.

Forkus, B; Ritter, S; Vlysidis, M; Geldart, K and Kaznessis, YN (2017). Antimicrobial probiotics reduce *Salmonella enterica* in turkey gastrointestinal tracts. Sci. Rep., 7: 1-9.

Galdeano, CM; Cazorla, SI; Dumit, JML; Vélez, E and Perdigón, G (2019). Beneficial effects of probiotic consumption on the immune system. Ann. Nutr. Metab., 74: 115-124.

Grabig, A; Paclik, D; Guzy, C; Dankof, A; Baumgart, DC; Erckenbrecht, J; Raupach, B; Sonnenborn, U; Eckert, J; Schumann, RR and Sturm, A (2006). *Escherichia coli* strain Nissle 1917 ameliorates experimental colitis via toll-like receptor 2- and toll-like receptor 4-dependent pathways. Infect. Immun., 74: 4075-4082.

Hansen, IS; Baeten, DL and den Dunnen, J (2019). The inflammatory function of human IgA. Cell. Mol. Life Sci., 76: 1041-1055.

Helmy, YA; Gloss, G; Jung, K; Kathayat, D; Vlasova, A and Rajashekara, G (2022). Effect of probiotic *E. coli* Nissle 1917 supplementation on the growth performance, immune responses, intestinal morphology, and gut microbes of *Campylobacter jejuni* infected chickens. Infect. Immun., 90: 1-16. https://doi.org/10.1128/iai.00337-22.

Hu, R; Lin, H; Li, J; Zhao, Y; Wang, M; Sun, X; Min, Y; Gao, Y and Yang, M (2020). Probiotic *Escherichia coli* Nissle 1917-derived outer membrane vesicles enhance immunomodulation and antimicrobial activity in RAW264. 7 macrophages. BMC Microbiol., 20: 1-13.

Huang, H; Park, CK; Ryu, JY; Chang, EJ; Lee, Y; Kang, SS and Kim, HH (2006). Expression profiling of lipopolysaccharide target genes in RAW264. 7 cells by oligonucleotide microarray analyses. Arch. Pharm. Res., 29: 890-897.

Jiang, Y; Kong, Q; Roland, KL; Wolf, A and Curtiss III, R (2014). Multiple effects of *Escherichia coli* Nissle 1917 on growth, biofilm formation, and inflammation cytokines profile of *Clostridium perfringens* type A strain CP4. Pathog. Dis., 70: 390-400.

Jöckel, S; Nees, G; Sommer, R; Zhao, Y; Cherkasov, D; Hori, H; Ehm, G; Schnare, M; Nain, M; Kaufmann, A and Bauer, S (2012). The 2′-O-methylation status of a single guanosine controls transfer RNA-mediated Toll-like receptor 7 activation or inhibition. J. Exp. Med., 209: 235-241.

Karamzadeh-Dehaghani, A; Towhidi, A; Zhandi, M; Mojgani, N and Fouladi-Nashta, A (2021). Combined effect of probiotics and specific immunoglobulin Y directed against *Escherichia coli* on growth performance, diarrhea incidence, and immune system in calves. Animal. 15: 1-8.

Lin, WY; Kuo, YW; Chen, CW; Huang, YF; Hsu, CH; Lin, JH; Liu, CR; Chen, JF; Hsia, KC and Ho, HH (2021). Viable and heat-killed probiotic strains improve oral

immunity by elevating the IgA concentration in the oral mucosa. Curr. Microbiol., 78: 3541-3549.

Liu, WQ; Tian, MX; Wang, YP; Zhao, Y; Zou, NL; Zhao, FF; Cao, SJ; Wen, XT; Liu, P and Huang, Y (2012). The different expression of immune-related cytokine genes in response to velogenic and lentogenic Newcastle disease viruses infection in chicken peripheral blood. Mol. Biol. Rep., 39: 3611-3618.

Luzina, IG; Keegan, AD; Heller, NM; Rook, GA; Shea-Donohue, T and Atamas, SP (2012). Regulation of inflammation by interleukin-4: a review of "alternatives". J. Leukoc. Biol., 92: 753-764.

Manzhalii, E; Hornuss, D and Stremmel, W (2016). Intestinal-borne dermatoses significantly improved by oral application of *Escherichia coli* Nissle 1917. World J. Gastroenterol., 22: 5415-5421.

Michael, H; Paim, FC; Langel, SN; Miyazaki, A; Fischer, DD; Chepngeno, J; Amimo, J; Deblais, L; Rajashekara, G; Saif, LJ and Vlasova, AN (2021). *Escherichia coli* Nissle 1917 enhances innate and adaptive immune responses in a ciprofloxacin-treated defined-microbiota piglet model of human rotavirus infection. Msphere. 6: 1-16.

Monteiro, RC (2014). Immunoglobulin A as an anti-inflammatory agent. Clin. Exp. Immunol., 178: 108-110.

Moyle, JR; de los Santos, FS; GR, H; Huff, WE; Rath, NC; Farnell, M and Fanatico, AC (2012). Early gastro-intestinal maturation in young Turkey poults. Int. J. Poult. Sci., 11: 445-452.

Pan, D and Yu, Z (2014). Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 5: 108-119.

Santhakumar, D; Rubbenstroth, D; Martinez-Sobrido, L and Munir, M (2017). Avian interferons and their antiviral effectors. Front. Immunol., 8: 49-65.

Sonnenborn, U (2016). *Escherichia coli* strain Nissle 1917—from bench to bedside and back: history of a special *Escherichia coli* strain with probiotic properties. FEMS Microbiol. Lett., 363: fnw212.

Suarez, DL; Miller, PJ; Koch, G; Mundt, E and Rautenschlein, S (2020). Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. In: Swayne, DE; Bouliane, M; Logue, CM; McDougold, LR; Nair V and Suarez, DL (Eds.), *Diseases of poultry*. (14 Edn.), Ames, Iowa, Iowa State University Press. PP: 109-166.

Takeuchi, T and Ohno, H (2022). IgA in human health and diseases: Potential regulator of commensal microbiota. Front. Immunol., 13: 1024330-1024342.

Ukena, SN; Westendorf, AM; Hansen, W; Rohde, M; Geffers, R; Coldewey, S; Suerbaum, S; Buer, J and Gunzer, F (2005). The host response to the probiotic *Escherichia coli* strain Nissle 1917: specific up-regulation of the proinflammatory chemokine MCP-1. BMC Med. Genet., 6: 1-13.

Vlasova, AN; Shao, L; Kandasamy, S; Fischer, DD; Rauf, A; Langel, SN; Chattha, KS; Kumar, A; Huang, HC; Rajashekara, G and Saif, LJ (2016). *Escherichia coli* Nissle 1917 protects gnotobiotic pigs against human rotavirus by modulating pDC and NK-cell responses. Eur. J. Immunol., 46: 2426-2437.

Wold, AE (2001). Immune effects of probiotics. Scand. J. Food Nutr., 45: 76-85.

Yan, F and Polk, DB (2011). Probiotics and immune health. Curr. Opin. Gastroenterol., 27: 496-507.

Yitbarek, A; Astill, J; Hodgins, DC; Parkinson, J; Nagy, É and Sharif, S (2019). Commensal gut microbiota can modulate adaptive immune responses in chickens vaccinated with whole inactivated avian influenza virus subtype H9N2. Vaccine. 37: 6640-6647.