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Evaluation of Acute Phase Response in Viral Interference between Live Vaccine Virus and a Virulent Newcastle Disease Virus

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Abstract

Newcastle disease (ND) is one of the most important viral diseases in the world that causes considerable damages to the poultry industry. This study aimed to investigate the acute phase response in homologous interference between Newcastle disease vaccine virus and a virulent Newcastle disease virus (NDV) in chickens. The experimental chicks received live vaccine virus either before or after challenge with virulent NDV to determine the stronger immune system stimulation and higher acute phase response. A total of 250 day-old Cobb-500 commercial broiler chickens were divided randomly into 5 equal groups (n = 50), and the chicks in each group were treated as follows: Group 1 received live vaccine at 22 days of age. Group 2 was vaccinated 24 hours prior to the challenge with NDV at 23 days of age. Group 3 was challenged with NDV at 23 days of age and received live vaccine 24 hours later. Group 4 was the negative control. Group 5 was challenged with NDV at 23 days of age. Blood samples were collected at intervals of 3, 6, 12, 18, 24, 48, 72 hours, and on days 6 and 9 after first inoculation in all the groups; and measurements of haptoglobin, serum amyloid A, ovotransferrin, adenosine deaminase, serum proteins (total protein, albumin, globulins), and gangliosides (total sialic acid, lipid-bound sialic acid, and protein-bound sialic acid) were carried out according to standard procedures. The results showed that the challenge with NDV led to a significant increase in inflammatory factors and acute phase response, whereas vaccination caused a mild increase in these parameters. At 24 hours, group 3 (post-challenge vaccinated chicks) showed a stronger immune system stimulation and higher acute phase response than group 2 (pre-challenge vaccinated chicks). To summarize, the results of this study indicated that challenge with NDV could lead to a significant increase in the inflammatory factors and acute phase response, whereas vaccination alone could cause a mild increase in these parameters. The challenge with NDV followed by vaccination resulted in a stronger immune system stimulation and higher acute phase response than vaccination followed by the challenge with NDV. According to these findings, vaccination of the chicks with a live vaccine soon after natural infection with virulent NDV may help the chicks to overcome the sequelae of the disease.

***Keywords*:** Acute phase response, live vaccine virus, Newcastle disease virus

Introduction

Newcastle disease (ND) is one of the most important viral diseases in the world that causes considerable damages to the poultry industry (Alexander, 2003). The Newcastle disease virus (NDV) belongs to the genus *Avulavirus* in the family *Paramyxoviridae*. The type and severity of diseases caused by this virus are often variable, making it difficult in recognizing them. The isolates and strains of the virus are very different, creating different disease faces in the same bird species (Alexander & Senne, 2008). Biosecurity and immunization of endangered birds are 2 major steps to prevent ND. One of the important goals in controlling ND is to prevent infection in sensitive birds or reduce the number of susceptible birds by vaccination. Vaccination is a widely used method in the control of viral diseases, including ND in the poultry production (Senne et al., 2004). Vaccination is used as a control tool in most countries where the poultry industry is commercialized, and the disease is endemic (Alexander, 2001). The

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increase in our knowledge about the process of creating safety protection is substantial in the development of new vaccines and implementation of a proper vaccination program (Norup et al., 2011). Vaccination reduces the serious consequences of the disease but cannot prevent the virus replication and shedding. However, the amount of virus proliferation and excretion diminishes with vaccination (Afsar et al., 2018; Capua et al., 2005). Birds may respond to the vaccination by developing humoral and cellular immune response. The development of the immune responses requires time, although the time needed to evoke different immune branches is not the same; humoral immunity takes the longest time, whereas cellular immunity takes the shortest (Sharma, 1999), and humoral immune response is more important in ND protection (Al-Garib et al., 2003). The replication of the NDV can be affected by the homologous and heterologous interference, a phenomenon in which replication of a virus in a cell often prevents the replication of another virus (Gelb et al., 2007). The importance of homologous and heterologous interference of NDV in pathogenesis and proliferation of virulent strains by non-pathogenic strains is described in ovo (Ge et al., 2012) and field conditions (Costa-Hurtado et al., 2014; Gelb et al., 2007).

In birds, like mammals, acute phase proteins (APPs) are synthesized in liver and secreted into blood; the production of APPs increases in acute stages of the infection (Nielsen et al., 1999; Juul-Madsen et al., 2003; Yazdani et al., 2015). Determining the concentration of APPs can be helpful in monitoring poultry health (O'Reilly & Eckersall, 2014). Several studies evaluated the changes in the levels of APPs in common poultry diseases (Asasi et al., 2013; Chamanza et al., 1999a; Chamanza et al., 1999b; Holt & Gast, 2002; Kokosharov, 2006; Mosleh et al., 2012; Mosleh et al., 2013; Nazifi et al., 2010; Nazifi et al., 2011; Rath et al., 2008; Xie et al., 2002b). Habibi et al. (2013) evaluated the levels of APPs in vaccinated and challenged birds by a wild NDV. Alterations of APPs in poultry infectious diseases have not been fully understood. Few reports are available regarding APP changes in the poultry vaccinated and challenged by ND vaccine.

This study aimed to investigate acute phase response in homologous interference of ND vaccine by experimental Newcastle infection in broiler chickens to determine which of the following results in stronger immune system stimulation and higher acute phase response: vaccination, then challenge with NDV or challenge with NDV, then vaccination.

Method

Virus and Vaccine Strain

The highly virulent NDV strain (Genebank Accession Number: JF820294.1; IVPI: 2.46) was used as a virus stock and propagated in the allantoic cavity of 10-day-old embryonated chicken eggs. The embryo infective dose (EID50) of harvested allantoic fluid was calculated as 10^8/mL per the method described by Reed and Muench (1938). To vaccinate the chickens, the commercial Villegas-Glisson/University of Georgia (VG/GA) ND vaccine (Avinew, Lot number: L439957) was used.

Animals and Experimental Design

A total of 250 1-day-old Cobb-500 commercial broiler chickens were divided randomly into 5 equal groups (n = 50) for 2 main reasons: first, the probability of losses after NDV challenge and second, to put down the effect of stress on the final results, that is, to prevent double sampling in each chick as the sampling method was heart puncture that could cause stress in chickens. The chicks in each group were treated as follows:

- Group 1 received live vaccine on day 22 of their age.
- Group 2 vaccinated on day 22 of their age and challenged with NDV 24 hours later at the age of 23 days.
- Group 3 challenged with NDV at 23 days of age and received live vaccine 24 hours later.
- Group 4 was negative control.
- Group 5 was challenged with NDV at 23 days of age.

Each group was reared in an isolated room of the animal research unit at the School of Veterinary Medicine, Shiraz University, and received standard feed and water *ad libitum* during the experiment. The hemagglutination inhibition (HI) test was used to measure maternal antibody against NDV in the sera of the chicks. At the age of 22 days (day of first inoculation), their mean HI titer was less than 3 log2. The animals were obtained commercially from the Parsian broiler chickens company (Tehran, Iran).

This study was approved by the state committee on animal ethics, Shiraz University, Shiraz, Iran. The recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes, were also followed.

Sampling

Blood samples were taken at intervals of 3, 6, 12, 18, 24, 48, 72 hours, and days 6 and 9 after first inoculation (challenge with NDV or vaccination with live vaccine) from the chicks of all groups. At each time interval, 5 chicks from each group were bled from heart (cardiac puncture). The sera were separated by centrifugation at 750 g for 15 min and stored at –20°C until used. After the experiment, the animals were euthanized using manual cervical dislocation or breaking the neck method, which is the most commonly applied method.

Evaluation of Haptoglobin (Hp) and Serum Amyloid A (SAA)

Serum levels of Hp and SAA were measured by a quantitative sandwich enzyme immunoassay using commercial chicken-specific kits (Shanghai Crystal Day Biotech, Shanghai, China). The analytical sensitivity of this test in serum is determined as .0156 mg/mL for Hp and .3 µg/mL for SAA by the manufacturer.

Ovotransferrin (OVT) Measurement

OVT was measured by a quantitative enzyme-linked immunosorbent assay method using a commercial chicken-specific kit (Abcam, Biotech, Life Sciences, Cambridge, England). The sensitivity of the OVT kit was .751 ng/mL. Both intra- and inter-assay precision of the OVT kit was CV<10%. The control serum recovery of the OVT kit was >85%.

Adenosine Deaminase (ADA) Measurement
ADA was assessed by an enzymatic-calorimetric assay kit (Diazyme Laboratories, Gregg Court, California, USA).

Measurement of Serum Proteins
Serum was analyzed for total protein (TP) by the Biuret method (Commercial kit; Pars Azmoon, Tehran, Iran), and for albumin (Alb) by the bromocresol green method (Commercial kit; Pars Azmoon, Tehran, Iran). Globulin was determined as the difference between serum TP and Alb. Cellulose acetate electrophoresis (15 minutes at 180 V using Elphor 5, Germany) was used to reveal serum proteins (albumin, α-globulin, β-globulin, and γ-globulin).

Gangliosides (TSA, LBSA, PBSA) Determination
Serum total sialic acid (TSA) concentration was determined by the thiobarbituric acid method. Lipid-bound sialic acid (LBSA) concentration was determined by the method described by Katopodis et al. (1982). Protein-bound sialic acid (PBSA) concentration was measured by subtracting LBSA from serum TSA.

Statistical Analysis
Statistical analysis was conducted using the Statistical Package for Social Sciences software version 16 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as means and standard errors. Means of each variable in the treatment groups and in various times were compared using mixed model analysis. In all the analyses, a $p < .05$ was considered as statistically significant.

Results

Figures 1-12 illustrate the patterns of changes in the acute phase response in time intervals after the experimental challenge with NDV and vaccination.

Haptoglobin
Hp concentration in groups 3 (challenged prior to vaccination), 2 (vaccinated prior to challenge), and 5 (challenge) initially showed an increase and then a decrease; the highest concentration of Hp in groups 3 (challenge + vaccine) and 5 (challenge) was observed at 48 and 72 hours, respectively; and in group 2 (vaccine + challenge) at 48 hours. In groups 4 (negative control) and 2 (vaccine + challenge), no significant changes were observed in the concentration of Hp over time. There was a significant difference in Hp concentration between the tested groups and the negative control group at 12, 18, 24, and 48 hours ($p < .05$) (Figure 1).

Serum Amyloid A (SAA)
The concentration of SAA in groups 1 (vaccine), 3 (challenge+-vaccine), and 5 (challenge) initially showed an increase and then a decrease; the highest concentration was found at 48 and 72 hours. In group 2 (vaccine+challenge), SAA concentration showed an initial increase, then a decrease on day 6, followed by an increase on day 9; the highest concentration was observed at 48 and 72 hours and on the 9th day. There was a significant difference in SAA concentration between the tested groups and the negative control group at all times ($p < .05$) (Figure 2).

Ovotransferrin (OVT)
OVT level in groups 1, 2, 3, and 5 (all groups except the negative control group) initially showed an increase and then a decrease; the highest OVT level in group 1 (vaccine) was observed at 48 and 72 hours, in group 3 (challenge+vaccine) at 48 hours, and in groups 2 (vaccine+challenge) and 5 (challenge) at 48 hours. In the negative control group, no significant change in OVT level was found over time. There was a significant difference in OVT level between the tested groups and the negative control group at all times, except at the 3rd and 6th hours ($p < .05$) (Figure 3).

Adenosine Deaminase (ADA)
ADA concentration in group 1 (vaccine) initially showed an increase and then a decrease; the highest concentration of ADA

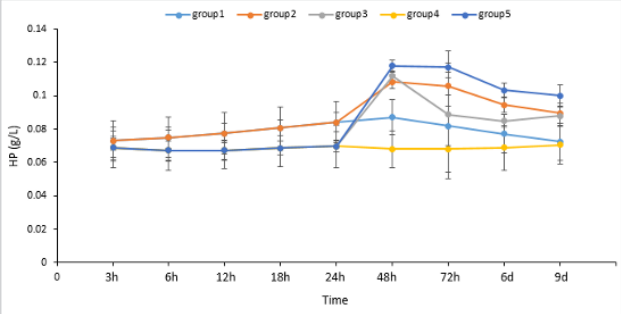

Figure 1
The Pattern of Changes in Haptoglobin (g/L) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

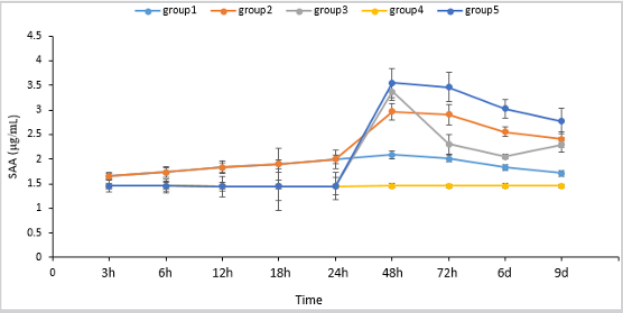

Figure 2
The Pattern of Changes in Serum Amyloid A (µg/mL) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

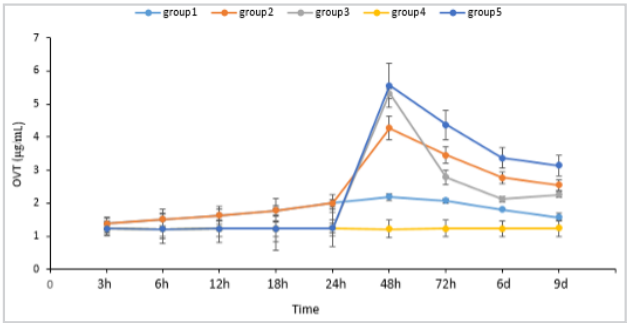

Figure 3
The Pattern of Changes in Ovotransferrin (µg/mL) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

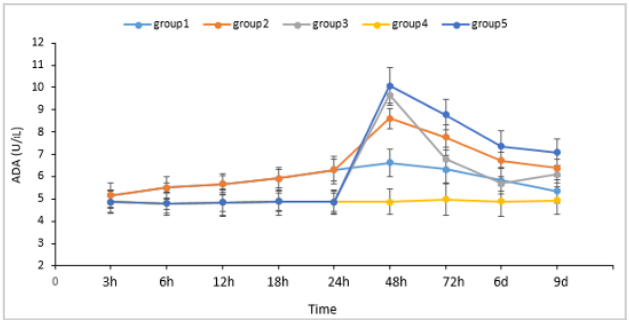

Figure 4
The Pattern of Changes in Adenosine Deaminase (U/L) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

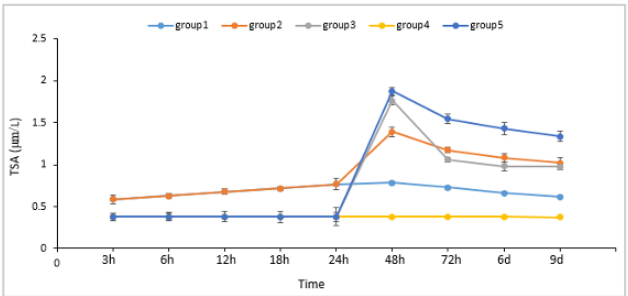

Figure 5
The Pattern of Changes in Total Sialic Acid (µmol/L) in the Sera of Chicks After Experimental challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

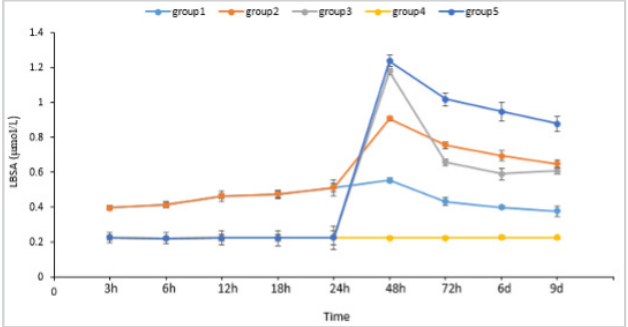

Figure 6
The Pattern of Changes in Lipid-Bound Sialic Acid (µmol/L) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

was found at 48 and 72 hours; there was no significant difference in ADA concentration among these times. The concentration of ADA in groups 3 (challenge+vaccine), 2 (vaccine+challenge), and 5 (challenge) initially showed an increase and then a decrease; the highest concentration of ADA was observed at 48 hours. There was a significant difference in ADA concentration between the tested groups and the negative control group at all times except at the 3rd hour and on the 9th day ($p < .05$) (Figure 4).

Total Sialic Acid

In groups 1 (vaccine), 3 (challenge+vaccine), and 5 (challenge), the concentration of TSA initially showed an increase and then a decrease; the highest TSA concentration in group 1 was observed at 48 hours and in groups 3 (challenge+vaccine) and 5 (challenge) at 48 hours. In addition, in group 2 (vaccine+challenge), the concentration of TSA initially showed an increase and then a decrease, whereas there was no significant difference in TSA concentration from 12 hours to 6 days. No significant change was found in the concentration of TSA in the negative control group over time. There was a significant difference in TSA concentration between the tested groups and the negative control group at all times ($p < .05$) (Figure 5).

Lipid-bound Sialic Acid (LBSA)

In all groups, except the negative control, the concentration of LBSA initially showed an increase and then a decrease; the highest LBSA concentration was observed at 48 hours. In the negative control group, no significant change in the concentration of LBSA was observed over time. There was a significant difference in LBSA concentration between the tested groups and the negative control group at all times ($p < .05$) (Figure 6).

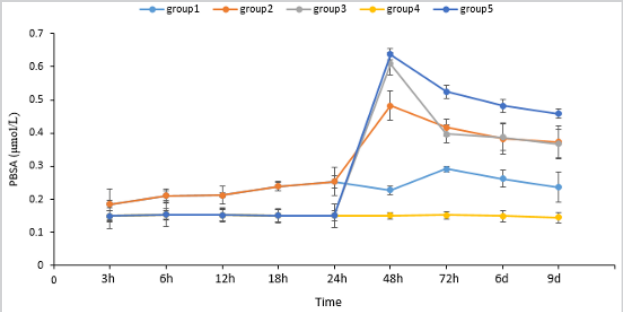

Figure 7
The Pattern of Changes in Protein-Bound Sialic Acid (µmol/L) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

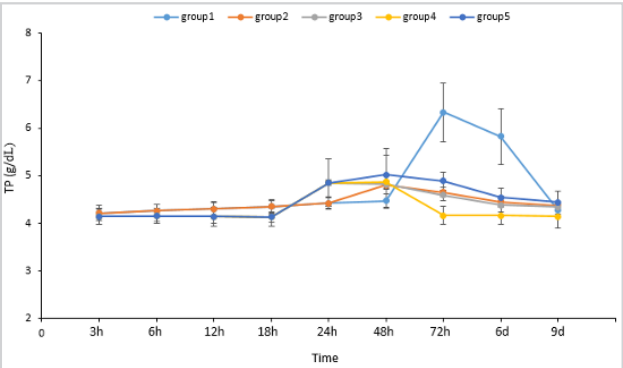

Figure 8
The Pattern of Changes in Total Protein (g/dL) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

Protein-bound Sialic Acid (PBSA)

In groups 1 (vaccine) and 5 (challenge), the concentration of PBSA initially showed an increase and then a decrease; the highest PBSA concentration in group 1 was observed at 72 hours and in group 5 (challenge) at 48 hours. In groups 3 (challenge+vaccine) and 2 (vaccine+challenge), the concentration of PBSA was constant until 18 hours; then, it showed an increase at 24 and 48 hours, followed by a decrease. In group 4 (negative control), no significant change in the concentration of PBSA was observed over time. There was a significant difference in PBSA concentration between the tested groups and the negative control group at all times except at the 3rd hour ($p < .05$) (Figure 7).

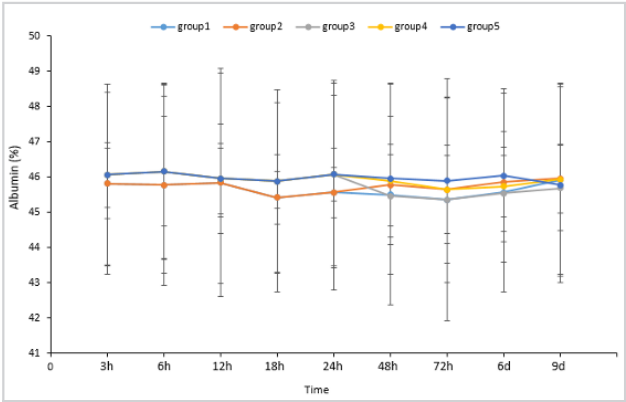

Figure 9
The Pattern of Changes in Albumin (%) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

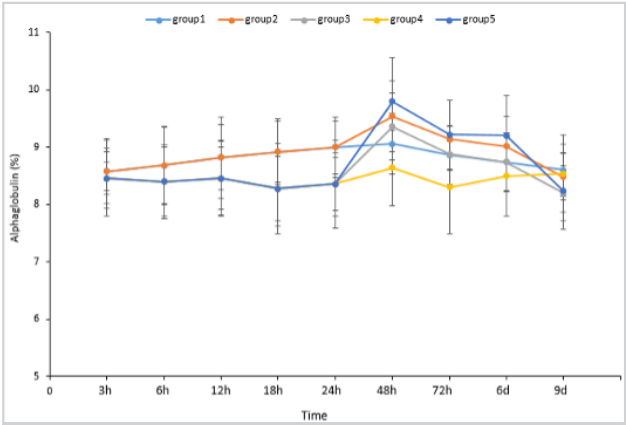

Figure 10
The Pattern of Changes in α-Globulin (%) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

Total Protein (TP)

The concentration of TP in group 1 (vaccine) was constant for up to 48 hours, then a sudden increase was observed at 72 hours (the highest TP concentration), followed by a decreasing trend. The concentration of TP in groups 3 (challenge+vaccine), 2 (vaccine+challenge), and 4 (negative control) initially showed an increase and then a decrease; the highest concentration of TP in groups 3 (challenge+vaccine) and 2 (vaccine+challenge) was ob-

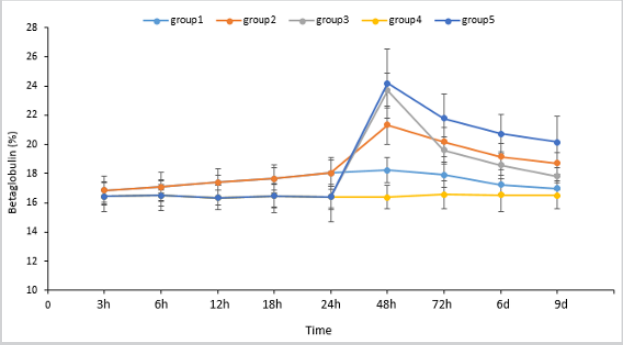

Figure 11
The Pattern of Changes in β-Globulin (%) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

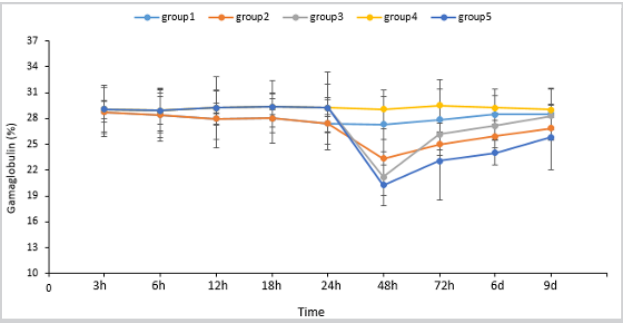

Figure 12
The Pattern of Changes in γ-Globulin (%) in the Sera of Chicks After Experimental Challenge with Newcastle Disease Virus and Vaccination. (Group 1: Received Live Vaccine on Day 22 of Age. Group 2: Vaccinated 24 Hours Prior to Challenge with NDV at 23 Days of Age. Group 3: Challenged with NDV at 23 Days of Age and Received Live Vaccine 24 Hours Later. Group 4: Negative Control. Group 5: Challenged with NDV at 23 Days of Age).

served at 24, 48, and 72 hours, and in group 4 (negative control) at 24 and 48 hours. In group 5 (challenge), the concentration of TP was constant for up to 18 hours and increased at 24 and 48 hours, followed by a decrease. No significant difference in TP concentration was found between the tested groups and the negative control group at all times, except at 18 and 72 hours (Figure 8).

Albumin (Alb)
In all the groups, no significant changes were observed in Alb concentration over time. Moreover, there was no significant difference in Alb concentration among all the groups and between the tested groups and the negative control group at all times (Figure 9).

α -globulin
In groups 1 (vaccine) and 4 (negative control), no significant changes were observed in α-globulin concentration over time. In groups 3 (challenge+vaccine), 2 (vaccine+challenge), and 5 (challenge), the concentration of α -globulin showed an increasing, but not a significant, trend from 12 hours to 6 days. There was no significant difference in the α-globulin concentration between the tested groups and the negative control group at all times (Figure 10).

β-globulin
In group 1 (vaccine), the concentration of β-globulin at 48 hours was significantly higher than at 3 hours ($p < .05$). The concentration of β-globulin in groups 3 (challenge+vaccine), 2 (vaccine+challenge), and 5 (challenge) initially showed an increase and then a decrease; the highest concentration of β-globulin in groups 3 and 5 was observed at 48 hours, and in group 2 at 48 and 72 hours. In the negative control group, no significant change in β-globulin concentration was found over time. There was no significant difference in β-globulin concentration between the tested groups and the negative control group at all times, except at 12 and 24 hours (Figure 11).

γ-globulin
In groups 1 (vaccine) and 4 (negative control), no significant change was observed in the concentration of γ-globulin over time. The concentration of γ-globulin in group 3 (challenge+-vaccine) initially showed a decrease and then an increase; the lowest γ-globulin concentration was found at 48 and 72 hours. In groups 2 (vaccine+challenge) and 5 (challenge), the concentration of γ-globulin initially showed a decrease and then an increase, whereas there was no significant difference in γ-globulin concentrations from 18 hours to 6 days. Moreover, there was no significant difference in γ-globulin concentrations between the tested groups and the negative control group at all times, except at 48 hours (Figure 12).

Discussion, and Conclusion and Recommendations

The strain and dose of the virus and the immunity of the bird are among the factors that affect the variation in ND clinical signs. In unvaccinated or poorly vaccinated birds, ND clinical signs can be found in the neural, gastrointestinal, reproductive, and respiratory systems (Dimitrova et al., 2017; Miller & Koch, 2013). Maternal antibodies against NDV show a progressive decrease until base levels after a few weeks (Cardoso et al., 2005; Gelb & Jackwood, 1998). The goals of vaccination in ND are to bring about a decrease in virus shedding and a reduction in the clinical signs (Dimitrova et al., 2017; Kapczynski et al., 2013). However, vaccination can be considered as a stressor causing a decrease in food intake, body weight gain, and nutrient digestibility (Kaab et al., 2018). Nishizawa et al. (2007) have evaluated the response of white Pekin ducks during an experimental vaccination and challenge in ND. They indicated that vaccination could interfere with NDV shedding and found that vaccination

against NDV was an important factor in reducing NDV shedding in the field. El-Tayeb et al. (2013) have determined the effect of maternal antibodies to NDV on vaccination with VG/GA vaccine. The minimum titer that interfered with vaccination was obtained on day 22 of the chick's age (less than 3 log2), showing the optimum time for vaccination with VG/GA.

A major part of the innate immune response present in all animal species is acute phase response that reduces the growth of infectious agents and maintains the normal function of an organ by preventing further damage and activating repair mechanisms (Cray et al., 2009; Firouzi et al., 2014; Hirvonen, 2000). Acute phase response indicates systemic activation in inflammatory processes but is not diagnostic of any specific disease (Rath et al., 2009). Several researchers have investigated acute phase response in chickens in common poultry diseases (Chamanza et al., 1999a, Chamanza et al., 1999b; Habibi et al., 2013; Holt & Gast, 2002; Mosleh et al., 2013; Nazifi et al., 2010; Nazifi et al., 2011; Xie et al., 2002b).

Part of the acute phase response includes the production of APP by hepatic origin. The assessment of APP levels is considered as a helpful tool in monitoring poultry health (O'Reilly & Eckersall, 2014).

Hp concentration was significantly higher, especially at 24 and 48 hours in group 5 (challenged with NDV) than in group 4 (negative control), revealing that challenge with NDV causes a significant increase in serum Hp. The concentration of Hp was significantly higher, especially at 24 and 48 hours in group 3 (challenged with NDV+vaccine) than in group 2 (vaccine+challenged with NDV). Milder and less changes in Hp concentration was found in group 2 (vaccine+challenged with NDV) than in group 3 (challenged with NDV+vaccine), showing that vaccination after challenge with NDV results in a higher and stronger Hp response than in vaccination before challenge. At times, there was no significant difference in Hp concentration, group 2 (vaccine+challenged with NDV) and group 1 (vaccine), indicating that vaccination then challenge with NDV compared with vaccination alone did not make much difference in Hp response.

The concentration of SAA in group 5 (challenged with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in SAA concentration. There was a significant increase in the concentration of SAA in group 3 (challenged with NDV+vaccine), especially at 48 hours, than in group 2 (vaccine+challenged with NDV). Milder and less changes in SAA concentration was found in group 2 (vaccine+challenged with NDV) than in group 3 (challenged with NDV+vaccine), showing that challenge with NDV then vaccination could result in a higher and stronger SAA response than with vaccination then challenge with NDV. There was no significant difference in SAA concentration when comparing groups 2 (vaccine+challenged with NDV) and 1 (vaccine), indicating that vaccination then challenge with NDV compared with vaccination alone did not make much difference in SAA response.

Haptoglobin is an α2-globulin and one of the APP whose serum levels increase during infections, inflammations, or tissue damages (Murata et al., 2004). SAA is an apolipoprotein of high-density lipoprotein and one of the major APP whose serum levels elevate following inflammation, physical stress, or at parturition (Murata et al., 2004). SAA and Hp have the most important changes to inflammation among the APP in many animal species (Piñeiro et al., 2007). In chickens, SAA is likely to be a reliable APP for diagnosing inflammatory lesions (Chamanza et al., 1999a; Chamanza et al., 1999b). SAA restrains the oxidative damage, employs immune cells in inflamed tissues, stops pyrexia, and modulates proinflammatory processes (Eriksen et al., 1993; Marques et al., 2017; O'reilly & Eckersall, 2014; Shainkin-Kestenbaum et al., 1991; Uhlar & Whitehead, 1999).

Firouzi et al. (2014) showed that there was a significant increase in SAA concentration in experimentally infected chickens with velogenic Newcastle virus than in healthy chickens. Habibi et al. (2013) have investigated the changes of Hp and SAA in village chickens challenged by a wild NDV. They showed that the concentrations of all variables, except Hp, were significantly higher in vaccinated and challenged birds than in the negative control group. Asasi et al. (2013) have shown that Hp and SAA concentrations of the birds challenged intranasally with infectious bronchitis virus were significantly higher than the healthy birds. The same results have also been shown by Nazifi et al. (2011) and Barbe et al. (2011).

The concentration of OVT in group 5 (challenged with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in OVT concentration. There was a significant increase in the concentration of OVT in group 3 (challenged with NDV+vaccine), especially at 48 hours, than in group 2 (vaccine+challenged with NDV). Milder and less changes in OVT concentration was found in group 2 (vaccine+challenged with NDV) than in group 3 (challenged with NDV+vaccine), showing that challenge with NDV then vaccination could result in a higher and stronger OVT response in comparison with vaccination then challenge with NDV. There was a significant difference in OVT concentration, especially at 48 hours (the range from 24 to 72 hours), between groups 2 (vaccine+challenge with NDV) and 1 (vaccine), indicating that vaccination then challenge with NDV could cause a greater OVT response than with vaccination alone.

Transferrins are found in birds as serotransferrin with hepatic origin and ovotransferrin with oviduct origin (Superti et al., 2007). OVT acts as an APP in birds and elevates in bacterial-, viral-, and chemical-induced inflammations. As long as the inflammation persists, OVT remains elevated (Hallquist & Klasing, 1994; Rath et al., 2008; Rath et al., 2009; Xie et al., 2002a; Xie et al., 2002b). The increased level of OVT during infection may be attributed to its role as an antioxidant against oxidative tissue damage (Giansanti et al., 2015), an immunomodulator (Xie et al., 2003), a preventer of infectious agents' growth (Giansanti et al., 2007), and a protectant in revitalizing homeostasis (Rath

et al., 2009). Moreover, OVT is associated with angiogenesis, a post inflammatory mechanism responsible for wound healing (Cermelli et al., 2000; Eming et al., 2007; Velazquez, 2007). OVT is regulated under inflammatory stress and can be considered as a diagnostic marker of infection and inflammation in chickens (Rath et al., 2009). Carlevaro et al. (1997) and Morgan et al. (2001) have reported that OVT could be upregulated in fibroblasts and chondrocytes in response to the inflammation and infection. Rath et al. (2009) have reported a significant increase in OVT serum levels in chickens infected with *Escherichia coli*, *Eimeria maxima*, and *Eimeria tenella*. Xie et al. (2002a) have reported that OVT is a major APP in chickens.

The concentration of ADA in group 5 (challenge with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in ADA concentration. There was a significant increase in the concentration of ADA in group 3 (challenged with NDV+vaccine), especially at 48 hours, than in group 2 (vaccine+challenged with NDV). Milder and less changes in ADA concentration was found in group 2 (vaccine+challenged with NDV) than in group 3 (challenged with NDV+vaccine), showing that challenge with NDV then vaccination could result in a higher and stronger ADA response than with vaccination then challenge with NDV. There was a significant increase in ADA concentration in group 2 (vaccine+challenged with NDV) than in group 1 (vaccine) at 24 and 48 hours, whereas no significant difference was observed at other times, indicating that vaccination then challenge with NDV could cause an ADA response than with vaccination alone.

ADA has a key role in the purine metabolism as it catalyzes the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine, resulting in regulation of extracellular adenosine level, an anti-inflammatory molecule. Adenosine acts as a sensor to the immune system during tissue damage and acute inflammation. Therefore, ADA is involved in inflammatory responses via adenosine adjustment and protects the host tissue from damage (da Silva et al., 2017; Franco et al., 1997; Frode & Medeiros, 2001; Kumar & Balachandran, 2009). ADA is involved in the cell-mediated immunity, and its activity can be considered as an important biomarker to determine the severity of inflammatory and immune responses in infections (Boiago et al., 2016). Boiago et al. (2016) have reported a significant decrease in ADA activity in serum and a significant increase in ADA activity in the liver tissue of laying hens naturally infected with *Salmonella gallinarum*. Da Silva et al. (2017) have reported that aflatoxin poisoning in quails increased the ADA activity. They reported that cell damage and inflammatory process owing to aflatoxin poisoning caused a decrease in the anti-inflammatory adenosine levels and resulted in an increase in ADA activity. In contrast, Lautert et al. (2014) have demonstrated a reduction in ADA activity in chickens intoxicated by mycotoxins (ochratoxin, zearalenone, and deoxynivalenol).

The concentration of TSA in group 5 (challenged with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in TSA concentration. The increase in TSA in group 3 (challenged with NDV+vaccine) was significantly more than in group 2 (vaccine+challenged with NDV), although significant changes were not found at all times; the increasing trend in TSA between groups 2 and 3 was close. This finding indicates that both vaccination then challenge with NDV and challenge with NDV then vaccination could cause a TSA response, whereas challenge with NDV then vaccination could result in a higher TSA response. The increase in TSA in group 2 (vaccine+challenged with NDV) was significantly higher than in group 1 (vaccine), indicating that vaccination then challenge with NDV could lead to a higher TSA response than with vaccination alone.

The concentration of LBSA in group 5 (challenged with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NVD could result in a significant increase in LBSA concentration. The increase in the concentration of LBSA in group 3 (challenged with NDV+-vaccine) was significantly more than in group 2 (vaccine+challenged with NDV) at 48 hours but not at other times. This result shows that neither vaccination then challenges with NDV nor challenge with NDV then vaccination caused any difference in the LBSA response, except at 48 hours. There was a significant increase in LBSA concentration in group 2 (vaccine+challenged with NDV) at all times than in group 1 (vaccine), indicating that vaccination then challenge with NDV could lead to a greater LBSA response than with vaccination alone.

The concentration of PBSA in group 5 (challenge with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in PBSA concentration. Although PBSA concentration was higher in group 3 (challenged with NDV+vaccine) than in group 2 (vaccine+challenged with NDV), there was no significant difference even at 24 to 72 hours, showing that both vaccination then challenge with NDV and challenge with NDV then vaccination could result in the same PBSA response. The increase in PBSA in groups 3 (challenged with NDV+vaccine) and 2 (vaccine+challenged with NDV) was significantly more than group 1 (vaccine), indicating that both vaccination then challenge with NDV and challenge with NDV then vaccination could cause a higher PBSA response than with vaccination alone.

Sialic acid (SA), an acetylated derivative of neuraminic acid, is widely distributed in mammals' tissue. Sialic acid is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids (Seyrek et al., 2008). The concentration of SA increases rapidly following the inflammatory and injury process. The measurement of serum SA concentration is of importance in the diagnosis and prognosis of inflammation and cancer (Citil et al., 2004). Firouzi et al. (2014) showed that there was a significant difference among TSA, LBSA, and

PBSA concentrations in experimentally infected chickens with velogenic Newcastle virus than in healthy chickens. Habibi et al. (2013) have investigated the TSA, LBSA, and PBSA in village chickens challenged by a wild NDV. They showed that the concentrations of all the variables were significantly higher in vaccinated and challenged birds than in negative control group. Asasi et al. (2013) have showed that TSA, LBSA, and PBSA concentrations of the birds challenged intranasally with infectious bronchitis virus were significantly higher than in the healthy birds. The same results have also been shown by Farsang et al. (2002), Keles et al. (2000), and Nazifi et al. (2011).

No significant difference in the concentration of TP was observed among all the groups at all times. The only change in TP concentration was found in group 5 (challenged with NDV) that showed a significant increase at 72 hours. There was no significant difference in Alb concentration among all groups at all times.

The most increasing trend in α–globulin concentration was found in groups 5 (challenged with NDV) and then group 3 (challenged with NDV+vaccine); the maximum incremental change was observed at 48 hours. There was no significant difference in α–globulin concentration between groups 2 (vaccine+challenged with NDV) and 1 (vaccine) and other groups.

The concentration of β-globulin in group 5 (challenged with NDV) was significantly higher, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV could result in a significant increase in β-globulin concentration. The increase in β-globulin concentration in group 3 (challenged with NDV+vaccine) was significantly more than in group 2 (vaccine+challenged with NDV), indicating that challenge with NDV then vaccination could result in a higher β-globulin response than with vaccination then challenge with NDV. The increase in β-globulin in groups 3 (challenged with NDV+vaccine) and 2 (vaccine+challenged with NDV) was significantly more than in group 1 (vaccine), showing that both vaccination then challenge with NDV and challenge with NDV then vaccination could cause a higher β-globulin response than with vaccination alone.

The concentration of γ-globulin in group 5 (challenged with NDV) was significantly lower, especially at 48 hours, than in group 4 (negative control), revealing that challenge with NDV results in a significant decrease in γ-globulin concentration. The decrease in γ-globulin concentration in group 3 (challenged with NDV + vaccine) was significantly more than in group 2 (vaccine+challenged with NDV), showing that challenge with NDV then vaccination results in a stronger γ-globulin response than with vaccination then challenge with NDV.

Conditions that cause alteration in serum proteins, like infections, could lead to an alteration in the protein roles (Eze et al., 2014). NDV can cause intestinal ulcers and hemorrhages that could lead to malabsorption and loss of proteins (Okorie-Kanu et al., 2016). The decrease in the level of plasma albumin, the most abundant plasma protein, leads to a decrease in the concentration of serum proteins (Harr, 2009; Okorie-Kanu et al., 2016). The loss of albumin in urine, diarrhea, or ulcers and the reduction in albumin production by liver because of insufficient intake of protein in diet may result in a decrease in serum albumin (Ihedioha & Chineme, 2005; Kaslow, 2011). Serum albumin shows a decrease in inflammatory conditions and anorexia and causes osmotic imbalance and dehydration (Lumeij, 2008; Petersen et al., 2004). In acute phase response in chickens, albumin concentrations decrease to 50%–75 % of normal concentrations (Adler et al., 2001; Grieninger et al., 1986; O'Reilly & Eckersall, 2014).

Eze et al. (2014) have investigated the effect of velogenic NDV on the immune responses and serum proteins in chickens. The serum total proteins in infected chickens were significantly higher than the controls on day 7 and 14 PI. By the day 21 PI, the infected chickens presented significantly lower total serum proteins than the control group. The serum albumin level in infected chickens was not significantly different when compared with the control throughout the experimental period. Okorie-Kanu et al. (2016) have investigated the blood biochemistry responses of cockerels experimentally infected with a velogenic NDV strain, KUDU 113. They showed a decreased concentration of total protein and albumin in vaccinated challenged and unvaccinated challenged birds compared with the unvaccinated unchallenged controls; this could be associated with the severity of enteritis that resulted in increased malabsorption and loss of protein. However, no significant difference in total protein concentration was found between vaccinated challenged and unvaccinated challenged birds. This finding could be associated with the vaccine protection that led to little effect on absorption and losses. They reported a significant decrease in total protein and albumin and a significant increase in globulin as early signs of velogenic NDV infection in chickens. This was indicative of the importance of vaccination because of the prevention of mortality and the reduction of pathologic effects on vaccinated challenged chickens. Talebi (2006) reported a significant decrease in the total proteins and albumin values in broilers vaccinated against NDV. The decrease in concentrations of total serum proteins and albumin was attributed to anorexia and enteritis caused by NDV. Anorexia was reported by Ezema et al. (2009) in vaccinated chickens challenged with a velogenic NDV. Chekwube et al. (2014) have assessed the effects of velogenic NDV on the immune responses and serum proteins in chickens. They reported a significant increase in total protein and serum globulins on days 7 and 14 post-infection.

In viral, bacterial, etc. infection, serum proteins have substantial roles and are an essential substrate for antibody synthesis (Eckersall, 2008; Kaslow, 2011; Nnadi et al., 2010; Oladele et al., 2005). Elevations in the level of antibodies, that are gamma globulin, are associated with the inflammatory conditions caused by microorganisms such as viruses (Okorie-Kanu et al., 2016). Eze et al. (2014) have investigated the effects of velogenic NDV on the immune responses and serum proteins in chickens. The serum globulins in infected chickens were signifi-

cantly higher than the control on days 7 and 14 PI but were significantly lower than the control on day 21 PI. The increase in globulin levels during NDV infection in chickens was reported by Snyder (2012) and Okorie-Kanu et al. (2016).

To summarize, the results of the present study indicated that challenge with NDV could lead to a significant increase in the inflammatory factors and acute phase response, whereas vaccination alone could cause a mild increase in these parameters. Challenge with NDV then vaccination could result in a stronger immune system stimulation and higher acute phase response than vaccination then challenge with NDV. On the basis of these findings, vaccination of the chicks with a live vaccine soon after natural infection with virulent NDV may help the chicks to overcome the sequelae of the disease.

Ethics Committee Approval: All animal experiments were approved by the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63; 4/10/2018). The recommendations of the European Council Directive (2010/63/EU) of September 22, 2010, regarding the standards in the protection of animals used for experimental purposes were also followed.

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