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RESEARCH ARTICLE

Evaluation of Antioxidant Parameters in Broiler Chicken after Vaccination and Experimental Challenge with Newcastle Disease Virus

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ABSTRACT

The aim of the present study was to evaluate the pattern of antioxidants in broiler chicken that were vaccinated and experimentally challenged with Newcastle disease (ND) virus. A total of 300 healthy day-old Cobb broiler chicks were divided randomly into four equal groups. The chicks in group 1 and 2 were vaccinated with live B1-ND vaccine. Those in group 2 were injected with a killed vaccine and group 3 chicks received only the adjuvant of killed vaccine. The birds in groups 1, 2 and 3 were challenged with a velogenic NDV and those in group 4 were kept as control. Sampling was done on days 1,2,3,7 after vaccination and on 1, 2, 3,7,14, 21 post challenge. The parameters measured included TAC, MDA, vitamins (A, E, C) and trace elements (Se, Cu, Zn). There was oxidative stress in all groups except control. Significant decrease in TAC was confirmed in all groups except control. The most oxidative stress was clarified in group 1 and the rate of decline for TAC was more significant. There were not specific differences in serum MDA levels between four groups but totally they were respectively higher in the 1-3 groups. According to the results, no significant changes in serum levels of trace elements and vitamins were observed. Although there were significant differences of these trace elements and vitamins between groups on some days, no specific trends were observed. In conclusion the changes indicate that maximum production of oxidative radicals occurs when live vaccine and virus are used concurrently that could be because of more possible inflammatory reaction compared with the other groups.

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INTRODUCTION

Newcastle disease virus (NDV) is the main cause of the Newcastle disease with major concerns in the poultry industry (Alexander and Senne, 2008; Sattar *et al.*, 2016). The NDV causes a serious respiratory and neurological disease and is an economically important infectious agent, causing substantial losses to the poultry industry (Mayo, 2002). The ND predominantly affects various kinds of birds including local, exotic and wild species (Sanda *et al.*, 2008). Different pathotypes of the virus including velogenic, mesogenic and lentogenic have been formerly characterized (Brown *et al.*, 1999). Very high rates of morbidity and mortality of the ND and also considerable reduction in the productivity of the affected birds were previously emphasized (Spradbrow, 1990). Vaccination is a widely used method in the control of viral diseases including ND in the poultry production (Senne *et al.*, 2004; Pansota *et al.*, 2013). As such, the vaccination of herds has been revealed the higher resistance to the field infection and also to reduce shedding of viral particles and eventually decline the transmission (Capua *et al.*, 2005). The substantial role of reactive oxygen species (ROS) in various bacterial, viral and fungal infections was clearly addressed in the literatures (Benzer and Yilmaz, 2009). Loss of equilibrium between amount of oxidant and anti-oxidant agents are leading to the oxidative stress (Benzer and Yilmaz, 2009). Based on their special activities, the anti-oxidant agents have been classified into two distinct groups; enzymatic and non-enzymatic groups. In the first group, various free radicals reducing agents such as vitamins A, E and C, have been categorized. Additionally, few cofactor agents are functioning as anti-oxidant compounds, the trace elements which indirectly involve in

the destruction of the toxic free radicals (Halliwell, 1994). Few trace elements such as zinc, copper and selenium are the main constructive factors of the anti-oxidant enzymes for instance; superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Georgieva *et al.,* 2011). In response to ROS elevations, SOD and GPX are among the main antioxidant enzymes involved in endogenous antioxidant defences in contradiction of ROS (Halliwell, 1994). In oxidative stress formation of different lipid peroxides like MDA (malondialdehyde) which are basically producing during various diseases are resulted from the lipid peroxidation of polyunsaturated fatty acids (PUFA), the process which eventually damage tissues and/or cell (Ostalowska *et al.,* 2006). Kadiam *et al*. (2011) showed that there was a significant increase in the lipid peroxidation levels in cerebrum, cerebellum and optic lobes of chicken exposed to NDV (Kadiam *et al.,* 2013). The possible mechanism of the influence of NDV on Vitamin A is via the changes in the metabolism of retinol binding protein (Clive *et al.,* 1991). Vitamin E treatment will ameliorate the antioxidant status in the NDV infected chickens (Kadiam *et al.,* 2011). Vitamin E supplementation had beneficial effects on chicken serum antibody development against Newcastle virus disease vaccine (Surai, 2002). Poultry have got the ability to synthesize and to modify Vitamin C which mainly caused by the environmental and pathological stressors (McDowell, 2000). Zinc is a cofactor of SOD and at its low concentration can considerably affecting the immune system both in human and animals (Dönmez *et al.,* 2012). Selenium is an essential trace mineral in immune system and is also a cofactor of GPX. In the case of ND infection, an increase in the antibody titre was shown in the presence of Selenium (Se) in diet (Hegazy and Adachi, 2000). The role of Se to produce immunoglobulins is crucial (Savaram *et al.,* 2013). The critical function of Zinc (Zn) and Copper (Cu) to accomplish numerous biological functions in both animal and plant cells has been clearly shown (Yazdankhah *et al.,* 2014). In addition, total antioxidant capacity (TAC) is a test used to measure the total antioxidant status. Therefore, instead of measuring a single antioxidant compound, the total antioxidant capacity of a sample is preferable (Kusano and Ferrari, 2008). The patterns of ample changes in the antioxidant process during challenging with Newcastle disease virus and vaccination against it in broiler chickens could provide comprehensive evidence on the pathogenesis of ND. Therefore, our study was focused to investigate the pattern of antioxidants in broiler chicken that were vaccinated and experimentally challenged with Newcastle disease virus.

MATERIALS AND METHODS

Experimental plan: 300 apparently healthy 1-day-old Cobb broiler chicks were randomly characterized into four equal experimental groups (n=75). They were tested for measurement of maternal antibody by HI test and accordingly chicks were vaccinated at day 20 of their age. The chicks in group 1 and 2 were vaccinated with live B1 ND vaccine. Those in group 2 were additionally injected with a killed vaccine simultaneously and group 3 chicks received only the adjuvant of the killed vaccine. The birds in these groups were challenged with a velogenic ND on day 8 after vaccination. Those in group 4 did not receive any vaccine and were treated as negative control group. Each group was bred in an isolated room and the chicks were obtained feed and water.

Animal ethics: This experiment was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. The recommendations of 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.

Virus and vaccines: A highly virulent NDV field strain (Gene bank accession Number: JF820294.1) was used for challenge study. Lentogenic NDV vaccines B1 (Batch number 08994018), inactivated ND (Batch number 26884842) vaccine and its adjuvant were provided by the Shiraz Branch of Razi Vaccine and Serum Research Institute (Iran).

Samples: Sampling was done on 1, 2, 3,7,14, 21 days post challenging and also on 1,2,3,7 days after vaccination. To separate serum, the whole blood taken from cardiac puncture was labeled, kept at room temperature to clot and centrifuged. The samples were finally kept at -20°C until further use.

Biochemical assays

Total antioxidant capacity (TAC): The commercial kit (Labor Diagnostika Nord (LDN) Com, Nordhorn, Germany) was employed to measure TAC. At the end, a color product of the chromogenic substrate (tetramethylbenzidine) was appeared. The change in color was measured calorimetrically at 450 nm and expressed as millimoles per liter (mmol/L).

Trace elements measurement: Serum samples were digested using combined perchloric and Nitric acid. Trace elements such as Cu, Zn and Se were measured by an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan) which were finally presented as ppm.

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Antioxidant vitamins: The HPLC method was employed to measure antioxidant vitamins. A commercial kit (ALPCO Diagnostics, USA) was used to calculate vitamin C, and the method of Johnson-Davis *et al.* (2009), was used to measure vitamin A and E.

Measurement of malondialdehyde (MDA): The modified HPLC method was employed to measure MDA. Final product was analyzed by UV spectrophotometer at 532 nm and values were finally expressed as mmol/L.

Statistical analysis: Statistical analysis was conducted using SPSS software (version16). Descriptive statistics were expressed as means and standard errors. Means of each variable in the treatment groups and in various times were compared using mixed model analysis. Each subject was considered as random effect in the model and time was introduced as repeated effect. Group, time, and their interaction were considered as fixed effects into the model. In significant cases, adjusted comparison of means was undertaken using Sidak post-hoc test. For all

variables, homogeneity of variances was checked using Leven's test. In the case of high variability of data and non-homogeneity of variances (data for copper), non-parametric tests were used. For comparison of copper between groups in each time point, Kruskal-Wallis analysis of variance was performed. Comparison of copper in successive times in each treatment group was conducted using Friedman test. In all analyses, a P-value less than 0.05 was considered as statistically significant.

RESULTS

In order to address the humoral response of the experimental chickens to the vaccination and challenge infection, serum samples were collected during the experiment and analyzed for HI antibody titer. All vaccinated birds developed antibodies in serum as a response to the vaccination. The increase in antibodies at 7 days post vaccination was seen in group 1 and 2 but no significant difference was observed. On day 2 after challenge significant difference was observed between group 2 and control. The antibody titer increased quickly after challenge with NDV and peaked 3 weeks after challenge.

In group 1 and 2 (vaccinated birds) mortality was low and at the end of the experiment mortality rates were 27.6 and 21.5% in group 1 and 2, respectively, but in group 3 mortality rate was 100%.

The pattern of changes in the serum level of TAC in all the experimental groups is shown in Fig. 1. The serum level of TAC showed significant changes in four groups except for the first three days of sampling. Based on our results, a considerable reduction in the serum levels of TAC in the group 1 and group 2 was observed. The highest level of TAC was observed in sera of group 4 (control) and then in groups 3 (adjuvant + challenge virus), 2 (live and inactivated vaccine + challenge virus) and 1 (live vaccine + challenge virus), respectively.

The serum level of MDA showed significant changes on the second, third and ninth days of sampling in all groups. Our results showed that groups 1 and 2 have greater amount of MDA than the groups 3 and 4. The highest level of MDA was observed in group 1 and then respectively in groups 2, 3 and 4. The pattern of changes in MDA is shown in Fig. 2.

Details of the alterations in the antioxidant trace elements (Zn, Se and Cu) in four experimental groups are presented in Fig. 3A-C. Our results revealed that Selenium showed significant changes in four groups except for the first day and the last day of sampling but there were no regular changes on different days. In contrast, the serum level of zinc only showed significant changes in four groups on the fourth, seventh and ninth days of sampling. The serum level of copper showed no significant changes in four groups and there were no regular changes on different days. There were consider-able variations in the concentration of three trace elements (Zn, Se and Cu) between four groups. Moreover, the pattern of changes in the serum level of antioxidant vitamins (A, E and C) in the groups is presented in Fig. 4A-C. Interestingly, results vary on different days in four groups. The serum level of vitamin A showed significant changes on the first, third, seventh, ninth and tenth days of sampling in four groups. Vitamin C showed significant changes on the first and fifth days of sampling in four groups.

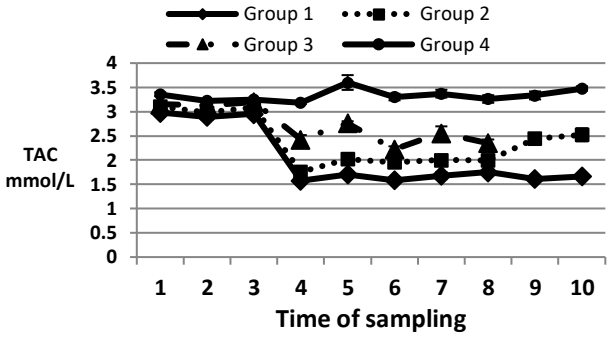

Fig. 1: Pattern of changes in TAC (mmol/L) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

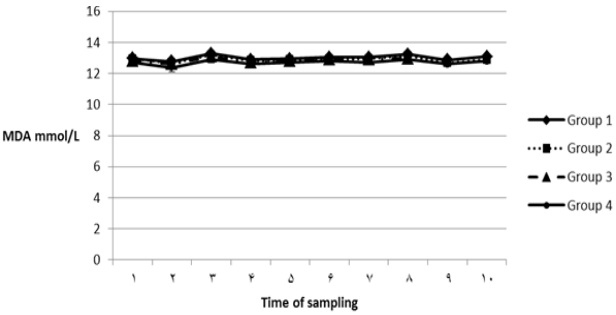

Fig. 2: Pattern of changes in MDA (mmol/L) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

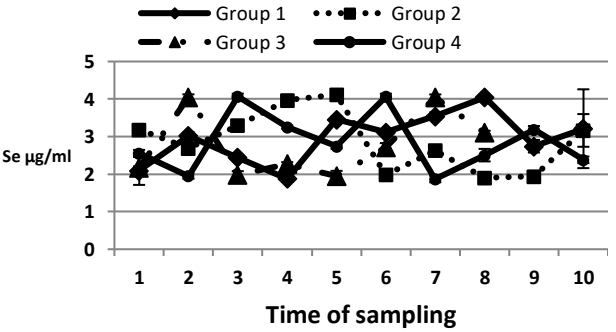

Fig. 3A: Pattern of changes in Se (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

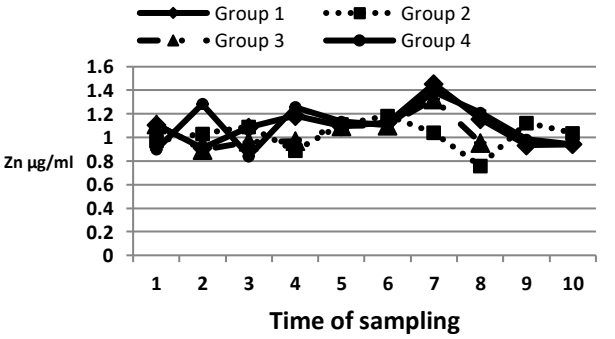

Fig. 3B: Pattern of changes in Zn (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

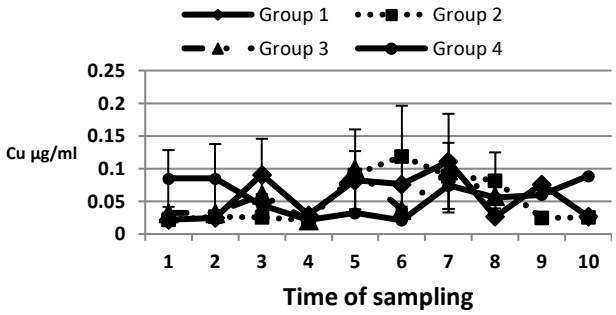

Fig. 3C: Pattern of changes in Cu (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

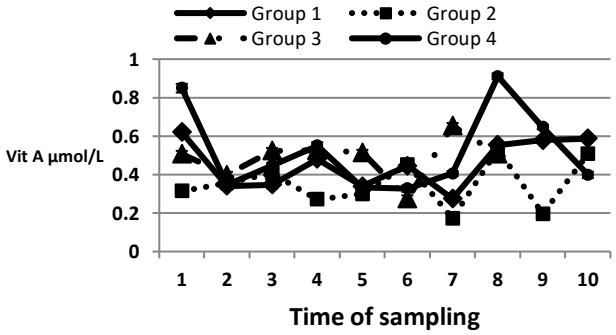

Fig. 4A: Pattern of changes in vitamin A (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

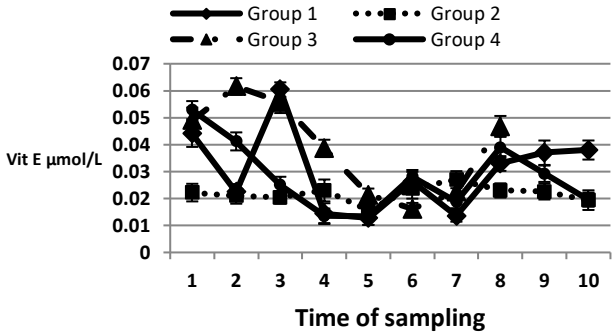

Fig. 4B: Pattern of changes in vitamin E (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

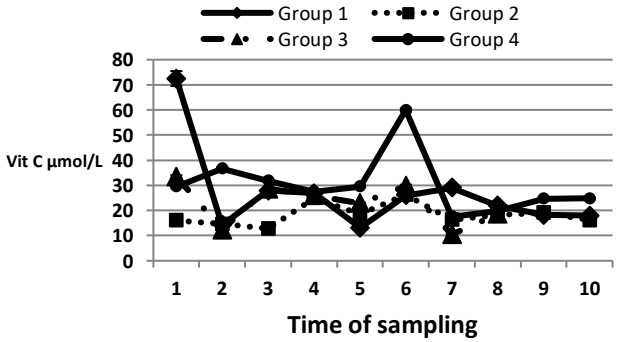

Fig. 4C: Pattern of changes in vitamin C (µg/ml) after experimental challenged with Newcastle disease virus and vaccination on different days (group 1: lived vaccine with virus, group 2: lived and inactivated vaccine with virus, group 3: adjuvant-virus, group 4: control).

Also, there were significant changes for vitamin E in four groups only on the eighth, ninth and tenth days of sampling. There were considerable variations in the concentration of three vitamins (A, E and C) between four groups.

DISCUSSION

Chicken defense against various diseases depends on the efficacy of the immune system responsible for elimination of foreign substances. Macrophage activation in different diseases is regularly accompanied by a so called "respiratory burst", an increase in the production of reactive oxygen species (ROS). Therefore, overproduction of ROS or impaired antioxidant defense can result in oxidative damage to host macromolecules. Newcastle disease challenge is often used to assess immuno-stimulating properties of various antioxidants (Surai, 2002). According to our data, there is oxidative stress in all the groups except control. The oxidative stress is likely due to challenge with virus or combined effect of challenging virus and vaccine that lead to inflammatory reactions. TAC has been considered as the cumulative functions of all the antioxidants and therefore its measurement represents each individual one (Serafini and Del Rio, 2004).

One of the protecting mechanisms contrary to free radicals is TAC, in which, the total capacity of anti-oxidants is expressed (Erel, 2004). So, it is one of the best indicators for investigating antioxidant capacity. In the present study significant decrease in TAC was confirmed in all the groups except control group. The reduction in TAC indicates an increased exposure to oxidative stress products. The major role of total antioxidant capacity in oxidative stress was formerly addressed by others. The level of TAC in groups 1, 2, 3 revealed a remarkable decrease, especially from the third to the tenth days of sampling. Rate of decrease of TAC in group 3 was lower than other groups. It implies that virus induces less oxidative stress in comparison with the use of vaccines and virus together. It might be suggested that the effect of virus alone could not induce more oxidative stress. The oxidative stress in group 2 is more than 3. It may result from confirmatory effect of using vaccine and virus together. In this study the most oxidative stress and inflammation was observed in group 1 and the rate of decline for antioxidant enzymes and TAC was more significant. Therefore, virus and live vaccine together induce more oxidative stress and probably reinforce counterpart effects to produce more oxidative stress and inflammation which infer that this condition promotes more immune system and consequently the maximum production of free radicals. The decreased level of TAC observed until the last day of sampling in groups 1, 2 and 3 implies that oxidative stress exists even in the late stage of infection.

MDA has been widely used for many years as a convenient biomarker for lipid peroxidation and oxidative stress (Ayala *et al*., 2014). The initial parameter to use as an indicator of oxidative stress for the damage of free radicals is the lipid peroxidation mainly affected the phospholipids of cell membrane, therefore, MDA is employed to measure the indicator of lipid peroxidation

caused by free radical damage (Enginar *et al.*, 2006). In this study there were not specific differences in the serum MDA levels between four groups but totally the higher blood levels of MDA were observed respectively in the 1, 2, 3 groups. The process resulted from increasing the production of free radicals and reduced antioxidant enzymes which resulted in lipid peroxidation. It implies that the effects of live vaccine and virus cause the most oxidative stress and subsequently significantly increased MDA compared with others. Also, as mentioned live and inactivated vaccine with virus cause more oxidative stress and MDA than the virus alone.

The principal antioxidant vitamins for tissue defense against free-radical damage include vitamins E, C and β-carotene (McDowell *et al.*, 2007). However, few antioxidant enzyme cofactors including trace elements; copper, manganese, selenium and zinc are essential to protect body from damage caused by free radicals during the oxidative stress (Chaturvedi *et al.*, 2004). In addition to antioxidant effects of vitamin E, selenium and carotenoids, they are considered as immuno-stimulating agents, the function which is more important to influence on growing and development, in the birds (Surai, 2002). Our results showed no significant changes in the serum level of non-enzymatic antioxidant agents including selenium, copper, zinc, vitamin C, A, and E during experimental challenged with Newcastle disease virus in broiler chicken. There were significant differences between groups on some days, but they have no specific trends. As the previous studies such as Anamika *et al.* (2008) showed, in response to elevations of reactive oxygen species, superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are the major antioxidant enzymes involved in endogenous antioxidant defenses against ROS. A subtle balance between production of free radicals and the antioxidant agents is not a synchronized process; thus, the imbalance (reduction in the construction of antioxidants and/or increase in the production of free radicals) caused the oxidative stress. Due to the crucial roles of antioxidant vitamins and trace elements, they are essential to protect tissues against oxidative stress (Revillard *et al.*, 1992). This suggests that probably the first defense against oxidative stress is antioxidant enzyme including SOD and GPX. Furthermore, these vitamins and trace elements have body storage and also poultry diet may have this non-enzymatic antioxidant which led to no significant changes in their serum levels.

Taken together, experimental challenged with Newcastle disease virus and vaccination in broiler chicken triggers oxidative stress on different days and it lasts until the late stage of infection. Probably the first defense against oxidative stress is enzymatic antioxidants. Besides the maximum production of oxidative radicals occurs when live vaccine and virus are used concurrently as a result of more inflammation.

Ethical approval: The study was conducted in agreement with Research Ethics Committee of Veterinary School of Shiraz University.

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Authors contribution: SN and HD conceived and designed the experiments; MA, SN, HD, MAL and MJT performed the experiments and analyzed the data; All authors contributed in finalization of this manuscript.

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