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Full Length Research Paper

The effects of *Echinacea purpurea* dried extract on humoral immune response of broiler chicks to Newcastle vaccination

Adel Feizi[1]* and Farhad Dadian[2]

[1]Department of Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[2]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

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Nowadays, using of live and killed vaccines is usually done to prevent Newcastle disease of poultry; however, some of the poultry farms are being encountered with this disease because the available vaccines do not produce enough antibody titers. In this research, an attempt was made to investigate the effect of using an immune stimulator *Echinacea purpurea* on antibody production against Newcastle vaccine. Four hundred and fifty (450) broiler chicks (Cobb) were divided into five groups and three replicates of 30 chicks per replicate. For six weeks, various doses of dry extract (17, 21, 25, 29 mg/kg) of *E. purpurea* were administered in drinking water to four treatment groups, and placebo was administered to the control group. All groups received Newcastle vaccines on days: 11, 19 and 38. Subsequently, on days 10, 25, 34 and 42, blood samples were taken from each group and Newcastle antibody titers were defined by HI test. This experiment showed that the use of *E. purpurea* in each of the foregoing doses had increasing effects on antibody titers, and this fact is significant between the control group and treatment groups. By using Duncan multiple range test, it was determined that this effect is significant in the case of 1st, 2nd and 3rd groups at 25th days results, but at 34th and 42nd days results, all groups show the same range of titers.

Key words: *Echinacea purpurea,* Newcastle vaccination, antibody titers, broiler chicks.

INTRODUCTION

Newcastle disease is one of the important diseases in the poultry industry and its intensity is different, depending on virus strain, species and the age of host, immunity condition, coincident infections with other organisms and so on (Saif, 2003). Viscerotropic velogenic Newcastle disease, which is the most severe form of the disease, is prevalent in Iran and threatens country's farms. Therefore, an immune modulator has been used in order to enhance immunity system. The herb *Echinacea purpurea* has been used since ancient times in North America and later expanded in Europe. Extract of this plant has many effects; the most important is that it modulates the immune system (Barret, 2003). Many mechanisms have been associated with modulation of the immune system including, activate phagocytosis (Bauer et al., 1989), fibroblasts stimulator (Schranner et al., 1989), increase in respiratory activity (Maas et al., 2005) and increasing leukocyte motility (Bodinet et al., 2002). Medical effects of the herb are antiviral (Eichler et al., 1994), antioxidant, anti-inflammatory, antibiotic (Barret, 2003) and some other effects on natural killer cells (Maas et al., 2005).

In some *in vitro* experiments, it was found that extract of this herb has antiviral activity against several viruses (Berman et al., 1998). Increased activity of natural killer cells under the effect of the herb has been proven by *in vitro* experiments. The herb is efficient in enhancing one of the prominent elements of inherent immunity system (Bodinet et al., 2004). Immunoglobulin or secreted

*Corresponding author. E-mail: a.feizi.iaut@gmail.com, a_feizi@iaut.ac.ir. Tel: +98 914 116 0795.

Table 1. The rate of dried herb were administered in drinking water in treatment groups (mg/kg).

Treatment group	T-group 1	T-group 2	T-group 3	T-group 4
Herb dosage (mg/kg)	17	21	25	29

antibodies by these cells are fundamentals for humoral immunity. Antibodies exist in most body fluids mainly, in serum or blood plasma. These antibodies enter reaction with microorganisms and results to their removal (Mayahi and Bouzarghmehrifard, 2000). This study aimed to investigate the effect of dried extract of *E. purpurea* on antibody titer obtained from Newcastle virus in broiler chicks and its relationship with humoral immunity, as well as evaluating the level of serum antibodies by HI test.

MATERIALS AND METHODS

Four hundred and fifty (450) Cobb broiler chicks were used in this study. They were divided into 5 groups and each group was replicated thrice with 30 birds per replicate. Four groups were selected as treatment groups and one group as the control group. The chicks were distributed in 3 m^2 pens in which floors were covered with straw.

Rearing

Pens, straw and equipment were disinfected with formaldehyde gas. Rearing was in standard condition and its duration was 42 days. Feeding method for all groups was conducted as free access.

Vaccination

B1 vaccine, with serial number 1211t and dead vaccine, with serial number P118307, both made by Razi research and serum producing institute, were used as eye drop and subcutaneous injection respectively on 11th day. La sota vaccine, made by Veternia Company, with serial number 5245047 was administered on the 19th day for Newcastle vaccine. Newcastle vaccine, made by Veternia Company, with serial number 5225054 was used on the 38th day.

E. purpurea treatment

Dried extract of *E. purpurea* obtained from Saha Company Iran, was used in 4 different periods in the treatment groups. Distilled water instead of the herb in identical condition was used for the control group. Also, total bacterial count and total mould and yeast was in standard level and free from *Staphylococcus aurous*, *Pseudomonas aeroginosa*, *Salmonella* and *E. coli*. Used dosages of the herb are shown in Table 1.

Feed conversion ratio (FCR) calculation

The grain was weighed at special hours, daily, and was placed in pens, separately. After removing the remaining grains of previous day, total remaining grains were weighed separately in order to calculate consumed grain by chicks. The chicks from each group were individually weighed weekly for calculating FCR, carefully. Note that in each group; calculating the total amount of feed and dividing it by the live weight, FCR was obtained.

Sampling

Blood sampling was conducted a day before the first vaccination and then, after three times vaccination (blood sampling was conducted by cutting off the chicks' head followed by sampling from wing area on the 10th day). The samples were transferred to the laboratory and the samples' serum were separated at 3,000 rpm centrifuging for 15 min, in order to do the HI test on serum samples. Altogether, samplings were done four times.

RESULTS

The results of HI titer on the 10th day showed that there is no significant difference between treatment and the control groups ($P > 0.05$). This result demonstrates the identical antibodies in all groups before Newcastle vaccine prescribing. HI titer on the 25th day was done so that after B1 vaccine prescribing and dead vaccine on the 11th day, followed by blood sampling on the 25th day, there is no significant difference among the treatment groups ($P > 0.05$).

The results show that all dosages have similar effect on HI titer increase which resulted by B1 and dead vaccine reactions. Based on the Orthogonal experiments, there was a significant difference between treatment and the control groups ($P < 0.05$); suggesting boosting antibody titer in treatment groups compared with the control group. Based on the data analysis by Duncan test for determining the relationship between different levels of medication and HI titer, resulted from B1 and dead vaccine reactions, first, second and third dosages had significant difference with fourth dosage. Based on the data obtained from HI test using one-way variance analysis of variance on the 34th day, the mean serum antibody was not significant among treatment groups ($P > 0.05$) but based on Orthogonal test there was significant difference between treatment and control the groups ($P < 0.05$). Based on the data analysis using Duncan test, in order to determine the relationship among different levels of medication and titer obtained by La sota vaccine, there was no significant relationship among HI titer of different levels of medication.

The results of HI titration on the 42nd day, following vaccination on the 38th day, are as follows: one-way analysis of variance showed that the mean serum antibody after vaccination was not significantly different

Table 2. The rate of Newcastle HI antibody titer obtained from groups on 10th, 25th, 34th and 42nd day (mean ± SD).

Day	*T-group 1	T-group 2	T-group 3	T-group 4	Control group
10	2.214±0.13[a]	2.225±0.15[a]	2.217±0.03[a]	2.213±0.09[a]	2.228±0.04[a]
25	5.142±0.54[a]	5.150±0.78[a]	5.480±0.19[a]	4.918±0.13[a]	4.018±0.12[b]
34	5.241±0.24[a]	5.711±0.32[a]	5.840±0.83[a]	5.690±0.17[a]	4.120±0.16[b]
42	6.520±0.11[a]	6.340±0.63[a]	6.780±0.20[a]	6.480±0.22[a]	5.610±0.01[b]

Different superscripts on means show significant difference (P < 0.05).

Table 3. Comparative study of mortality percentage and FCR of treatment and control group.

Group	Mortality percentage	Feed conversion ratio
T - group 1	1.68[a]	1.610[a]
T - group 2	2.5[b]	1.653[a]
T - group 3	1.43[a]	1.615[a]
T - group 4	2.60[b]	1.642[a]
Control group	3.45[c]	1.780[b]

Different superscripts on means show significant difference (P < 0.05).

among treatment groups (P > 0.05) but based on Orthogonal test, significant difference was observed between treatment and the control groups (P < 0.01). Based on the data analysis using Duncan test for determining the relationship among different levels of medication and antibody titer obtained from La sota vaccine, there was no significant difference among obtained HI titers from different levels of medication, and all treatment groups had increased antibody titer in similar extent (Table 2).

Table 3 demonstrates mortality percentage in the treatment and control groups. Based on statistical analysis using one-way analysis of variance, there was significant difference among T - group 1 and T - group 3, with T - group 2 and T - group 4 (P < 0.05). Furthermore, based on comparative analysis of Orthogonal test, the difference between treatment and control groups was significant (P < 0.01). Based on the data analysis using Duncan test in order to determine the relationship among different level of medication and mortality percentage among treatment groups, there is more difference about groups 1 and 3. The FCR between treatment and control is shown in Table 3. Based on statistical studies, there was no significant difference among treatment groups (P > 0.05) but the difference between treatment groups and the control group is significant (P < 0.05).

DISCUSSION

Newcastle disease is one of the important diseases in poultry industry and its intensity is different, dependent on virus strain, species and the age of host, immunity condition, coincident infections with other organisms, and so on (Alexander, 2003). Viscerotropic velogenic Newcastle disease which is most severe form of the disease is prevalent in Iran and threatens country's farms. Most of the vaccination programs produce no complete immunity against Newcastle disease; so for obtaining high antibody titer in order to prevent birds form the disease, strengthening immunity compounds are suggested.

Based on studies, the effects of this herb extract on immune modulation have been proved (Allen, 2003; Barret, 2003). Barret (2003) showed, by *in vitro* experiments that *E. purpurea* increases activity of natural killer cells as the first defensive layer against viral infected cells; therefore has effective influence on inherent immunity system (Barret, 2003). Chaves et al. (2007) and Linda et al. (2002) showed the herb's effects on inhibition of hyaluronidase activity, stimulation of adrenal activity and stimulation of interferon production in treatment groups (Chaves et al., 2007; Linda et al., 2002). Based on the studies conducted by Skwarek et al. (1996), *E. purpurea* dried extract can stimulate Interferon (IFN) in cell culture viral infected animals studies in the case of increased immunity stimulation.

Based on the studies conducted by Zhai et al. (2007), antiviral and antibiotic effects of the herb have been proved; therefore, significant reduction in mortality rate of treatment groups compared with the control group accounts for the herb's properties because the control of bacterial infections especially *E. coli*, have an important role in reducing mortality rate. This research showed that

dried extract of *E. purpurea* can stimulate the immune system and increase the immune response of Newcastle vaccination. These finding are compatible with Allen et al. (2003), Bodinet et al. (2004), Frieier et al. (2003) and Mass et al. (2005) study results. Based on the results obtained from the present study, the effect of any four dosages of the herb was observed in increased rate of HI antibody titer. Furthermore, mortality percentage reduction and FCR improvements was seen in treatment groups compared with the control group; that the least rate of mortality was related to treatment groups 1 and 3, and the best FCR related to treatment group 1. Then, it can be concluded that the use of the herb's extract leads to increase immunity level and the rate of antibody titer obtained from vaccination against Newcastle virus. The extract causes a reduction in the complications and mortality rate of the disease, as well as stress reduction that leads to increase immunity and disease reduction. Therefore, we suggest that the rate of 25 mg/kg *E. purpurea* dried extract should be used in broiler chicks' drinking water because it produced the highest Newcastle HI antibody titer and best performance.

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