Evaluating the effect of *Hypericum perforatum* on antibody titers obtained from B1 and La Sota vaccines in broiler chicks with HI test

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Accepted 30th May, 2012

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 titres. In this research, an attempt was made to investigate the effect of using an immune stimulator named *Hypericum perforatum* on antibody production against Newcastle vaccine. 450 broiler chicks (Ross 308) were divided into five groups and three replicates of 30 chicks per replicate. For six weeks, various doses of dry extract (16, 20/5, 24/5 and 28/5 mg/kg) of *H. perforatum* 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 titres were defined by HI test. This experiment showed that the use of *H. perforatum* in each of the foregoing doses, had increasingly effects on antibody titres 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 titres.

**Key words:** *Hypericum perforatum*, B1, La Sota, antibody titers, HI test and broiler chicks.

**INTRODUCTION**

Newcastle disease is one of the important diseases in poultry industry that 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). Viscerotrophic velogenic Newcastle disease which is the most severe form of the disease is prevalent in Iran and treats country's farms. Therefore, an immunity stimulant was used in order to enhance immunity system. The herb *Hypericum perforatum* or St John’s Wort is one of the Hypericaceae families (Re et al., 2003). Medical effects of the herb are antibiotic (Mennini and Gobbi, 2004), antiviral (Meruelo et al., 1988), antioxidant (Benedi et al., 2004), anti-stress (Franklin et al., 2004), anticancer (Hostanska et al., 2003), anti-depression, and some other effects on natural killer cells (Helgason et al., 2000).

In some *in vitro* experiments, it was found that Hypericin available in this herb has antiviral activity against several viruses (Jacobson et al., 2001; Lau et al., 1998). Increased activity of natural killer cells under the effect of the herb has been proven by *in vitro* experiments. Regarding that, these cells are of inherent immunity and considered as the first defensive layer against infected cells by virus; so the herb is efficient in enhancing one of the prominent elements of inherent immunity system (Lau et al., 1998). Immunoglobulin or secreted 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 causes to their removal (Chauhan, 1993; Mayahi and Bouzarghmehrifard, 2000). This study aimed to investigate the effect of dried extract of *H. perforatum* on antibody titer obtained from Newcastle vaccination in broiler chicks and its relationship with humoral immunity as well as evaluating the rate of serum antibodies by HI test.

**MATERIALS AND METHODS**

450 Ross 308 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 1 group as the control group. The chicks were distributed in 3 m² pens which floors was covered with straw.

**Keeping and rearing**

Pens, straw and equipment were disinfected with formaldehyde gas. Rearing has been 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 1210t and dead vaccine, with serial number P118306, 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 Co., with serial number 5245046 was administered on 19th day for Newcastle vaccine. Newcastle vaccine, made by Veternia Co., with serial number 5225053 was used on 38th day.

**Medicine prescribing**

Dried extract of *H. perforatum* obtained from Saha Co. Iran, was used in 4 different periods in treatment groups. Distilled water instead of the herb in identical condition was used for control group. The extract was standardized based on the rate of chikuric acid and minimum acid content was 1.4%. Also total bacterial count and total mould and yeast was in standard level and free from *Staphylococcus aurous*, *Pseudomonas aeroginosa*, *Salmonella* and *Escherichia coli*. Used dosages of the herb were as shown in the Table 1.

**FCR calculating**

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 divide it by 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 to sampling from wing area on 10th day). The samples were transferred to the laboratory and the samples’ serum separated by 3000 rpm centrifuging for 15 min in order to do the HI test on serum samples. Altogether, samplings were done 4 times; so 420 samples were obtained for experiments.

**RESULTS**

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

The results of HI titration on 10th day showed that there is no meaningful difference between treatment and control groups (P > 0.05). This result demonstrates the identical antibodies in all groups before Newcastle vaccine prescribing. HI titration on 25th day was so after B1 vaccine prescribing and dead vaccine on 11th day followed by blood sampling on 25th day, there is no significant difference among all groups (P > 0.05). The results showed that all dosages have similar effect on HI titration increase resulted by B1 and dead vaccine reactions. Based on Orthogonal experiments, there was a significant difference between treatment and control groups (P < 0.05); suggesting boosting antibody titer in treatment groups compared with control group. Based on data analysis by Duncan Test for determining relationship between different levels of medication and HI titer resulted from B1 and dead vaccine reactions. Based on Orthogonal experiments, there was a significant difference between treatment and control groups (P < 0.05); suggesting boosting antibody titer in treatment groups compared with control group. Based on data analysis by Duncan Test for determining relationship between different levels of medication and HI titer resulted from B1 and dead vaccine reactions. Based on Orthogonal experiments, there was a significant difference between treatment and control groups (P < 0.05); suggesting boosting antibody titer in treatment groups compared with control group. Based on data analysis by Duncan Test for determining relationship between different levels of medication and HI titer resulted from La sota vaccine, there was no meaningful relationship among HI titer of different levels of medication. The results of HI titration on 42nd day following vaccination on

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>T-group 1</th>
<th>T-group 2</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Herb dosage</td>
<td>16</td>
<td>20/5</td>
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38th day are as follows: one-way analysis of variance showed that the mean serum antibody after vaccination was not significantly different among groups (P > 0.05), but based on Orthogonal Test, significant difference was observed between treatment and control groups (P < 0.01). Based on 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 meaningful difference among the ones obtained in HI titers from different levels of vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful difference among the vaccine, there was no meaningful 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studies on rats that the extract of the herb can reduce brain's cortisol and corticosterone. Mentioned findings about rats stress reduction conform to the present study findings; such that the herb's extract caused mortality and FCR reduction in treatment groups. Based on 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 control group; that the least rate of mortality 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 disease. The extract causes to reduce 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 24/5 mg/kg  that leads to increase immunity and disease reduction.

REFERENCES


