Evaluation The Effect Of Hypericum Perforatum Dried Extract On Antibody Titer Obtained From Newcastle Vaccine In Broiler Chicks

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Abstract: Nowadays using of lived and killed vaccines is usual due to prevention of Newcastle disease of poultry; however, some of the poultry farms are encountering with this disease, because the available vaccines do not produce high antibody titers. According to direct correlation of antibody titers against virus and the protection rate against disease, in this research an attempt is made on investigating the effect of usage of an immunostimulator named Hypericum perforatum on antibody production against Newcastle vaccine. 450 broiler chicks (Cobb) divided to five groups and three replicates by including 30 chickens per replicate. For six weeks various doses of dry extract of Hypericum perforatum is prescribed in water to four treatment groups and placebo is prescribed to control group. All groups received Newcastle vaccines on day: 11, 19 and 38. Subsequently, on days 10, 25, 34 and 42 blood samples is taken from each group (7 chicks from each replicate) and Newcastle antibody titers is defined by HI test. This experiment showed that the use of Hypericum perforatum in each of foregoing doses, has suitable effects on antibody titer increase and this fact is significant between the control group and treatment groups (P<0.05). By using Duncan multiple range test, it is determined that this effect is significant in the case of 1st, 2nd and 3rd groups at 25th day's results, but at 34 and 42 day's results all groups show a same range of titers. It is also revealed that this use of Hypericum perforatum induces FCR improvement and mortality rate decrease which is significant in comparison treatment groups with control group (P<0.05). This effect is significant in the case of 1st and 2nd groups for FCR and in the case of 1st group for mortality rate.

Key words: Hypericum perforatum; Newcastle antibody titre; broiler chicks

INTRODUCTION

Newcastle disease is one of the important diseases in poultry industry that its intensity is different depend on virus strain, species and the age of host, immunity condition, coincident infections with other organisms, and so on Saif, Y.M., (2003):. Viscerotropic velogenic New castle disease which is most sever 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 Jhon'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., 2033), 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 proved 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 humeral immunity. Antibodies exist in most body fluids mainly in serum or blood plasma. Bird's exposure to microorganisms causes to stimulate especial antibodies. These antibodies enter reaction with microorganisms and causes to their removal (Chauhan, H.V.S. 1993; Mayahi, M. Bouzargmehrifard, M.H. 2000). The study aimed to investigate the effect of dried extract of Hypericum perforatum on titer obtained from Newcastle vaccine in broiler chicks and its relationship with humeral immunity as well as evaluation the rate of serum antibodies by HI test.

MATERIALS AND METHOD

450 Cobb broiler chicks were used in this study. They were divided to 5 groups and each group was divided to 30 chicks with 3 replications. Four groups were selected as treatment groups and 1 group as the control group. The chicks were distributed in three m² pens which floors covered with straw.
A: keeping and rearing:
Pens, straw, and equipments 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.

B: Vaccination:
B1 vaccine, made by Razi research and serum producing institute, with serial number 1210t and dead vaccine, made by Razi research and serum producing institute, with serial number P118306 were used as eye drop and subcutaneous injection on 11th day and La sota vaccine, made by Veternia Co., with serial number 5245046 on 19th day for Newcastle vaccine. Newcastle vaccine, made by Veternia Co., with serial number 5225053 was used on 38th day.

C: Medicine prescribing:
Dried extract of Hypericum 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 E.coli. Used dosages of the herb are as following table:

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>T-group 1</th>
<th>T-group 2</th>
<th>T-group 3</th>
<th>T-group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herb dosage</td>
<td>17</td>
<td>21/5</td>
<td>25/5</td>
<td>29/5</td>
</tr>
</tbody>
</table>

D: FCR calculating:
The grain was weighted at especial hours, daily and was placed in pens, separately. After removing the remaining grains of previous day, total remaining grains were weighted separately in order to calculate consumed grain by chicks. The chicks were individually weighted weekly for calculating FCR carefully.

E: 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 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 AND DISCUSSION

<table>
<thead>
<tr>
<th>group day</th>
<th>T-group 1</th>
<th>T-group 2</th>
<th>T-group 3</th>
<th>T-group 4</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2/214±0/014²</td>
<td>2/222±0/02²</td>
<td>2/214±0/014⁴</td>
<td>2/214±0/08⁴</td>
<td>2/236±0/03³</td>
</tr>
<tr>
<td>25</td>
<td>5±0/76⁶</td>
<td>5/19±0/22⁴</td>
<td>5/333±0/17⁶</td>
<td>4/761±0/84⁴</td>
<td>3/952±0/16⁶</td>
</tr>
<tr>
<td>34</td>
<td>5/904±0/44⁴</td>
<td>5/813±0/36⁶</td>
<td>6/0±0/14⁴</td>
<td>5/809±0/22⁴</td>
<td>5/142±0/14⁴</td>
</tr>
<tr>
<td>42</td>
<td>6/512±0/20⁴</td>
<td>6/123±0/27⁷</td>
<td>6/812±0/17⁷</td>
<td>6/311±0/21⁷</td>
<td>5/544±0/7⁹</td>
</tr>
</tbody>
</table>

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

Based on results obtained from table 2, titration result that was conducted on 10th day and before vaccination, 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 that after B1 vaccine prescribing and dead vaccine on 11th day followed by blood sampling on 25th day, there is no significant statistical difference among all groups (P > 0.05). The results shows that all dosages have similar effect on HI titration increasing resulted by B1 and dead vaccine reactions. Based on Orthogonal experiments, there is meaningful difference between treatment and control groups (P <
0.05); suggesting increased more antibody titer in treatment groups compared with control group. Based on data analysis by Duncan Test for determining relationship between different levels of medicine and HI titer resulted from B1 and dead vaccine reactions, first, second and third dosages have meaningful difference with fourth dosage. Based on data obtained from HI test using one-way variance analysis on 34th day, the mean serum antibody isn't meaningful among all groups on 19th day (P > 0.05) but based on Orthogonal Test there is meaningful difference between treatment and control groups (P < 0.05). Based on data analysis using Duncan Test in order to determine the relationship among different levels of medicine and titer obtained by La sota vaccine, there is no meaningful relationship among HI titer of different levels of medicine and all groups has been increased antibody titer identically. The results of HI titration on 42nd day following vaccination on 38th day are as follows: one-way variance analysis shows that the mean serum antibody after vaccination is not meaningfully different among groups (P > 0.05) but based on Orthogonal Test, significant meaningful difference is observed between treatment and control groups (P < 0.01). Based on data analysis using Duncan Test for determining the relationship among different levels of medicine and antibody titer obtained from La sota vaccine, there is no meaningful difference among obtained HI titers from different levels of medicine and all treatment groups have increased antibody titer in similar extent.

Table 3: comparative study of treatment and control group functions

<table>
<thead>
<tr>
<th>group</th>
<th>Mortality percentage</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T – group 1</td>
<td>1/5⁵</td>
<td>1/602²</td>
</tr>
<tr>
<td>T – group 2</td>
<td>2⁹</td>
<td>1/638⁴</td>
</tr>
<tr>
<td>T – group 3</td>
<td>1/5⁵</td>
<td>1/644²</td>
</tr>
<tr>
<td>T – group 4</td>
<td>2⁹</td>
<td>1/631⁴</td>
</tr>
<tr>
<td>Control group</td>
<td>4/83³</td>
<td>1/764¹</td>
</tr>
</tbody>
</table>

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

Table 3 demonstrates mortality percentage in treatment and control groups. Based on statistical analysis using one-way variance, there is meaningful difference among all groups (P < 0.05). Furthermore, based on comparative analysis of Orthogonal Test, the difference between treatment and control groups is meaningful (P < 0.01).

Based on data analysis using Duncan Test in order to determine the relationship among different level of medicine and mortality percentage among treatment groups, there is more difference about groups 1 and 3. The rate of FCR between treatment and control has been shown in table 3. Based on statistical studies there is no meaningful difference among all groups (P > 0.05) but the difference between treatment groups and control group is meaningful (P < 0.05).

**Discussion and conclusion:**

Newcastle disease is one of the important diseases in poultry industry that its intensity is different depend on virus strain, species and the age of host, immunity condition, coincident infections with other organisms, and so on (Alexander, D.J. 2003). Viscerotropic velogenic Newcastle disease which is most sever form of the disease, is prevalent in Iran and treats country's farms. Most of vaccination programs produce no complete immunity against Newcastle disease; so for obtaining high antibody titer in order to prevent saver form the disease, strengthening immunity compounds are suggested. Helgason et al. showed by in vitro experiments that Hypericum Perforatum Increases activity of natural killer cells as the first defensive layer against viral infected cells; therefore has effective influence on inherent immunity system (Helgason et al., 2000). Hostanska et al. (2003) showed the herb's effects on malignant cancer cells in human body. Prince et al. proved antivirusing effects of the herb against cattle diarrhea virus by in vitro experiments (Prince et al., 2000). Meruelo et al. identified antivirusing effects of the herb against leukemia (Meruelo et al., 1988). Tang et al., (1990) showed that Hypercin available in the extract of the herb is an antivirus against leukemia. Kraus et al., (1990) proved hypercin effect on horses' infectious anemia. Lavie et al., (1989) identified that Hypercin is an antivirus against attenuator virus of mice immunity; also, Lenard et al proved hypercin antiviral function against vesicoloestomatit (Lenard et al., 1993).

The role of Hypericum perforatum extract in stimulating immunity system for increasing Antibody titer because of vaccination, which investigated in the present study on in vitro models(human and mouse) conforms to Helgason et al., (2000) studies in the case of increased immunity stimulation. Based on studies conducted by Mennini et al., (2004) antiviral and antibiotic effects of the herb have been proved; therefore, meaningful reduction in mortality rate of treatment groups compared with 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.
Trofimiuk et al., (2005) showed that the herb reduces the mice disorders resulted by chronic stresses, also El-sherbing et al., (2003) proved anti-stress effects of the herb on mice brains. It was found in Franklin et al., studies (2004) 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's 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 New castle 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. Finally, the extract leads to reduce mortality percentage and improvement of poultries' function.

REFERENCES


