The Effect of Antibiofin® on the Immune Response Against Avian Influenza Subtype H9N2 Vaccine in Broiler Chickens

Forough Talazadeh1*, Mansoor Mayahi1, Marziye Naghavi2

1Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Abstract

Background: Some herbs such as thyme (Thymus vulgaris) are rich in flavonoids, act as antioxidants, and may improve the immune function.

Objectives: This study was performed to investigate the effects of Antibiofin® (mostly including Thymus vulgaris) in drinking water on immune response against avian influenza (AI) subtype H9N2 vaccine of broiler chickens.

Materials and Methods: One hundred eighty one-day-old broiler chickens were purchased and divided into 4 equal groups. Chickens of groups A and B received 0.1% and 0.2% Antibiofin® respectively in their drinking water. Chickens of group C did not receive Antibiofin® but were vaccinated against AI. Chickens of group D were not vaccinated against influenza disease and did not receive Antibiofin®. All groups except group D were vaccinated with AI-ND killed. Blood samples were collected before vaccination as well as after vaccination on days 14, 21 and 28, and antibody titer against influenza disease vaccine was determined by hemagglutination inhibition (HI) test.

Results: The results of this study showed that receiving Antibiofin® at 0.1% and 0.2% concentrations, 14 and 28 days after vaccination, could increase the specific antibody titer against avian influenza subtype H9N2 vaccine compared to the control group.

Conclusions: Antibiofin® enhanced the systemic antibody response against avian influenza subtype H9N2 vaccine in broiler chickens.

Received January 20, 2016; Revised May 3, 2016; Accepted May 15, 2016

Background

Influenza viruses belong to the family of Orthomyxoviridae, and are divided into three types of A, B, and C. These viruses are segmented, and have negative-strand RNA. All three types of influenza viruses can infect humans, but only the type A viruses can infect birds and are referred to as avian influenza viruses (AIVs). Type A influenza viruses are divided into subtypes based on the hemagglutinin (HA) and neuraminidase (NA) proteins. Ten NA subtypes (N1–N9) and 17 HA subtypes (H1–H16) are described currently. Since H9N2 virus and human influenza viruses have similar receptor binding epitopes, hence they have a broader host range, and can infect humans. In addition, H9N2 AIV infection in chickens is latent and easily overlooked; thereby increasing the chance of infecting humans. For turkeys and chickens, clinical signs reflect abnormalities in the digestive, respiratory, reproductive, and urinary organs. In breeders and layers, hens may show decreased egg production and increased broodiness. In addition, domestic poultry may show generalized clinical signs including decreased feed and water consumption, occasionally diarrhea, ruffled feathers, huddling, listlessness, lethargy, and decreased activity. Nowadays, using antibiotics at sub-therapeutic levels has caused concerns about antibiotic residues in the animal productions which lead to the development of drug-resistant bacteria in animals and human. Thus, at the beginning of 2006, in the European Union, medical and public concerns focused on the complete deletion of the antibiotics from animal food. Therefore, in poultry industry, it is important to replace antibiotic growth promoters in the food by other substances. Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut. Recently some substances such as phytotherapeutic feed additives, prebiotics, and probiotics have been used instead of antibiotics. Herbal plants have some effects on animal immune system including stimulation and suppression of the indicators of non-specific defense mechanism, and humoral and cellular im-
munities. Nutrition is a critical determinant of immune responses.\textsuperscript{10,11} Natural products can be used as immunostimulants. Herbal plants mostly exert their beneficial effects on animal immune system by their secondary metabolites.\textsuperscript{12} The immunostimulating activities of many of herbal plants have been studied in chicken, human, and mouse cell lines.\textsuperscript{13-15} For example, the steroidal sapoin of ginseng can stimulate the immune system by the production of cytokines (TNF-α, IL-2, IL6, and INF-γ), lymphocyte activity, and macrophage activation.\textsuperscript{16} \textit{Gingko biloba} can mediate the production of inflammatory cytokines because it has flavonoids and terpenes.\textsuperscript{17} Saponins can stimulate humoral and cellular immunities.\textsuperscript{18} In some studies, they induced the production of cytokines such as interferons and interleukins.\textsuperscript{19,20} The immunostimulant activity of saponins may be based on aldehyde groups or branched sugar chains\textsuperscript{21} or an acyl residue bearing the aglycone.\textsuperscript{22} The immunomodulatory effects of herbal plant polysaccharides have also been widely studied.\textsuperscript{23,24} Qiu and Cui\textsuperscript{24} reported that administration of four Chinese herbs including Astragalus root, Achyranthes root, Isatis root, and Chinese Yam can significantly enhance the antibody titer in vaccinated chickens. Beta-sitosterol and its glycoside can have immune modulating activities.\textsuperscript{25} This phytosterol complex may target the TH1 and TH2 cells, improving T-lymphocyte and natural killer cell activities.\textsuperscript{26} Moreover, Chinese herbs can help the development of immune organs such as the spleen and thymus\textsuperscript{27} as well as increase of antibody production. Some herbs that are full of flavonoids such as thyme (\textit{Thymus vulgaris}) increase the activity of vitamin C, act as antioxidants, and seem to improve the immune function.\textsuperscript{27,28} Carvacrol and thymol are the main phenolic components in \textit{Thymus vulgaris}.\textsuperscript{29} This study was conducted to investigate the effect of different levels of Antibiofin\textsuperscript{®} (mostly including \textit{Thymus vulgaris} and other extracts such as \textit{Salvia officinalis}, \textit{Satureja hortensis}, and \textit{Agastache foeniculum}) on systemic antibody responses against influenza disease vaccine in broiler chickens.

**Objectives**

This study was performed to survey the effect of Antibiofin\textsuperscript{®} (mostly including \textit{Thymus vulgaris}) in drinking water on immune response against AI subtype H9N2 vaccine of broiler chickens.

**Materials and Methods**

**Thyme Extract**

Antibiofin\textsuperscript{®} (mostly including \textit{thymus vulgaris} and other extracts such as \textit{Salvia officinalis}, \textit{Satureja hortensis}, and \textit{Agastache foeniculum}) was purchased from Parsimendaru Company, commercially as solution.

**Experimental Design**

One hundred eighty one-day-old broiler chicks, Ross strain, were purchased and 20 chicks were randomly bled for determination of vaccination time. The remaining chicks were divided into 4 equal groups and each group was divided into 4 subgroups including 10 chicks. Chicks of groups A and B received 0.1% and 0.2% Antibiofin\textsuperscript{®}, respectively, in their drinking water from one week before vaccination till 2 weeks after vaccination. Chicks in group C did not receive Antibiofin\textsuperscript{®} but were vaccinated against AI. Chicks of group D were not vaccinated against influenza disease and did not receive Antibiofin\textsuperscript{®}. All groups except group D were vaccinated with AI-ND killed vaccine (subtype H9N2) subcutaneously in the back of the neck on 9 days old.

**Blood Collection and Serological Tests**

Ten chicks of each group were randomly bled and blood samples were collected before vaccination as well as after vaccination on days 14, 21 and 28 and antibody titer against influenza disease vaccine was determined by HI test. Blood samples were obtained from the brachial vein and sera were separated and frozen at -20°C until the serological tests were performed. Serum samples were analyzed by hemagglutination inhibition (HI) test to detect antibodies against AIV according to Alexander et al.\textsuperscript{30}

**Microplate Hemagglutination Inhibition Assay**

Beta procedure of microplate HI test was performed in U-bottomed 96-well microtiter plates to determine the antibody level of the sera of different groups. One percent of chicken erythrocytes were used in this test. The test was conducted using constant 4HA unit AIV virus and diluted.\textsuperscript{30}

**Statistical Analysis**

The titers obtained by HI test were subjected to SPSS version 18.0. One-way Analysis of variance (ANOVA) LSD test was performed to determine the significant differences in HI titers of chickens of each group after vaccination. Means were compared at a significance level of 5%.

**Results**

According to Table 1, 14 days after vaccination, there was significant difference between group D and all other groups (\textit{P}<0.05). There was also significant difference between groups A and C and between groups B and C, and antibody titers in groups A and B were higher than those in group C (\textit{P}<0.05). Twenty-one days after vaccination, there was significant difference between group D and all other groups (\textit{P}<0.05). Twenty-eight days after vaccination, there was significant difference between group D and all other groups (\textit{P}<0.05). Twenty-eight days after vaccination, there was significant difference between groups A and C and between groups B and C (\textit{P}<0.05), and antibody titers in groups A and B were higher than those in group C. The results of present study showed that 14 and 28 days after vaccination, receiving 0.1% and 0.2% Antibiofin\textsuperscript{®} significantly increased the specific antibody response to AIV compared to all groups.

**Discussion**

Improving immunity in poultry production is of very
importance as it prevents common serious diseases. A variety of different factors such as vaccination failure or vaccination quality, content of experimental diets, and effect of some immunosuppressive diseases can induce immunodeficiency. The studies of the immune system have shown that some herbs such as coneflower (Echinacea purpurea) are most effective in immune system improvement, because of stimulation of non-specific immune system. It is thought that certain flavonoids, polysaccharides, and isobutylamides of Echinacea can enhance the immune system. \(^{31}\) Herbs like thyme (Thymus vulgaris) that are full of flavonoids, can extend the activity of vitamin C, act as antioxidants, and may enhance the immune responses. \(^{32,33}\)

The results of present study showed that 14 and 28 days after vaccination, receiving 0.1% and 0.2% Antibiofin®, significantly increased the specific antibody response to AI vaccine compared to all groups \((P<0.05).\)

In disagreement with our results, Teymouri Zadeh et al studied the effect of thymus vulgaris extract on antibody responses to red blood cell and Newcastle disease virus. They reported that there was no difference between 0.1% Thymus vulgaris extract received by birds and control group. \(^{34}\) Albumin to globulin ratio, heterophile to lymphocyte ratio, and antibody titer against sheep red blood cell, Influenza and Newcastle viruses in broilers treated with 5 and 10 g/kg thyme powder showed no significant differences with control birds. \(^{35}\) Rahimi et al reported that dietary thyme extract (0.1%), soluble in water, did not affect immune system compared to control group \((P<0.05).\) \(^{36}\) The small effects of thyme extract on immune system in some researches are probably related to the type of thyme, dose of additives, vaccination schedule, and stimulating material that was used in their study.

The results of this study were in agreement with the findings of Al-Ankari et al. who found that the use of herbal mint (Mentha longifolia) in broiler chicken diets increased antibody titers against NDV, suggesting that essential oil stimulates the immune system. \(^{37}\) Furthermore, the results were in agreement with the findings of Mahmoodi Bardzardi et al who found that myrtle essential oil (MEO) at 200 mg/kg was more effective in increasing the antibody titers against Newcastle disease virus and AI virus. \(^{38}\)

**Conclusions**

The results of this study showed that receiving Antibiofin® at 0.1% and 0.2% concentrations, 14 and 28 days after vaccination, could increase the specific antibody titer against AI subtype H9N2 vaccine compared to the control group.

**Acknowledgments**

The authors are grateful to Shahid Chamran University of Ahvaz, Ahvaz, Iran, for supporting this study.

**Authors Contributions**

Study concept and design: Forough Talazadeh, Mansoor Mayahi, and Marziye Naghavi. Analysis and interpretation of data: Forough Talazadeh and Mansoor Mayahi. Drafting of the manuscript: Forough Talazadeh. Critical revision of the manuscript for important intellectual content: Forough Talazadeh and Mansoor Mayahi. Statistical analysis: Forough Talazadeh.

**Conflict of Interest Disclosures**

None.

**References**


11. Chandra RK. Nutrition and the immune system: an

---

**Table 1. Effect of Antibiofin® on HI Antibody Titer Against AI Vaccine**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (0.1%)</td>
<td>6.1 ± 0.53</td>
<td>5.1 ± 0.31&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.2 ± 0.94&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.5 ± 0.9&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>B (0.2%)</td>
<td>6.1 ± 0.53</td>
<td>5.2 ± 0.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.3 ± 0.65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.6 ± 0.83&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>C (vaccinated)</td>
<td>6.1 ± 0.53</td>
<td>4.2 ± 0.51&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>4.7 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 ± 0.79&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>D (unvaccinated)</td>
<td>6.1 ± 0.53</td>
<td>1.7 ± 0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.7 ± 0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.7 ± 0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The column with different superscripts are significantly different \(( P<0.05)\), *Mean ± standard deviation.*


