International Journal of
Enteric
Pathogens

Int J Enteric Pathog. 2016 August;4(3):e39396

Original Article

http://enterpathog.com

doi 10.15171/ijep.2016.04

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

Forough Talazadeh[1*], Mansoor Mayahi[1], Marziye Naghavi[2]

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

***Corresponding Author:**
Forough Talazadeh,
Tel: +98-6113330073;
Fax: +98-6113360807;
Email: ftalazade@gmail.com

Published Online May 27, 2016

Keywords: Avian influenza virus (AIV), Thyme extract, Immune response, Antibiofin®, Broiler chickens

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.[1] 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.[1] 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.[2] 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.[3-5] Therefore, in poultry industry, it is important to replace antibiotic growth promoters in the food by other substances.[6] Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut.[7] Recently some substances such as phytogenic feed additives, prebiotics, and probiotics have been used instead of antibiotics.[8,9] 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.[10,11] Natural products can be used as immunostimulants. Herbal plants mostly exert their beneficial effects on animal immune system by their secondary metabolites.[12] The immunostimulating activities of many of herbal plants have been studied in chicken, human, and mouse cell lines.[13-15] For example, the steroidal saponin 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.[16] *Gingko biloba* can mediate the production of inflammatory cytokines because it has flavonoids and terpenes.[17] Saponins can stimulate humoral and cellular immunities.[18] In some studies, they induced the production of cytokines such as interferons and interleukins.[19,20] The immunostimulant activity of saponins may be based on aldehyde groups or branched sugar chains[21] or an acyl residue bearing the aglycone.[20] The immunomodulatory effects of herbal plant polysaccharides have also been widely studied.[22,23] Qiu and Cui[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.[24] This phytosterol complex may target the TH1 and TH2 cells, improving T-lymphocyte and natural killer cell activities.[25] Moreover, Chinese herbs can help the development of immune organs such as the spleen and thymus[26] as well as increase of antibody production. Some herbs that are full of flavonoids such as thyme (*Thymus vulgaris*) increase the activity of vitamin C, act as antioxidants, and seem to improve the immune function.[27,28] Carvacrol and thymol are the main phenolic components in *Thymus vulgaris*.[29] This study was conducted to investigate the effect of different levels of Antibiofin® (mostly including *Thymus vulgaris* and other extracts such as *Salvia officinalis*, *Satureja hortensis*, and *Agastache foeniculum*) on systemic antibody responses against influenza disease vaccine in broiler chickens.

Objectives

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

Materials and Methods

Thyme Extract

Antibiofin® (mostly including thymus vulgaris and other extracts such as *Salvia officinalis*, *Satureja hortensis*, and *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®, respectively, in their drinking water from one week before vaccination till 2 weeks after vaccination. Chicks in group C did not receive Antibiofin® but were vaccinated against AI. Chicks 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 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.[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.[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 ($P<0.05$). 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 ($P<0.05$). Twenty-one days after vaccination, there was significant difference between group D and all other groups ($P<0.05$). Twenty-eight days after vaccination, there was significant difference between group D and all other groups ($P<0.05$). There was also significant difference between groups A and C and between groups B and C ($P<0.05$), 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® significantly increased the specific antibody response to AIV compared to all groups.

Discussion

Improving immunity in poultry production is of very

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

Groups	Days Postvaccination			
	0	14	21	28
A (0.1%)	6.1 ± 0.53	5.1 ± 0.31^{cd*}	5.2 ± 0.94^{d}	5.5 ± 0. 9 ^{cd}
B (0.2%)	6.1 ± 0.53	5.2. ± 0.6^{cd}	5.3. ± 0.65^{d}	5.6. ± 0.83 ^{cd}
C (vaccinated)	6.1 ± 0.53	4.2 ± 0.51^{abd}	4.7 ± 0.78^{d*}	4.8 ± 0.79^{abd}
D (unvaccinated)	6.1 ± 0.53	1.7 ± 0.53^{abc}	–^{abc}	–^{abc}

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

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

1. Zhu X, Yu W, McBride R, et al. Hemagglutinin homologue from H17N10 bat influenza virus exhibits divergent receptor-binding and pH-dependent fusion activities. Proc Natl Acad Sci U S A. 2013;110:1458-1463. doi:10.1073/pnas.1218509110.
2. Swayne DE, Halvorson DA. Influenza In: Saif YM, Fadly AM, Glisson JR, MCDougald LR, Nolan LK, Swayne DE, eds. Diseases of Poultry (Vol. 1).12th ed. Ames, Iowa: Blackwell publishing; 2008:153-174.
3. Cervantes H. Banning antibiotic growth promoters: Learning from the European experience. Poult Int. 2006;45:14-15.
4. Nollet L. AGP alternatives-part I. EU close to a future without antibiotic growth promoters. World Poult. 2005;21(6):14-15.
5. Wakeman GW. AGP alternatives- Part II. Dietary strategies to influencebacterial microflora. World Poult. 2005;21:28-29.
6. Bach Knudsen KE. Development of antibiotic resistance and options to replace antimicrobials in animal diets. Proc Nutr Soc. 2001;60(3):291-299. dx.doi.org/10.1079/pns2001109.
7. Vahdatpour T, Nikpiran H, Babazadeh D, Vahdatpourand S, Jafargholipour MA. Effects of Protexin®, Fermacto® and combination of them on blood enzymes and performance of Japanese quails (Coturnix Japonica). Annals of Biological Researches. 2011;2:283-291.
8. Patterson JA, Burkholder KM. Application of prebiotics and probiotics in poultry production. Poult Sci. 2003;82(4):627-631. dx.doi.org/10.1093/ps/82.4.627.
9. Ricke SC. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult Sci. 2003;82(4):632-639. dx.doi.org/10.1093/ps/82.4.632.
10. Kirk CK. Interaction between nutrition and infections disease infectious disease. In: Calnek BW, ed. Diseases of Poultry. Ames, IA: Iowa State University Press; 1997:73-74.
11. Chandra RK. Nutrition and the immune system: an

introduction. Am J Clin Nutr. 1997;66:460S-463S.
12. Hashemi SR, Zulkifli I, Hair Bejo M, Farida A, Somchit MN. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. Int J Pharmacol. 2008;4(5):352-360. doi:10.3923/ijp.2008.352.360.
13. Shan BE, Yoshida Y, Sugiura T, Yamashita U. Stimulating activity of Chinese medicinal herbs on human lymphocytes in vitro. Int J Immunopharmacol.1999;21(3):149-159. dx.doi.org/10.1016/s0192-0561(98)00074-5.
14. Cao LZ, Lin ZB. Regulatory effect of Ganoderma lucidum polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells in vitro. Acta Pharmacol Sin. 2003;24(4):312-326.
15. Lin ZB, Zhang HN. Anti-tumor and immunoregulatory activities of Ganoderma lucidum and its possible mechanisms. Acta Pharmacol Sin. 2004;25(11):1387-1395.
16. Tan BKH, Vanitha J. Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr Med Chem. 2004;11:1423-1430. dx.doi.org/10.2174/0929867043365161.
17. Li XY. Immuno-modulating components from Chinese medicines. Pharm Biol. 2000;38:33-40.
18. Oda K, Matsuda H, Murakami T, Katayama S, Ohgitani T, Yoshikawa M. Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biol Chem. 2000;381(1):67-74.
19. Jie YH, Cammisuli S, Baggiolini M. Immunomodulatory effects of Panax ginseung ca MEYER in the mouse. Agents Actions. 1984;15(3):386-391. dx.doi.org/10.1007/bf01972376.
20. Kensil CR. Saponins as vaccine adjuvants. Crit Rev Ther Drug Carrier Syst. 1996;13(1-2):1-55.
21. Bomford R, Stapleton M, Winsor S, et al. Adjuvanticity and ISCOM formation by structurally diverse saponins. Vaccine. 1992;10(9):572-577. dx.doi.org/10.1016/0264-410x(92)90435-m.
22. Nair PKR, Melnick SJ, Ramachandran R, Escalon E and Ramachandran C. Mechanism of macrophage activation by (1,4)-α-D-glucan isolated from Tinospora cordifolia. Int Immunopharmacol. 2006;6(12):1815-1824. dx.doi.org/10.1016/j.intimp.2006.07.028.
23. Chen W, Zhang W, Shen W, Wang K. Effects of the acid polysaccharide fraction isolated from a cultivated Cordyceps sinensis on macrophages in vitro. Cell Immunol. 2010;262:69-74. dx.doi.org/10.1016/j.cellimm.2010.01.001.
24. Qiu Y, Cui B. Effects of four polysaccharides on antibody titer and T-lymphocyte in vaccinated chicken. J Nanjing Agric Univ. 2008;31:77-78.
25. Bouic PJ, Lamprecht JH. Plant sterols and sterolins: A review of their immune-modulating properties. Altern Med Rev. 1999;4:170-177.
26. Gao X, Wu W. The effect of dang-gui (Angelica sinensis) and ferulic acid on mice immune system. Chin J Biochem Pharm. 1994;15:107-110.
27. Manach F, Regerat F, Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr Res. 1996;16(3):517-44. dx.doi.org/10.1016/0271-5317(96)00032-2.
28. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem. 1996;7(2):66-76. dx.doi.org/10.1016/0955-2863(95)00168-9.
29. Masada Y. Analysis of oils by gass chromatography and mass spectrometry. New York: Johan Wiley and Sons; 1976.
30. Alexander DJ. A review of avian influenza in different bird species. Vet Microbiol. 2000;74:3-13.
31. Rehman J, Dillow JM, Carter SM, Chou J, Le B, Maisel AS. Increased production of antigren-specific immunoglobulins G and M following in vivo treatment with medicinal plants Echinacea angustifolia and Hydratis canadensis. Immunol Lett. 1999;68(2–3):391-395. dx.doi.org/10.1016/s0165-2478(99)00085-1.
32. Cook NC, Samman S. Flavonoids chemistry, metabolism, cardio protective effects and dietary sources. J Nutr Biotecnol. 1996;7:66-76.
33. Manach F, Regerat F, Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr Res.1996;16(3):517-544. dx.doi.org/10.1016/0271-5317(96)00032-2.
34. Teymouri Zadeh Z, Rahimi S, Karimi Torshizi MA, Omidbaigi R. The effects of Thymus vulgaris L., Echinacea purpurea (L.) Moench., Allium sativum L. extracts and virginiamycin antibiotic on intestinal microflora population and immune system in broilers. Iranian Journal of Medicinal and Aromatic Plants. 2009;25(1):39-48.
35. Toghyani M, Tohidi M, Gheisari A, Tabeidian S. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr J Biotechnol. 2010;9(40):6819-6825.
36. Rahimi S, Teymouri Zadeh Z, Karimi Torshizi MA, Omidbaigi R, Rokni H. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. Journal of Agricultural Science and Technology. 2011;13:527-539.
37. Al-Ankari AS, Zaki MM, Al-Sultan SI. Use of habek mint (Mentha longifolia) in broiler chicken diets. Int J Poul Sci. 2004;3:629-634.
38. Mahmoodi Bardzardi M, Ghazanfari S, Salehi A, Sharifi SD. Growth performance, carcass characteristics, antibody titers and blood parameters in broiler chickens fed dietary myrtle (Myrtus communis) essential oil as an alternative to antibiotic growth promoter. Poult Sci J. 2014;2(1):37-49.