



# Immune response of broiler chickens supplemented with pediatric cough syrup including thyme extract in drinking water against influenza vaccine

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## ABSTRACT

**Introduction:** This study was conducted to investigate the effects of thyme extract in drinking water on immune response of broiler chickens.

**Methods:** A total of 245-day-old broiler chicks were purchased and 20 chicks were bled for determination maternal antibody and remaining chicks were divided into 5 equal groups. Chickens of group A, B and C received 0.1%, 0.15% and 0.2% of Pediatric Cough Syrup including thyme extract respectively in drinking water for all of the experimental period. Chickens of group D were not received Pediatric Cough Syrup but vaccinated against Influenza disease. Chickens of group E were kept as control group and were not received Pediatric Cough Syrup and Influenza disease vaccine. Chickens of group A, B, C and D were vaccinated with AI-ND killed vaccine (subtype H9N2), subcutaneously in neck back. Blood samples were collected before vaccination as well as on days 14, 28 and 35 after vaccination. Ten chickens of each group were bled randomly and antibody titer against influenza vaccine virus was determined by hemagglutination inhibition (HI) test.

**Results:** The results of the present study showed that Pediatric Cough Syrup including thyme extract at 0.2%, increased the specific antibody response against Influenza vaccine virus compared to all groups.

**Conclusion:** Pediatric Cough Syrup including thyme extract can improve the specific antibody response against Influenza vaccine virus.

### Implication for health policy/practice/research/medical education:

Utilization of immunostimulants is a solution to improve the immunity of animals and decrease their susceptibility to infectious disease. Herbs that are rich in flavonoids such as thyme (*Thymus vulgaris*) may extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function.

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## Introduction

Herbal plants have some effects on animal immune system including stimulation and suppression of the indicators of non-specific defense mechanism, humoral and cellular immunity. A critical determinant of immune responses is nutrition (1,2). The immunostimulating activities of a lot of herbal plants have been studied in chicken, human, and mouse cell lines (3-5). For example, steroidal ginseng saponins can stimulate immune system by production of cytokines (TNF- $\alpha$ , IL-2, IL6, and INF- $\gamma$ ), lymphocyte and macrophage activities (6). Due to possessing flavonoid and terpene compounds, *Ginkgo biloba* leaves are able to mediate production of inflammatory cytokines (7).

Saponins have the ability to stimulate humoral and cellular immunity (8) and induce production of cytokines such as interferons and interleukins (9,10). The immunostimulant activities of saponins are considered to be based on aldehyde groups or branched sugar chains (11) or an acyl residue bearing the aglycone (10). Immunomodulatory effects of polysaccharide-based herbal plants have also been widely studied (12,13). Qiu and Cui reported that administration of four Chinese herbs obtained from Astragalus root, Achyranthes root, Isatis root and Chinese Yam, could significantly enhance the antibody titers in vaccinated chicken. Beta-sitosterol and its glycoside are found to have profound immune modulating activities

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(14). This phytosterol complex targets the TH1 and TH2 cells, resulting in improved T-lymphocyte and natural killer cell activities (15). Chinese herbs can also help to develop immune organs, such as the spleen and thymus (16) as well as increase in antibody production. Some herbs such as thyme (*Thymus vulgaris*) are full of flavonoids. Carvacrol and thymol are the main phenolic components of *T. vulgaris* (17). Studies have shown that thyme plant (oil and its major constituents) possesses a strong antibacterial effect against *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus subtilis*, *Salmonella sonnei*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella typhimurium*, *Bacillus cereus*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella enteric* (18-24). In addition, performance promoting effects of essential oil, extract, powder or principal components of thyme have been demonstrated in poultry (25-30). Influenza viruses belong to the family Orthomyxoviridae. They are divided into three types, A, B, and C with segmented, negative-strand RNAs (31). All three types of influenza can infect humans, but only the type A viruses can infect birds and are collectively referred to as avian influenza (AI) viruses. Type A influenza viruses are divided into subtypes based on the hemagglutinin (HA) and neuraminidase (NA) proteins. Currently, 10 NA subtypes (N1-N9) and 17 HA subtypes (H1-H16) have been described (31). Due to the similar receptor binding epitopes with human influenza viruses, H9N2 virus will have a broader host range to infect humans. In addition, H9N2 AIV infection in chickens is latent and easily overlooked, increasing its chance of infecting humans. In 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.

Domestic poultry might show generalized clinical signs including decreased feed and water consumption, occasionally diarrhea, ruffled feathers, huddling, listlessness, lethargy, and decreased activity (32). This study was conducted to study the immune response of broiler chickens supplemented with Pediatric Cough Syrup including thyme extract in drinking water against Influenza vaccine.

## Materials and Methods

### Chickens

A total of 245-day-old broiler chicks (Ross 308) were procured. All chickens were divided into 5 groups and raised under standard conditions. Chickens of group A, B, C and D were submitted to vaccinations against AIV.

### Vaccine

AI-ND killed vaccine (subtype H9N2).

### Thyme extract

Pediatric Cough Syrup (*T. vulgaris* aqueous extract) was purchased commercially as solution (Dineh Iran- The Group of Pharmaceutical, Hygienic & Food industries).

The product contained 5 mg thymol/5 mL of the solution.

### Experimental design

Chickens of groups A, B and C received 0.1%, 1.5% and 0.2% of Pediatric Cough Syrup including thyme extract respectively in drinking water for all of the period of experiment. Chickens of group D did not receive Pediatric Cough Syrup but vaccinated against Influenza disease. Chickens of group E were kept as control group and did not receive Pediatric Cough Syrup and influenza vaccine. Chickens of groups A, B, C and D were vaccinated with AI-ND killed vaccine (subtype H9N2) subcutaneously of neck back.

### Blood collection and serological tests

Blood samples were collected before vaccination as well as on days 14, 28 and 35 post vaccination. Ten chickens of each group were bled randomly. Then, an antibody titer against influenza vaccine was determined by hemagglutination inhibition (HI) test. Blood samples were drained from the brachial vein and sera were separated, identified and frozen at -20°C until the serological tests were performed. Serum samples were analyzed by the HI test to detect antibodies against influenza vaccine.

### Microplate hemagglutination inhibition assay

Beta procedure of micro-plate HI test was performed in U-bottomed 96-well microtiter plates with 1% chicken erythrocytes to determine the antibody level of the sera samples collected from the chicks of different groups. The test was conducted using constant 4HA unit AIV and diluted.

### Statistical analysis

The titers obtained by HI were analyzed by SPSS software version 18.0. A 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

Based on the results obtained from Table 1, 14 days after vaccination, there was a significant difference between group C and all groups. Twenty-eight and 35 days after vaccination, antibody titers in groups A, B, and C were higher than group D, but there were no significant difference between groups A, B, C as compared to group D. Fourteen, 28 and 35 days after vaccination, group C showed the highest antibody levels. The results of present study suggest that Pediatric Cough Syrup including thyme extract is able to improve the specific antibody response against Influenza vaccine virus.

### Discussion

Natural products can be used as immune-stimulants. Herbal plants exert their beneficial effects on animal immune system mostly by secondary metabolites (33).

**Table 1.** Effect of probiotic on HI antibody titer against avian influenza disease virus

Days post-vaccination groups	0	14	28	35
A (0.1%)	6.7±0.29	2.37±0.9 <sup>c*</sup>	4±0.8 <sup>e</sup>	3.04±0.2 <sup>e</sup>
B (0.15%)		2.19±0.35 <sup>c</sup>	4.1±0.9 <sup>e</sup>	3.25±0.53 <sup>e</sup>
C (0.2%)		3.25±0.9 <sup>abde</sup>	4.3±0.9 <sup>e</sup>	3.47±0.4 <sup>e</sup>
D (vaccinated)		2.05±0.7 <sup>c</sup>	3.5±0.3 <sup>e</sup>	2.9±0.37 <sup>e</sup>
E (unvaccinated)		1.85±0.35 <sup>c</sup>	-	-

The column with different superscripts are significantly different ( $P < 0.05$ ).

\*Mean ± standard deviation.

The results of the present study showed that thyme extract at 0.2%, was able to improve the specific antibody response against Influenza vaccine virus, as compared to all other groups. In contrast to our results, Teymouri-Zadeh et al reported that there was no difference between *T. vulgaris* extract received birds and control group, when they studied the effect of *T. vulgaris* extract on antibody responses to red blood cell and Newcastle disease virus (34). Toghyani et al reported that 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 had no significantly differences with control birds (35). Furthermore, in disagreement with our results, Rahimi et al reported that dietary thyme extract (0.1%) soluble in water increased performance and lactic acid counts and reduced *E. coli* numbers but did not affect immune system compared with control group ( $P < 0.05$ ) (36). The weak results of thyme extracts on the immune system in some researches are probably related to type of thyme, the dose of additives, and also vaccination program times and stimulator materials used in their studies.

The results are 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. This suggests that the plant's essential oil may stimulate the immune system (37). Also the results of the present study are 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 antibody titers against Newcastle disease virus and avian influenza virus (38).

### Conclusion

The results of the present study show that Pediatric Cough Syrup including thyme extract is able to improve the specific antibody response against Influenza vaccine virus.

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### Authors' contributions

Study concept and design: FT and MM. Analysis and interpretation of data: FF and MM. Drafting of the manuscript: FT. Critical revision of the manuscript for

important intellectual content: FT and MM. Statistical analysis: FT

### Conflict of interests

The authors declared no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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