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Effect of *Thymus vulgaris* extract on systemic antibody responses against Influenza and Newcastle disease vaccine in broiler chickens

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ABSTRACT

Background & Aim: The recent study was conducted to investigate the effects of Thyme extract in drinking water on immune response of broiler chickens.

Experimental: A total of 245 day-old broiler chicks were purchased and 20 chicks were bled for determination maternal antibody and remaining chicks divided into 5 equal groups. Chickens of group A, B and C received 0.1%, 0.15% and 0.2% of Thyme extract in drinking water for all of the period of experiment, respectively. Chickens of group D did not receive Thyme extract but vaccinated against Newcastle and Influenza diseases. Chickens of group E were kept as control group and did not receive Thyme extract and Newcastle and Influenza diseases vaccines. Chickens of group A, B, C and D were vaccinated with live Newcastle vaccine (B1 strain) intraocularly and AI-ND killed vaccine (subtype H9N2) subcutaneously of 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 Newcastle and Influenza vaccine virus was determined by HI test.

Results & Discussion: Results indicted that the extract had no effect on antibody response against Newcastle vaccine virus, but 14 days after vaccination, receiving of 0.2% of extract, significantly increased the specific antibody response against Influenza vaccine compared to all groups.

Industrial and practical recommendations: Herbs that are rich in flavonoids such as thyme (*Thymus vulgaris*) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function.

1. Introduction

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, medical and public concerns focused on the complete removal of the antibiotics from animal feed in the European Union at the beginning of 2006 (Nollet, 2005; Wakeman, 2005; Cervantes, 2006). Therefore, poultry industry has been looking for the substances that could replace antibiotic growth promoters (AGP)

in the feed (Bach Knudsen, 2001). Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut (Vahdatpour *et al*., 2011). Recently some alternative components such as probiotics, prebiotics, organic acids and phytogenics fed additive have been introduced instead of antibiotics (Patterson and Burkhoder, 2003; Ricke, 2003). Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead, 2003). Medicinal plants and their essential oils have been used extensively in food products, perfumery, dental and oral products due to their different medicinal properties (Suppakul *et al*., 2003). Also in poultry production, it's very important to improve immunity so as to prevent infectious diseases. A variety of such factors as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is a solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Liu, 1999). Herbs that are rich in flavonoids such as thyme (*Thymus vulgaris*) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach *et al*., 1996; Cook and Samman, 1996). *Thymus vulgaris* is a medicinal herb in the *Lamiaceae* family, cultivated worldwide for culinary, cosmetic perennial and medical purposes. This species has special functions such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant (Hertrampf, 2001; Abu-Darwish *et al*., 2009). Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in Thymus vulgaris (Masada, 1976). Thyme (extract, oil, and the major components) have shown antibacterial activity against the *Clostridium botulinum, Clostridium perfringens*, *Bacillus subtilis*, *Salmonella sonnei*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella typhimurium*, *Bacillus cereus*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella enteric* reported in previous literatures (Nevas *et al*., 2004; Fan and Chen, 2001; Tabak *et al*., 1996; Juven *et al*., 1994; Ultee *et al*., 2000; Friedman *et al*., 2002; Thakare, 2004). Performance promoting effects of essential oil, extract, powder or principal components of thyme have been demonstrated in poultry (Al-Kassie, 2009; cross *et al*., 2007, Al-Mashhadani *et al*., 2011; Lee *et al*., 2003; Bolukbasi and Erhan., 2007; El-Ghousein and El-Beitawi., 2009). For example, supplementation of 200 ml Thyme extract per 1000 liter drinking waters in broiler chickens led to improvement of body weight gain and feed conversion ratio (Feisi and Bijanzad., 2010). Moreover, Rahimi *et al.* (2011) reported that dietary Thyme extract solution (0.1%) in water increased the performance and *lactic acid* counts whereas reduced the *Escherichia coli* numbers ($p < 0.05$). A variety of factors such as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Liu, 1999). Herbs that are rich in flavonoids such as thyme (*Thymus vulgaris*) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach *et al*., 1996; Cook and Samman, 1996). The current study was conducted to evaluate the potential of applying different levels of Thyme extract (*Thymus vulgaris*) on systemic antibody responses against Influenza and Newcastle disease vaccine in broiler chickens.

2. Materials and Methods

2.1. 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 and NDV.

2.2. Vaccinia

Hitchner B1 vaccine Cevac®, AI- ND killed vaccine (subtype H9N2).

2.3. Thyme extract

Thymus vulgaris aqueous extract was purchased commercially as solution (Dineh Iran - The Group of Pharmaceutical, Hygienic & Food industries (P.J.S)). The product contained 5 mg thymol per 5 ml of the solution.

2.4. Experimental design

Chickens of group A, B and C received 0.1%, 0.15% and 0.2% of Thyme extract respectively in drinking water for all of the period of experiment. Chickens of group D did not receive Thyme extract but vaccinated against Newcastle and Influenza diseases. Chickens of group E were kept as control group and did not receive Thyme extract and Newcastle and Influenza diseases vaccines. Chickens of group A, B, C and D were vaccinated with live Newcastle vaccine (B1 strain) intraocularly and AI-ND killed vaccine (subtype H9N2) subcutaneously of neck back.

2.5. 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 and antibody titer against Newcastle and Influenza vaccine virus was determined by 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 Hemagglutination inhibition test (HI) to detect antibodies against AIV and NDV (Alexander *et al*., 1983).

2.6. Microplate heamagglutination inhibition (HI) 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 ND virus and diluted.

2.7. Statistical analysis

The titers obtained by HI were submitted to analysis of variance using the Statistical Package for social Sciences (SPSS) version 18.0 program. One Way ANOVA LSD Test were 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%.

3. Results and discussion

Results of Table 1, indicated that before vaccination, there was no significant difference between all groups. On the other hand, 14, 28 and 35 days after vaccination, there was no significant difference between groups A, B, C as compared to the group D ,but groups A, B, C and D had significant difference as compared to the group E ($P < 0.05$). The results of present study showed that Thyme extract had no effect on antibody response against Newcastle vaccine virus.

The results of Table 2, indicated that before vaccination, there was not any significant difference between all groups, whereas 14 days after vaccination, there was significant difference between group C and all groups. Antibody titers in groups A, B, and C were higher than group D and E on the 28 and 35 days after vaccination, but there was not any significant difference between groups A, B, C as compared to group D. 14, 28 and 35 days after vaccination, group C has the highest antibody levels. The results of present study showed that14 days after vaccination, receiving of 0.2% of Thyme extract, significantly increased the specific antibody response to avian Influenza vaccine compared to all groups. In poultry industry, it's very important to improve immunity so as to prevent infectious diseases. A variety of such factors as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Liu, 1999). Herbs that are rich in flavonoids such as thyme (*Thymus vulgaris*) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach *et al*., 1996; Cook and Samman, 1996). In agreement with our results, Teymouri Zadeh *et al.* (2009) reported no significant difference in antibody responses to red blood cell and Newcastle disease viruses no between 0.1% *thymus vulgaris* extract received birds and control group (Teymouri Zadeh *et al*., 2009). None of the immune related parameters such as antibody titer against Newcastle, Influenza viruses and sheep red blood cell, heterophil to lymphocyte ratio and albumin to globulin ratio were differed significantly in broilers treated with 5 and 10 g/kg thyme powder while compared with control birds (Toghyani *et al*., 2010). Furthermore, Rahimi *et al*. (2011) 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 ($P<0.05$) (Rahimi *et al*.,2011).

Table 1. The effect of Thyme extract on HI antibody titer against Newcastle disease virus in broiler chicks.

groups	Days after vaccination			
	0	14	28	35
A (0.1% **)**	6.7±0.29	2.37±0.9 ^{C*}	4±0.8^{E}	3.04±0.2^{E}
B (0.15% **)**	6.7±0.29	2.19±0.35 ^{C}	4.1±0.9^{E}	3.25±0.53^{E}
C (0.2% **)**	6.7±0.29	3.25±0.9 ^{ABDE}	4.3±0.9^{E}	3.47±0.4^{E}
D (vaccinated)	6.7±0.29	2.05±0.7 ^{C}	3. 5±0.3^{E}	2.9±0.37^{E}
E (unvaccinated)	6.7±0.29	1.85±0.35 ^{C}	-	-

Different superscripts in the same column represent significant difference ($p < 0.05$).
*Mean ± standard deviation

Table 2. Effect of probiotic on HI antibody titer against avian Influenza disease virus.

groups	Days after vaccination			
	0	14	28	35
A (0.1% **)**	6.05±0.8	4.1±0.87^{e*}	5.54±0.82 ^{e}	4.72±0.9 ^{e}
B (0.15% **)**	6± 0.15	4.6±0.96 ^{e}	5.09±0.75 ^{e}	4.5±0.89^{e}
C (0.2% **)**	6.13±0.57	4.6±0.84 ^{e}	5.02±0.9^{e}	4.5±0.42 ^{e}
D (vaccinated)	5.9±0.33	4± 0.85 ^{e}	5.1±0.56 ^{e}	4.63±0.48 ^{e}
E (unvaccinated)	6.1±0.19	1.7±0.82^{abcd}	- ^{abcd}	- ^{abcd}

Different superscripts in the same column represent significant difference ($p < 0.05$).
*Mean ± standard deviation

The beneficial effects of thyme plant on bacterial and fungal activities and also potent antioxidant properties of major components of thyme essential oil such as thymol and carvacrol has been reported (Vincent 2002; Basilico and Basilico 1999). Considering the thyme characteristics, we anticipated that an increase in immune response of chicks would be observed. The lower results of thyme extract on immune system is probably related to the dose of additives, type of thyme, possess and preparation period and also vaccination program times and stimulator material that used in our study. Regarding this fact that a few reports are available on the impact of thyme or thyme component on bird immune response, more studies will be needed to investigate thyme extract immonomodulatory properties and principal components (Thymol and carvacol) on broiler health.

4. Conclusions

In conclusion, the results of the present study showed that supplementation of 0.1, 0.15 and 0.2% thyme extract in drinking water did not improve the systemic antibody responses against Newcastle disease vaccine in broiler chickens in the whole experimental period but 14 days after vaccination, receiving of 0.2% of Thyme extract significantly increased the specific antibody response against Influenza vaccine virus compared to all groups.

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