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Study on Immunomodulatory Activity of Dietary Garlic in Chickens Vaccinated against Avian Influenza Virus (Subtype H_9N_2)

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Abstract: Fresh garlic powder was evaluated for ability to potentiate the immune response of broiler chicks to Avian Influenza Virus (AIV) vaccine. For this purpose, 280 day-old chicks (Ross 308) were randomly allocated to 4 groups A, B (52 each) C and D (88 each). The birds in groups A and B were given control diet during the experiment, but those in groups C and D received diet supplemented with 1 and 3% garlic powder, respectively. After 2nd bleeding, half of the chicks in groups C and D were separated as groups E and F and fed control diet thereafter. On 9th day of age, the chicks in all groups except A were immunized subcutaneously against AIV (subtype H_9N_2) with a commercial oil-based inactivated vaccine (Merial, France). Fifteen chicks from each group were bled on days 14, 24 and 34 post vaccination and also 5 just before vaccination. The sera were used for antibody titration against AIV by both HI and ELISA tests. The results showed that antibody levels were considerably higher in the vaccinated chicks than those in the non-vaccinated control throughout the experimental period ($p<0.05$) but not affected by the treatment ($p>0.05$). In addition, the removal of garlic from diet had no significant ($p>0.05$) effect on serum titer. It is suggested that diet supplementation with garlic powder can not stimulate the humoral response of chickens against AIV vaccine.

Key words: Garlic, immunity, humoral, immunomodulator, broiler, H_9N_2

INTRODUCTION

Recent investigations are beginning to clarify the immunomodulatory properties of herbal substances in human and animals. Chinnah *et al.* (1992) reported a significantly higher antibody titer to Newcastle Disease Virus (NDV) as well as a sustained antibody level to Infectious Bursal Disease Virus (IBDV) in chickens; when they added a polydispersed beta-(1,4)-linked acetylated mannan (acemannan) to killed vaccines. A positive effect of oil extracted propolis on humoral immunity of broilers to ND, IBD and AI viruses was also observed by Taheri *et al.* (2005). As well, *in vitro* study elucidated that Echinacea extract has the ability to stimulate macrophage phagocytosis and NK cell synthesis of interferon-gamma (Groom *et al.*, 2007). Along with the evidence, Sullivan *et al.* (2008) found that oral administration of *E. purpurea* reduces bacterial burden in the spleen of mice infected by *Listeria monocytogenes*, demonstrating its efficacy *in vivo*.

Various preparations of garlic (*Allium sativum L.*) have been also reported to possess antimicrobial (Corzo-Martinez *et al.*, 2007) and immunomodulatory (Kyo *et al.*, 2001) activities in human. Animal studies showed that mice injected with garlic extracts had a significant increase of delayed type hypersensitivity response, but not of antibody response, to sheep Red Blood Cell (RBC) (Ghazanfari *et al.*, 2002). Moreover, Patya *et al.* (2004) reported that allicin, the main active component in garlic, has the potential to exhibit anti-tumor activity in mice. In a study performed by Tatara *et al.* (2008) an increased lysozyme and ceruloplasmin activity after Aged Garlic Extract (AGE) and allicin treatment was observed in early-weaned piglets. On the other hand, little is known about the interrelationship of garlic and chickens' immune system. Gabor *et al.* (1998) reported that in-water application of a liquid mixture of feed acidifiers, garlic and microbial cell extracts augmented the serological response of chickens to the vaccines prepared from inactivated ND and IBD viruses, but our previous study in chickens has showed that dietary garlic had no effect on haematological profile and humoral immunity to live NDV vaccine (Jafari *et al.*, 2008). However, in view of this point that immunomodulatory property could be antigen-dependent (Chinnah *et al.*, 1992; Taheri *et al.*, 2005) and of the global importance of AIV (subtype H_9N_2) and its responsibility for great losses of commercial poultry in Iran, an attempt was made to evaluate the effect of fresh garlic, in powder form, on serological response of broilers to AIV vaccine.

MATERIALS AND METHODS

Experimental design: From February to March 2008, a total of 280 day-old broiler chicks (Ross 308) were housed in poultry research section at Shahid Chamran University (Iran) and randomly allocated to 4 groups A, B (52 each) C and D (88 each). The birds in groups A and B received control mash diet (based on corn and

soybean) during the experiment, but those in groups C and D were fed diet supplemented with 1 and 3% fresh garlic powder, respectively. Furthermore, to evaluate the effect of consumption period of garlic on immune response, half of the chicks in groups C and D were separated after 2nd bleeding as groups E and F and were given control diet until the end of the experiment. All chicks were reared under sanitary conditions and fed for 6 weeks on diet formulated to meet the nutrient requirements of broiler (NRC, 1994). The feed and water were provided *ad libitum*. On 9th day of age, the chicks in all groups except A were immunized subcutaneously against AIV (subtype H_9N_2) with 0.25 mL of a commercial oil-based inactivated vaccine (Merial, France) into the lower back of the neck. Fifteen chicks from each group were bled via brachial vein on days 14, 24 and 34 post vaccination (pv) and also 5 just before vaccination. After collecting blood from chicks, they were marked with leg bands, so that they are not reused for blood collection. The separated sera by centrifugation (1000 rpm, 5 min) were stored at -20°C until the end of the experiment. The AIV-specific antibody levels were measured by conventional hemagglutination-inhibition test (4 HA unit of Ag) as per OIE standards (2008) and enzyme-linked immunosorbent assay (Synbiotic kit, USA). Statistical analysis was performed using one-way analysis of variance (Petrie and Watson, 2006). Differences showing $p<0.05$ were considered statistically significant.

Dietary garlic preparation: High quality garlic bulbs were purchased from local markets, peeled and cut into smaller pieces. Then, they were dried in oven (ISUZU, Japan) at 50-60°C to produce powder (Lawson, 1996). The prepared diets were stored at room temperature and used within utmost 3 days.

RESULTS AND DISCUSSION

The results of Table 1 and 2 show that maternal antibody titer to AIV significantly decreased with age in the non-vaccinated control chicks (group A) reached to a very low level at 14 days post vaccination and was not detectable afterwards, whereas the titers in vaccinated groups after a short-term decrease ($p<0.05$) remained at an almost constant level without any significant change ($p>0.05$) until 43 days of age. This finding indicated that humoral immune response to AIV was elicited after vaccination. In vaccinated chicks, decrease of serum titer in the 14 days after immunization could be due to the delay in immunostimulation by killed vaccine and formation of Ag-Ab complex in the presence of high maternal antibody, precipitating its elimination.

It was observed in the current study that there were no difference ($p>0.05$) among vaccinated groups in anti-AIV titers (Table 1, 2). In addition, the removal of garlic from diet had no significant ($p>0.05$) effect on serum titer. The lack of humoral specific response after garlic treatment

Table 1: Effect of dietary garlic on serum HI titer[1] (Log_2) in broiler chicks vaccinated against AI virus

Experimental groups	Days after vaccination			
	0*	14	24	34
A (0% garlic)[2]	5.6±0.23[a]	2.5±0.19[c]	0[d]	0[d]
B (0% garlic)	5.4±0.24[a]	4.1±0.21[b]	4.0±0.32[b]	3.6±0.25[b]
C (1% garlic)	5.6±0.24[a]	4.3±0.27[b]	3.9±0.35[b]	3.7±0.35[b]
D (3% garlic)	5.4±0.22[a]	4.4±0.28[ab]	4.1±0.27[b]	3.7±0.31[b]
E (0% garlic)[3]	-	-	3.8±0.32[b]	3.6±0.32[b]
F (0% garlic)[3]	-	-	4.0±0.25[b]	3.8±0.32

[b-d]Values within columns/rows with no common superscript differ significantly ($p<0.05$), [1]Values represent means±SE for each treatment; n = 5 (before vaccination), n = 15 (after vaccination). [2]Non-vaccinated control group, [3]Derived from treated groups after 2nd bleeding and were fed control diet thereafter, *9 days of age

Table 2: Effect of dietary garlic on serum ELISA titer[1] in broiler chicks vaccinated against AI virus

Experimental group	Days after vaccination			
	0*	14	24	34
A (0% garlic)[2]	4092±370[a]	1169±159[c]	0[d]	0[d]
B (0% garlic)	3909±351[a]	2657±243[b]	2665±334[b]	2366±262[b]
C (1% garlic)	3995±264[a]	2743±245[b]	2608±350[b]	2257±298[b]
D (3% garlic)	3841±178[a]	2817±239[b]	2719±245[b]	2415±281[b]
E (0% garlic)[3]	-	-	2663±227[b]	2381±304[b]
F (0% garlic)[3]	-	-	2629±228[b]	2374±253

[b-d]Values within columns/rows with no common superscript differ significantly ($p<0.05$), [1]Values represent means±SE for each treatment; n = 5 (before vaccination), n = 15 (after vaccination). [2]Non-vaccinated control group, [3]Derived from treated groups after 2nd bleeding and were fed control diet thereafter, *9 days of age

is in agreement with what described by Ghazanfari *et al.* (2002). The researchers injected mice intraperitoneally using 2 sources of garlic (freshly-prepared and commercial tablet extracts) for 5 days at doses of 1-300 mg kg^{-1}, but did not find any increase in anti-SRBC antibody level in comparison to control group. The present observation is also in accordance with our earlier study (Jafari *et al*., 2008) where inclusion of fresh garlic powder to the diet of broiler chicks proved to be unable to make any significant change in the hematological profile and anti-NDV antibody level. The obtained results may be somewhat supported by the study performed by Dorhoi *et al.* (2006) where standardized ethanol extract of garlic did not stimulate the proliferation of lymphocytes taken from laying hens and even impaired the phagocytic capacity of monocyte-derived macrophage culture at the concentration of 200 mg L^{-1}. However our data are in contrast to what reported by Gabor *et al.* (1998) where a liquid product, developed using feed acidifiers, garlic and microbial cell extracts, made a significant rise in serological response of broilers to inactivated NDV vaccine when it was applied in a concentration of 1 mg L^{-1} in drinking water beginning 2-3 days before parenteral vaccination and continued for 17-20 days. This discrepancy likely relates to the presence of other components in the product applied by Gabor *et al.* (1998) or to the type of garlic preparation. It is well-known that different garlic

preparations yield different active components and even with various amounts (Corzo-Martinez *et al*., 2007; Lanzotti, 2006). Therefore, the other possible reason for the negative results is that the immunologically active constituents may have not been in sufficient amount to stimulate humoral specific immunity when garlic powder is added to diet up to level of 3%. Besides, the effect of bird strain on the response to a given garlic preparation should not be ignored. For example, Chowdhury *et al*. (2002) investigated the effect of sun-dried garlic paste on reproduction parameters in different strains of laying hens and found significantly different responses in some traits among the strains.

With due attention to the results obtained from the current study, it is suggested that diet supplementation with garlic powder can not enhance the serological response of broilers to AIV vaccine. However, an overall judgement about immunomodulatory properties of garlic in chickens needs more studies with other garlic preparations, particularly purified active components like allicin and also an evaluation of humoral non-specific defense mechanisms.

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