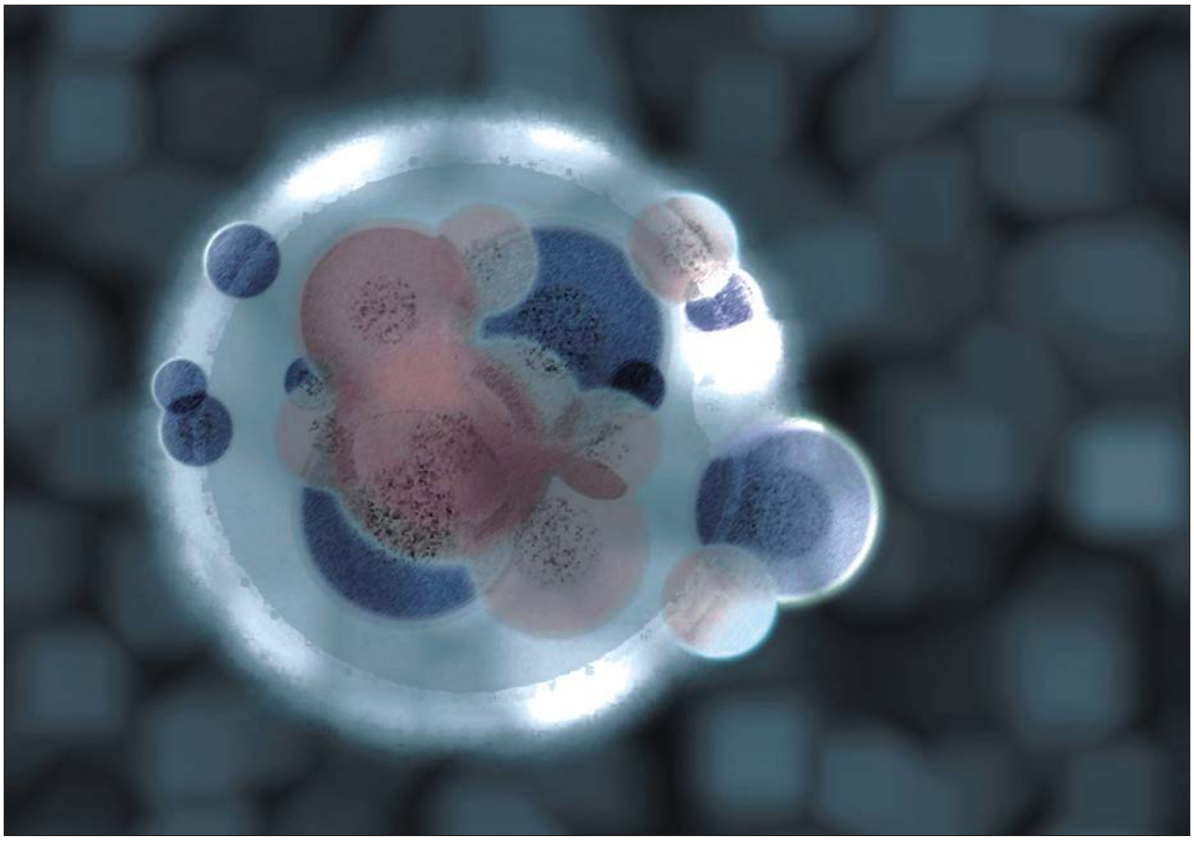

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Effect of Dietary Garlic on Serum Antibody Titer Against Newcastle Disease Vaccine in Broiler Chicks

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Abstract: The aim of this study was to investigate the effect of garlic, in powder form, on serological response of broilers to NDV (Newcastle Disease Virus) vaccine. Two hundred and eighty, day-old, Ross chicks were randomly divided into groups A, B (52 each), C and D (88 each). The birds in groups A and B received control diet during the experiment, but those in groups C and D were given control 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 afterwards. At 9 days of age, all groups except A were inoculated with a killed NDV vaccine (Merial, France) subcutaneously and with B_1 strain (Merial, France) by eye drop. Fifteen chicks were bled from each group on days 14, 24 and 34 post vaccination and also five just before vaccination. The sera were assayed for antibody against NDV by both HI and ELISA tests. The results showed that antibody titers were significantly higher in vaccinated chicks than in non-vaccinated chicks ($p<0.05$), but there were no significant effects of treatment on antibody level ($p>0.05$). Furthermore, the removal of garlic from diet had no effect on serum titer ($p>0.05$). It is concluded that diet supplementation with garlic powder can not enhance the serological response of broilers to NDV vaccine.

Key words: Garlic, Newcastle disease virus, immunomodulator, broiler chicks

INTRODUCTION

Historically, garlic (*Allium sativum* L.) has been used worldwide for medicinal applications. In the last decades, several clinical reports, including meta-analysis, have demonstrated the hypocholesterolemic and hypotensive effects of garlic in humans (Durak *et al*., 2004). *In vitro* studies have also documented that various garlic preparations have antifungal (Lemar, *et al*., 2005), antiparasitic (Coppi *et al*., 2006), antiviral (Terrasson *et al*., 2007) and antibacterial (Lee *et al*., 2008) properties against human's pathogens.

Animal studies showed that allicin, the main biologically active component in garlic, has the potential to exhibit anti-tumor activity in mice (Patya *et al*., 2004). Moreover, Ghazanfari *et al*. (2002) injected mice intraperitoneally with different doses of garlic extracts and found a significant increase of delayed type hypersensitivity response, but not of antibody response, to sheep Red Blood Cell (RBC). The positive response of humoral non-specific defense mechanisms like lysozyme and ceruloplasmin activity after Aged Garlic Extract (AGE) and allicin treatment was reported in early-weaned piglets in a study performed by Tatara *et al*. (2008). But the literature is very limited regarding the effect of garlic on immune system in broilers. Gabor *et al*. (1998) reported that a liquid product containing feed acidifiers, garlic and microbial cell extracts augmented the serological response to the vaccine prepared from inactivated NDV. The literature shows that studies made on garlic have been accompanied with different results in some cases. For example, Birrenkott *et al*. (2000) reported that 3% garlic powder did not have any significant effect on serum and yolk cholesterol in laying hens fed diets for 8 months, whereas Yalcin *et al*. (2006) observed a significantly lower serum and yolk cholesterol ($p<0.01$) when laying hens were given for 22 week diets containing 1% garlic powder. Although the reason for this is unknown, it likely relates to preparation methods, the stability of chemical components and the duration of the study (Amagase *et al*., 2001). Thus, the aim of the study was to investigate the effect of garlic, in powder form, on serological response of broilers to NDV vaccine.

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MATERIALS AND METHODS

Experimental design: From February to March 2008, a flock of 280 day old, Ross × Ross broiler chicks were housed in poultry research section at Shahid Chamran University (Iran) and randomly divided into groups A, B (52 each), C and D (88 each). The birds in groups A and B received control mash diet during the experiment, but those in groups C and D were fed control diet supplemented with 1 and 3% garlic powder, respectively. Also, 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 fed for 6 weeks on broiler diet formulated to meet NRC requirements. This diet was available *ad libitum*, along with water. At 9 days of age, all groups except A were inoculated with a killed NDV vaccine (Merial, France) subcutaneously and with B_1 strain (Merial, France) by eye drop. Fifteen chicks were bled via wing vein from each group on days 14, 24 and 34 post vaccination and also five just before vaccination. After collecting blood from chicks, they were marked with leg bands, so that they are not reused for blood collection. The sera were stored at -20°C until the end of the experiment. The NDV-specific antibody levels were measured by conventional hemagglutination-inhibition test (4 HA unit of Ag) according to Thayer and Beard (1998) 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 to 60°C to produce powder. Prepared diets were stored at room temperature and used within utmost three days.

RESULTS AND DISCUSSION

The results of Table 1 and 2 show that maternal antibody titer to NDV significantly decreased with age in the non-vaccinated control chicks (group A) and reached to a very low level at the age of 23 days, whereas it had a noticeable increase ($p<0.05$) in all vaccinated groups until 43 days of age. This finding indicated that humoral immune response to NDV was elicited after vaccination.

It was observed in the current study that there were no difference ($p>0.05$) among vaccinated groups in anti-NDV titers (Table 1, 2). The lack of humoral specific response after garlic treatment is in agreement with what described by Ghazanfari *et al.* (2002). The researchers injected mice intraperitoneally using two 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. Also, 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 present 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. Although garlic powder represents the composition of garlic cloves better than any other type of processed garlic, some changes do occur during processing. Therefore, the other possible

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

Experimental groups	Days after vaccination			
	0*	14	24	34
A (0% garlic)[2]	3.6±0.4[a]	1.4±0.16[c]	0[e]	0[e]
B (0% garlic)	3.8±0.2[a]	5.4±0.29[b]	6.3±0.16[c]	7.1±0.27[d]
C (1% garlic)	3.4±0.4[a]	5.6±0.27[b]	6.5±0.18[c]	7.0±0.23[d]
D (3% garlic)	2.8±0.6[a]	5.2±0.38[b]	6.1±0.19[c]	6.8±0.25[d]
E (0% garlic)[3]	-	-	6.1±0.21[c]	7.1±0.13[d]
F (0% garlic)[3]	-	-	6.5±0.20[c]	6.9±0.22[d]

[a-e]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, *Nine days of age

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

Experimental groups	Days after vaccination			
	0*	14	24	34
A (0% garlic)[2]	397±75[a]	0[e]	0[e]	0[e]
B (0% garlic)	385±99[a]	2178±468[b]	4801±706[c]	8078±815[d]
C (1% garlic)	420±72[a]	2203±231[b]	5326±654[c]	7076±622[d]
D (3% garlic)	310±120[a]	1885±251[b]	6005±526[c]	8133±627[d]
E (0% garlic)[3]	-	-	5701±633[c]	8141±650[d]
F (0% garlic)[3]	-	-	5057±477[c]	7061±953[d]

[a-e]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, *Nine days of age

reason for the negative results is that the active constituents may have not been sufficient to stimulate humoral specific immunity when garlic powder is added to diet up to level of 3%; although the effect of bird strain on the response to a specific garlic preparation should not be ignored. 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.

In view of the results obtained from the current study, it is concluded that diet supplementation with garlic powder can not enhance the serological response of broilers to NDV vaccine. However, an overall judgement about immunomodulatory properties of garlic in chickens needs further studies with other garlic preparation, especially purified active components like allicin and also an evaluation of humoral non-specific defense mechanisms.

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