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Effect of Dietary Garlic on Immune Response of Broiler Chicks to Live Newcastle Disease Vaccine

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Abstract: This study was designed to determine the effect of garlic powder on humoral immune response of broilers against NDV (Newcastle Disease Virus) vaccine. Two hundred and forty, two-day-old, Ross chicks were randomly assigned into 4 groups of 60 birds each. Chicks in groups 1 and 2 were given control mash diet during the experiment (6 week), but those in groups 3 and 4 were fed on control diet supplemented with 1 and 3% garlic powder, respectively. All groups except number 1 were vaccinated by eye-drop with B1 strain (Pestikal, Croatia) at 9 and 18 days of age. Ten blood samples were taken from each group on days 0, 14, 24 and 34 after first vaccination. The serum antibody level against NDV was measured by both HI and ELISA tests. The EDTA-mixed blood samples were examined for total and differential leukocyte count. The results showed that antibody titers in vaccinated chicks were significantly more than in non-vaccinated chicks ($p<0.05$), but not influenced by the diet ($p>0.05$). A significant increase of total leukocyte and percentage of lymphocytes was observed in vaccinated chicks 14 days after vaccination, but there were no difference ($p>0.05$) among vaccinated groups. It is concluded that inclusion of garlic powder to the diet of broilers don't have any beneficial effect on humoral immune response to live NDV vaccine.

Key words: Garlic, immune response, blood parameters, broiler

INTRODUCTION

Garlic was believed in the folklore of many cultures to be effective in curing ailments (Amagase *et al*., 2001). In the last decades, several clinical reports, including meta-analysis, have described the hypocholesterolemic effect of garlic in humans (Stevinson *et al*., 2000). *In vitro* studies have also shown that various garlic preparations have antibacterial (Bakri and Douglas, 2005), antiviral (Weber *et al*., 1992), antifungal (Lawson, 1996) and antiparasitic (Ankri *et al*., 1997) properties against human's pathogens.

Animal studies suggested that allicin, the main biologically active component in garlic, has the potential to exhibit anti-tumor activity in mice (Patya *et al*., 2004). Also, 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 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 when laying hens were fed diets for 8 month, whereas Chowdhury *et al*. (2002) observed a significantly lower serum and yolk cholesterol when laying hens were fed for 6 week on diets supplemented with 2-10% sun-dried garlic paste. 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). Therefore, the first objective of this study was to further study the effect of garlic, in

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powder form, on serological response of broilers to live NDV vaccine. The second objective was to examine the quantitative changes of blood leukocytes as functional parameters in immune responses.

MATERIALS AND METHODS

Experimental design: From October to November 2007, two hundred and forty, two-day-old, male Ross x Ross broiler chicks were housed in poultry research section of Shahid Chamran University (Iran) and randomly divided into 4 groups of 60 birds each. Chicks in groups 1 and 2 were given control mash diet (based on corn and soybean meal), but those in groups 3 and 4 were fed on control diet supplemented with 1 and 3% garlic powder, respectively. All chicks were fed for 6 week on rations formulated to meet the nutrient requirements of broilers (National Research Council, 1994). Feed and water were provided *ad libitum*. All birds except those in group 1 were vaccinated by eye-drop with B1 strain (Pestikal, Croatia) at 9 and 18 day of age. Ten blood samples were taken via wing vein from each group on days 0, 14, 24 and 34 after first vaccination. The sera were stored at -20°C until the end of the experiment. The assessment of NDV-specific antibody levels were made by conventional hemagglutination-inhibition test (4 HA unit of Ag) as per Thayer and Beard (1998) and enzyme-linked immunosorbent assay (Synbiotic kit, USA). EDTA-mixed blood samples were examined for total and differential leukocyte count using Natt and Herrick solution and Wright staining according to Dein (1986). The data were analyzed as one-way design by analysis of variance (Petrie and Watson, 2006). The significance of data was set at $p<0.05$.

Preparation method for dietary garlic: High quality garlic bulbs were purchased from local markets, peeled and cut into smaller pieces. Then, they were dried in oven at 50 to 60°C to produce powder. Diets were prepared the following day and were stored at room temperature for a maximum of two weeks.

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 1) and reached to a very slight level at the age of 23 days, whereas it had a remarkable increase ($p<0.05$) in all vaccinated groups until 33 days of age. This finding suggested that humoral immune response to NDV was elicited after vaccination.

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

	Days after vaccination			
Diet	0*	14	24	34
0% garlic[2]	3.0±0.32[a]	1.3±0.31[b]	0[c]	0[c]
0% garlic	2.9±0.38[a]	4.2±0.30[c]	5.8±0.26[b]	5.6±0.26[b]
1% garlic	3.2±0.37[a]	4.1±0.26[c]	5.6±0.31[b]	5.5±0.27[b]
3% garlic	3.4±0.41[a]	4.3±0.25[c]	5.7±0.30[b]	5.5±0.31[b]

[a-c]Values in columns/rows with no common superscript differ significantly ($p<0.05$), [1]Values represent means±SE for each treatment; n = 10. [2]Non-vaccinated control group, *First vaccination at 9 days of age

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

	Days after vaccination			
Diet	0*	14	24	34
0% garlic[2]	412±53[a]	0[b]	0[b]	0[b]
0% garlic	397±56[a]	1377±179[c]	2639±348[d]	2449±279[d]
1% garlic	484±72[a]	1332±162[c]	2801±312[d]	2650±191[d]
3% garlic	459±62[a]	1368±117[c]	2839±305[d]	2509±370[d]

[a-d]Values in columns/rows with no common superscript differ significantly ($p<0.05$). [1]Values represent means±SE for each treatment; n = 10. [2]Non-vaccinated control group. *First vaccination at 9 days of age

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). Ghazanfari *et al.* (2002) 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. However 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 (Gabor *et al.*, 1998). This discrepancy likely relates to the difference in the type of vaccine (live vs. killed), the type of garlic preparation or the presence of other components in the product applied by Gabor *et al.* (1998). Also, the strain of bird may affect the response to a given garlic preparation. Chowdhury *et al.* (2002) studied 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. Garlic powder represent the composition of garlic cloves better than any other type of processed garlic, however some changes do occur during processing. Therefore, the other possible reason for the negative results is that the active constituents may not have been sufficient to stimulate humoral immunity when garlic powder is added to diet up to level of 3%.

A significant increase of total leukocyte and percentage of lymphocytes was observed in vaccinated chicks 14 days after vaccination (Table 3, 4),

Table 3: Effect of dietary garlic on total leukocyte count[1] in broiler chicks vaccinated against ND virus

	Days after vaccination			
Diet	0*	14	24	34
0% garlic[2]	17468±1325[a]	19610±1697[a]	21320±1726[b]	2212±1735[b]
0% garlic	7389±1408[a]	26850±1895[b]	22480±1680[b]	22540±1512[b]
1% garlic	16856±1418[a]	26395±1782[b]	23120±1675[b]	23345±1578[b]
3% garlic	16995±1516[a]	27320±1986[b]	21985±1802[b]	21899±1771[b]

[a-c]Values in columns with no common superscript differ significantly ($p<0.05$). [1]Values are number of WBC/μL and represent means±SE; n = 10. [2]Non-vaccinated control group. *First vaccination at 9 days of age

Table 4: Effect of dietary garlic on differential leukocyte count[1] in broiler chicks vaccinated against ND virus

		Garlic supplementation of diet (%)			
Days after vaccination	Diff. WBC (%)	0*	0	1	3
0**	Lym	69.6±2.9[a]	71.6±3.1[a]	68.4±1.7[a]	70.1±1.9[a]
	Mono	4.0±0.7[a]	3.6±0.7[a]	3.7±0.5[a]	3.6±0.4[a]
	Hetero	25.0±1.5[a]	23.0±1.6[a]	26.7±2.1[a]	24.4±1.8[a]
	Eosino	1.4±0.3[a]	1.8±0.4[a]	1.2±0.3[a]	1.9±0.4[a]
14	Lym	72.9±1.8[a]	78.1±3.1[b]	77.3±2.4[b]	77.9±3.1[b]
	Mono	6.0±0.9[a]	4.7±0.7[a]	5.5±0.6[a]	4.6±0.6[a]
	Hetero	18.5±1.7[a]	15.3±1.5[a]	15.7±1.1[a]	15.7±1.4[a]
	Eosino	2.6±0.7[a]	1.9±0.5[a]	1.5±0.5[a]	1.8±0.4[a]
24	Lym	73.8±2.8[a]	76.1±2.3[a]	75.1±1.8[a]	75.9±2.7[a]
	Mono	7.0±0.8[a]	6.6±0.8[a]	7.5±1.0[a]	6.9±0.7[a]
	Hetero	16.4±0.9[a]	14.8±0.9[a]	15.4±1.0[a]	15.1±1.1[a]
	Eosino	2.8±0.6[a]	2.5±0.5[a]	2.0±0.5[a]	2.1±0.6[a]
34	Lym	74.0±2.7[a]	74.9±2.2[a]	73.5±1.7[a]	75.1±2.6[a]
	Mono	8.2±0.9[a]	7.1±0.7[a]	8.5±1.1[a]	7.6±1.2[a]
	Hetero	14.5±0.8[a]	15.1±0.8[a]	15.0±1.0[a]	14.8±0.7[a]
	Eosino	3.3±0.5[a]	2.9±0.7[a]	3.0±0.8[a]	2.5±0.5[a]

[a-b]Values in rows with no common superscript differ significantly ($p<0.05$). [1]Values represent means±SE for each treatment; n = 10. *Non-vaccinated control group. **First vaccination at 9 days of age

suggesting that immune response was activated by vaccine, but there were no difference ($p>0.05$) among vaccinated groups, being in agreement with serological finding. To the best of our knowledge, the effect of dietary garlic on hematological parameters in broilers has not been previously reported in the literature, although Dorhoi *et al.* (2006) reported that the 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 200 mg L^{-1}.

Considering the results obtained from the current study, it is concluded that inclusion of garlic powder to the diet of broilers does not have any beneficial effect on serological response against NDV.

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